Advances in Flavours and Fragrances
From the Sensation to the Synthesis

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From the Sensation to the Synthesis

Edited by

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Preface

This book is a compilation of sixteen of the twenty papers presented at the 2001 RSC/SCI flavours and fragrances conference at Scarman House, University of Warwick.

The meeting was spaced over two and a half days and saw speakers and delegates from all corners of the world exchanging ideas and information.

The aim of the meeting was to bring together scientists from both industry and the academic world, who have a common interest in the chemistry of flavours and fragrances. The subject matter was intentionally broad, covering areas such as biochemistry of receptors/structure activity relationships, analytical techniques, natural products/essential oils, organic and bioorganic chemistry, and flavours/foods. The book is divided into the same sections as the original meeting.

The chapters contained in this book have been rapidly edited and proof read by the editor only. Every effort has been made to ensure that no mistakes are present but inevitably it is likely that some still exist! The editor also asks that the reader is understanding of the fact that most chapters have been written by people who are not native English speakers.

Finally, I would like to thank everybody who contributed to the 2001 conference and made it such a success.

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STRUCTURE ACTIVITY RELATIONSHIPS AND THE SUBJECTIVITY OF ODOUR SENSATION

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1 INTRODUCTION

Structure activity relationships (SAR) are one of the most useful sets of tools in both pharmaceutical and fragrance research. Ever since Amoore carried out his studies and formulated his theory of odour recognition, chemists have been looking at the shape of molecules and their associative possibilities to find clues that would explain perceived odour sensations. How difficult it is to go down this research path in finding new chemical entities with interesting odour qualities is clear from the broad variety of odours the human nose is able to detect and identify. I will now attempt to explain how complex the activity side of SAR can be and what the consequences of this complexity are.

In this context I will again follow up the question which Pieter Aarts recently put at the top of an article [1], although he was dealing with a totally different subject: "The Optimal Fragrance - Lucky Shot or Organised Hunt?"

The sense of smell is even able to discriminate between the antipodes of chemical structures like R- and S-carvone or R- and S-p-menthene-8-thiol [2]. When a perfume layman, like a chemist, tries to verify the reported odour descriptions, he becomes aware that the difference between the odours of chemically similar substances is dependent on purity, concentration in your nose, your sniffing technique, the way the air streams through your nose [3], and much more.

As Charles Sell tells us in a remarkable report about structure/odour correlations entitled "The Mechanism of Olfaction and the Design of Novel Fragrance Ingredients" [4], it is sometimes a trace impurity which fundamentally changes the scent of a substance or a mixture of substances.

2 AMOORE'S CONCEPT OF PRIMARY ODOURS

Let us start with John E. Amoore's [5] theory of odour reception (figure 1), which is based on specific anosmia and the concept of primary odours. What I understand about his idea is that he tried to find chemical structures by using the holes in the olfactory epithelium and a negative selection of substances that were reported as resulting in specific anosmia.

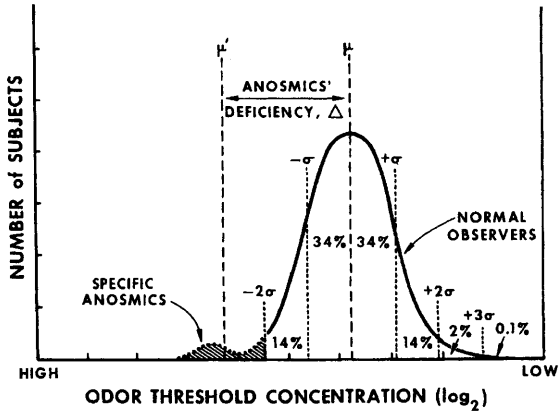


Figure 1 *John E. Amoore's theory of odour reception*

In terms of SAR, this would mean he was searching for chemicals with no activity. And from the shape of the molecules he found in this way he tried to reconstruct a receptor site which could in size and shape accept this chemical structure (figure 2). The goal of his studies was a classification of odours by collecting groups of similar molecules, which could fit, specifically into the same receptor. Amoore was limited in his approach to the choice of known substances and he was also dependent on the odour descriptions he was given by the experts. My opinion in this context is that Amoore could never definitely know whether a substance, which would bind to the same specific receptor, would cause the same odour sensations and associations. In other words, he grouped various chemicals together, guided by the similar odour descriptions for those materials.

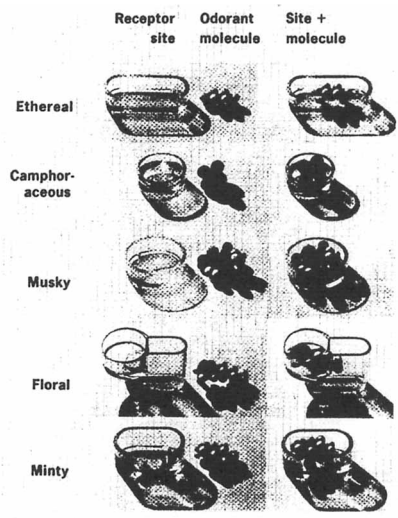


Figure 2

3 SPECIFIC ANOSMIA AND THE CONCEPT OF PRIMARY ODOURS

I have to admit at this point that I have a problem. My problem is with specific anosmia, which is the basis of Amoore's theory of olfaction. The way Amoore measured specific anosmia demonstrated the usefulness of his approach and proved the reality of this phenomenon. However, the results are not useful to classify scents; they only caused chemists to focus on molecules for which there would probably be a specific receptor in the nasal mucous membrane. When a chemist looks at the structures found by Amoore they are surprised to find four small molecules like trimethylamine and isobutyric aldehyde, alongside two very large molecules like androstenone and pentadecanolide (figure 3).

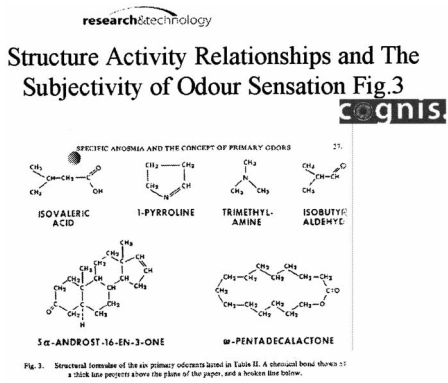


Figure 3 *Structure activity relationships and the subjectivity of odour sensation*

Those who are able to smell androstenone with 19 carbon atoms describe it as reminding them of stale sweat. Isovaleric acid, a molecule with 5 carbon atoms, is almost officially said to smell sweaty. So, am I to believe that a molecule with 19 carbon atoms is bound to the same specific receptor as a similarly smelling compound containing 5 carbon atoms? The Amoore approach is most interesting because, when you think about it, in the end it doesn't tell you much about the structural side of SAR, nor does it tell you much about the activity on the side of the receptor, but it raises the question of what specific anosmia means. What is the sense of lacking receptors?

When we at Cognis were searching for new sandalwood substances, I noticed that I became anosmic to Sandelice®; first only on Fridays, then later all the time (figure 4).

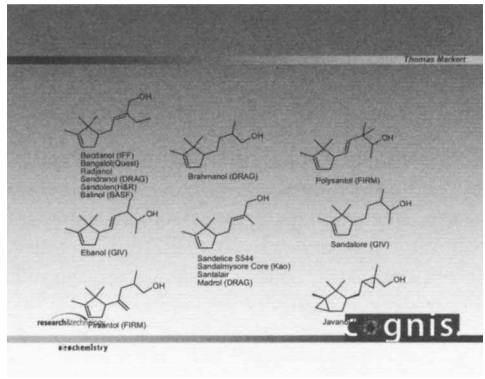


Figure 4 *Sandelice, lost in a forest of numerous sandalwood trees*

Then I noticed that my anosmia was a hyperosmia. I was so sensitive to Sandelice® that I had the odour impression for a fraction of a second and then my nose had adapted. So adaptation can also look like anosmia. By contrast, I am truly anosmic to androstenone. True specific anosmics smell the impurities in the compounds. So, although I'm training

on our androstenone sample, to me it smells a little bit cedar-woody but not at all like urine or stale sweat. Others nearly had their noses blasted off when they opened the bottle. So I consider that the purity of our androstenone sample is very good.

3.1 What anosmics smell?

When the results of The Smell Survey were published by National Geographic in 1987 [6], I thought how unhappy the 1.2% of people who were suffering from total anosmia must feel. I thought those people would neither smell nor taste anything so delicious as truffles or foie gras. This is by far not the case. I learned from one of my neighbours who lives a few houses away from ours that her bulbus olfactorius had been severed in a car crash. But she is still able to taste and to smell. - Though she might need more cigarettes or beer to have the same activity effect as osmic people. - And I wondered how this could happen without the ability to smell. Then I read [7] about people who, though lacking a sense of smell, were able to cook, detect dry or humid air, and more. At least taste is working well in anosmics.

By thinking about the odour impressions of people lacking olfaction, I found the explanation for some unusual odour descriptions. What do you think a powdery or dusty scent should mean? Would it be a powder or dust that would enter your nose? We were once purifying the essential oil of pinus longifolia and when I smelled the fractions, I immediately imagined smelling powdered bellpepper from a pepper pot. The visual picture of a liquid in a distillation bulb did not fit the odour impression of a powder. Then suddenly I had an idea about what could be the explanation for this curious phenomenon. Like every mucous membrane, the olfactory epithelium is sensitive to touch as well. In other words, your nose does not just smell things, it also feels them (figure 5).

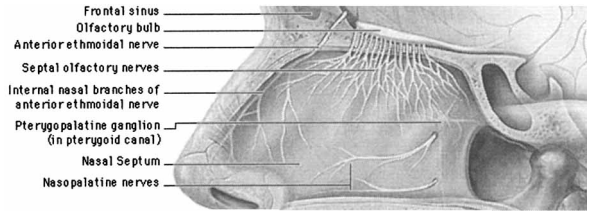


Figure 5

Our sense of taste is based on touch. The only reason we boil our soup or coffee is that we like it hot. Umami (monosodium glutamate) is discussed as a fifth taste quality but it could also work as a transporter of tastemakers (my personal name for taste enhancers) – comparable to the odour binding proteins – by distributing aroma components in your mouth. The result is called mouthfeel. Touch, pain, or trigeminal reception is what anosmics smell, and probably osmics as well.

3.2 The Kallmann syndrome

Kallmann's syndrome is a neuronal migration defect, which also affects olfactory system development. To test the functioning of olfaction with patients suffering from Kallmann's syndrome, doctors use common fragrance materials. In this way it was found that many fragrances have a strong trigeminal component. Anosmic patients were able to assign odour descriptions to fragrances without using olfactorial nerves. So the information must

have been transported by a nerve other than the bulbus olfactorius. In his report about "Trigeminal Perception of Odorant Quality in Congenitally Anosmic Subjects" [8], Matthias Laska presents a list of compounds eliciting strong trigeminal responses, which sounds like Amoore's list of primary odours (figure 6).

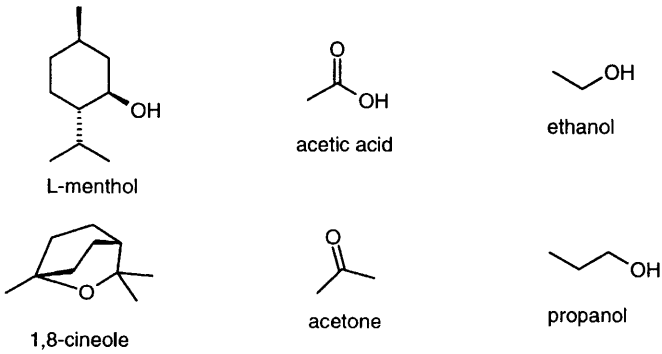


Figure 6

Again there are four smaller molecules like acetic acid, acetone or ethanol and two larger entities (-)-menthol and 1,8-cineole. The existence of trigeminal sensation, which I would like to call feeling chemicals, is well known from von Skramliks' [9] experiments that were published in 1926. He found a trigeminal component in odorants by monorhinal application. When the test person could detect whether the odorant entered the left or right nostril this was by trigeminal irritation, because olfactorial reception is not able to identify the direction from which the smell has approached the nasal cavity. To test the extent of the trigeminal nerve stimulus within a sniffing process, monorhinal application is recommended.

So what do we learn from those results?

4 THE ACTIVITY SIDE OF SAR REASSESSED

In a report called "Clinical Testing of Olfaction Reassessed" A.J.Pinching [10] speaks of the "poor smell vocabulary of most humans, which was regarded as a barrier to interpretation of olfactory tests. However it has become clear that the great majority of odours have a trigeminal component to their detection." Let us now take a closer look at odour descriptions. In SAR they represent the activity part of the relationship, and the accuracy of this part should be as scientific as the knowledge about the chemical structure. But this is by no means the case. I do not dispute the trigeminal component of odour sensation. What I think is rather that you have a pain sensation in your odour description that does not come from an olfactory reception site. That means that many impressions may stem from stimulating trigeminal nerve endings and you consider them to be your odour reception, not knowing or even wanting to know that you as an individual suffer from specific anosmia regarding this particular scent.

As a perfumer you cannot tell everybody that you are not able to smell floral or musk substances, because you would have all the marketing people crowding round trying to sell you those substances. That was how I learned that a little cedarwood effect could be my reception of androstenone. This is enough to live with, but not enough to detect truffles, which contain markers similar to androstenone.

Methyl dihydrojasmonate (figure 7) is said [11] to smell less intensive as its purity increases. When you have perceived this substance once, you have the impression of blossoming flowers everywhere in nature, especially in springtime. The sensation is not a smell for me but a kind of radiation, which conjures up the picture of a sunbeam sizzling your nose into a springtime feeling. Substances with the same effect, later evaluated by innocent perfumers, were always attributed the quality "smells like paint". So which activity would you propose to search for a receptor for methyl dihydrojasmonate, the paint or the flowery activity?

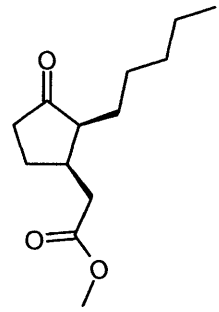


Figure 7 *cis-Methyldihydrojasmonate (Hedione, FIRM)*

Being aware of how difficult it is to describe and identify odours, many companies have invented descriptor systems, which they put in a graph showing the intensity for each of 160 descriptors of an aroma (figure 8).

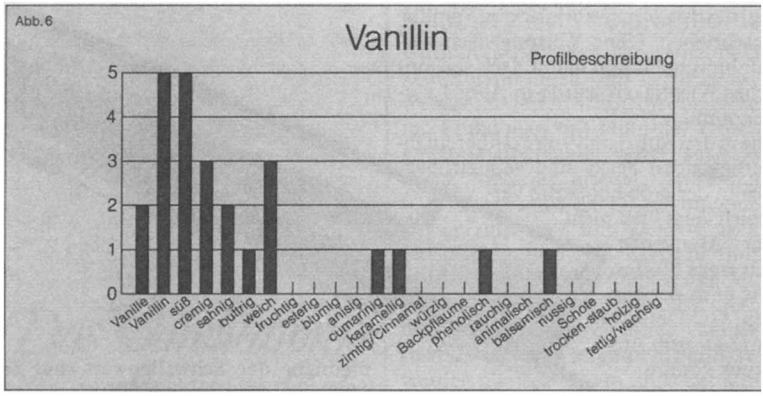
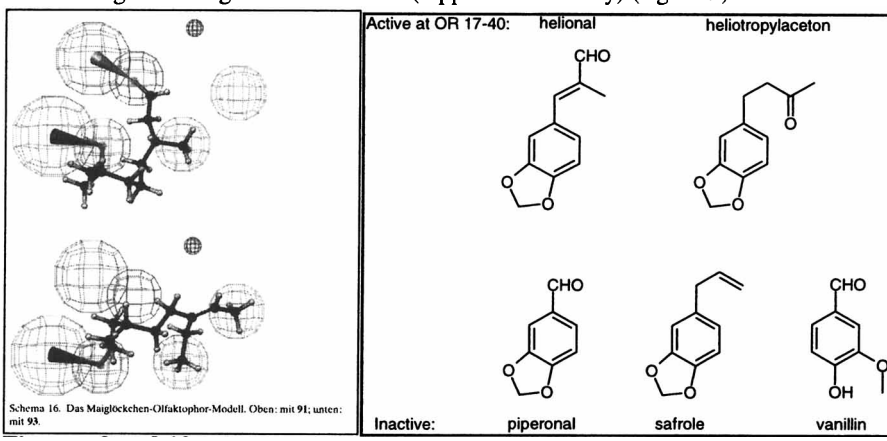


Figure 8

This is a method used to characterise scents more accurately. This is also the way the so-called "electronic noses" work. With their different sensors they adsorb or oxidise vapours of organic material and identify the vapour composition by pattern analysis with neural networks. However, the electronic noses do not give any odour description that could be used in SAR analyses. They are only able to discriminate between headspaces that they have stored.

4.1 Activity in a chemical sense

This is highlighted in an excellent review about "trends in fragrance chemistry" [12] in the German magazine Angewandte Chemie (Applied Chemistry) (figure 9).



Figures 9 and 10

What Givaudan researchers make visible and sniffable in this review is a olfactophor model; i.e. known molecules with known scents were put together in one olfactophor, similar molecules with other scents were used to define certain exclusion zones around the olfactophor. This olfactophor model was used to explain scents of new materials that belonged, olfactorily, to the same family. So nobody except the concept users can know what came first, the concept or the molecule ... or the chicken or the egg?

Why don't chemists use the knowledge of, for example, the research results of Hanns Hatt [13] (figure 10) who reports that the first cloned human olfactory receptor "OR 17-40 exhibits a remarkable ability to discriminate structurally closely related molecules like helional and piperonal. Interestingly, to humans, both chemicals smell differently as well." He wonders about the thresholds of some odorants in mammals, especially in humans, which can be as low as a few parts per million. Such high sensitivity is not observed with cloned receptors. Multiple factors may explain the higher sensitivity observed in vivo, including the presence of odorant binding proteins in the nasal mucus. In one figure (figure 11, Diagram B in [13] p.122), the protein encoded by the human OR 17-40 is presented as traversing the plasma membrane seven times).

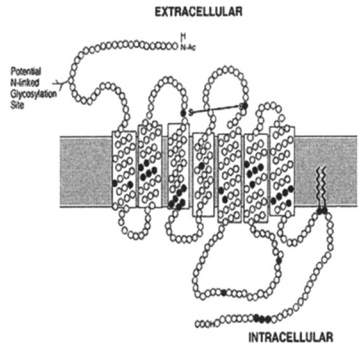


Figure 11

The finding that the odorant receptors react more sensitively in vivo to odorants than in vitro is analogous to what Amoore found with his dilution method. Some people were extremely sensitive to some molecules, which he recognised as primary odours. The success in isolating and identifying human receptors may mean that special sensitivity to special odorants has nothing to do with receptors but with those multiple factors that may explain higher sensitivity in vivo like a vomeronasal organ.

4.2 The quality of fragrances reassessed

Dietrich Kastner tells us [14] that taste and smell apparently may not be qualities of molecules. This is true in a philosophical sense because it is our mind that makes perception happen by offering the basic conditions for our ability to perceive in time and space. Immanuel Kant therefore created the term a priori. At the point when Hatt was wondering why in his mind structurally related molecules like helional and piperonal smelled different, Kastner knew that from the nearly 20,000 substances which he smelled and characterised, he hadn't found any 2 odorants with a totally identical scent. The conclusion from this observation was, that odour is what we make in our mind out of the reception of odorants. On the other hand, this would mean that the same molecule would also smell different to the same nose at different locations and occasions. This is what I myself am wondering about, since I found in many cases that new synthesised molecules smelled different in Krefeld, where our perfumers work, compared to Holthausen, where I work. This I can explain through what I think is trigeminal nerve stimulation dependency. From small molecules to bigger ones is like switching trigeminal reception to olfactorial perception.

When Günther Ohloff once held a lecture in Düsseldorf about his "triaxial rule of odour sensation in the ambergris odorants family" he was asked during the discussion about the odours of hydrogen cyanide or hydrogen sulphide. His remarkable answer was: "What you perceive from those molecules is not an odour reception" (figure 12, "The nose as spectroscopist" [19]) This is also my theory in explaining the subjectivity of odour reception: Smaller molecules are felt through irritation of trigeminal nerve endings. Examples of these touch sensations are the cooling effect of (-) menthol or the burning sensation of chilli capsaicin or the stinging of acetic acid, the mucous layer membrane wrinkling of acetone, or the pain sensation of carbonic acid. As the studies of impulses with congenitally anosmic subjects have shown, they are well able to receive odour sensation from small molecules and can even make statements about the qualities of the trigger (figure 13).

Figure 12

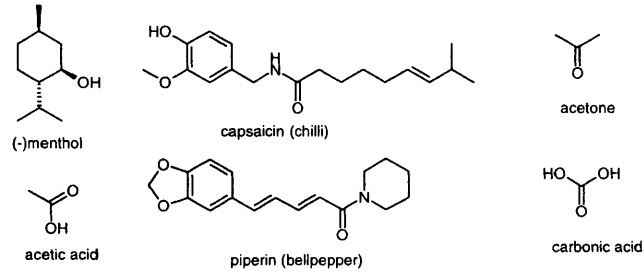


Figure 13

This is a very common phenomenon, though we normally are not aware of it. For example, the difference between the taste of alcohol-free and alcohol-containing beer is made by ethanol. Or the effect of champagne compared to the effect of wine on your hangover is caused by carbon dioxide via the trigeminal nerve, which means a measurable activity in the pain centre of the brain. The individual has the impression of becoming drunk sooner than by consuming the same amount of wine.

This is also the explanation for different odour sensations of the same molecule in different places. Humidity and temperature are influencing factors for odour reception. Humidity means that odorants and receptors are covered by different amounts of water and therefore may have different scents. Perfumers know the importance of humidity from GC-sniffing and normal people know that it is very difficult to taste wine properly during flights. This is because the air conditioning dries the mucous membranes and food and drink is not tasty any more, except champagne and spirits. These have carbonic acid and ethanol, which is enough to compensate the climate effect. Thus, the Lufthansa sommelier recommends drinking lots of water before tasting wine whilst on board.

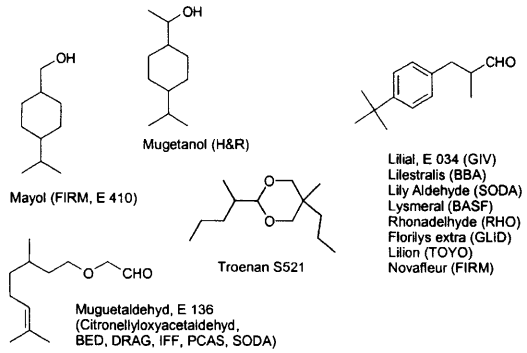


Figure 14

The reason why aldehydes in general have more intensive scents than the corresponding alcohols is in my opinion the trigeminal sensation. This clearly does not influence the low threshold of aldehydes like vanillin, as trigeminal reception is not as sensitive as odour reception. Many people do not perceive anything when sniffing acetals like Troënan® [15,16] (figure 14) but have strong "odour" responses from aldehydes of the same muguet family, like with Bourgeonal®. It is well known that aldehydes might be oxidised, forming the corresponding acid on their way to the receptors, and it might be that the acids are responsible for triggering stinging impulses.

4.3 Ways of explaining subjectivity in the perception of odorants

From tests with anosmics we learned that there are trigeminal sensations in chemoreception which chemists do not normally take into account when searching for new structures using SAR methods. In my opinion, it is mostly larger molecules where a stringent SAR approach is useful, for example in the musk, amber or sandalwood family. It seems as if those molecules with a more complex structure lack trigeminal distribution in odour perception. It is therefore well known that people who are not able to smell macrocycles are able to perceive PCMs or nitromusk molecules (figure 15) and vice versa.

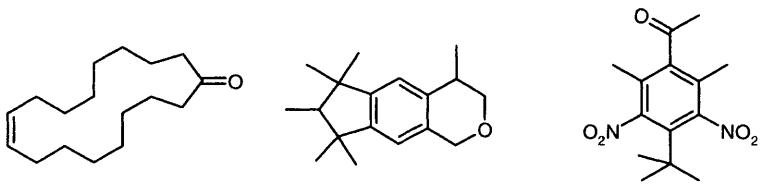


Figure 15 *A macrocyclic musk (mcm), polycyclic musk (pcm), and nitro musk (nm)*

It seems also as if musks are normally ketones and sandalwoods are normally alcohols, but there are still some exceptions, just as there are exceptions to the triaxial rule in the family of ambergris molecules. There will always be a degree of uncertainty in assigning a chemical structure to a particular perception. Expert perfumers are trained to find odour descriptors that make it possible for them to identify the same molecule again and again. The odour descriptors themselves are normally not enough to tell chemists what kind of molecule was received. This turns out to be worse with mixtures. Consumers and chemists too, to a certain extent, also associate scents with individual memories like the smell of granny's bathroom or some other situation in their youth. The most narcotic smell of my youth is Opium from Yves Saint Laurent with which I was taught disco dancing at school. Therefore, odour description in itself is, between the extremes, both too general and too individual. Climate differences and ethnic odour preferences are well known.

The most intriguing question in my opinion is that of the sense of specific anosmia. As an expert perfumer or chemist I cannot work with fragrances which have an odour only for me but for many other people have little or no scent. Lack of receptors for specific substances is normal in every individual and Amoore could measure this phenomenon by his dilution method. He used his results in forming his concept of primary odours to find out about receptors. When I consider this, I have to say that this would be the right way in SAR. You have to search for substances to which there is a high proportion of anosmics. Because this is the only way you can be sure that you have a compound at hand which binds to a specific odour receptor. This concept, but with a different explanation, led to the patents for Timberol® and Norlimbanol®(figure 16).

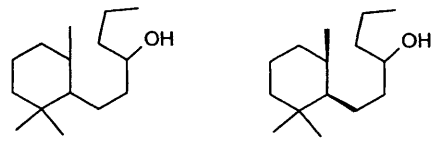


Figure 16

The high amount of anosmics to those substances was claimed to be an indicator of high substantivity or fixating power [17, 18]. So specific anosmia should be the only argument to test the attractiveness of new compounds. It is the only way to be sure of having compounds that fit perfectly into one receptor site. Perfumistic evaluation and selling of those fragrances is especially difficult, for obvious reasons. With this knowledge in mind some perfumers use mixtures of, for example, PCMs and macrocyclic musks so

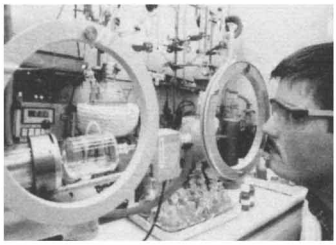
that anosmics in one of those areas have an impression of the other similar-smelling odorant. Does this also mean that the different musks do not bind to the same receptor?

5 CONCLUSION

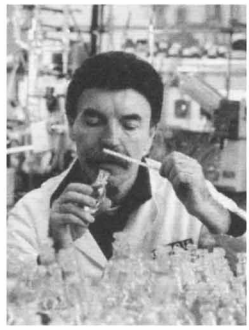
Subjectivity in odour sensation could be described as mixture of trigeminal and odour sensation, where smaller molecules could influence especially the emotional part of odour perception, the likes and dislikes. People lacking specific receptors partly take trigeminal odour responses for odour sensation and might thus have different interpretations of those scents than other people have. So the field where the chemical senses are essential for life is the most difficult to speak about generally. The landscape of transcribed and expressed receptor proteins in humans seems to be as complex as the immune system, and this should be considered in SAR studies as well. Therefore, my not very optimistic – and maybe not very scientific – view of SAR is that it is not able to predict new odour molecules and not even the odour of existing ones. And what is a revolution in medical science, that the first human odour receptors are already known, will not lead to the construction of new chemical entities, because one still needs a known fragrance to find an unknown receptor. It could be more difficult to find new aroma chemicals for known receptors. As Charles Sell put it [4] "there are multiple factors which influence recognition of fragrance ingredients". In searching for the differences in odour perception and identification, the most difficult problem will be answering the question why we obviously do have so many different receptors and also lack so many common receptors. Is our nose of such little importance in modern times that we have lost the ability to perceive special scents? Or is it a matter of individuality that we became aware of the incomparability of odour reception? The multiplicity of facets in chemical reception may have led D.Kastner to the idea that smell is not a quality of fragrance molecules. His argument is that no 2 molecules out of 20,000 smelt the same [13], but I think the more likely argument for his hypothesis would be that a highly purified substance may well give different odour impressions on different occasions. If this were the case, the conclusion would have been that odour is not a chemical quality.

That molecules of the same kind sometimes give a sensation as if you could feel the shape of the molecule is unbelievable. But it is in my mind the one and only argument speaking for the fact that odours are qualities of molecules. The general public, unaware of the chemistry involved, knows that "tastes differ" and this is also true for colours, or was it pain.

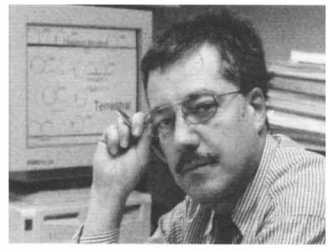
Finally we are the crew:



Marc Speitkamp



Volker Porrmann



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RELATIONSHIP OF ODOUR AND CHEMICAL STRUCTURE IN 1- AND 2-ALKYL ALCOHOLS AND THIOLS

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1 INTRODUCTION

In recent years, with the explosion of new tastes and combinations of tastes, food has taken on more than simply the functional role of the maintenance of life. Flavour is one of most significant factors in taste. Among the constituents of flavours, some compounds influence the characteristics of flavour greatly. They are called "key compounds", and therefore it is very important for flavour companies to research and develop them. In order to analyse odour-structure correlation, the methods based on concepts of quantitative structure-activity relationships (QSAR) [1-13] and comparative molecular field analysis (CoMFA) have been mainly used. The relationship of odour and chemical structure in 1- and 2-alkyl alcohols and thiols having carbon number from 5 to 11 as synthetic flavour materials was investigated using sensory evaluation. The respective odour characteristics were analysed by plotting radar charts. The obtained data was also treated with a principal component analysis in order to investigate the relationship between the odour and chemical structure.

2 EXPERIMENTAL

2.1 1- and 2-alkyl alcohols and 1-alkyl thiols

The following commercial reagents were used: 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol, 1-decanol, 1-undecanol, 2-pentanol, 2-hexanol, 2-heptanol, 2-octanol, 2-nonanol, 2-decanol, 2-undecanol, 1-pentanethiol, 1-hexanethiol, 1-heptanethiol, 1-octanethiol, 1-nonanethiol, 1-decanethiol and 1-undecanethiol.

2.2 Synthesis of 2-alky thiols

Bromination of 2-hexanol, 2-heptanol, 2-nonanol and 2-undecanol with PBr_3 yielded the respective bromides.[14, 15] The bromides were reacted with NaSH to give 2-hexanethiol, 2-heptanethiol, 2-nonanethiol and 2-undecanethiol respectively.[16] The thiols, 2-pentanethiol, 2-octanethiol and 2-decanethiol were prepared from the corresponding bromides by

reaction with NaSH. The 2-alkyl thiols were purified by distillation and analysed by GC, GC-MS, FT-IR, ^{1}H-NMR and ^{13}C-NMR. The purities of these thiols were over 97%.

2.3 NMR and GC-MS

^{1}H-NMR spectra were obtained with a JNM-EX 270 spectrometer (JEOL, Tokyo, Japan) at 270 MHz. ^{13}C-NMR spectra were obtained with a JNM-EX 270 spectrometer (JEOL, Tokyo, Japan) at 67.5 MHz.

GC-MS analysis was performed on a Hewlett-Packard HP6890 series. The chromatograph was equipped with a TC-WAX column (60 m x 0.25 mm with 0.25 μm film) and was programmed from 70°C (5 min) to 240°C at 3°C/min; injector temperature, 240°C; detector temperature, 240°C. The detector ionisation potential was 70eV.

2-Pentanethiol: ^{1}H-NMR: δ 0.91 (t, 3H, J = 7.3), 1.33 (d, 3H, J = 6.6), 1.36-1.59 (m, 4H), 1.48 (d, -SH, J = 6.3), 2.90-3.00 (m, 1H) ppm. ^{13}C-NMR: δ 13.7, 20.6, 25.6, 35.3, 43.0 ppm. MS: *m/z* (%) = 104 (M^+, 44), 71 (40), 70 (M^+ - H_2S, 32), 61 (66), 60 (15), 59 (12), 55 (58), 47 (16), 45 (11), 43 (100), 42 (30), 41 (57), 39 (41), 29 (28).

2-Hexanethiol: ^{1}H-NMR: δ 0.90 (t, 3H, J = 7.1), 1.27-1.60 (m, 6H), 1.33 (d, 3H, J = 6.6), 1.48 (d, -SH, J = 5.9), 2.88-2.98 (m, 1H) ppm. ^{13}C-NMR: δ 14.0, 22.4, 25.6, 29.6, 35.6, 40.6 ppm. MS: *m/z* (%) = 118 (M^+, 38), 85 (26), 84 (M^+ - H_2S, 22), 69 (29), 61 (62), 60 (17), 59 (10), 57 (17), 56 (38), 55 (39), 47 (10), 43 (100), 42 (35), 41 (66), 39 (31), 29 (24).

2-Heptanethiol: ^{1}H-NMR: δ 0.89 (t, 3H, J = 6.9), 1.25-1.62 (m, 8H), 1.33 (d, 3H, J = 6.6), 1.48 (d, -SH, J = 5.9), 2.88-2.98 (m, 1H) ppm. ^{13}C-NMR: δ 14.0, 22.5, 25.6, 27.1, 31.5, 35.6, 40.8 ppm. MS: *m/z* (%) = 132 (M^+, 28), 99 (2), 98 (M^+ - H_2S, 22), 70 (33), 69 (29), 61 (58), 60 (18), 59 (10), 57 (100), 56 (74), 55 (42), 43 (42), 42 (20), 41 (77), 39 (28), 29 (31).

2-Octanethiol: ^{1}H-NMR: δ 0.89 (t, 3H, J = 6.8), 1.28 (br s, 6H), 1.33 (d, 3H, J = 6.6), 1.35-1.59 (m, 4H), 1.48 (d, -SH, J = 5.9), 2.88-2.95 (m, 1H) ppm. ^{13}C-NMR: δ 14.0, 22.6, 25.6, 27.4, 29.0, 31.7, 35.6, 40.9 ppm. MS: *m/z* (%) = 146 (M^+, 30), 113 (2), 112 (M^+ - H_2S, 26), 84 (25), 83 (40), 82 (10), 71 (55), 70 (74), 69 (30), 61 (71), 60 (23), 59 (11), 57 (79), 56 (56), 55 (78), 47 (11), 43 (76), 42 (41), 41 (100), 39 (33), 29 (41).

2-Nonanethiol: ^{1}H-NMR: δ 0.88 (t, 3H, J = 6.8), 1.27 (br s, 8H), 1.33 (d, 3H, J = 6.6), 1.36-1.59 (m, 4H), 1.48 (d, -SH, J = 6.3), 2.88-2.98 (m, 1H) ppm. ^{13}C-NMR: δ 14.1, 22.6, 25.6, 27.4, 29.2, 29.3, 31.8, 35.6, 40.9 ppm. MS: *m/z* (%) = 160 (M^+, 26), 127 (3), 126 (M^+ - H_2S, 25), 98 (15), 97 (34), 85 (24), 84 (29), 83 (25), 82 (10), 71 (43), 70 (44), 69 (44), 61 (64), 60 (21), 59 (11), 57 (52), 56 (69), 55 (78), 47 (10), 43 (91), 42 (37), 41 (100), 39 (31), 29 (44).

2-Decanethiol: ^{1}H-NMR: δ 0.88 (t, 3H, J = 6.6), 1.27 (br s, 10H), 1.33 (d, 3H, J = 6.6), 1.35-1.59 (m, 4H), 1.48 (d, -SH, J = 6.3), 2.88-2.98 (m, 1H) ppm. ^{13}C-NMR: δ 14.1, 22.6, 25.6, 27.4, 29.2, 29.3, 29.5, 31.8, 35.6, 40.9 ppm. MS: *m/z* (%) = 174 (M^+, 23), 141 (3), 140 (M^+ - H_2S, 26), 112 (12), 111 (20), 98 (15), 97 (32), 85 (26), 84 (23), 83 (26), 82 (10), 71 (33), 70 (55), 69 (53), 61 (61), 60 (19), 59 (10), 57 (72), 56 (72), 55 (81), 43 (88), 42 (30), 41 (100), 39 (27), 29 (43).

2-Undecanethiol: ^{1}H-NMR: δ 0.88 (t, 3H, J = 6.8), 1.27 (br s, 12H), 1.33 (d, 3H, J = 6.9), 1.43-1.57 (m, 4H), 1.48 (d, -SH, J = 5.9), 2.88-2.98 (m, 1H) ppm. ^{13}C-NMR: δ 14.1, 22.7, 25.6, 27.4, 29.3 (2C), 29.5 (2C), 31.9, 35.6, 35.6, 40.9 ppm. MS: *m/z* (%) = 188 (M^+, 18), 155 (3), 154 (M^+ - H_2S, 23), 111 (18), 98 (12), 97 (28), 85 (20), 84 (25), 83 (33), 82 (11), 71 (31), 70 (49), 69 (51), 67 (10), 61 (52), 60 (16), 57 (70), 56 (53), 55 (76), 43 (80), 42 (27), 41 (100), 39 (26), 29 (44).

3 RESULTS AND DISCUSSION

3.1 Odour characteristics

Odour characteristics of 1- and 2-alkyl alcohols and thiols were described respectively by using ten sensory descriptive terms: sweet, fruity, tropical, floral, fresh, refreshing, spicy, roasty, fishy and oily. The odour strength in each descriptive term was scored as 0 none; 1 weak; 2 a little weak; 3 normal; 4 a little strong; 5 strong. The average score from assessment of 1- and 2-alkyl alcohols and thiols by three trained flavourists was plotted on a radar chart [17] (Figure 1).

For the 1-alkyl alcohols, 1-hexanol had the strongest fresh and refreshing factors. 1-heptanol had a strong fruity factor. 1-octanol had a stronger fruity factor than 1-heptanol, and also had a strong fresh factor. 1-decanol and 1-undecanol had a stronger oily factor than the other molecules. Fruity and floral factors decreased from a peak at 1-octanol. The fresh factor decreased from a peak at 1-hexanol. The oily factor increased according to increasing carbon number.

For the 2-alkyl alcohols, the sweet factor of 2-decanol and 2-undecanol was markedly weaker than the other molecules. The fruity factor decreased from a peak at 2-heptanol. Tropical and floral factors decreased from a peak at 2-octanol. The fresh factor gradually decreased in relation to increase of the number of carbons. A strong refreshing factor was observed with 2-pentanol and 2-hexanol, but it decreased in relation to an increase in the number of carbon atoms. Fishy and oily factors increased according to increasing carbon number.

For the 1-alkyl thiols, sweet and tropical factors decreased from a peak at 1-heptanethiol. The floral factor was scarcely noticed in any compounds, although 1-decanethiol had little of it. The refreshing factor was also scarcely noticed in all compounds. The spicy factor increased according to a decrease in the number of carbons.

For the 2-alkyl thiols, sweet, fruity and tropical factors of 2-pentanethiol and 2-hexanethiol were weak, but strong in other compounds such as 2-heptanethiol which was very strong. The fruity and tropical factors of 2-octanethiol were also very strong. The floral factor was very weak in all compounds, but increased according to an increasing number of carbons. The fresh factor was slightly felt in all compounds except 2-pentanethiol and 2-hexanethiol. The refreshing factor was extremely weak in almost all compounds, but was slightly felt in 2-heptanethiol, 2-octanethiol and 2-nonanethiol. The spicy factor was slightly felt in 2-pentanethiol, 2-hexanethiol and 2-undecanethiol, but was almost negligible for the other compounds. The fishy factor was very strong in 2-pentanethiol and 2-hexanethiol and relatively weak in the other compounds.

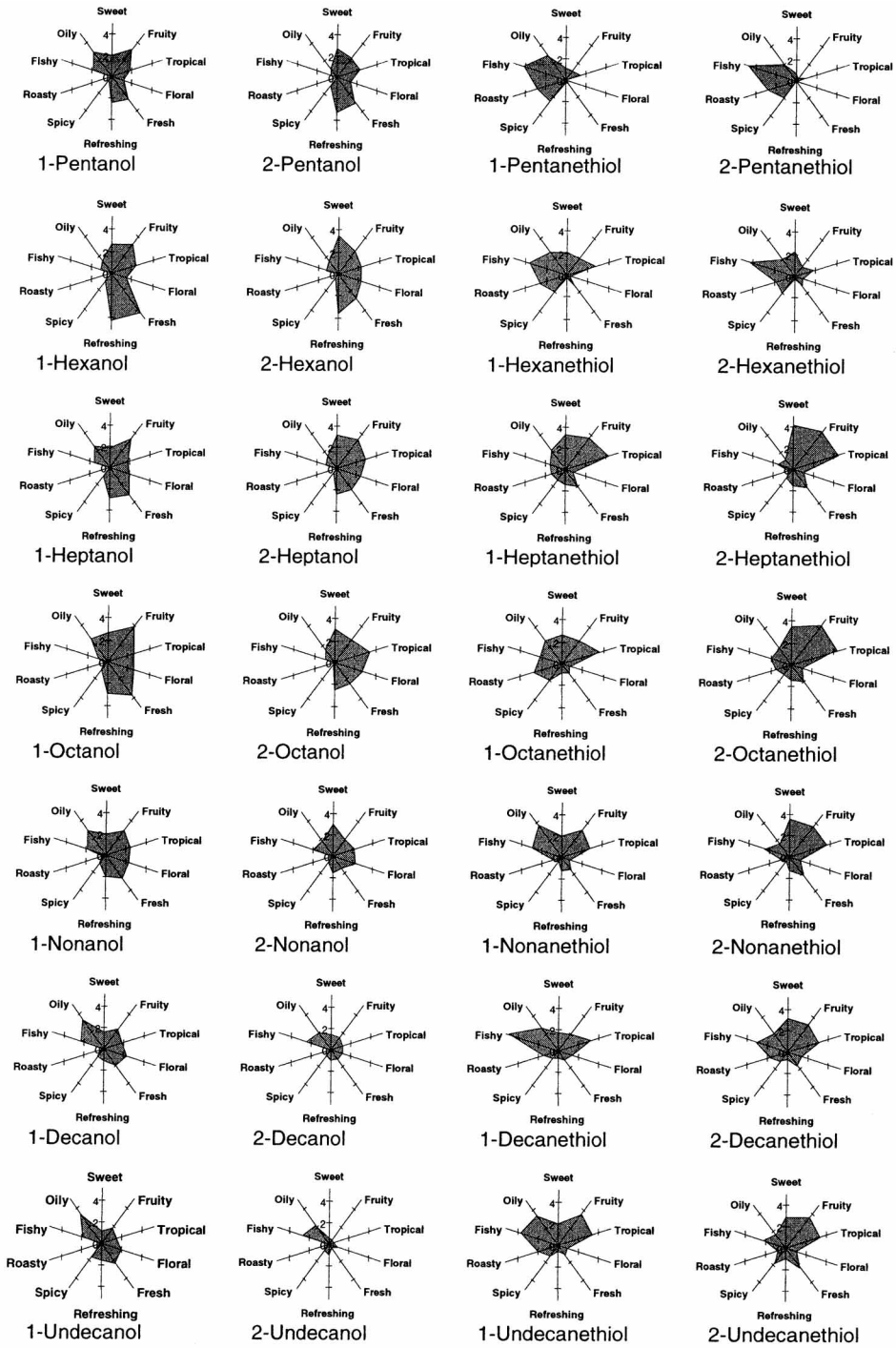

Figure 1 *Odour profiles of 1- and 2-alkyl alcohols and thiols*

3.2 Principal component analysis

The relationships between odour and chemical structure in 1- and 2-alkyl alcohols and thiols was investigated by analysis of the data using principal component analysis.[18, 19]

The result of this analysis with 1- and 2-alkyl alcohols is shown figure 2. The contribution of principal component 1 (PC 1) and 2 (PC 2) summed up to nearly 70% (Table 1). Fruity, tropical, fresh, sweet and refreshing factors contributed to the plus side of PC 1, while fishy and oily factors contributed to the minus side. Oily, roasty and floral factors contributed to the plus side of PC 2, while the refreshing factor contributed to the minus side. Pentanols, hexanols, heptanols and octanols were positioned on the plus side of PC 1, while nonanols, decanols and undecanols were on the minus side. The change of odour between 1-alkyl alcohols and 2-alkyl alcohols was more contributed to PC 2 than PC 1. In addition, 1-alkyl alcohols were positioned on the plus side of PC 2, while 2-alkyl alcohols were on the minus side. The result indicates that the oily and floral factors in 1-alkyl alcohols are stronger than those in 2-alkyl alcohols.

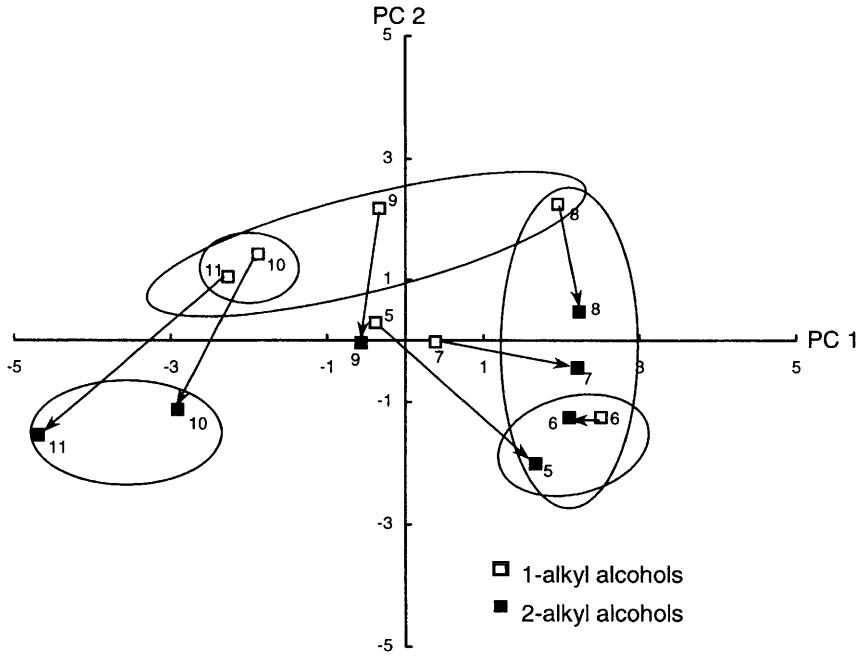

Figure 2 *The score-plots on the plane of PC 1 vs. PC 2 in 1- and 2-alkyl alcohols*

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Sweet	0.896	-0.070	-0.336	0.081	-0.040	0.166
Fruity	0.795	0.411	0.192	-0.284	0.169	-0.139
Tropical	0.872	0.284	-0.266	0.134	-0.038	-0.157
Floral	0.503	0.610	-0.345	0.431	0.193	0.102
Fresh	0.796	0.156	0.548	-0.146	-0.025	0.065
Refreshing	0.859	-0.249	0.361	-0.162	-0.029	0.176
Spicy	-0.326	0.166	0.743	0.535	-0.132	-0.009
Roasty	-0.351	0.758	-0.196	-0.211	-0.462	0.062
Fishy	-0.922	0.046	-0.141	-0.101	0.219	0.124
Oily	-0.499	0.734	0.299	-0.163	0.244	0.046
Eigenvalue	2.269	1.362	1.213	0.840	0.640	0.372
Proportion	51.495	18.558	14.707	7.063	4.091	1.383
Cumulative proportion	51.495	70.054	84.761	91.824	95.915	97.297

Table 1 *Eigenvalues and eigenvectors of the correlation matrix in 1-and 2-alkyl alcohols*

The result of 1- and 2-alkyl thiols is shown figure 3. The contribution of PC 1 and 2 summed up to nearly 83% (Table 2). Floral, refreshing, fruity, fresh and sweet factors contributed to the plus side of PC 1, while fishy, roasty and spicy factors contributed to the minus side. Oily, fishy and floral factors contributed to the plus side of PC 2, whilst the spicy and sweet factors contributed to the minus side. Pentanethiols and hexanethiols were positioned on the minus side of PC 1. The change of odour between 1-pentanethiol and 2-pentanethiol was relatively little. The change of odour between 1-hexanethiol and 2-hexanethiol was also relatively little. 2-Heptanethiol, 2-octanethiol and 2-nonanethiol were positioned on the plus side of PC 1. The reason for this is that 2-alkyl thiols have stronger sweet, fruity and tropical factors than the 1-alkyl thiols, and 1-alkyl thiols have a stronger oily factor than the 2-alkyl thiols. The changes of odour from 1-nonanethiol, 1-decanethiol and 1-undecanethiol to the corresponding 2-alkyl thiols were very large, and they had a high contribution for both PC 1 and PC 2. The reason for this is that 1-nonanethiol, 1-decanethiol and 1-undecanethiol have stronger oily and fishy factors than the corresponding 2-alkyl thiols, while 2-nonanethiol, 2-decanethiol and 2-undecanethiol have stronger sweet, floral, fresh and spicy factors than the corresponding 1-alkyl thiols. The changes of odour from 1-heptanethiol to 2-heptanethiol and from 1-octanethiol to 2-octanethiol contributed more to PC 1 than PC 2. The reason for this is that 2-heptanethiol and 2-octanethiol have much stronger sweet, fruity, tropical, floral, fresh and refreshing factors than the corresponding 1-alkyl thiols, and have weaker spicy roasty and oily factors. These results show that 2-alkyl thiols with a carbon number in the range of 7 to 11 have stronger fruity, fresh, tropical and sweet factors than the corresponding 1-alkyl thiols. It also indicated that 2-alkyl thiols have a brighter odour than 1-alkyl thiols.

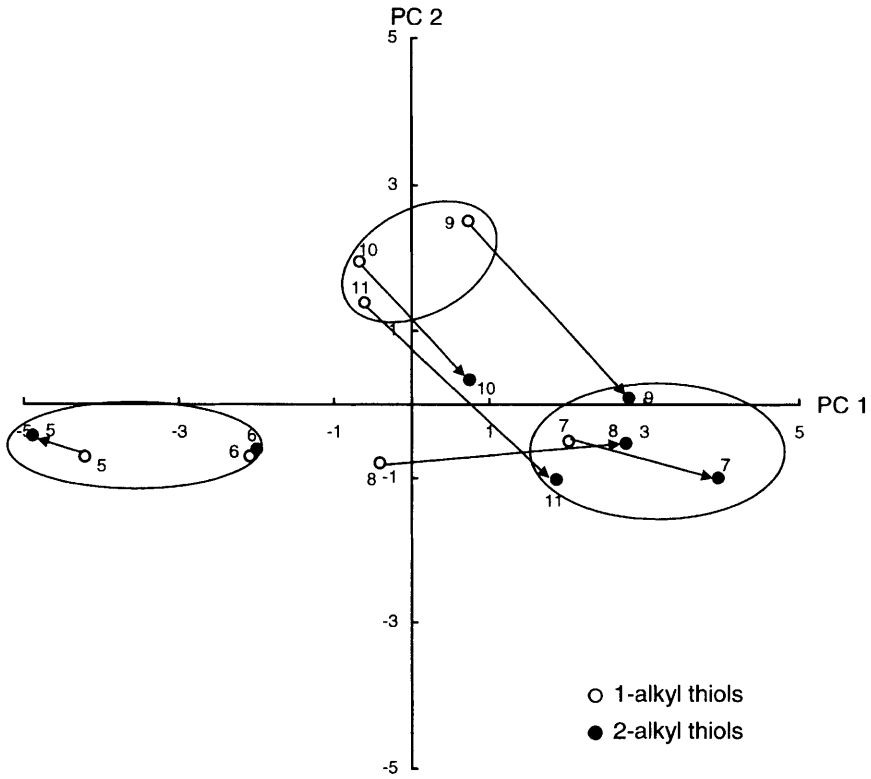

Figure 3 *The score-plots on the plane of PC 1 vs. PC 2 in 1- and 2-alkyl thiols*

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Sweet	0.933	-0.292	0.030	0.058	-0.079	0.144
Fruity	0.944	0.037	-0.216	0.145	0.048	-0.065
Tropical	0.924	-0.033	-0.197	0.244	-0.076	-0.143
Floral	0.748	0.360	0.390	0.364	0.075	0.008
Fresh	0.944	-0.146	0.059	-0.027	0.231	0.091
Refreshing	0.934	0.048	-0.186	-0.225	-0.111	-0.130
Spicy	-0.692	-0.639	-0.147	0.119	0.243	-0.110
Roasty	-0.849	-0.239	-0.229	0.355	-0.186	0.065
Fishy	-0.820	0.346	0.416	0.060	0.012	-0.111
Oily	-0.392	0.690	-0.587	0.052	0.133	0.058
Eigenvalue	2.639	1.141	0.932	0.643	0.444	0.320
Proportion	69.664	13.018	8.682	4.140	1.970	1.027
Cumulative proportion	69.664	82.682	91.364	95.504	97.474	98.500

Table 2 *Eigenvalues and eigenvectors of the correlation matrix in 1-and 2-alkyl thiols*

The result for the analysis of all compounds is shown in figure 4. The contribution of PC 1 and 2 summed up to nearly 74% (Table 3). Looking at the odour correlation between 1-alkyl alcohols and 1-alkyl thiols, the changes of odour among the compounds with a carbon number range from 5 to 9 contributed more to PC 1 than PC 2. The changes of odour for pentyl and hexyl compounds had high contribution for PC 1.This is due to the fact that 1-pentanol and 1-hexanol have stronger fresh and refreshing factors, whilst the corresponding thiols have stronger roasty and spicy factors. 1-Heptanol and 1-octanol have very strong floral and fresh factors, whilst the corresponding thiols have a stronger roasty factor. 1-Nonanol has strong floral and fresh factors, whilst 1-nonanethiol has stronger oily and fishy factors. The changes of odour for decyl and undecyl compounds contributed more to PC 2 than PC 1. This is due to 1-decanol and 1-undecanol having stronger floral and fresh factors, whilst the corresponding thiols have stronger tropical, roasty and fishy factors. Looking at the odour correlation between 2-alkyl alcohols and 2-alkyl thiols, the changes of odour from 2-pentanethiol and 2-hexanethiol to the corresponding alcohols contributed to PC 1, as already seen in the case of the 1-alkyl compounds. The rational behind this is that 2-pentanol has stronger fresh and refreshing factors than 2-pentanethiol, whilst 2-pentanethiol has stronger roasty and fishy factors. Comparing 2-hexanol and 2-hexanethiol, 2-hexanol has a stronger refreshing factor, whilst 2-hexanethiol has stronger spicy and fishy factors. The changes of odour for 2-heptanethiol, 2-octanethiol, 2-nonanethiol, 2-decanethiol and 2-undecanethiol contributed to PC 2 because 2-heptanol, 2-octanol and 2-nonanol have a very stronger floral factor, whilst the corresponding thiols have stronger fruity and tropical factors. Also, 2-decanethiol and 2-undecanethiol have stronger sweet, fruity, tropical and spicy factors than the corresponding alcohols. These results indicated that alkyl thiols have stronger fruity, sweet and tropical factors than the corresponding alcohols, with a carbon number above 7.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Sweet	0.657	0.684	-0.175	-0.135	0.062	0.011
Fruity	0.657	0.613	0.369	0.064	-0.092	0.006
Tropical	0.378	0.877	0.182	-0.129	0.053	-0.010
Floral	0.744	-0.396	0.158	-0.177	0.470	0.102
Fresh	0.872	-0.066	0.060	0.419	-0.026	0.192
Refreshing	0.875	-0.270	-0.098	0.279	-0.148	0.114
Spicy	-0.717	0.302	-0.164	0.529	0.257	-0.076
Roasty	-0.796	0.495	-0.019	0.159	0.075	0.134
Fishy	-0.899	0.077	0.051	-0.195	-0.047	0.356
Oily	-0.424	-0.198	0.865	0.122	-0.007	-0.050
Eigenvalue	2.285	1.492	1.007	0.826	0.577	0.462
Proportion	52.200	22.274	10.149	6.816	3.329	2.131
Cumulative proportion	52.200	74.474	84.624	91.439	94.768	96.899

Table 3 *Eigenvalues and eigenvectors of the correlation matrix in 1-and 2-alkyl alcohols and thiols*

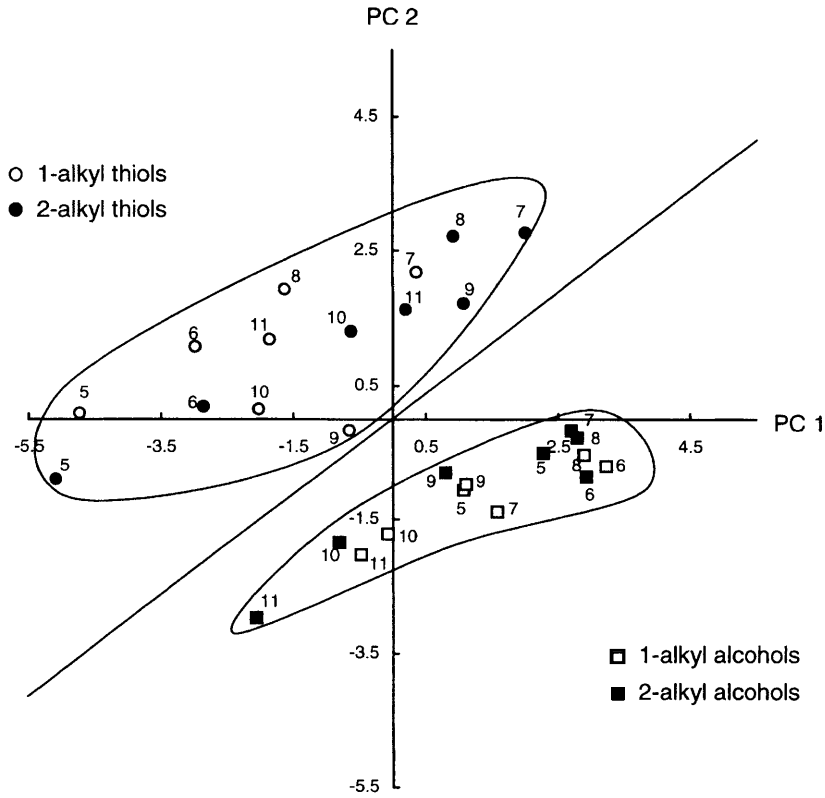

Figure 4 *The score-plots on the plane of PC 1 vs. PC 2 in 1- and 2-alkyl alcohols and thiols*

4 CONCLUSION

The relationship between odour and chemical structure in alkyl alcohols and thiols, which have a carbon number range from 5 to 11, was investigated. The results indicated that odour characteristics classified alcohols and thiols to carbon number 5 and 6, or 7 and 8, or 10 and 11. Compounds with 9 carbons, in short, nonyl compounds had both odour characters, which are found in the groups of heptyl and octyl compounds, and decyl and undecyl compounds. In alkyl alcohols, 1-alkyl alcohols with a carbon number from 5 to 8 had a stronger oily factor than the corresponding 2-alkyl alcohols, whilst 2-alkyl alcohols had a stronger sweet factor. 1-Alkyl alcohols with a carbon number from 9 to 11 had stronger fresh and oily factors, but 2-alkyl alcohols had little odour character. For the alkyl thiols, 1-alkyl thiols with a carbon number of 5 or 6 had stronger fruity, tropical and oily factors than the corresponding 2-alkyl thiols. The 2-alkyl thiols had a stronger fishy factor. This was reversed in thiols with a carbon number of 7 or 8. 1-Heptanethiol and 1-octanethiol had stronger fishy and oily factors than the corresponding 2-alkyl thiols. 2-Heptanethiol and 2-octanethiol had stronger sweet, fruity, tropical, floral and fresh factors,

and had a very bright odour. In thiols with a carbon number from 9 to 11, 1-alkyl thiols had stronger spicy, roasty and oily factors than the corresponding 2-alkyl thiols. The 2-alkyl thiols had stronger sweet and fresh factors.

From the result of the principal component analyses, it was shown that the change tendencies of odour from 1-pentanol, 1-hexanol, 1-decanol and 1-undecanol to the corresponding 1-alkyl thiols are similar to those from 2-alkyl alcohols to 2-alkyl thiols. The change tendencies of odour from 1-heptanol and 1-octanol to the corresponding 1-alkyl thiols differ from those of 2-heptanol and 2-octanol to the corresponding 2-alkyl thiols. It is seen that the change in tendencies of odour from 1-alkyl compounds to 2-alkyl compounds and from alkyl alcohols to alkyl thiols have a regular trend. In addition, it indicated that 2-heptanethiol and 2-octanethiol have a brighter and unique odour when compared to other alkyl thiols.

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Analytical

NEW DEVELOPMENTS IN SORPTIVE EXTRACTION FOR THE ANALYSIS OF FLAVOURS AND FRAGRANCES

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1 INTRODUCTION

Developments in separation sciences gained momentum in recent years and there are hardly any analytical challenges left that can not be tackled with state-of-the-art pressure- or electrodriven separation methods. For a large number of applications sample preparation is still the bottleneck for high accuracy, precision and sample throughput, and new developments in this respect are more than welcome.

In recent years, several new sample preparation methods have been introduced for gas chromatographic analysis like gum phase extraction (GPE) for gaseous samples, solid phase extraction (SPE) and solid phase microextraction (SPME) for liquid samples, ultrasonic (UE) and accelerated solvent extraction (ASE) for solid samples, etc. For an overview on recent developments in sample preparation for chromatographic analysis, we refer the reader to a recent review article.[1]

Sorptive extraction (SE) in its different forms is slowly but surely taking a unique position in sample preparation. Sorptive extraction refers to extraction of organic compounds from a sample matrix being it a gas, a liquid or a solid, into the bulk of a polymeric retaining phase. The mechanism is based on partitioning or dissolution in a polymer that is above it's glass transition temperature and behaves as a liquid. This differs from adsorptive extraction in which compounds are retained on the surface by temporary bonds to active sites of the adsorbent like the inorganic materials charcoal or silica gel or the organic materials Tenax, polyurethane foam and divinylbenzenestyrene copolymer. In sorptive extraction the silicone rubber polydimethylsiloxane (PDMS) is mostly used as enrichment phase (sorbent). PDMS is also the best known GC stationary phase. Features of PDMS for enrichment include: PDMS/gas distribution coefficients are known from GC retention times or can be calculated, PDMS/water distribution coefficients are very close to octanol/water distribution coefficients that are tabulated as log P or can be calculated by e.g. software programs like LOGWIN[2], high inertness and thermal stability (from -100°C to 350°C), good enrichment performance for polar and reactive analytes, and last but not least degradation products of PDMS are low in intensity, very specific i.e. cyclic dimethylsilicones, and can easily be identified and differentiated from target solutes with a mass spectrometer (MS).

Sorptive extraction has already been studied intensively and forms the basis of the enrichment techniques open tubular trapping (OTT), solid phase microextraction (SPME), and gum phase extraction (GPE) in the breakthrough and equilibrium mode. In OTT a capillary column with a thick PDMS layer (up to 100 µm) is used for enrichment of gaseous and liquid samples.[3,4,5,6] SPME uses a special syringe with a fused silica fibre coated with a PDMS or polyacrylate (PA) layer.[7,8] In GPE PDMS particles [9,10,11] are employed. After enrichment by OTT, SPME or GPE, the solutes are thermally desorbed on-line with a capillary GC system. The sensitivity that can be obtained with the above-mentioned techniques solely depends on the amount of sorbent employed. In the case of OTT and SPME the amount of PDMS available for sorption is very low (typically 0.5 µl for SPME and 1.75 µl/10 cm for OTT), thus causing limited sensitivity. In GPE with particles (typically 300 µl) this problem is circumvented but intensive drying is needed for the enrichment from water samples and volatile analytes are thus lost.

