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Medicinal Natural Products

A Biosynthetic Approach

Second Edition

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Paul M Dewick

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*Dedicated to the many hundreds of
pharmacy students I have taught over
the years, who, unknowingly, have
encouraged me to write this text*

CONTENTS

1	ABOUT THIS BOOK, AND HOW TO USE IT	1
	The Subject	1
	The Aim	1
	The Approach	2
	The Topics	2
	Be Selective	2
	To Learn, or to Understand?	3
	Conventions Regarding Acids, Bases, and Ions	3
	Nomenclature	3
	Some Common Abbreviations	4
	Further Reading	4
2	SECONDARY METABOLISM: THE BUILDING BLOCKS AND CONSTRUCTION MECHANISMS	7
	Primary and Secondary Metabolism	7
	The Building Blocks	8
	The Construction Mechanisms	12
	Alkylation Reactions: Nucleophilic Substitution	12
	Alkylation Reactions: Electrophilic Addition	13
	Wagner–Meerwein Rearrangements	15
	Aldol and Claisen Reactions	15
	Schiff Base Formation and the Mannich Reaction	18
	Transamination	20
	Decarboxylation Reactions	20
	Oxidation and Reduction Reactions	24
	Dehydrogenases	24
	Oxidases	25
	Mono-oxygenases	25
	Dioxygenases	27
	Amine Oxidases	28
	Baeyer–Villiger Oxidations	28
	Phenolic Oxidative Coupling	28
	Glycosylation Reactions	29
	Some Vitamins Associated with the Construction Mechanisms	30
	Vitamin B ₁ , Vitamin B ₂ , Vitamin B ₅ , Vitamin B ₆ , Vitamin B ₁₂ , Vitamin H	
	Further Reading	33
3	THE ACETATE PATHWAY: FATTY ACIDS AND POLYKETIDES	35
	Saturated Fatty Acids	36
	Unsaturated Fatty Acids	40
	Fixed Oils and Fats, Evening Primrose Oil	
	Acetylenic Fatty Acids	47
	Branched-Chain Fatty Acids	48
	Echinacea	

Prostaglandins	51
<i>Prostaglandins, Isoprostanes</i>	
Thromboxanes	58
<i>Thromboxanes</i>	
Leukotrienes	58
<i>Leukotrienes</i>	
Aromatic Polyketides	60
Cyclization: Simple Phenols	61
Structural Modifications: Anthraquinones	63
<i>Senna, Cascara, Frangula, Allied Drugs, Hypericum/St John's Wort</i>	
C-Alkylation Reactions	72
<i>Mycophenolic Acid, Khellin and Cromoglicate</i>	
Phenolic Oxidative Coupling	76
<i>Griseofulvin</i>	
Oxidative Cleavage of Aromatic Rings	78
Starter Groups Other Than Acetate	79
<i>Poison Ivy and Poison Oak, Aflatoxins, Cannabis, Tetracyclines, Anthracycline</i>	
<i>Antibiotics</i>	
Macrolides and Polyethers	92
Extender Groups other than Malonate	92
<i>Macrolide Antibiotics, Avermectins, Polyene Antifungals, Tacrolimus and Sirolimus,</i>	
<i>Ansa Macrolides</i>	
Cyclization Through Diels–Alder Reactions	111
<i>Mevastatin and other Statins</i>	
Genetic Manipulation of the Acetate Pathway	114
Further Reading	117
 4 THE SHIKIMATE PATHWAY: AROMATIC AMINO ACIDS AND	
PHENYLPROPANOIDS	121
Aromatic Amino Acids and Simple Benzoic Acids	121
<i>Folic Acid (Vitamin B₉), Chloramphenicol</i>	
Cinnamic Acids	130
Lignans and Lignin	132
<i>Podophyllum</i>	
Phenylpropenes	135
<i>Volatile Oils Containing Principally Aromatic Compounds</i>	
Benzoic Acids from C₆C₃ Compounds	141
Coumarins	142
<i>Dicoumarol and Warfarin, Psoralens</i>	
Styrylpyrones	147
<i>Kava</i>	
Flavonoids and Stilbenes	149
Flavonolignans	151
<i>Silybum marianum</i>	
Isoflavonoids	154
<i>Phyto-oestrogens, Derris and Lonchocarpus</i>	
Terpenoid Quinones	158
<i>Vitamin E, Vitamin K</i>	
Further Reading	164