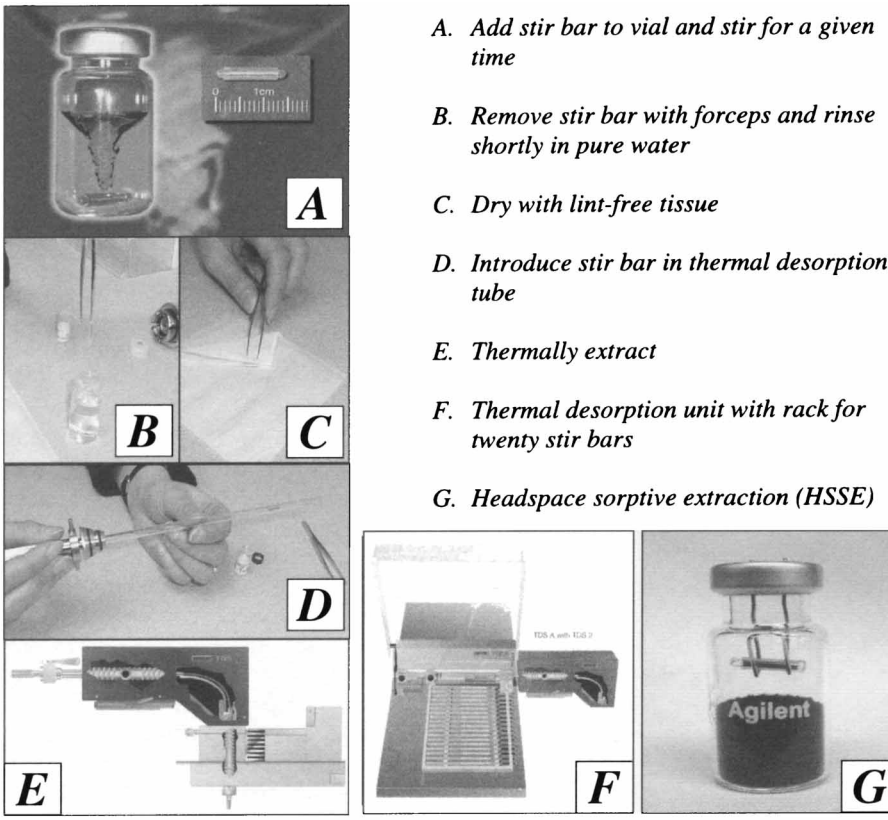
The most recently introduced sorptive extraction techniques are stir bar sorptive extraction (SBSE)[12] and head space sorptive extraction (HSSE).[13] SBSE and HSEE present solutions to most of the above mentioned problems and are simple, robust, sensitive and solvent less sample enrichment methods. In both SBSE and HSSE, a stir bar consisting of a magnetic core encapsulated in glass, is coated outside with a thick layer of PDMS. In SBSE, the stir bar is placed in an aqueous sample while in HSSE the stir bar is placed in the headspace above a solid material. The extraction mechanism is the same as for SPME but because of the large increase in sorbent phase (typically 25 µl to 125 µl *vs* 0.5 µl for SPME) sensitivity is up to 100 times higher. Commonly, after extraction of the analytes of interest from the sample matrix, the stir bar is thermally desorbed and the analytes are transferred to a cold (-100°C) programmed temperature vaporisation (PTV) injector for refocusing followed by capillary GC/MS or GC/AED analysis. SBSE and HSSE have been applied for the analysis of micropollutants in water samples[12], for the determination of the corkiness flavour in wine[14], for pesticide analysis[15], for the determination of the preservative benzoic acid in foodstuffs[16] and for the determination of PCB's in human sperm.[17] As an alternative, the stir bars can be desorbed by liquid extraction followed by conventional or large volume injection in capillary GC. Liquid desorption can also be applied for solutes that are not amenable to GC analysis. The extracts are then analysed by liquid chromatographic or electrophoretic techniques.

In this contribution, the principle of SBSE and HSSE is presented and their applicability in flavour and fragrance research illustrated with some typical analyses covering the broad application range of SBSE and HSSE like the analysis of flavour carriers in tea, beer, yoghurt and bananas, with the analysis of off-flavours in beer, and with the analysis of the bitter compounds in beer.

2 METHOD AND RESULTS

Stir bars coated with 25 to 125 µl PDMS are commercially available from Gerstel GmbH (Mulheim a/d Ruhr, Germany). Thin PDMS coatings are applied for the enrichment of semi-volatiles and thermolable solutes while thick coatings are used for SBSE enrichment of volatiles and for HSSE. SBSE extraction of a liquid sample is performed by placing a suitable amount of sample in a headspace vial or another piece of glassware e.g. an Erlenmeyer flask, depending on the chosen volume and the sensitivity to be reached. The

stir bar is added and the sample is stirred for 30 to 120 min. Full equilibration is not necessary for quantitative analysis. For HSSE, the stir bar is hung in a closed vial over the solid material for a given time. After enrichment, the stir bar is removed and introduced in an empty glass thermal desorption tube (187 mm L x 4 mm i.d.) and transferred to a thermal desorption unit. Desorption temperatures depend primarily on the volatility of the analytes of interest, and are between 150 to 300°C at which the stir bar is desorbed for 5 to 15 min under a flow of helium. Cryofocussing in a PTV inlet is required to obtain narrow inlet bands in the capillary GC analysis. Typical manipulations in SBSE are illustrated in Figure 1.



A. *Add stir bar to vial and stir for a given time*

B. *Remove stir bar with forceps and rinse shortly in pure water*

C. *Dry with lint-free tissue*

D. *Introduce stir bar in thermal desorption tube*

E. *Thermally extract*

F. *Thermal desorption unit with rack for twenty stir bars*

G. *Headspace sorptive extraction (HSSE)*

Figure 1 *Different manipulations and devices for SBSE and HSEE*

For thermal desorption a TDS-2 or a TDS-A system was used (Gerstel GmbH, Mulheim a/d Ruhr, Germany). The thermal desorption unit was mounted on a HP 6890 GC/MSD (Agilent Technologies, Wilmington, DE, USA) equipped with a CIS 4 PTV inlet (Gerstel). As an alternative to thermal desorption, liquid desorption may be used as will be described for the analysis of the bitter acids in beer by micellar electrokinetic chromatography (MEKC).

The first application of SBSE concerns the analysis of two tea samples with different flavours namely a "herb-mix" flavour (Figure 2A) and a raspberry flavour (Figure 2B).

The tea's samples were prepared by simply adding boiling water. At 60°C, 10 ml samples were placed in headspace vials and SBSE extraction was performed on 25 µl PDMS stir bars during 90 minutes. The stir bars were then thermally desorbed by programming the thermodesorption unit from its initial temperature, 20°C, at 60°C/min to a final temperature of 240°C at which the stir bar was desorbed for 10 minutes. During thermal desorption the TDS-2 was operated in the splitless mode so that the entire amount of desorbed analytes flows towards the cryotrap. The desorbed solutes were cryofocussed at -150°C using liquid nitrogen in an empty glass tube. After desorption, the PTV was programmed to 280°C for re-injection of the trapped compounds. Since the tea samples were found to be rather concentrated, PTV re-injection was performed in the split mode at a split ratio of 1:20. The analytical column applied was a HP-5 MS (30 m L x 0.25 mm i.d. x 0.25 µm d_f), which was operated in the constant flow mode at a flow of 1 ml/min helium. The GC oven temperature was programmed from 40°C, which was kept for 1 min, at a rate of 5°C/min to 300°C. The detector used was a mass spectrometer. The herb-mix tea contains several terpenoids with anethole (peak 2) as main compound. The raspberry tea is characterised by high concentrations of amyl propionate (peak 7), cis-3-hexenyl propionate (peak 8) and α-ionone (peak 11). Surprisingly, *iso*- and *n*-butylphthalate (peaks 13 and 14) are present in relatively high concentrations. The occurrence of the phthalates is not so strange because they are known as aroma keepers *i.e.* they are responsible for a slower release of the aroma solutes.

Fatty matrices (milk, fresh cheese, yoghurt, *etc.*) have also been analysed with SBSE. A typical example is shown in Figure 3 representing the profile for yoghurt flavoured with strawberries. For this application, the yoghurt sample was diluted 1:1 with distilled water and extracted during 60 minutes at 1400 rpm with a 55 µl PDMS stir bar. The column was a Stabilwax (30 m L x 0.25 mm i.d. x 0.25 µm d_f). The GC oven temperature was programmed from 40°C (1 min) at a rate of 5°C/min to 240°C. The compounds responsible for the strawberry flavour namely ethyl-2-methylbutyrate (peak 3) and γ-decalactone (peak 10) are clearly present. It is surprising that the lipid matrix did not disturb the SBSE enrichment for this quality control application.

HSSE is similar to SPME for gaseous samples but uses a larger amount of sorbent. The performance of HSSE is illustrated with the analysis of the aroma carriers in bananas (Figure 4). A slice of 1 g of an unripe and a ripe banana was put in a 250 ml Erlenmeyer and HSSE sampling was performed for 60 minutes using a 55 µl PDMS coated stir bar. Thermal desorption of the HSSE stir bar was performed and for cryofocusing of the analytes prior to injection, a PTV injector with liquid nitrogen cooling was applied. In the liner of the PTV a small plug of Tenax (ca. 20 mg) was placed. Splitless thermal desorption was done by ramping the TDS from 40°C to 250°C at a rate of 60°C/min and holding the upper temperature for 5 min. During thermal desorption, the PTV was cooled at –150°C and then ramped to 250°C at a rate of 600°C/min. The injector was operated in the split (1/50) mode. Capillary GC analyses were performed on a 30 m L x 250 µm I.D. x 1 µm HP-1 column (Agilent Technologies), with helium as carrier gas. The oven was programmed from 30°C (1 min) to 300°C at a rate of 10°C/min. The mass spectrometric detector was operated in the scan mode (m/z 20-400). The green unripe banana profile (A) is going over into the typical banana aroma profile (B) in which 2-pentanol acetate and 2-methyl-1-methylbutyl and 2-methyl-3-methylbutyl propionate play an important role.

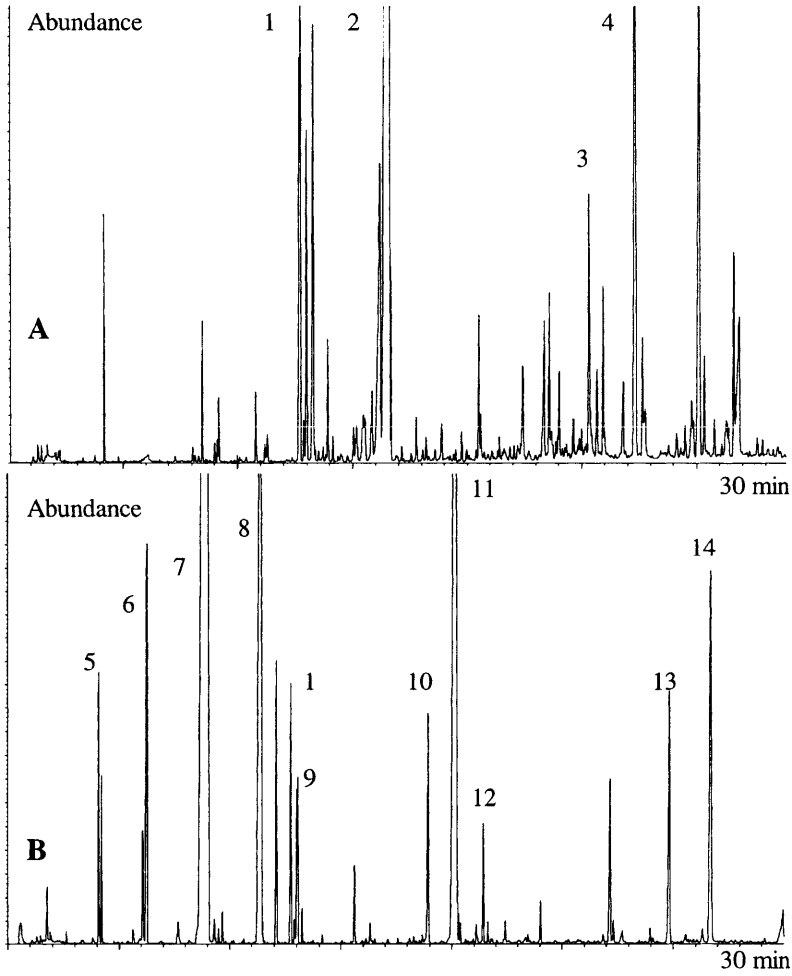


Figure 2 *SBSE-TD-CGC/MS of a herb-mix flavoured (A) and a raspberry flavoured tea (B). Compounds: 1, menthone; 2, anethole; 3, bisabolol oxide B; 4, bisabolol oxide A; 5, butyl acetate; 6, amyl acetate; 7, amyl propionate; 8, cis-3-hexenyl propionate; 9, iso-menthone; 10, damascone; 11, α-ionone; 12, β-ionone; 13, di-isobutylphthalate; 14, dibutylphthalate.*

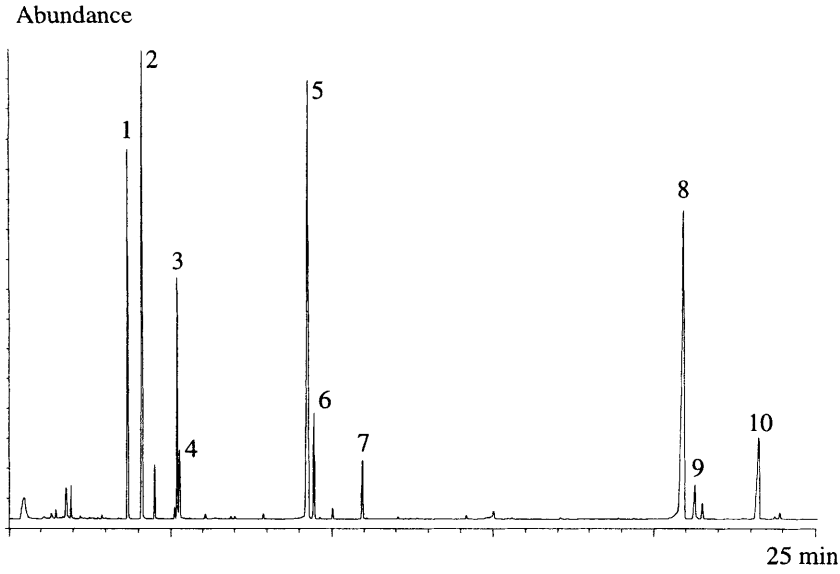


Figure 3 *SBSE-TD-CGC/MS of yoghurt flavoured with strawberries. Compounds: 1, methyl-2-methyl butyrate; 2, ethyl butyrate; 3, ethyl-2-methyl butyrate; 4, cis-3-hexenol A; 5, ethyl caproate; 6, cis-3-hexenyl acetate; 7. isoamyl butyrate; 8, methyl cinnamate; 9, vanilline; 10, γ-decalactone.*

The elucidation of the "hoppy aroma note" in beer has always been a challenging task. The main reason without any doubt is the lack of sensitive and selective analytical methods. In the brewery industry, organoleptic panels very often identify a hoppy note in beers, but analytical evidence cannot support their observations. SBSE was evaluated for the enrichment of hop derived solutes in beers. Beer extraction was performed by placing a 10 ml beer sample in a 20 ml headspace vial, adding a stir bar of 24 µl PDMS and stirring at 700 rpm during 45 min. The following conditions were used: thermal desorption 20°C - 60°C/min - 240°C (10 min); splitless mode ; 50 ml/min desorption flow; PTV : -150°C (cryofocussing temp) - 600°C/min - 280°C (2 min) ; split mode (1/20); column 30 m x 0.25 mm i.d. x 0.25 µm Stabilwax, carrier gas 1 ml/min helium; oven temp. 40°C (1 min) - 5°C/min - 240°C (30 min). Detection was by 1:1 effluent splitting to MSD operated in the full scan mode and to an PFPD detector.

The chromatograms obtained by parallel MS and PFPD (S-mode) detection for a beer after stir bar sorptive extraction are very complex. The enriched compounds range from

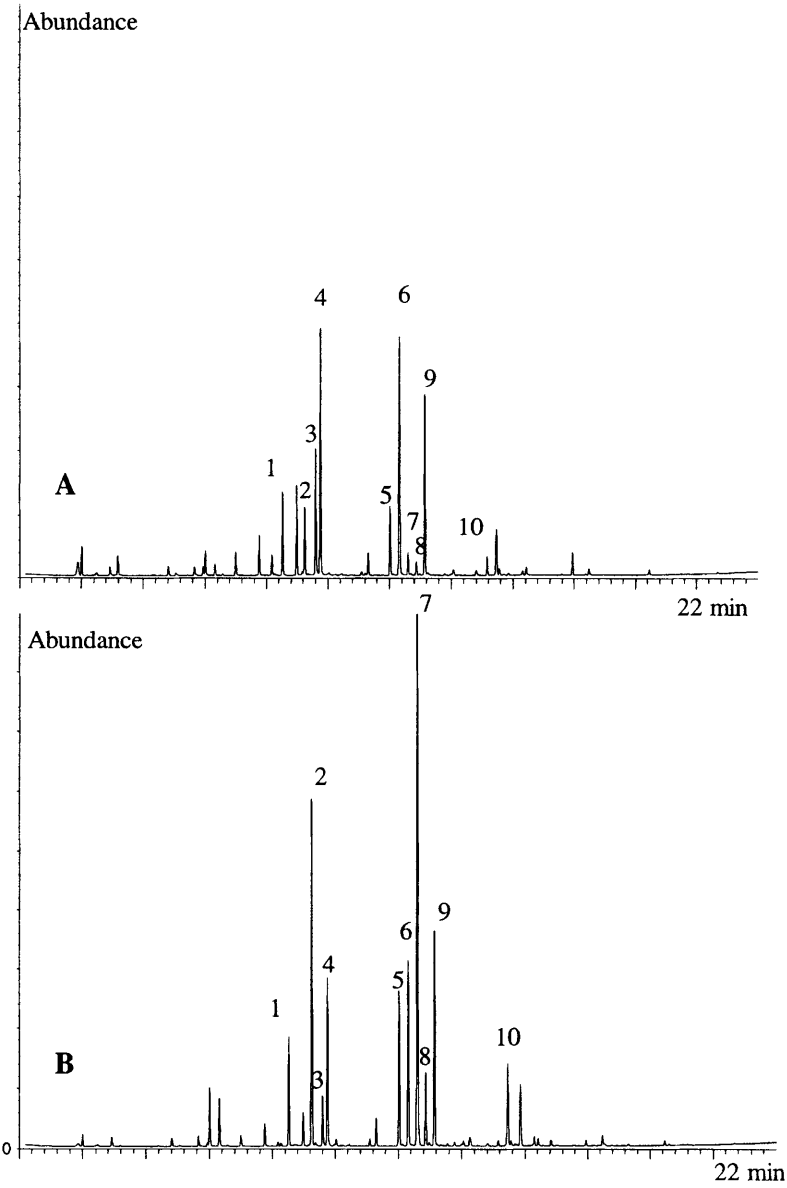


Figure 4 *HSSE-TDS-CGC/MS of a (A) unripe and (B) ripe banana. Compounds: 1, butyl acetate; 2, 2-pentanol acetate; 3, 1-hexanol; 4, 1-butanol-3-methyl acetate; 5, butyl butyrate; 6, hexyl acetate; 7. 2-methyl-1-methylbutyl propionate; 8, 2-methyl-3-methylbutyl propionate; 9, hexyl butyrate; 10, 2-ethylcyclohexyl butyrate.*

acetaldehyde to palmitic acid and thus cover a wide volatility range. Different sulphur compounds were detected such as dimethylsulphide, methylthioacetate, dimethyldisulphide, methional and 2-(2-furfanyl)thiazole. Hop derived solutes in the aromagram could be identified in different beers by ion extraction.

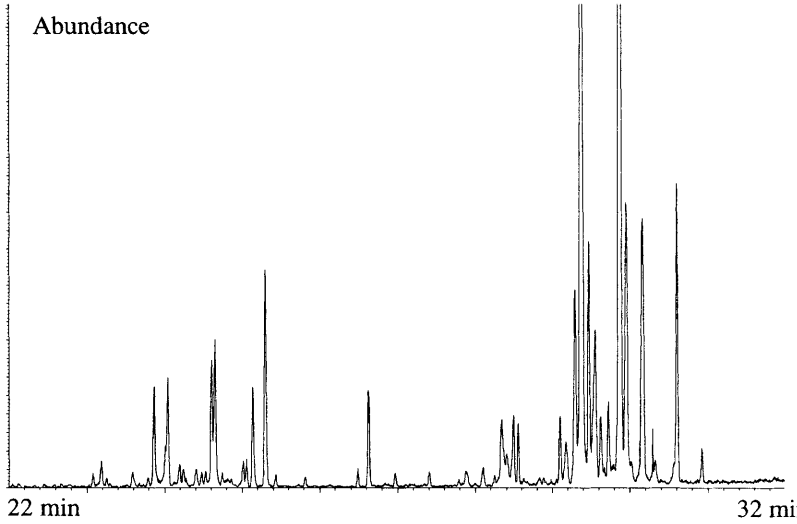


Figure 5 *SBSE-TD-CGC/MS ion extraction chromatogram at 204 m/e of a "hoppy" beer*

Figure 5 shows part of the extracted ion chromatogram at m/e 204 and important sesquiterpenes and sesquiterpenoids like humulene and β-damascenone could be detected. Moreover off-flavour compounds, such as the "sunstruck" flavour that originates from the bitter acids, could be detected and quantified at the ppt level. This is shown in Figure 6. A fresh beer sample was analysed directly after opening the bottle and after irradiation with UV-light. The peak at 9.34 min increases as function of irradiation time and this compound could be identified by full scan mass spectrometry as 3-methyl-but-2-ene-thiol (insert Figure 6). It is known that this compound is responsible for the "sunstruck" flavour of beer at the ppt level. Stir bar sorptive extraction opens new perspectives for the quality control of beer (ppm level), for the elucidation of hop derived aroma compounds in beer (ppb and sub-ppb level) and for ultra-trace analysis of important off-flavour compounds such as 3-methyl-but-2-ene-thiol.

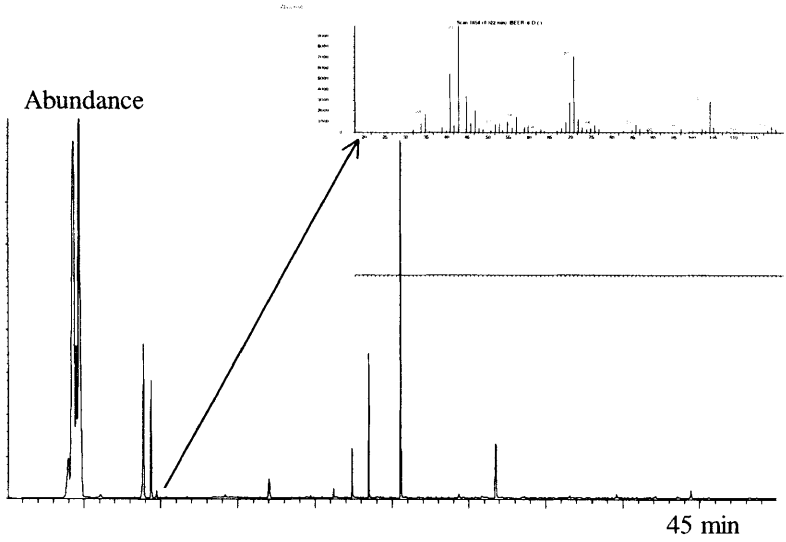


Figure 6 *SBSE-TD-CGC/PFPD-MS. Identification of the sunstruck flavour*

The bitter taste of beer is derived from hop (*Humulus lupus L.)* or hop extracts added to the wort during brewing. In the boiling process the hop α-acids or humulones are converted into the iso-α-acids or isohumulones. These products are not only responsible for the bitter taste of beer, but also exhibit bacteriostatic properties and furthermore play an essential role in the foam stability of beer as well as in the formation of off-flavours like the sunstruck flavour. Liquid chromatography (LC) is intensively used for the analysis of α- and β-acids in hops[18,19] and of iso-α–acids in beer.[20,21] Problems related with interaction of the solutes with trace metals in the chromatographic system resulting in poor resolution and quantitation have been reported. [20,21] The electrodriven separation technique micellar electrokinetic chromatography (MEKC)[22,23] has been evaluated for the analysis of the hop acids and the iso-α-acids. For the analysis of the iso-α-acids in beer, a pre-concentration step is normally performed although direct beer injection in MEKC is possible. Enrichment is commonly carried out by liquid-liquid extraction or by solid phase extraction on reversed phase materials. SBSE-LD has been applied for the MEKC analysis of the bitter compounds in beer. The SBSE-LD-MEKC procedure is as follows. Beer (25 ml) is poured in a headspace vial of 40 ml and 1 ml of 6 M HCl is added. A stir bar containing 25 µl PDMS is introduced and the sample is stirred at 1500 rpm at 25°C for 60 minutes. The stir bar is dipped in bidistilled water and dried on a tissue paper. The stir bar is then placed in 2 ml of acetonitrile containing the internal standard ethylparaben at 8 mg/l and is stirred at 1500 rpm at 25°C for 60 minutes. 0.5 ml is taken and evaporated to dryness under a nitrogen stream. The residue is dissolved in 0.5 ml bidistilled water and the sample is injected. MEKC analysis was performed on a HP^{3D} CE capillary electrophoresis system equipped with diode array detection (Agilent Technologies, Waldbronn, Germany). Data analysis was done with the ChemStation (Rev. A.06.01) software (Agilent). Bare fused silica capillaries (Composite Metal Services Ltd., Worcester, UK) with an internal

diameter of 75 µm and a total length of 77.7 cm was used (L_{eff} = 55.7 cm). The buffer consisted of 50 mM phosphate (pH 7.6) containing 40 mM sodium dodecylsulphate (SDS). Injection was performed hydrodynamically for 2 s. The MEKC method provides more than adequate resolution for the separation of the 6 iso-α-acids within a sufficiently short time. Interference from other beer components is eliminated when using SBSE for sample preparation. This is evident from figure 7 which shows a comparison between a sample obtained by direct injection of beer (A) and by SBSE-liquid desorption (B). The repeatability of the MEKC analysis was found to be adequate as can be seen from the data in Table 1. The RSD (%) for the areas (measured as normalised areas, i.e. peak area divided by migration time) of the hop acids were calculated relative to the normalised area for ethylparaben.

	Relative areas	
Acid	RSD(%)* migration time	RSD(%)* Relative normalised area
cis-cohumulone	0,14	4,50
trans-cohumulone	0,15	5,52
cis-adhumunlone	0,16	4,21
cis-humulone	0,23	5,33
trans-adhumulone	0,19	4,12
trans-humulone	0,26	3,46
ethylparaben	0,40	-

* n = 5

Table 1 *Repeatability of SBSE-LD-MEKC for the bitter compounds in beer.*

3 CONCLUSION

Sorptive extraction is a powerful technique for flavour and fragrance analysis. SBSE and HSEE are two novel techniques broadening the applicability of sorptive extraction. More developments like passive sampling, "enfleurage" on PDMS, etc. are expected in the near future.

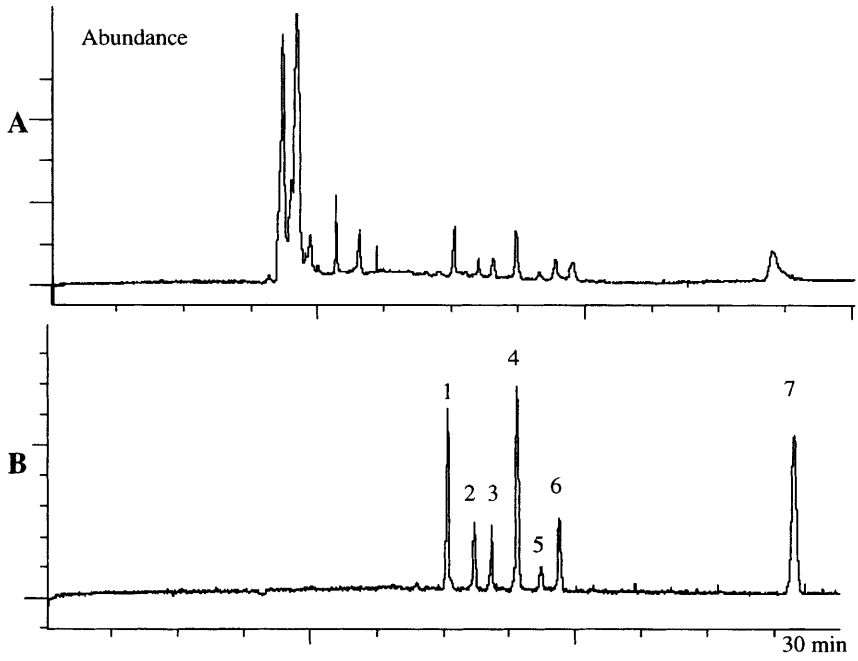


Figure 7 *The bitter compounds in beer by direct injection (A) and SBSE-LD-MEKC (B).*
Compounds: 1, cis-cohumulone; 2, trans-cohumulone; 3, cis-adhumulone, 4, cis-humulone; 5, trans-adhumulone, 6, trans-humulone; 7, ethylparaben (internal standard).

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APPLICATION OF CHROMATOGRAPHIC AND SPECTROSCOPIC METHODS FOR SOLVING QUALITY PROBLEMS IN SEVERAL FLAVOUR AROMA CHEMICALS

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1 INTRODUCTION

The confusion in the interpretation of the molecular structure of some aroma chemicals causes many misunderstandings with the users of these products. Several important cases, *i.e.* keto-enol tautomerisation of α-diketones, isomerisation of α-angelica lactone and the exact substitution pattern of pyrazines, will be discussed.

The analytical methods used to unravel the correct molecular structures were GC, GC-MS and ^{15}N-NMR spectroscopy especially polarisation-transfer for sensitivity enhancement (INEPT) and 2D-correlation techniques.

2 α-DIKETONES

α-Diketones are an important group of aroma chemicals that are characterised by two adjacent keto functionalities. Their structure consists of 4 to 7 carbon atoms as shown in table 1.

The flavour characteristics of α-diketones range from creamy, sweet, buttery, caramely and milky, to fruity, roasted and burnt. This group of natural products occur in many food flavours, butter, milk, fruits, wine, roasted products, vinegar, yoghurt, coffee, raspberry and cheddar cheese. These materials are applied to dairy flavourings (*e.g.* margarine), ice cream flavours, butter, strawberry, caramel, butterscotch, brown sugar, custard and cream. The α-diketones are also used as key raw-materials for pyrazines, *e.g.* diacetyl for 2,3-dimethylpyrazine, 3,4-hexanedione for 2,3-diethylpyrazine etc.

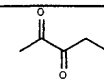
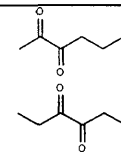
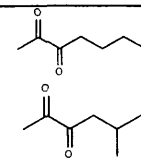
4 Carbon atoms	2,3-Butanedione diacetyl	
5 Carbon atoms	2,3-Pentanedione acetyl propionyl	
6 Carbon atoms	2,3-Hexanedione acetyl butyryl 3,4-Hexanedionedipropionyl	
7 Carbon atoms	2,3-Heptanedione acetyl valeryl 5-Methyl-2,3-hexanedione acetyl isovaleryl	

Table 1 *α-Diketones*

2.1 On the keto-enol tautomerism of α-diketones

The GC analysis of certain diketones has caused some confusion amongst several of our clients. The following explanation should help remove this confusion.

Aldehydes and ketones exist in solution as equilibrium mixtures of two isomeric forms, the **keto** form and the **enol** form. We have noticed that this equilibrium in 3,4-Hexanedione (figures 1 & 3) especially, but also in the other α-diketones, is dependent on the GC conditions, the insert, the insert filling and age, the column, etc.

It has been shown that when injecting the material under different GC conditions, the equilibrium changes, especially when using an apolar column, *e.g.* HP-5. When a polar column, *i.e.* HP-FFAP was used, only the **keto** form was detected, thus summing up the area of both peak areas that are seen when using an apolar column. Even when using an apolar column with different inserts, the same material would give either 2 peaks or 1 peak (which is the sum of both areas). The enol form can be readily trapped by reaction with BSTFA (*N,O*-Bis(trimethylsilyl)trifluoroacetamide)(figures 2 & 4).

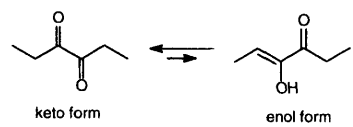


Figure 1 *The tautomers of 3,4-hexanedione which are seen in GC analysis*

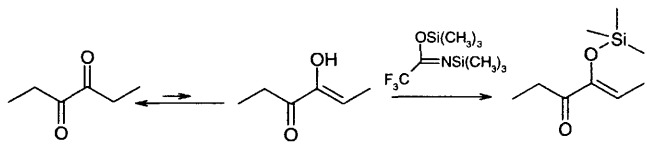


Figure 2 *The enol form can react with BSTFA*

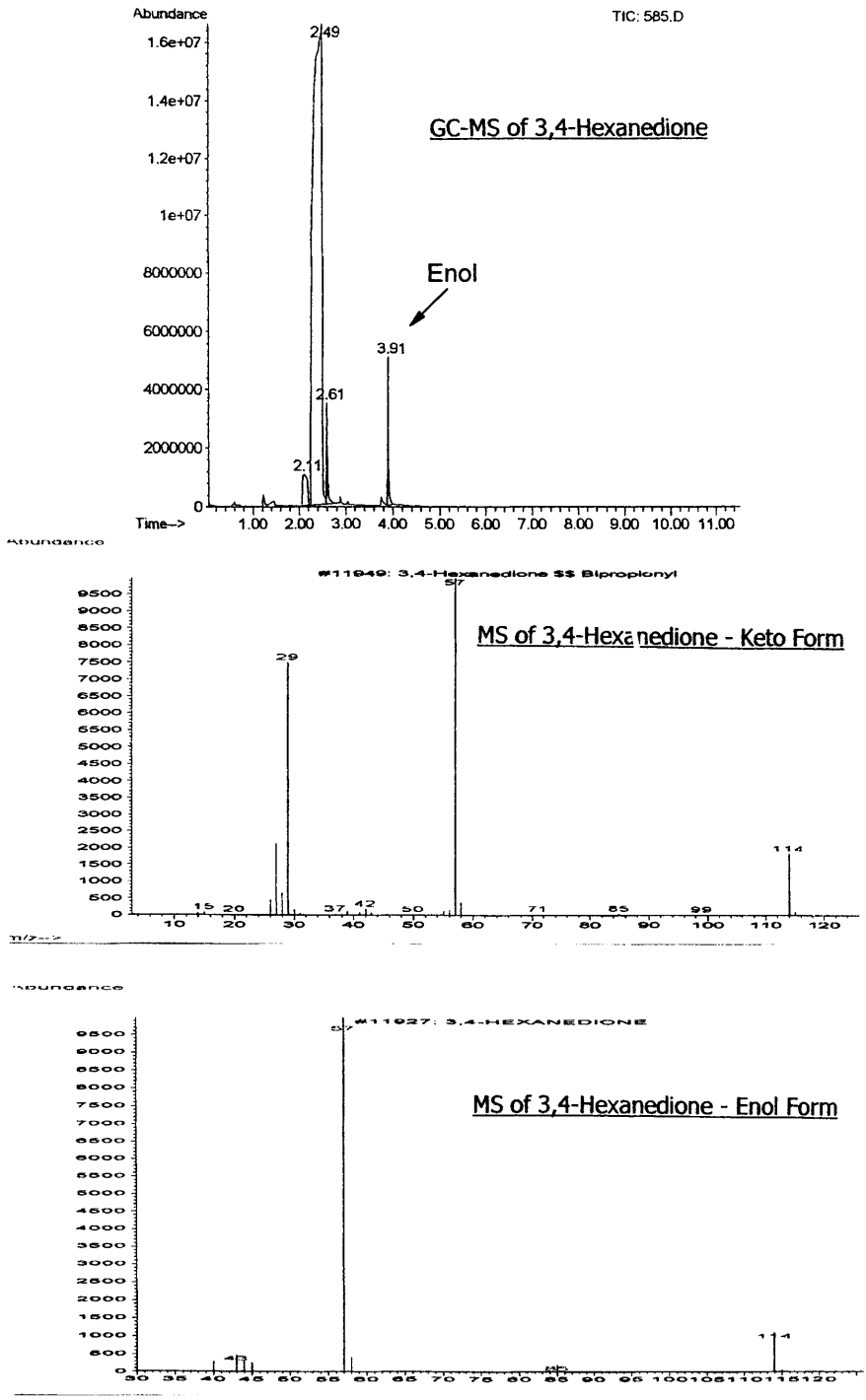

Abundance
TIC: 585.D
2.49
GC-MS of 3,4-Hexanedione
Enol
3.91
2.61
2.11
Time-->
#11949: 3,4-Hexanedione $$ Bipropionyl
MS of 3,4-Hexanedione - Keto Form
#11927: 3,4-HEXANEDIONE
MS of 3,4-Hexanedione - Enol Form

Figure 3

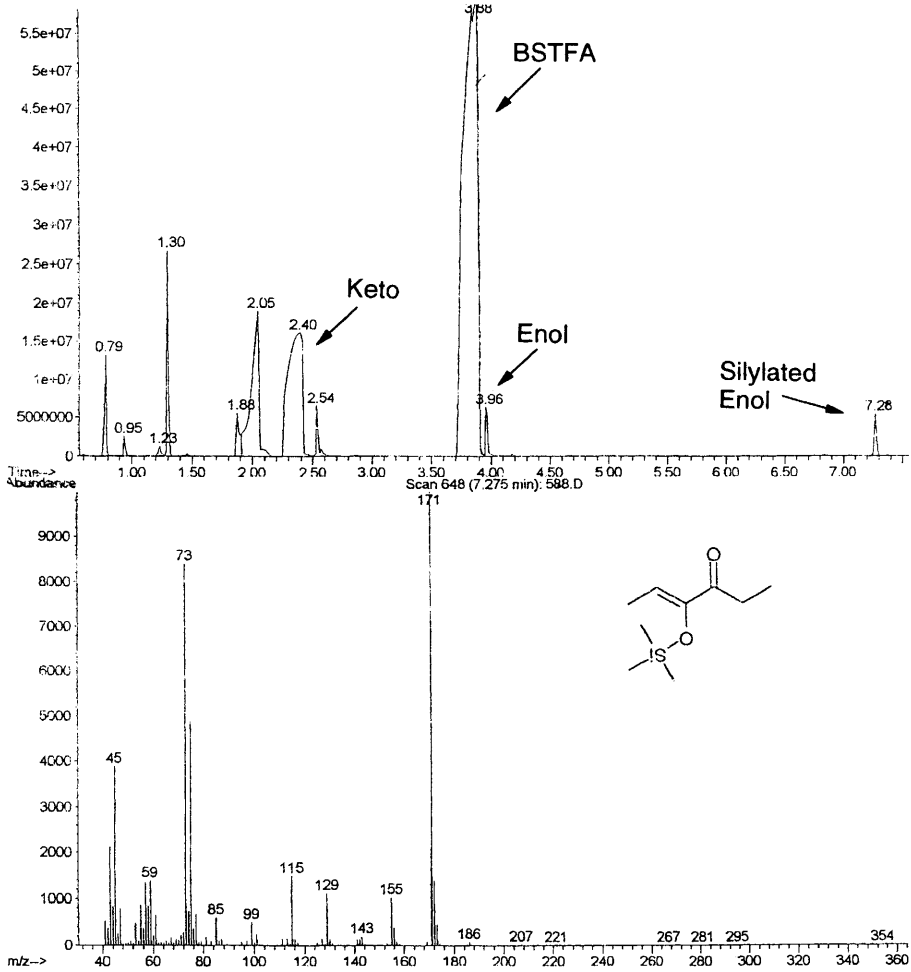


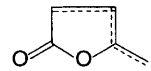
Figure 4

As a consequence of these results, one has to take into account the sum of the 2 peaks (keto and enol), when calculating the purity of α-diketones, and not only the major keto form peak.

3 α-ANGELICA LACTONE

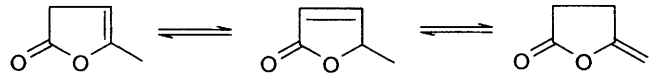
α-Angelica lactone occurs in grape (dried), bread, soybean and liquorice. The material has a sweet, creamy, coconut, and vanilla flavour with hay and coumarin-like nuances. It is applied in aromatic and dairy formulations; coconut, vanilla, molasses, coumarin, cream, milk, nut and tobacco flavours.

Angelica lactone (5-Methyl-2,3H-furanone) can be described by the following structure:



Angelica Lactone

Practically, this molecule exists in an equilibrium of 3 isomers (figure 5) of which the major isomer is α.



α-Angelica Lactone β-Angelica Lactone γ-Angelica Lactone

Figure 5 *The 3-isomers of angelica lactone*

As can be seen, there is a possibility for the double bond electrons to migrate. This phenomenon is affected by different parameters, *e.g.* temperature, pH, etc. When a sample of α-angelica lactone is injected onto a gas chromatograph (figure 6), it is influenced by the injector temperature, the form and acidity of the insert, the column conditions, its history and other parameters.

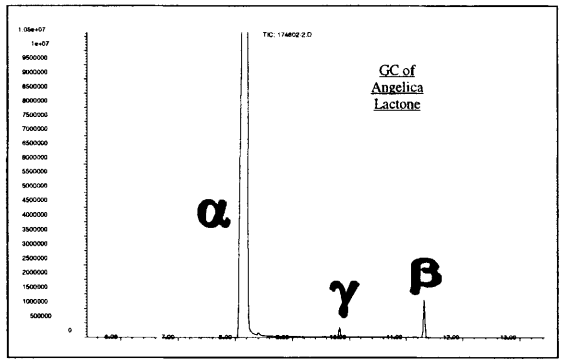


Figure 6

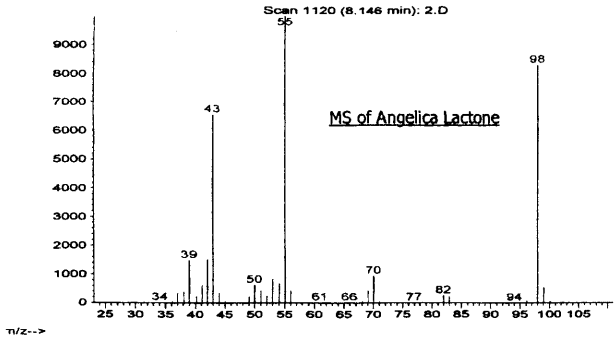


Figure 7

Due to its sensitivity, α-Angelica lactone isomerises during a GC run to give a certain isomeric partition, as shown in figure 8.

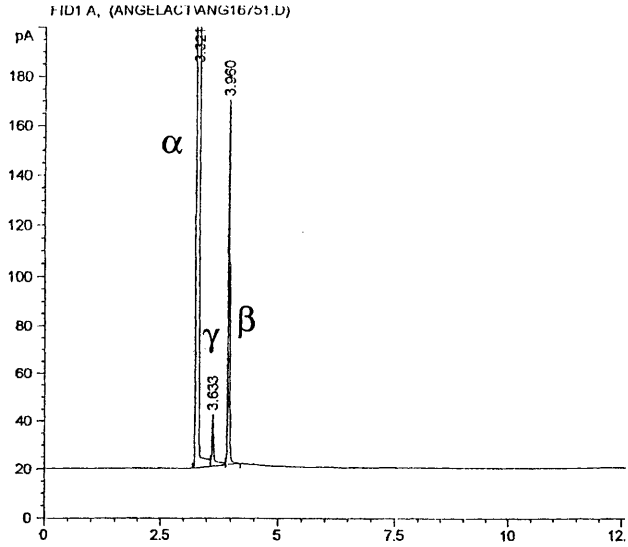


Figure 8

Another result of this phenomenon that can be observed (figure 8) is the cohesion of the peaks, which arises from the isomerisation taking place on column, during the GC run. Figures 9 and 10 show the same material after being injected after an injection of 5 µL diethylamine, to change the column pH.

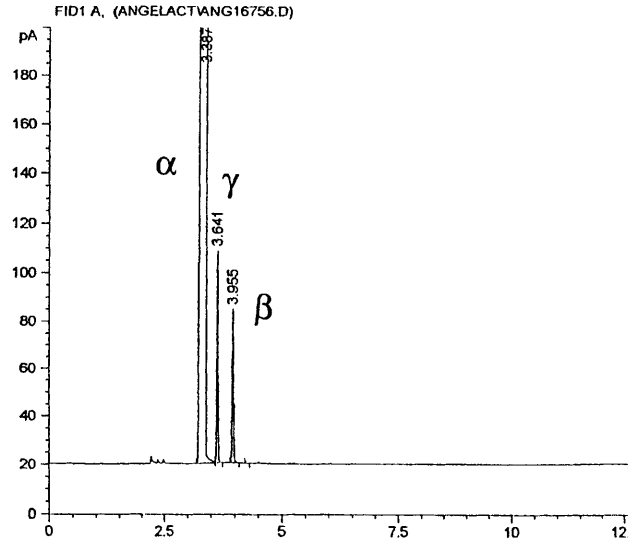


Figure 9 *GC of α-angelica lactone after diethylamine injection*

	α	γ	β
Before $HNEt_2$ Injection	93.7%	1.1%	5.3%
After $HNEt_2$ Injection	98.6%	0.7%	0.6%

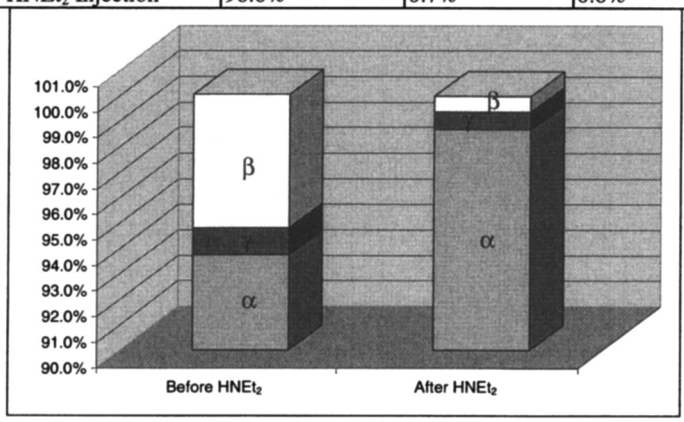


Figure 10 *shows the ratio of isomers before and after diethylamine injection*

As in the former example, one has to take into account that different partition patterns of the three isomers peaks of angelica lactone are obtained in relation to different parameters in the GC, *e.g.* temperature, pH, etc.

4 ON THE SUBSTITUTION PATTERN OF PYRAZINES

Pyrazines are materials obtained during the Maillard reaction as by-products of the browning reaction of sugars and proteins or amino acids. These reactions occur during roasting, cooking, baking, etc. of different food products. The importance of these materials motivated organic chemists to synthesise and use them in the flavour industry as ingredients in flavour formulations for roasted nuts, meat flavours, etc.

By 1970, the first pyrazines obtained GRAS status in the US, for use as flavouring substances. However, as a result of the lack of modern analytical tools, the exact structures were not defined. For example, a mixture of isomers of methyl-methoxy- pyrazines was described in an non-exact manner, namely 2-methoxy-3(5 or 6)-methyl- pyrazine.

The problem of the exact structure of several commercially significant pyrazines arises when examining the product lists of some important pyrazine manufacturers. The ambiguous pyrazines are mainly those which contain a substituent at position number 5 or 6, in addition to a substituent at position number 2, for example 2-methyl-5 or 6-methoxy-pyrazine (figure 11).

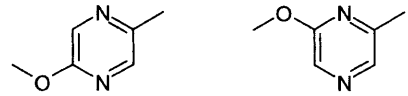


Figure 11 *2-Methyl-5-methoxypyrazine and 2-methyl-6-methoxypyrazine*

In this work, the correct substitution pattern of disubtituted pyrazines was evaluated. The method which was used to elucidate the structures of these rather simple, but puzzling molecules was nitrogen or ^{15}N-NMR spectroscopy[1].

The substitution pattern of di- or trisubstituted pyrazines can be elucidated by a combination of NMR methods (figures 12 & 13), especially in mixtures by gradient selected $^{1}H,^{15}N$ HMBC experiments (figure 13) at natural abundance level. In the case of disubstituted pyrazines it allows one to distinguish between 2,3-/2,5- and 2,6-disubstituted isomers. Another advantage is the determination of and assessment of ^{15}N chemical shifts even in relatively low concentrations. The reliability of this method was verified by experimental data resulting from ^{1}H NMR and long-range heteronuclear correlation experiments such as FLOCK or $^{1}H,^{13}C$ HMBC experiments. Carbon-carbon coupling constants derived from INADEQUATE experiments were taken into account as additional tests. Thirty-one commercially available di- and trisubstituted pyrazines were measured in order to identify the substitution pattern and to obtain the chemical shift information.

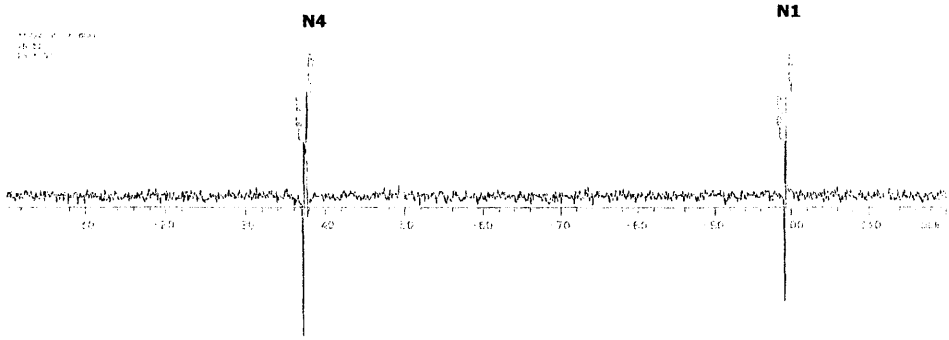


Figure 12 *INEPT spectrum of 2-methyl-5- or 6-propoxypyrazine*

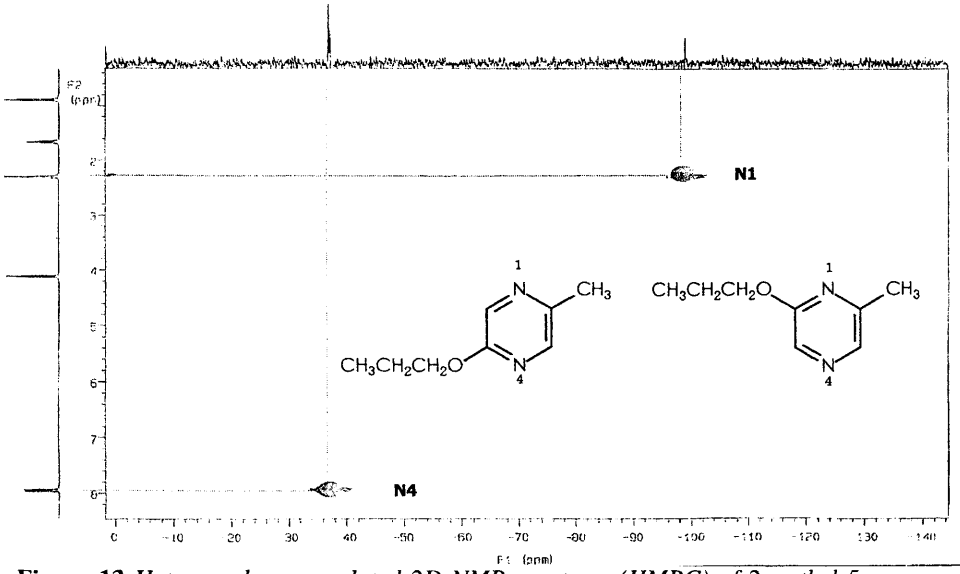
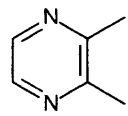
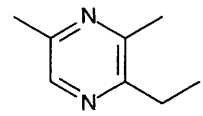
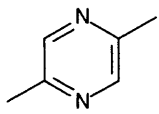
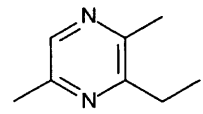
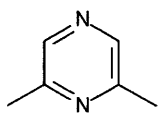
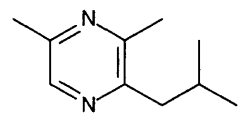
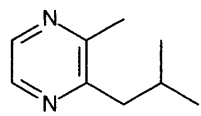


Figure 13 *Heteronuclear correlated 2D NMR spectrum (HMBC) of 2-methyl-5-propoxy- or 2-methyl-6-propoxypyrazine*

Figures 14 to 19 show the range of ^{15}N chemical shifts that can be expected for various functional groups. Figure 20 details proton, carbon, and nitrogen shifts for several well-known pyrazines. The effect of substitution on the NMR of pyrazines is detailed in figures 21 and 22.

Alkylpyrazine	N-1	N-4	Alkylpyrazine	N-1	N-4
	-46.6	-46.6		-48.1	-45.6
	-45.8	-45.8		-44.8	-46.8
	-44.5	-47.2		-45.1	-46.0
	-48.1	-46.4			

Alkyl groups (only) place the ^{15}N chemical shifts in the range of -44 to -48 ppm.

Figure 14 *^{15}N NMR data of di- and trisubstituted alkyl pyrazines*

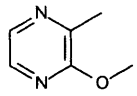
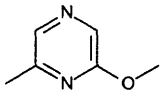
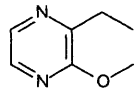
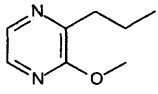
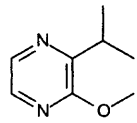
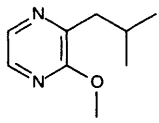
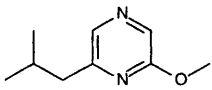
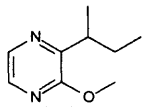
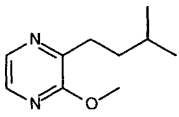
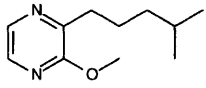
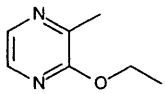
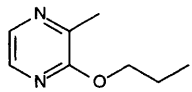
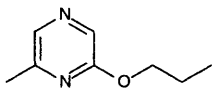
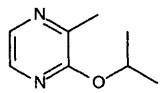
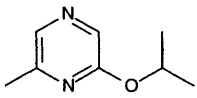
Alkoxypyrazine	N-1	N-4	Alkoxypyrazine	N-1	N-4
	-100.9	-41.7		-94.4	-37.9
	-100.9	-43.6		-100.1	-42.8
	-99.8	-45.7		-100.8	-40.3
	-94.6	-38.6		-100.3	-44.5
	-100.8	-42.6		-100.4	-42.0
	-93.5	-38.3		-100.7	-42.1
	-93.6	-38.3		-99.8	-42.4
	-93.0	-41.7			

Figure 15 *^{15}N NMR data of substituted alkoxy pyrazines*

Alkoxy groups place the ^{15}N chemical shifts in the range of -93 to -101 ppm. Alkyl groups in presence of alkoxy substituents place the ^{15}N chemical shifts in a range of -38 to -46 ppm.

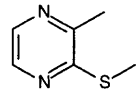
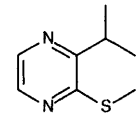
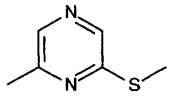
Thioalkoxypyrazine	N-1	N-4	Thioalkoxypyrazine	N-1	N-4
	-60.7	-50.0		-59.0	-54.0
	-58.4	-46.6			

Figure 16 *^{15}N-NMR data of substituted thioalkoxy pyrazines*

Thioalkoxy groups place the ^{15}N chemical shifts in the range of -58 to -61 ppm. The alkyl groups in presence of thioalkoxy substituents place the ^{15}N chemical shifts within the range of -46 to -54 ppm.

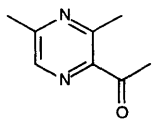
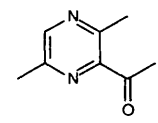
Acetylpyrazine	N-1	N-4	Acetylpyrazine	N-1	N-4
	-42.3	-40.6		-44.1	-38.6

Figure 17 *^{15}N-NMR data of disubstituted acetyl pyrazines*

Acetyl groups place the ^{15}N chemical shifts in the range of -42 to -44 ppm. The alkyl groups in presence of acetyl substituents place the ^{15}N chemical shifts within the range of -38 to -44 ppm.

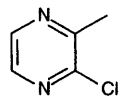
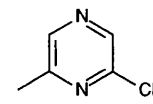
Chloropyrazine	N-1	N-4	Chloropyrazine	N-1	N-4
	-52.0	-37.0		-52.9	-35.1

Figure 18 *^{15}N-NMR data of di- and substituted chloro pyrazines*

Chloro groups place the ^{15}N chemical shifts in the range of -52 to -53 ppm.The alkyl groups in presence of chloro substituents place the ^{15}N chemical shifts within the range of -37 to -53 ppm.

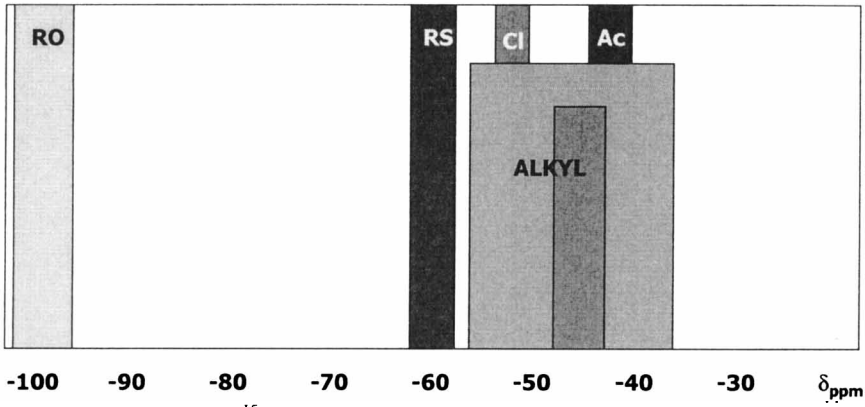


Figure 19 *Summary of ^{15}N-NMR chemical shifts for substituted pyrazines*

R	δ (^{15}N) [ppm]		δ (^{1}H) [a] [ppm]				δ (^{13}C) [ppm]				
	N1	N4	H3	H5	H6	R	C2	C3	C5	C6	R
H	-43.9	-43.9	8.60	8.60	8.60		145.2	145.2	145.2	145.2	
CH_3	-45.2	-44.1	8.471	8.382	8.462	2.576	154.2	144.9	141.9	143.9	21.6
OCH_3	-97.6	-37.3	8.236	8.117	8.089	3.971	160.6	136.0	136.5	140.6	53.5
SCH_3	-59.4	-47.4	8.465	8.192	8.365	2.574	157.7	143.5	139.1	143.8	12.6
O=C-CH_3	-45.6	-41.0	9.236	8.753	8.646	2.728	147.7	143.5	147.8	143.6	25.8 [b]

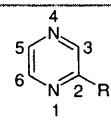


Figure 20 *^{15}N, ^{1}H and ^{13}C NMR shift data of pyrazine and four monosubstituted pyrazines*

To summarize:

- Alkyl substituents cause the nitrogen atoms to resonate in the range of -35 to -55 ppm.
- Alcoxy groups cause the nitrogen atoms to resonate in the range of -93 to -101 ppm.
- Thioalcoxy substituents cause the nitrogen atoms to resonate in the range of -58 to -61 ppm.
- Acetyl groups cause the nitrogen atoms to resonate in the range of -42 to -44 ppm.
- Chlorine atoms cause the nitrogen atoms to resonate in the range of -52 to -53 ppm.

Substituent	N-1 (ppm)	C-3 (ppm)	H-3 (ppm)
H	-43.9	145.2	8.6
CH_3	-45.2	144.9	8.47
OCH_3	-97.6	136.0	8.24
SCH_3	-59.4	143.5	8.47
$COCH_3$	-45.6	143.5	9.24

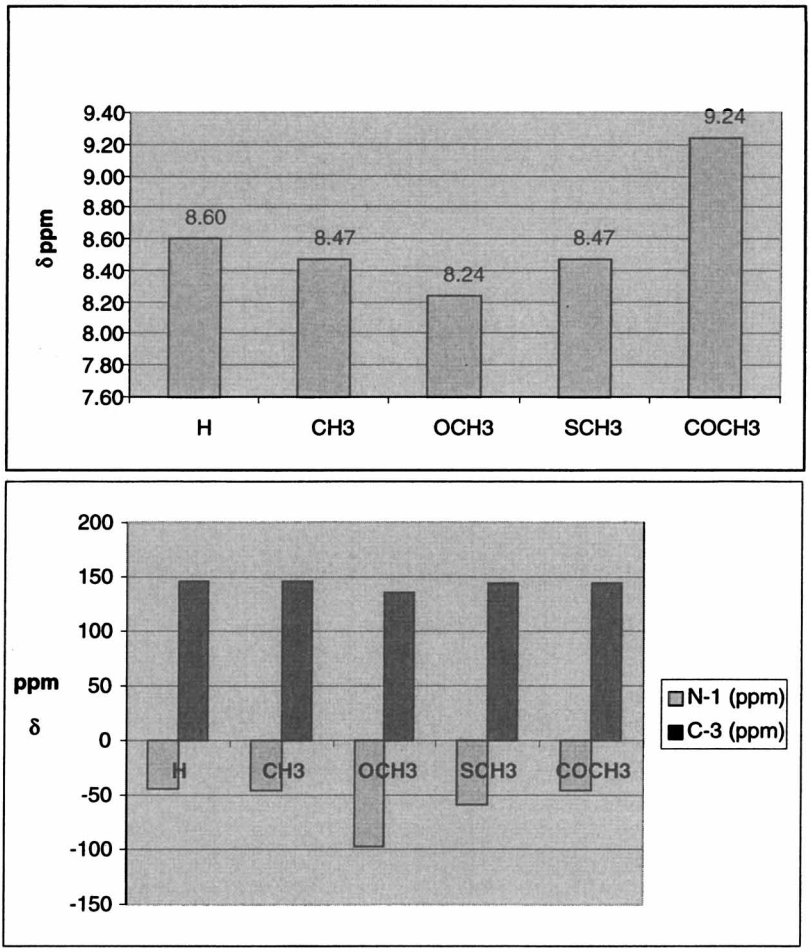


Figure 21 *Effect of electronic properties of substituents on pyrazines chemical shifts*

The largest low-frequency shift of N-1 was found in 2-methoxypyrazine which is attributed to the strong π-donating nature of the methoxy group.

Substituent	H-3 ^{1}H-NMR	C-3 ^{13}C-NMR	N-1 ^{15}N-NMR
CH_3	Upfield	Upfield	Upfield
OCH_3	Upfield	Upfield	Upfield
SCH_3	Downfield	Downfield	Downfield
$COCH_3$	Downfield	Downfield	Downfield

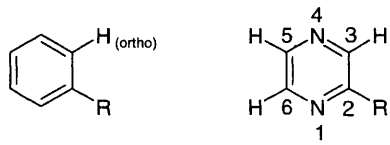
Figure 22 *The effect on the chemical shifts of substituted pyrazines*

Similar trends are observed in the chemical shifts movements of H-3 (in the ^{1}H-NMR), C-3 (in the ^{13}C-NMR) and N-1 (in the ^{15}N-NMR) resonances:

Methyl and methoxy groups shift upfield, thiomethoxy and acetyl groups shift downfield.

4.1 Effect on Chemical Shifts of Substituted Pyrazines vs. Benzenes

When comparing the effect on the chemical shift of the *ortho* hydrogen atom of a pyrazine and a benzene ring, the opposite effect is seen (figure 22).



Substituehnt	H (*ortho*)	Effect	H-3	Effect
CH_3	-0.16	Downfield	+0.13	Upfield
OCH_3	-0.46	Downfield	+0.36	Upfield
$COCH_3$	+0.62	Upfield	-0.64	Downfield

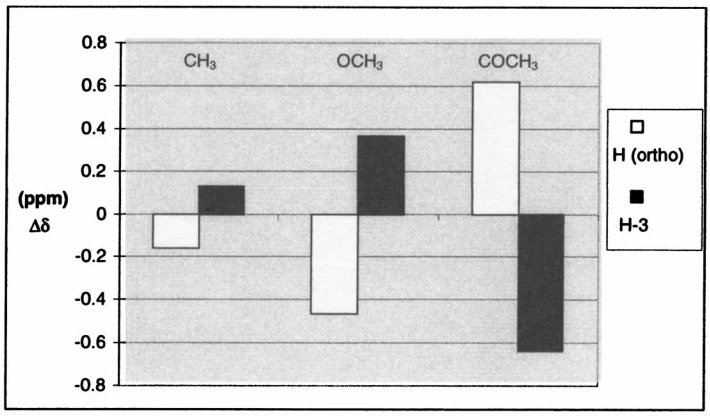


Figure 23 *Effect on chemical shifts of substituted pyrazines vs. benzenes*

5 SUMMARY

- While calculating the purity of α-diketones, one has to take into account the sum of the 2 peaks (keto and enol), and not only the major keto form peak.

- Angelica lactone (5-Methyl-2,3H-furanone) exist as an equilibrium of three isomers of the double bond, which show different GC patterns under different GC conditions.

- ^{15}N NMR data of 31 different pyrazines was measured and evaluated. Chemical shifts ranges were determined for alkyl, alcoxy, thioalcoxy, acetyl and chloro substituents.

- The effect of electronic properties of substituents on chemical shifts of pyrazines in ^{15}N, ^{13}C and ^{1}H NMR spectra was studied.

- The combined data enabled us to better understand the ^{15}N-NMR of pyrazines and to evaluate and predict their structure more accurately.

References

1. Studies Towards Structure Determination of Substituted Pyrazines, Zviely, A. Kern, I. Gozlan and R. Frim, Perfumer & Flavorist, 23, 27, 1998.

Natural Products and Essential Oils

COMMERCIAL ESSENTIAL OILS: TRUTHS AND CONSEQUENCES

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1 INTRODUCTION

For an essential oil to be a genuine commercial essential oil it must be isolated from a whole plant or plant part of known taxonomic origin by physical means only.

The physical methods used are distillation (steam, steam/water and water) or expression (also known as cold pressing, a unique feature for citrus peel oils). There is one other method of oil isolation known as maceration/distillation that is specific to a very limited number of essential oil plants. In the process, the plant material is macerated in warm water to release the enzyme-bound essential oil. Examples of oils produced by maceration are onion, garlic, wintergreen, bitter almond, etc. A sub- or supercritical CO_2 extract does not constitute an essential oil, even if it has been fractionated to remove the non-volatile and waxy components found in the extract. This product is best described as a volatile concentrate. Obviously, if any extracting solvent is used to isolate the volatiles such as a halohydrocarbon, the end product is always an extract. There is one further product that has been classified as an oil (although in this author's mind this is a mixture of an oil and the wood thermal degradation products) and that is destructive, dry or empyreumatic distillation.

If we consider a partial inventory of the vegetable kingdom, it is estimated that, although there are approximately 17,500 aromatic plants in the world, only about 300 are used as raw material sources in the flavour and fragrance industries world-wide. These plants are used to produce essential extracts, oleoresins, concretes, absolutes, resinoids, pomades, tinctures, etc. Of these materials, ca. 50% are produced from cultivated plants while the other 50% are obtained either as by-products of a primary industry or are harvested in the wild (wild-crafted). In addition, many of the raw materials are not distilled or extracted within close proximity of where they were grown or harvested.

Wild populations of aromatic plants can either be homogeneous or heterogeneous. Individual members of a population can differ both in essential oil yield and their quantitative composition. However, oil compositional differences in commercial oils is more complex than described above. There are several reasons why essential oils can have differing chemical compositions, although the taxonomic origin of the plant from which the oil was isolated is known, e.g.

1. Genetic: clone, hybrid, cultivar or population specific taxon variation
2. Ontogenetic: variation in the growth stage of the plant harvested for oil isolation.
3. Extrinsic: effect of climate, soil, season, time, disease or insect damage, geographical origin, etc.
4. Infraspecific: variation in chemotype of plants harvested (wild plants but also some seed sown plants)
5. Endogenous: within plant variation which can be affected by how much of plant was harvested
6. Processing: variation in the method of oil isolation
7. Exogenous: addition of components either prior to distillation or directly into the oil

2 REASONS FOR DIFFERING CHEMICAL COMPOSITION

2.1 Genetic

As expected the genotype of the plant from which the oil is obtained has a marked effect on the oil composition. Some cultivated aromatic plants are reproduced clonally; however, different clones of the same botanical species can and are often used for oil production. An example of this can be seen in the oil composition of three cultivars of lavandin produced in France, which are reproduced clonally (see Table 1).

Compound	Abrialis	Grosso	Super
1,8-cineole	6.7-10.4	5.2-10.2	2.0-4.9
(Z)-β-ocimene	2.1-2.6	0.7-1.2	1.3-1.4
(E)-β-ocimene	4.0-5.5	0.5-0.6	1.9-2.4
camphor	8.2-12.2	5.9-8.2	3.8-6.0
linalool	19.6-39.6	25.7-34.1	29.3-31.0
linalyl acetate	18.6-28.0	26.2-36.7	30.4-45.0
lavandulyl acetate	1.1-2.7	2.0-2.4	1.6-2.5
terpinen-4-ol	1.0-1.2	2.8-3.6	1.4-1.6
lavandulol	0.5-1.0	0.8-2.4	0.4-0.9
α-terpineol	0.5-1.0	1.0-1.2	0.4-0.5
borneol	2.4-3.7	2.0-3.6	1.6-2.3

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Table 1 *Comparative Composition of Commercial Lavandin Oils*

An example of marked captionin oil composition due to clonal difference can be seen with *Tagetes minuta* (Table 2).

The effect of cultivar on oil composition can be seen with *Salvia officinalis* (Table 3). A more complex situation of variation in oil composition is found with geranium (Table 4). Geranium is a hybrid that has been produced from the cross between *Pelargonium graveolens* L'Herit ex Arton or *P. radens* H. E. Moore with *P. capitatum* (L.) L'Herit. Until someone performs a DNA fingerprint on the plant material used to produce the various oils their true hybrid origin will remain unknown.

	Pre-Flowering		Early Flowering		Full Flowering		Post Flowering	
	Clone 1[1]	Clone 2[2]	Clone 1	Clone 2	Clone 1	Clone 2	Clone 1	Clone 2
(Z)-β-ocimene	16.9-17.8	2.0	21.9-33.3	20.4	35.3	19.0	41.3-45.9	11.8
dihydrotagetone	46..4-51.3	16.5	30.0-36.0	22.9	30.5	31.3	14.8-24.1	33.4
(E)-tagetone	1.4-3.2	16.9	17.5-24.1	11.4	2.2	7.3	7.5-8.2	3.7
(Z)-tagetone	18.5-22.4	21.5	1.3-1.4	23.4	16.9	29.7	18.2-20.4	25.8
(Z)-tagetenone	1.5-4.4	0.1	6.9-8.6	8.3	6.9	0.2	0.3-0.4	0.6
(E)-tagetenone	2.0-3.0	0.4	1.5-1.9	2.2	1.8	0.2	2.0-3.6	0.4

1 Lawrence 2 Chalchat et al. (1995)

Table 2 *Ontogenetic Effect on Composition of Oils of Two Clones of Tagetes minuta*

	Hungarian[1]	Czech[1]	French[1]	Yugoslavian*[1]	Albanian*[2]
α-pinene	3.4	6.2	1.9	2.0-6.6	1.1-5.2
camphene	4.3	2.6	2.6	1.7-8.5	4.6-7.2
β-pinene	1.4	3.0	1.9	0.7-3.4	0.6-1.5
1,8-cineole	9.5	4.8	11.0	5.8-22.5	7.4-19.6
α-thujone	22.4	22.8	26.4	7.2-36.3	16.6-39.8
β-thujone	2.2	7.1	5.7	3.9-40.1	4.2-7.8
camphor	25.9	25.6	19.9	7.0-23.1	17.2-30.6
α-humulene	9.6	5.5	4.0	0.8-2.9	2.1-7.4

1 Chalchat et al. (1998) 2 Asllani (2000)

Table 3 *Percentage Composition of Commercial Oils of Salvia officinalis*

Compound	Algeria	Australia	China	Egypt
linalool	5.3-6.3	4.6	3.6-3.9	6.5-9.9
cis-rose oxide	0.7-1.1	0.4	1.0-1.4	0.9-1.0
trans-rose oxide	0.3-0.4	0.2	0.5-0.7	0.3-0.4
menthone	0.9-1.3	0.2	1.4-2.4	0.5-1.3
isomenthone	5.3-5.4	7.6	5.4-5.7	5.4-7.0
citronellol	22.9-27.9	31.7	36.5-39.1	24.8-33.0
geraniol	17.1-25.0	9.8	8.7-8.9	14.0-18.0
citronellyl formate	7.6-9.4	12.8	9.2-12.5	6.5-7.4
geranyl formate	5.9-6.5	3.4	1.9-2.1	3.1-4.8
guaia-6,9-diene	t-0.3	4.6	6.5-6.8	0.3-0.5
10-epi-γ-eudesmol	4.2-5.4	-	-	4.0-5.7
	India	**Morocco**	**Reunion**	
linalool	7.0-9.0	5.6-9.9	9.1-10.8	
cis-rose oxide	0.6-1.2	0.8-1.3	0.4-0.6	
trans-rose oxide	0.3-0.5	0.3-0.6	0.3-0.4	
menthone	0.4-0.6	0.8-2.1	0.4-1.1	
isomenthone	5.3-7.7	4.2-5.6	8.1-9.5	
citronellol	24.4-29.0	18.6-28.0	20.7-23.2	
geraniol	17.1-21.9	18.6-20.6	18.1-20.7	
citronellyl formate	6.0-8.6	6.0-7.6	7.4-10.4	
geranyl formate	3.7-6.3	4.1-6.0	5.5-5.9	
guaia-6,9-diene	0.1-0.2	0.2-0.5	5.8-6.9	
10-epi-γ-eudesmol	3.8-6.4	2.5-5.2	-	

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Table 4 *Comparative Percentage Composition of Selected Compounds in Geranium Oil*

Chromosome No.	Ploidy	Pollen Grain Size	Prochamazulene Biotype
2n=18	diploid	<231.42 μm	+
2n=36	tetraploid	21.65-23.46 μm	+
2n=54	hexaploid	23.89-25.96 μm	-
2n=72	octaploid	>26.43 μm	-

Oswiecimska (1973, 1974)

Table 5 *Polyploidy in Achillea millefolium*

Compound	Diploid Oil	Hexaploid Oil	Octaploid Oil
sabinene	15.3	12.4	1.2
β-pinene	7.5	11.5	1.0
1,8-cineole	1.0	1.1	14.3
linalool	<0.1	0.1	26.2
camphor	0.1	1.0	6.6
terpinen-4-ol	1.8	5.1	1.7
β-caryophyllene	22.7	7.5	1.9
germacrene D	11.4	6.8	1.3
caryophyllene oxide	0.8	6.0	1.3
(E,E)-farnesol	-	6.5	-
chamazulene	24.6	-	0.4

Hofmann et al (1992)

Table 6 *Percentage composition of major components of diploid, hexaploid and octaploid forms of Achillea millefolium*

Compound	Ontogenetic Stage				Cilantro Oil
	Flowering Initiation	Full Flowering	Green Fruit	Brown Fruit	
α-pinene	0.1	0.3	1.6	5.1	3.1
limonene	t	t	0.2	1.3	1.3
γ-terpinene	0.1	0.4	1.6	6.3	4.2
octanal	1.2	0.9	0.4	0.4	0.5
nonanal	0.5	0.1	0.1	0.1	0.3
decanal	30.0	11.9	6.2	1.6	10.4
camphor	0.1	0.5	2.2	2.4	1.2
linalool	0.3	17.5	40.9	60.4	33.6
(E)-2-decenal	20.6	46.5	30.2	3.9	21.7
dodecanal	3.3	1.0	0.5	0.4	1.0
(E)-2-undecenal	2.6	1.4	0.8	0.2	0.9
geranyl acetate	4.2	0.8	1.0	1.6	1.2
tridecanal	3.1	2.0	1.1	0.5	1.2
(E)-2-dodecanal	7.6	6.0	4.8	2.5	3.6
geraniol	0.2	0.4	0.9	1.4	0.8
tetradecanal	0.7	0.1	0.1	0.2	0.1
(E)-2-tridecenal	0.5	0.1	0.1	0.1	0.1
(E)-2-tetradecenal	4.5	1.7	1.6	1.7	1.1

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Table 7 *Ontogenetic Influence on Oil Composition of Whole Plants of Coriandrum sativum*

A further example worth mentioning is yarrow (*Achillea millefolium* L. ssp. *millefolium*) because it exists in various polyploidal forms. Polidy or the existence of polyploids means that the cells of yarrow can contain more than two sets of chromosomes. As the quality of yarrow oil is judged by its chamazulene content, ploidy is of great importance. In the

1970s, it was determined that diploids and tetraploids contained achillicin a prochamazulene precursor, whereas higher polyploids did not. It was also shown that the existence of the prochamazulene precursor and pollen grain size were correlated (Table 5). This correlation was confirmed by Hofman et al. (1992) as shown in Table 6.

2.2 Ontogenetic

Many plants, particularly herbaceous plants grown in temperate zones of the world, exhibit growth effects on their oil composition. Although this phenomenon is generally well known, the magnitude of variation can be quite dramatic. Also, it must be realised that an ontogenetic effect is influenced by extrinsic conditions. Again, this is more pronounced in the temperate and sub-tropical zones. Examples of ontogenetical effects on the oil composition of whole coriander plants (Table 7) and two clones of *Tagetes minuta* (Table 2) can be seen.

2.3 Extrinsic

The extrinsic conditions such as climate, water, sunlight, day length, pressure, nutrients, diurnal fluctuation, soil type, disease and insect damage, etc. can have an effect on the composition of an oil. An example of the effect of extrinsic conditions on the composition of Midwest (USA) peppermint oil, both within a season (1988, 1993 or 1997) and over the three seasons is shown in Table 8. Similarly, the effect of seasonal variation on the composition of clary sage oil produced in North Carolina over 4 years can be seen in Table 9.

The effect of disease on the composition of an oil produced from an Indian cultivar of geranium can be seen in Table 10. These differences, although not major except for geraniol, could be the result of necrotic leaves, thereby changing the leaf/stem ratio in the distillation charge.

	Season			3 Season Range
Compound	**1988**	**1993**	**1997**	
limonene	1.4-1.7	1.4-1.7	1.0-2.6	1.0-2.6
1,8-cineole	5.6-6.7	5.6-6.2	3.6-6.7	3.6-6.7
menthone	18.1-29.1	24.5-29.9	13.2-34.6	13.2-34.6
menthofuran	0.8-5.3	1.6-3.0	0.2-2.9	0.2-5.3
isomenthone	3.0-4.3	3.8-4.3	2.0-4.6	2.0-4.6
menthyl acetate	2.0-5.9	2.9-3.6	1.9-8.8	1.9-8.8
menthol	33.1-41.1	33.2-37.5	34.1-54.4	33.1-54.4
germacrene D	2.1-3.5	3.2-2.6	0.9-2.1	0.9-3.5
piperitone	0.5-0.6	0.5-0.6	0.3-0.7	0.3-0.7

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Table 8 *Comparative Percentage Composition of Selected Components in Midwest Peppermint Oils Over Three Different Seasons*

Compound	1979	1981	1982	1984
myrcene	0.9	0.1	1.5	1.8
limonene	0.4	0.1	0.8	0.6
(Z)-β-ocimene	0.3	0.4	0.5	0.7
(E)-β-ocimene	0.4	0.2	1.0	1.4
linalool	27.6	22.3	24.1	25.3
linalyl acetate	44.4	53.4	47.6	44.9
α-terpineol	3.6	4.5	4.7	5.4
germacrene D	2.3	2.8	2.6	3.6
neryl acetate	2.9	3.5	3.2	3.0
nerol	0.6	1.0	1.0	1.1
geraniol	2.7	2.5	2.7	3.3

Lawrence

Table 9 *Effect of Extrinsic Conditions on Clary Sage Oil Composition*

Compound	Healthy Plants Oil	Diseased Plants Oil
linalool	10.5	9.5
cis-rose oxide	0.5	0.8
trans-rose oxide	0.4	0.5
menthone	0.8	0.9
isomenthone	7.7	8.9
citronellol	28.5	31.4
geraniol	24.3	12.4
citronellyl formate	5.0	6.2
geranyl formate	2.1	1.9
10-epi-γ-eudesmol	5.7	8.2
citronellyl tiglate	1.3	3.9
geranyl tiglate	1.2	3.0

Rajeswara Rao et al. (2000)

Table 10 *Effect of Disease on Geranium Oil Composition*

2.4 Infraspecific

The existence of infraspecific (within a species) chemical differences in the essential oils of some aromatic plants is well known. Such plants are generally referred to as chemotypes or chemo-varieties. In the context of this manuscript, a chemotype is a morphologically identical plant that possesses different essential oil from the essential oil normally encountered.

The reason why the existence of infraspecific differences is of importance to the essential oil industry is that many plants that are used to produce an oil are collected from the wild. If all of the plants collected are of the same chemotype a uniform oil composition can be obtained; however, this is not always the situation.

Compound	Percentage	Compound	Percentage
α-pinene	0.6-1.0	trans-sabinene hydrate	t-0.5
myrcene	1.0-2.8	linalool	4.0-7.0
α-terpinene	0.9-2.6	terpinen-4-ol	0.7-2.7
γ-terpinene	5.0-10.3	thymol	36.0-55.0
p-cymene	15.0-28.0	carvacrol	1.2-4.0

Table 11 *Thymus zygis – ISO/AFNOR Specifications*

Spanish thyme oil illustrates this infraspecific point well. Examination of the ISO/AFNOR Standard (Table 11) reveals that the oil of commerce is rich in thymol.

However, *Thymus zygis* (Löfl.) L. is collected from the wild, and it exists in three subspecies forms that are morphologically very similar. In addition, these morphologically similar subspecies have been found (Velasco Negueruela and Alonso 1984, Salgueiro and Proença da Cunha 1989, Proença da Cunha and Salgueiro 1991, Salgueiro et al. 1993, Saez 1995, and Sanchez Gomez et al. 1995) to exist in a number of chemotypic forms (Table 12). It is no wonder that some discernible variations have been found in commercial Spanish thyme oils.

	Chemotypes	ssp. *gracilis*	ssp. *sylvestris*	ssp. *zygis*
1	thymol	✔	✔	✔
2	carvacrol	-	✔	✔
3	thymol/carvacrol	✔	✔	✔
4	linalool	✔	✔	✔
5	linalool/trans-sabinene hydrate	✔	-	-
6	linalool/α-terpineol	✔	-	-
7	linalool/terpinen-4-ol	✔	-	-
8	linalool/thymol	-	✔	-
9	geraniol/geraniol acetate	-	✔	✔
10	camphene/geranyl acetate	✔	-	-
11	1,8-cineole/linalool	-	✔	-
12	1,8-cineole/thymol	-	✔	-
13	1,8-cineole/linalool	-	✔	-
14	1,8-cineole/linalool/thymol	-	✔	-
15	α-terpineol/α-terpinyl acetate	-	✔	✔
16	myrcenol	-	-	✔

Table 12 *Chemotypes of Thymus zygis subspecies*

The existence of polychemism can also be found with *Hyssopus officinalis* L. ssp. *officinalis*. Examination of the oils containing bicyclic components (Table 13) reveals that none of the oils produced from thirty-five strains of *H. officinalis* grown in North Carolina met the ISO/AFNOR standard. This data set represents an excellent example of the effects of infraspecific variation on hyssop oil.

Compound	1(15)*	2(15)*	3(2)	4(3)	ISO/AFNOR
β-pinene	7.4-15.4	8.5-20.3	16.6-17.2	7.7-8.1	13.5-23.0
1,8-cineole	<0.1	<0.1	<0.1	18.8-29.3	-
pinocamphone	26.4-62.8	0.2-24.9	0.2	0.1-18.8	5.5-17.5
pinocarvone	0.1-3.4	0.1-0.4	19.8-20.0	0.1-1.0	-
isopinocamphone	2.5-24.7	22.0-61.5	30.8-32.4	15.9-23.4	34.4-50.0

* no. of strains

1. pinocamphone > isopinocamphone > β-pinene > pinocarvone
2. isopinocamphone > pinocamphone >β-pinene > pinocarvone
3. isopinocamphone > pinocarvone > β-pinene > pinocamphone

4 (i) isopinocamphone > 1,8-cineole > pinocarvone and
(ii)1,8-cineole > isopinocamphone > pinocarvone

Table 13 *Comparative Percentage Composition of Major Components of Oils of Thirty-five Strains of Hyssopus officinalis and ISO/AFNOR Standard for Oil*

2.5 Endogenous

All essential oil bearing plants possess a variation in the oil composition across the plant. This within-plant variation is often ignored by essential oil scientists and, as a result, erroneous conclusions can be drawn from the analysis of an oil isolated from small quantities of plant material. This is not critically important for commercial essential oils but there is a ramification that can effect an oil composition. For example, examination of the data presented in Table 14 shows the variation in oil composition of oils produced from leaves collected from different stalk positions of *Mentha pulegium* L. compared with a typical commercial oil. As can be seen, compositional differences across the plant are very evident.

Compound	Lower Leaves	Middle Leaves	Upper Leaves	Typical Pennyroyal Oil
α-pinene	1.3	0.7	0.4	0.4
β-pinene	1.0	0.4	0.3	0.3
limonene	0.3	0.2	0.2	0.4
3-octanol	1.3	2.1	2.2	0.7
menthone	15.3	18.3	3.6	2.3
isomenthone	10.3	22.6	2.5	0.8
neomenthol	35.4	8.6	0.4	0.4
neoisomenthol	6.2	1.2	0.2	0.1
pulegone	7.0	37.7	85.3	86.7

Strengele (1994)

Table 14 *Comparative Composition of Mentha pulegium oil by Stalk Position*

Compound	Percentage
menthyl heptenone	nd-4.5
(E)-β-ocimene	nd-5.8
citronellal	0.7-39.0
citronellol	nd-6.3
neral	0.4-31.0
geraniol	nd-22.6
methyl citronellate	nd-4.9
geranial	1.1-41.1
geranyl acetate	nd-3.3
β-caryophyllene	nd-19.9
germacrene D	nd-26.2
germacrene D-4-ol	nd-2.9
caryophyllene oxide	nd-23.5

nd = not detected. Lawrence

Table 15 *Percentage Composition of the Major Components of Melissa officinalis oils*

The percentage composition of the major components of melissa oil can vary widely (Table 15). In pursuance with the concept that the oil composition of different leaf positions might explain the reasons why the neral + geranial content and citronellal contents vary widely in commercial melissa oil. Hose et al. conducted a study on the ontogentic effect of oil composition variation. They examined the oil composition of leaves of *M. officinalis* obtained from different stem positions on a single plant and compared these to the oil composition of the whole plant. As can be seen in Table 16,

there were dramatic differences in the oil composition between the leaf oils produced from the different stem positions. It was concluded that the composition of the leaf oil was dependent on the position of the leaves on the stem and not, as had been previously assumed, the age of the plant. To complicate this further, Hose et al. found that there were also age dependent changes in oil composition that took place within the secretory structures on the leaf. They found that although the sum of citronellal, neral and geranial remained approximately constant, neral and geranial were converted to citronellal as the leaf aged. The varied oil composition of melissa is of distinct importance for the pharmaceutical and aromatherapy use of the plant (antispasmodic and sedative properties) because the biological activity of neral and geranial has been found to be greater than citronellal.

Compound	Whole Plant Oil	Basal Leaf Oil	Middle Leaf Oil	Top Leaf Oil
(E)-β-ocimene	4.6	1.3	2.6	2.2
citronellal	22.8	52.4	25.6	1.1
neral	10.1	<0.1	3.2	11.1
geraniol	3.4	-	<0.1	1.8
methyl citronellate	2.2	17.9	1.9	-
geranial	16.3	0.5	7.1	26.1
β-caryophyllene	10.4	7.3	17.5	13.0
germacrene D	10.5	6.3	21.6	20.0
germacrene D-4-ol	2.3	2.5	7.9	4.9

Hose et al. (1997)

Table 16 *Comparative Percentage Composition of Major Components of the Leaf Oil of Melissa officinalis Produced From Leaves of Different Stem Positions*

2.6 Processing

Commercial oils are distilled either by water distillation (hydrodistillation), steam and water distillation or steam distillation. Hydrodistillation and steam and water distillation are still used in many developing countries for oil isolation because the stills are less expensive to construct and the process does not require the high cost of a boiler (e.g., satellite steam generator). In developed countries most oils are isolated by steam distillation. As with all commercial processes the parameters of use can have an effect on the end product. A comparison between the three distillation processes can be seen in Table 17. Steam distillation offers several advantages over hydrodistillation or steam and water distillation.

The effects of different distillation parameters on oil composition can be seen in three examples. First, a comparison of an oil produced from dried *Origanum majorana* under vacuum, a hydrodistilled oil and a number of commercially available majoram oils reveals some significant differences in composition as can be seen in Table 18. The effect of steam versus hydrodistillation on the oil composition of coriander is less dramatic as can be seen in Table 19.

Parameter	Hydrodistillation	Steam & Water Distillation	Steam Distillation
• Oil yield	Lowest	Medium	Highest
• Speed of distillation	Lowest	Medium	Highest
• Formation of still notes	Highest	Medium-high	Lowest
• Loss of oxygenated constituents	Highest (phenols dissolve in water)	Medium-low (refluxing a problem)	Lowest
• Susceptibility to hydrolysis	Highest (pH increase in water)	Medium-high[a] (refluxing a problem)	Lowest
• Susceptibility to polymerisation	Highest (prolonged heat)	Medium to high (thermal conductivity of still wall)	Lowest

[a] Wetness of plant material can affect hydrolysis

Lawrence

Table 17 *Processing: Comparison of Hydrodistillation, Steam & Water Distillation and Steam Distillation*

Compound	Commercial Oil	Hydrodistilled Oil	Vacuum Microwave Distilled Oil[a]
sabinene	2.0-5.3	2.9	4.6
α-terpinene	0.9-7.0	3.0	0.9
p-cymene	0.9-9.5	3.5	8.2
γ-terpinene	3.2-10.2	7.6	11.1
cis-sabinene hydrate	2.4-6.7	0.7	4.6
trans-sabinene hydrate	7.1-14.0	6.9	8.2
linalool	2.3-5.6	2.0	2.9
terpinen-4-ol	16.4-36.3	48.2	29.8
α-terpineol	3.8-8.3	7.8	4.6
cis-sabinene hydrate acetate	-	-	4.6
linalyl acetate	1.9-5.4	2.4	2.2
terpinen-4-yl acetate	2.3-5.4	2.0	0.9

[a]Plants dried prior to distillation

Lawrence

Table 18 *Effect of Processing on Herb Oil of Origanum majorana*

Compound	Hydrodistilled Oil	Steam Distilled Oil
α-pinene	1.2	3.7
camphene	0.2	0.6
sabinene	0.1	0.1
β-pinene	0.1	0.4
myrcene	0.5	0.8
limonene	1.1	2.0
γ-terpinene	2.2	4.1
p-cymene	0.3	0.7
terpinolene	0.3	0.5
linalool	81.8	75.7
camphor	4.8	4.5
geranyl acetate	2.0	3.2
geraniol	3.2	2.0

Bandoni et al. (1998)

Table 19 *Effect of Distillation Process on Oil Composition of Coriander*

Compound	Fresh Plant Oil	Dried Plant Oil
myrcene	1.7	0.1
limonene	0.8	0.2
(Z)-β-ocimene	0.7	t
(E)-β-ocimene	1.4	0.1
linalool	28.6	19.1
linalyl acetate	44.9	60.2
α-terpineol	3.1	2.5
germacrene D	2.6	2.0
neryl acetate	1.0	1.7
geranyl acetate	1.9	3.0
nerol	1.2	0.5
geraniol	3.3	1.2

Lawrence

Table 20 *Comparative Composition of Clary Sage Oil Produced from Fresh and Dried Plant Material by Steam Distillation*

Compound	Typical Oil	Aged Oil	Well-Aged Oil
α-pinene	1.0-6.5	2.3	0.5
myrcene	0.4-1.0	0.1	-
γ-terpinene	2.2-5.1	0.1	t
p-cymene	0.3-3.0	3.8	4.9
cis-linalool oxide†	0.1-0.4	4.9	14.1
trans-linalool oxide†	0.1-0.3	4.3	12.3
camphor	2.1-4.4	nd	6.3
linalool	68.9-83.7	64.0	38.1

Bandoni et al. (1998)
† furanoid form nd = not determined

Table 21 *Comparative Percentage Composition of Aged Coriander Oil*

The effect of dryness of plant material on the on the oil composition of clary sage oil produced by steam distillation can be seen in Table 20. An example of age as it relates to oil composition can also be seen for coriander (Table 21).

2.7 Exogenous

A number of years ago, the lavandin producing areas of Provence was visited during distillation season. On the visit to a distillation site, it was observed that the distiller was "wetting down" his lavandin flowers once they were compressed in the still. When questioned why this process was performed the distiller said that it helped him increase his yield. Examination of the liquid used to "wet down" the flowers revealed a mixture of synthetic linalool and linalyl acetate. As a result, it is not surprising that the yield was increased. It is quite likely that this was an isolated incident; nevertheless, the addition of a "wetting mixture" was also found to have been applied in dillweed oil production in the United States. The wetting mixture in this case was (+)-limonene and racemic carvone. Before discussing the addition of materials to the oil or adulteration, the term oil standardisation requires attention.

3 OIL STANDARDISATION

The term standardisation is often used in the essential oil industry to indicate that a coupage (a blend of components from other sources) has been added to an oil so that the seasonal or geographical variation of the oil blend in minimised so that oil composition consistency is maintained from shipment to shipment. In some situations the essential oil seller compounds a coupage so that the end product oil is specific to a customer.

In their discussion on mint oil standardisation, Moyler and Moss (1988) noted that commercial gain was not a valid reason for blending mint oils. They stated that the sole purpose was to achieve consistency in the commercial availability and supply of mint oils irrespective of crop year and origin. As a result, oil blends should not have a geographical origin on the label or they should be labelled as with other natural flavours added (WONF). Oil standardisation for consistency can, therefore, be either:

- A blend of oils produced from the same botanical source irrespective of the geographical origin of the oils
- An oil with other natural ingredients added
- A blend of oils with other natural ingredients added

The only difference between these three is that the first one is a genuine oil while the other two are WONF oils.

3.1 Adulteration

The addition of both natural and synthetic components to an oil either to standardise it or for financial gain when the oil is labelled as a genuine oil of known geographical origin or just a genuine oil is unfortunately not an uncommon practice in the essential oil industry.

Adulteration can take many forms such as:

- The addition of synthetic (foreign) compounds unrelated to the oil composition
- The addition of synthetic compounds related to the oil composition
- The addition of oils of or fractions of oils of similar composition to all or part of the oil.
- The addition of natural compounds produced enzymatically or from other oil sources.
- The addition of more than one of the above

3.2 Analytical procedures for determining whether an oil is natural/genuine

There are a number of analytical procedures used to determine whether an oil is natural/genuine:

- Unstable isotope measurement
- Stable isotope measurement ($^{13}C/^{12}C$, D/H)
- Site-specific natural isotope fractionation (SNIF-NMR)
- Foreign component identification
- Hydrocarbon or oxygenated component quantitation
- Oxygen heterocyclic quantitation (Citrus oils)
- Selected ion monitoring of MS of known impurity
- Component ratio measurement
- Chiral GC analysis
- Any combination of the above methods

Although it is beyond the scope of this manuscript to discuss the analytical procedures in detail, the theory behind some of the procedures does require some explanation.

3.2.1 Unstable Isotope Measurement. According to Culp and Noakes (1990), differentiation between synthetic compounds produced from fossil fuels and natural compounds can be achieved by measuring the unstable ^{14}C isotope content.

In 1962, Friedlander and Kennedy reported that the formation of ^{14}C in the upper atmosphere occurred through the interaction of cosmic radiation with nitrogen. As CO_2 is an atmospheric gas, a certain proportion of $^{11}CO_2$ naturally exists in the atmosphere. As plants fix CO_2 through photosynthesis, a portion of $^{14}CO_2$ is fixed. In a report by Bricout and Koziet (1978), they showed that all recent plant materials have a well-established ^{14}C content as their source of carbon is atmospheric CO_2. They noted that prior to 1950 the ^{14}C activity of CO_2 was ca. 13.6dpm/gC; however, this activity increased to ca. 26 dpm/gC in 1964 as a direct result of nuclear weapons testing. By the late 1980s, the activity had decreased slightly to ca. 15 dpm/gC following the Limited Test Ban Treaty. Due to their extreme age, petroleum (fossil fuel) based synthetic compounds are devoid of ^{14}C because the isotope has a half-life of 5730 years. Such a difference can, therefore, be used to unequivocally differentiate/characterize between a natural compound and a synthetic compound produced from fossil fuels.

However, according to Culp and Noakes (1990) a natural essential oil component cannot be distinguished from its synthetic counterpart by this test alone for two major reasons. One is that many so-called synthetic compounds have natural materials as their starting product. A second is that the simulation of a natural ^{14}C activity could be produced by the addition of a ^{14}C-labeled compound. Theoretically, a measured ^{14}C activity of a known oil component could be compared with the expected activity for a certain year as a determinant of year of harvest; however, this has not really been used to any great extent. ^{14}C-activity has been used sparingly to determine whether petroleum based materials have been added to natural benzaldehyde (Krueger 1987), cinnamaldehyde (Hoffman and Salb 1989) and ethyl butyrate (Byrne et al. 1986). This analytical procedure requires the isolation of the natural component in question followed by the liquid scintillation measurement of the unstable radioactive ^{14}C isotope.

3.2.2 Stable Isotope Measurement – Carbon. As a consequence of photosynthesis, plants are selectively enriched with ^{12}C and depleted in ^{13}C relative to atmospheric CO_2, which contains approximately $^{13}CO_2$ (1.1%) and $^{12}CO_2$ (98.9%), as a reflection of their environment and metabolism (O'Leary 1981). This means that the depletion of ^{13}C is different depending upon the photosynthetic pathway utilised by the plant.

There are three possible photosynthetic pathways known in plants. The first, which is known as the Calvin pathway (C-3 pathway) is used by most plants (Calvin and Bassham 1962). For C-3 plants atmospheric CO_2 is fixed by carboxylation of ribulose 1,5-disphosphate to yield 3-phosphoglycerate. A second pathway, which is known as the Hatch Slack pathway (Hatch and Slack 1966, C-4 pathway) is used only by a few aromatic plants such as *Cymbopogon* species. For C-4 plants atmospheric CO_2 is fixed by carboxylation of phosphoenolpyruvate, which occurs with a lower carbon isotope fractionation than the CO_2 pathway. The third pathway, (Bender 1971), which is known as the Crassulacean Acid Metabolism pathway (CAM-pathway), is used by *Vanilla planifolia* and other succulent plants. For CAM plants atmospheric CO_2 is fixed at night by carboxylation of phosphoenolpyruvate with malic acid accumulation. During the day

malic acid is decarboxylated and the liberated CO_2 is fixed by ribulose 1,5-diphosphate. The two steps in the CO_2 fixation are influenced by environmental factors.

Stable carbon isotopic measurement is performed with specially designed and modified mass spectrometric equipment so that the isotopic ratio of the CO_2 obtained from an isolated essential oil component can be compared with the CO_2 obtained from a standard or a standard CO_2. The result is a unique ratio of $^{13}CO_2$:$^{12}CO_2$. For essential oil constituents this ratio is more commonly encountered as a $\delta^{13}C$ value per mil ($^{o}/_{oo}$) or parts per thousand deviation of the sample isotopic ratio, which is expressed as follows:

$$\delta^{13}C\ (^{o}/_{oo}) = \left[\frac{^{13}CO_2 / {}^{12}CO_2\ (\text{sample})}{^{13}CO_2 / {}^{12}CO_2\ (\text{standard})} - 1 \right] \times 1{,}000 \quad \text{(Bender 1971)}$$

Examples of the use of this technique to determine genuineness can be seen in Table 22.

Components Determined	Oil	Reference
• β-pinene, limonene, γ-terpinene, nerol, geraniol, neral, geranial, neryl acetate, geranyl acetate	Lemon	Braunsdorf et al. (1993)
• octanal, nonanal, decanal, dodecanal	Orange	Braunsdorf et al. (1993)
• myrcene, limonene, γ-terpinene, p-cymene, linalool, geraniol	Coriander	Frank et al. (1995)
• neral, geranial, citronellal, nerol, geraniol, geranyl acetate, β-caryphyllene	Melissa, citronella, lemongrass, lemon	Hener et al. (1995)
• linalool, linalyl acetate, α-terpineol	Neroli, petitgrain, bergamot	Mosandl & Juchelka (1997)
• α-thujene, α-pinene, sabinene, myrcene, octanal, limonene, γ-terpinene, terpinolene, linalool, α-sinensal, methyl N-methyl anthranilate	Mandarin	Faulhaber et al. (1997)
• α-pinene, β-pinene, myrcene, limonene, octanal, linalool, linalyl acetate, geranyl acetate	Bitter orange	Juchelka (1998)

Table 22 *Use of Stable Isotope Ratio Measurements ($^{13}C/^{12}C$) to Examine Oil Genuineness*

3.2.3 Stable Isotope Measurement – Hydrogen. It is known that as water evaporates from the oceans and lakes it is progressively depleted of deuterium (D, the heavier H isotope) as the evaporation/condensation cycle proceeds. According to Craig (1961), this phenomenon is most evident in the mountains and polar regions where water vapour is condensed at higher altitudes and latitudes with the associated temperature decrease thereby depleting deuterium in both cloud and rain water. Isotopic fractionation of D occurs in plants as water evaporates through leaf stomata through the normal evapotranspiration cycle. However, according to Yapp and Epstein (1980) the D/H ratio is only affected to only a minor extent by this phenomenon in comparison with the relative D/H ratio in the local water and the local environment in which the plants are grown. Although there is not a clear distinction between the isotope ranges of various hydrogen pools, δD variations occur as a direct result of photosynthetic pathways, biochemical subgroups and botanical species (Culp and Noakes 1990).

δD $^{o}/_{oo}$ values can be used to differentiate between natural and synthetic sources of major essential oil constituents. In the case of δD $^{o}/_{oo}$ ratios they can be measured against one or two standards such as Standard Mean Oceanic Water (SMOW), which has a δD $^{o}/_{oo}$ value set at zero and/or Standard Light Antarctic Precipitation (SLAP), which has a δD $^{o}/_{oo}$ value of –428 (Gonfiantini 1978).

δD values are obtained using a similar calculation as used for $\delta^{13}C$ values e.g.,

$$\delta D\ (^{o}/_{oo}) = \left[\frac{D/H \text{ sample} - D/H \text{ standard})}{D/H \text{ (standard)}} \right] \times 1{,}000$$

In 1978, Bricout and Koziet determined the δD values for (E)-anethole from a variety of sources showing that synthetic anethole (Table 23) was easily differentiated from natural sources. They also showed how synthetic L-menthol, linalool and neral/geranial could be differentiated from their natural counterparts (Table 24).

Anethole Source	**δD Value SMOW**
sweet fennel oil	-86
bitter fennel oil	-91
star anise oil	-84 to –96
synthetic	-45

Bricout & Koziet (1978)

Table 23 *δD $^{0}/_{00}$ Values for (E)-anethole*

Constituent		**δD_{SMOW} Value**
L-menthol	ex Peppermint	-394
	ex Cornmint	-358
	Synthetic	-196 to –242
linalool	ex Coriander	-269
	ex Thyme	-257
	ex Petitgrain	-244
	Synthetic	-170
neral/geranial	ex Lemongrass	-276
	ex Bitter orange	-258
	ex *Litsea cubeba*	-251
	Synthetic	-174

Bricout & Koziet (1978)

Table 24 *δD Values for Specific Oil Constituents*

3.2.4 Site-specific Natural Isotope Fractionation. As a way to exploit the joint structural and quantitative dimensions of proton NMR spectroscopy, Martin and Martin (1981, 1985) determined that deuterium was far less randomly distributed in organic molecules than was once thought. They found that large variations characterised in the deuterium contents of different molecular sites were related to specific enzymatic or biochemical (kinetic) or thermodynamic isotope effects. To examine this phenomenon Martin et al. (1982) pioneered the technique used to determine site-specific natural isotope fractionation using NMR and gave it the acronym SNIF-NMR. With this technique direct measurement of isotopic ratios of protons at several positions in a single molecule is performed, thereby enhancing the performance of an isotopic method to draw an irrefutable conclusion as to the naturalness of a specific component.

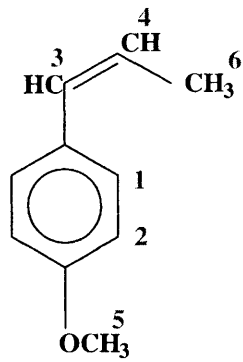


Figure 1 *(E)-anethole contains six non-equivalent sites associated with six monodeuterated isotopomers. Ratios of the protons at each site with those at other sites throughout the molecule leads to site specific natural isotope fractionation (SNIF NMR) which results in the ability to differentiate between the natural and synthetic anethole (Martin et al.1982).*

Since the values of the specific D/H isotopic ratios measured by D-NMR are sensitive to the method of purification and to instrumental conditions, internal ratios are also taken into account. For example with (E)-anethole (see Figure 1), the isotopic distributions of the aromatic, ethylenic and methyl sites can be characterised by the ratios 2/1, 4/3 and 6/5, respectively (Martin et al. 1982).

3.2.5 Foreign Components. A comparative capillary GC profile on both a polar and non-polar column of a sample oil and a genuine oil will readily reveal the existence of a "stranger" peak that can be quickly identified by a combination of GC/MS and retention indices.

If the unrelated constituent is a non-volatile additive, add a known weight of a selected internal standard and re-run the GC profile and determine whether the internal peak is found in the amount close to expected. If not, then the oil was adulterated with a non-volatile components. It should be noted that the internal standard should be carefully selected so that its retention index will not coincide with any of the oil components. Examples of some unrelated synthetic components that have been identified in some commercial oils can be seen in Table 25.

3.2.6 Hydrocarbon or Oxygenated Component Quantitation. Because oils can be quite complex, it is useful to separate the oxygenated components from the hydrocarbons by column or flash chromatography to examine their genuineness. It is easier to examine the quantitative data for each group and determine their reproducibility. Also, if chiral GC is planned this procedure simplifies the mixture somewhat. An example of the hydrocarbons and oxygenated constituents of lemon oil can be seen in Tables 26-27.

Oil	Adulterant	Reference
cassia	dibenzyl ether	Lawrence
rose	dibutyl phthalate	Lawrence
geranium	dibenzyl phthalate	Lawrence
cinnamon	benzyl benzoate	Lawrence
citronella	diethylene glycol monoethyl ether	Lawrence
juniperberry	hercolyn D	Lawrence
nutmeg	ethyl palmitate	Lawrence
palmarosa	triacetin	Lawrence (1976)
patchouli	propylene glycol hexylene glycol hercolyn D, etc.	Bruns (1978)
Canaga vetiver	hexylene glycol	Peyron et al. (1975)

Table 25 *Examples of Synthetic (foreign) Compounds Unrelated to the Oil Compositions that have been found as Oil Adulterants*

Compound	Sicilian Oil	Californian Oil
α-thujene	0.41	0.39
α-pinene	1.74	1.75
camphene	0.05	0.06
sabinene	1.70	1.83
β-pinene	10.25	11.14
myrcene	1.60	1.58
α-phellandrene	0.04	0.05
α-terpinene	0.22	0.17
p-cymene	0.13	0.25
limonene	65.16	65.93
(Z)-β-ocimene	0.05	0.05
(E)-β-ocimene	0.11	0.09
γ-terpinene	9.28	8.31
terpinolene	0.41	0.35

Chamblee et al. (1991)

Table 26 *Selected Monoterpene Hydrocarbons in Lemon Oil*

Compound	Sicilian Oil	Californian Oil
octanal	0.10	0.08
1,8-cineole	0.05	0.04
nonanal	0.13	0.13
linalool	0.18	0.14
citronellal	0.11	0.08
decanal	0.05	0.05
neral	1.25	0.73
geranial	2.02	1.14
perillaldehyde	0.02	0.03
undecanal	0.02	0.03
neryl acetate	0.51	0.57
geranyl acetate	0.38	0.49

Chamblee et al. (1991)

Table 27 *Selected Oxygenated Constituents in Lemon Oil*

Another way to determine the authenticity of an oil is to examine its unique sesquiterpene hydrocarbon content as can be seen in Table 28 for the citrus oils.

Sesquiterpene HC	Bergamot	Bitter Orange	Grape-fruit	Lemon	Lime	Mandarin	Orange
α-copaene	-	-	13.4	-	-	3.7	8.5
β-cubebene	-	-	13.3	-	-	2.5	7.0
β-caryophyllene	24.0	27.8	38.2	13.8	11.8	32.2	6.8
trans-α-bergamotene	21.9	-	-	24.5	16.5	-	-
(Z)-β-farnesene	4.1	3.7	1.4	1.6	1.7	-	9.0
germacrene D	4.9	51.8	9.8	-	4.0	2.8	6.3
valencene	-	-	-	-	-	-	26.5
α-selinene	-	-	-	-	1.0	10.6	-
(E,E)-α-farnesene	-	0.9	-	-	-	40.9	5.4
β-bisabolene	32.7	-	-	37.1	38.5	-	-
δ-cadinene	-	-	13.0	-	-	3.9	10.9

G. Dugo et al. (1999)

Table 28 *Comparative Sesquiterpene Hydrocarbon Content of Citrus Oils*

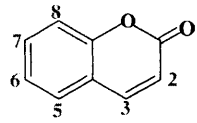
Recently, Feger et al. (2000) also examined the sesquiterpenes in citrus oils; however, they determined the germacrene content of the oils (Table 29), which can also be used to determine oil genuineness.

Citrus Oil	A	B	C	D	Bicyclo
bergamot	0.01	0.01	0.02	0.05-0.07	0.01
grapefruit	0.02-0.03	-	-	0.07-0.11	0.02-0.04
lemon	t	-	-	-	0.04-0.12
lime distilled	-	0.04-0.08	-	-	-
lime key (A/B)	0.36-0.46	0.78-0.90	0.50-0.59	0.30-0.41	-
lime Persian	0.12-0.18	0.16-0.19	0.09-0.12	0.07-0.16	-
mandarin	t-0.02	t	<0.01-0.01	0.02-0.04*	t-0.01
orange bitter	t-0.01	0.01-0.02	0.01-0.02	0.07-0.11*	t-0.01
orange sweet	t-0.02	-	-	0.07-0.33*	t-0.01
tangerine	0.03	0.08-0.10	0.05-0.07	0.06-0.0	0.01-0.02

Feger et al. (2000) *co-elutes with valencene

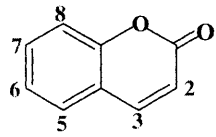
Table 29 *Percentage of Germacrenes in Citrus Oils*

3.2.7 Oxygen Heterocyclic Quantitation in Citrus Oils. Cold pressed citrus oils are known to contain a few percent of non-volatile oxygen heterocyclic compounds such as coumarins, psoralens and flavones. The major types of components found can be seen in Figures 2-4.



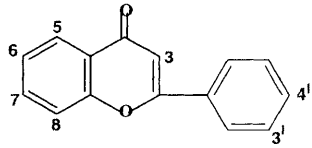
Herniarin:	7-methoxycoumarin
Osthol:	7-methoxy-8-isopentylcoumarin
Citropten:	5,7-dimethoxycoumarin
Aurapten:	7-geranyloxycoumarin
Meranzin:	7-methoxy-8(2^1,3^1-epoxy)isopentenylcoumarin
Isomeranzin:	7-methoxy-8(2^1-oxo)isopentenylcoumarin
Meranzin hydrate:	7-methoxy-8(2^1,3^1-dihydroxy)isopentenylcoumarin
Epoxyaurapten:	7-(6^1,7^1-epoxy)geranyloxycoumarin

Figure 2 *Coumarins in Citrus Oils*



Bergapten:	5-methoxypsoralen
Bergamottin:	5-geranyloxypsoralen
Imperatorin:	8-isopentenyloxypsoralen
Phellopterin:	5-methoxy-8-isopentenyloxypsoralen
Isopimpinellin:	5,8-dimethoxypsoralen
Onidillin:	5-isopentenyloxy-8-methoxypsoralen
Expoxybergamottin:	5-(6',7'-epoxy)geranyloxypsoralen
Epoxybergamottin hydrate:	5-(6',7'-dihydroxy)-geranyloxypsoralen
Oxypeucedanin:	5-(2',3'-epoxy)-isopentyloxypsoralen
Oxypeucedanin hydrate:	5-(2',3'-dihydroxy)-isopentyloxypsoralen
Byakangelicin:	8-(2',3'-dihydroxy)-isopentyloxy-5-methoxypsoralen
Byakangelicol:	8-(2'3'-epoxy)-isopentyloxy-5-methoxypsoralen

Figure 3 *Psoralens in Citrus Oils*



tangeretin:	5,6,7,8,4'-pentamethoxyflavone
sinensetin:	5,6,7,3',4'-pentamethoxyflavone
nobiletin:	5,6,7,8,3',4'-hexamethoxyflavone
tetra-O-methylscutellarein:	5,6,7,4'-tetramethoxyflavone

Figure 4 *Methoxyflavones in Citrus Oil*

Examples of these can be seen in Tables 30-31. In Table 32, it can be deduced that the commercial bitter orange oils have been adulterated with sweet orange oil as all of the coumarins and psoralens are reduced except meranzin hydrate and the levels of heptamethoxyflavone and tetra-O-methylscutellarein are increased.

Compound	Orange	Mandarin Oil
tangeretin	38-73	148-339
3,3',4',5,6,7,8-heptamethoxyflavone	55-123	16-69
nobiletin	34-80	36-151
tetra-O-methylscutellarein	21-42	3-11
3',3',4',5,6,7-hexamethoxyflavone	6-23	-
sinensetin	5-16	1-4
Mondello et al. (1993)	mg/100g oil	

Table 30 *Methoxyflavone Content of Orange and Mandarin Oils*

Finally in Table 33, it can be deduced that the bergamot oil has been adulterated with lime oil because of the increase in 5-geranyoxy-7-methoxy-coumarin and the addition of herniarin.

Compound	Key A	Key B	Persian
bergamottin	3157-3283*	3154	2221-3918
5-geranyloxy-7-methocoumarin	3065-4045	4093	1943-3780
onidillin	25-35	24	5-8
citropten	491-632	484	326-569
herniarin	86-96	74	339-594
bergapten	100-124	89	158-250
isopimpinellin	350-365	331	169-293
oxypeucedanin	-	144	210-328

*mg/100g oil P.Dugo et al. (1998)

Table 31 *Main Oxygen Heterocyclic Components in Lime Oil*

Compound	Spanish	Italian	Commercial Oils
osthol	366-371*	154-184	105-197
bergapten	71	52-73	27-45
epoxybergamottin	304-328	188-322	0-127
meranzin	307-332	788-1172	0-401
isomeranzen	208-213	154-211	69-126
tangeretin	95-99	59-156	34-78
heptamethoxyflavone	23-25	5-14	4-30
nobiletin	76-85	34-87	16-46
tetra-O-methylscutellarein	5-8	10-18	0-24
epoxybergamottin hydrate	24-42	13-45	24-43
meranzin hydrate	18-39	10-70	31-98

*mg/100g oil P.Dugo et al. (1996)

Table 32 *Main Oxygen Heterocyclic Components in Bitter Orange Oil*

Compound	C.P. oil	Recovered oil	Bergapten-free oil	Commercial oil
bergamottin	1000-2750*	930-1660	1170-1620	680-1160
5-geranyloxy-7-methoxycoumarin	80-270	110-180	150-200	180-370
citropten	120-350	90-220	0-50	100-130
herniarin	-	-	-	+
bergapten	110-320	130-290	0-90	40-100

*mg/100g oil Mondello et al. (1993)

Table 33 *Main Oxygen Heterocyclic Components in Bergamot Oil*

3.2.8 Selected Ion Monitoring of MS of Known Impurity. Agnel and Teisseire (1984) determined adulteration in lavender oil with synthetic linalool and linalyl acetate by determining the existence of dihydrolinalool (1.5-2.0%) and dehydrolinalool (0.05-0.10%) in linalool and dihydrolinalyl acetate (1.5-2.0%) and dehydrolinalyl acetate (0.05-0.10%); however, this was difficult if the amounts of synthetic linalool and linalyl acetate added to the oil were quite low. Frey (1988) determined that through the use of selected ion monitoring (SIM) using fragments m/e 138, 123 and 73 the addition of as little as 2% synthetic linalool and linalyl acetate could be determined in commercial samples of neroli and bergamot oils. He also determined that as phenylpentadienal was found as an impurity of synthetic cinnamaldehyde using fragments m/e 158, 129 and 128, the addition of as little as 2% synthetic cinnamaldehyde could be determined in a commercial sample of Chinese cassia oil.

3.2.9 Component Ratio Measurement. A number of years ago Lawrence et al. (1989) used component ratios rather than direct quantitative data to differentiate between peppermint oils produced from the same clone in different regions of the Midwest and far western United States. To simplify the component ratio data obtained from the analyses of ca. more than 50 samples from each growing area selected, component ratios were presented as polygons in a pattern recognition pictorial representation. More recently, this data was expanded to include two seasons data for peppermint (*Mentha piperita* L.) so that regional authenticity could be monitored. However, the data for peppermint was also used to determine adulteration of U.S. peppermint with Indian peppermint oil (Table 34). Also, with the use of other component ratios the adulteration of US peppermint oil with dementholised oil obtained from *M. arvensis* L. f. *piperascens* Malinv. ex Holmes (Table 35). Component ratios have also been used to determine adulteration in a number of citrus oils as shown in Tables 36-38.

Component Ratio	Midwest	Willamette	Madras	Yakima	Idaho
cineole/menthol x 50	✔	✔	✔	-	-
cineole/germacrene D x 2/3	✔	✔	✔	-	✔
menthone/piperitone x 1/10	✔	✔	✔	✔	-
isomenthone/piperitone x 2/5	-	✔	-	✔	-

Lawrence (1999)

Table 34 *Component Ratio that can be successfully used to detect adulteration of U.S. Peppermint Oil (1993) with Indian Peppermint Oil*

Component Ratio	Midwest	Willamette	Madras	Yakima	Idaho
menthone/piperitone x 1/10	✔	-	-	-	-
cineole/limonene	-	✔	✔	✔	-
isomenthone/piperitone x 2/5	✔	-	-	-	-
menthyl acetate/germacrene D x2	-	✔	-	-	-
cineole/menthofuran	-	-	-	✔	-
menthofuran/isomenthone x2	-	-	-	✔	✔

Lawrence (1999)

Table 35 *Component Ratio than can be successfully used to detect adulteration of U.S. Peppermint Oil (1993) with Chinese or Indian Dementholised Cornmint Oil*

Ratios	Genuine oil	2 Adulterated commercial oils
citronellal/terpinen-4-ol	0.167-1.875	0.119-0.136
octyl acetate/α-terpineol	0.842-4.742	0.500-0.561
γ-terpinene/sabinene + β-pinene	0.661-1.279	0.670-0.733
trans-sabinene hydrate acetate/ α-terpineol	0.704-3.323	0.303-0.354

Verzera et al. (1996)

Table 36 *Component Ratio of Genuine and Two Adulterated Bergamot Oils*

Compound		Orange Terpenes added to Lemon Oil		
Ratio	Lemon Oil	5%	10%	20%
δ-3-carene	0-0.008	0.008-0.014	0.012-0.022	0.020-0.032
δ-3-carene/α-terpinene	0-0.044	0.044-0.086	0.069-0.146	0.104-0.254
δ-3-carene/camphene	0.005-0.129	0.145-0.274	0.226-0.468	0.328-0.744

Verzera et al. (1978)

Table 37 *Detection of Lemon Oil Adulterated with Orange Terpenes*

Compound	Bitter Orange Oil	Orange Oil Added 3%	Orange Oil Added 5%	Orange Terpenes Added 3%	Orange Terpenes Added 5%
δ-3-carene	Max 0.006	0.005-0.009	0.008-0.012	0.006-0.007	0.009-0.011
δ-3-carene/camphene	Max 1.092	0.714-1286	1.000-1.714	0.857-1.000	1.111-1.375
δ-3-carene/terpinolene	Max 0.875	0.500-0.900	0.777-1.091	0.750-1.000	0.900-1.125

P. Dugo et al. (1996)

Table 38 *Bitter Orange Oil Adulterated with Orange Oil or Orange Terpenes*

3.2.10 Chiral GC Analysis. Many components of essential oils possess one or more asymmetric carbon atoms that exhibit optical activity. These chiral compounds of natural origin are generally found in a characteristic enantiomeric distribution because they evolve via enzymatically controlled biosynthetic synthesis.

The advent of chiral GC columns and multidimensional GC techniques in which the components to be examined for enantiomeric distribution are "heart cut" from a standard capillary GC column and the analyses are performed on the chiral column has become almost a routine procedure for determining adulteration in commercial essential oils. Examples of the use of enantiomeric distributions to determine adulteration in coriander, mandarin and neroli oils can be seen in Tables 39, 40 and 41, respectively, to name just a few.

Compound	Genuine Coriander Oil	Commercial Oils 1	2	3	4
(4S)-(-)-limonene	50	38	12	7	15
(4R)-(+)-limonene	50	62	88	93	85
(3S)-(+)-linalool	10	33	27	34	20
(3R)-(-)-linalool	90	67	73	66	80

Frank et al. (1995)

Table 39 *Percentage Composition of Commercial Coriander Oil Adulterated with (+)-Limonene and Racemic Linalool*

Compound	Lemon Oil	Mandarin Oil	Mandarin Oil/Lemon Oil 99:1	90:1
(1R,5R)-(+)-β-pinene	5.1	97.6	90.3	50.2
(1S,5S)-(-)-β-pinene	94.9	2.4	9.7	49.8
(1R,5R)-(+)-sabinene	15.1	78.8	75.0	45.6
(1S,5S)-(-)-sabinene	84.9	21.2	25.0	54.4
(4R)-(+)-limonene	98.1	98.0	98.0	97.8
(4S)-(-)-limonene	1.9	2.0	2.0	2.2
(3S)-(+)-linalool	28.5	86.9	80.7	73.1
(3R)-(-)-linalool	71.5	13.1	19.3	26.9
(4S)-(+)-terpinen-4-ol	19.7	11.4	14.4	15.7
(4R)-(-)-terpinen-4-ol	80.3	88.6	85.6	84.3
(4R)-(+)-α-terpineol	22.6	28.9	26.5	25.6
(4S)-(-)-α-terpineol	77.4	71.3	73.5	74.4

Mondello et al. (1998)

Table 40 *Chiral Analysis Used to Determine Addition of Mandarin Oil in Lemon Oil*

Compound	Neroli Oil	Commercial Oils	
		1	2
(1R,5R)-(+)-α-pinene	4.6-13.6	11.9	13.1
(1S,5S)-(-)-α-pinene	86.4-95.4	88.1	86.9
(1R,5R)-(+)-β-pinene	0.1-0.8	2.9	4.0
(1S,5S)-(-)-β-pinene	99.2-99.9	97.1	96.0
(4R)-(+)-limonene	93.1-98.5	97.1	96.7
(4S)-(-)-limonene	1.5-6.9	2.9	3.3
(3S)-(+)-linalool	9.4-29.2	27.4	36.9
(3R)-(-)-linalool	70.8-90.6	72.6	63.1
(3S)-(+)-linalyl acetate	1.8-4.6	37.2	11.4
(3R)-(-)-linalyl acetate	95.4-98.2	62.8	88.6
(4S)-(+)-terpinen-4-ol	35.3-49.0	35.1	58.8
(4R)-(-)-terpinen-4-ol	51.0-64.7	64.9	41.2
(4R)-(+)-α-terpineol	69.1-72.3	60.2	71.9
(4S)-(-)-α-terpineol	27.7-30.9	39.8	28.1
(R)-(-)-nerolidol	0.4-1.8	1.6	33.4
(S)-(+)-nerolidol	98.2-99.6	98.4	66.6

Mondello et al. (1994)

Table 41 *Enantiomeric Distribution of Selected Compounds in Neroli Oils*

Compound (Threshold ppm)	Concentration in ppm	
	Orange Oil	Mandarin Oil
octanal[1] (0.00007)	3590 (47%)	1870 (25%)
linalool[2] (0.0008)	5460 (6%)	1320 (2%)
nonanal[3] (0.0001)	500 (5%)	320 (3%)
citronellal[4] (0.046)	490	320
decanal[1] (0.0001)	3360 (31%)	970 (9%)
neral[4] (0.074)	780	90
geranial[1] (0.0032)	1200	480
thymol[4] (0.0155)	-	590
dodecanal[4] (0.055)	500	300
methyl N-methyl anthranilate[4] (0.00115)	-	4580 (4%)
β-sinsensal[3] (0.00005)	340 (6%)	-
α-sinsensal[3] (0.00005)	250 (5%)	3050 (57%)

Percentages in parentheses refer to importance to overall aroma of orange and mandarin oils.
[1]Buttery et al (1978) [2]Padrayuttawat et al. (1997) [3]Ohloff (1994) [4]Devos et al. (1990)

Table 42 *Comparative Selected Oxygenated Constituents of Orange and Mandarin Oils*

4 CONSEQUENCES

The consequences of using commercial essential oils that are not genuine are well known. However, there is a tendency to ignore the fact that they may be adulterated either with synthetic (foreign) components unrelated to the oil composition (the worst case scenario), synthetic compounds related to the oil composition or the addition of oils of a similar composition, fractions of other oils or natural compounds from other sources. The resultant blended oil will, therefore, have a reduced olfactory character, will be less potent than the genuine oil and will have less impact on its end product which defeats its purpose for use. Examination of selected highly odoured constituents of orange and mandarin oils (Table 42) shows the concentration and threshold of these constituents. If the concentration in ppm is divided by its threshold (also in ppm) the percentage importance of

these constituents reveals that in orange oil the major components responsible for the odour character of this oil are octanal, decanal, linalool, β-sinensal, α-sinensal and nonanal. Similarly, α-sinensal, octanal, decanal, methyl N-methyl anthranilate, nonanal and linalool are the major components responsible for the odour of mandarin oil.

	Concentration in ppm		
Compound (Threshold ppm)	**Bitter Orange Oil**	**Petitgrain**	**Neroli**
octanal[1] (0.00007)	1910 (49%)	-	-
nonanal[2] (0.0001)	360 (6%)	500 (58%)	-
decanal[1] (0.0001)	1940 (35%)	-	-
α-terpineol[2] (0.035)	590	56300 (19%)	17900 (1%)
nerol[3] (0.029)	70	9900 (4%)	6900
neral[3] (0.074)	-	4300	4100
geranial[1] (0.0032)	980	3800 (14%)	6500 (4%)
indole[3] (0.000032)	-	-	600 (36%)
methyl anthranilate[3] (0.00115)	-	-	1100 (2%)
neryl acetate[4] (2.0)	240	17300	9200
geranyl acetate[4] (0.15)	1140	31600	13800
methyl N-methyl anthranilate[3] (0.00115)	-	-	31800 (53%)
(E)–nerolidol[2] (0.012)	830	800	17600 (3%)
linalool[5] (0.0008)	3300 (7%)	32,550,000*	15,590,000*
linalyl acetate[3] (0.0089)	11,730 (2%)	62,570,000*	9,760,000*

*Major odour characters of petitgrain and neroli oils. Percentages in parentheses refer to importance to overall aroma of bitter orange and the odour characters of petitgrain and neroli oils.
[1]Buttery et al (1978) [2]Ohloff (1994) [3]Devos et al. (1990) [4]Yang et al (1992) [5]Padrayuttawat et al. (1997)

Table 43 *Comparative Selected Oxygenated Constituents of Bitter Orange, Petitgrain and Neroli Oils*

Using this same procedure of dividing the concentration (pppm) by the threshold (ppm) for bitter orange (Table 43), it can be seen that the most important odour characters in the oil are octanal, decanal, linalool, nonanal and linlyl acetate. Also in this same table, the odour characters for petitgrain and neroli are obviously linalool and linalyl acetate; however, the other odour characters of lesser importance in these oils are nonanal, α-terpineol, geranial and nerol for petitgrain oils and methyl N-methyl anthranilate, indole, geranial, (E)-nerolidol, methyl anthranilate and α-terpineol in neroli oil. Because of the importance of linalool and linalyl acetate in petitgrain and neroli oils, the addition of racemic linalool as an adulterant can greatly affect the potency of these oils as (+)-linalool has a detection threshold of an order of magnitude higher than the naturally occurring (-)-linalool.

In Tables 44-45 a similar treatment of lemon oil and three oils of grapefruit can be seen. It is important to note in the grapefruit oil example that the naturally occurring (+)-nootkatone has a threshold of 1 ppb, whereas its (-)-enantiomer has a threshold of 1 ppm. Hence, any addition of racemic nootkatone can drastically reduce the odour character of a grapefruit oil.

It is hoped from this discussion of oil composition variation and the methods used to determine genuineness or adulteration that there is a realisation that diluents or adulterants have a deleterious effect on the odour intensity of the oil. Finally, it is hoped that the users of commercial essential oils will become more demanding about their purchase and use of genuine essential oils so that genuine oils become the norm rather than the exception.