5 THE MEVALONATE AND DEOXYXYLULOSE PHOSPHATE PATHWAYS:	
TERPENOIDS AND STEROIDS	167
Hemiterpenes (C₅)	172
Monoterpenes (C₁₀)	172
<i>Volatile Oils Containing Principally Terpenoid Compounds</i>	
Irregular Monoterpenes	186
<i>Pyrethrins</i>	
Iridoids (C₁₀)	187
<i>Valerian</i>	
Sesquiterpenes (C₁₅)	191
<i>Feverfew, Chamomile and Matricaria, Artemisia annua and Artemisinin, Gossypol, Trichothecenes</i>	
Diterpenes (C₂₀)	203
<i>Taxus brevifolia and Taxol (Paclitaxel), Ginkgo biloba, Forskolin</i>	
Sesterterpenes (C₂₅)	212
Triterpenes (C₃₀)	212
Triterpenoid Saponins	219
<i>Liquorice, Quillaia, Ginseng</i>	
Modified Triterpenoids	225
Tetraterpenes (C₄₀)	226
<i>Vitamin A</i>	
Higher Terpenoids	231
Steroids	232
Stereochemistry	232
Cholesterol	233
<i>Cholesterol</i>	
Steroidal Saponins	237
<i>Dioscorea, Fenugreek, Sisal, Sarsaparilla, Yucca</i>	
Cardioactive Glycosides	241
<i>Digitalis purpurea, Digitalis lanata, Strophanthus, Convallaria, Squill, Toxic Plants</i>	
Phytosterols	252
<i>Soya Bean Sterols, Fusidic Acid</i>	
Vitamin D	257
<i>Vitamin D</i>	
Bile Acids	260
<i>Bile Acids</i>	
Adrenocortical Hormones/Corticosteroids	262
Semi-Synthesis of Corticosteroids	264
<i>Corticosteroid Drugs</i>	
Progestogens	267
<i>Progestogen Drugs</i>	
Oestrogens	
<i>Oestrogen Drugs, Aromatase Inhibitors, Oestrogen Receptor Antagonists</i>	
Androgens	282
<i>Androgen Drugs</i>	
Further Reading	285

6 ALKALOIDS	291
Alkaloids Derived from Ornithine	292
Pyrrolidine and Tropane Alkaloids	292
<i>Belladonna, Stramonium, Hyosycamus, Duboisia, Allied Drugs, Hyoscyamine, Hyoscine and Atropine, Coca</i>	
Pyrrolizidine Alkaloids	305
Alkaloids Derived from Lysine	307
Piperidine Alkaloids	307
<i>Lobelia</i>	308
Quinolizidine Alkaloids	309
Indolizidine Alkaloids	310
Alkaloids Derived from Nicotinic Acid	311
Pyridine Alkaloids	311
<i>Vitamin B₃, Tobacco, Areca</i>	
Alkaloids Derived from Tyrosine	315
Phenylethylamines and Simple Tetrahydroisoquinoline Alkaloids	315
<i>Catecholamines, Areca, Lophophora, Curare</i>	
Modified Benzyltetrahydroisoquinoline Alkaloids	327
<i>Opium</i>	329
Phenethylisoquinoline Alkaloids	341
<i>Colchicum</i>	
Terpenoid Tetrahydroisoquinoline Alkaloids	343
<i>Ipecacuanha</i>	
Amaryllidaceae Alkaloids	345
<i>Galanthamine</i>	
Alkaloids Derived from Tryptophan	346
Simple Indole Alkaloids	347
<i>5-Hydroxytryptamine (Serotonin), Psilocybe</i>	
Simple β -Carboline Alkaloids	349
Terpenoid Indole Alkaloids	350
<i>Rauwolfia, Catharanthus, Iboga, Nux-vomica, Ellipticine</i>	
Quinoline Alkaloids	359
<i>Cinchona, Camptothecin</i>	
Pyrroloindole Alkaloids	365
<i>Physostigma</i>	
Ergot Alkaloids	368
<i>Ergot, Morning Glories</i>	
Alkaloids Derived from Anthranilic Acid	376
Quinazoline Alkaloids	377
Quinoline and Acridine Alkaloids	377
Alkaloids Derived from Histidine	379
Imidazole Alkaloids	379
<i>Pilocarpus</i>	
Alkaloids Derived by Amination Reactions	381
Acetate-derived Alkaloids	381
<i>Conium maculatum</i>	
Phenylalanine-derived Alkaloids	382
<i>Ephedra, Khat</i>	
Terpenoid Alkaloids	385
<i>Aconite</i>	