Compound (Threshold ppm)	Concentration in ppm Lemon Oil Sicilian	Lemon Oil Californian
octanal[1] (0.00007)	1000 (35%)	800 (33%)
octanol[2] (0.011)	300	100
linalool[3] (0.0008)	2000 (6%)	1400 (5%)
nonanal[2] (0.0001)	1300 (31%)	1300 (37%)
citronellal[4] (0.046)	1100	800
decanal[1] (0.0001)	500 (12%)	500 (14%)
nerol[4] (0.029)	150	300
neral[4] (0.074)	12500	7300
geranial[1] (0.0032)	20200 (15%)	11400 (10%)
neryl acetate[5] (2.0)	5100	5700
geranyl acetate[5] (0.15)	3800	4900
nootkatone[2] (0.0001)	-	-

Percentages in parentheses refer to importance to overall lemon oil aroma.
[1]Buttery et al. (1978) [2]Ohloff (1994) [3]Padrayuttawat et al. (1997) [4]Devos et al. (1990) [5]Yang et al. (1992)

Table 44 *Comparative Selected Oxygenated Constituents of Lemon Oil*

Compound (Threshold ppm)	Concentration in ppm Grapefruit Oil Duncan	Grapefruit Oil Marsh	Grapefruit Oil Red Blush
octanal[1] (0.00007)	2300 (27%)	5800 (63%)	3400 (54%)
octanol[2] (0.011)	700	1400	1400
linalool[3] (0.0008)	4500 (5%)	2500 (2%)	2000 (6%)
nonanal[2] (0.0001)	700 (6%)	600 (5%)	500 (6%)
citronellal[4] (0.046)	-	-	-
decanal[1] (0.0001)	2200 (18%)	2600 (20%)	2600 (29%)
nerol[4] (0.029)	300	200	300
neral[4] (0.074)	600	600	500
geranial[1] (0.0032)	800	1000	700
neryl acetate[5] (2.0)	50	50	100
geranyl acetate[5] (0.15)	800	500	600
nootkatone[2] (0.0001)	5400 (44%)	1300 (10%)	700 (8%)

Percentages in parentheses refer to importance to overall grapefruit oil aroma.
[1]Buttery et al. (1978) [2]Ohloff (1994) [3]Padrayuttawat et al. (1997) [4]Devos et al. (1990) [5]Yang et al. (1992)

Table 45 *Comparative Selected Oxygenated Constituents of Grapefruit Oil*

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STABLE ISOTOPES FOR DETERMINING THE ORIGIN OF FLAVOUR AND FRAGRANCE COMPONENTS: RECENT FINDINGS.

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1 INTRODUCTION

During the last couple of decades, the use of stable isotopes has developed in parallel with that of very sensitive and precise methods for their detection. Such methods rely on physical characteristics of these species: mass differences are measured by isotopic mass spectrometry, resonance differences exhibited by nuclei are measured by NMR, and photon emission from excited atoms is measured by atomic emission spectrometry.

Nowadays, isotopic ratios of hydrogen, carbon, nitrogen, oxygen and sulphur are mostly determined using two methodologies: the global stable isotope ratio analysis (SIRA) by mass spectrometry, and the quantitative positional isotopic ratio analysis by NMR. These ratios have been widely used for assessment of the origin, mainly natural versus synthetic, of defined isolated compounds in flavour mixtures [1,2]. Both methods have their own advantages and disadvantages, and should preferably be used in combination. In any case, definitive information is obtained only if comprehensive databases have been compiled beforehand, using samples of known origin. The possible effects of isotope fractionation during isolation procedures should not be overlooked either.

2 NATURAL vs SYNTHETIC AND STABLE ISOTOPES

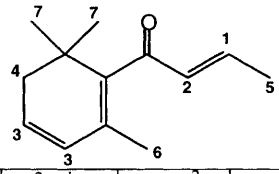
Stable isotope methods have gained increasing importance in the authenticity assessment of flavour compounds. Measurement of carbon and hydrogen stable isotope ratios, which required multi-milligram quantities of pure compounds when using "off-line" methods, became more straightforward and convenient with the introduction of commercial "on-line" GC-IRMS instruments in 1990[2]. The latest GC-"P"-IRMS instrumentation involves a pyrolysis interface ("P"), which allows one to measure oxygen isotope ratios. Over the 10 past years, a significant amount of data have been published on the differentiation of flavour and essential oil components, from (or versus) their synthetic (or so-called "nature-identical") counterparts[3]. Therefore, these data are considered as an authentication tool only. In this respect, a further example of this possibility is provided by the case of (*E*)-β-damascenone, a ubiquitous key flavour contributor in natural flavours. When isolated from

rum (made from cane sugar), damascenone shows a ^{13}C enrichment that is typical of a C4 plant, whereas the same material obtained by a biotechnological process from a hydroxylated carotenoid precursor present in a C3 plant would display a $^{13}C/^{12}C$ ratio rather similar to that of synthetic damascenone (Table 1).

	$\delta\ ^{13}C$ (‰ caption)
natural (rum)	- 16.2 ± 0.2
natural (biotech)	- 31.9 ± 0.6
synthetic (fossil)	- 29.9 ± 0.2

Table 1 *$^{13}C/^{12}C$ ratio for different sources of damascenone*

Some additional information is given by the measurement of $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios, but definitive information is provided by Site-Specific Natural Isotope Fractionation NMR (SNIF-NMR) (table 2).



origin	$\delta\ ^{18}O$ [a]	$\delta\ ^{2}H$ [b]	Site **1** $^{2}H/^{1}H$	Site **2** $^{2}H/^{1}H$	Site **3** $^{2}H/^{1}H$
natural (rum)	-7.7	-162	48	34	84
synthetic	20.9	-126	137	217	145

[a] ‰ (SMOW); [b] ‰ ± 1 (SMOW)

Table 2 *SNIF-NMR values for different sources of damascenone*

Not only may stable isotopes be used for determining the origin of natural products, as in the case of biotechnology-derived isolates (see below), but they can be also used to extract more intimate clues which may help to characterize some parameters involved in fermentation processes. Hence, we reported for the first time in 1988 that 6-pentyl-α-pyrone (6-PP), a metabolite of *Trichoderma viride*, can be characterized by carbon SIRA[4]. As expected, when fed with either grape juice sugars (a C3 plant) or corn steep liquor (a C4 plant) as a sole carbon source, the fungus biosynthesises 6-PP with specific $^{13}C/^{12}C$ ratios typical of source (Table 3).

carbon source	$\delta\ ^{13}C$ ‰ ± 0.2 (PDB)
grape juice sugars	-27.1
corn steep liquor	-12.4
synthetic	-28.8

Table 3 *Carbon SIRA of 6-PP of different origins*

Therefore, one can see that the isotopic signature of *de novo* fermentation-derived products can be tailor made. Indeed, as of today, very little has been published on the use of stable isotopes as tracers to support manufacturing claims in flavours and fragrances. Interestingly, a recent patent[5] claims the use of perfume or flavour ingredients labelled with ^{2}H, ^{15}N, ^{18}O and ^{13}C for tracing formula duplication, a method that had been

suggested long ago by Brazier[6]. However, there is little doubt that isotope labelling is used today in drug tagging for counterfeit control (at least to a certain extent), and in forensic applications (explosives, illicit drugs etc.).

3 STABLE ISOTOPES AND BIOGENETIC CONSIDERATIONS

Fruit flavour volatiles may be generated through different biosynthetic pathways. Pineapple, banana and apple flavour volatiles have been investigated by carbon SIRA[7]. Pineapple is an example of a plant following the unusual Crassulacean Acid Metabolism (CAM). Therefore, typical ^{13}C enrichment is observed for all the volatile aroma components. Measurement of the relative concentration and carbon SIRA of flavour components even allows one to characterize the fruits according to their geographical origin and variety (Table 4).

Compound	Queen Victoria		Smooth Cayenne	
	$\delta^{13}C$ (‰)	rel. %	$\delta^{13}C$ (‰)	rel. %
Methyl 2-methylbutyrate	-19.1 ± 0.35	6.60	-20.5 ± 0.22	8.25
Diethyl malonate	n.m.	0.13	-16.4 ± 0.2	3.10
Ethyl 2-methylbutyrate	-19.3 ± 0.35	9.40	n.d.	n.m.
Methyl hexanoate	-20.9 ± 0.6	13.8	-22.6 ± 0.2	38.9
Methyl 3-methylthiopropionate	n.m.	4.2	-25.2 ± 0.2	11.7
Ethyl hexanoate	-20.5 ± 0.23	21.7	n.m.	2.0
Methyl octanoate	-23.0 ± 1.0	5.1	-19.7 ± 0.5	3.0
Methyl 3-acetoxyhexanoate	n.m.	0.2	-22.1 ± 0.6	8.7
ethyl octanoate	-24.0 ± 1.4	10.4	n.m.	0.5

n.m.: not measured; n.d.: not detected

Table 4 *Carbon isotopic deviations of some pineapple volatile compounds*

In the case of purple passionfruit (*Passiflora edulis* Sims), a C3 plant, the usual carbon isotope deviations are observed for all the flavour components, but, interestingly, the putative mevalonic route to Edulan I and β-ionone is associated with a significant ^{13}C depletion[8] (Table 5).

compound	$\delta^{13}C$ (‰)	compound	$\delta^{13}C$ (‰)
ethyl butyrate	-35.6 ± 0.3	Edulan I	-37.9 ± 0.6
ethyl hexanoate	-31.9 ± 0.7	β-ionone	-35.0 ± 0.4
n-octyl butyrate	-32.2 ± 0.6		
hexyl hexanoate	-30.4 ± 0.8		

Table 5 *Carbon isotopic deviations of certain purple passion fruit components*

4 STABLE ISOTOPES AS CLUES OF MANUFACTURING TECHNIQUES EMPLOYED FOR NATURAL FLAVOUR ISOLATES

4.1 Raspberry ketone

Deuterium SIRA finds some usefulness in the case of raspberry ketone since the natural modification is significantly enriched in deuterium, relative to the synthetic counterparts. Using SNIF-NMR, Martin *et al.*[9] had even shown more precisely that in the case of anethole and other *p*-coumaric-related phenylpropanoids, the natural products exhibit a high aromatic deuterium enrichment. This is understandable if we bear in mind that the aromatic ring originates from photosynthesized carbohydrates having a high overall D/H content. Consequently, raspberry ketone would be expected to display the same feature, and this is indeed the case, as we show in Table 6.

	sum of aromatic D/H molar fractions
Natural raspberry ketone	0.441 (mean)
Synthetic raspberry ketone	0.355 (mean)

Table 6 *D/H content in raspberry ketone*

It had also been shown previously that compounds which derive from *p*-coumaric acid (anethole, estragole, vanillin...) are usually deuterium-enriched in the α-position with respect to the hydroxyl or methylated hydroxyl group (meta from the C_3 side chain) as a consequence of a specific isotope effect operating during the aromatization step of prephenic acid into tyrosine, the precursor of *p*-coumaric acid (figure 1).

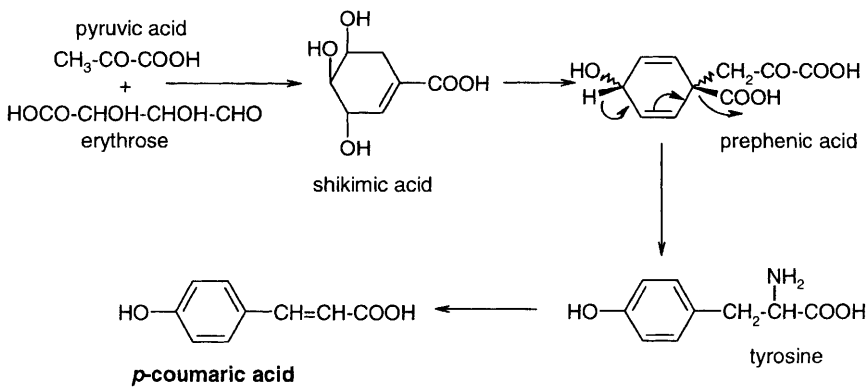
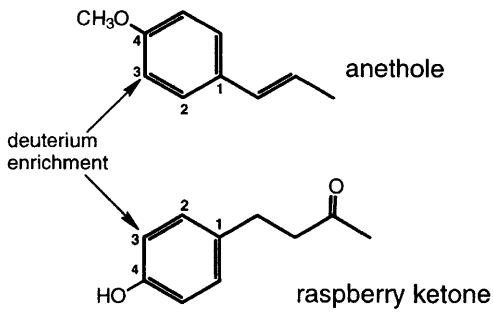


Figure 1 *Overview of route to p-coumaric acid from pyruvic acid and erythrose*

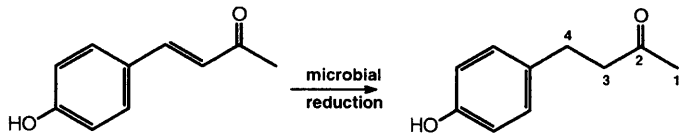
Again, this feature is also present in the raspberry ketone: when obtained from natural precursors, it exhibits a higher D/H content on site 3, ortho relative to the hydroxyl group, than on site 2. In aromatic rings of synthetic origin (anethole, raspberry ketone etc.), the D/H ratios are practically identical on both sites 2 and 3 (Table 7).



	D/H molar fraction f_2 (mean values)	D/H molar fraction f_3 (mean values)
Natural anethole (fennel)	0.164	0.228
Synthetic anethole	0.176	0.170
Natural raspberry ketone	0.190	0.247
Synthetic raspberry ketone	0.175	0.175

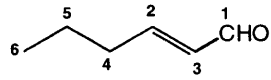
Table 7 *D/H values in anethole and raspberry ketone*

Concerning the specific aspect of the double bond reduction of the raspberry ketone precursor, one should be able to distinguish between bioconversion and catalytic hydrogenation processes, even though the final result seems to be the same. SNIF-NMR would be expected to provide definitive information on the mode of reduction that is used to convert the unsaturated precursor into raspberry ketone. We were able to perform tedious deuterium natural abundance measurements on carbon atoms α (C3) and β (C4) relative to the carbonyl group, and we could establish that site β is significantly enriched relative to site α, in all products generated through biological means[10]. Thus, in these cases, the ratio of deuterium molar fractions on site β versus site α is 1.16 ± 0.02, which appears to be quite sufficient for determining the mode of reduction that has been used.



4.2 *E*-2-hexenal

The biochemical oxidative degradation of unsaturated C-18 fatty acids into C-6 "green" compounds is very well documented. Among these compounds, *E*-2-hexenal is of great interest since it occurs as such in many fruit species, and it is also generated upon tissue disruption during fresh plant processing. Today, this feature is used for the biotechnological production of both *E*-2-hexenal and *Z*-3-hexenol or *E*-2-hexenol.



(*E*)-2-hexenal

Table 8 summarizes the deuterium abundance values recorded by SNIF-NMR on two selected sites 1 and 3 of the *E*-2-hexenal molecule from various origins.

Origin	Site 1 (D/H, ppm)	Site 3 (D/H, ppm)
Synthetic	123	95
Tomato fruit juice	77	142
Natural a	74	132
Natural b	65	136
Natural c	63	164
Natural d	67	153
Natural e	67	157
Alfalfa leaf	63	116
Microbial oxidation of natural E-2-hexenol	78	171
Chemical oxidation of natural E-2-hexenol	232	172

Table 8 *D/H values for E-2-hexenal of different origins*

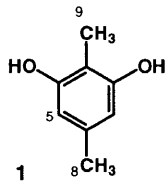
Not only can the SNIF-NMR method provide a clear-cut image of the natural origin of *E*-2-hexenal, but, as in the case of raspberry ketone, it can also allow one to distinguish products obtained from natural *E*-2-hexenol by oxidation using either a microbial process, for example using *Candida boidinii*[11], or a chemical reaction. Indeed, in the case of the chemical oxidation, the deuterium abundance at site 1 bearing the aldehyde function of *E*-2-hexenal is dramatically increased, whereas in the case of the bio-converted product, it is similar to that of natural counterparts directly isolated from plants. Interestingly, it has been shown recently that when *E*-2-hexenol and *E*-2-hexenal co-exist in a given plant-derived mixture, the global deuterium content is lower in the aldehyde than in the alcohol[12].

5 THE CASE OF A NATURAL RAW MATERIAL FOR FRAGRANCES: LICHEN RESINOIDS

As of today, about 3000 tons per year of lichen are processed into resinoids, mostly by French manufacturing companies located in the Grasse area. The raw material belongs to two main species: *Evernia prunastri* (oak moss) and *Pseudevernia furfuracea* ("tree moss"). Mono-aromatic compounds derived from depsides are among the main odour-donating compounds in both species. Some of them (e.-g. **2**) have been available to perfumers as synthetic chemicals for more than 25 years. Lichens are symbiotic organisms of fungi and algae, and appear to utilize C3 photosynthesis[13]. We were able to confirm that this is indeed the case, in taking β-orcinol **1**, methyl β-orcinolcarboxylate **2**, atranol **3** and methyl haematommate **4** as examples. Not surprisingly, a significant deuterium depletion is again observed in the natural products, relative to their synthetic modifications (Table 9).

compound	origin	$\delta^{13}C$ ‰ ± 0.2 (PDB)	D/H ± 0.2 (ppm)
1	natural	- 31.3	128.5
2	natural	- 30.1	123.1
3	natural	- 28.4	139.0
4	natural	- 29.1	132.3
1	synthetic	- 32.0	142.6
2	synthetic	- 29.7	148.0
3	synthetic	- 33.6	140.4
5	synthetic	- 31.3	136.3

Table 9



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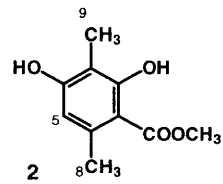

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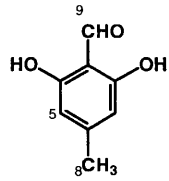

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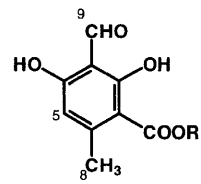
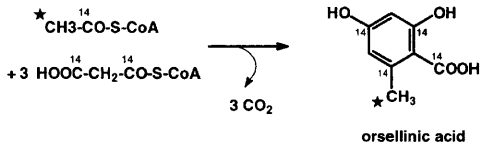


4 : R = CH_3
5 : R = C_2H_5

For the first time, we could measure the site specific deuterium abundances in depsidic compounds, though not without some technical difficulties due to the relatively large amounts of pure compounds required, and problems related to their concentration in the solvent, and to the internal standard. Partial results are depicted in Table 10:

Compound	-CHO D/H (ppm)	-$COOCH_3$ D/H (ppm)	8-CH_3 D/H (ppm)	9-CH_3 D/H (ppm)
Natural 1	-	-	111.2 ± 1.7	96.4 ± 1.0
Natural 2	-	98.0 ± 1.6	112.8 ± 0.9	92.8± 1.2
Natural 3	112.5 ± 7.4	-	106.6 ± 1.0	-
Synthetic 1	-	-	125.6 ± 0.2	145.3 ± 1.1
Synthetic 2	-	143.7 ± 4.3	126.5 ± 0.6	142.4 ± 2.2
Synthetic 3	102.0 ± 6.6	-	127.9 ± 0.5	-

Table 10



orsellinic acid

Figure 2

It is noteworthy that both the 9-CH_3 in **2** and the methanol involved in the esterase-mediated esterification of natural β-orcinolcarboxylic acid are similarly deuterium-depleted. The C_1 fragment incorporation to form the 9-CH_3 in **2** and then the CHO group in **3** or **4**, is thought to be achieved by formate prior to the completion of the aromatic ring[14]. Therefore, although a possible isotopic effect during esterification cannot be ruled out, these preliminary results are consistent with the involvement of these two one-carbon units (formic acid and methanol) at a later stage during the biosynthesis of **2**. As the biosynthetic route to orsellinic acid (figure 2) involves one acetyl-coenzyme-A C_2 unit and three malonyl-coenzyme-A C_3 units, it is clear that the 8-CH_3 of **2** originates from one initial acetic acid unit. As a result, significant deuterium abundance discrepancies are observed for the 8-CH_3 and 9-CH_3 groups in **1** and **2**.

It should be stressed that such considerations, which are based solely on a few preliminary measurements, would warrant further confirmation starting from many different samples from various origins. Work in this direction is currently in progress.

6 ACKNOWLEDGMENT

The author thanks G. J. Martin for fruitful discussions.

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FRAGRANT ADVENTURES IN MADAGASCAR
The analysis of fragrant resin from *Canarium madagascariense*

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1 INTRODUCTION

Natural Products Research in the fragrance industry is increasingly about the discovery of new scents rather than the identification of natural ingredients. To discover new natural scents you have to search for them in their native environment. One of the most unique botanical environments on earth is the rainforest of Madagascar where 80% of the 1200 plant species are endemic. It was the combination of this and the number of plants that are grown and extracted for their perfume in Madagascar that lead us to choose this location for a fragrance prospecting expedition. One of the most remote relics of original rainforest is the Montagne d'Ambre in the far north of Madagascar. Its name even suggests there may be some historical connection with fragrant materials.

Several species were found which were of fragrance interest, notably *Canarium madagascariense*, a close relative of elemi which exudes a very fragrant resin. The resin from this species has been examined by GC-olfactometry and GCMS and compared to that of elemi. Elemi gum is collected from several species but mainly *C.luzonicum* (Miqu) A.Gray, (syn. *C.commune* Wild) in Malaysia and the Philippines. The resinoid and essential oil are important fragrance ingredients valued for both their odour and fixative properties[1,2]. The resin of *C.madagascariense* is not traded as a fragrance ingredient, although we heard evidence that it is used locally both as incense and medicially.

2 METHODS

2.1 Sample collection

Samples of the resin from *C.madagascariense* were collected in the Montagne D'Ambre region of Madagascar in October 2000 with the collaboration of E.S.S.A (Ecole Supérieure des Sciences Agronomiques) and A.N.G.A.P(l'Association Nationale pour la Gestion des Aires Protégées). The resin which is a clear golden to dark brown colour as it is exuded from wounds in the bark of the tree dries to a white crystalline mass. The fresh resin has an interesting and unusual fragrance similar to frankinsence but with strong

citrus, lime and juniper top notes. A sample of elemi (*C. luzonicum*) from the in-house reference collection was selected for comparative analysis.

2.2 Extraction and analysis

The resin was extracted with a 80:20 mixture of hexane:acetone, filtered and concentrated. A second sample was dissolved in ethanol, filtered and concentrated. Both samples were analysed by GC-MS and GC-olfactometry.

GC-olfactometry was carried out using an HP5890 fitted with an OPTIC® injector and equipped with an effluent splitter, a custom built smelling port and an odour response button to record the detection of an odour electronically.
Column: HP5 25m x 0.32mm x 0.52um
Carrier: He @ 40cm/sec
Temp. program: 50° 3°/min. 280°(5min)

GC-MS analysis was accomplished using a Varian3400 GC coupled to a Finnigan ITS40 ion trap mass spectrometer.
Column: HP ultra2 (Hewlett Packard), 50m x 0.2mm 0.33µm,
Injector: 270°
Carrier: Helium at 1.6ml/min
Temp. program: 50° 2°/min. 270°
Scan range: 35 - 450 amu

Identifications were made by reference to both in-house and commercial libraries of mass spectra [3], with confirmation by relative retention indices (RRI).

Comparative GC-FID analyses were carried out using an HP6890 equipped with an FID and an OPTIC® injector.
Column: HP5 25m x 0.2mm x 0.33um
Carrier: Hydrogen @ 40cm/sec
Temp. program: 50° 10°/min. 280°(5min)

Relative retention idices (RRI) were calculated on this HP5 column using n-alkanes from C7 (heptane) to C30 (triacontane).

3 RESULTS

3.1 GC-olfactometry

A typical annotated chromatogram is shown in figure 1. The description of the odour associated with each peak is recorded and the odour response chromatogram is annotated with the descriptions. The majority of peaks had the familiar odours of the monoterpenoids. A less familiar odour, but one consistent with the rich woody odour of the resin itself was recorded at 15.0 min. From the odour descriptions obtained by GC-olfactometry it is possible to ascertain which of the components present make an important contribution to the fragrance of the original sample.

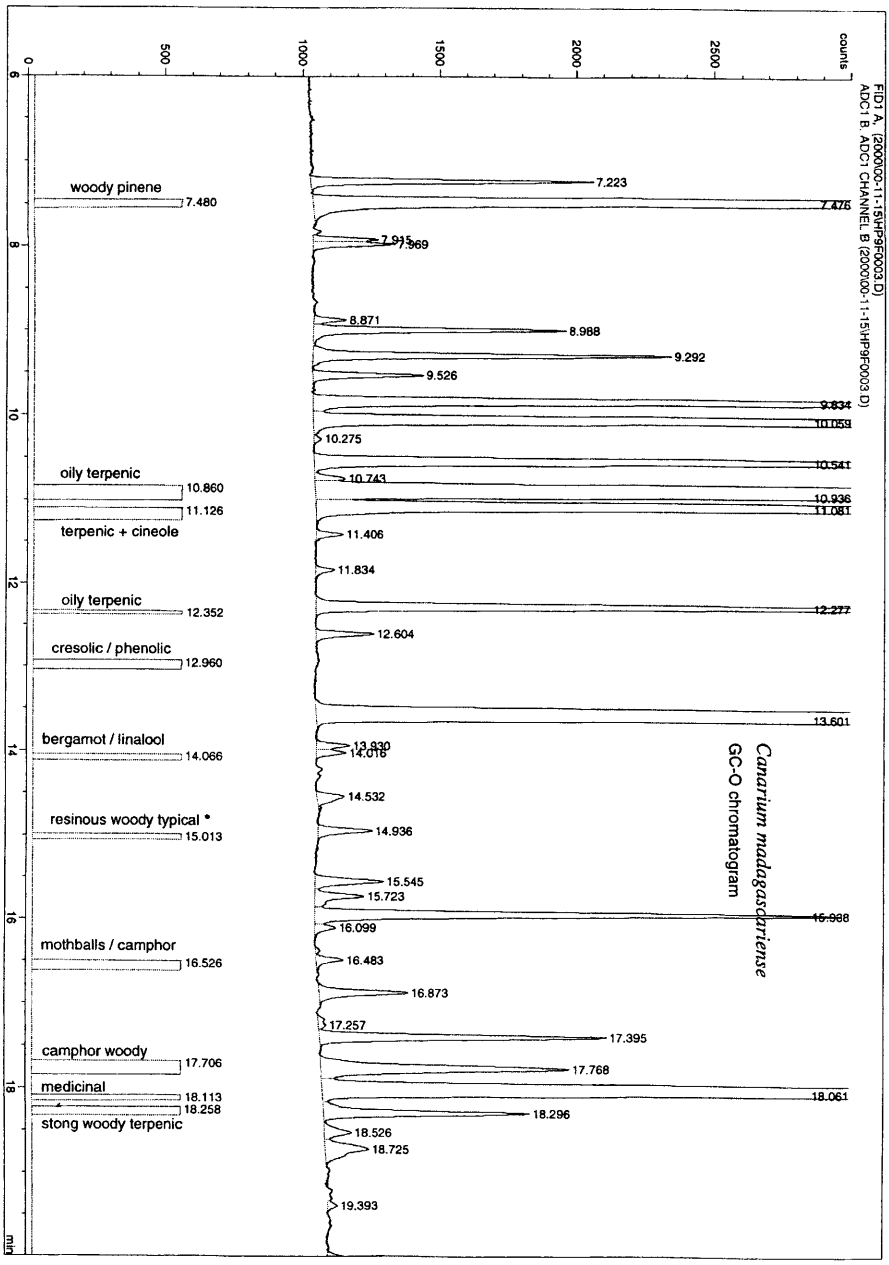

Figure 1 *Annotated GC olfactogram of Canarium madagascariense resin.*

3.2 GC-MS results

Having completed the olfactory assessment, the sample was analysed by GC-MS, with particular attention being paid to those areas of the chromatogram for which interesting odours had been recorded. The peak at RT 15.0min was identified as cis p-menth-2-en-1-ol and a trace of the trans isomer was found at RT 15.5min co-eluting with another compound. Although not readily available, this material has been previously reported as having a woody, sandalwood, minty, lime odour somewhat similar to 4-terpineol. This description is consistent with that from GC-olfactometry and lead us to conclude that it makes an important contribution to the overall fragrance of the resin. The reference sample of elemi was also analysed by GC-MS and the full results are given in Table 1.

Chromatograms of the extracts from the two species are given in Figure 2. The significant differences between the two species are the presence of elemol, elemicin and limonene in elemi oil and the greater proportion of p-cymene and terpinolene in *C.madagascariense* extract. Both samples contain alpha and beta phellandrenes and previous anlayses have indicated higher levels of phellandrenes in elemi[4] but as these materials are not particularly stable the proportion in the oil has more to do with the age, treatment and history of the resin or oil sample than the botanical source.

Table 1 *Composition of extract of C.madagascariense and C.luzonicum*

RRI	Compound name	Canarium *C.madagascariense* %RPA	Elemi *C.luzonicum* %RPA
930	alpha-thujene	0.60	0.07
938	alpha-pinene	10.70	0.28
951	alpha fenchene	0.12	
954	camphene	0.16	tr
977	sabinene	0.06	3.35
982	beta-pinene	0.55	0.14
992	myrcene	0.75	0.60
1009	alpha-phellandrene	5.48	8.01
1015	3-carene	tr	0.04
1021	alpha-terpinene	2.76	0.17
1029	para-cymene	21.74	1.44
1033	limonene	2.00	59.40
1034	beta-phellandrene	5.00	tr
1037	1,8-cineole	0.05	tr
1039	cis-ocimene	0.03	0.28
1049	trans-ocimene	tr	0.26
1063	gamma-terpinene	1.62	0.12
1072	trans-sabinene hydrate	0.10	0.29
1074	para-cresol	tr	
1091	terpinolene	14.65	0.52
1101	linalol	0.06	0.06
1121	fenchol	tr	
1123	cis-2,8-menthadien-1-ol		0.04
1129	cis-p-menth-2-en-1ol	0.10	0.02
1147	1,3,8-p-menthatriene	0.12	
1140	trans-p-menth-2-en-1-ol	0.10	tr
1142	limonene epoxide		0.06
1151	trans-beta-terpineol		tr
1155	camphor	0.90	0.02
1174	cis-beta-terpineol		0.02
1176	borneol	0.18	0.02

1184	terpinen-4-ol	0.54	0.24
1190	para-cymen-8-ol	0.61	0.07
1197	alpha-terpineol	6.51	1.78
1204	gamma-terpineol	0.01	
1210	cis-sabinol	0.10	0.19
1214	trans-piperitol	0.05	0.02
1226	cis-carveol		0.07
1231	nerol		0.05
1238	trans-carveol		0.06
1250	carvone	tr	0.06
1257	linalyl acetate	0.08	
1264	piperitone	0.16	0.05
1295	thymol	0.14	
1305	carvacrol	tr	tr
1311	terpinene hydrate	0.51	
1345	delta-elemene		0.02
1347	trans-piperitol acetate	0.10	
1359	terpinyl acetate	0.45	
1359	alpha-cubebene		0.02
1385	alpha-copaene	0.09	0.12
1403	beta-copaene	tr	0.12
1407	methyl eugenol		0.26
1431	caryophyllene		0.21
1446	trans-alpha bergamotene	0.23	
1472	humulene		0.11
1499	germacrene D	0.18	0.11
1536	delta-cadinene	0.24	0.17
1561	elemicin		4.00
1564	elemol		15.00
1610	guaiol		0.24
1666	gamma eudesmol		0.17
1668	beta-eudesmol		0.35
1670	valerianol		0.24
1688	bulnesol		0.06
	Total identified	77.8	99.0

%RPA data from integrated FID chromatogram. Identities from GCMS. tr – trace <0.01%rpa
RRI on HP5 column using n-alkanes.

Table 1 Continued *Composition of extract of C.madagascariense and C.luzonicum*

4 CONCLUSIONS

Although *C.madagascariense* is in the same botanical family and genus as elemi (*C.luzonicum*), it is clear from both the olfactory data and the analytical data that the odour and composition of the resin from these two species is distinctly different. This is the first detailed analysis of the resin from *C.madagascariense* and provides the information from which a new perfumery accord has been created that replicates the fragrance of this resin from the rainforest of Madagascar.

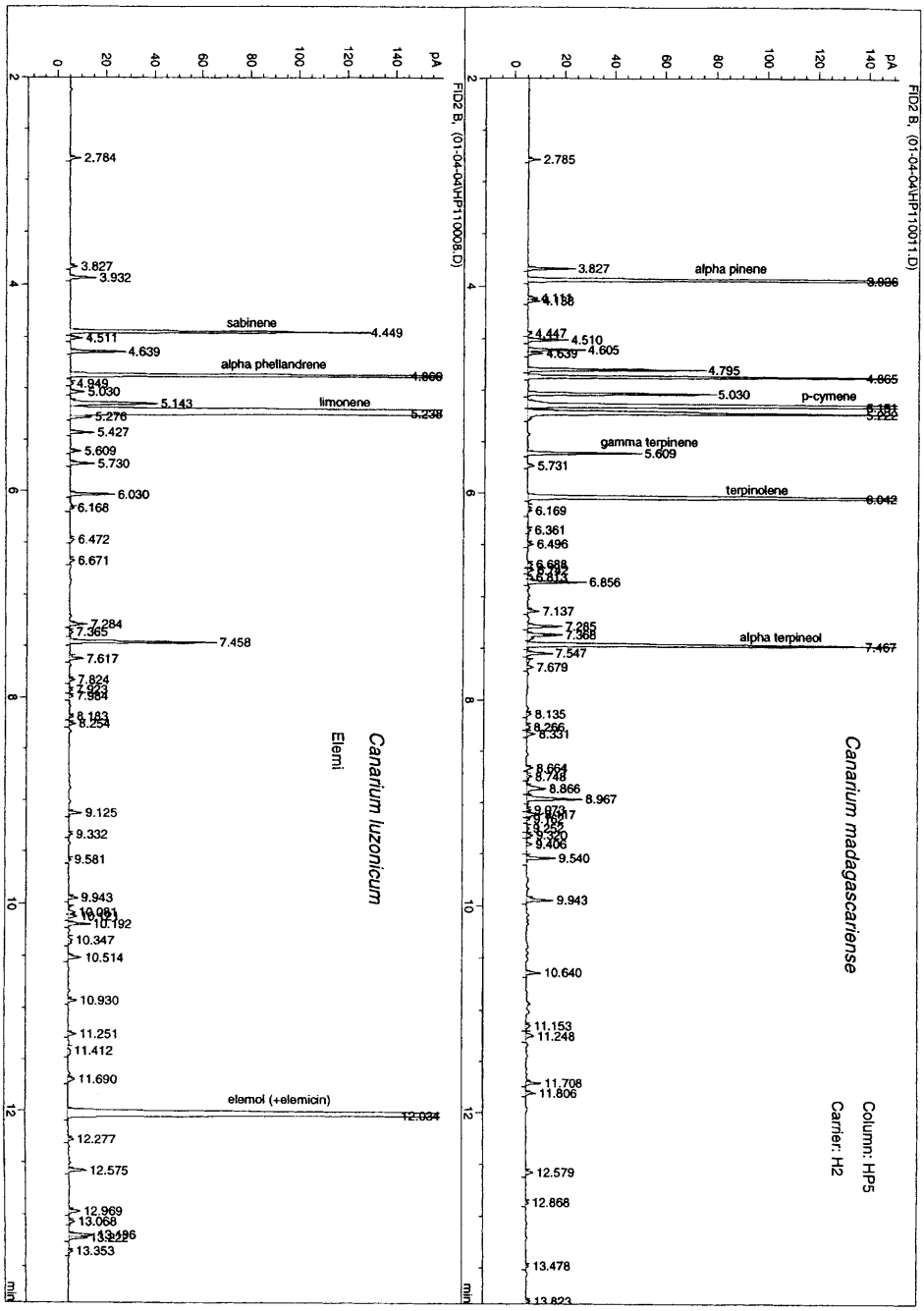

Figure 2 *Comparison of extracts of C.luzonicum and C.madagascariense*

The discovery of this fragrant resin and the many other scented flowers, leaves, and barks that were found in the forests of Madagascar during our expedition illustrate the importance of such ventures in the discovery and creation of new fragrances. Only by travelling to regions rich in unusual flora with a team of fragrance and botanical experts can one successfully discover such a variety of new scents. Through the identification of the plants and the analysis of the extracts, we can discover the secrets of the natural chemistry of these fragrant materials. With advances in modern technology and the skills of the creative perfumer it is possible to bring these scents from the rainforest to the consumer without the need to remove any plants from their native habitat.

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THE EFFECT OF MICROGRAVITY ON THE FRAGRANCE OF A MINIATURE ROSE, "OVERNIGHT SCENTSATION" ON SPACE SHUTTLE (STS-95)

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1 INTRODUCTION

Everybody knows that gravity governs the universe. It is due to gravity that we stand, fruits fall and leaves flutter to the ground. NASA has sent man to the moon and into space for the last 30 years to study the effect of micro gravity on the physiology of astronauts.

During previous shuttle missions, seeds and tubers were grown in space, but to our knowledge, no one has ever sent a living flowering plant into space to study the effect of microgravity on the production of the aroma constituents of a flower.

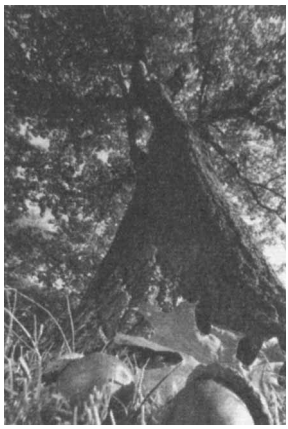


Figure 1 *Oak tree*

The plant, (Figure 1), is the only living entity that grows against the effects of gravity, a phenomenon known as "gravitropism". With this in mind, it would be very interesting to know how a flowering plant responds to microgravity. NASA provided us with the opportunity to study the effects of microgravity on the aroma of a flower on STS-95 Space Shuttle Mission.

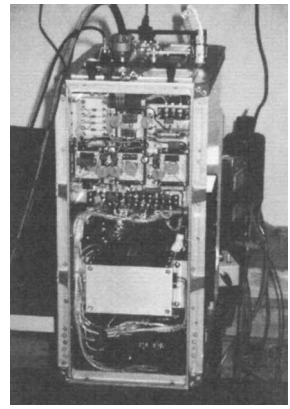


Figure 2 *Astroculture unit*

2 EXPERIMENTAL

To send a living plant into conditions of microgravity, one needs to design a proper chamber, which could hold the plant in space and provide all of its biological requirements. This chamber, known as the ASTROCULTURE™ unit, (Figure 2), was developed and built by Dr. Zhou and his associates of WCSAR. Despite the large appearance of this unit, the plant-growing chamber is only 5 inches by 8 inches in dimension.

This chamber is equipped with LED lighting for photosynthesis, a substrate medium (arcelite), a reservoir containing nutrient solution, as well as humidity and temperature controls. This unit provides all of the earthly growth requirements of a living plant. Once the chamber was ready, much careful consideration was given to the choice of flowering plant to be sent into space.

To send a flower to space, the following criteria had to be met:

1. Since we are a Flavour and Fragrance Company, the plant must have a flower with an aesthetically pleasant and suitable aroma for making the perfume.
2. The plant has to fit in the growing chamber which is 5" x 8".
3. The flower's life span has to be 10 days from bud to bloom.
4. The plant must bloom during October-November, 1998.

So, what was that unique flower which would fulfil all of these requirements?

Figure 3 *Hybrid tea-roses in garden*

After much thought, a rose plant was selected to be sent into space because of the following reasons:

1. The rose is the queen of all flowers. Every person on this earth adores the rose.
2. It is the national flower of the United States of America, established by President Ronald Reagan in 1986.

Being an American involved in research on an American space flight, what could be better than to send America's national flower, the rose, into space? However, traditional rose plants are at least 2 to 4 feet high and cannot be accommodated in the plant chamber, (Figure 3). For this reason, we looked for another kind of rose i.e. a miniature rose.

Figure 4 *Miniature roses in IFF Greenhouse*

But another problem came to our hand. Most miniature roses do not have any aroma. Therefore, we searched for a miniature rose with a pleasant aroma. After screening many miniature roses, we found a hybrid miniature rose, known as "Overnight Scentsation", (Figure 4), not only because of its beauty but also for its sensational aroma. This rose has an intense green rosy aroma, which is not found in regular roses. Now we had to grow this rose so that it blooms in the cold months of October/November.

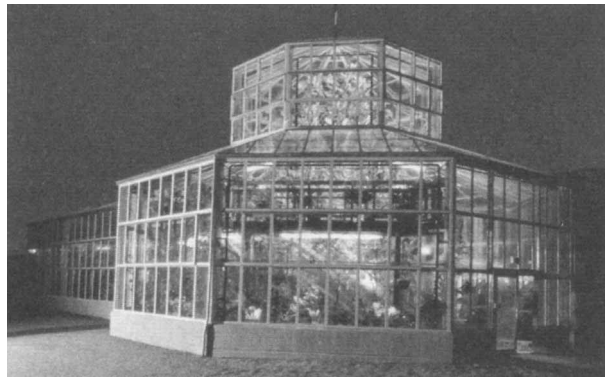


Figure 5 *IFF Greenhouse*

Figure 5 shows the special IFF greenhouse where light, humidity and temperature are controlled by computers. We have grown this miniature rose in this special IFF greenhouse, where they bloom throughout the year.

Now that the ASTROCULTURE™ unit has been built, the rose has been selected and grown in the greenhouse, only one more important aspect of the project remains to be solved, that is how to analyse the aroma of the living rose in space.

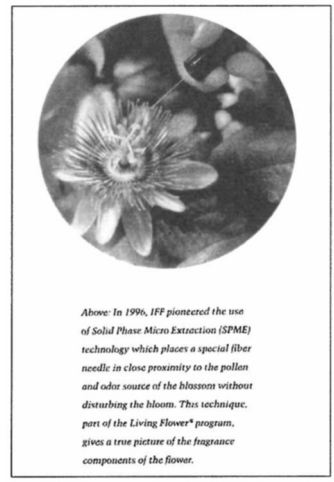


Figure 6 *SPME Living Flower Sampling Technology*

To do that, another unique IFF technology was used, Living Flower technology. Let me describe this very simple but unique method. In 1992-1993, we developed a simple technique to analyse a living flower without picking, touching or harming the flower in any way. A device known as an SPME needle containing a glass fibre coated with liquid absorbent is placed above the flower petals without touching the flower. Depending upon the intensity of the aroma of the flower, the needle is kept there for 0.5 - 2 hours. The molecules surrounding the flower are absorbed onto the needle and introduced into an instrument called a gas chromatogram - mass spectrometer for subsequent analysis (Figure 6).

Now, we had to adopt this sampling method in the ASTROCULTURE™ unit.

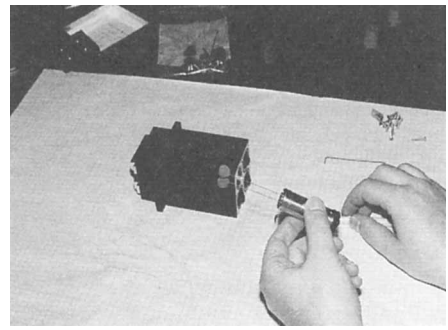


Figure 7 *SPME sampling unit*

A precise metal holder as shown in this picture, containing eight SPME needles was developed so that the rose could be analysed over four consecutive days. This sampling device was fitted within the ASTROCULTURE™ unit in such a way that when the rose bloomed the SPME needle would extend above the rose (Figure 7).

After devising this analytical method, we performed preliminary experiments with the miniature rose in this Astroculture™ unit on earth.

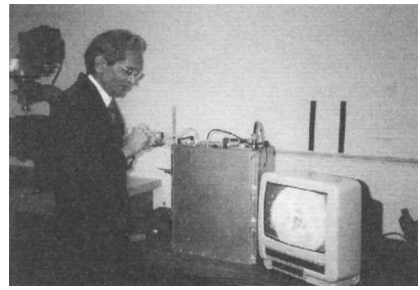


Figure 8 *Ground Simulation experiment*

This is the Ground Simulation experiment where the rose was analysed from bud to full bloom over four consecutive days, (Figure 8). This method of analysis was taught to Astronaut Mr. John Glen, who actually performed this experiment in space. Now let's look at the comparative analysis of the living rose (Overnight Scentsation) in the green house and in the Astroculture™ unit.

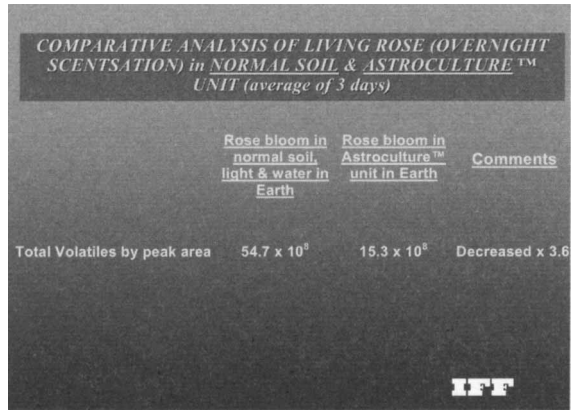


Figure 9 *Comparative Analysis of living rose (Overnight Scentsation) in NORMAL SOIL & ASTROCULTURE UNIT (average of 3 days)*

We found that the total amounts of the volatile contents produced by the flower in the Astroculture™ unit have decreased by a factor of 3 when compared to the total amounts of the volatile contents produced by the flower in the green house, (Fig. 9).

Figure 10 shows the differences in the constituents of the rose grown in the greenhouse and in the Astroculture™ unit.

COMPARATIVE ANALYSIS OF LIVING ROSE (OVERNIGHT SCENTSATION) in NORMAL SOIL & ASTROCULTURE™ UNIT (average of 3 days)

Compound	Rose bloom in normal soil, light & water in Earth	Rose bloom in Astroculture™ unit in Earth	Comments
Cis-3-Hexenyl/Hexyl Acetate	3.5	1.1	Decreased x 3
Rose oxide	0.7	0.3	Decreased x 2
3,5-Dimethoxy Toluene	0.3	0.7	Increased x 2
Theaspirane	1.5	6.7	Increased x 5
Phenyl ethyl acetate	14.2	24.7	Increased x 2
Phenyl ethyl alcohol	33.7	31.0	*No change*
Citronellol	15.3	9.1	Decreased x2
Geraniol	11.7	0.9	Decreased x12
Methyl geranate	0.2	0.3	*No change*
Methyl Eugenol	1.5	2.9	Increased x 2
Dihydro β-ionol	4.4	5.2	*No change*
Hydrocarbons (C15-C21)	9.2	10.0	*No change*
Acids (C12-C16)	0.1	3.2	Increased x32

IFF

Figure 10 *Comparative Analysis of living rose (Overnight Scentsation) in NORMAL SOIL & ASTROCULTURE TM UNIT (average 3 days)*

This shows that the flower's aroma has changed in this Astroculture™ unit. We may ask why? Answer is simple. We have changed the growing conditions of the flower, particularly the environmental atmosphere. We are of opinion that like a human being, when the flower is placed in a closed chamber, it is under stress and the physiology of the plant has probably changed, hence the aroma is also changing. Please remember that we have to compare this ground simulated Astroculture™ unit experiment with the space experiment.

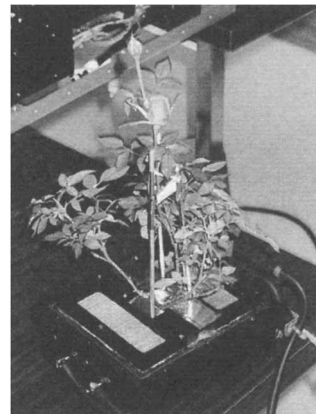


Figure 11 *Rooting tray with rose plant and 2 buds*

Everything is ready for the space experiment. Now we are waiting for the final launch day on October 29th 1998. This rose plant with 2 rose buds was placed in the plant-growing chamber of the ASTROCULTURE™ unit, and the SPME needle box was positioned in such a way that as the rose bloomed, the SPME needle could be positioned above the rose petals. As you can see, the stem of the rosebud was tied with a thread on a metal pole so that the rosebud does not bend or fall due to the tremendous thrust of the launch. By the way, this is the only work I have done, (Figure 11).

Figure 12 *Shuttle launch*

Finally, the much awaited launch day came! On the 29th of October, 1998, this shuttle containing the rose plant with two rose buds was launched, (Figure 12). Unless you personally see the launch, you probably cannot imagine the tremendous thrust the shuttle produced at the moment of launching. I literally prayed to God to keep the rose bud intake on the plant! And God granted my prayers!

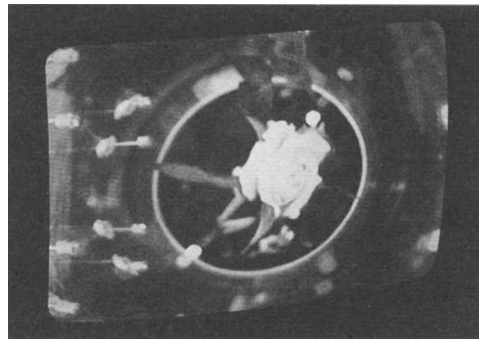


Figure 13 *Rose is blooming-video picture*

This is the actual video picture of the rose after 2 days in space. As you can see in the picture, the rose has bloomed in space and was analysed by John Glenn and other astronauts for four consecutive days by SPME needles as seen here, (Figure 13).

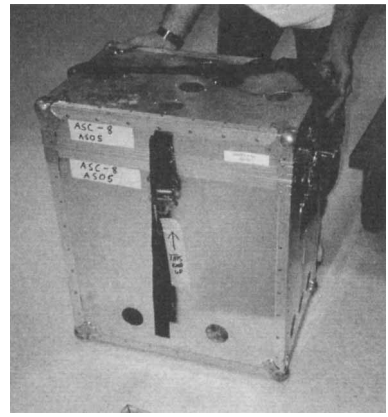


Figure 14 *Shuttle Module returning from space*

After nine days in microgravity, the rose came back to the earth in this module (Figure 14).

Figure 15 *Astroculture* ™ *unit*

Figure 15 shows laboratory assistants opening the box to take out the rose plant after 9 days in space. You can probably imagine the intense tension of our minds at this moment!

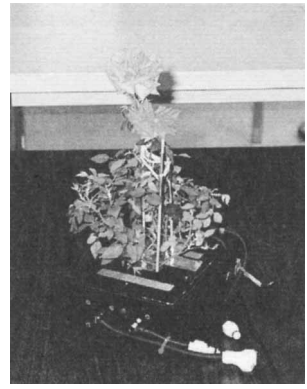


Figure 16 *Flowers when returned from space*

The box is opened, and the rose plant is alive. Figure 16 is a picture of the rose as it came from space. You can see that the flowers are fully open.

Figure 17 *Close-up of the flowers from space*

Figure 17 is a close-up picture of blossoms as grown in space. Careful examination revealed that more new shoots and leaves had been produced. The flower also smelled good.

All eight SPME needles were analysed by GC-MS. The Space data was compared with our ground simulation experiment. Now let us see the results.

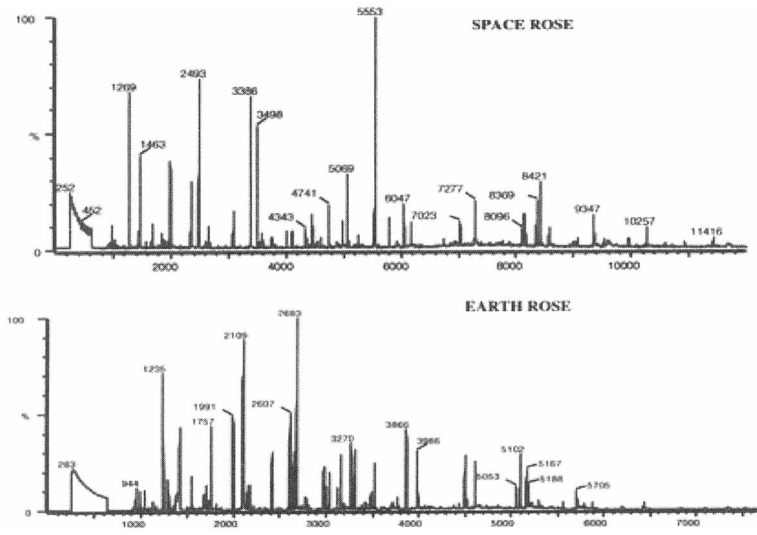


Figure 18 *GC/MS profile of space-rose and earth-rose*

Looking at figure 18, the top is the space rose - GC/MS profile and the bottom is that of the ground simulated experiment in earth. One can easily see the differences in the constituents of the Space rose verses Earth rose.

Let us now see the quantitative differences in the constituents of both, space and earth rose.

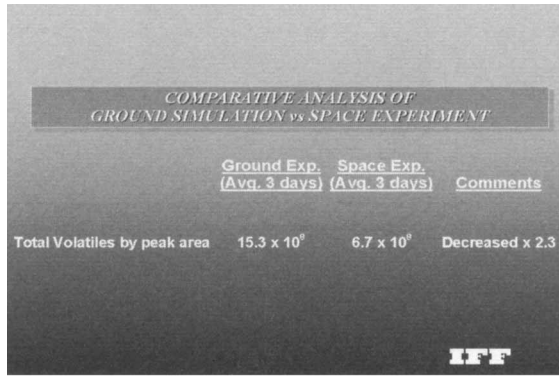


Figure 19 *Total volatiles of ground experiment and space experiment*

The very first thing we found was that the total amount of volatiles produced by the flower in space has been significantly reduced when compared to the amount of volatiles produced by the flower in the Astroculture™ unit on earth, (Figure 19). Although it has not been scientifically determined why the Space rose produces less aroma, one could speculate that based upon the increased growth of the foliage, that more nutrients were available to the lower branches than to the top of the plant where the flower grows. Or under microgravity – just like human beings, the plant was under stress. Human beings loose muscle, the flower lost the aroma! Now let us see the differences in constituents of the both flowers.

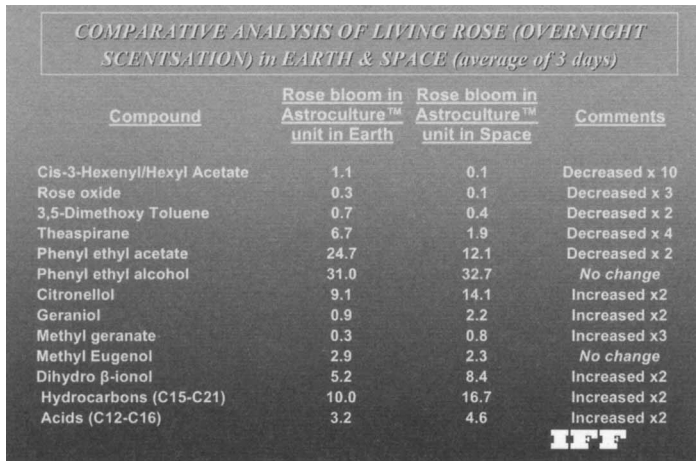


Figure 20 *Individual components*

Figure 20 shows the changes in the constituents of both roses. The green note constituents such as *cis*-3-hexenyl acetate, hexyl acetate and rose oxide have been drastically reduced. The heart of the rose constituents' viz. phenyl ethyl alcohol, citronellol, geraniol, and methyl geranate have been increased. At the same time, non-polar hydrocarbons also increased.

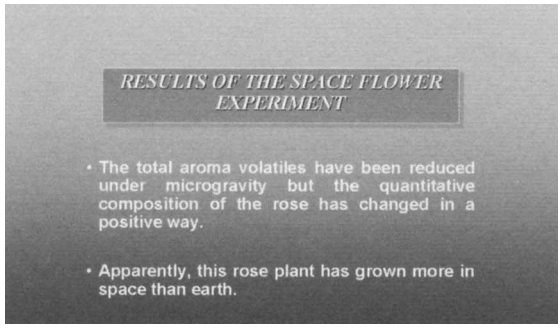
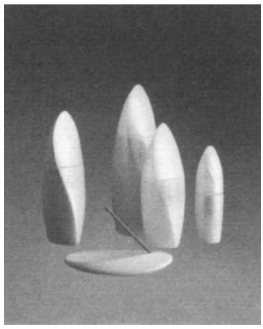


Figure 21 *Results of space flower experiment*

Figure 21 summarises our findings. From these results, I would like to predict that plants probably would grow more in other terrestrial places like Mars, where the gravity is less than that on earth, as long as proper plant growth conditions are met. The flower will also provide an aesthetic aroma but this will be different that experienced on earth.

This experiment also poses a very important question to my mind. Since the plant grows more and produces a different but more pleasant aroma under micro gravity, there must be another force that governs the life of the plant. Could it be **God or Gravity?**



I would like to leave this question to all the distinguished Natural Product Chemists to solve this natural mystery. Although we may not solve the mystery, it brought happiness in the minds of people around the world. Last September, Shiseido, the famous company of Japan introduced a new fragrance, utilising the Space Rose fragrance, called ZEN. I am sure you know that ZEN means, ultimate Bliss, (Figure 22).

Figure 22 *Shiseido's Zen fragrance*

Acknowledgements

We would like to thank all NASA officials for providing this opportunity to work with WCSAR. In this respect, we would also like to thank Mr. Robert Meyers, Mr. Sam Durst and Mr. Perry Sandstrom at WCSAR who assisted in building the Astorculture Unit™. Last but not least, we would like to thank our colleague, Mr. Robert Trenkle for devising the original method of SPME.

Organic and Bioorganic Chemistry

AMBERGRIS FRAGRANCE COMPOUNDS FROM LABDANOLIC ACID AND LARIXOL

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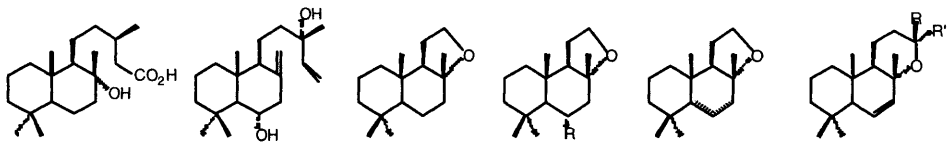
1 INTRODUCTION

Labdanes form a large group of diterpenes, consisting of a substituted decalin system, with a substituted side chain at C_9[1]. The C_9 side chain may contain various functional groups as can be seen from labdanolic acid (**1**) and larixol (**2**), but often a tertiary methyl-vinyl-alcohol functionality at C_{13} as in larixol is encountered.



labdanolic acid (**1**) | larixol (**2**) | Ambrox® | Ambrox® derivatives | Ambroxenes | Δ^6-Ambra-oxides

Figure 1

Labdane diterpenoids are available from nature and have been used frequently as starting material for the synthesis of other natural products. Most of the chemistry of labdanes has been developed for the preparation of Ambrox® and Ambrox®-like compounds (see figure 1)[2].

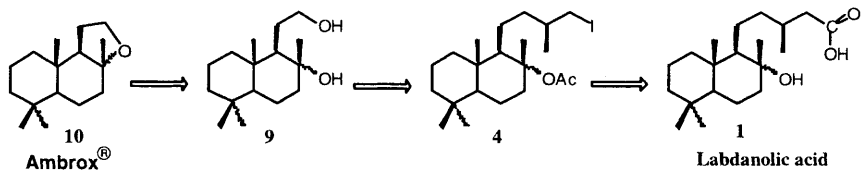
Since ancient times, ambergris has been one of the most highly valued perfumery materials[3]. Ambergris is a metabolic product of the sperm whale (*Physeter macrocephalus L.*), which accumulates as concretions in the gut. (-)-Ambrox® (**10**), the commercially most important constituent of natural ambergris, was recognised as the prototype of all ambergris odourants[4]. For this reason, various synthetic routes to (-)-Ambrox® and its racemate have been developed[5]. The price of (-)-Ambrox® is relatively high, which induces a continuing search for new syntheses[6] of this compound and its derivatives, preferably starting from cheap, abundantly available natural labdanes. Sclareol is nowadays the industrially used starting material for the preparation of (-)-Ambrox®[7]. Labdanolic acid (**1**) and larixol (**2**) are both available from labdanum gum[7] and larix

terpentine[8] respectively, but have found little use as starting material in syntheses of Ambergris odour compounds. In our laboratory, we have investigated possible applications of these two labdanes[9].

2 AMBROX®

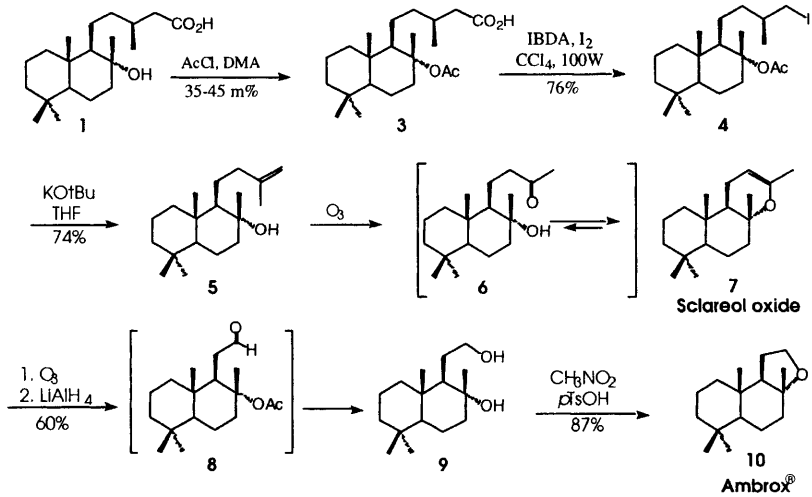
2.1 Ambrox® from labdanolic acid

Labdanolic acid (**1**) is the main component (ca. 40%) in the acidic fraction of the *n*-hexane extract of *Cistus ladaniferus L.* ("Rock-rose")[7]. The oxidative degradation of the C_9 side-chain of labdanolic acid can in principle provide suitable synthons for the synthesis of (-)-Ambrox® (**10**). The degradation of labdanolic acid or its methyl ester is not an easy task because the carboxyl group is the only available functional group in the side chain. Lead tetraacetate is the most commonly used decarboxylating reagent and moderate yields of the acetate were obtained[10]. We have investigated the iododecarboxylation of labdanolic acid as the most significant step in its conversion into Ambrox® (Scheme 1).



Scheme 1

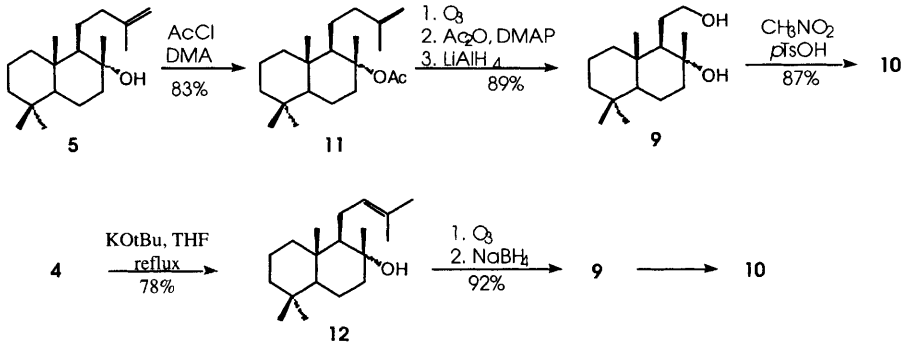
The commercial extract from *Cistus ladaniferus L.*[11] was obtained by steam-distillation of the twigs and leaves of the plant, or by treatment of the plant with hot neutral or alkaline water. However, these conditions easily caused dehydratation of the tertiary hydroxyl group, which resulted in a mixture of labdenic acids. To prevent this dehydration the dried twigs and leaves of the *Cistus ladaniferus L.* were soaked with *n*-hexane. The hexane solution was separated from the solid material and after evaporation of the solvent a sticky labdanum gum was obtained. The gum was dissolved in ether and extracted with NaOH solution. The basic solution was acidified, extracted, and after concentration a crude labdanolic acid was obtained. The purification of this crude labdanolic acid appeared to be difficult, but after conversion of the C_8 tertiary alcohol group into its acetate **3**, the purification of the acetate proved to be relatively easy. The iododecarboxylation[12] of **3** was achieved by irradiation with a 100W tungsten lamp and the iodide **4** was obtained in a good yield of 76%.



Scheme 2

Because iodide **4** is not a stable compound it was treated directly with KO*t*Bu in THF at *room temperature* to cause dehydroiodination and hydrolysis of the acetate at C_6 which resulted in compound **5** in a yield of 74%. Ozonolysis of the double bond in **5** and reduction of the intermediate ozonides gave the methyl ketone **6**, which immediately cyclised to sclareol oxide **7**. Sclareol oxide was ozonolysed and reduced directly to afford diol **9** in 60% overall yield from **5**. Stirring of diol **9** in nitromethane in the presence of *p*-toluenesulfonic acid gave Ambrox® (**10**) in 87% yield (Scheme 2).