Steroidal Alkaloids	388
<i>Solanum Alkaloids</i>	
Purine Alkaloids	394
<i>Caffeine, Theobromine, and Theophylline, Coffee, Tea, Cola, Cocoa, Maté Tea, Guarana</i>	
Saxitoxin and Tetrodotoxin	396
<i>Saxitoxin, Tetrodotoxin</i>	
Further Reading	398
7 PEPTIDES, PROTEINS, AND OTHER AMINO ACID DERIVATIVES	405
Peptides and Proteins	405
Ribosomal Peptide Biosynthesis	407
Peptide Hormones	410
Thyroid Hormones	410
<i>Thyroxine, Calcitonin</i>	
Hypothalamic Hormones	411
<i>Thyrotrophin-Releasing Hormone, Luteinizing Hormone-Releasing Hormone, Growth Hormone-Releasing Hormone/Factor, Somatostatin</i>	
Anterior Pituitary Hormones	413
<i>Corticotropin, Growth Hormone, Prolactin, Gonadotrophins</i>	
Posterior Pituitary Hormones	413
<i>Oxytocin, Vasopressin</i>	
Pancreatic Hormones	416
<i>Insulin, Glucagon</i>	
Interferons	417
<i>Interferons</i>	
Opioid Peptides	417
Enzymes	419
Nonribosomal Peptide Biosynthesis	421
Peptide Antibiotics	423
<i>Cycloserine, Polymyxins, Bacitracins, Tyrothricin and Gramicidins, Capreomycin, Vancomycin and Teicoplanin, Bleomycin, Cyclosporins, Streptogramins, Dactinomycin</i>	
Peptide Toxins	433
<i>Death Cap (Amanita phalloides), Ricin, Botulinum Toxin, Microcystins, Snake Venoms</i>	
Modified Peptides: Penicillins, Cephalosporins, and Other β-Lactams	422
Penicillins	422
<i>Penicillins</i>	
Cephalosporins	444
<i>Cephalosporins, Cephameycins, Carbacephem</i>	
Other β -Lactams	450
<i>Clavulanic Acid, Carbapenems, Monobactams</i>	
Cyanogenic Glycosides	455
Glucosinolates	457
Cysteine Sulphoxides	460
<i>Garlic</i>	
Further Reading	461

8 CARBOHYDRATES	465
Monosaccharides	465
Oligosaccharides	469
<i>Monosaccharides and Disaccharides, Vitamin C</i>	
Polysaccharides	473
<i>Polysaccharides</i>	
Aminosugars	477
Aminoglycosides	478
<i>Aminoglycoside Antibiotics, Streptamine-containing Antibiotics,</i> <i>2-Deoxystreptamine-containing Antibiotics, Acarbose, Lincomycin and Clindamycin</i>	
Further Reading	486
Index	487

1

ABOUT THIS BOOK, AND HOW TO USE IT

An introductory chapter briefly describing the subject, the aim, the approach and the topics. Students are offered advice on how to select material for study, and encouraged to understand the information rather than learn the factual material. General information on ionization in biochemicals and on nomenclature is given, together with a list of common abbreviations.

THE SUBJECT

This book has been written primarily for pharmacy undergraduates to provide a modern text to complement lecture courses dealing with pharmacognosy and the use of natural products in medicine. Nevertheless, it should be of value in most other courses where the study of natural products is included, although the examples chosen are predominantly those possessing pharmacological activity.

For centuries, drugs were entirely of natural origin and composed of herbs, animal products, and inorganic materials. Early remedies may have combined these ingredients with witchcraft, mysticism, astrology, or religion, but it is certain that those treatments that were effective were subsequently recorded and documented, leading to the early Herbals. The science of pharmacognosy – the knowledge of drugs – grew from these records to provide a disciplined, scientific description of natural materials used in medicine. Herbs formed the bulk of these remedies. As chemical techniques improved, the active constituents were isolated from plants, were structurally characterized, and, in due course, many were synthesized in the laboratory. Sometimes, more active, better tolerated drugs were produced by chemical modifications (semi-synthesis), or by total synthesis of analogues of the active principles.

Gradually synthetic compounds superseded many of the old plant drugs, though certain plant-

derived agents were never surpassed and remain as valued medicines to this day. Natural drugs derived from microorganisms have a much shorter history, and their major impact on medicine goes back only about 60 years to the introduction of the antibiotic penicillin. Microbially produced antibiotics now account for a very high proportion of the drugs commonly prescribed. There is currently a renewed interest in pharmacologically active natural products, be they from plants, microorganisms, or animals, in the continued search for new drugs, particularly for disease states where our present range of drugs is less effective than we would wish. Herbal remedies are also enjoying a revival as many sufferers turn away from modern drugs and embrace ‘complementary medicine’.

THE AIM

Many modern university pharmacy courses include a pharmacognosy component covering a study of plant-derived drugs, and traditionally this area of natural products has been taught separately from the microbially derived antibiotics, or the animal-related steroidal and prostanoid drugs. These topics usually form part of a pharmaceutical chemistry course. The traditional boundaries may still remain, despite a general change in pharmacognosy teaching from a descriptive study to a phytochemical-based approach, a trend towards integrating pharmacognosy within pharmaceutical

chemistry, and the general adoption of modular course structures. A chemistry-based teaching programme encompassing all types of natural product of medicinal importance, semi-synthetic derivatives, and synthetic analogues based on natural product templates, is a logical development, and one we have practised at Nottingham for several years. This coursebook provides a suitable text for such a programme, and attempts to break down the artificial divisions.

THE APPROACH

This book establishes a groundwork in natural product chemistry/phytochemistry by considering biosynthesis – the metabolic sequences leading to various selected classes of natural products. This allows application of fundamental chemical principles and shows the relationships between the diverse structures encountered in nature, thus giving a rationale for natural products and replacing the traditional descriptive approach with one based more on deductive reasoning. Subdivision of the topics is predominantly via biosynthesis, not class or activity, and this provides a logical sequence of structural types and avoids a catalogue effect. There is extensive use of chemical schemes and mechanism, with detailed mechanistic explanations being annotated to the schemes, as well as outline discussions in the text. Extensive cross-referencing is used to emphasize links and similarities. As important classes of compounds or drugs (indicated by an asterisk) are reached, more detailed information is then provided in the form of short separate monographs in boxes, which can be studied or omitted as required, in the latter case allowing the main theme to continue. The monograph information covers sources, production methods, principal components, drug use, mode of action, semi-synthetic derivatives, synthetic analogues, etc, as appropriate. Those materials currently employed as drugs are emphasized in the monographs by the use of bold type.

THE TOPICS

A preliminary chapter is used to outline the main building blocks and the basic construction mechanisms employed in the biosynthesis of natural products. Many of these fundamental principles

should be familiar, having been met previously in courses dealing with the fundamentals of organic chemistry and biochemistry. These principles are then seen in action as representative natural product structures are described in the following chapters. These are subdivided initially into areas of metabolism fed by the acetate, shikimate, mevalonate and deoxyxylulose phosphate pathways. Remaining chapters then cover alkaloids, peptides and proteins, and carbohydrates. The book tries to include a high proportion of those natural products currently used in medicine, the major drugs that are derived from natural materials by semi-synthesis, and those drugs which are structural analogues. Some of the compounds mentioned may have a significant biological activity which is of interest, but not medicinally useful. The book is also designed to be forward looking and gives information on possible leads to new drugs. A selection of supplementary reading references is provided at the end of each chapter; these are limited as far as possible to recent review articles in easily accessible journals rather than books, and have been chosen as student friendly.