An obvious way to circumvent the unstable sclareol oxide was the synthesis of **9** *via* a Criegee rearrangement[13] of the ozonide of **5**, but this approach was unsuccessful since the methyl ketone **6** was obtained as the result of a normal ozonolysis of **5**. To prevent the interference of the alcohol at C_8 during the ozonolysis, the group was again protected as its acetate and now the ozonolysis and Criegee rearrangement worked well (Scheme 3). After reduction of the intermediate with $LiAlH_4$, diol **9** was obtained in 89% overall yield from compound **11**. This diol was transformed into (-)-Ambrox® as described in Scheme 2. However, the shortest route was found when the dehydrohalogenation of iodide **4** is performed with KO*t*Bu in *refluxing* THF, or in DMSO at room temperature. The intermediate alkene **5** isomerised *in situ* to **12** (Scheme 3), and ozonolysis of the double bond followed by reduction of the intermediate ozonides with sodium borohydride gave diol **9** in 92% overall yield from **12**.

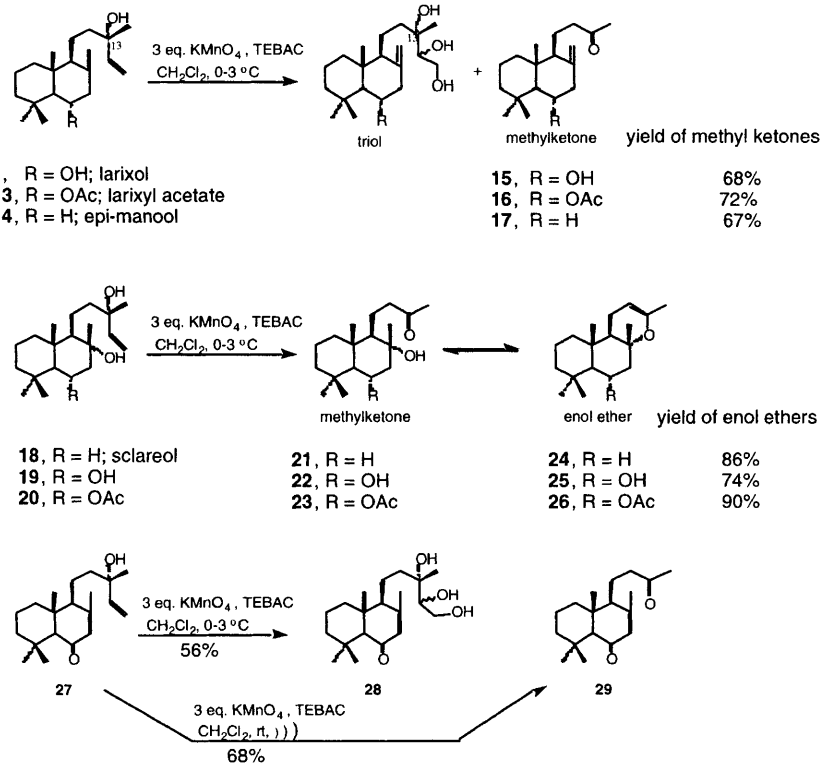


Scheme 3

In this way it was demonstrated that Ambrox® can be obtained in a short procedure of 4 steps in 47% overall yield starting from the acetate of labdanolic acid. The main drawback of the method is the necessary application of iodine in the decarboxylation reaction. When a cheaper procedure can be found for this reaction, labdanolic acid (**1**) will become a good alternative starting material for the industrial preparation of Ambrox®.

2.2 Ambrox®-like compounds from larixol

For the bicyclic part of the labdanes to be of use in the synthesis of other natural products the C_9 side chain has to be shortened. Several studies to oxidise the side chain have been reported in the literature for larixol (**2**) and epimanool (**14**)[14], but many do not use cheap oxidants, are irreproducible or do not contain an easy work-up. Also, some special oxidation procedures, which have been developed for sclareol (**18**)[15], do not proceed in the same way for larixol due to the absence of the hydroxyl group at C_8 in the latter. This C_8 hydroxyl group participates in intermediates, which are formed during the oxidation of sclareol, and such participation is not possible in epimanool (**14**), larixol (**2**), and its acetate **13**. Later, the presence of the secondary hydroxyl group at C_6 in the latter two compounds and the double bond at C_8 may cause selectivity problems. A modification of the method of Ogino *et al*[16], using anhydrous permanganate in the presence of a phase-transfer catalyst, was investigated for the oxidation of the labdanes **2** and **13**, **14**, **18-20,** and **27** that are depicted in Scheme 4[14f].

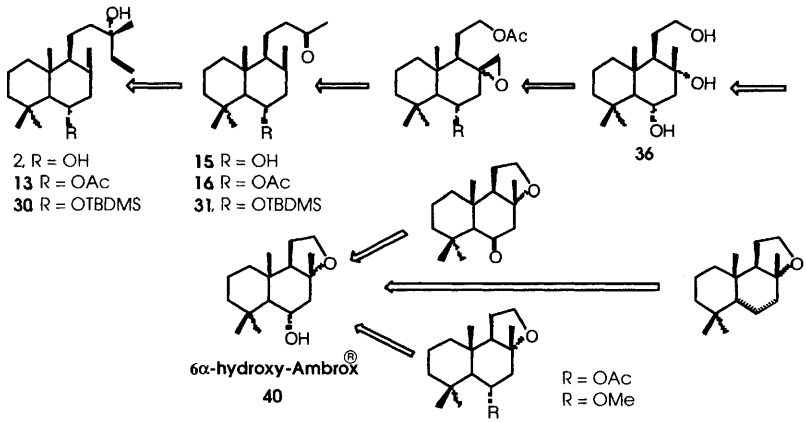


Scheme 4

The oleoresin of the Venice larch terpentin of the *Larix decidua Miller* (*L. europaea D. C.*) consists mainly of larixol, *epi*-manool and larixyl acetate **13**[17]. To facilitate purification, the extract was hydrolysed to give larixol as the main constituent, which could be obtained in pure form via crystallisation from cyclohexane. The most apolar compound in the residual mother liquor is *epi*-manool[18], which could be isolated by chromatography. Compound **19** was obtained from larixol in 64% overall yield, after epoxidation of the exocyclic double bond with oxone and reductive opening of the epoxide using $LiAlH_4$. Compound **27**[9c] was synthesised from larixol in 73% overall yield by oxidation with pyridinium chlorochromate (PCC) to the C_6-ketone, followed by base catalysed isomerisation of the exocyclic double bond using methanolic sodium methoxide.

The oxidation of the labdanes **2** and **13**, **14**, **18-20** and **27** was investigated using standard methods with 1.5 or 3 equivalents of permanganate, and the results of the latter method are summarised in Scheme 4. From these results it became clear that generally good to high yields of single products could be obtained. In addition, the influence of the substituent at C_8 on the product formation became evident. When an exocyclic double bond was present at C_8 a reasonable selective oxidation of the double bond in the side chain could be achieved to give the methyl ketones in good yields. When a hydroxyl group was present at C_8, this group had a strong tendency to react with the methyl ketone in the side chain, and cyclic enol ethers were isolated as the main reaction products in high yield. These enol ethers were usually not very stable and with many oxidation systems further oxidation took place to afford compounds with a shortened side chain at C_9. The application of sonication accelerates the oxidation appreciably, and shortened the reaction time from 14 to 2 hours, but the products and the yields were not affected. The ratio between triols and methyl ketones was pH dependent, with alkaline pH favouring the triols.

The oxidation of the C_9 side-chain of larixol or its acetate provided suitable synthons for the synthesis of Ambrox®-like compounds. 6□-Hydroxy Ambrox can be considered to be a key intermediate in such syntheses and the conversion of larixol into this intermediate has been investigated first as is indicated in scheme 5.

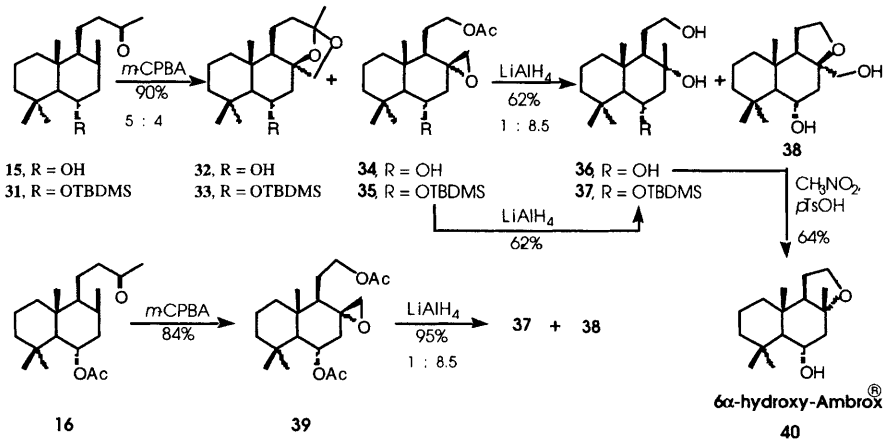


Scheme 5

An obvious way to achieve further breakdown of the obtained methyl ketones to a functionalised two carbon side chain is the Baeyer Villiger oxidation. This reaction was investigated for three derivatives, containing an hydroxyl group, a

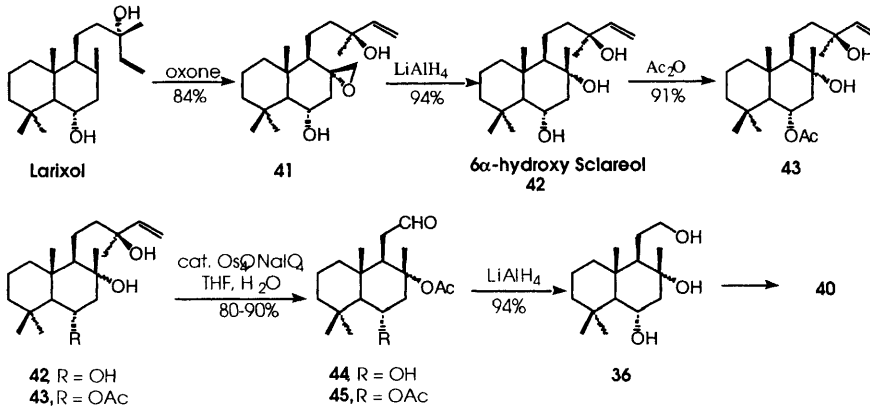
*tert.*butyldimethylsilyloxy group or an acetate at C_6 (Scheme 5). It is known that methyl ketones give acetates upon Baeyer Villiger oxidation, but under these conditions double bonds may be epoxidized as well. Reduction of the acetate and the epoxide moiety should be possible in one step to give the triol **36**, which may be cyclised to 6α-hydroxy Ambrox® **40**.

When methyl ketone **15** was treated with *m*-CPBA in dichloromethane at room temperature the expected epoxy-acetate **34** was formed along with the acetal **32** in an almost equal yield (Scheme 6). Apparently, epoxidation of the double bond is faster than the Baeyer Villiger oxidation of the methyl ketone and a subsequent reaction of the epoxide with the carbonyl group gives the acetal **32**. A similar mixture of compounds **33** and **35** was obtained in the Baeyer Villiger oxidation of the TBDMS protected compound **31** with *m*-CPBA. On the other hand, when the acetate **16** was treated with *m*-CPBA the Baeyer Villiger product **39** was obtained in a good yield of 84% (Scheme 6).



Scheme 6

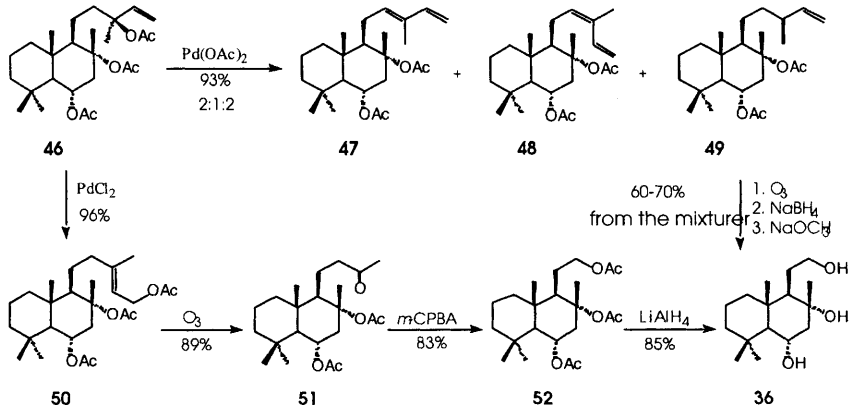
The epoxy-acetates **34** and **39** were treated with $LiAlH_4$ to reduce the epoxide to the desired tertiary hydroxyl group at C8, and also simultaneously reduce the acetates. However, the reduction of these two epoxy-acetates gave the cyclic ether **38** as the main product with the desired triol **36** being obtained only as a minor component. Obviously the acetates are reduced first, and then the alkoxide in the side-chain attacks the epoxide to form the cyclic ether **38**. In contrast the reduction of compound **35** with $LiAlH_4$ proceeded well and gave a 62% yield of diol **37**. The triol **36** could be cyclised in 64% yield to 6α–hydroxy-Ambrox® **40** after stirring in nitromethane in the presence of *p*-toluenesulfonic acid. Although incidental transformations in this sequence proceeded in good yield, no overall high yield conversion of the methyl ketones to 6α-hydroxy Ambrox® could be achieved and therefore other routes were investigated.



Scheme 7

The two major difficulties in the former route were connected with the lack of selectivity in the Baeyer Villiger oxidation and in the reduction of the resulting epoxy-acetates. To avoid these problems, the exocyclic double bond at C_8 in larixol was first converted into a hydroxy-methyl group by oxidation with oxone followed by reduction of the epoxide **41** with $LiAlH_4$ to give 6α-hydroxy-sclareol **42** (scheme 7). In this case no competitive formation of cyclic ethers was observed. The C_6-hydroxyl group in **42** could be protected selectively as its mono acetate **43**, and both compounds were oxidised with a catalytic amount of OsO_4 and an excess of $NaIO_4$ to afford the aldehydes **44** and **45** respectively, as the major products in high yield. When the aldehydes were reduced with $LiAlH_4$ triol **36** was obtained in 94% yield.

Two other routes starting from 6α-hydroxy-sclareol **42**, based on the palladium catalysed elimination or isomerisation of acetates in the side chain, followed by ozonolysis, were also investigated (scheme 8). Protection of the secondary and the tertiary hydroxyl groups in **42** as acetate groups could be achieved with acetyl chloride in N,N-dimethylaniline to give triacetate **46** in high yield. When **46** was treated with a catalytic amount of palladium acetate in the presence of triphenylphosphine at 100 °C, a mixture of three dienic acetates **47**, **48** and **49** in a ratio of 2:1:2 was obtained respectively in over 90% yield[19]. Ozonolysis of this mixture gave an aldehyde, which was reduced and saponified to give triol **36** in a good yield.



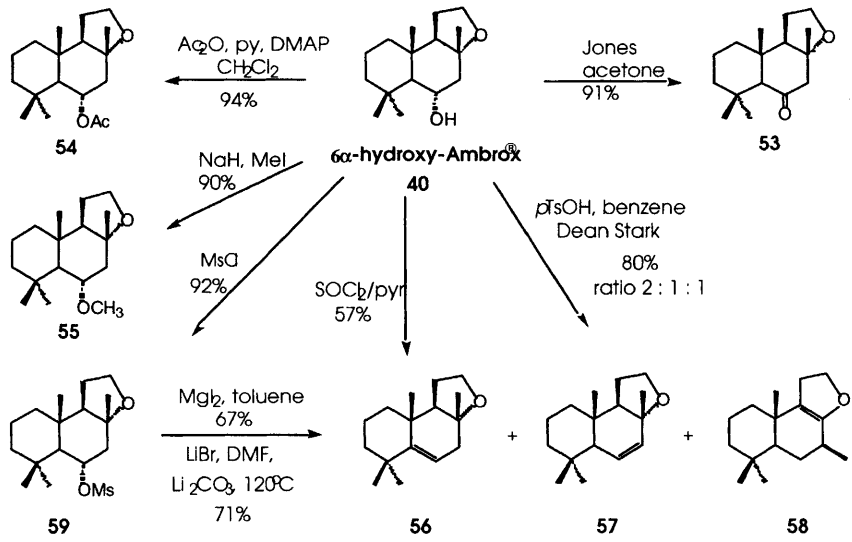
Scheme 8

Triacetate **46** could also be isomerised in nearly quantitative yield to the allylic acetate **50**[19] (Scheme 8). Ozonolysis of the double bond in the side chain gave methyl ketone **51,** which was transformed into triacetate **52** by Baeyer Villiger oxidation, again in a good 83% yield. Complete reduction of **52** with $LiAlH_4$ gave triol **36** in 85% yield. The same triol can also be obtained from triacetate **52** by saponification.

When the results, obtained in the conversion of larixol into 6α-hydroxy-Ambrox® are compared, it can be concluded that the route described in Scheme 7 gives the highest yield in the shortest number of steps. On the other hand, the second route of scheme 8 is the easiest one to perform and has our preference.

2.3 Other Ambrox® derivatives

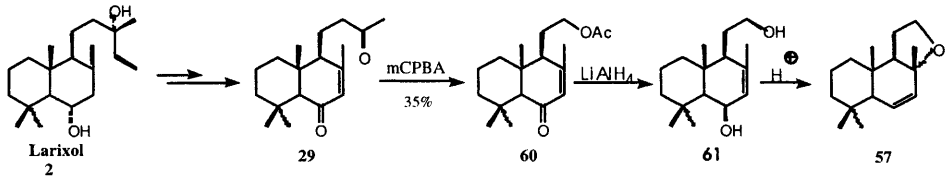
6α-Hydroxy Ambrox® **40** was used as the key intermediate for the preparation of a number of simple Ambrox® derivatives. Treatment of **40** with Jones' reagent in acetone produced 6-oxo-Ambrox® **53** in 91% yield. Conversion of **40** to its acetate **54** was achieved by acetic anhydride and DMAP in pyridine and dichloromethane in 94% yield. The methyl ether **55** was obtained in 91% yield after treatment of **40** with sodium hydride and methyl iodide. When **40** was treated with *p*-toluenesulfonic acid in benzene under Dean Stark conditions a non-separable mixture of three compounds **56, 57,** and **58** was formed in a ratio of 2:1:1. The structures of **56** and **57** were confirmed by independent synthesis (*vide infra*), the structure of compound **58** is still tentative. This pleasant smelling mixture was separated on a GC-MS apparatus and on a GC-sniff apparatus, and all three compounds showed a pleasant smell, with the Δ^5-alkene **56** as the most interesting one.



Scheme 9

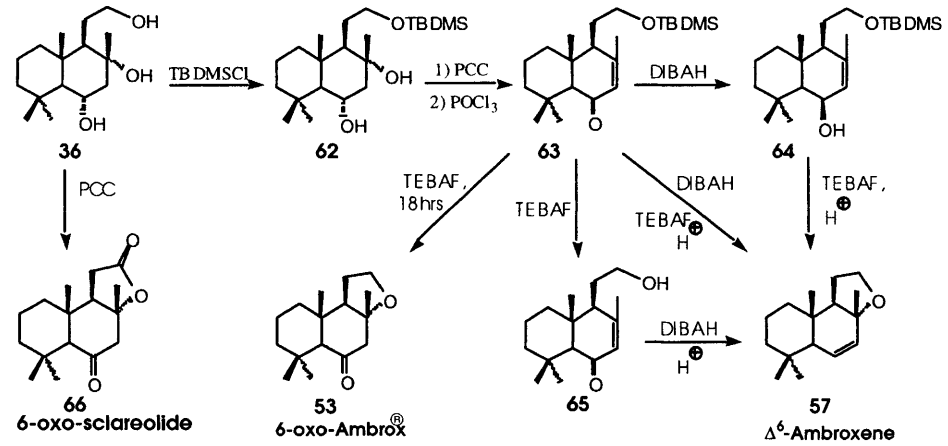
This Δ^5-alkene **56** was produced in a more selective way by conversion of **40** to mesylate **59** and treatment of this mesylate with magnesium iodide in toluene or by treatment with lithium bromide and lithium carbonate in *N,N*-dimethylformamide (DMF) at 120°C. This afforded the Δ^5-alkene **56** in about 70% yield, with preservation of its pleasant smell.

A selective synthesis of Δ^6-Ambroxene **57** proved to be more difficult and laborious. Several approaches were investigated, and ultimately the one via ring closure of allylic alcohol **61** proved to give the best results (Scheme 10). Three reaction sequences were investigated for the conversion of larixol into diketone **29**[10d] and the best one was to first oxidise the side chain in larixol with $KMnO_4$ to methyl ketone **16** (see Scheme 4), followed by oxidation of the hydroxyl group at C_6 and isomerisation of the double bond. In this way diketone **29** was obtained in 56% overall yield. However, in our hands the Bayer-Villiger reaction of **29** to **60** could be accomplished only in a moderate 30% yield, although several attempts were undertaken to improve this reaction[20]. The carbonyl and acetate group in **60** could be reduced in moderate yield in one operation using $LiAlH_4$. The cyclisation of the obtained diol could be achieved after acidification of the reaction mixture to give the Δ^6-ambroxene **57** with the correct configuration at C_8, and this result encouraged us to look for a better synthesis of the intermediate diol **61**.



Scheme 10

The oxidative breakdown of the side chain to an ethylene hydroxy moiety can be accomplished in several ways as was described in schemes 7 and 8. Selective protection of the primary hydroxyl group in the side chain of triol **36**, followed by oxidation of the secondary hydroxyl group at C_6 and elimination of the tertiary hydroxyl group at C_8, gave the enone **63** in 67% overall yield[21] (scheme 11).



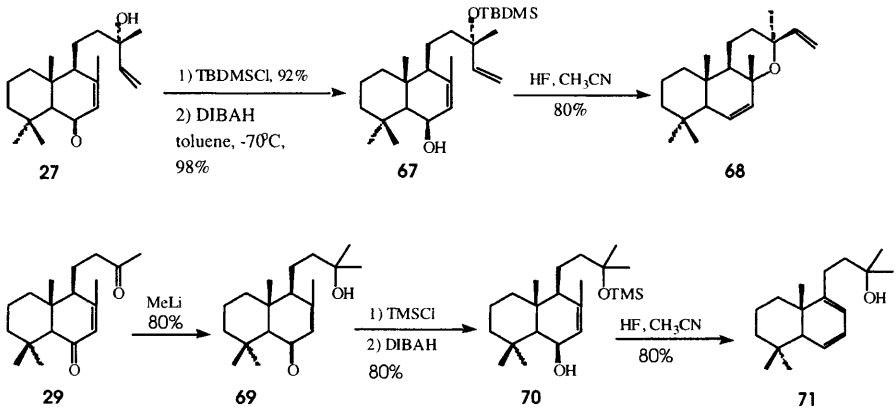
Scheme 11

A selective synthesis of Δ^6-Ambroxene **57** from enone **63** now proved to be possible in three simple steps. Reduction of the carbonyl group at C_6 with DIBAH in toluene at low temperature, deprotection of the hydroxyl group in the side chain of **64** and acid catalysed cyclisation afforded Δ^6-Ambroxene **57** in high yield. The first two reactions could also be

carried out in the reversed sequence, and the second two could be carried out in one pot. Oxidation of triol **36** with PCC led in one step to 6-oxo sclareolide **66**. Deprotection of the hydroxyl group in **63** led to cyclisation to 6-oxo Ambrox® **53**[22](see Scheme 11).

The approach described in Scheme 11 seemed suitable for the *selective* synthesis of other Δ^6 Ambrox®-like compounds as well[9e]. The allylic hydroxyl group at C_6 can be abstracted easily and the resulting mesomeric carbocation can be intercepted by the hydroxyl group in the side chain. The enones **27** and **29**, obtainable in a few reaction steps from larixol, are good intermediates to try out the possibilities of this approach for the synthesis of several C_{13} substituted Δ^6-tricyclic tetrahydropyranyl ethers (Δ^6-Ambra-oxides) (Schemes 12-14).

The enone **27** could be synthesised easily from larixol by oxidation of the hydroxyl group at C_6, followed by base catalysed isomerisation of the exocyclic double bond to the conjugate endocyclic position[9c] (Scheme 12). Reduction of the carbonyl group at C_6 often requires protection of the hydroxyl group in the side chain, this was the case in the reduction of **27**. On the other hand, the acid catalysed deprotection of the hydroxyl group in the side chain often proceeded along with cyclisation as was demonstrated in the synthesis of compound **68**. Deprotection of the highly hindered TBDMS group in **67** with TBAF could not be accomplished.

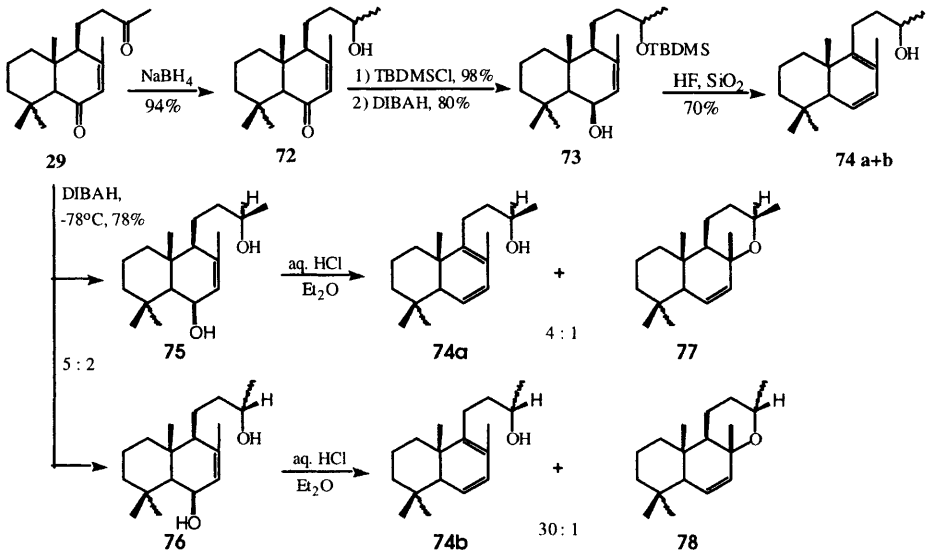


Scheme 12

Oxidation of the side chain in **27**, gave diketone **29** (see also Scheme 10) in which the non-conjugated carbonyl group in the side chain could be manipulated selectively. Addition of methyl lithium to **29**, protection of the hydroxyl group in the side chain, and reduction of the carbonyl group at C_6 afforded the allylic alcohol **70**. However, when this compound was subjected to the combined deprotection cyclisation reaction, the diene-alcohol **71** was isolated as the only product and no cyclisation was observed.

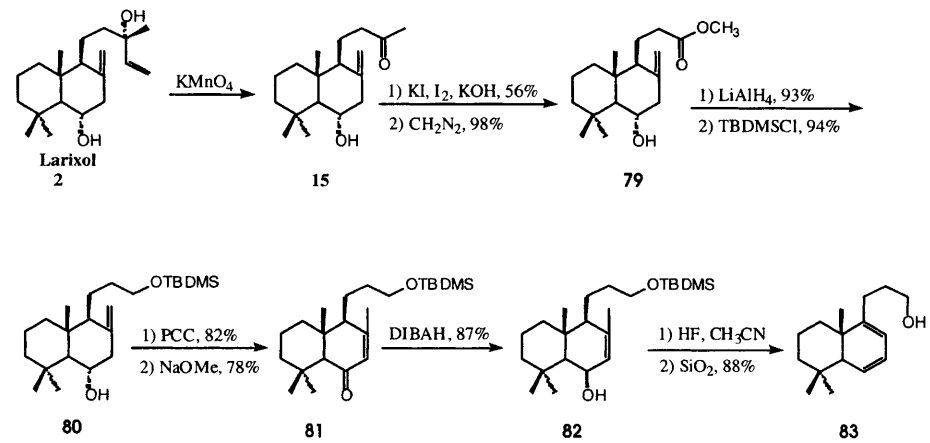
Similar behaviour was observed with the C13-monomethyl analogues (Scheme 13). Selective reduction of the carbonyl group in the side chain of **29** gave an unseparable mixture of the C13- mono methyl compounds **72**. A similar reaction sequence consisting of protection of the side chain, reduction of the C6-carbonyl group, and deprotection provided again a mixture of C_{13}-mono-methyl diene-alcohols **74a** and **74b**. The reduction of both carbonyl groups in diketone **29** could be carried out simultaneously using DIBAH, and now the intermediate diols **75** and **76** could be separated by chromatography. Aqueous acid treatment of the separate isomers did give some of the cyclised product **77** and **78**

respectively, but the elimination reaction was the predominant one[23]. Attempts to cyclise the dienes were unsuccessful.



Scheme 13

Finally, the cyclisation of the C_{13} unsubstituted analogue was investigated (Scheme 14). For its preparation, the side chain of larixol had to be shortened to methyl ketone **15**, followed by an iodoform reaction to yield the acid **79**. Reduction of the corresponding ester and selective protection of the primary hydroxyl group in the side chain afforded **80**.



Scheme 14

Allylic alcohol **82** could be prepared in the usual way by oxidation of the hydroxyl group at C_6, isomerisation of the double bond, and reduction of the enone **81**. Deprotection and acid catalysed cyclisation of **82** again resulted in just dehydration to the diene-alcohol **83** with no cyclised product being observed[23].

So it turned out that the successful cyclisation of the Δ^7, 6β-hydroxyl compound **61** to a five membered cyclic ether, e.g. Δ^6-Ambroxene **57**, was not matched by an equally successful cyclisation of the corresponding homologues to the six membered cyclic ethers, e.g. the Δ^6-Ambra-oxides. In all but one of the latter cases, elimination of the 6β-hydroxyl group to the corresponding Δ^6,Δ^8-dienes was observed as the major reaction.

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THE SYNTHESIS OF FRAGRANT CYCLOPENTANONE SYSTEMS

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1 INTRODUCTION

Cyclopentanones have long been recognised as having attractive olfactory properties and we have been particularly interested in developing new synthetic routes to these molecules and related analogues. A class of interesting 5-membered ring compounds includes calythrone **1** (Figure 1), a naturally occurring cyclopentene-1,3-dione β-triketone[1,2] with a floral odour, reminiscent of *cis*-jasmone. The structurally related compound 2-butyl-4,5-dimethylcyclopent-4-ene-1,3-dione **2** was first identified[3] during the synthesis of bovilide[4]. Whilst **2** was noted to possess a buttery jasmine odour, the olfactory properties of related analogues was unknown.

The methyl jasmonates[5], the (±) *trans*-isomer **3** and (±) *epi*-isomer **4** shown, are key well known natural products used widely in the formulation of many perfumes. Methyl epijasmonate **4** possesses superior olfactory properties compared to *trans*-methyl jasmonate **3**, and the *epi*-isomers have also been shown to have greater biological activities.[6,7] The unnatural analogue methyl dihydrojasmonate **5** is a constituent of famous fragrances[8] and magnolia ketone **6** has also been reported to possess fragrant properties.[9]

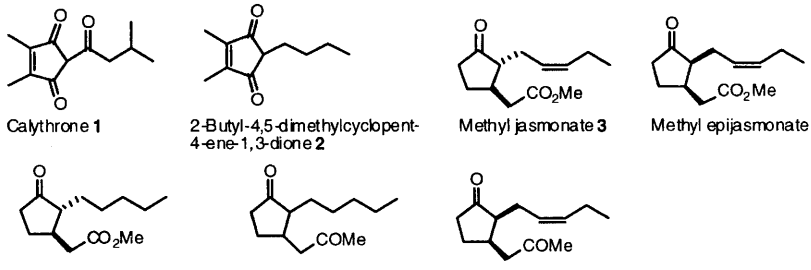


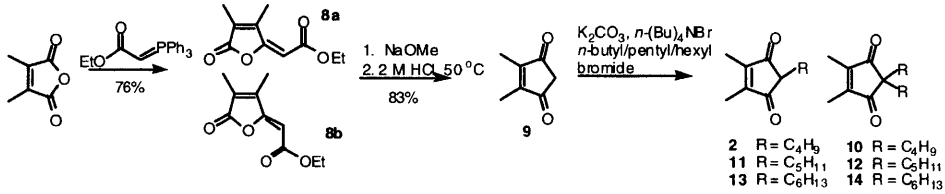
Figure 1 *Structures 1-7*

With our interests in the fragrance properties of new cyclopentanones and with a desire to investigate new synthetic routes to these compounds we focussed our attention on the synthesis of **2** and analogues, methyl epijasmonate **4** and the magnolia ketone analogue **7**, and finally investigations into short syntheses of both enantiomers of *trans*-methyl jasmonate **3** and *trans*-methyl dihydrojasmonate **5**. The results from these investigations are described below.

2 RESULTS AND DISCUSSION

2.1 2-Alkylated Cyclopentene 1,3-Diones[10]

During the synthesis of bovilide, which is found in green tea leaves,[11] 2-butyl-4,5-dimethylcyclopent-4-ene-1,3-dione **2** was identified as a side product[3]. Investigations into the synthesis of **2** and analogues were performed for potential use in perfumery applications. Two strategies were used and are outlined below: firstly the synthesis of **2** and related analogues *via* a 2-ylidenebutenolactone to identify the compounds with the strongest olfactory properties; secondly, the synthesis of **2** using a Pauson-Khand and selective oxidative strategy.



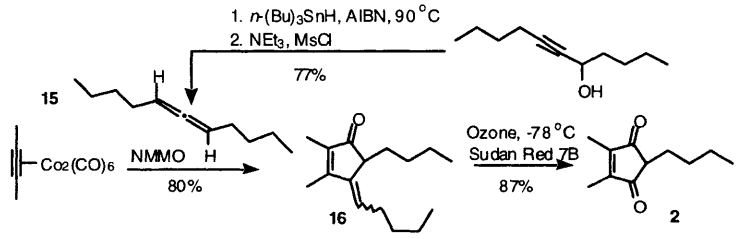
Scheme 1 *Synthesis of 2-alkylated cyclopentene 1,3-diones*

The synthetic route shown in Scheme 1 was initially explored, due to previous reports on the rearrangement of ylidenebutenolactones[2,12] and our aim of generating analogues *via* the key intermediate 4,5-dimethylcyclopent-4-ene-1,3-dione **9**. The reaction between 2,3-dimethylmaleic anhydride and ethoxycarbonylmethylenetriphenylphosphorane in toluene gave the butenolactones **8a** and **8b** in 76% yield, and an isomeric ratio of *E*:*Z* of 2:3.[13,14] The geometric mixture of isomers was not problematical due to the rearrangement in the next step. Accordingly, the mixture of **8a** and **8b** was treated with sodium in methanol and then dilute HCl to effect the ring opening and closure, followed by hydrolysis of the ester and decarboxylation to generate 4,5-dimethylcyclopent-4-ene-1,3-dione **9**, in an overall yield of 83%.[15] We found that the use of methoxide, rather than ethoxide, led to enhanced yields in this system.

Problems are often encountered in the selective *C*-alkylation of 1,3-dicarbonyl compounds due to competing self condensation and *O*-alkylation reactions. We initially wished to access quantities of analogues of **2**, both mono- and dialkylated materials, for fragrance analysis purposes. Several alkylation procedures were explored including the use of alumina impregnated with sodium ethoxide,[16] however, this led to the formation of self condensation products only. The use of a biphasic procedure using tetrabutylammonium bromide as a phase transfer catalyst[17] proved more promising and using bromobutane led to the formation of **2** and **10** in 57% and 11% yields respectively. Attempts to improve the yield of the monoalkylated compound in particular were not

successful, however it enabled access to these materials. Therefore, using the same procedure with pentyl- and hexylbromide the corresponding analogues (**11-14**) were generated. Analysis revealed that compound **11** possessed a much weaker jasmine odour than **2**, and that **13** was odourless. By comparison, the dialkylated compounds, **10**, **12**, **14** had weaker buttery odours. Thus 2-butyl-4,5-dimethylcyclopent-4-ene-1,3-dione **2** still possessed the strongest odour characteristics, and therefore a novel alternative route to **1** was sought to avoid the non-selective low yielding alkylation procedure.

The Pauson-Khand reaction is a powerful synthetic method to construct five membered rings using allenic compounds together with alkyne cobalt complexes.[18,19] This would enable the formation of the five membered ring, however, oxidative cleavage would be required to reveal **2**. The route explored is outlined below in Scheme 2.



Scheme 2 *Use of the Pauson-Khand to generate 2-butyl-4,5-dimethylcyclopent-4-ene-1,3-dione 2*

Dicobalthexacarbonylbut-2-yne complex was prepared from but-2-yne.[19,20] Undec-6-yn-5-ol[21,22] was prepared in 87% yield from the reaction between the anion of 1-hexyne and *n*-valeraldehyde at low temperature. This was stored under nitrogen to avoid aerial oxidation to the corresponding ketone, undec-6-yn-5-one, which possessed a strong pineapple odour. Undec-6-yn-5-ol was then heated with tributyltin hydride and AIBN to give the hydrostannylation product which was directly converted into the mesylate, and underwent elimination to form 5,6-undecadiene **15**.[22,23] The Pauson-Khand reaction was then performed using *N*-methylmorpholine *N*-oxide monohydrate and 4-pentylidene-5-butyl-2,3-dimethylcyclopent-2-enone **16** was readily formed in 80% yield.[19]

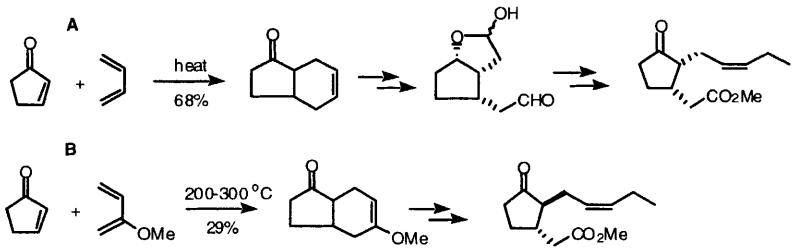
The selective oxidation of the trisubstituted exocyclic alkene was initially attempted using catalytic osmium tetraoxide/sodium periodate oxidation,[24,25] however, despite exploring a range of conditions low yields were achieved. The use of an ozonolysis procedure together with an azo-dye (Sudan Red 7B[26]) to indicate completion of the trisubstituted alkene bond cleavage successfully generated **2** in an isolated yield of 87%. Overall, using one synthetic route we were able to assess the olfactory properties of several analogues and have prepared the most interesting compound using an alternative synthesis.

2.2 Methyl Epijasmonate 4 and *Cis*-3-(2-Oxopropyl)-2-(pent-2*Z*-enyl)-cyclopentan-1-one 7[27]

Methyl epijasmonate **4**, in particular the (+)-enantiomer is known to have numerous biological functions.[7] Despite the ready epimerisation of epijasmonate to the thermodynamically more stable *trans*-isomer **3**, with its much greater odour characteristics it still has potential for use in fragrance applications for example *via* a pro-fragrance strategy. Furthermore, we were interested in the fragrance properties of analogues of

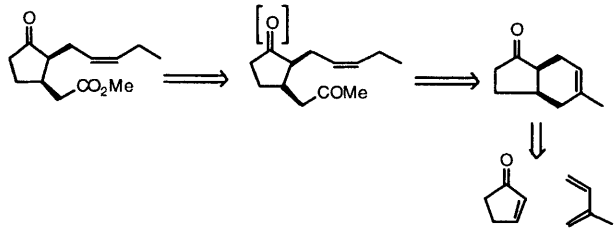
magnolia ketone[9,28]. We therefore focussed on the development of an efficient synthetic route, to (±)-methyl epijasmonate **4** and (±)-*cis*-3-(2-oxopropyl)-2-(pent-2Z-enyl)-cyclopentan-1-one **7** (the unsaturated, epi-analogue of magnolia ketone), that could also be adapted to potentially generate enantioselective samples.

One of the most efficient methods of establishing the *cis*-stereochemistry on the five membered ring is *via* a Diels-Alder reaction. Previous routes have been reported to the jasmonates using cyclopentenone and a Diels-Alder strategy including Torii's[29] (**A**, Scheme 3) who used butadiene and subsequently generated a hemiacetal to establish the stereochemistries. A patent also describes the synthesis of methyl *trans*-jasmonate using 2-alkoxybutadiene[30] (**B**, Scheme 3). The key cycloaddition steps were not high yielding because regio-isomers were generated together with isomeric products.[29,31,32,33]



Scheme 3 *Previous routes to the jasmonates using a cycloaddition reaction*

The difficulties that can be encountered with such a Diels-Alder reaction using butadienes have been investigated with the reaction proceeding to give a mixture of cycloadducts including double bond shifted isomerised products.[31] Other synthetic approaches reported to epijasmonate include a free radical approach to establish the *cis*-stereochemistry by Knochel[34], and Montforts'[35] and Bestmann's[36] enantioselective syntheses using chiral synthons with subsequent removal of the chiral directing group. We wished to develop a general route for the construction of *cis*-substituted cyclopentanones, but using readily available starting materials (see scheme 4).

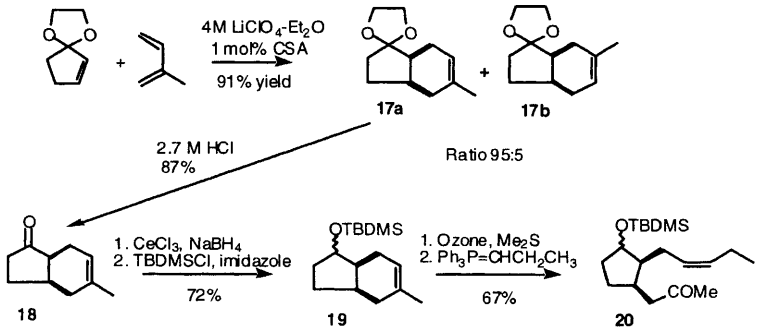


Scheme 4 *Strategy to synthesise epijasmonate using isoprene*

The use of isoprene as a diene was ideal due to its low cost, and compared to 2-alkoxybutadienes is less prone to polymerisation under Lewis acid conditions. Furthermore, such a strategy would allow the direct generation of **6**. The reaction between cyclopentenone and isoprene was initially explored using aluminium chloride as the Lewis acid catalyst, however, the best reaction regioselectivity we observed was 5:1, *para*-isomer: *meta*-isomer in 49% yield (literature, ratio 10:1, 76% yield).[33]

An alternative, more selective procedure was required, and we noted that Grieco has reported acid catalysed ionic Diels-Alder reactions in high yields and regioselectivities

using the ethylene ketals of cyclohexenones as dieneophiles, in 4.0-5.0 M solutions of lithium perchlorate together with camphor sulfonic acid (CSA).[37] Accordingly, the ethylene ketal of 2-cyclopenten-1-one[38] was reacted with isoprene under these conditions and readily gave the required adduct **17** in excellent yields (see Scheme 5). The regioselectivity was 95:5 for **17a**:**17b** (*para*-isomer: *meta* isomer) by ^{1}H NMR. Fortunately, the minor regioisomer was readily removed using column chromatography to give **17a** in 85% isolated yield. We noted that this reaction has also been reported but in lower yields by Vankar *et al*, who has performed the reaction with lithium perchlorate in nitromethane (73% yield) or using Nafion-H as an acid catalyst (68% yield).[39]



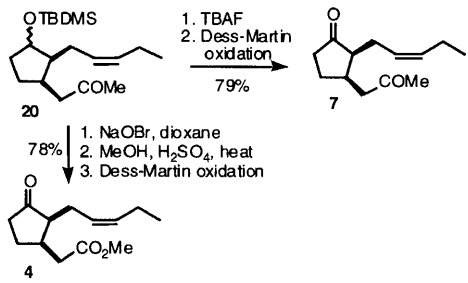
Scheme 5 *Synthesis of the key intermediate 20*

The direct ozonolysis of **17a** to cleave the double bond resulted in the formation of a complex mixture of products. It was evident that the ketal was also being cleaved during the ozonolysis, so it was necessary to deprotect the ketal and reduce and protect the resulting ketone to ensure that no epimerization would take place during the remaining synthetic steps. Deprotection of the ketal was carried out using dilute hydrochloric acid (2.7 M) at room temperature to give **18**. We noted that if this reaction was performed at higher temperatures migration of the double bond occurred yielding a mixture of isomers. The reduction of the ketone was carried out using several reducing agents, however, our preferred method was using cerium (III) chloride heptahydrate[40] with sodium borohydride which generated the diastereoisomeric alcohols in 91% yield and a ratio of 10:1 by ^{1}H NMR. Since one diastereoisomer was formed in much greater quantities than the other it eased characterisation of the compounds in subsequent steps because at no stage was it possible to readily separate the diastereoisomers. Indeed, it was not necessary since both diastereoisomers would provide the required *epi*-compound after a late stage oxidation.

After protection of the alcohols in 81% yield, the silyl ether **19** was then reacted with ozone treatment followed by a reductive work-up (dimethyl sulfide) to afford the methyl ketone intermediate. The Wittig coupling was carried out using the unstabilised ylid (supernatant solution), generated *in situ* from *n*-propyl phosphonium bromide and sodium hexamethyldisilylazide, to which was added the freshly prepared aldehyde. The use of such 'salt free' conditions is known to give rise to predominantly Z-alkenes, and the key intermediate **20** was formed in 67% over two steps.[41]

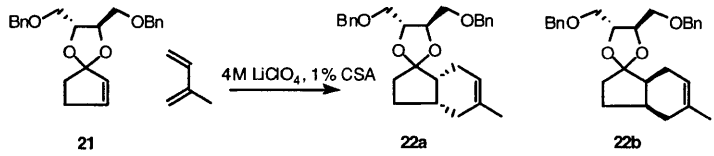
The conversion of **20** into *cis*-3-(2-oxopropyl)-2-(pent-2Z-enyl)-cyclopentan-1-one **6** was readily achieved (see Scheme 6). The deprotection of **20** with TBAF, and oxidation of the resulting alcohol under mild conditions utilising freshly prepared Dess-Martin periodinane[42] gave **7** in 79% yield over 2 steps. The material possessed a sweet floral odour.

Alternatively the methyl ketone **20** was converted to the corresponding acid using a sodium hypobromite in a dioxane/water mixture for the haloform reaction. This simultaneously led to the removal of the silyl protecting group in 89% overall yield. Methylation of the acid afforded the methyl cucurbate diastereoisomers in 92% yield and finally, oxidation of the cucurbate was carried out, again using Dess-Martin periodinane, to readily generate methyl epijasmonate **4** in a yield of 78% over the 3 steps.



Scheme 6 *Synthesis of methyl epijasmonate **4** and cis-3-(2-oxopropyl)-2-(pent-2Z-enyl)-cyclopentan-1-one **7***

This methodology was able to generate methyl epijasmonate and cis-3-(2-Oxopropyl)-2-(pent-2Z-enyl)-cyclopentan-1-one in overall yields of 29%, and we were interested to establish whether the route could be adapted to generate enantioenriched samples. Whilst it is envisaged that the ionic Diels-Alder goes *via* an oxygen allyl cation which is stabilised by the lithium ion, it was not known whether a homochiral ketal or the allyl cation generated would have any facial directing effects on the approaching diene. Accordingly, the dibenzyl ketal **21** was prepared from cyclopentenone and (2*S*,3*S*)-1-benzyloxy-4-(2,4-cyclohexadienylmethoxy)butane-2,3-diol.[43,44] When this was reacted with isoprene in the presence of lithium perchlorate and 1% CSA unfortunately the two diastereoisomers **22a** and **22b** were formed in equal quantities.[43] However, diastereoselectivites have been reported when performing ionic Diels-Alder reactions with chiral acetals under alternative conditions, for example using $TiCl_2(i\text{PrO})_2$.[45] Although we did not investigate this further it is possible that this approach could lead to the selective formation of products.



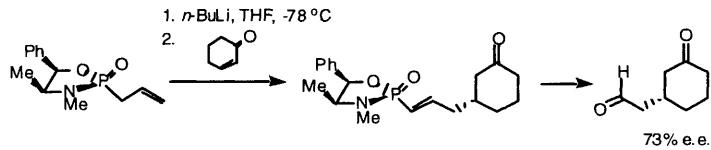
Scheme 7 *Homochiral ketal approach*

2.3 Enantioselective Syntheses of Methyl Dihydrojasmonates 5 and Methyl Jasmonates 3[46]

Methyl *trans*-jasmonate and methyl *trans*-dihydrojasmonate are widely used in the formulation of many perfumes.[8] There is keen interest in the enantioselective syntheses of these compounds and several syntheses have been reported. For example, (-)-**3a** and (+)-

3b have been prepared using an enantiopure precursor and Posner's Pummerer rearrangement strategy[47] and the dihydrojasmonates (-)-**5a** and (+)-**5b** using a solid-liquid asymmetric phase transfer catalysis where a range of e.e.s was obtained depending on the phase transfer catalyst and reaction conditions utilised.[48] However, many of the syntheses reported are multistep and therefore overall low yielding.

With the intense interest in this class of compounds there is a continuing desire to develop versatile, short, selective syntheses. We were interested in developing a strategy, which involved the conjugate addition of chiral phosphonamide anions to α,β-unsaturated carbonyl compounds with subsequent removal of the chiral template. Additions using chiral phosphonamide anions have been reported by, for example, Haynes,[49] Hanessian who used C_2-symmetrical phosphonamides,[50] Hua[51] and Denmark who used 1,3,2-oxazaphosphorane 2-oxides[52]. Hua *et al* has reported the addition of extended lithium anions of chiral phosphonamides to several cyclic α,β-unsaturated carbonyl compounds such as 2-cyclopentenone.[51] The products generated were subsequently converted into β-substituted acids and aldehydes with enantioselectivities of 28-98% e.e. The selectivities observed depended on the enone, the phosphonamide used and *N*-alkyl group.[51] As shown in Scheme 8 the use of phosphonamides derived from (1*R*,2*S*)-ephedrine led to the formation of products in approximately 70% d.e. However, more variable selectivities were observed for those prepared from (1*R*,2*R*)-norpseudoephedrine with an *N*-^{i}propyl group (22-88% d.e.).[51]



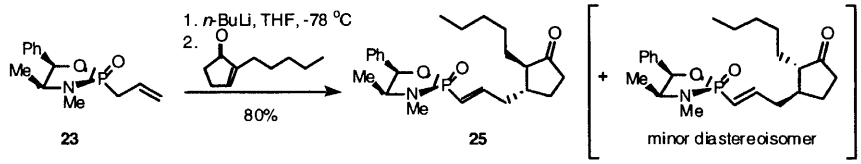
Scheme 8 *Hua's use of chiral phosphonamides to generate a β-substituted aldehyde*[51]

The introduction of an alkyl group at the α-position on the enone system is likely to enhance facial stereodifferentiation, with chelation of the lithium ion with the cyclopentenone on the O=P-O face of the phosphonamide (due to the *N*-alkyl group present). This would result in the formation of adducts in overall higher diastereoselectivities than reported by Hua and would provide rapid access to the jasmonates.

The chiral phosphonamides, (2*R*,4*S*,5*R*)-3,4-dimethyl-5-phenyl-2-(2-propenyl)-1,3,2-oxazaphospholidin-2-one **23**, and (2*S*,4*S*,5*R*)-3,4-dimethyl-5-phenyl-2-(2-propenyl)-1,3,2-oxazaphospholidin-2-on **24**, were used since Hua had reported similar selectivities with both diastereoisomers which we hoped to achieve. These were readily prepared from 2-propene-1-phosphonyl dichloride and (1*R*,2*S*)-ephedrine in a 1:1 ratio and were readily separated by flash column chromatography as a solid and an oil.[53] Our initial studies focused on the use of the enone, 2-pentyl-2-cyclopenten-1-one.

Deprotonation of the phosphorous template **23** and addition of 2-pentyl-2-cyclopenten-1-one led to the formation of **25** as the major addition product together with a minor diastereoisomer in 80% yield (Table 1). The use of template **24** similarly led to the formation of **26** and a minor diastereoisomer in 86% yield. The separation of the diastereoisomeric products was investigated but not successful.

After the highly selective reaction with 2-pentyl-2-cyclopenten-1-one the reaction was then performed using 2-(2-pentynyl)-2-cyclopenten-1-one[54] which generated **27** and **28** together with minor diastereoisomers in 76% and 83% yield, respectively.



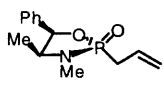
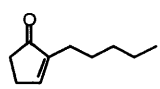
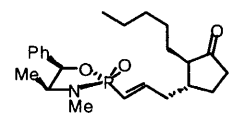
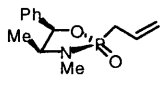
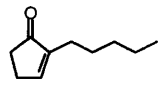
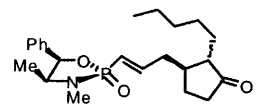
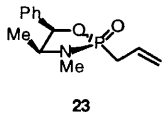
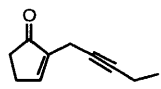
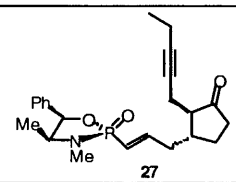
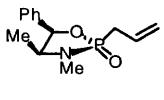
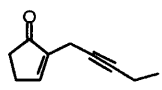
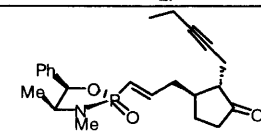
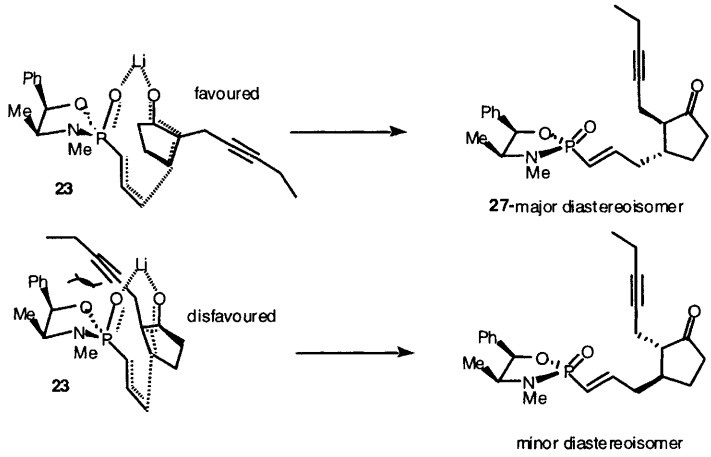
Phosphonamide	Cyclopentenone	Major Diastereoisomer	Major: minor diastereoisomeric ratio	Yield
 **23**		 **25**	95:5	80%
 **24**		 **26**	93:7	86%
 **23**		 **27**	94:6	76%
 **24**		 **28**	92:8	83%

Table 1 *The use of templates **23** and **24** and selectivities observed in additions to α-substituted cyclopentenones*

The high reaction selectivities observed using both cyclopentenones, compared to those reported by Hua who observed approximately 70% d.e.s, can be rationalised by considering the higher facial stereodifferentiation obtained due to the presence of an alkyl chain at the α-position (Figure 2). Thus, as shown the favoured reaction involves addition onto the *Si* face of the α-substituted cyclopentenone and the presence of the alkyl group at the α-position enhances the selectivity due to unfavourable steric interactions encountered on additions to the *Re* face.

Efficient removal of the template was critical to the success of this synthetic strategy, and initial experiments with **25** and **26** explored the use of both oxidative conditions (hydrogen peroxide) and reductive conditions (dimethyl sulfide). The later generated 3-

oxo-2-pentanylcyclopentyl ethanal in 62% yield whilst the use of hydrogen peroxide gave a mixture of inseparable compounds. A stepwise oxidation of the aldehyde with subsequent methyl ester formation was carried out generating (+)-**5b** in only 20% yield

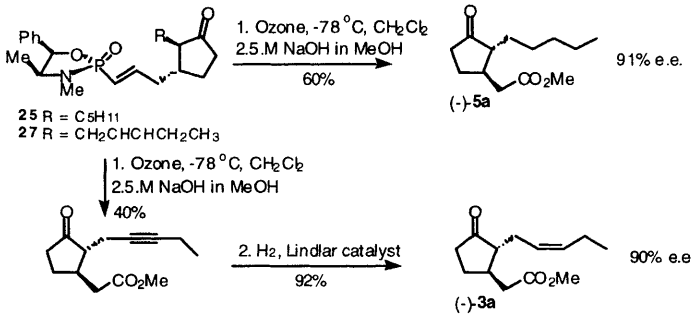


from **26**.

Figure 2 *The use of template 23 and 24 facial stereodifferentiations*

A more direct procedure was required, and the ozonolysis was then carried out in a 2.5 M solution of sodium hydroxide in methanol.[55] This directly afforded (-)-methyl dihydrojasmonate (-)-**5a** in 60% yield from **25** (Scheme 9) and (+)-**5b** in 57% yield from **26** in 91% e.e. and 85% e.e. respectively.

Due to the presence of the alkyne moiety in **27** and **28** care had to be taken during the ozonolysis to avoid oxidation of the alkyne. Whilst again ozonolyses under reductive conditions was considered, the corresponding aldehyde was generated in 39% yield. Therefore, the reaction was carried out as before but was closely followed by tlc analysis to indicate maximal product formation and the reaction was rapidly quenched. The corresponding methyl esters were then isolated in 39-40% yield and subsequent Lindlar reduction afforded (-)-**3a** and (+)-**3b** in 92% yield and 90% e.e. and 84% e.e., respectively.



Scheme 9: *Removal of phosphonamide templates*

In summary, we have used the 1,4-addition of chiral 2-propenylphosphonamide anions to α-substituted cyclopentenones in high diastereoselectivities to selectively generate (-) and (+)-methyl dihydrojasmonate (**5a** and **5b**) and (-) and (+)-methyl jasmonate (**3a** and **3b**) *via* a short synthetic route. Such a synthetic strategy could be used to readily synthesise further jasmonate analogues.

3 CONCLUSION

The synthesis of compounds **2**, **3a**, **3b**, **4**, **5a** and **5b** and **7** have been described. The methodology developed has enabled the synthesis of new compounds for fragrance analysis as well as investigating new synthetic routes to important extensively used fragrance compounds. The synthetic routes described can also be used in the preparation of further analogues and have several advantages over previously reported methods.

Acknowledgements

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DESIGNING DAMASCONE- AND IONONE-LIKE ODORANTS

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1 INTRODUCTION

" *Put on your red dress*
and slip on your high heels
and some of that sweet perfume
it sure smells good on you ..."
J. Gill, 'My, My, My' [1]

When it comes to smelling '*sweet*' or '*powdery*', ionones are the perfumery materials of choice. Discovered as early as 1893 by Tiemann and Krüger,[2] the ionones became central to perfumery and fragrance chemistry, and thus inspired the synthesis of numerous analoga and derivatives. In a broader sense, β-damascenone and other damascones, Iso E Super®, Koavone®, Timberol® and also Georgywood® can be considered ionone analoga, too. Figure 1 places these odorants in a common timeline.[3]

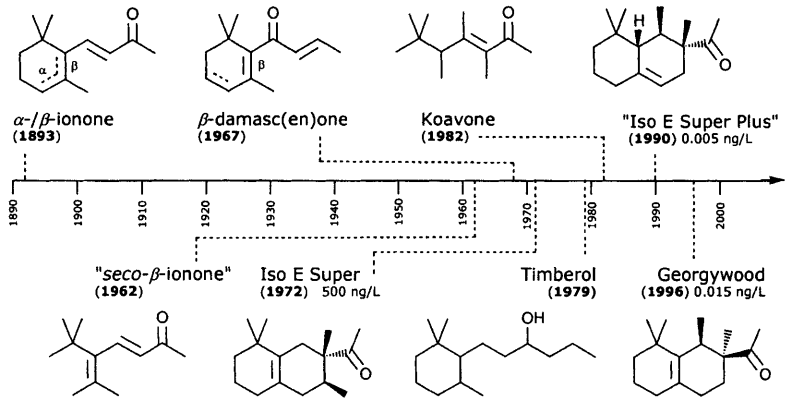

Figure 1 *The evolution of ionones, damascones and their analoga and derivatives*

The ionone analoga in Fig. 1 cover a broad range of odour notes from floral (*β*-damascenone) *via* animalic (Timberol®) to woody characteristics (Georgywood®). In the syntheses of these odorants, ionones served as structural or chemical templates, and thus principles of rational design are well illustrated in this family.

2 *SECO*-IONONES

Acyclic compounds of the pseudoionone series were long known to possess no distinct odour characteristics. Therefore, Jitkow and Bogert[4] first varied the substituents on the cyclohexenyl ring; and they stated in 1941 that the typical violet odour of *β*-ionone does critically depend on a trimethyl-substituted cyclohexenyl ring. Of these three methyl groups, two have to be adjacent to the 3-oxo-1-butenyl chain, so in position 1 and 5 according to the trivial numbering in Fig. 2. Accordingly, two bulky hydrophobic groups flanking the 3-oxo-1-butenyl substituent seemed necessary. But then again, was a cyclohexenyl ring really crucial?

In 1962, Sestanj[5] imaginarily cut out ring atom C-2 and C-3 of *β*-ionone, and proposed 5-isopropyl-6-methyl-3,5-heptadien-2-one as an interesting target molecule that could retain the odour characteristics of the parent *β*-ionone. He synthesised this 1(2),3(4)-*diseco*-*β*-ionone starting from 3-isopropyl-4-methyl-1-pentyn-3-ol, and indeed found the compound to possess an odour similar to that of *β*-ionone.[5] The physical data were also reminiscent to those of *β*-ionone; however, as one could expect the boiling point of 104–108°C/10 mm was significantly lower: The derived *seco*-ionone was more diffusive, while close in odour to its parent compound.

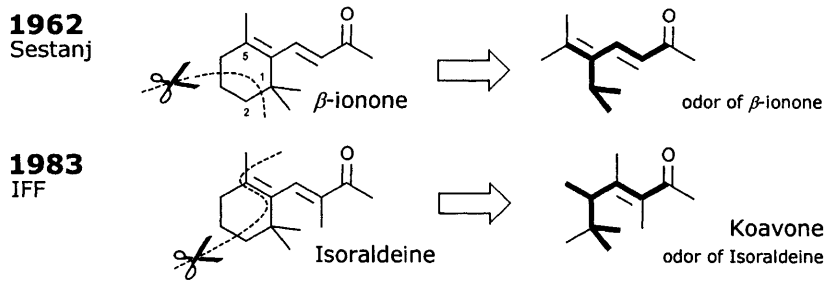

Figure 2 *The seco-concept in the design of ionones*

The idea of Sestanj was taken up in the early 1980s by chemists of IFF, who chose Isoraldeine® (8-methylionone) as parent structure. Employing ethyl methyl ketone instead of acetone in the classical ionone synthesis, 8-methylionone had also been discovered by Tiemann in 1893.[6] It is more intense than the genuine ionones, possesses a very fine odour reminiscent of violets with a slightly woody-vetiver tonality, and became therefore the most popular violet odorant in perfumery.

Dissecting the bonds between C-2(C-3) and C-4(C-5), transposing the C-5 methyl group to C-7, and hydrogenating the endocyclic double bond, leads from Isoraldeine® to Koavone®, which was still quite close in odour to Isoraldeine® and 10-methylionone. This odour similarity was also rationalized by a superposition analysis on computer;[7] one of the first applications of molecular modelling in the flavour and fragrance industry.

3 *CYCLO*-IONONES

Omitting '*unnecessary*' atoms, while retaining all structural features that code a given odour note, furnishes more volatile and more flexible molecules. These can povide more diffusivity and impact in a perfume. Often however, more substantivity is desired, and this on the contrary requires higher molecular weight, besides lower thresholds that make up for the lower vapour pressure.

Introducing additional rings in a molecule is an interesting strategy to devise more substantive odorants. In addition to increasing the molecular weight, a ring system can rigidify the molecule; thus allowing better insight into the receptor geometry. This insight can then inspire further structure modifications, and thereby guide the synthesis of more potent odorants.