BE SELECTIVE

Coverage is extensive to allow maximum flexibility for courses in different institutions, but not all the material will be required for any one course. However, because of the many subdivisions and the highlighted keywords, it should be relatively easy to find and select the material appropriate to a particular course. On the other hand, the detail given in monographs is purposely limited to ensure students are provided with enough factual information, but are not faced with the need to assess whether or not the material is relevant. Even so, these monographs will undoubtedly contain data which exceed the scope of any individual course. It is thus necessary to apply selectivity, and portions of the book will be surplus to immediate requirements. The book is designed to be user friendly, suitable for modular courses and student-centred learning exercises, and a starting point for later project and dissertation work. The information presented is as up to date as possible, but undoubtedly new research will be published that modifies or even contradicts some of the statements made. The reader is asked always to be critical and to maintain

a degree of flexibility when reading the scientific literature, and to appreciate that science is always changing.

TO LEARN, OR TO UNDERSTAND?

The primary aim of the book is not to rely just on factual information, but to impart an understanding of natural product structures and the way they are put together by living organisms. Rationalization based on mechanistic reasoning is paramount. The sequences themselves are not important; the mechanistic explanations for the processes used are the essence. Students should concentrate on understanding the broad features of the sequences, and absorb sufficient information to be able to predict how and why intermediates might be elaborated and transformed. The mechanistic explanations appended to the schemes should reinforce this approach. Anyone who commits to memory a sequence of reactions for examination purposes has missed the point. Of course, passing exams is probably the main reason why students are prompted to read this book, and the retention of some factual information will be essential. There is no alternative to memory for some of the material covered in the monographs, but wherever possible, information should be reduced to a concept that can be deduced, rather than remembered. The approach used here should help students to develop such deductive skills.

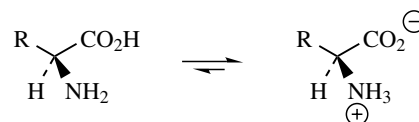
CONVENTIONS REGARDING ACIDS, BASES, AND IONS

In many structures, the abbreviation **P** is used to represent the phosphate group and **PP** the diphosphate (or pyrophosphate) group:



At physiological pHs, these groups will be ionized as shown, but in schemes where structures are given in full, the unionized acids are usually depicted. This is done primarily to simplify structures, to eliminate the need for counter-ions, and to avoid mechanistic confusion. Likewise, amino

acids are shown in unionized form, although they will typically exist as zwitterions:



Ionized and unionized forms of many compounds are regarded as synonymous in the text, thus acetate/acetic acid, shikimate/shikimic acid, and mevalonate/mevalonic acid may be used according to the author's whim and context, and have no especial relevance.

NOMENCLATURE

Natural product structures are usually quite complex, some exceedingly so, and fully systematic nomenclature becomes impracticable. Names are thus typically based on so-called trivial nomenclature, in which the discoverer of the natural product exerts his or her right to name the compound. The organism in which the compound is found is frequently chosen to supply the root name, e.g. podophyllotoxin and peltatins from *Podophyllum peltatum*. Name suffixes might be -in to indicate 'a constituent of', -oside to show the compound is a sugar derivative, -genin for the aglycone released by hydrolysis of the sugar derivative, -toxin for a poisonous constituent, or may reflect chemical functionality, such as -one or -ol. Traditionally -ine is always used for alkaloids (amines). Structurally related compounds are then named as derivatives of the original, using standard prefixes, such as hydroxy-, methoxy-, methyl-, dihydro-, homo-, etc for added substituents, or deoxy-, demethyl-, demethoxy-, dehydro-, nor-, etc for removed substituents, positioning being indicated from systematic numbering of the carbon chains or rings. Some groups of compounds, such as steroids, fatty acids, and prostaglandins, are named semi-systematically from an accepted root name. In this book, almost all structures depicted in the figures carry a name; this is primarily to help identification and, for the student, structural features are more pertinent than the names used. It will soon become apparent that drug names chosen by pharmaceutical manufacturers are quite random, and have no particular relationship to the chemical structure.

We are also currently experiencing a transitional period during which many established drug names are being changed to recommended international non-proprietary names (rINN); both names are included here, the rINN preceding the older name.

SOME COMMON ABBREVIATIONS

ACP	acyl carrier protein
ADP	adenosine diphosphate
Api	apiose
Ara	arabinose
ATP	adenosine triphosphate
B:	general base
CoA	coenzyme A as part of a thioester, e.g. acetyl-CoA (CH ₃ COSCoA)
CDP	cytidine diphosphate
CTP	cytidine triphosphate
Dig	digitoxose
DMAPP	dimethylallyl diphosphate (dimethylallyl pyrophosphate)
Enz	enzyme
FAD	flavin adenine dinucleotide
FADH ₂	flavin adenine dinucleotide (reduced)
FMN	flavin mononucleotide
FMNH ₂	flavin mononucleotide (reduced)
FPP	farnesyl diphosphate (farnesyl pyrophosphate)
Fru	fructose
Gal	galactose
GFPP	geranylfarnesyl diphosphate (geranylfarnesyl pyrophosphate)
GGPP	geranylgeranyl diphosphate (geranylgeranyl pyrophosphate)
Glc	glucose
GPP	geranyl diphosphate (geranyl pyrophosphate)
HA	general acid
HMG-CoA	β-hydroxy-β-methylglutaryl coenzyme A
HSCoA	coenzyme A
IPP	isopentenyl diphosphate (isopentenyl pyrophosphate)
LT	leukotriene
Mann	mannose

NAD ⁺	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide (reduced)
NADP ⁺	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
O	oxidation – in schemes
P	phosphate – in text
P	phosphate – in structures
PEP	phosphoenolpyruvate
PG	prostaglandin
PLP	pyridoxal 5'-phosphate
PP	diphosphate (pyrophosphate) – in text
PP	diphosphate (pyrophosphate) – in structures
Rha	rhamnose
Rib	ribose
SAM	S-adenosyl methionine
TDPGlc	thymidine diphosphoglucose
TPP	thiamine diphosphate (thiamine pyrophosphate)
TX	thromboxane
UDP	uridine diphosphate
UDPGlc	uridine diphosphoglucose
UTP	uridine triphosphate
W–M	Wagner–Meerwein rearrangement
Xyl	xylose
<i>hν</i>	electromagnetic radiation; usually UV or visible
Δ	heat

FURTHER READING

Pharmacognosy, Phytochemistry, Natural Drugs

Books

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2

SECONDARY METABOLISM: THE BUILDING BLOCKS AND CONSTRUCTION MECHANISMS

Distinctions between primary and secondary are defined, and the basic building blocks used in the biosynthesis of secondary natural products are introduced. The chemistry underlying how these building blocks are assembled in nature is described, subdivided according to chemical mechanism, including alkylation reactions, Wagner–Meerwein rearrangements, aldol and Claisen reactions, Schiff base formation and Mannich reactions, transaminations, decarboxylations, oxidation and reduction reactions, phenolic oxidative coupling, and glycosylations.

PRIMARY AND SECONDARY METABOLISM

All organisms need to transform and interconvert a vast number of organic compounds to enable them to live, grow, and reproduce. They need to provide themselves with energy in the form of ATP, and a supply of building blocks to construct their own tissues. An integrated network of enzyme-mediated and carefully regulated chemical reactions is used for this purpose, collectively referred to as **intermediary metabolism**, and the pathways involved are termed **metabolic pathways**. Some of the crucially important molecules of life are carbohydrates, proteins, fats, and nucleic acids. Apart from fats, these are polymeric materials. Carbohydrates are composed of sugar units, whilst proteins are made up from amino acids, and nucleic acids are based on nucleotides. Organisms vary widely in their capacity to synthesize and transform chemicals. For instance, plants are very efficient at synthesizing organic compounds via photosynthesis from inorganic materials found in the environment, whilst other organisms such as animals and microorganisms rely on obtaining their raw materials in their diet, e.g. by consuming plants. Thus, many of the metabolic pathways are concerned with degrading materials taken in as food, whilst

others are then required to synthesize specialized molecules from the basic compounds so obtained.