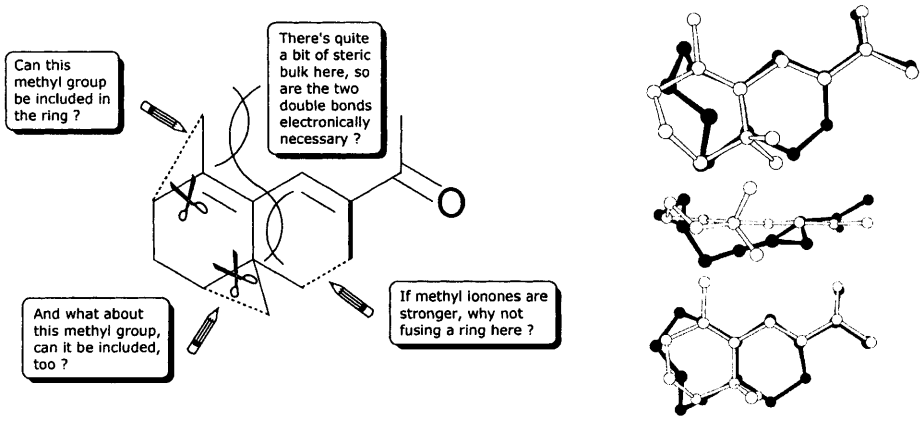

Figure 3 *Designing cyclo-ionones at the desktop and on the computer*

Fig. 3 presents the initial thoughts and molecular-modeling calculations that preceded the synthesis of 1-{bicyclo[6.4.0]dodec-1(8)-en-10-yl}ethan-1-one.[8] The steric bulk of the *gem*-dimethyl and the 8-methyl group of Isoraldeine® was mimicked by fusing a six-membered ring. The remaining 5-methyl group was then found to be best superimposed by a cyclooctene ring. This simplified the synthetic access by Diels–Alder reaction of but-3-en-2-one with bis(methylene)cyclooctane, speculating that the double-bonds were more of conformational than electronical importance. Indeed, the designed target compound showed the typical fruity-woody, violet odour of β-ionone.[8]

1-{Bicyclo[6.4.0]dodec-1(8)-en-10-yl}ethan-1-one was, however, not only interesting for giving an idea about the molecular dimensions of the ionone receptor, but also as a structural link to the woody family, *e.g.* Iso E Super®. An interesting new reaction[8] led regio- and distareoselectively to a potent woody-ambery odorant, reminiscent of Georgywood® and Iso E Super® (Fig. 4). In the presence of Wilkinson's catalyst, 4-methylenespiro[2.7]decane reacted with 3-methylbut-3-en-2-one to provide (*r*-9,*c*-10)-1-{9,10-dimethylbicyclo[6.4.0]dodec-1(8)-en-10-yl}ethan-1-one, which possesses an intense woody-ambery odour with an excellent threshold of 0.01 ng/L air. By introduction of just two methyl groups one can switch the odour note from typical violet-like to woody-ambery. Further derivatives[8] provided additional insight into the structure–odour correlation of these two odour notes.

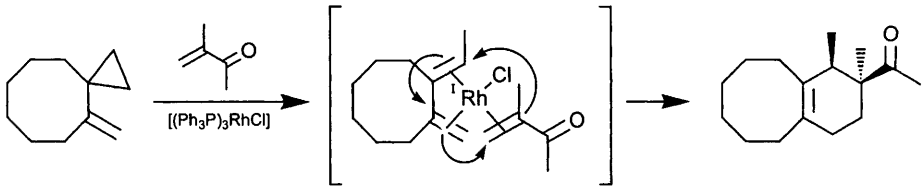

Figure 4 *Synthesis of (r-9,c-10)-1-{9,10-dimethylbicyclo[6.4.0]dodec-1(8)-en-10-yl}ethan-1-one — a powerful odorant reminiscent of Iso E Super®*

4 ISO E SUPER® AND GEORGYWOOD®

Iso E Super®, as a better quality of Isocyclemone E® was called later by IFF, was for instance used in 18% in »Trésor« (Lancôme, 1990), in 25% in »Fahrenheit« (Dior, 1988), in 35% in »Déclaration« (Cartier, 1998), and even in 43% in »Feminité du Bois« (Shiseido, 1992).[9] It is synthesized by Diels–Alder reaction of myrcene with 3-methylpent-3-en-2-one and subsequent acid-catalyzed cyclization as outlined in Fig. 5.

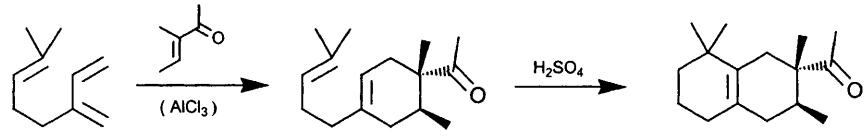

Figure 5 *Industrial synthesis of Iso E Super®*

However, GC–olfactometry at Givaudan showed that not the main product of Fig. 5, but a *ca.* 5% constituent determines the characteristic woody-ambery odour of the commercial material.[9] While the theshold of the main compound of Iso E Super® is about 500 ng/L, this minor constituent possesses an odour threshold of only 0.005 ng/L; and thus, became known as "Iso E Super Plus" at Givaudan internally.

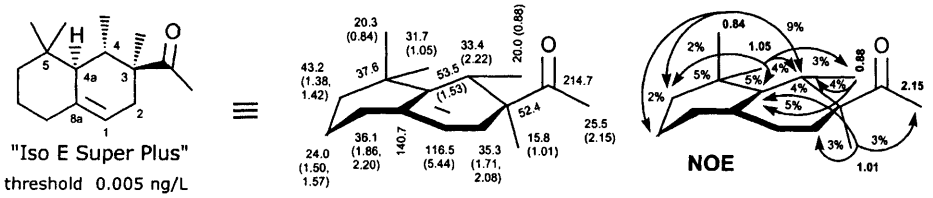

Figure 6 *Structure elucidation of the powerful impurity of Iso E Super®*

By reaction of commercial Iso E Super® with peracetic acid and repeated column chromatography, this powerful minor constituent could be isolated,[9] and INADEQUATE as well as NOEDIFF experiments led to the assignment of the structure depicted in Fig. 6. This structure was proved by a directed synthesis starting from α-ionone.[9] Its formation in the

industrial synthesis of Iso E Super® can be rationalised by acid-catalysed rearrangement of the endocyclic double bond during the cyclisation step.

However, the structure of the powerful minor constituent turned out to be too difficult to be produced on an industrial scale; and therefore, numerous derivatives were synthesised, aiming at an easier access. Georgywood® (Fig. 7) turned out to be the best of these derivatives. Its industrial synthesis by Diels–Alder reaction of homomyrcene with methyl isopropenyl ketone is straightforward, and it was introduced into perfumery in 1996.[10] »Golden Moments« (P. Presley, 1999) with 5% of Georgywood is a recent example for its use in perfumery.[10]

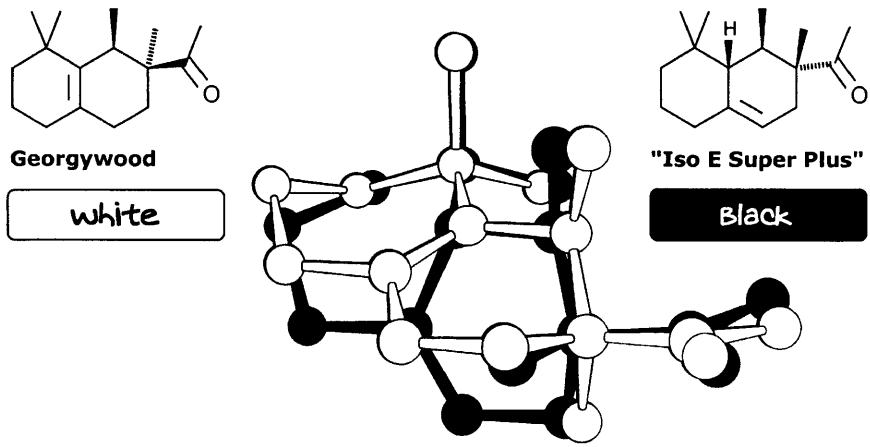

Figure 7 *Superimposing Georgywood® on the lead structure "Iso E Super Plus"*

In order to arrive at the same spatial orientation of the substituents, the configuration of the acetyl-bearing carbon of Georgywood® is inverted. As is depicted in Fig. 7, Georgywood® superimposes much better on "Iso E Super Plus" than one would expect bearing their two-dimensional structural formulae in mind.

Quite recently, Erman and coworkers at Millenium were able to increase the content of "Iso E Super Plus" by employing phosphoric acid in methanol.[11] It is, however, still a long way to the production of commercial "Iso E Super Plus" at affordable price, and Georgywood® constitutes a benchmark difficult to beat.

5 TIMBEROL®

A diastereomeric mixture of tetrahydroionols only possesses a very weak and uncharacteristic odour. But when the side chain is elongated by just two carbon atoms, a surprisingly powerful powdery, woody-floral odorant of animalic, steroid-type tonality results, which has been successfully introduced into perfumery as Timberol® by Dragoco.

Though actually Timberol® has not much in common with Ambrox® in terms of odour, the motivation for its synthesis may have very well been the design of a 9(10),12(13)-*diseco*-8-desmethyl Ambrox®, as sketched in a report by Brunke, Rojahn and Warnecke.[12] However, the 9-methyl-Timberol, which structurally resembles Ambrox® even closer, is by far weaker than Timberol®, and smells only floral-sweet and fatty; so has

nothing in common with the odour of Ambrox®. In addition, the C5-stereochemistry of the most active stereoisomer of Timberol®, which recently has been introduced into perfumery as Dexnorlimbanol by Firmenich, does not match the configuration of Ambrox®

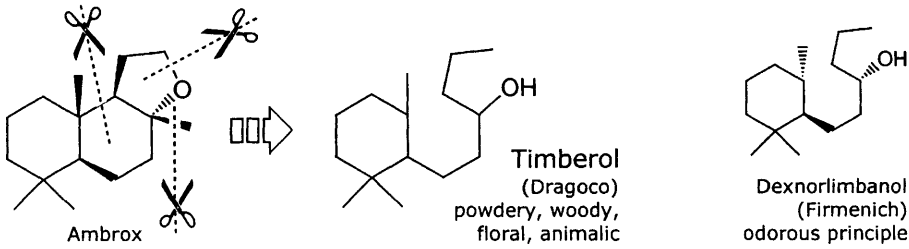

Figure 8 *A possible idea behind the synthesis of Timberol®, the design of a 9(10),12(13)-*diseco*-8-desmethyl Ambrox®?*

But even though in terms of odour and stereochemistry this *seco*-design was not successful, it may have helped to find the way to a new powerful woody-floral, animalic odorant, which –with respect to the weak and uncharacteristic tetrahydroionol– probably no one would have expected.

6 THE DAMASCONE FAMILY

Like with the ionones, the first structure modifications in the damascone family were systematic permutations and modifications of the cyclohexenyl substituents.[13] And as it is summarised in Fig. 9 quite a few of these analoga found their way into perfumery.

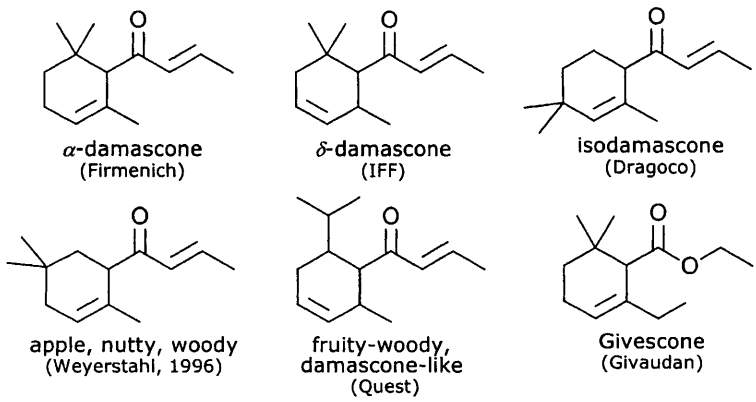

Figure 9 *The damascone family so far*

However, all these analoga possess rather different odour notes, and α- and β-damascone as well as β-damascenone remained benchmarks difficult to beat. The importance of α-damascone is also documented in the elaboration of a stereoselective

synthesis of (*S*)-(–)-α-damascone, which could be carried out on industrial scale.[14] That this elegant process, which utilises (1*R*,2*S*)-(–)-*N*-isopropylephedrine as a chiral auxiliary in an enantioselective protonation, still has not been employed technically, might be due to the fact that both antipodes smell relatively similar. The (*R*)-(+)-isomer is certainly more apple-like, and also possesses an unpleasant cork-stopper nuance, but both enantiomers share the same main odour characteristics, and their thresholds differ '*just*' by a factor of 70.

It should therefore still be possible to find more powerful damascone odorants, for which one would expect a higher chiral discrimination on the receptor. If a chiral odorant is '*perfectly*' complementary to the chiral proteinogenic receptor site, its enantiomer should be odorless, or weaker by at least a factor of 1000.

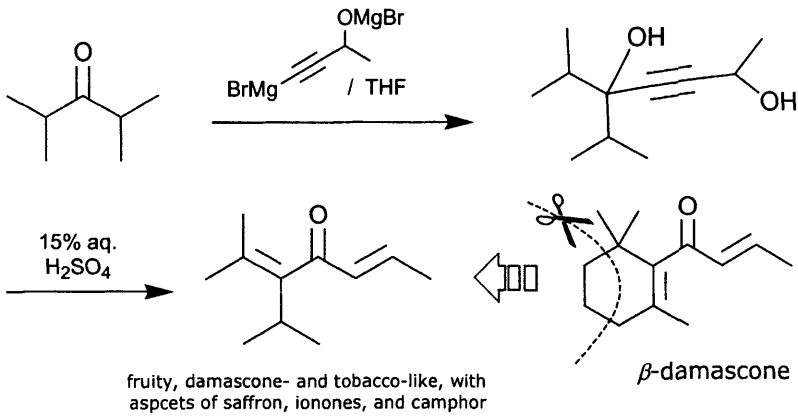

Figure 10 *The* seco-*damascone patented by Takasago*

In 1978, even before the introduction of Koavone (Fig. 2), 2-methyl-3-isopropylhepta-2,5-dien-4-one, a 2(3),4(5)-*diseco*-β-damascone, had already been described in a patent of Takasago.[15] Grignard reaction of diisopropyl ketone with μ-(3-butyn-2-olato)bis(magnesium bromide) furnished 5-isopropyl-6-methylhept-3-yne-2,5-diol, which was transformed into the target structure by means of a Rupe rearrangement (Fig. 10).[15] This *seco*-damascone possesses fruity, damascone- and tobacco-like odour characteristics, which are indeed quite close to the parent β-damascone. However, 2-methyl-3-isopropylhepta-2,5-dien-4-one was never introduced into perfumery.

7 SUBSTITUTED OCT-2-EN-4-ONES

We found this interesting *seco*-damascone by GC–olfactometry as a *ca.* 1.1% trace constituent of a very complex, crude reaction product. At first, no reasonable structure could be proposed for this trace constituent on the basis of the chemistry involved or the GC/MS data.[16] Thus, a *ca.* 370 μg sample was isolated by preparative GC, but HMBC and NOESY NMR spectra did not allow the unambiguous assignment of the structure. While there was no doubt about a hepta-2,5-dien-4-one skeleton with one tetrasubstituted double bond bearing a methyl and an isopropyl group, the relative positions of the latter remained

unclear. Besides 2-methyl-3-isopropylhepta-2,5-dien-4-one, 5,6,7-trimethylocta-2,5-dien-4-one was therefore also a possible structure for this trace constituent. When we synthesised both compounds, it turned out that the trace constituent of the reaction mixture was indeed the 2-methyl-3-isopropylhepta-2,5-dien-4-one that had been patented by Takasago (Fig. 10). Yet, when we finally had also the 5,6,7-trimethylocta-2,5-dien-4-one in hands, we found that this also possessed a damascone odour, and in fact was much stronger and by far superior to that of the trace constituent.

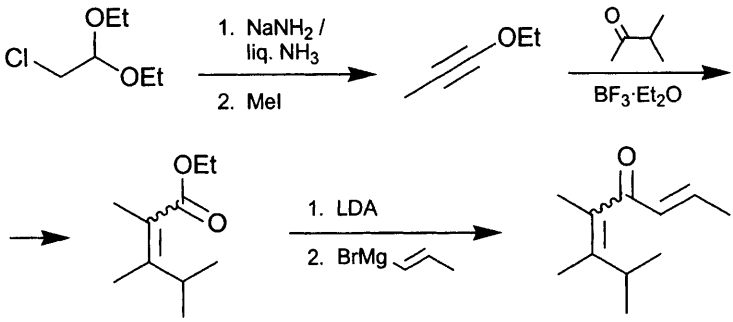

Figure 11 *Synthesis of (2E,5E/Z)-5,6,7-trimethylocta-2,5-dien-4-one*

Figure 11 details our synthesis of 5,6,7-trimethylocta-2,5-dien-4-one, which commenced with the preparation of 1-ethoxypropene from 2-chloro-1,1-diethoxyethane. After treatment with sodium amide in liquid ammonia, the resulting sodium acetylide was alkylated with iodomethane. Addition of 1-ethoxypropene to methyl isopropyl ketone in the presence of boron trifluoride diethyl etherate furnished in 59% yield the ethyl 2,3,4-trimethylpent-2-enoate. This was converted in the next step to the target compound by a Grignard reaction with prop-1-en-1-ylmagnesium bromide and *in-situ* trapping of the resulting dienone as lithium enolate.[16] (2*E*,5*E*/*Z*)-5,6,7-Trimethylocta-2,5-dien-4-one possesses a very powerful and diffusive, fruity-rosy, *β*-damascone-like odour, reminiscent of apples, plums, raisins, and other dried fruits. Contrary to what one would expect with the previous *seco*-structures in mind, these interesting odour characteristics are however mainly due to the (2*E*,5*Z*)-configurated *cis*-isomer, while the (2*E*,5*E*)-configurated *trans*-isomer is almost not detectable for some people.

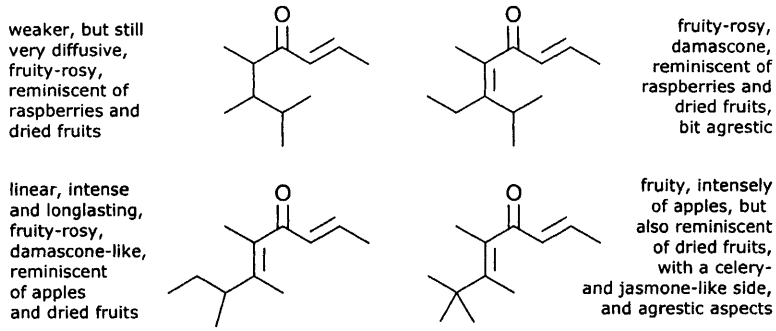

Figure 12 *Some derivatives with odour descriptions*

Therefore, 5,6,7-trimethylocta-2,5-dien-4-one can not be considered a *seco*-damascone, anymore, and it thus was very exciting to explore the structure–odour correlation of related oct-2-en-4-ones. In Figure 12, four analoga are shown, together with their respective odour descriptions. Even the partially hydrogenated derivative still possesses a fruity-rosy odour, reminiscent of raspberries and dried fruits. In the dienone analogs, the strength of the (5*E*)-isomers increases when the C6-substituent becomes more bulky, and finally the (5*E*)-isomers become even stronger than the (5*Z*)-isomers.

However, (2*E*,5*Z*)-5,6,7-trimethylocta-2,5-dien-4-one, discovered by serendipity, still constitutes the most powerful and most pleasant damascone-like odorant of this series. As with the powerful impurity in Iso E Super®, serendipity therefore continues to play an essential part in fragrance chemistry.

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CREATION OF FLAVOURS AND THE SYNTHESIS OF RAW MATERIALS INSPIRED BY NATURE'

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1 INTRODUCTION

1.1 The concept of Generessence

History – The flavour industry was born in the 1800s with the availability of herb and spice extracts, manufacture of simple chemicals and the distillation of simple essential oils. A flavour at this time would contain a mix of an extract (violet leaves), essential oils (coriander) and simple chemicals (vanillin).

In the earlier part of the 1900s developments in the chemical industry meant that new flavour chemicals were becoming available. At this time very little was known about the chemical composition of flavours in nature so flavour chemicals were used simply because they happened to smell of a particular food product.

In the mid 1900s flavour companies targeted useful chemicals in terms of odour and made every possible variation. Aldehyde C16 is a good example of a component isolated from this type of research. Also at this time the first serious attempts were made to analyse the chemical composition of food. Chemists relied on bucket chemistry and identification of just one chemical was a major undertaking. One important chemical discovered in this way was raspberry ketone, the structure of which was initially kept a closely guarded secret. The invention of GC/MS opened up the possibility of detailed analysis of the flavour of natural food. However attempts to recreate flavours from these first analyses was a failure due to the lack of fine detail although this work did give rise to the discovery of many novel materials such as hexenols and pyrazines. Flavours became recognisable and the modern flavour industry was born.

Traditional flavours are based around key components with some occasional addition of new compounds found in analyses. These flavours do not however represent the true compositions of the original food, so as a result may be unbalanced and inflexible. For example, they will work well in the application for which they were designed, but may not be automatically used in another without modification.

Generessence® flavours- a new approach

The concept of Generessence® is to re-create as exactly as possible the flavour as nature intended.

Only component volatile compounds found in the target food are allowed in the flavour formulation. This enforced discipline forces the flavourist to be truly creative and not rely on traditional flavour ingredients.

Once the qualitative part is correct the quantitative part follows and the result is a truly natural flavour character which is not distorted by dosage, is water and / or oil soluble and can be applied to all applications.

2 INSTRUMENTATION

2.1 GC

2.1.1 Non-polar. The extracts were analysed on a HP6890 GC with split/splitless injection and a Flame Ionisation Detector (FID). 1µL of extract was injected onto a SGE BPX-5 chromatographic column (50m x 0.22 mm ID x 0.25µm film thickness) in the split mode. The injection temperature was 220°C. The GC oven was run using temperature programming, with an initial temperature of 40°C held for 0.5 minutes ramped at 3°C/min to a final temperature of 290°C held for 5 minutes. The detector temperature was 320°C.

2.1.2 Polar. The extracts were also injected onto a HP 6890 GC with split/splitless injection and flame ionisation detector (FID) fitted with a SGE BP21 chromatographic column (50m x 0.22 mm ID x 0.25µ film thickness) using the same injection and detection techniques. The GC oven was run using temperature programming with an initial temperature of 40°C held for 0.5 minutes ramped at 3°C/min to a final temperature of 240°C and held for 20 minutes.
All data was collected and stored on the Labsystems Atlas data system.

2.2 Mass Spectroscopy

For analysis on both BPX-5 phase and BP 21, the sample was introduced via a Hewlett Packard 5890 GC into a Micromass Prospec magnetic sector mass spectrometer. GC oven conditions were the same as the conditions outlined above. The mass spectrometer was operated in EI mode scanning from m/z 500 to m/z 33 @ 1 second per scan. Spectra were analysed on the OPUS data system using the Bush Boake Allen in-house library and the commercial Wiley6 and NIST libraries.

2.3 Infra Red

Run as a neat liquid between sodium chloride plates on a Perkin-Elmer System 2000 FTIR spectrophotometer.

2.4 NMR

Run in deuterochloroform solution on a JEOL GSX-270 FTNMR spectrometer with deuterium lock and 5mm $^{1}H/\ ^{13}C$ dual probe. The ^{1}H spectrum was referenced against TMS as internal reference and the ^{13}C spectrum was referenced against the central peak of the deuterochloroform triplet as 77.0ppm. Chemical shifts are quoted in ppm. (δ).

2.5 Headspace

Headspace was collected onto tubes containing 160mg Tenax TA resin.
Thermally desorbed at 220°C for 5 min in a Perkin Elmer ATD 400.
ATD internal cold trap at -30°C, trap fired at 250°C for 0.8 min and sample passed to GC column via a fused silica transfer line.

2.6 Sulphur detector

Sievers 350B SCD. Probe fitted to HP5890 FID. Sample split 1:1 between SCD and FID.

3 COMPONENT IDENTIFICATION

Quantified components were identified by comparison of their mass spectra and linear retention time indices[1] with authentic reference compounds. Authentic reference compounds were synthesised as required and confirmed by spectral methods (^{1}H-NMR, ^{13}C NMR, IR, MS).

4 SCOPE OF DISCUSSION

A total of 193 Generessence® analytical projects have been undertaken. For the purpose of this discussion and by way of example, we shall outline just a few based on three target samples, beef, mango and hazelnut.

5 SAMPLE PREPARATION

5.1 Mango

Pulp and skin of organoleptically selected mangos (434g) were homogenised and extracted with dichloromethane for five hours using several small soxhlet extractors. The combined extracts were initially concentrated down on a rotary evaporator. High boiling and non-volatile matter was removed using a bulb to bulb distillation apparatus, collecting the volatiles in a dry-ice cooled collection bulb. The distillate was further concentrated by blowing down with a stream of nitrogen gas.

5.2 Grilled beef

5.2.1 Headspace. Slices of lean sirloin beef were cooked under an electric grill for approximately 3 minutes each side until medium-well done. The hot meat was immediately placed inside a specially designed glass apparatus with integral condenser, preventing moisture from entering the Tenax traps and allowing meat flavoured condensation to be collected for future extraction. Headspace was collected on each slice for 15 minutes after which the sample was replaced with a freshly grilled slice. This process was repeated to give a total sampling time of 4 hours.

5.2.2 Steam distillate. Slices of the grilled sirloin beef were cooked as described above. The meet was minced and steam distilled in a Nickerson and Likens apparatus[2] for 4 hours with dichloromethane as solvent. The extract was concentrated using an air condenser.

5.2.3 Liquid/ liquid extract of condensation. Condensation collected during the headspace sampling was found to have a strong meaty odour. As such this was extracted into dichloromethane and analysed by GC-MS.

5.3 BBQ beef

5.3.1 Headspace. Half-inch thick slices of lean sirloin beef were barbecued on a charcoal garden barbecue until medium–well done. Each freshly cooked steak was placed in the collection apparatus described above. The headspace was collected for a total of 5 hours on successive steaks, each steak being replaced after 20 minutes to maintain the "freshly cooked" aroma.

5.3.2 Steam distillate. Slices of the BBQ sirloin beef were cooked as described above. The meat was minced and steam distilled in a Nickerson and Likens apparatus[2] for 4 hours with dichloromethane as solvent. The extract was concentrated using an air condenser.

5.3.3 Liquid/liquid extract of condensation. As with the Grilled beef the condensation collected during headspace sampling was extracted into dichloromethane and analysed by GC-MS.

5.4 Beef and onion

Cubed beef and diced onions were cooked together to identify the composition of the flavour of the combined materials. The analyses included a headspace sample collected during the final minutes of cooking and a sample obtained by simultaneous distillation extraction of the cooked mixture.

Cubes of beef were browned on all sides and mixed with lightly sautéed onions, covered with water and simmered (covered) for approximately three hours. Headspace samples of the cooking vapours were collected onto Tenax through a small condensate trap to control moisture. At the end of the cooking time the mixture was steam distilled into pentane-ether using a Nickerson and Likens apparatus[2]. The extract was concentrated by air condenser.

Additionally, this analysis provided an opportunity to characterise novel flavour components generated from the precursor ingredients of the two food substrates.

5.5 Hazelnuts

The hazelnuts used for this work were chosen from a wide selection available by a panel of flavourists.

5.5.1 Untoasted hazelnuts (steam distillate). The nuts were shelled, chopped and steam distilled into dichloromethane for 2 hour using a Nickerson and Likens apparatus[2]. The resulting extract was concentrated using an air condenser and analysed by GC, GC-MS and GC fitted with a sulphur detector.

5.5.2 Untoasted hazelnuts (headspace). Freshly shelled hazelnuts were roughly chopped, placed in a shallow dish and enclosed within a Rilsan bag. Headspace was collected for 3.5 hours.

5.5.3 Toasted hazelnuts. Headspace and steam distillation were prepared as above after toasting the hazelnuts in an oven for 8 minutes at 240°C

6 HIGHLIGHTS OF THE VOLATILE COMPONENTS IDENTIFIED

6.1 Mango

A total of 262 components were identified and quantified, 7 of which were a direct result of the sulphur analysis. The analysis contained many terpene hydrocarbons, a notable observation was ocimene being the major component in the Alphonso mango whilst terpinolene was the major component in the Philippine mango. In both cases the level of the other terpene hydrocarbon was negligible. All analyses contained a wide range of straight and branched chain saturated aliphatic ether esters, whilst cis-3-hexenyl esters, 3-hydroxy ethyl esters, methyl ketones, (E)-2-unsaturated aldehydes and lactones were also observed.

Comparison of the trace obtained by normal detection and using the sulphur detector is shown in figures 1 and 2.

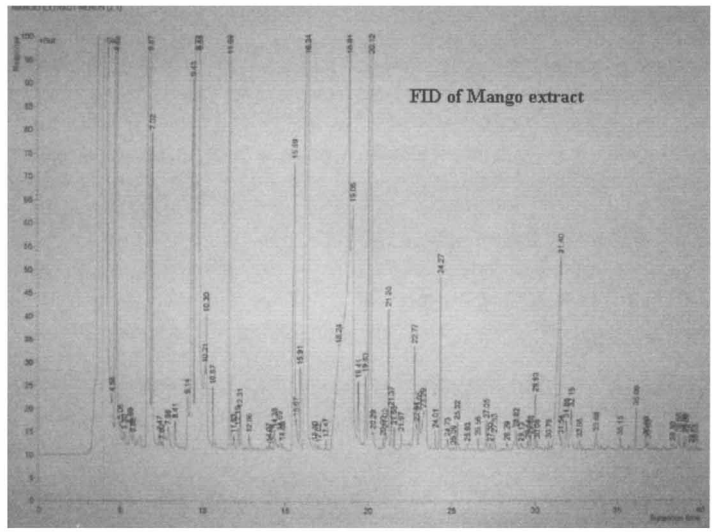


Figure 1

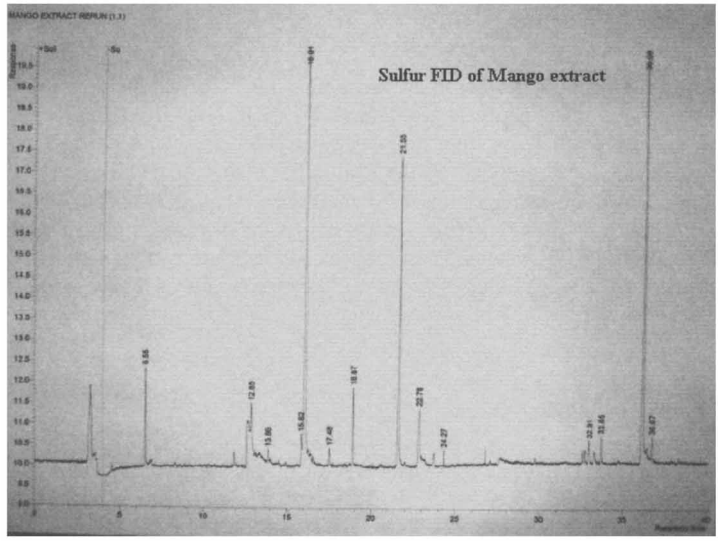


Figure 2

Component	CAS	Recent reports in nature	Retention index[3]
(E)-2-(Z)-6-nonadienal	557-48-2	Chung et al[4]	765
acetic acid	64-19-7		187
butyric acid	107-92-6		397
(Z)-3-hexenol	928-96-1		457
(Z)-ocimene	3338-55-4		636
(E)-ocimene	3779-61-1		650
ethyl acetate	141-78-6		200
ethyl butyrate	105-54-4		400
2,5-dimethyl-4-oxo-4,5-dihydrofuran-3-yl butyrate	114099-96-6	New identification	1024
4-acetoxy-2,5-dimethyl-2,3-dihydrofuran-3-one	4166-20-5		851
4-methoxy-2,5-dimethyl-3(2H)-furanone	4077-47-8		669
terpinolene	586-62-9	Stojanova et al, Menon et al, Verzera et al[5]	693
methyl thioacetate	1534-08-3		294
2-(methylthio) ethanol	5271-38-5	Karagiannis[6]	450
methyl thiobutyrate	2432-51-1	Song et al[7]	495
methyl (methylthio) acetate	16630-66-3	Weenen[8]	517
ethyl (methylthio) acetate	4455-13-4	Werkoff et al[9]	593
ethyl 3-mercaptobutyrate	156472-94-5	New identification[10]	642

Table 1 *A selection of significant or interesting components in mango*

6.2 Beef (combination of the analyses of BBQ beef, grilled beef and beef and onion.)

A total of 528 components were identified and quantified in the 8 analyses, including 220 components that were sulphur and /or nitrogen containing.

A wide range of alkyl pyrazines was identified, along with a variety of 2-acetyl 5 and 6 ring-hetercycles. Sulphur components included trithianes, dithiolanes, 2-methyltetrahydro furan-3-thiol, alpha mercapto ketones, alkyl and allyl sulphides, disulphides and higher sulphides, and particularly of interest furfuryl propyl disulphide[11].

Among oxygen containing moieties a wide range of gamma and delta–lactones, branched long chain saturated chain aldehydes, (Z)-2-aldehydes, C5 to C12 (E)-2-aldehydes, C7 –C11 dienals and 2,3-diketones were identified. Aliphatic aldehydes C3 -C18 with C14 - C18 aldehydes constituting a large proportion of the volatile components. A number of acids were also noted.

Component	CAS	Recent reports in nature	Retention index*
Cis,cis-2,4,6-triethyl-1,3,5-trithiane	53897-58-8	New identification	1253
2,5-dimethyl-3-ethylpyrazine	13360-65-1	Choi et al[12]	693
2,5-dimethylpyrazine	123-32-0		519
2-acetyl-2-thiazoline	29926-41-8	Zehentbauer et al and Ong et al[13]	729
2-acetylpyrrole	1072-83-9	Mahatheeranont et al[14]	683
2-acetylthiazole	24295-03-2	Chung et al[4] and Naf et al[15]	636
2-ethyl-4-methyl-1,3-dithiolane[16]	17564-27-1	New identification	757
2-methyltetrahydrofuran-3-one	3188-00-9	Chung et al[4]	414
2-methyltetrahydrofuran-3-thiol[16]			516 563
3-mercaptobutan-2-one	40789-98-8		424
3-mercaptopentan-2-one	67633-97-0		511
4-acetyl-gamma-butyrolactone	29393-32-6	Naf et al[17]	756
bovolide	774-64-1	Naf et al[15]	1143
decan-3-one	928-80-3		795
diallyl disulphide	2179-57-9		697
dimethyl disulphide	624-92-0		341
dimethyl trisulphide	3658-80-8		583
dipropyl disulphide	629-19-6		723
dipropyl trisulphide	6028-61-1		950
furfuryl propyl disulphide[10]	252736-36-0	New identification	1008
methional	3268-49-3		522
methyl mercaptan	74-93-1		25
methylpyrazine	109-08-0		428
Thialdine[16]	638-17-5	Chung[18]	831
(E)-2-(E)-4-decadienal	25152-84-5	Naf et al[15]	936
trimethylpyrazine	14667-55-1		609

Table 2 *A selection of significant or interesting components in Beef*

6.3 Hazelnut

A total of 336 components were identified and quantified. Unsurprisingly, it was observed that pyrazines, pyridines and pyrroles were identified in the toasted hazelnuts. A range of aliphatic alcohols and aldehydes, furans, 2,4-dienals, (E)-2-aldehydes, methyl substituted C-6 and C-7 saturated and unsaturated ketones, as well as methyl alkyl ketones were found in both cooked and uncooked hazelnuts.

Component	CAS	Recent reports in nature	Retention index	Toasted	Un-toasted
Pyrazines					
2,3-dimethylpyrazine	5910-89-4		523	√	√
2,5-dimethylpyrazine	123-32-0		519		√
trimethylpyrazine	14667-55-1		609		√
2-ethyl-3-methylpyrazine	15707-23-0		610		√
5-methyl-6,7-dihydrocyclopentapyrazine	23747-48-0		757		√
Saturated ketones					
2-methylhexan-3-one[20]	7379-12-6	Froehlich and Schreier[19]	434	√	√
3-methylheptan-4-one[20]	15726-15-5	Ross et al[21]	530	√	√
5-methylhexan-2-one	110-12-3	Valeroet al[22]	451		√
5-methylheptan-3-one	541-85-5	Baser et al[23]	542	√	
Unsaturated ketones					
2-methyl-(Z)-4-hexen-3-one	50396-99-1	New Identification	438		√
2-methyl-(E)-4-hexen-3-one[20]	50396-90-2	New Identification	484		√
5-hepten-2-one	6714-00-7	Kayser[24]	494	√	√
5-methyl-1-hepten-4-one	81925-80-6	New Identification	523	√	
5-methyl-(E)-3-hepten-2-one	5090-16-4	El-Saharty[25]	595	√	√
6-methyl-(E)-3-hepten-2-one	20859-10-3	New Identification	600		√
5-methyl-(Z)-2-hepten-4-one	103070-07-1		530	√	√
5-methyl-(E)-2-hepten-4-one	102322-83-8	Blanch[26]	578	√	√
5-methyl-(E)-3-hexen-2-one	5166-53-0	Shiota[27]	500	√	√
3-penten-2-one	625-33-2	Chung et al[4]	333	√	√
Aldehydes					
2,4-decadienal	2363-88-4	Song et al[28]	936	√	√
hexanal	66-25-1	Valero[20]	403	√	√
3-methylbutanal	590-86-3	Valero[20]	241	√	√
Sulphur containing					
2-(methylthio) acetaldehyde	23328-62-3	Werkhoff et al[29]	368	√	√
4-(methylthio) butan-2-one	34047-39-7	Werkhoff et al[26]	604		√
4-(methylthio) pentan-2-one	143764-28-7	Werkhoff et al[26]	633		√
Others					
2-acetyl pyridine	1122-62-9	Chung et al[4]	649		√
methyl pyrrole-2-carboxylate	1193-62-0				
octan-2,3-dione	585-25-1	Chevance[30]	589	√	√

Table 3 *A selection of significant or interesting components in Hazelnut*

6.4 Sensory evaluation

Where practicable components were identified as target compounds for synthesis by GC odour.[31] Evaluation of synthesised components was undertaken globally by an expert panel of flavourists. Evaluations were performed in water, salt water or sugar water at a concentration consistent with the threshold of the material. Results were presented on a hedonic scale, with evaluation in application undertaken on selected candidates.

7 SYNTHESIS OF REFERENCE COMPONENTS

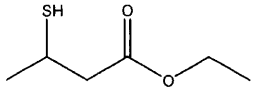
Authentic reference components were synthesised by established synthetic procedures: 2,5-Dimethyl-4-oxo-4,5-dihydrofuran-3-yl butyrate, 3-mercaptopropyl propionate, 2-mercaptopropyl propionate, ethyl 4-mercaptobutyrate, ethyl 3-mercaptobutyrate, 2,4,6-triethyl-1,3,5-trithiane, 2-ethyl-4-methyl-1,3-dithiolane, octan-2,3-dione, 4-(methylthio) butan-2-one, 4-(methylthio) pentan-2-one, 4-methyl-3-hepten-2-one, 5-methyl-3-hepten-2-one, 6-methyl-(E)-3-hepten-2-one, 3-methylheptan-4-one, 5-hepten-2-one, 2-methyl-(Z)-4-hexen-3-one, 2-methyl-(E)-4-hexen-3-one, 3-mercaptopropyl acetate, 3-hydroxypropyl thioacetate, 1,2,4-trithiolane, thialdine, ethyl 2-methyl-2-pentenoate, 3-methylheptan-4-one, 2-methylhexan-3-one, 4-acetyl-gamma-butyrolactone, bovolide, furfuryl propyl disulphide, cis-1-pentenyl formate, 3-(allylthio) propanal, 12-methyltridecanal. Components were isolated by distillation, chromatography or recrystallisation and verified by spectral analysis.

7.1 Compounds identified and synthesised from analysis of the volatile components in mango[32]

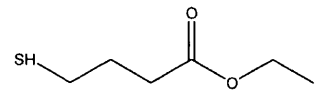
2,5-Dimethyl-4-oxo-4,5-dihydrofuran-3-yl butyrate was identified for the first time in nature and confirmed by comparison with an authentic standard. This material has been found to be useful in a range of fruity flavours in addition to mango, imparting a more 'natural' or less 'candy' note to the creation.

Key sulphur component:
The sulphur trace showed a component in the same place as a 'mango' tropical odour was observed by a GC-odour experiment. This component MW 148 was therefore identified as a synthetic target.

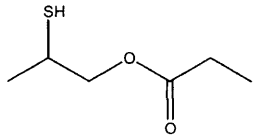
From the analytical data collated from the Mango Generessence® analysis, retention time, odour, GS-MS, GC-IR it was not possible to explicitly define a synthetic target. Therefore four structures 1-4 were proposed (figure 3).



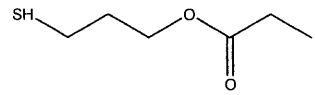
Ethyl 3-mercaptobutyrate **1**



Ethyl 4-mercaptobutyrate **2**



2-Mercaptopropyl propionate **3**



3-Mercaptopropyl) propionate **4**

Figure 3

Two of these structures possess the 1,3-oxygen, sulphur odourophore[33] so commonly observed in compounds with a tropical odour or natural occurrence. Thus these were the preferred candidates, although all four were synthesised to validate the identification beyond dispute.

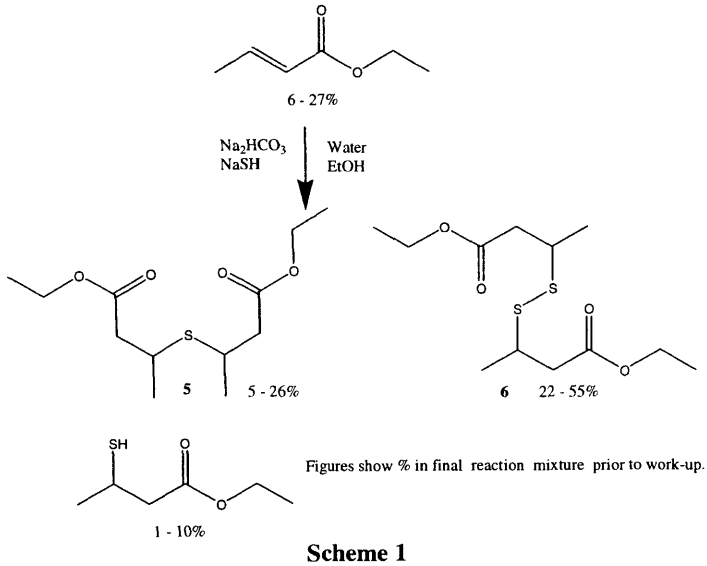
Comparison of analytical data with the authenticated synthetic standard[34] confirmed the 'active' nature identical target molecule as ethyl 3-mercaptobutyrate 1[10].

7.1.1 Synthesis of the target molecule 1. Initially addition of sodium hydrogen sulphide to ethyl crotonate in aqueous sodium bicarbonate solution was attempted, but successful reaction conversion was not accomplished at ambient or 50°C. Reaction of ethyl crotonate with hydrogen sulphide in hexane was found to be unsatisfactory, with or without aluminium chloride addition. It was found that reaction of sodium hydrogen sulphide with ethyl crotonate in aqueous sodium bicarbonate solution was moderately successful under slight pressure (scheme 1).

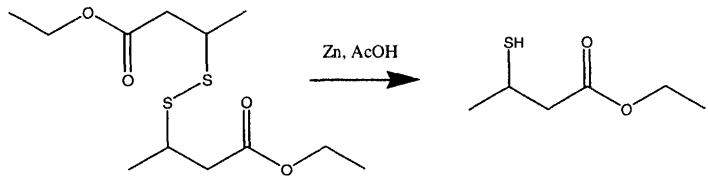
The isolated yield of ethyl 3-mercaptobutyrate 1 by this method was typically only 3% and the product ratio was clearly variable. To improve the yield, reduction of dimer 6 was undertaken (scheme 2) which was accomplished in moderate yield and made the overall yield of product from ethyl crotonate 15%.

Once the target compound had been confirmed as the key ingredient in the mango analysis, organoleptic evaluation, formulation trials and application tests confirmed that the compound was indeed a key mango and tropical component in both flavour and fragrance applications.

At this time steps were taken to improve the synthesis both in terms of ease and yield. Attempts to form 6 using the chemistry described were unsatisfactory as large quantities of the monosulphide 5 were always formed. It was found that addition of sulphur to the reaction mixture enabled formation of disulphide 6 as the major product in good conversion (ca. 70%) and in moderate yield (ca. 65% of theory). After reduction of 6 to the target mercaptan 1 the overall yield of distilled material was 29%.



Scheme 1



Scheme 2

As a 'spin off' from this analysis a useful intermediate was made en-route to target 2. This compound ethyl 4-(thioacetoxy) butyrate[10] has also been found of use as an artificial flavouring ingredient.

7.2 Compounds identified and synthesised from analysis of the volatile components in beef[35]

Although not specifically discussed in this work a Generessence® analysis of fried onion has been undertaken as part of the program. The results below demonstrate some of the differences in the synthesised components identified when beef is cooked with onion rather than in isolation.

The following component was identified in beef, beef and onion and fried onion:
octan-2,3-dione.

The following components were identified in beef, but not in beef and onion or fried onion:

4-acetyl-γ-butyrolactone, cis-bovolide.

The following components were identified beef and onion and fried onion, but not in beef: 2-ethyl-4-methyl-1,3-dithiolane, 2,4,6-triethyl-1,3,5-trithiane.

The following components were identified beef and onion but not in beef or onion: furfuryl propyl disulphide, 3-(allylthio) propanal[36]

The following component was identified beef, beef and onion but not in onion: Thialdine.

Of the above mentioned components, furfuryl propyl disulphide[10], 2-ethyl-4-methyl-1,3-dithiolane and 2,4,6-triethyl-1,3,5-trithiane are believed to be newly identified components in nature.

7.3 Compounds identified and synthesised from analysis of the volatile components in hazelnut[37]

Two of the compounds synthesised for use in hazelnut application, namely 6-methyl-(E)-3-hepten-2-one and (E/Z)-2-methyl-4-hexen-3-one[20] were newly identified in nature. One of these 2-methyl-(E)-4-hexen-3-one is covered by a Haarmann and Reimer Patent[20] and although found to be organoleptically viable was not used in final creations.

6-Methyl-(E)-3-hepten-2-one, and 5-methyl-3-hepten-2-one were made by standard aldol chemistry. (E/Z)-2-methyl-4-hexen-3-one was synthesised by reaction of 2-bromopropane with 2-butenal under Grignard conditions followed by oxidation of the resultant 2-methyl-4-hexen-3-ol with pyridinium chlorochromate to furnish the required product. The previously identified[24] 5-hepten-2-one was made by ethyl acetoacetate condensation with crotyl bromide followed by hydrolysis and decarboxylation.

4-(Methylthio) butan-2-one and 4-(methylthio) pentan-2-one were prepared my addition of methyl mercaptide to the corresponding unsaturated ketone.

Octan-2,3-dione (also found in the analysis of beef and in many other Generessence® analyses) was found to be an interesting component for use in the nut, cereal, meat and baked categories.

Several of the ketones outlined in figure 3 were found to be cheaper than 5-methyl-(E)-2-hepten-4-one Filbertone™ and of key importance to the flavour. One component, 6-methyl-(E)-3-hepten-2-one was indeed found to be essential to a good natural topnote flavour. Removal of either this component or 5-methyl-(E)-2-hepten-4-one greatly reduced the organoleptic authenticity of the final flavour, particularly when used in application.

8 ACKNOWLEDGMENTS

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35 Beef analysis includes a variety of different analyses, for the purpose of this discussion it includes Grilled beef, Barbequed beef and fried and onion.
36 Tentative identification, along with 3-(propenylthio) propanal.
37 Components identified in analysis of either or both toasted or untoasted Hazelnuts

Flavours/Foods

NEW RESULTS ON THE FORMATION OF IMPORTANT MAILLARD AROMA COMPOUNDS

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1 INTRODUCTION

Flavours produced during thermal food processing, such as baking or roasting, are universally known and are widely appreciated by man. Because usually the raw materials do have rather bland flavours, it is quite obvious that the aroma compounds must be formed by a chemical degradation of odourless precursors present in the food. One of the first scientists who gave a more general idea on the reaction generating this type of food aromas was Louis Camille Maillard. In his early studies[1,2] he reacted amino acids in the presence of reducing carbohydrates and was able to generate different odours besides brown colour. Because of the pioneering work performed later by Amadori[3], Heyns[4] and, in particular, by Hodge[5], the first stages of the complex reaction cascade, today known as the "Maillard-reaction (MR)", are basic knowledge in food chemistry and, in particular, the primary reactions have been introduced in many textbooks.

One of the first comprehensive reaction schemes was published by Hodge[5] about 50 years ago and named after him as the "Hodge-scheme" (Figure 1). It is suggested that, first, the carbohydrate, e.g., an aldose, reacts with an amine to yield the glycosylamine, which forms an 1,2-eneaminol and yields either the 3-deoxyosone by elimination of water or undergoes a rearrangement into the Amadori-compound. This, in turn should form the 2,3-enediol which may eliminate the amine, thereby generating a methyl α-dicarbonyl intermediate, which is known today as the 1-deoxyhexosone. The deoxyosones will either eliminate a further molecule of water or, alternatively, the carbohydrate skeleton will be cleaved.

Although the Hodge scheme is frequently used to explain the early stages of the Maillard reaction, it does not take into consideration that Amadori compounds and deoxyosones do mainly exist in the less reactive cyclic conformation[6] and, furthermore, it does not give detailed information on the formation of volatile flavour compounds in the "advanced" stages of the reaction.

Systematic sensory evaluations performed on thermally processed Maillard-type mixtures have shown that the amino acid reacted clearly determines the type of aroma generated. E.g. proline was characterized as a precursor of cereal, cracker-like aromas, leucine generates chocolate-type odours and cysteine produces flavour impressions similar to those of processed meat[7-9]. This principle of generating flavours by heating mixtures of amino acids and carbohydrates is today widely used in the manufacturing of the so-called "process flavourings".

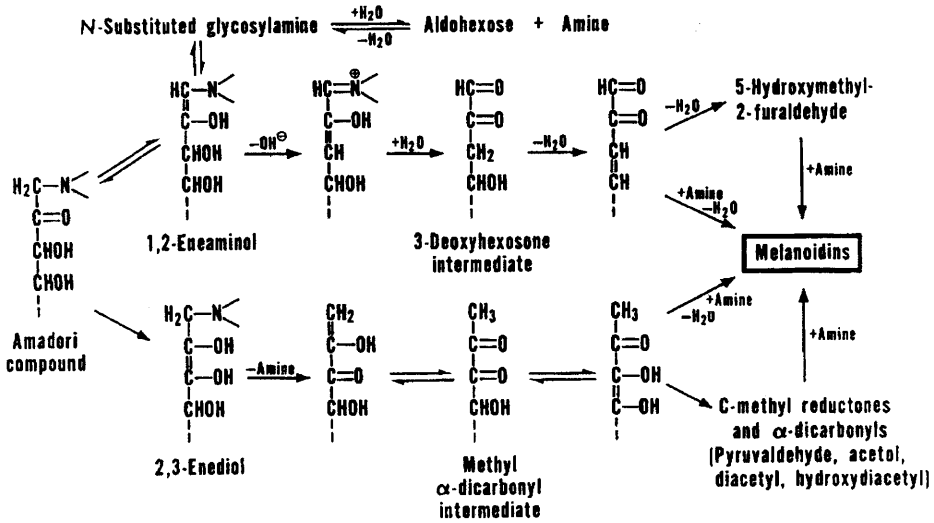


Figure 1. *The so-called "Hodge-scheme" of the Maillard reaction*[5]

However, most of these products are made by an empirical rather than a systematic approach. A main reason for this is the fact that often neither the key aroma compounds evoking the characteristic aromas of processed foods nor the odours generated in processed Maillard mixtures have been systematically characterized. However, to optimise either the flavour formation in a food itself or in a reaction flavour, this knowledge is a prerequisite to focus the investigations on reaction pathways leading to the formation of only the key aroma compounds.

The application of an Aroma Extract Dilution Analysis on flavour extracts followed by quantitations and a calculation of odour activity values (OAV: ratio of concentration to odour threshold) has been proven as a useful approach to detect the most odour-active compounds in foods or model systems[10]. Among the odourants identified in processed foods by the OAV approach, some compounds generated by Maillard-type reactions have gained much interest due to their characteristic flavour qualities and, also, their high odour potencies. Examples are the following: In fresh wheat bread crust[11,12] as well as in freshly popped corn[13,14], 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine were characterized as key aroma compounds. On the other hand, studies on Maillard-type model mixtures containing the amino acid proline have shown that both intensely roasty, popcorn-like smelling odourants are formed as the key aroma compounds[15,16].

The caramel-like smelling 4-hydroxy-2,5-dimethyl-3(2H)-furanone (4-HDF) has been identified as an important aroma compound in many processed foods based on a combination of analytical and sensory approaches (Table 1)[12,17-21]. Furthermore, 4-HDF has also been characterized as a key odourant in sugar containing model systems independently from the amino acid reacted[22-24].

Another group of important food odourants are the *Strecker* aldehydes which are generated by an oxidative decarboxylation of amino acids[25,26]. Due to their low odour thresholds, in particular, methional, 2- and 3-methylbutanal, phenylacetaldehyde and methylpropanal have been identified as important contributors to several food aromas[11,13,17,19]. Model reactions performed recently have, however, shown that besides the *Strecker* aldehydes, the corresponding flavour-active *Strecker* acids may also be generated.

Food	Conc. (µg/kg)	OAV[a]
Roasted coffee[17]	131000	13100
Cooked beef meat[18]	9075	908
Rye bread crust[12]	4310	315
French fries[19]	2591	111
Wheat bread crust[12]	1920	140
Popcorn[20]	1370	100
Barley malt[21]	820	60

[a] Odour activity values were calculated by dividing the concentration by the odour threshold in the respective matrix.

Table 1. *Odour activity values of 4-hydroxy-2,5-dimethyl-3(2H)-furanone in several foods*

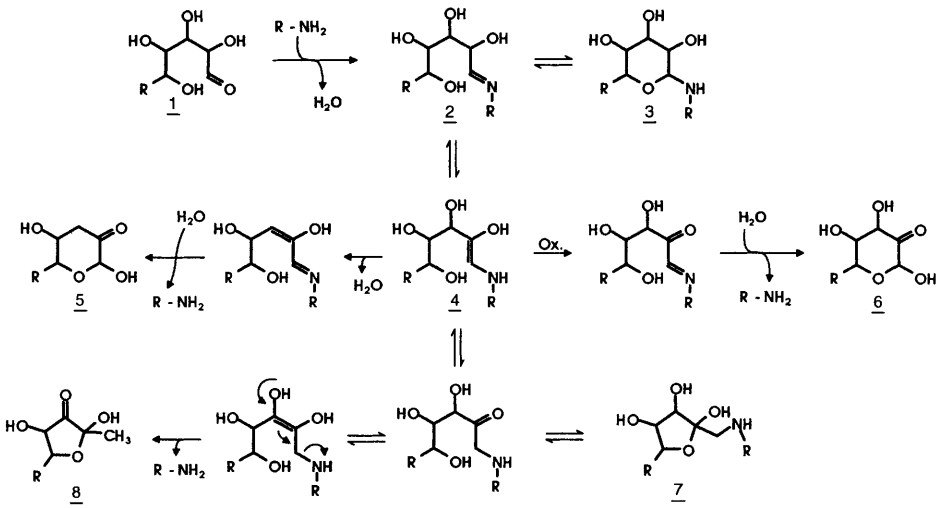
A great number of comprehensive reviews on the Maillard reaction have previously been published, e.g.[27-32]. While some papers focused exclusively on the mechanistic aspects of the MR, a few recent papers also gave valuable information on the correlation between the MR and food flavour compounds[30-32]. Because the knowledge on the formation mechanisms of the above mentioned aroma compounds has been much extended within the recent years, the intention of the following chapter is to discuss the formation pathways of some Maillard-type aroma compounds established as key food aroma compounds mainly based on results obtained in the authors lab.

2 GENERAL ASPECTS OF THE MAILLARD REACTION

In Figure 2, the reactions taking place in the primary stage of the MR are displayed based on Hodge's scheme, but taking into consideration mainly the cyclic forms of the respective intermediates. The Schiff base (2 in Figure 2) formed from a hexose and an amino compounds must not necessarily cyclise into the glycosylamine (3 in Figure 2). The key intermediate is probably the 1,2-eneaminol (4 in Figure 2). This compound has three possibilities to react further. Either an oxidation may take place leading to an α-oxo imine, which after hydrolysis, forms the hexosone (6 in Figure 2). Compound 4 may also eliminate water, yielding the 3-deoxyosone after hydrolysis of the α-oxo imine formed. The third possibility is the Amadori rearrangement yielding either the Amadori compound (7 in Figure 2) or the 1-deoxyosone (8 in Figure 2) after ß-elimination of the amino compound.

None of the reaction intermediates shown in Figure 2 are stable and, therefore, the so-called "advanced" Maillard reaction will take place during further heat treatment. In general, the following types of reactions must be considered:

- dehydration reactions maintaining the carbohydrate skeleton
- retro-*Aldol* reactions leading to fission products
- *Aldol*-type reactions of fissions products previously formed
- substitution of oxygen by nitrogen or sulphur
- redox reactions
- *Strecker* reaction



(1: carbohydrate; 2: Schiffbase; 3: Glycosylamine; 5: 3-Deoxyosone; 6: Glucosone; 8: 1-Deoxyosone; 7: *Amadori* product)

Figure 2. *Primary reaction pathways of the Maillard reaction*

To illustrate the two first mentioned reactions in more detail, the formation of furfural (I in Figure 3) from the 3-deoxyosone of a pentose or norfuraneol (II in Figure 3) from the 1-desoxyosone is shown in Figure 3. Furthermore, a retro-*Aldol* cleavage of different α-diketones is exemplified in Figure 4 yielding various fission products, e.g., 2-oxopropanal or glyoxal.

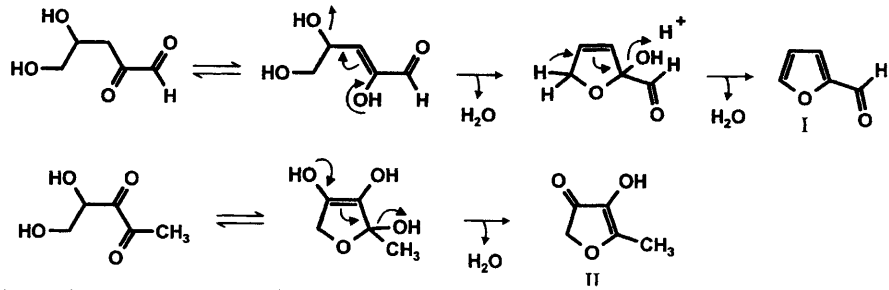


Figure 3. *Formation of furfural (I) and norfuraneol (II) from the 3- and the 1-deoxyosone of pentoses*

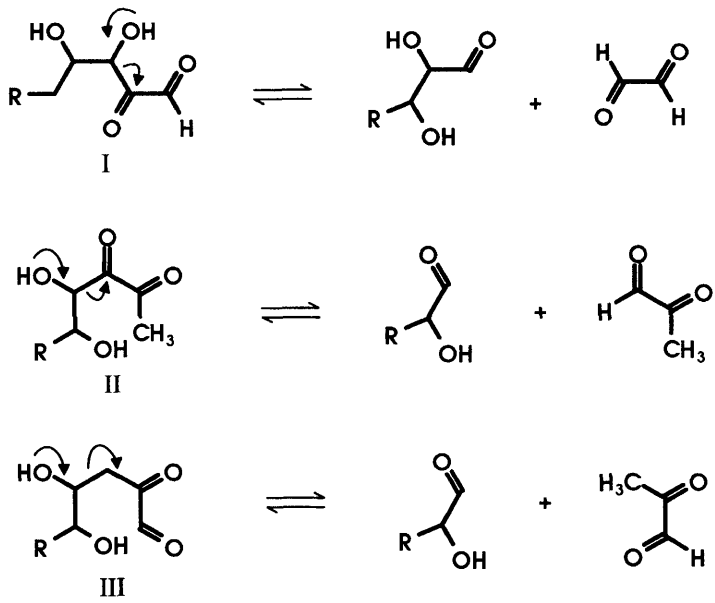


Figure 4. *retro-Aldol cleavage of a glycosone (I) or the 1- (II) and the 3-deoxyosone (III), respectively*

3 FORMATION OF 4-HYDROXY-2,5-DIMETHYL-3(2H)-FURANONE (4-HDF)

4-Hydroxy-2,5-dimethyl-3(2H)-furanone (4-HDF) is not very stable and can easily be adsorbed by the food matrix or by silica during column separation and work-up manipulations. So, an important prerequisite in performing studies on the formation of such flavour compounds is an exact method for quantitation. Previously we have established[33] that a stable isotope dilution assay using a carbon-13 labelled isotopomer of 4-HDF (100 % labelling) as the internal standard is the method of choice and is, therefore, used throughout our experiments. The differentiation between the labelled internal standard [$^{13}C_2$]-4-HDF and the flavour compound is done by mass chromatography (Figure 5).

It is well known that rhamnose, a 6-deoxyhexose, is an effective precursor of 4-hydroxy-2,5-dimethyl-3(2H)-furanone. As shown in Figure 6, its formation is easily possible from the cyclic form of the 1-deoxyrhamnosone. However, rhamnose is not present ubiquitously in foods and, compared to glucose or fructose, its concentration is generally much lower.

In a study on the precursors of 4-HDF in wheat bread crust or bakers yeast, respectively, we could earlier identify fructose-1,6-biphosphate and glucose-6-phosphate as effective precursors of 4-HDF[20]. As shown in Figure 7, acetylformoin, formed by elimination of phosphate from the 1-deoxyhexosone-6-phosphate, is suggested as the key intermediate. Its disproportionation (self-oxidation) immediately results in 4-HDF formation.

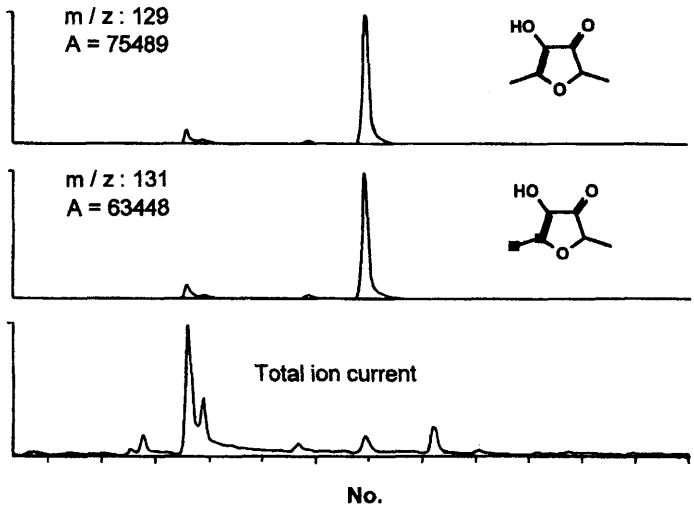


Figure 5. *Quantification of 4-hydroxy-2,5-dimethyl-3(2H)-furanone by a stable isotope dilution analysis in combination with mass chromatography*

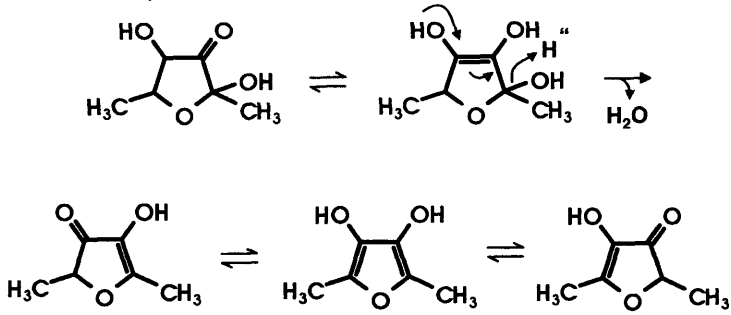


Figure 6. *Formation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone from the 1-deoxy-rhamnose*

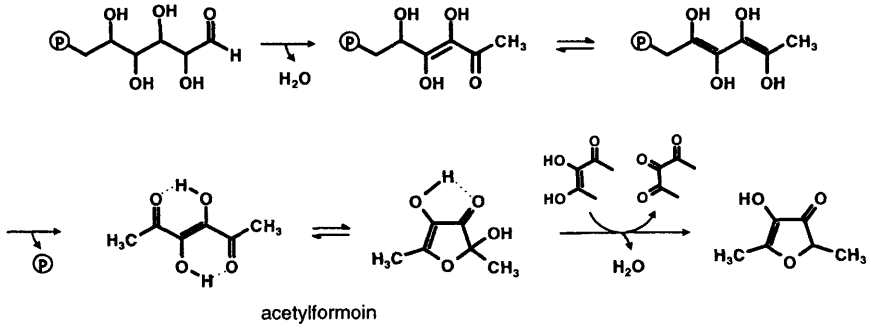


Figure 7. *Proposed formation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone from glucose-6-phosphate via acetylformoin as the key intermediate*[20]

In a very recent model study on the formation of 4-HDF from acetylformoin we found that the presence of proline much increased the yields of the flavour compound from acetylformoin[34]. It is assumed that acetylformoin reacts in its cyclic form yielding the Schiff base with proline. Dehydration and decarboxylation then yields 1-pyrroline and 4-HDF in a *Strecker*-type reaction[34].

In a further model experiment, we recently compared the effectiveness of ribose, glucose and rhamnose in forming 4-HDF in the presence of the amino acid cysteine. The results indicated (Table 2) that generally increasing the pH also increased the yields of 4-HDF independently from the carbohydrate used. However, it is interesting to note that 4-HDF, a six-carbon compound, can be formed from a pentose. This gave us the idea that an *Aldol*-type reaction of fission products, e.g., 2-oxopropanal and 2-oxopropanol may also generate the 4-HDF via 2,5-dioxo-3,4-dihydroxyhexane as the key intermediate (Figure 8). In this type of reaction no reduction (disproportionation) of an intermediate is necessary. Quantitative model studies on 2-oxopropanal/2-oxopropanol mixtures confirmed this assumption, thereby corroborating the reaction pathway. The formation was favoured at higher values and about 1.1 mol % of 4-HDF were formed at pH 7.0[35].

Carbohydrate	Amount (µg) formed at pH		
	3	5	7
Ribose	0.8	19	208
Glucose	2.3	80	788
Rhamnose	1140	19800	79860

[a] The carbohydrate (10 mmol) and cysteine (3.3 mmol) were reacted at 145°C for 20 min in phosphate buffer (100 mL; 0.5 mol/L; pH 5.0).

Table 2. *Influence of the pH on the formation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (4-HDF) from carbohydrates (Hofmann and Schieberle, unpublished results)*[a]

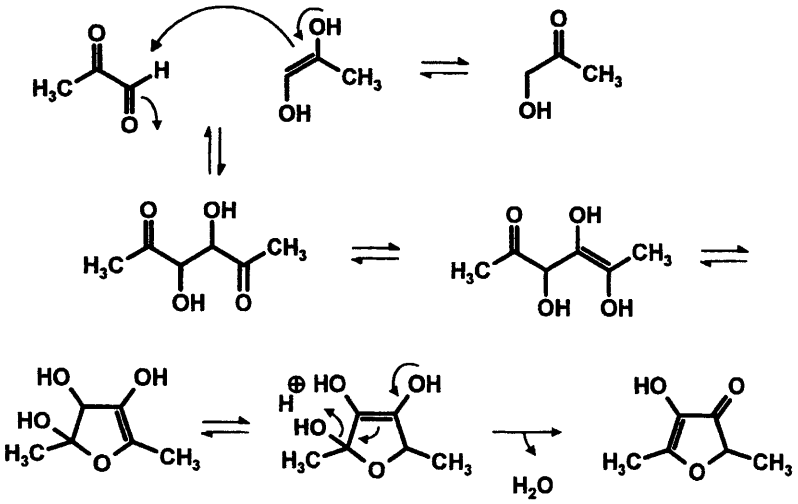


Figure 8. *Formation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone by an Aldol reaction of 2-oxopropanal and 2-oxopropanol (hydroxyacetone)*

From these studies the question arose whether and to what extent the 4-HDF is formed from hexoses, such as glucose or fructose, either via acetylformoin or via carbohydrate fission products.

By application of a so-called carbohydrate modul labelling experiment[36] we could recently show that in the formation of flavour compounds in the MR very often different pathways are operating and, that water is an important modifier: while under dry-heating conditions, 4-HDF was formed from proline and glucose only via the C-6 pathway (Figure 7), in the presence of water about 62 % of the 4-HDF was found to be formed from carbohydrate fission products. Because in both model reactions acetylformoin showed a similar isotopic pattern as the 4-HDF[34], 2-oxopropanol is obviously not the only C-3 intermediate. Based on the results obtained[34], 1,3-dihydroxy-2-propanone was suggested as another very efficient intermediate in 4-HDF generation. A partially labelled 4-HDF was formed from a 1+1 mixture of labelled and unlabelled glucose and, furthermore, partially labelled acetylformoin was also detected[34], the following reaction pathway is obvious: 2-oxopropanal is condensed with dihydroxyacetone yielding a C-6 compound which forms acetylformoin upon elimination of water (Figure 9). This is finally "reduced" in a *Strecker* reaction with proline.

In general, the data indicates that the reaction conditions used in the Maillard reaction may not only change the yields of certain aroma compounds but may also change the formation pathways leading to the flavour compound under investigation.

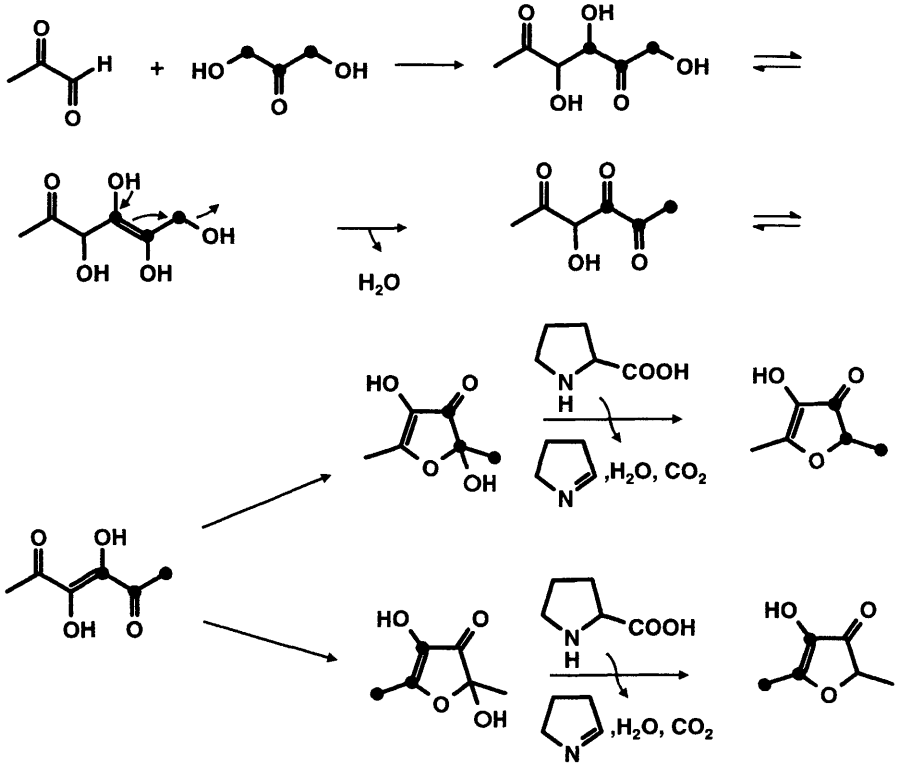


Figure 9. *Formation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone by a retro-Aldol reaction of 2-oxopropanal and dihydroxyacetone via acetylformoin as the key intermediate*[34]

4 FLAVOUR CONTRIBUTION AND FORMATION OF 2-ACETYLTETRA-HYDROPYRIDINE

Hunter et al.[37] were the first who described 2-acetyltetrahydropyridine as volatile constituent of a thermally treated proline/dihydroxyacetone mixture. Later on the compound was identified as an important odourant in wheat bread crust[11] and popcorn[13]. As indicated in Table 3, the odourant is the most odour-active among the 3 aroma compounds eliciting a roasty, popcorn-like aroma in popcorn. In a model study on a thermally treated proline/glucose mixture, we have recently show that the same three odourants also contributed to the aroma of the model mixture thereby confirming this amino acid as the precursor of the three flavour compounds[23].

By application of the carbohydrate modul labelling approach[36] we have recently shown that a 1+1 mixture of 2-acetyltetrahydropyridine and [$^{13}C_3$]-2-acetyltetrahydro-pyridine is formed from a 1+1 mixture of glucose and [$^{13}C_6$]-glucose[34]. This experiment corroborated our previous data indicating that a ring opening of proline occurs during ACTHP formation incorporating 3 carbon atoms from a carbohydrate fission product[38].

Odourant	Conc. (µg/kg)	Odour threshold (µg/kg in starch)	OAV[a]
2-Acetyltetrahydropyridine	1207	0.054	22351
2-Acetyl-1-pyrroline	57	0.0073	7808
2-Propionyl-1-pyrroline	21	0.010	2100

[a] OAVs were calculated by dividing the concentrations by the odour thresholds.

Table 3. *Odour activity values (OAV) of selected roasty, popcorn-like smelling odourants in popcorn*[14]

In a previous paper we had suggested that 1-pyrroline, which may be formed by a reaction of proline with acetylformoin[34], reacts with 2-oxopropanol in an *Aldol*-type reaction to yield 2-(1-hydroxy-2-oxo-3-propyl)pyrrolidine (Figure 10) which after enolisation and water elimination directly yields the ACTHP. This reaction pathway has been proven by model experiments[38] and about 1 mol % of ACTHP was formed when 1-pyrroline and 2-oxopropanal were reacted.

However, the following labelling experiments showed that 2-oxopropanol is not the only intermediate in ACTHP formation. Synthesis of the 1-[$^{13}C_1$]-labelled Amadori compound of proline and glucose (100 % label) and its degradation by a thermal treatment in water generated a 1+1 mixture of $^{13}C_1$-ACTHP (labelled in the methyl group) and unlabelled ACTHP[34]. Contrary, only 30 % of the precursor 2-oxopropanol was labelled. So, an additional pathway generating ACTHP must exist. Based on the obtained results it could be concluded that acetylformoin is also a key intermediate in ACTHP formation. Its *Strecker*-type reaction with proline, cyclisation, hydrolytic ring opening and elimination of 2-oxopropanal yields 2-(1-hydroxy-2-oxo-3-propyl)pyrrolidine (Figure 11). This intermediate was recently synthesized by us and proven to be a key intermediate in ACTHP formation[38].

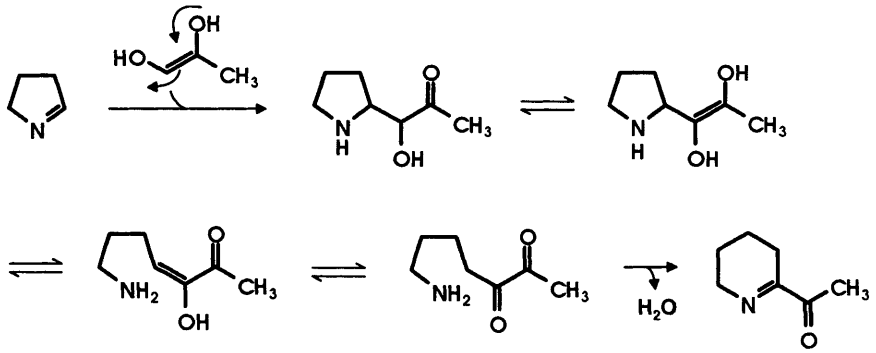


Figure 10. *Formation of 2-acetyltetrahydropyridine from 1-pyrroline and 2-oxopropanol (hydroxy-2-propanone)*

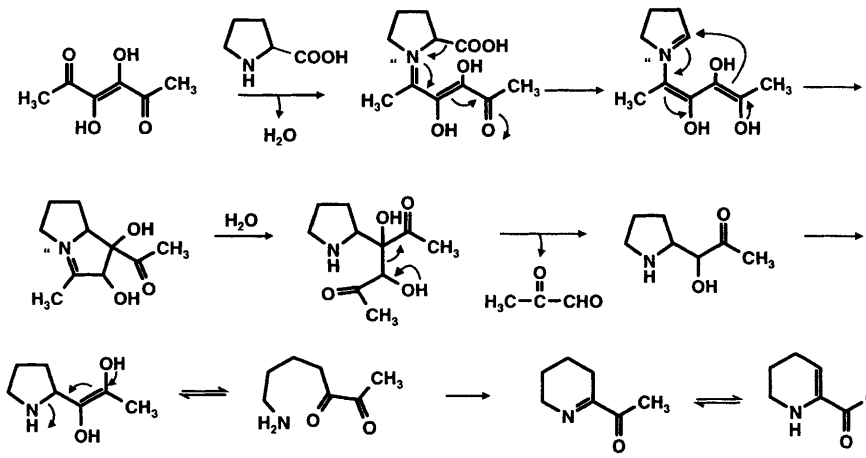


Figure 11. *Formation pathway leading from acetylformoin and proline to 2-acetyltetrahydropyridine*

5 AROMA COMPOUNDS FORMED BY THE *STRECKER* REACTION

Once hexosones or deoxyosones as well as fission products of carbohydrates with an α-diketo structure have been formed, a *Strecker* reaction will compete with the primary reaction pathways shown in Figure 1. The *Strecker* reaction starts with the formation of an imine from the amino acid and an α-dicarbonyl compound (Figure 12). The azavinylogous ß-ketoacid formed (I) easily decarboxylates yielding intermediate II which is finally hydrolysed into an α-aminoketone and the *Strecker* aldehyde (V). Although about 20 amino acids may occur naturally in foods, only the following six amino acids are important precursors of food odourants. The reason is that the aldehydes formed have quite low odour thresholds:

- Valine → 2-Methylpropanal
- Leucine → 3-Methylbutanal
- Isoleucine → 2-Methylbutanal
- Methionine → Methional

- Phenylalanine → Phenylacetyldehyde
- Alanine → Acetaldehyde

By application of analytical studies in combination with sensory experiments, *Strecker* aldehydes have been characterized as contributors to many food aromas, such as wheat bread crust[11], roasted coffee[17] or French fries[19], and recently we have found (Table 4) that four *Strecker* aldehydes, namely 2- and 3-methylbutanal, methional and methylpropanal were among the eight most important odourants of a special barley malt[21].

Very often also the corresponding acid, in particular, 2- and 3-methylbutanoic acid and phenylacetic acid, additionally contribute to the overall aromas of foods and it is generally believed that the acids are formed by oxidation from the respective aldehydes. However, a very recent experiment using labelled phenylacetaldehyde clearly showed that this assumption is wrong[39]. To shade some light on the formation of the acids in course of a *Strecker* reaction, first, the time course of the formation of phenylacetaldehyde and phenylacetic acid in a glucose/phenylalanine mixture was studied. The results showed that the formation of both compounds started immediately with heating, but with increasing the reaction time, the formation of the acid was favoured[39]. Further model studies indicated the crucial role of free oxygen in the formation of, in particular, the acid (Table 5). In the absence of oxygen (models A; Table 5) always the aldehyde formation was favoured. However, in the presence of oxygen (models B; expts. 1 und 3; Table 5) acid formation was favoured. Addition of copper ions (expt. 2; Table 5) enhanced this effect.

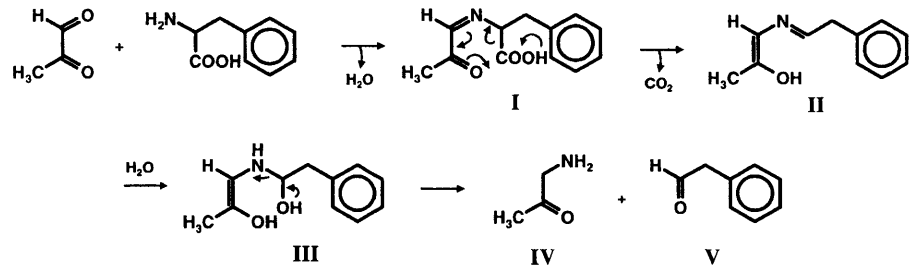


Figure 12. *Strecker degradation of L-phenylalanine initiated by 2-oxopropanal*

Odourant	Conc. (µg/kg)	Odour threshold (µg/kg starch)	OAV
3-Methylbutanal	7500	32	235
2-Methylbutanal	6900	53	130
Methional	35	0.27	130
2-Methylpropanal	4000	56	70

Table 4. *Concentrations and odour activity values (OAVs) of selected key odourants in barley malt*[21]

Expt.	α-Dicarbonyl	PA (μmol/mmol)		PAA (μmol/mmol)	
		model A[b]	model B[c]	model A[b]	model B[c]
1	2-Oxopropanal	11.1	10.2	4.7	20.4
2	2-Oxopropanal[d]	n.a.	10.0	n.a.	27.1
3	Glyoxal	9.2	8.3	2.2	12.2
4	3-Deoxyosone	8.4	7.8	1.5	3.3

[a] A solution of the α-dicarbonyl (1.0 mmol) and L-phenylalanine (1.0 mmol) in phosphate buffer (10 mL; 0.1 mol/L; pH 7.0) was refluxed for 60 min in a closed vial.
[b] Oxygen was absent.
[c] Oxygen was present.
[d] The reaction was performed in the presence of oxygen and copper (II) ions (0.05 mmol $CuSO_4$).
n.a., not analysed.

Table 5. *Amounts of phenylacetaldehyde (PA) and phenylacetic acid (PAA) generated from L-phenylalanine in the presence of different α-dicarbonyls[a] – Influence of oxygen and copper ions*

Based on these results, the formation pathway shown in Figure 13 was proposed. The first steps of the *Strecker* reaction are the same as shown in Figure 12. However, intermediate III contains an eneaminol structure which has recently been shown to be susceptible to air oxidation[40]. The oxidation step, which may be catalysed by traces of metal ions, leads to an α-oxo imine (V in Figure 13) which, after enolisation is hydrolysed to yield phenylacetic acid.

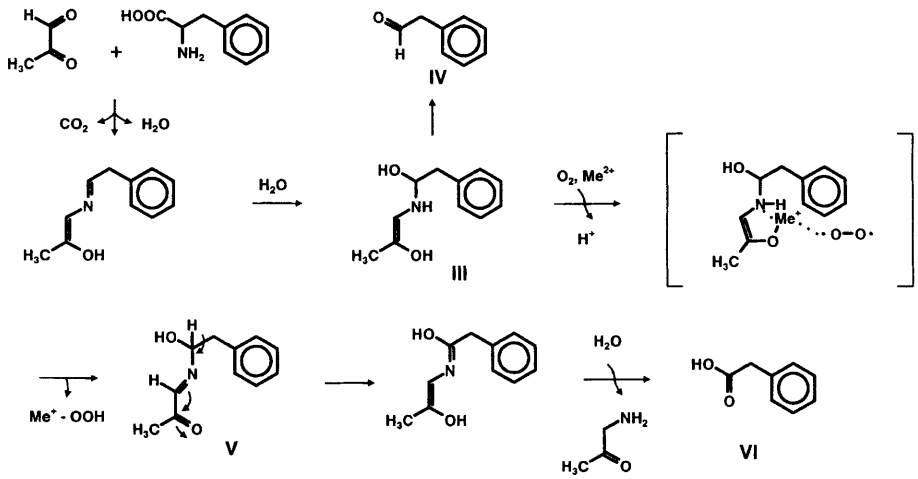


Figure 13. *Proposed reaction pathway leading to the formation of phenylacetaldehyde (IV) and phenylacetic acid (VI) from 2-oxopropanal and L-phenylalanine*

Interestingly, if the *Strecker* degradation of phenylalanine was catalysed by the 3-desoxyosone, compared with 2-oxopropanal, the acid concentration was not as significantly enhanced (expt. 4; Table 5). This fact can be explained by the reaction pathway shown in Figure 14. Contrary to 2-oxopropanal, the 3-deoxyosone exists preferably as a cyclic ketone. This intermediate may also form a Schiff base with the amino acid leading to dehydration and decarboxylation. However, the 3-amino-

dihydropyranone ring structure is not susceptible to oxidation, thus favouring the formation of the aldehyde.

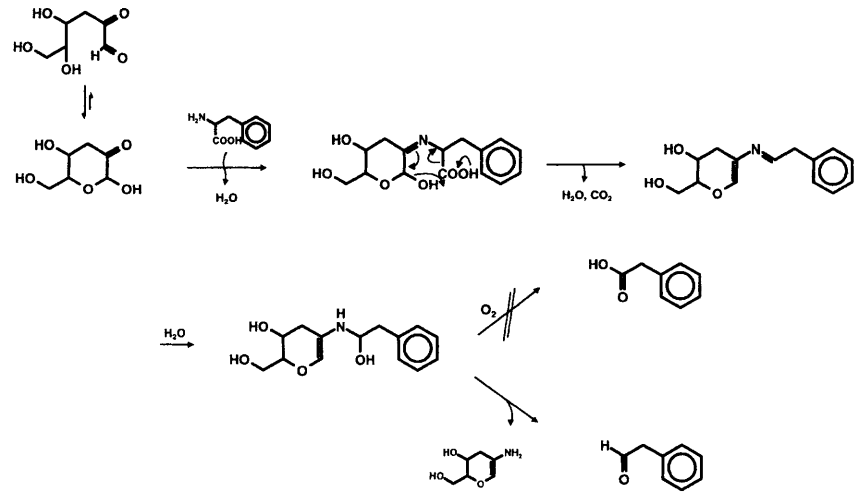


Figure 14. *Proposed mechanism for the preferred formation of phenylacetaldehyde from the 3-deoxyosone and L-phenylalanine*

In a very recent study[41] we showed that the presence of oxygen generally enhances the yields in a *Strecker* reaction, in particular, when Amadori products were reacted (Table 6). However, besides being more effective precursors, the Amadori products also favoured the generation of aldehydes compared to binary reaction mixtures containing glucose and phenylalanine (Table 6).

Exp.	ARP-Phe		Glc/Phe	
	PA	PAA	PA	PAA
I[a]	0.6	0.3	0.4	0.2
II[b]	5.5	3.0	1.4	1.8
III[c]	13.8	7.6	2.6	3.9

[a] The precursors (1 mmol each) were heated (100°C) in phosphate buffer (10 mL; 0.5 mol/L; pH 7.0) for 120 min in a closed vial under an atmosphere of argon.

[b] Argon was replaced by air oxygen.

[c] Heating was performed in an air atmosphere and in the presence of copper (II) ions (0.05 mmol $CuSO_4$).

Table 6. *Concentrations (μmol/mmol) of phenylacetaldehyde (PA) and phenylacetic acid (PAA) generated upon thermal treatment of either N-(1-deoxy-D-fructosyl)-L-phenylalanine (ARP-Phe) or glucose/phenylalanine (Glc/Phe)*

This data led to the proposal of a reaction pathway yielding a "*Strecker*" aldehyde in a reaction different from the classical *Strecker* reaction. It is assumed that the Amadori compound (I in Figure 15), an α-aminoketone, is directly oxidised into the corresponding α-iminoketone (II in Figure 15). Hydrolysis of this intermediate may yield the glucosone (III in Figure 15) thereby re-generating the amino acid. However, cyclisation of the oxidized Amadori compound (IIb in Figure 15) followed by a decarboxylation/ dehydration yields intermediate IV which is finally hydrolysed into phenylacetaldehyde.

6 CONCLUSIONS

In general, the results of our studies show that the presence of a Maillard-type flavour compound in a food does not tell us much detail about the way it was formed. So, from the chemical standpoint, it is not very helpful to focus simply on carbohydrates and amino acids as the precursors in the MR. The most effective intermediates and reaction pathways have to be discovered, because the "traditional" published ways for the Maillard reaction cannot be regarded as the one and only way that a food flavour compound can be formed.

In particular, if one intends to optimise the yields of a Maillard-type aroma either in a food or a reaction flavouring the following systematic concept may be useful:

- Characterise the key Maillard-type odourants in foods
- Increase knowledge on the flavour potential of a food by quantitative correlations of precursors present in the food and amounts of flavour compounds formed thereof by a thermal treatment
- Investigate the formation of single odourants in model system by means of labelling experiments and synthesised intermediates
- Enhance the yields of key aroma compounds by either enhancing the precursor/ intermediate concentrations in the food itself or by reacting precursors outside the food

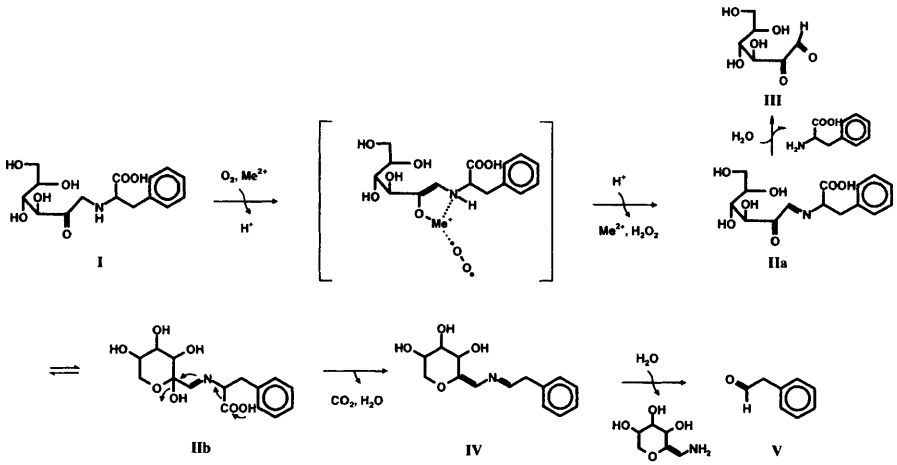


Figure 15. *Formation of phenylacetaldehyde via an oxidative degradation of N-(1-deoxy-D-fructosyl-L-phenylalanine (ARP-Phe)*

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OUT OF AFRICA : THE CHEMISTRY AND FLAVOUR PROPERTIES OF THE PROTEIN THAUMATIN

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1 INTRODUCTION

In 1848 a British army surgeon, WF Daniell, working in Western Africa noticed the local use of a fruit to sweeten fermented palm wine. Further investigation revealed that part of this fruit was intensely sweet and so he collected specimens for identification and classification by Kew Gardens in London. Upon return to England Dr Daniell presented a paper to the Pharmaceutical Society of Great Britain where he referred to this fruit as the "Miracle Fruit of Western Africa". Kew later named the fruit Thaumatococcus daniellii.

Since the death of Dr Daniell in 1865 Thaumatococcus daniellii has been largely ignored until the 1970's when extensive research and product development led to the identification and isolation of the key sweet component, a protein now known as Thaumatin.

2 PRODUCTION

Thaumatococcus daniellii grows freely below the canopy of the secondary rainforest of Western Africa and is effectively a weed that grows on the forest floor. It is widely distributed throughout the region growing in Ivory Coast, Nigeria, Ghana and Cameroon. Dr Daniell referred to it growing in Soudan this is an old name for part of Southern Nigeria and not to be confused with the Republic of Sudan we know today.

Figure 1 *Thaumatococcus daniellii fruit on the floor of the rainforest*

After collection of the fruit the high protein aspect or Aril is cut out and frozen before shipping to the UK for extraction.

Figure 2 *Cutting out the arils from Thaumatococcus daniellii fruit in Ivory Coast*

Once in the UK the production operation commences with washing and then aqueous extraction of the protein from the Aril. The liquor is then concentrated by reverse osmosis before freeze drying to produce the effectively pure protein, Thaumatin.

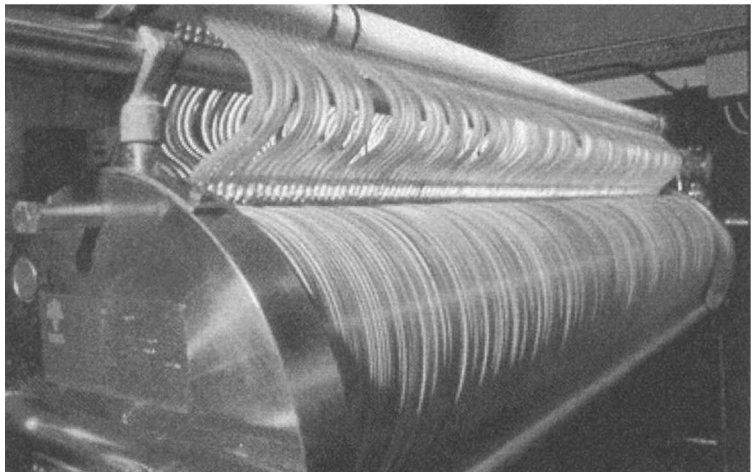

Figure 3 *Concentration and purification of Thaumatococcus daniellii extract*

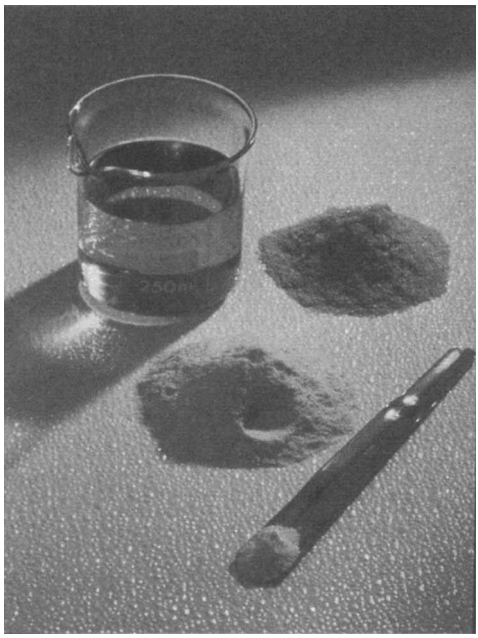

Figure 4 *Pure Thaumatin*

3 BIOCHEMICAL STRUCTURE AND PHYSICAL PROPERTIES OF THAUMATIN

Thaumatin is composed of a mixture of closely related proteins known as the thaumatins. The predominant protein is Thaumatin I (T_I) followed by Thaumatin II (T_{II}, maximum 45%).

Both T_I and T_{II} are proteins of 207 amino acids, with a molecular weight of 22,209 and 22,293. This is quite small for a protein but it is similar in size to the caseins in milk (i.e. casein 23,000), to the enzymes trypsin (23,800) and papain (20,900). It is, however considerably larger than insulin (11,466) and the polypeptide hormones.

The sequence of amino acids is shown in figure 1. All the common amino acid is alanine. The N-terminal ammonium group is not acetylated or converted into a pyrrolidene carboxyl group, this latter feature being often found in extracellular proteins such as immunoglobulins, venoms or hormones. The C-terminal carboxyl group is also unsubstituted by an amide group. This rare event is occasionally found in venoms and hormones.

The relative abundance in T_I and T_{II} respectively of the basic amino acids arginine (12 or 13 residues) and lysine (11 residues) plus the weakly basic asparagine (10 or 8 residues) and glutamine (4 or 5 residues) compared to the acidic amino acids, aspartic acid (12 or 13 residues) and glutamic acid (6 residues) is responsible for the high overall isoelectric point of around pH 11. This isoelectric point is high compared to most proteins indicating the relatively dense accumulation of the basic residues at the hydrophilic surface of the protein. Protamines and histomes have high isoelectric points but they have much higher content of arginine (70-80% in protamines, T_I has 8.5%) and have a much lower molecular weight (circa 5000).

3.1 Tertiary Structure of The Thaumatins

A striking feature of the thaumatins is the 8 disulphide bridges which give a crosslinked network of amino acid chains thus conferring unusual and extremely useful stability to heat and pH denaturation.

Disulphide bridges are common in proteins that travel or operate in extracellular space as digestive enzymes, immunoglobulins and milk proteins. For example, lysozyme has 5 disulphide bridges, comprising 7.2% of the protein (almost identical to T_I: 7.4 % cysteine), trypsin has 6 and bovine serum albumen, 17 disulphide bridges.

The presence of Cys-Cys at positions 158 and 159 in the sequence is another frequently encountered structural element (e.g. human serum albumen) which forms the basis of linking three chain segments close together again conferring stability.

The basic amino acids lysine and arginine and the dicarboxylic amino acids aspartic acid and glutamic acid also contribute to the tertiary structure through salt linkages.

3.2 Quaternary Structure

T_I and T_{II} have only 1 polypeptide chain and thus normally have no oligomeric forms or sub units and hence no regular quaternary structure. The type of amino acid residue and their linkages and sequence suggest ready enzymolysis and digestion by human gastric and intestinal proteases and peptidases. This has been confirmed in vivo by rat digestibility and human volunteer studies. (Data on File at Britannia Natural Products)

3.3 Solubility

Thaumatin is freely soluble in cold water too greater than 20% solution (200 mg/ml) whereas normal levels of use are 0.00005 - 0.0005 % by weight.

In alcohols such as ethanol and isopropyl alcohol, 12% solutions can be obtained in 60% alcohol and prehydration in a little water will allow solubility in up to 90% alcohol. Gels can be formed at high concentrations of Thaumatin in alcohol. Solubility is good (at least 10%) in aqueous glycerol, propylene glycol and polyols such as sorbitol (all 60-80% in 40-20% water). Thaumatin is not suitable in typical organic and aprotic solvents.

3.4 Calorific Value

The extreme effectiveness of Thaumatin allows practical use levels to be effectively non-calorific. Although per gram it has the normal calories of a protein (4.1 cal/g, or 17 kJ/g), a typical use as a flavour modifier would have no measurable calories.

4 SAFETY AND LEGISLATION

Thaumatin is metabolised like any other protein and is recognised as completely safe. It is a naturally occurring protein extracted from a fruit and meets all the legislative requirements as a natural food ingredient. It is also recognised and certified as meeting the requirements of both Kosher and Halal authority's.

Generally speaking there is world-wide approval for Thaumatin as a natural food ingredient and more specific approval for use a sweetener and flavour enhancer. In the USA Thaumatin is designated FEMA GRAS (Generally Recognised As Safe) and has the

FEMA Number 3732. It is widely approved FEMA and recognised by the FDA for about 30 applications in food and beverages.

In Europe approval is given for use both as a sweetener and as a flavour enhancer with the designation E 957.

In Japan Thaumatin is a natural ingredient approved for general use in food and pharmaceuticals.

The FAO/WHO Joint Expert Committee on Food Additives (JECFA) have declared Thaumatin's allowable daily intake (ADI) as not specified. In so doing recognising and acknowledging the safe non-toxic character of the protein.

4.1 Oral Care

Thaumatin has a very important property which makes it extremely useful for oral care products (as well as sweet confectionery), it is non-cariogenic .i.e. it does not promote the cause of dental caries. Thus it has proved a very popular ingredient in oral hygiene products.

5 ESSENTIAL PROPERTIES

Many of the interesting flavour and food properties of Thaumatin can be traced back to it's unique sweetness profile. Recognised as the sweetest natural substance Thaumatin has a slow onset of sweetness followed by a very elongated taste and sweetness profile.

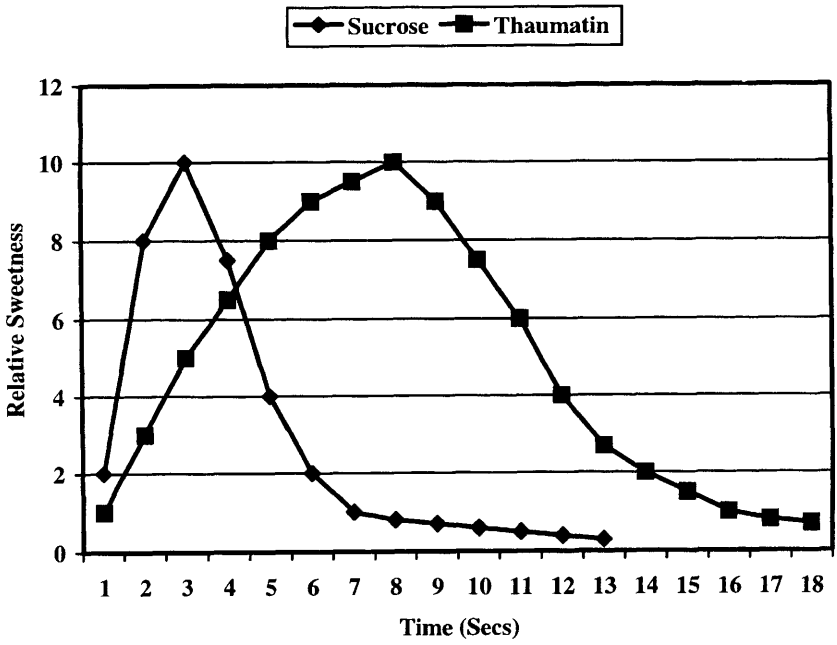

Figure 5 *Sweetness profile of Thaumatin*

5.1 Sweetness Intensity

Thaumatin is well known as the sweetest Natural substance and is indeed mentioned in the Guinness Book of Records as such, being 6000 times sweeter than a 1% sucrose solution. The sweetness intensity measurement does in fact alter with the concentration of the comparison solution. So, comparing Thaumatin with a 10% solution of sucrose may give a figure closer to 3000 times it's sweetness.

5.2 Synergy with other Intense Sweeteners

Thaumatin shows excellent synergy with other intense sweeteners and polyols typically contributing say 1% or 2 % of total sweetness to the combination but resulting in a total overall perceived sweetness increase of 10% to 20%.

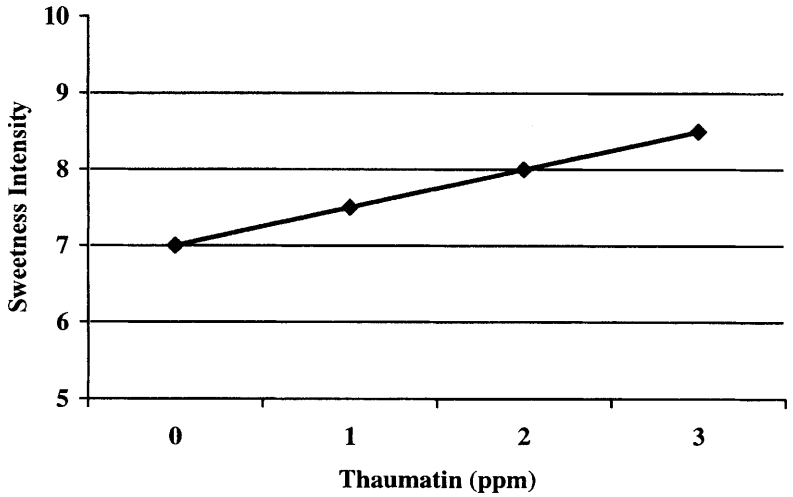

Figure 6 *Thaumatin sweetness synergy*

Best results are seen with Sweeteners where the further enhancement of the sweetener system occurs through masking of the bitter and metallic after taste contributed by products such as Saccharin for example.

5.3 Flavour Modification

Thaumatin can show significant flavour modification over a wide range of concentration levels and a variety of applications.

Essentially the slow onset of sweetening effect and it's longevity are the key factors in this flavour improvement and enhancement. Beware however that the wrong characters are not enhanced in a complex mixture thus leading to a loss of performance.

At levels above 30 ppm, when used on its' own, Thaumatin will exhibit a faint liquorice taste and slight cooling effect in the mouth. When working with Thaumatin one should be aware of this as the liquorice perception can be quite longstanding and may interfere with later work and evaluation of Thaumatin's effects in other formulations.

Thaumatin (ppm or mg/Kg)	Effect	Examples
0.2 – 0.5	Enhances Feed Flavours and Masks Bitterness	Animal Feed & Pet Food, Saccharin Masking & Synergy
0.5 – 5.0	Modifies Flavour & Enhances Aroma	Flavourings & Coffee
30 – 200	Prolongs Flavour & Intensifies Sweetness	Chewing Gum & Hard Candies
50 – 150	Masks Extreme Bitterness & Contributes Sweetness	Pharmaceutical & Oral care Products

Table 1 *Broad application levels for flavour enhancement*

Flavour Characteristic	Normal Perception Threshold (%)	Reduction of Threshold with 0.5 ppm Thaumatin
Peppermint	0.00006	10
Menthol	0.00006	3 – 5
Coffee Extract	0.01	3 – 4
Chocolate Flavour	0.0048	2
Milk Base	0.048	2
Vanilla essence	0.0048	2
Orange Essence Oil	0.0048	2
Apple Aroma	0.0024	2
Chicken Extract	0.072	1.5 – 2
Beef Extract	0.0045	2
Lemon Essence Oil	0.02	2 –8
Strawberry Flavour	0.00024	1.5 – 2
Mustard Seed Oil	0.0006	2
Tuna Fish	0.0048	1.5 – 2
Perilla	0.0024	4

Table 2 *Examples of flavour enhancement*

Successful applications of this flavour enhancement effect can be seen with Mint flavours particularly in oral care and chewing gum confectionery. From Table 2 above it will have been noted that there is a significant reduction in the threshold of perception when Thaumatin is applied with mint characters including peppermint oil, spearmint oil and menthol. This can enable lower volumes of mint oils and flavours to be used in the application or as is often the case with chewing gum to prolong the perception of flavour in the product.

5.4 Mouth Feel improvement with Thaumatin

A very interesting and notable fact is that of the improvement of mouth feel as well as the enhancement of flavour in many applications. This is very noticeable and useful in the application in low fat dairy products. Often sensed as being watery and lacking in body a small addition of Thaumatin to a low fat formulation can give the perception of a much greater fat content. By adding Thaumatin to the flavour system or fruit preparation that

may be used to flavour a yoghurt for example can modify the perception of the creaminess and fat content of the product base and add an enhanced flavour effect.

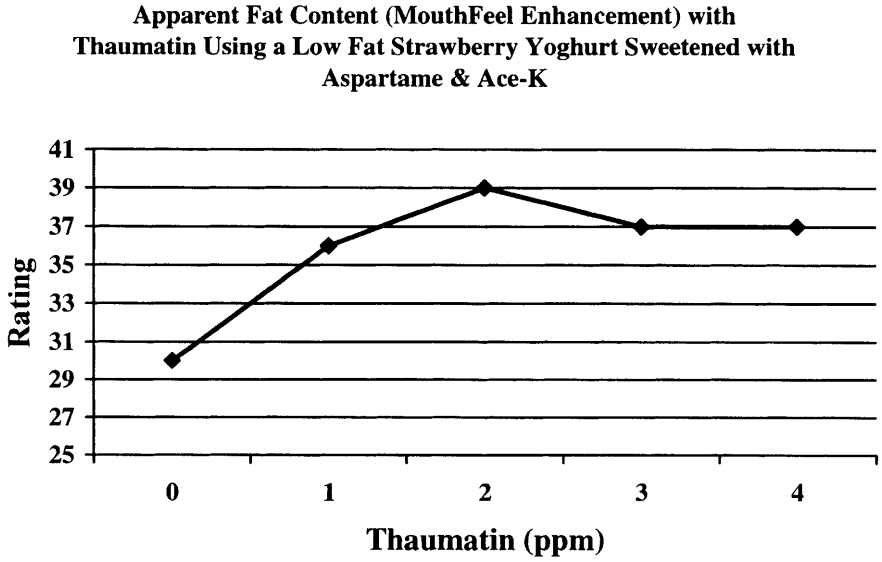

Figure 7 *Thaumatin effects on mouth feel*

Figure Seven also serves to demonstrate the importance of finding the correct level for the addition of Thaumatin in each application. There are no exact rules for the use of Thaumatin except to say that each product is different and that Thaumatin will have an optimum level of performance in every application.

Here we can see that around 2 ppm of Thaumatin gave the optimum mouthfeel enhancement in this particular combination of low fat dairy product and strawberry flavour. Increasing the level of Thaumatin does not necessarily lead to improved performance. It is most likely here that the panel has started to focus on the negative aspects now being improved and brought forward through the increased addition of Thaumatin. For example, an aspect of the strawberry flavour the panel find unpleasant (say butyric notes) may now being brought forward.

These effects, then, can be used to advantage in product applications such as Vitamin and Mineral Tables, OTC Products, Nutraceuticals, Active Pharmaceutical Preparations, Soya Peptides and masking of ingredient degradation products.

5.5 Bitterness Masking with Thaumatin

The late onset of sweetness and the ability of Thaumatin to enhance flavours means that it can also demonstrate the ability to mask the aftertaste and bitterness of materials. A particularly useful benefit when working with both Pharmaceutical and Nutraceutical ingredients.

The benefit of using Thaumatin in Soya containing foodstuffs is demonstrated in Figure 8.

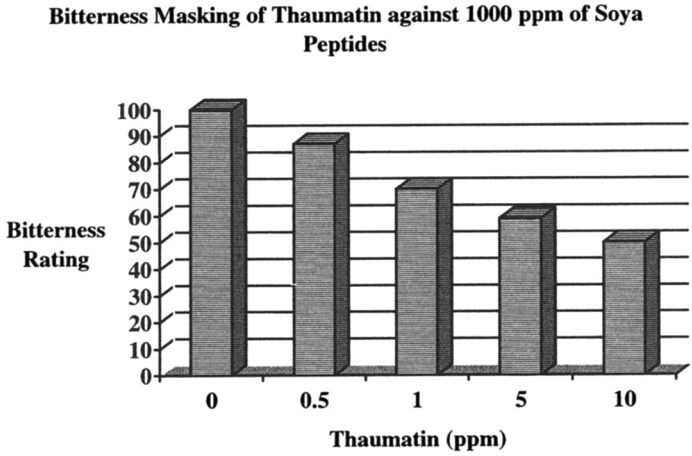

Figure 8 *Bitterness masking of Thaumatin*

A good example of this in a successful application is the use of Thaumatin in combination with a Lemon Flavour in beverage systems. This combination of Lemon Oil based flavour in an aqueous environment exposed to light and air will almost certainly lead to oxidation of the aldehydes present to give a cardboard like off-note. This character is easily detected and will give the final beverage an unacceptable short shelf life often of only a few weeks.

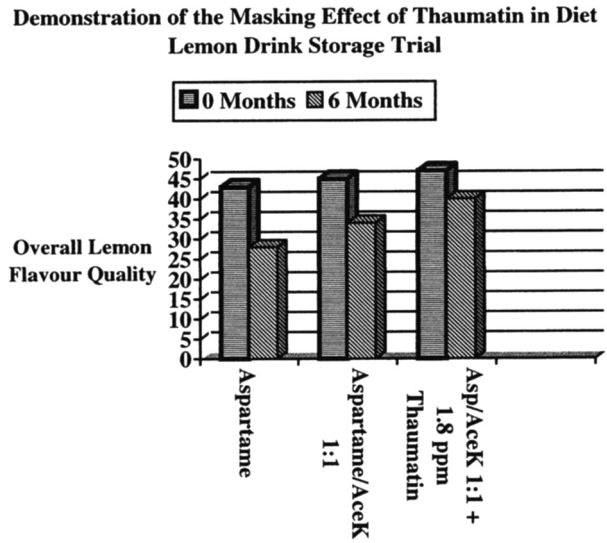

Figure 9 *Off-note flavour masking with Thaumatin*

Using Thaumatin in the sweetener system works by masking these off-notes. Although it does not prevent the oxidation of the Citral, by preventing the detection of the degradation products it enables the product to be perceived as acceptable and thus lengthens the shelf life.

6 APPLICATIONS

6.1 Flavours

The use of Thaumatin at low levels (typically less than 2 ppm at use level) in flavour formulations will greatly enhance the immediate impact and the longevity of perception as well as having particular synergistic effects with specific individual components.

Especially noteworthy is the impact with Mint oils and Menthol.

6.2 Flavour Enhancers

Whilst having a flavour enhancement impact of it's own Thaumatin has been shown to have a major synergism with Monosodium Glutamate (MSG) allowing the reduction of MSG by 80% without noticeably lowering the perceived flavour enhancement effect.

6.3 Chocolate

Thaumatin has the right combination of effects to make a major impact on the flavour of Chocolate. Firstly, there is the ability to enhance the creamy nature of dairy products and boost the apparent fat content thus giving a smoother mouthfeel to the product. Add to this the masking of the bitter notes associated with chocolate and the overall improvement is significant.

6.4 Cereals

The use of Thaumatin to reduce the sugar content of children's cereals is a relatively new application but of course one with great merit. Technically the Thaumatin can be either dusted on (which may affect the appearance of the cereal) or be included in the formulation before cooking and extrusion. Thaumatin has great heat stability and may be extruded with other materials without loss of performance.

Not only can the sugar level be reduced but the cereal flavour is enhanced with the further benefits including : Improved mouthfeel when eaten with low fat milk; further flavour improvement of fruit pieces; greater appeal for chocolate flavour cereal as bitter notes are masked and creamy chocolate flavour is improved. This combination also tends to make the cereal more appealing to children.

6.5 Pet Food and Animal Feed

The use of Thaumatin in both pet food and animal feed is fairly well explored. It does appear that Thaumatin does however possess the ability to be appealing to both cats and dogs an unusual property in a pet food ingredient. It has been shown that pets prefer food with Thaumatin and can detect doses measured in parts per billion.

Thaumatin is widely used in animal feed applications as a powerful natural sweetener and flavour enhancer.

6.6 Tea and Coffee

The main components of both Tea (Caffeine and Tannic acid) and Coffee (Caffeine) are extremely bitter and astringent so one would expect Thaumatin to improve palatability by masking this bitterness. However, Thaumatin has a great impact when milk is added especially noticeable with coffee of course. Thaumatin also has a significant role to play with the latest trends in iced tea and coffee drinks and particularly with those that are dairy based, contributing to all aspects of the product through masking bitter notes to improving mouthfeel and creaminess and enhancing added flavour character.

6.7 Alcoholic Beverages

Thaumatin works well in alcoholic beverages at levels around 5 ppm it noticeably smoothes flavour characters such as gin and at higher levels contributes to sweetness. In complex mixtures, Martini and Pimm's like products, that include herbal extracts Thaumatin can help in bringing out and reinforcing character such as orris root or

In low alcohol products such as shandys or fruit juice containing new age beverages for example Thaumatin can help give the impression that there is a higher concentration of alcohol present.

6.8 Over the Counter Medicines and Pharmaceuticals

Thaumatin has proved extremely useful in masking long-lasting bitter aftertastes of active materials in oral medicines particularly those used in cough remedies and pain relief.

Reasonably high levels (220 – 500 ppm) of Thaumatin may need to be used but will prove effective with problem products such as Paracetamol, Codeine and the intensely bitter and metallic after taste left with B vitamins for example.

The flavours that tend to be used in these products, mint, menthol, aniseed wintergreen etc, also readily lend themselves to enhancement with Thaumatin.

Each formulation will have to be examined for the optimum level of Thaumatin of course since the overall effect will depend on the total active ingredients and the sweetener system being employed.

Again the non-cariogenicity of Thaumatin gives it further advantage in children's medicines.

7 CONCLUSION

Thaumatin is a very complex and intensely sweet protein, natural and safe in use, with excellent characteristics for use in flavours.

Whilst possessing a flavour of it's own at levels above 30 ppm, when it reveals the cooling, liquorice mouthfeel, at lower levels it displays great synergy with flavours and the ability to enhance the impact of a variety of flavour components and foods.

Acknowledgements & Bibliography

The practical work referred to above has been mostly carried out by Hayley Roth and Steve Pearce at Britannia Natural Products Limited and represents a summary of our current understanding of the use of Thaumatin in flavour work. However, we are indebted to those that provided the sound basis to work upon.

These people include our current colleagues at Britannia, who have provided great support and encouragement, as well as the former employees of the Talin Company and Tate & Lyle who carried out much of the early research and generated data on potential applications for Thaumatin. Much of that work is referred to below.

Please contact either of us for further information or help and advice on how to use Thaumatin in your food and flavour formulations.

For further reading and a more detailed historic and technical treatise on Thaumatin we have provided the following list of references.

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STABILITY OF THIOLS IN AN AQUEOUS PROCESS FLAVOUR

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1 INTRODUCTION

Process flavourings, which are generally prepared by thermal treatment of an aqueous solution of reducing sugars and amino acids, show considerable flavour instability in aqueous solution. To overcome this instability, process flavours are generally spray-dried. The resulting powder has sufficient flavour stability, if temperature and humidity do not get too high. The stability in water is so limited however, that a great deal of effort in planning is required, to prevent significant flavour loss, before the product is spray-dried. Especially thiols, which are essential for a good meaty character in savoury process flavours, are particularly unstable. This urged us to look in more detail at flavour instability of a model thiol containing process flavour in aqueous solution.

Thermal treatment of a solution of ribose and cysteine results in the formation of several specific character impact components with a meaty overall impression [Hofmann, 1995a and 1995b; Schieberle and Hofmann, 1996]. The use of cysteine in combination with a pentose is responsible for the formation of very potent sulphur containing meat flavour compounds, such as 2-methyl-3-furanthiol (MFT), 3-mercapto-2-pentanone (3MP), 2-mercapto-3-butanone (MB), and 2-furfurylthiol (FFT) [Hofmann, 1995a and 1995b]. Some of these compounds were similar to those identified in beef [Gasser and Grosch, 1988] and were recently quantified in several types of heated meat [Kerscher and Grosch, 1998]. In the study described here the ribose + cysteine based process flavour reported by Hofmann was used (1995a&b), as a model for meaty process flavours. As thiols are known for their reactivity/instability such as oxidation and involvement in nucleophilic or radical reactions, we assumed that the degradation of these compounds is a likely cause of the limited shelf-life or flavour instability of savoury process flavours.

The interaction of thiol and disulphide flavour compounds with food components has been studied by Mottram (1996), which indicated a.o. that these compounds undergo redox reactions with proteins. Hofmann (1996) and Guth (1995) reported model studies on the oxidative stability of odour-active thiols and disulphides, respectively. Eisenreich (1994; 1995) has reported studies on antioxidative activities of volatile sulphur-containing heterocyclic compounds confirming their reactivity in free radical oxidation reactions.

The study described in this paper was designed to determine the (in)stability of the most important character impact components in an aqueous solution of the model process flavour described by Hofmann (1995a&b), and to investigate why some of the character

impact components are unstable. A more extensive study has been published recently by Seeventer (2001).

2 EXPERIMENTAL PROCEDURES

2.1 Materials

2-Methyl-3-furanthiol and 2-mercapto-3-butanone (10% solution in triacetin) were obtained from Oxford Chemicals (Cleveland, U.K.), 2-furfurylthiol, maltol, and D(-)-ribose from Aldrich (Steinheim, Germany), L-cysteine and 2,5-dimethyl-3-methylfuranthiol from Quest International (Naarden, The Netherlands)

2.2 Analytical instruments

A Fisons 8000 series gas chromatograph type 8130, equipped with a HP-5 capillary column (50 m x 0.32 mm; 1.05 μm film), a flame ionisation detector (FID), and a Fisons AS 800 autosampler was used for GC analysis. The GC was run with an injector temperature of 225 °C and detector temperature of 250 °C. The oven temperature was programmed from 65 °C to 120 °C at 3 °C/min and from 120 to 250 °C at 40 °C/min. Chrom-Card for Windows (Fisons Instruments) was used for data handling. HRGC mass spectrometry was performed using a Finnigan MAT TSQ 70 mass spectrometer in the electron impact mode at 70 eV. Analysis of cysteine using capillary electrophoresis was performed with a Hewlett Packard 3D CE.

2.3 Procedure for the preparation of the aqueous model process flavouring

A solution of 180 mmol D-ribose and 60 mmol L-cysteine in 1800 ml phosphate buffer (0.5 M, pH 5.0) was heated in a two litre autoclave from room temperature to 130 °C in 10 min and was kept at 130 °C for 20 min, followed by rapid cooling with tap water.

2.4 Storage of the aqueous model process flavouring

Portions of 100 ml were stored in the dark at 50 °C in closed bottles both under air and argon atmosphere. At certain time intervals, after addition of maltol as the internal standard, the aqueous reaction mixtures were extracted with dichloromethane-diethyl ether 7/3 v/v (20 ml). The extract was dried (Na_2SO_4), carefully concentrated to a volume of 2 ml at room temperature and at a pressure of 200 mbar using a rotary evaporator, and finally concentrated under a stream of argon to 1.0 ml and subjected to HRGC analysis.

2.5 Storage of reconstituted model process flavourings

A stock solution in ethanol of 2-methyl-3-furanthiol, 2-mercapto-3-butanone, and 2-furfurylthiol was added to an aqueous phosphate buffered solution (0.5M; pH 5.0) to obtain a final concentration of 50 μM of each of these components. This is a concentration approximately 10 fold higher than found in the aqueous model of Hofmann (1995a and 1995b). Either ribose or/and cysteine were added in various concentrations, typically in the range of 25-250 mM. Volumes of 5 ml of these solutions were stored in the dark at 50 °C in closed glass containers (15 ml) under an air atmosphere. At certain time intervals, after

addition of maltol as the internal standard, the aqueous solutions were extracted with dichloromethane-diethylether 7/3 v/v (2.0 ml). The extract was dried (Na_2SO_4) and analysed by HRGC.

All plots of relative area against storage time showed a linear decrease, indicating zero-order or pseudo-zero order kinetics for the compounds investigated. In all storage experiments described in this report, zero-order models were used based on visual assessment and the coefficient of determination (R^2) obtained from regression analysis. The relative decrease in the starting concentration was calculated by linear regression, using the least squares method.

2.6 Deuterium exchange experiment with 2-methyl-3-furanthiol and bis(3-furanyl) disulphide

^{1}H-NMR was used to follow the deuterium exchange of 2-methyl-3-furanthiol and bis(3-furanyl) disulphide at room temperature in CH_3OD solution containing DCl during several days.

3 RESULTS AND DISCUSSION

3.1 Flavour stability of the aqueous model process flavouring

The stability in an accelerated storage test at 50 °C of some important flavour compounds in the aqueous model process flavouring [Hofmann, 1995a and 1995b; Schieberle and Hofmann, 1996] is shown in table 1 (entry 1). 2-Methyl-3-furanthiol (MFT) was found to be the most unstable component. Furthermore, an interesting observation was the fact that there was almost no difference between storage of the aqueous model process flavouring under air or argon atmosphere (data not presented here), probably due to an anti-oxidative effect of the matrix. The anti-oxidative properties of the matrix components cysteine and products formed in the Maillard reaction are well known. Entry 2 shows the results obtained from a storage experiment of a reconstituted mixture of flavour compounds in aqueous solution, using concentrations which are approximately tenfold higher than in the original process flavour. The concentrations were increased to allow more facile analysis of the components of interest.

We anticipated that the presence of residual cysteine and/or ribose could have an effect on the stability of some of the character impact components of the model process flavour. The effect of additional cysteine and ribose on the stability of the flavour impact components was therefore investigated in the reconstituted mixture (Table1, entries 3-5). Residual cysteine and ribose concentrations were determined in a freshly prepared model process flavour, and were found to be 15 mM and 25 mM, respectively.

Entries 3 and 4 of table 1 show the results obtained when the reconstituted mixture was exposed to accelerated storage conditions with addition of ribose and cysteine respectively. Comparison of entry 2 with entry 3, shows that ribose addition has no effect on stability. A stabilising effect of the addition of cysteine was clearly present (entry 4). Comparison of entry 4 with entry 5 shows that, when both ribose and cysteine are present (entry 5), an even larger stabilising effect on MFT and the furanones can be observed, than with cysteine alone.

	1[a]	2[b]	3[b]	4[b]	5[b]
	model proces flavouring	no addition	+ 250 mM ribose	+ 150 mM cysteine	+ 150 mM cysteine + 250 mM ribose
Compound	Decrease in concentration (% per day)				
2-mercapto-3-butanone (MB)	< 20	> 90	> 90	< 30	< 30
2-methyl-3-furanthiol (MFT)	< 60	> 90	> 90	< 80	< 50
2-furfurylthiol (FFT)	n.d.[c]	> 90	> 90	< 50	< 50

[a] According to the procedure of Hofmann (1995a and 1995b): a phosphate buffered (0.5 M, pH 5.0) solution of D-ribose (100 mM) and L-cysteine (33 mM) was heated in an autoclave from room temperature to 130 °C in 10 min and was kept at 130 °C for 20 min. Character impact components were formed in the order of concentrations of around 5 µM.

[b] 50 µM of each character impact component present in aqueous phosphate buffered solution (0.5 M; pH 5.0).

[c] Not determined.

Table 1. *Stability at 50 °C of five character impact components of the aqueous model process flavour (entry 1) and reconstituted models (entry 2-5): influence of ribose and cysteine on the decrease in concentration (% per day).*

Because of the stabilising effect of cysteine on the model process flavouring, the stability of the reconstituted aqueous mixture with character impact components in concentrations of 50 µM was investigated at various cysteine concentrations. Figure 1 shows the stability of 3-mercapto-2-butanone (MB), 2-furfurylthiol (FFT), and 2-methyl-3-furanthiol (MFT) at various cysteine concentrations. Interestingly the decrease in concentration of MFT versus the cysteine concentration has a clear minimum, which is different from what is observed for MB and FFT. The stability of MFT shows an optimum at a cysteine concentration of around 50 mM.

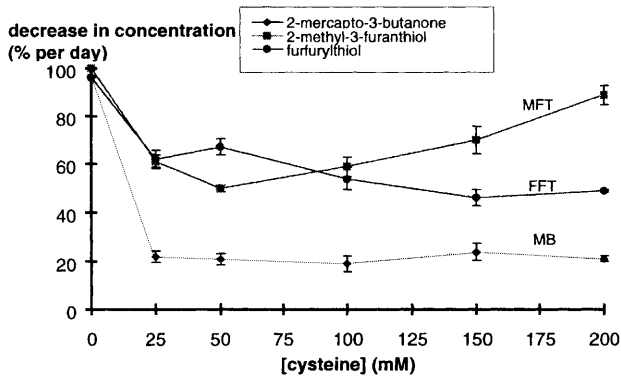

Figure 1. *Stability of a reconstituted model process flavouring: influence of [cysteine] at pH 5.0 (0.5 M phosphate).*

Based on these results we suggest that the observed stabilisation of MFT by additional ribose (see table 1; entries 4 and 5) was caused by the decrease in the cysteine concentration due to the formation of the thiazolidinecarboxylic acid derived from cysteine and ribose. The

actual concentration of cysteine after addition of ribose (250 mM) will be lower than 150 mM, as a result of the formation of this thiazolidinecarboxylic acid.

The results shown in figure 1 suggest that the mechanism of degradation of MFT in the presence of cysteine is slightly different from that of MB and FFT. Apart from an oxidative degradation pathway via (mixed) disulphide formation, another pathway leading to non-volatile or polar degradation products by reaction of MFT with a.o. cysteine may be possible, which would account for the decreased stability of MFT at higher cysteine concentration.

Evidence for the exceptional reactivity of MFT came from the storage of a mixture of only the three thiols MB, FFT, and MFT in phosphate buffered solution (0.5 M; pH 5.0) in the absence of cysteine. The amount of MB and FFT at the beginning was equal to the total amount of both thiols and (mixed) disulphides at the end of storage. This was absolutely not the case for MFT. After storage a large amount of MFT was not detected, neither as thiol nor as (mixed) disulphide, using GC-analysis. When the same experiment was performed with 2,5-dimethylfuranthiol (DMFT) instead of MFT, the total amount of DMFT at the beginning was equal to the total amount of both DMFT and (mixed) disulphides at the end of storage (data not presented).

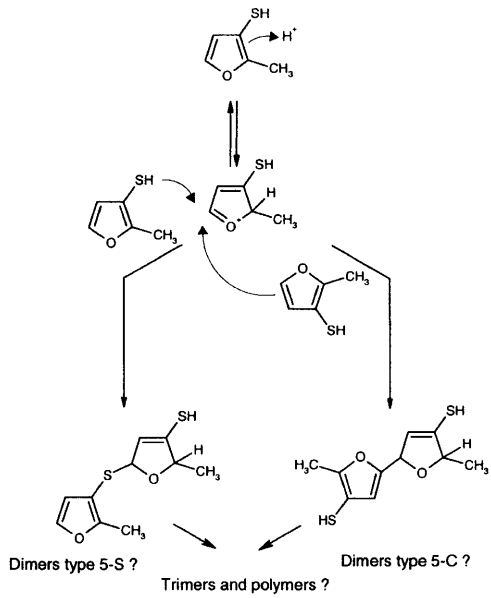

Figure 2. *Proposed mechanism for polymerisation of 2-methyl-3-furanthiol*

We think this can be explained by the greater ability of MFT to undergo oligomerisation/polymerisation. We know that MFT easily undergoes polymerisation, when stored neat at ambient temperature, as some of the commercial samples already contain considerable amounts of polymeric material. Therefore, we hereby propose a hypothetical mechanism for the polymerisation of MFT as shown in Figure 2, which can explain these observations. Protonation at the 2-position leads to an electrophilic species, which would easily react with a nucleophile. As the thiol group of MFT is a good nuclophile, protonation would catalyse dimerisation/polymerisation. Apart from the reaction of MFT with itself, other thiols, when present in a large enough concentration, could react as well, e.g. cysteine. This mechanism could explain the different effect of the cysteine concentration on the stability of

MFT in comparison to other thiols such as MB and FFT (Figure 1). In the case of DMFT, polymerisation at the 5-position would be more difficult due to steric hindrance caused by the 5-methyl group.

If the left part of the mechanism is of practical importance, coupling with other thiols would be expected as well. Since in the reconstituted mixture experiments no loss of the other thiols was observed, we must conclude that this part of the proposed mechanism is not significant.

3.2 Deuterium exchange experiment with MFT and bis(3-furanyl) disulphide

To confirm our hypothesis of the degradation mechanism of MFT in aqueous solution, a deuterium exchange experiment with MFT was performed. Using ^{1}H-NMR, some proton-deuterium exchange was observed at the 4-position, but to a much larger extent at the 5-position (fig. 3) when MFT was kept in CH_3OD solution containing DCl at room temperature. This is in agreement with the preference of electrophilic substitution at the position next to the hetero-atom of aromatic heterocycles. However, except for the formation of the corresponding disulphide, no evidence for the formation of dimers was found, as was expected according to the mechanism in figure 2. An explanation for this could be that in the model NMR experiment a methanolic solution had to be used instead of an aqueous phosphate buffered (0.5 M; pH 5) solution.

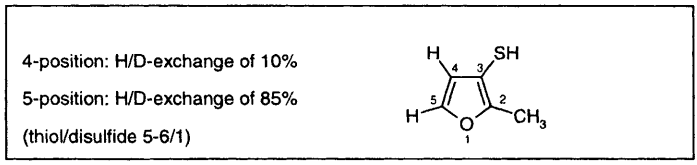

Figure 3. *Deuterium exchange experiment with 2-methyl-3-furanthiol in CH_3OD/DCl during 64 days at room temperature.*

To obtain deuterium exchange data for the 2-positon of 3-furanthiols, the synthesis of 3-furanthiol was synthesised (Hofmann, 1995a) according to figure 4. Deuterium exchange experiments with this compound could provide evidence for the proposed mechanism of the degradation of MFT (fig. 2). Some difficulties were encountered during the isolation of 3-furanthiol. The purity of the isolated compound was not satisfactory (^{1}H-NMR), possibly caused by the inherent instability of 3-furanthiol.

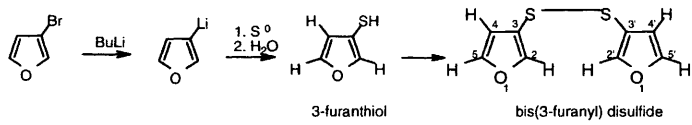

Figure 4. *Synthesis of bis(3-furanyl) disulphide.*

However, bis(3-furanyl) disulphide was isolated in a pure form and was used for the deuterium exchange experiment. The high exchange rate at the 2- and 2'-positions was remarkable, when compared to that of the other positions (fig. 5). The preference for exchange at the 2- and 2'-positions is the result of the directing and activating effect of the S-atom linked to C-3 and C-3'. These results suggest that 2-protonation is facile in MFT, and is possibly a crucial step in the degradation of MFT as shown in figure 2. Addition of nucleophiles to the 5-position of protonated MFT will most likely proceed via an ionic mechanism. Nevertheless, other mechanisms (e.g. radical mechanism) could play a role in the degradation of MFT.

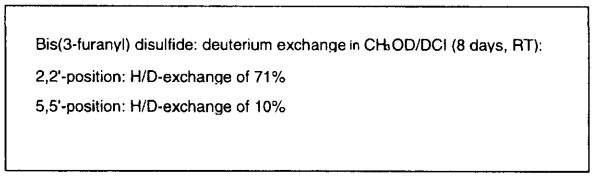

Figure 5. *Deuterium exchange experiment with bis(3-furanyl) disulphide.*

4 CONCLUSIONS

The data presented indicates that in meat process flavours based on cysteine and ribose, 2-methyl-3-furanthiol is the one of least stable character impact components. The instability is not due to disulphide formation, but appears to result from electrophilic coupling reactions, resulting in products which cannot be detected by GC. H-D exchange showed that H-5 exchanges much faster than any other proton in MFT, suggesting that the reactivity of the 5-position is responsible for the rapid decomposition of MFT. This is in agreement with the lower stability of MFT in comparison with 2,5-dimethyl-3-furnathiol. Residual cysteine stabilises MFT and other thiols.

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HIGH IMPACT AROMA CHEMICALS

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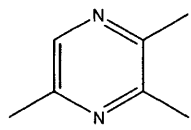
1 INTRODUCTION

The demand for 'high impact chemicals' has been driven by the twin engines of increased consumer sophistication in the market for flavours and by improvements in the analytical techniques needed to identify character impact molecules. Consumers are no longer content simply with apple and orange but look to passion fruit and mango and the availability of the fruit itself means that flavour must closely match the 'real thing'. Fortunately, developments in the flavour and fragrance industry have gone hand-in-hand with advances in the chemical sciences. From the nineteenth century, which saw the identification and synthesis of key materials such as cinnamaldehyde and vanillin, to more recent decades, advances such as the so-called 'hyphenated techniques', in particular GC-MS and GC-Olfactometry, have enabled flavour chemists to identify the compounds present in natural materials. Some of these compounds, though present at only trace levels, are key contributors to the odour and flavour of natural materials. This has been augmented recently by the use of Solid Phase Microextraction (SPME) to capture the aroma chemicals at source, such as the IFF' 'Living Flower®' and 'Living Flavour®' technologies, Givaudan's 'Taste-Trek™' studies on aroma chemicals emitted by plants in the rain forest canopy and IFF/BBA's Generessence® programme. Many of the materials identified are the 'high impact aroma chemicals' which will be discussed in this article.

The term 'high impact aroma chemical' is one which many of us can understand but for which there is no dictionary definition. I will set four key criteria which, for the purpose of discussion, will constitute a 'high impact aroma chemical';

Low odour threshold. This is an 'obvious' feature but there is no absolute definition of 'low' to which we can turn! So for the purpose here, I have set 'low odour threshold' to be less than ten parts per billion (10 ppb, or 10 in 10^9). Some apparently odorous compounds fall out by this definition; for example 2,3,5-trimethylpyrazine **1** has an odour threshold[1] of around 1000 ppb and cannot be considered 'high impact'. However the 2-alkoxy-3-alkypyrazines such as 2-methoxy-3-methylpyrazine **2**, which has an odour threshold of only 5 ppb, would constitute a high impact material by our definition.



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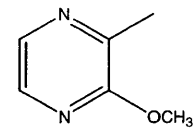

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Figure 1 *Contrasting pyrazines*

Character impact. The material should have recognisable character, even at the low levels that such a material would be used at. This criterion eliminates many esters, such as ethyl 2-methylbutyrate **3**, which has an odour threshold of only 0.1 ppb, but at low levels has only a vague 'fruitiness', which may be pleasant but not 'characteristic'.

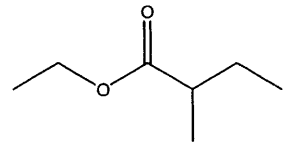

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Figure 2 *Esters, odourous but low impact?*

Desirable character impact. Although many chemicals have a powerful odour this odour is not always a desired one! For example 2,4,6-trichloroanisole **4** is a highly odorous metabolite of a fungus that attacks paper. However it is highly improbable that this can be considered a 'high impact aroma chemical' as it is unlikely that a flavourist or perfumer will have a brief to recreate the aroma or taste of mouldy books!

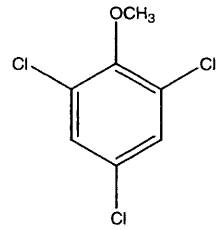

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Figure 3 *An unwanted odour*

This is of course a matter of context, as many aroma chemicals are repellent when neat or in high concentration, but in the correct context contribute to the desired effect. For example the nature of 4-mercapto-4-methyl-2-pentanone **5** can be gleaned from its' common name of 'Cat Ketone'; however, it is also a key component of sauvignon grape[2]. Depending on the context it can be used to re-create the bouquet of a fine Cabernet Sauvignon or the 'atmosphere' of where the local alley cats have marked their territory!

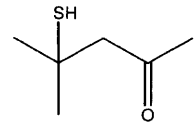

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Figure 4 *Chat ou vin?*

Available to the flavourist. There are three aspects here:

Regulatory. The material should not be 'forbidden' in the context it is to be used; for flavour use the material should be nature identical, preferably FEMA GRAS. Natural status may be important, especially for the USA where the 'nature identical' category is lacking.

Stability. Many materials can be manufactured but have insufficient 'shelf-life' to be useful in a finished flavour. For example *cis*-3-hexenal **6** is a key aroma chemical emitted by cut grass and other vegetation; it has a low odour threshold (0.25 ppb) and a powerful, desirable green character. Unfortunately it is chemically reactive, and readily rearranges to the more stable conjugated form, giving the more familiar *trans*-2-hexenal **7**. Even the 'half-life' of the isolated material may not be enough to guarantee a useful level of stability. The rearrangement is 'prototropic' and hence catalysed by both acid and base; the rate will be increased by a factor of ten for each pH unit away from its 'optimum' stability point.

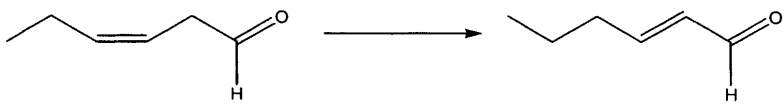

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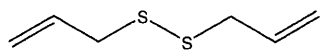
Figure 5 *Rearrangement of hexenals*

Economics. Many high impact chemicals are relatively expensive, which reflects the small market volume and the difficulties associated with manufacturing and handling such materials. However, the material must be commercially available at a price which enables a flavourist or perfumer to 'add value' to their formulation by its use; if the material is 'captive', the internal costings must not be prohibitive. In short, despite high prices, the high impact of these materials gives 'more fizz for your buck'. An example of this can be seen from garlic chemistry. The major component of garlic oil is allyl disulphide **8** (2-propenyl disulphide). The isomeric 1-propenyl disulphide **9** is also present (*cis*- and *trans*-forms) but whereas the former is readily synthesised, and hence cheap and readily available, no suitable route for large-scale preparation of the latter exists at present. Laboratory syntheses have been reported, but the costs of material made in such a way mean that any advantage in the flavour is outweighed by a vast increase in costs; the added value is insufficient.



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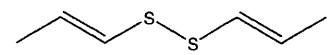

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Figure 6 *Contrasting garlic disulphides*

For the purpose of this discussion, 'high impact chemicals' are those which fulfil these criteria. Such compounds are powerful materials, highly active at low levels; their uplifting effect makes them the Viagra® of the Flavor and Fragrance Industry.

1.1 A note on identification of High Impact Aroma Chemicals

It was noted in the introduction that new analytical techniques have enabled chemists to identify aroma chemicals down to lower and lower levels. It is a tribute to the remarkable ingenuity of chemists of the 19th and early 20th centuries that it was possible to identify any materials, when the only techniques available were elemental analysis by combustion, molecular weight determination by freezing point depression / boiling point elevation, and 'proof by synthesis'. At the risk of using a cliché, "people today have it easy"! As can be seen on the 'time line' below, the identification of many powerful aroma chemicals took place during the sixties and seventies, as GC and GCMS became available.

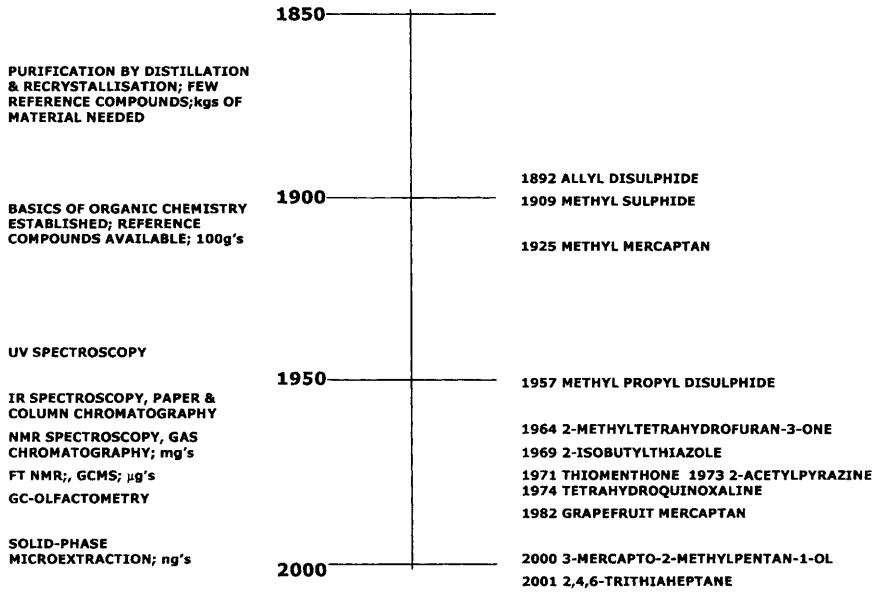

Figure 7 *A Time-Line for high impact aroma chemicals*

2 USES

High Impact Chemicals have been identified in many foodstuffs and have many applications, to the extent that a simple listing would make dull reading. However, many key flavour 'notes' are associated with high impact aroma chemicals, as illustrated by the "flavour wheel" below (figure 8), running from the sweet and fruity notes through to savoury and alliaceous. The range of chemical functionalities is also wide, with sulphur-containing molecules particularly prominent. The order in the wheel is not random; the East – West division is largely between 'sweet' and 'savoury'. Clockwise from 'truffle' to vegetable', the materials are formed by biogenesis in plants, whereas most of the remainder, in the Southern part of the wheel, are Maillard products formed in cooked foods. The materials in the 'Eastern sector' of the wheel, from 'green, grassy' to 'smoky', are of interest to the perfumer as well.

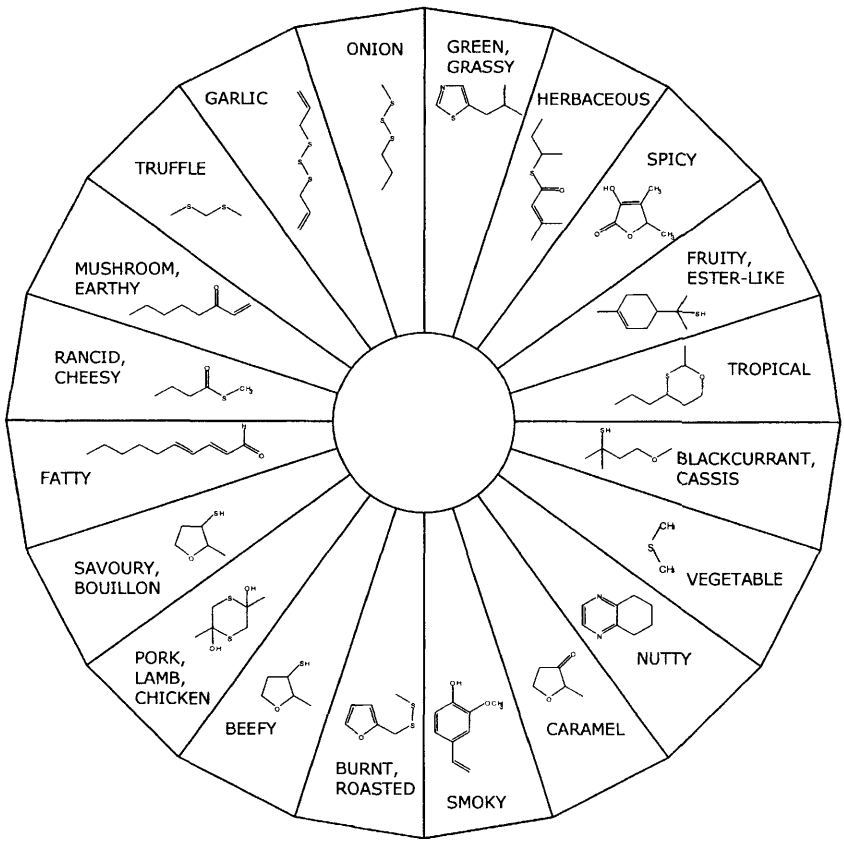

Figure 8 *A Flavour Wheel for high impact aroma chemicals*

2.1 Green, grassy

Here the traditional molecules are the 'hexenyl' compounds, the so-called C_6 wound compounds, produced by all green tissues as a consequence of the peroxide defence mechanism, where linoleic acid acts as "trap" for peroxy radicals and is in turn cleaved. As noted above, the true high impact chemical in this group is the unstable *cis*-3-hexenal **6**, the initial cleavage product of linoleic acid. The stable aroma chemicals *trans*-2-hexenal **7** (leaf aldehyde) and *cis*-3-hexenol **10** (leaf alcohol) are widely used; with odour thresholds of 17 and 70 ppb respectively, they are 'borderline cases' as high impact chemicals, though their distinctive character is in their favour. A fresh greenness is also associated with the more odorous 2-isobutylthiazole **11** (odour threshold 3 ppb). This molecule is released by tomato vine and has both tomato and more general green (string bean, geranium leaf) character, especially on dilution.

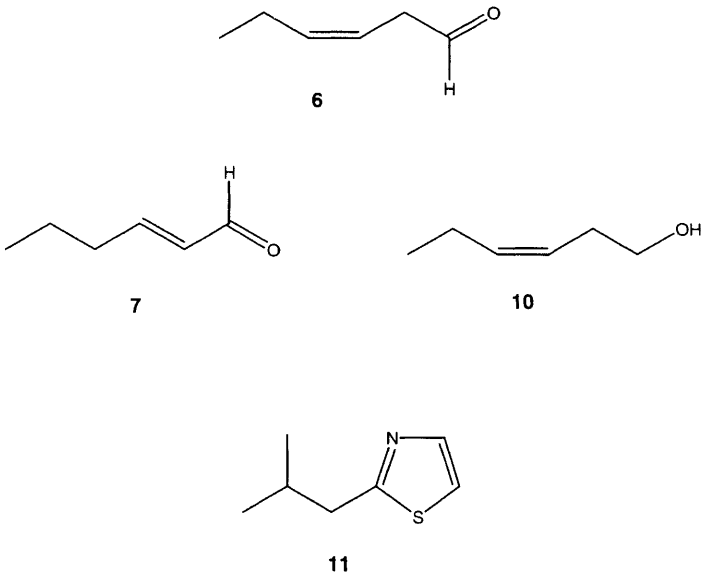

Figure 9 *Green, grassy compounds*

2.2 Herbaceous

This is a very wide-ranging concept with a large overlap with the fragrance area. A series of thioesters, typified by sec-butyl 3-methylbut-2-enethioate **12** are found in galbanum oil, and the key odourants of coriander are long-chain unsaturated aldehydes such as *trans*-2-dodecenal **13**.

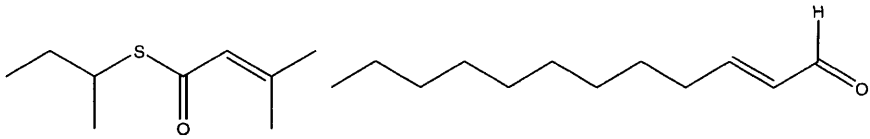

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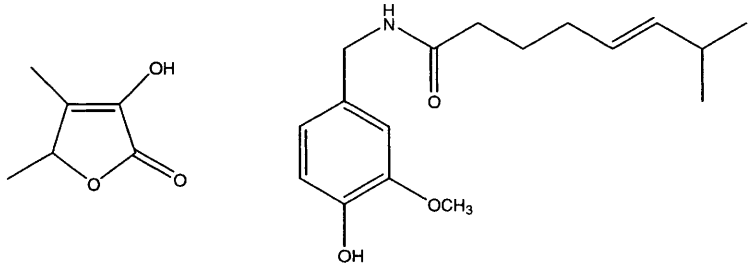
Figure 10 *Molecules with herbaceous odours*

2.3 Spicy

Spicy can refer to both 'strongly-flavoured' or 'hot'. For the former, an excellent example is the 'fenugreek lactone' or sotolone **14**, which has an intense and very persistent 'curry' aroma – it's persistence includes a remarkable tendency to linger on the skin should one have accidental contact with it! For 'hot' or 'piquant' we have capsaicin **15**; whilst it's effect is as a 'sensation' via the trigenimal system, the intensity of it's effect on the tongue (or any other part of the body it has contact with!) is sufficiently intense to consider it to be a high impact chemical!

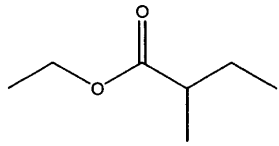


14 15

Figure 11 *Spicy chemicals*

2.4 Fruity, ester-like

The esters are obvious candidates here, but as noted above whilst many have low odour thresholds (ethyl butyrate 1 ppb, ethyl isobutyrate 0.1 ppb, ethyl 2-methylbutyrate **3** 0.1 ppb, ethyl hexanoate 1-3 ppb), they lack the character which would make them truly high impact. Instead we may illustrate fruity notes with what may be the ultimate high impact aroma chemical, *p*-1-menthen-8-thiol **16**, the Grapefruit mercaptan. This has the remarkably low threshold of ~ 10^{-5} ppb, and retains it's character even at low levels. At high concentrations, the molecule simply has a sulphurous, almost rubbery odour common to mercaptans, and requires dilution to at least 0.001% before the fresh grapefruit juice character can be recognised. As an illustration of what an odour threshold of 10^{-5} ppb means, one tonne of grapefruit mercaptan could odourise 100,000 km^3 of water, a Great Lakes volume of liquid!



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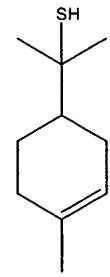

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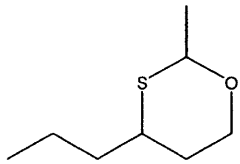
Figure 12 *Fruity molecules*

2.5 Tropical

This is one of the most important areas for high impact aroma chemicals. Analysis of passionfruit and durian has shown the presence of many powerful sulphur compounds, a large number of which were included in FEMA's GRAS 18 list in 1998. Possibly the best known is tropathiane, 2-methyl-4-propyl-1,3-oxathiane **17**,(odour threshold ~3 ppb) ; 3-mercapto-1-hexanol **18** and a number of acylated derivatives were included in FEMA's GRAS 18 list, as were thioesters such as the thiohexanoate **19** and thioisovalerate **20**.



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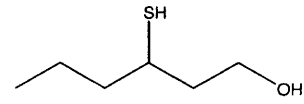

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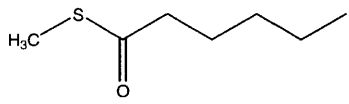

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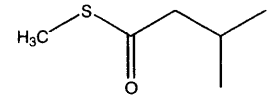

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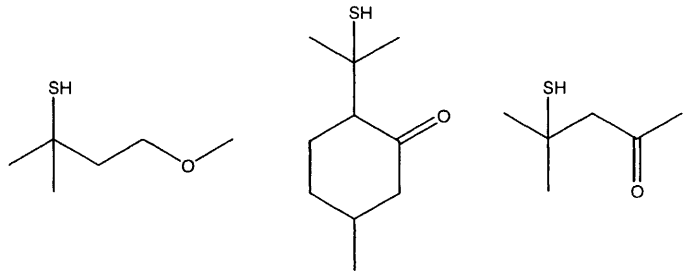
Figure 13 *Sulphur compounds for tropical notes*

2.6 Blackcurrant

This is a very popular flavour in Europe associated with many health-related products ('nutraceuticals' or functional foods) and with alcoholic drinks (Cassis liqueur, and added as a 'cordial' to some spirits). The key material in blackcurrant is 2-methoxy-4-methyl-4-butanethiol **21**; it is also a key component contributing a fruity flavour to olive oil[3]. Two other materials have been used to recreate the rather catty note of blackcurrant; p-menthathiolone **22**, the main odour-active ingredient of Buchu leaf oil, and 4-mercapto-4-methyl-2-pentanone **5**, the 'Cat Ketone' mentioned earlier;



21 22 5

Figure 14 *'Catty' mercaptans for blackcurrant*

2.7 Vegetable

This is obviously rather a large category! A compound of major importance is the ubiquitous dimethyl sulphide **23** (DMS, methyl sulphide, odour threshold ~3 ppb). When pure, this has a clean, crisp sweetcorn odour. Some material on the market lacks this note and has unpleasant, sulphurous, rotten cabbage odours; GCMS on such material has shown the presence of dimethyl disulphide and methyl ethyl sulphide. Other powerful compounds for vegetable note are 3-methylthiopropanal **24** (methional, odour threshold 0.2 ppb) and its homologue 3-methylthiobutanal **25**. On a more specific note, we should mention 2-isobutyl-3-methoxypyrazine **26**, the 'bell pepper', main character impact compound found in green or bell peppers, with it's very low odour threshold of 0.002 ppb.

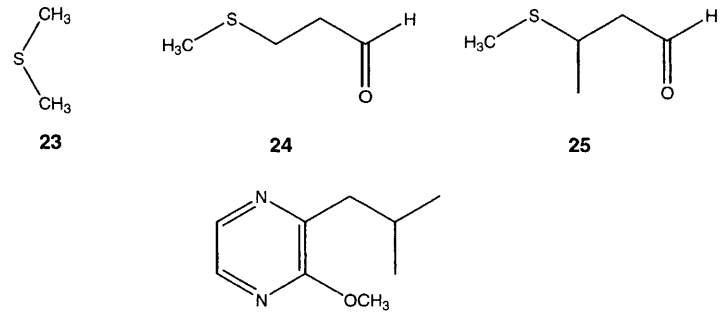


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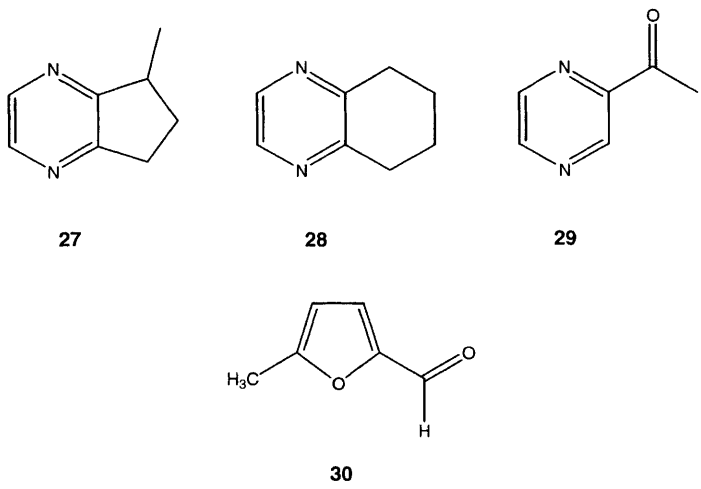
Figure 15 *Molecules for vegetable aromas*

2.8 Nutty

'Nuttiness' is associated with pyrazines; whilst it is part of the character of almost all pyrazines, it is particularly associated with the 'higher' pyrazines such as methyldihydrocyclopentapyrazine **27** ('Maple lactone pyrazine) and 5,6,7,8-tetrahydroquinoxaline **28** (THQ). 2-Acetylpyrazine **29** is very reminiscent of popcorn; whilst it's odour threshold is rather high at 62ppb, its persistent character earns it membership of the 'high impact club'. Some pyrazines are present in the 'raw' nut, whereas others are formed in roasted nuts by the Maillard reaction between amino-acids

and sugars. This also generates furans, such as 5-Methylfurfural **30**, with an almond, marzipan aroma.



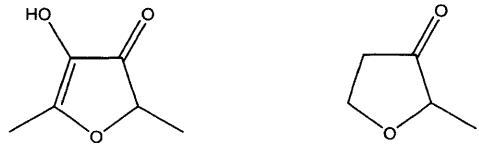
27 **28** **29**

30

Figure 16 *"Nutty" aroma chemicals*

2.9 Caramel

Caramelisation occurs on the heating of sugars and carbohydrates. This is the typical Maillard reaction sequence, which generates furans with caramel odours. The ubiquitous hydroxydimethylfuranone **31** has a sweet, 'cotton-candy' aroma and a low odour threshold of 0.04ppb. 2-Methyltetrahydrofuran-3-one **32** (coffee furanone) is less odorous but has a very pleasant, sweet caramel character.

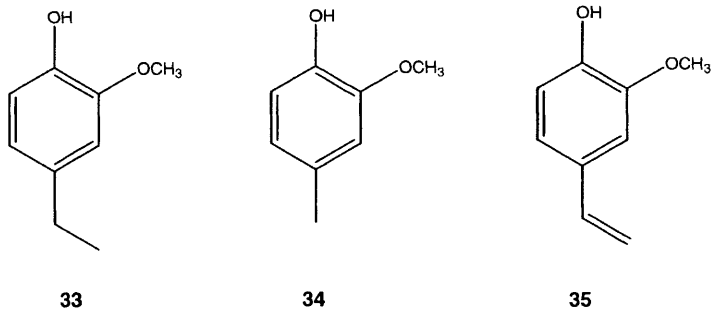


31 **32**

Figure 17 *Molecules for 'caramel' odours*

2.10 Woody, smoky

Guaiacols are very important in this area. 4-Ethyl- and 4-methylguaiacols, **33** and **34**, have rather phenolic, medicinal odours with threshold value of 90 and 50 ppb respectively, but more important is 4-vinylguaiacol **35** (2-methoxy-4-vinylphenol, MVP). This has a spicy, clove-like smokiness particularly associated with smoked ham, and a low odour threshold of only 3 ppb. It is also available in a natural form.

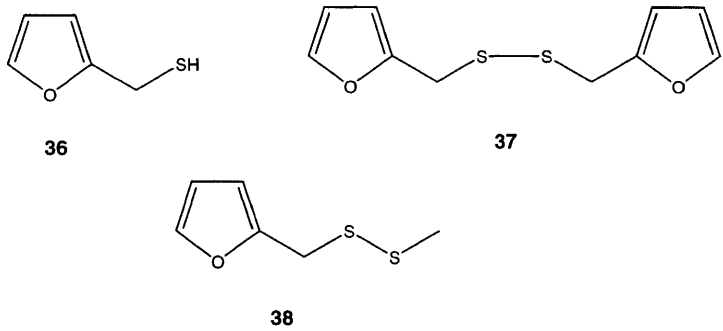


33 34 35

Figure 18 *Guaiacols for smoke flavours*

2.11 Roasted, burnt

This sector is associated with cooked food; in this and the following sectors the high impact chemicals are those produced in the Maillard Reaction. These are 'secondary' products formed from reaction of 'primary' Maillard products such as Strecker aldehydes with sulphur sources such as cysteine. Shieberle has used the term 'advanced' or 'extended' Maillard products for such materials. For roasted and burnt notes derivatives of furfuryl mercaptan **36** are paramount. The mercaptan itself, with an odour threshold of 0.005ppb, was the first high impact aroma chemical to be identified. It exhibits one of the classic phenomena associated with high impact chemicals, the change in the nature of the odour with concentration. At low concentrations (0.01-0.5ppb) the material has a roasted, coffee aroma, becoming burnt and sulphurous in the range 1-10ppb. The neat material has no coffee odour, only an unpleasant oily smell resembling gasoline. Derivatives of furfuryl mercaptan tend to be somewhat less odorous; the disulphide **37** (dithiodimethylenedifuran) is much less 'obnoxious', and the mixed disulphide furfuryl methyl disulphide **38** has a pleasant sweet coffee (Mocha) aroma; the latter has an odour threshold of 0.04ppb.



36 37

38

Figure 19 *Furfuryl mercaptan derivatives for coffee and roasted notes*

2.12 Meaty, beefy

This is the province of 2-methylfuran-3-thiol **39** (MFT) and its derivatives. The thiol, it's disulphide **40**, mixed disulphide **41** and thioether **42** have all been found in beef; the odour threshold of the disulphide has been reported as being as low as 2 x 10^{-5} ppb, but our

experience of working with these materials indicates that this odour threshold may be due to residual thiol. MFT itself has an initially rather chemical odour, becoming more meaty on dilution. The disulphide has more recognisable character, a rich 'aged beef', 'prime rib' aroma. The GRAS 19 thioether **42** has more 'roasted' character.

Other mercaptans have beef character. 3-Mercapto-2-butanone **43** and 3-mercapto-2-pentanone **44** are commonly found in 'beef' Maillard reactions; the latter has an odour threshold of 0.7ppb.

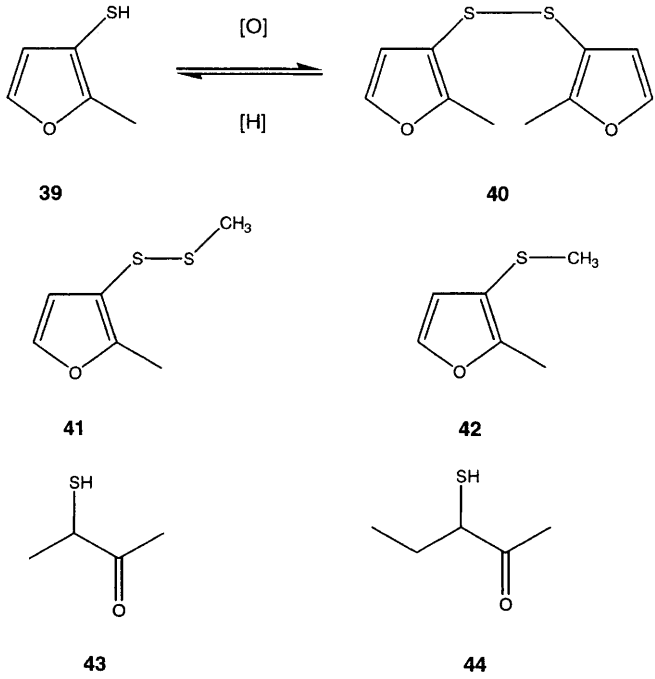


Figure 20 *Sulphur compounds for beef*

2.13 Other Meats

Whilst 2-methyl-3-furanthiol is important in other meats as well as beef, in particular pork, other high impact chemicals also occur. Mercaptopropanone dimer **45** has an intense 'chicken broth' odour and the unsaturated aldehyde *trans*-2-*trans*-4-decadienal **46** is very reminiscent of chicken fat. The latter has been implicated in the observation that whereas 2-methyl-3-furanthiol **39** has been found in chicken, its intensely beefy disulphide **40** is not found or is at a much lower level. It has been proposed that this be due to oxidants being scavenged by unsaturated aldehydes such as **46** and hence not being available for the oxidation of **39** to **40**. 2,5-Dimethylfuranthiol **47** has been reported to be present in chicken, but other work has failed to confirm this. The author's experience of the this material is that it is more prone to oxidation than 2-methyl-3-furanthiol, and this may be preventing its detection. When a sample of neat **47** is left in the laboratory exposed to the air it rapidly becomes cloudy due to droplets of water formed as the by-product of aerial oxidation. A compound with excellent pork character is pyrazineethanethiol **48**. This has not yet been reported in nature, and may be an 'analytical quirk'. Since vinylpyrazine **49**

has been found in pork (and other meats), and pork is rich in sulphur compounds, including hydrogen sulphide, it is difficult to see how **48** can't be formed!

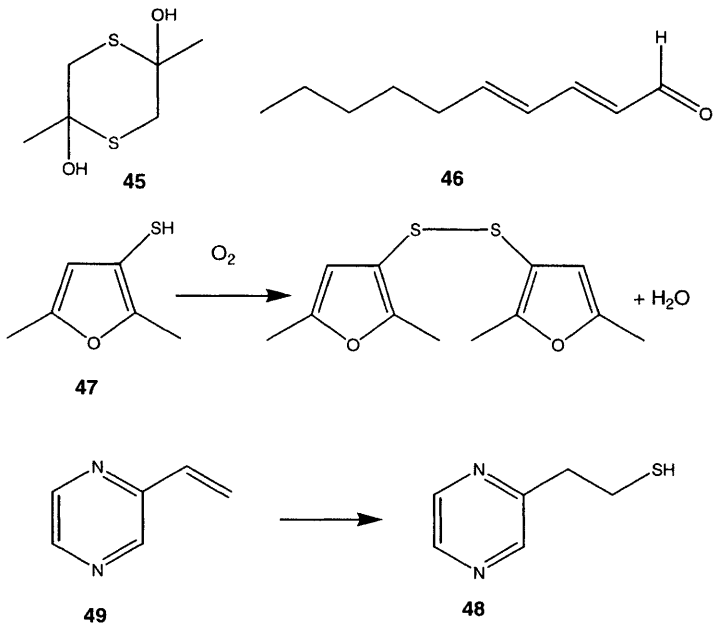


Figure 21 *Pork chemistry*

Lamb character is associated with two acids, 4-methyloctanoic **50** and 4-methylnonanoic acid **51**; whilst these have the higher odour thresholds of other carboxylic acids, their sharp-fatty aromas give them at least honorary membership of the high impact club!

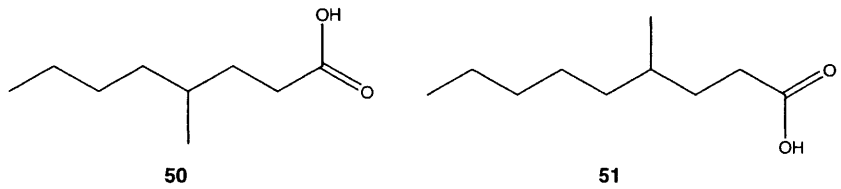
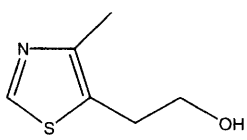


Figure 22 *Lamb acids*

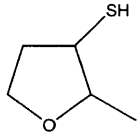
2.14 Bouillon, HVP

The aroma chemical associated with this is 4-methylthiazole-5-ethanol **52** (Sulfurol). However, it has a reported odour threshold of over 10,000ppb, so is scarcely a high impact chemical. It is also a well-known phenomenon that apparently identical batches of sulfurol have different odours, with the desirable 'meaty' note not always present. A possible candidate for this impurity is 2-methyltetrahydrofuran-3-thiol **53**. This is an intensely 'savoury' molecule, with brothy, casserole, boiled meat notes and even allium overtones.

Its carbon, oxygen-sulphur framework is actually the same as that in sulfurol; it may be a degradation product or a by-product formed during the synthesis of sulfurol.



52



53

Figure 23 *Savoury, bouillon compounds*

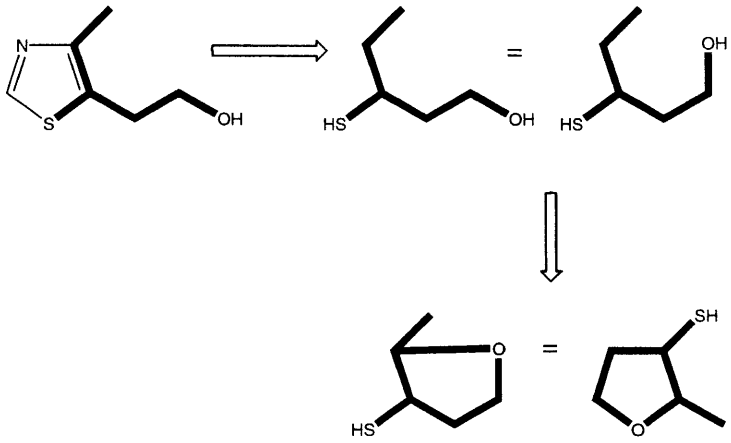


Figure 24 *Structural relationship between sulphurol and 2-methyltetrahydrofuran-3-thiol*

2.15 Fatty

Not at first sight the most desirable of characters, but fattiness is key character in foodstuffs (as those forced to eat low-fat or reduced fat foodstuffs know to their cost) in terms of both flavour and 'mouth-feel'. Aldehydes have very fatty notes, in particular *trans*-2-nonenal **54** and *trans*-2-*trans*-4-decadienal **46**; the latter is reminiscent of chicken fat and has an odour threshold of 0.07 ppb. A molecule with great potential in this area is 12-methyltridecanal **55**. This 'tallowy' material is found in beef fat and appears to originate from micro-organisms in the rumen of cattle[4]. It is absorbed by the gut as plasmalogens, and released only when the beef is heated over a long period, e.g. stewing; briefly roasting the meat does not release this chemical. Hence, with the use of this material we have the potential to create a boiled or stewed beef flavour well differentiated from roasted or fried beef.

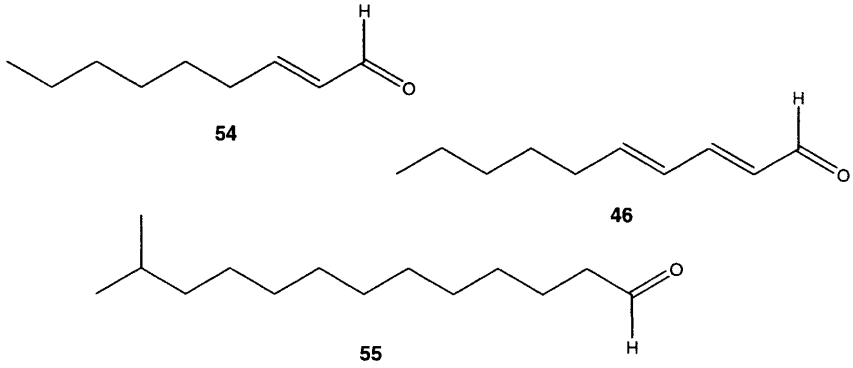


Figure 25 *Fatty aldehydes*

2.16 Cheesy, rancid

'Cheesiness', desirable or otherwise, is often associated with acids, but these have quite high odour thresholds, e.g. valeric acid **56**, which has a nauseating sweaty-cheesiness at high concentration, but also the mercifully high odour threshold of 3000 ppb! However, such is the character of these that the impact is greater than the odour threshold might imply. Unsaturated acids such as *trans*-2-hexenoic acid **57** have more powerful, acrid odours; several *trans*-2-enoic acids (*trans*-2-hept, oct- and non-enoic acid) were included on the GRAS 19 list. Simple thioesters such as methyl thiobutyrate **58** and methyl (2-methyl)thiobutyrate **59** also have an intense cheesy-sweet-fruity odour;

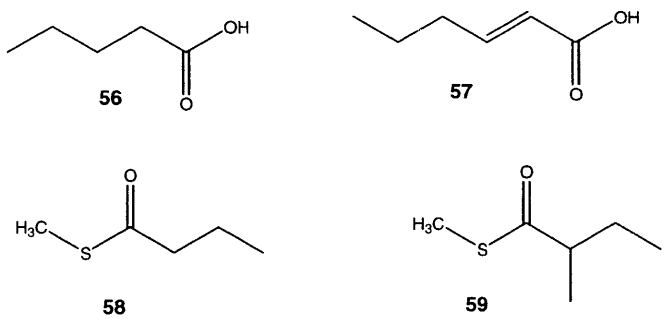
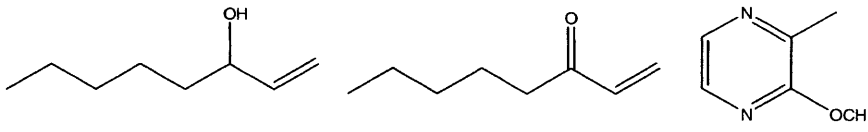


Figure 26 *Molecules for cheesy, rancid notes*

2.17 Mushroom, earthy

Here we have a 'classical' high impact aroma chemical, 1-octen-3-ol **60**, with an odour threshold of only 1ppb and very characteristic of mushroom. However, this is not the whole story as the related 1-octen-3-one **61** has a threshold some two hundred times lower at only 0.05ppb! This has a very fresh wild mushroom aroma. It has also been identified as a powerful odorant in materials as diverse as elder flower[5], raspberry and chocolate. 'Earthiness' is also associated with some pyrazines, especially 2-methyl-3-methoxypyrazine **2**.

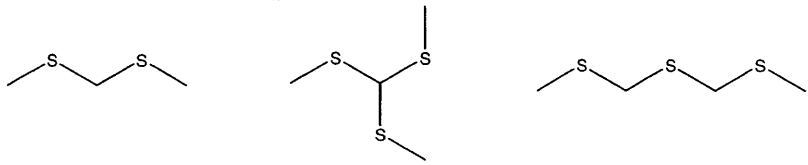


60 61 2

Figure 27 *Compounds for mushroom and earthy aromas*

2.18 Truffle

The black truffle is perhaps the most select member of the fungal food family. Whilst it contains familiar volatiles such as 1-octen-3-ol **60**, the key character impact material is bis(methylthio)methane (2,4-dithiapentane, truffle sulphide) **62**. This has the very powerful earthy-alliaceous aroma associated with the truffle; also present is tris(methylthiomethane) (3-methylthio-2,4-dithiapentane, methylidynetris(methyl sulphide), 'manxane') **63**, with an aroma more reminiscent of the white truffle. Also very recently identified in white truffle is the isomer of **63**, 2,4,6-trithiheptane **64**.

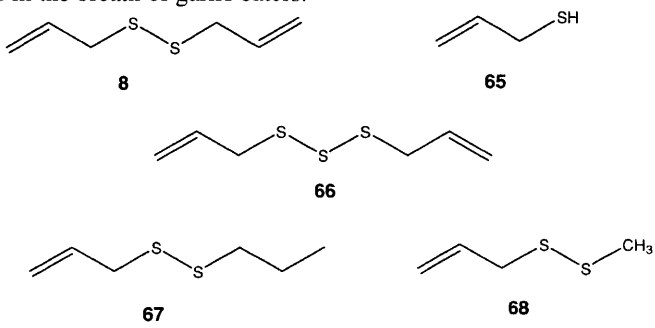


62 63 64

Figure 28 *Truffle volatiles*

2.19 Garlic

Garlic is rich in sulphur compounds, especially allyl compounds; indeed, the commonly used term 'allyl' for 'prop-2-enyl' derives from *allium sativum* or garlic. The major component of garlic oil is allyl disulphide **8**, with the mercaptan **65** and higher sulphides such as the trisulphide **66** and mixed disulphides such as **67** and **68** also present. Allyl methyl disulphide **68** is particularly 'pungent' and has been detected at unexpectedly high concentrations in the breath of garlic eaters.



8 65 66 67 68

Figure 29 *Aroma chemicals for garlic*

2.20 Onion

As with garlic, onion is high in sulphur compounds, but mostly these are saturated compounds such as the methyl and propyl sulphides **69** – **73**. These have less harsh, 'sweeter' notes compared to the allyl compounds. Recently two new highly odorous mercaptans were identified in onion[6], 3-mercapto-2-methylpentan-1-ol **74**, an onion- and leek-like material with an odour threshold of 0.15 ppb, and 3-mercapto-2-methylpentanal **75**, more pungent and meaty, with an odour threshold of 0.95 ppb.

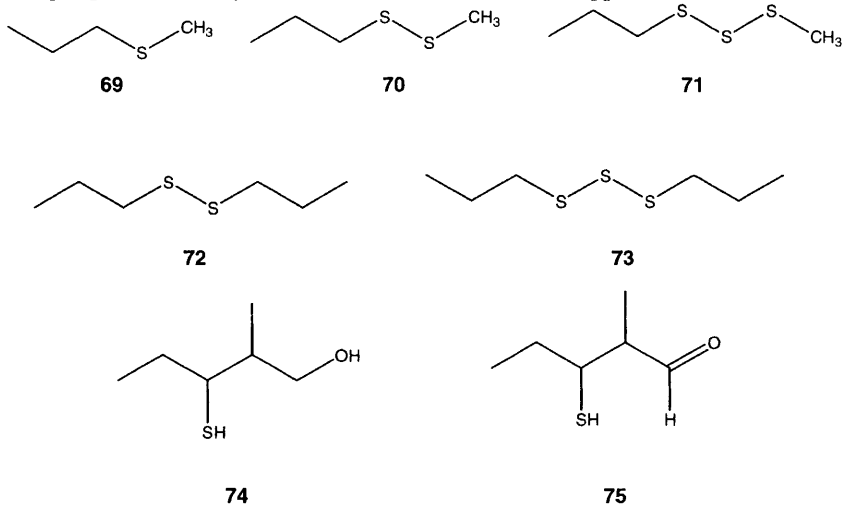


Figure 30 *Old and new molecules from onion*

Some of the higher allium sulphides may be formed by chemical transformations; when unsymmetrical disulphides are treated with base, 'disproportionation' takes place to form a statistical mixture of the symmetrical and unsymmetrical disulphides. This is presumed to occur via nucleophilic attack on the disulphide bond, as shown below. Traces of thiols are excellent catalysts for this reaction, which gives the possibility of this being facilitated by cysteine in foodstuffs and flavours;

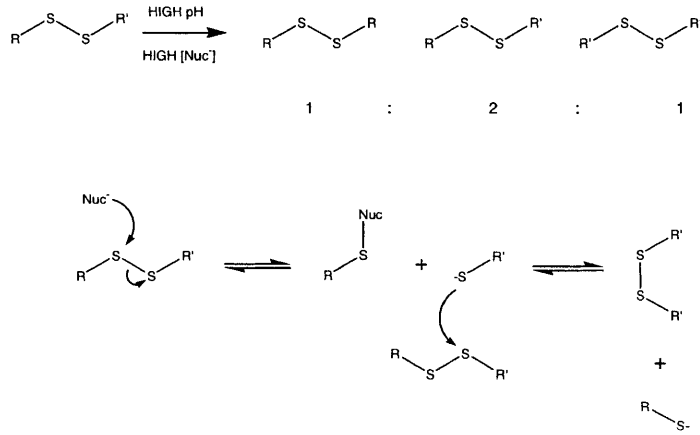


Figure 31 *Disproportionation of disulphides*

Trisulphides are even more sensitive, rapidly forming a symmetrical mixture of di-, tri-, and tetra-sulphides at pH 9, presumably by a similar pathway;

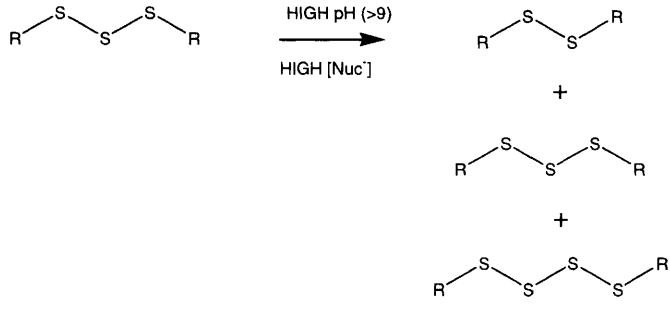
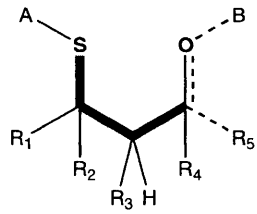


Figure 32 *Disproportionation of trisulphides*

3 THE "STINKOPHORE"; STRUCTURE-ODOUR RELATIONSHIPS IN HIGH IMPACT AROMA CHEMICALS

Very few structure-odour relationship studies have been conducted in the flavour area, in part because the usage of materials in this area is dominated less by activity and more by the issue of 'nature-identical' (and/or natural). There is little value in designing the world's 'truffliest' molecule if, in the end, it cannot be used. We might also comment that nature has done rather well in making high impact chemicals herself anyway! However, there are some conclusions that we can draw. The first is the 'Tropical' olfactophore, the 1,3-oxygen-sulphur relationship. We see this in many powerful tropical, fruity and vegetable aroma chemicals;



A=H, SCH_3, ring
B=H, CH_3, Acyl, absent if carbonyl
R_1, R_2 =H, alkyl,
R_3 =H, alkyl, ring
R_4=H, CH_3, ring, OR
R_5=H, absent if carbonyl

Figure 33 *The tropical olfactophore*

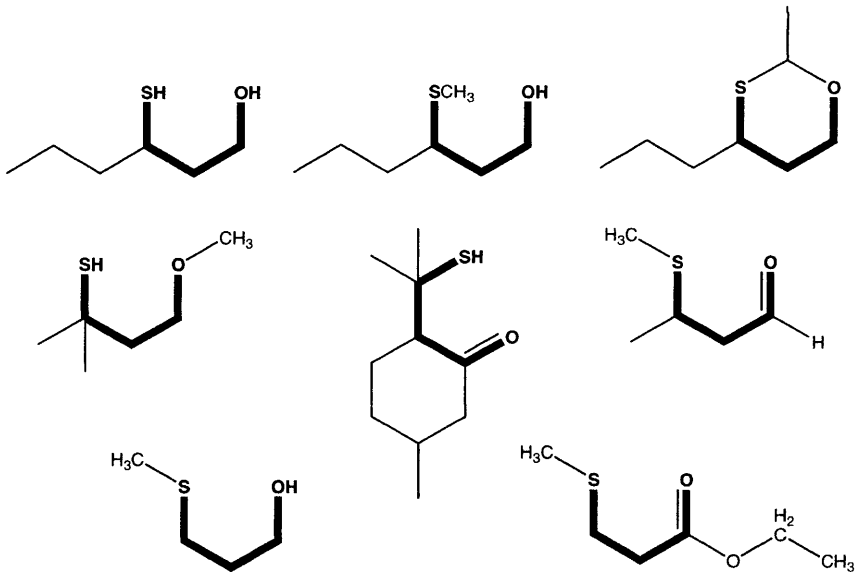


Figure 34 *Molecules showing the tropical olfactophore*

The high frequency with which we see this functionality probably reflects the biosynthetic route, i.e. Michael addition of a sulphur nucleophile to an α,β,-unsaturated carbonyl compound followed by further transformations (reduction, acylation, alkylation) at both the sulphur and oxygen functionality's.

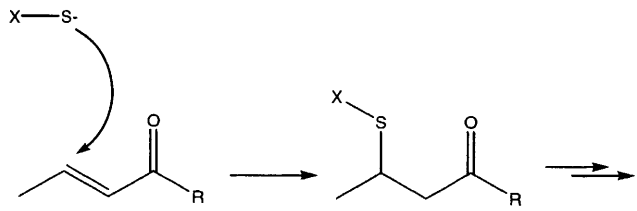


Figure 35 *Biosynthesis of the tropical olfactophore*

Interconversion between functionalities on the oxygen and sulphur takes place readily. When S-acetyl-3-mercaptohexanal (**76**) is reduced with sodium borohydride, the O-acetyl alcohol **78** is produced along with the expected S-acetyl compound **77**, and smaller amounts of the de- and di-acylated products **79**, **80**;

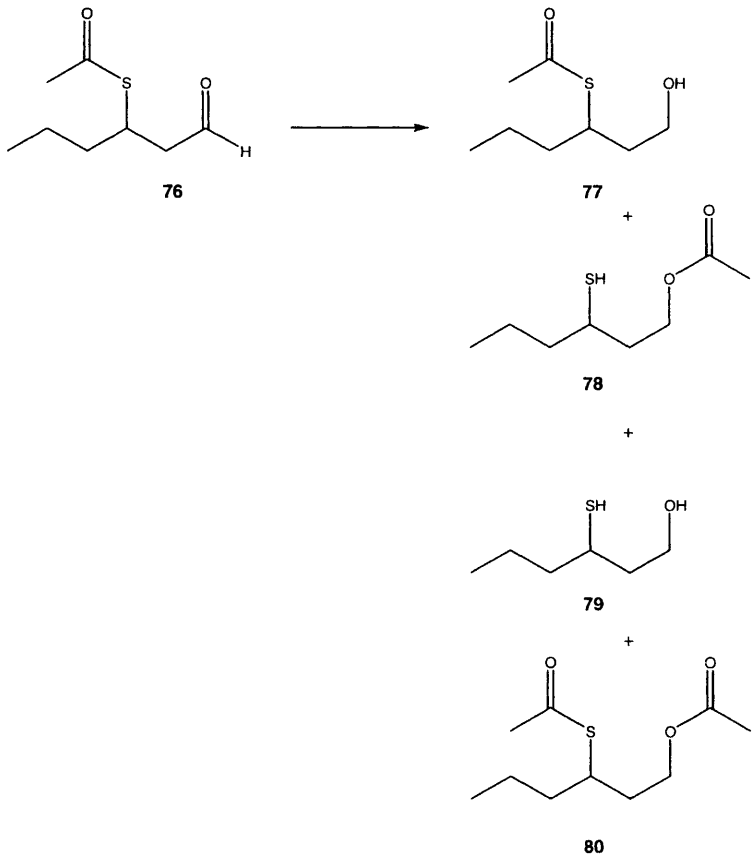


Figure 36 *An unexpected acyl transfer*

This "OAc-SAc Shuffle" takes place due to the facile formation of the 6-membered transition state;

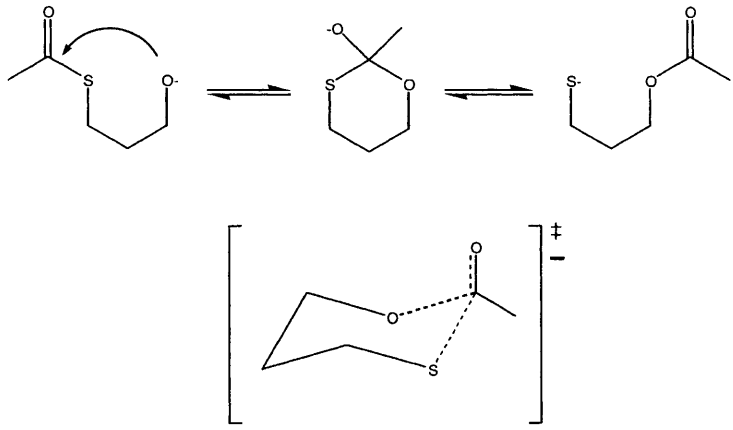
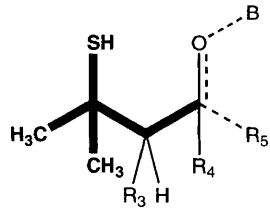


Figure 37 *The OAc-SAc shuffle*

We have a more specific olfactophore for the catty, blackcurrant area, where the three key compounds all have a tertiary ("1,1-Dimethyl") mercaptan;



A=H, SCH_3, ring
B=H, CH_3, Acyl, absent if carbonyl
R_3 =H, alkyl, ring
R_4=H, CH_3, ring, OR
R_5=H, absent if carbonyl

Figure 38 *The catty olfactophore*

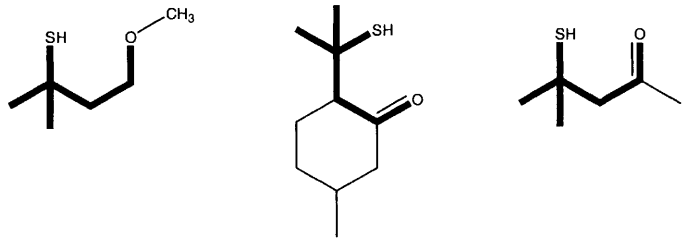
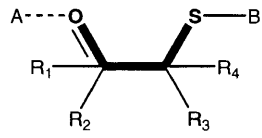


Figure 39 *Molecules showing the catty olfactophore*

This leads us to the possibility of building a molecule smelling more like cat's urine than anything nature has produced, should we so desire...

A more tentative olfactophore is the 1,2-oxygen, sulphur relationship in meaty, savoury compounds, usually formed as "advanced" Maillard products;



A=ring, absent if carbonyl
B=H, CH_3, Acyl, SR
R_1=ring, absent if carbonyl
R_1, R_2, R_3=ring, H, alkyl

Figure 40 *The savoury olfactophore*

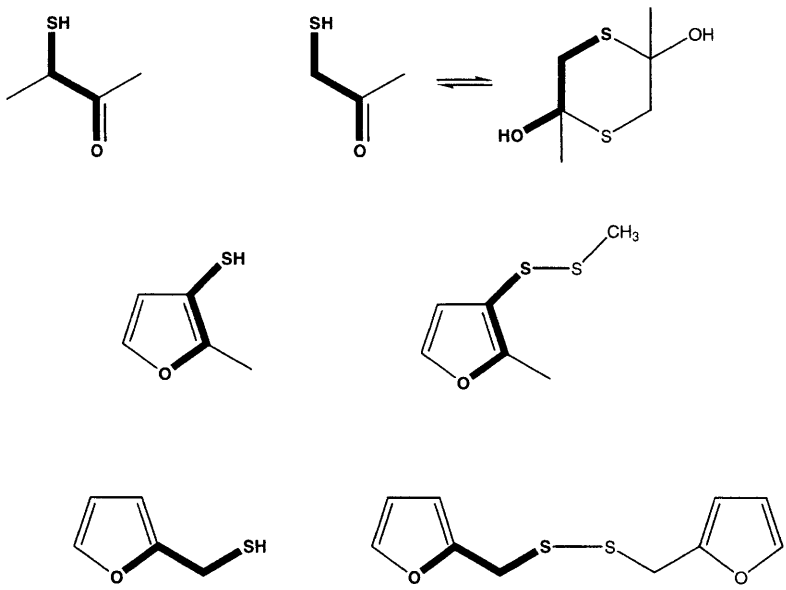
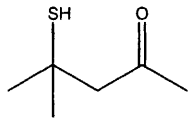


Figure 41 *Molecules showing the savoury olfactophore*

4 THE FUTURE

There are three areas where developments are continuing. The first is in synthetic chemistry; materials that are interesting but too expensive for use at present may become available at a 'useable' price due to the discovery of a viable synthetic route. The cycle of discovery, synthesis, and manufacture with falling prices has occurred since the 19th century work on cinnamaldehyde and vanillin. A second area involves further analytical work, which may be in examination of new 'exotic' foodstuffs or re-evaluation of familiar materials. For example, the cat ketone **5** was recently found to be an unexpectedly important odourant in grapefruit[7]. We are now able to go 'further down in the noise' on a gas chromatography and identify materials at lower levels but even higher odour thresholds. For example dimethyl selenide (**81**) has been detected in garlic and garlic breath[8]. Handling and manufacture of such materials may require 'glove-box' techniques more familiar from radiation chemistry! The third area that may have an important effect on the usage of high impact aroma chemicals is research being carried out on delivery systems. A number of very interesting aroma chemicals are also highly reactive and have a short half-life in normal formulations. Systems which can trap and release such materials could enable their use for the first time; examples include the related 2-acetyl-1-pyrroline **82** (basmati rice)[9] and 2-acetyltetrahydropyridine **83** (bread crust);



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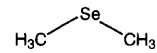
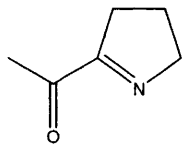
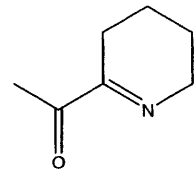


81



82



83

Figure 42 *Future high impact aroma chemicals?*

5 A NOTE ON 'ASSOCIATIONS'

Whilst the human nose is an unsophisticated instrument compared with that of some animals, it remains a more powerful organ that we sometimes realise. It appears to have a 'hot line' to the brain, and our ability to associate odours with people and with places is well known. The 'impact' of some of the materials discussed in this paper makes them very effective in this and some of the 'associations' that people have made when shown these materials are 'greengrocers' for 2-isobutylthiazole, presumably due to it's tomato notes, 'the cinema' for 2-acetylpyrazine (via it's popcorn odour!) and 'fields at 6a.m.' for 1-octen-3-one. Some twenty-five years ago the commentator had a job starting early in the morning and would go out to pick mushrooms in an adjacent field. Some associations are very personal and depend on very individual circumstances; whereas most commented on the blackcurrant, fruity aroma of 4-methoxy-2-methyl-2-butanethiol, to a colleague with a six month old baby, it was wet diapers.

6 WHY?

A final question that may be asked is simply 'why are we so sensitive to these aroma chemicals?'

The functions of our senses of smell and taste are threefold;

1. To find/attract a mate
2. To find/identify food
3. To avoid toxins

It is actually quite difficult clearly explain our responses in these simple terms.

1. To find/attract a mate

This is probably the least relevant here! Whilst the extreme sensitivity of insects to sex pheromones is well studied, the fact that these aroma chemicals are found in foodstuffs is something of a complication. Sexual attraction between an animal and its food is unlikely to be a successful evolutionary strategy.

2. To find/identify food

At first sight this is the 'obvious' explanation! However, most of the high impact aroma chemicals are formed only when food is cooked. Since the cooking of food is of very recent provenance in evolutionary terms, it is unlikely that we have evolved any physical features to respond to this. Some high impact materials are found in fruits, but again, the chemical may only be released when the fruit is actually being eaten. For example allyl disulphide and the other allium sulphides are only released only when the tissues of the garlic clove have been damaged, and a cow certainly doesn't smell of roast beef!

3. To avoid toxins

There are three main sources of toxins;
Those present in the environment
Those produced as an organisms waste
Those produced by the decomposition of food.

It is this latter area which gives a clue that this may be the cause of the response to these high impact chemicals. The group of compounds to which we have the greatest sensitivity is mercaptans, which are produced by the decay of cysteine and methionine in proteins. This may be the origin of our response to the simple materials such as hydrogen sulphide, methyl mercaptan and simple alkyl thiols. The enhanced response to mercaptans such as 2-methyl-3-furanthiol and p-menthene-8-thiol may simply be that these happen to trigger the receptors more easily. This is a coincidental response and not a specific 'design'. To use an analogy from pharmaceutical chemistry, morphine happens to fit our endorphin receptors in the brain with great efficacy, but it is not suggested that we have evolved to develop morphine addiction!

Our response to these molecules appears to have a very 'primitive' origin; we have yet to meet an individual with specific anosma to these materials. The ability to respond to chemicals in our surroundings, is the primary sense. We now differentiate taste and smell, but to the simplest organisms it is as one. Even the simplest and most primitive organisms, the prokaryotic bacteria and archea, have this sense. This leads us to a fascinating possibility; there is much evidence that life evolved in a sulphur-rich environment, where a sulphur compound would be a nutrient or a toxin, depending on concentration. Does our love for roast beef and truffles have its ultimate origins in the days when the only course on the menu was the primordial soup?

7 CONCLUSION

As the great 17th Century philosopher Rene Decartes might have said, "odorato ergo sum".

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