Despite the extremely varied characteristics of living organisms, the pathways for generally modifying and synthesizing carbohydrates, proteins, fats, and nucleic acids are found to be essentially the same in all organisms, apart from minor variations. These processes demonstrate the fundamental unity of all living matter, and are collectively described as **primary metabolism**, with the compounds involved in the pathways being termed **primary metabolites**. Thus degradation of carbohydrates and sugars generally proceeds via the well characterized pathways known as glycolysis and the Krebs/citric acid/tricarboxylic acid cycle, which release energy from the organic compounds by oxidative reactions. Oxidation of fatty acids from fats by the sequence called β -oxidation also provides energy. Aerobic organisms are able to optimize these processes by adding on a further process, oxidative phosphorylation. This improves the efficiency of oxidation by incorporating a more general process applicable to the oxidation of a wide variety of substrates rather than having to provide specific processes for each individual substrate. Proteins taken in via the diet provide amino acids, but the proportions of each will almost certainly vary from the organism's requirements. Metabolic pathways are thus available to

interconvert amino acids, or degrade those not required and thus provide a further source of energy. Most organisms can synthesize only a proportion of the amino acids they actually require for protein synthesis. Those structures not synthesized, so-called essential amino acids, must be obtained from external sources.

In contrast to these primary metabolic pathways, which synthesize, degrade, and generally interconvert compounds commonly encountered in all organisms, there also exists an area of metabolism concerned with compounds which have a much more limited distribution in nature. Such compounds, called **secondary metabolites**, are found in only specific organisms, or groups of organisms, and are an expression of the individuality of species. Secondary metabolites are not necessarily produced under all conditions, and in the vast majority of cases the function of these compounds and their benefit to the organism is not yet known. Some are undoubtedly produced for easily appreciated reasons, e.g. as toxic materials providing defence against predators, as volatile attractants towards the same or other species, or as colouring agents to attract or warn other species, but it is logical to assume that all do play some vital role for the well-being of the producer. It is this area of **secondary metabolism** that provides most of the pharmacologically active natural products. It is thus fairly obvious that the human diet could be both unpalatable and remarkably dangerous if all plants, animals, and fungi produced the same range of compounds.

The above generalizations distinguishing primary and secondary metabolites unfortunately leave a 'grey area' at the boundary, so that some groups of natural products could be assigned to either division. Fatty acids and sugars provide good examples, in that most are best described as primary metabolites, whilst some representatives are extremely rare and found only in a handful of species. Likewise, steroid biosynthesis produces a range of widely distributed fundamental structures, yet some steroids, many of them with pronounced pharmacological activity, are restricted to certain organisms. Hopefully, the blurring of the boundaries will not cause confusion; the subdivision into primary metabolism ($\equiv$ biochemistry) or secondary metabolism ($\equiv$ natural products chemistry)

is merely a convenience and there is considerable overlap.

THE BUILDING BLOCKS

The building blocks for secondary metabolites are derived from primary metabolism as indicated in Figure 2.1. This scheme outlines how metabolites from the fundamental processes of photosynthesis, glycolysis, and the Krebs cycle are tapped off from energy-generating processes to provide biosynthetic intermediates. The number of building blocks needed is surprisingly few, and as with any child's construction set a vast array of objects can be built up from a limited number of basic building blocks. By far the most important building blocks employed in the biosynthesis of secondary metabolites are derived from the intermediates acetyl coenzyme A (acetyl-CoA), shikimic acid, mevalonic acid, and 1-deoxyxylulose 5-phosphate. These are utilized respectively in the **acetate**, **shikimate**, **mevalonate**, and **deoxyxylulose phosphate** pathways, which form the basis of succeeding chapters. **Acetyl-CoA** is formed by oxidative decarboxylation of the glycolytic pathway product pyruvic acid. It is also produced by the β -oxidation of fatty acids, effectively reversing the process by which fatty acids are themselves synthesized from acetyl-CoA. Important secondary metabolites formed from the acetate pathway include phenols, prostaglandins, and macrolide antibiotics, together with various fatty acids and derivatives at the primary/secondary metabolism interface. **Shikimic acid** is produced from a combination of phosphoenolpyruvate, a glycolytic pathway intermediate, and erythrose 4-phosphate from the pentose phosphate pathway. The reactions of the pentose phosphate cycle may be employed for the degradation of glucose, but they also feature in the synthesis of sugars by photosynthesis. The shikimate pathway leads to a variety of phenols, cinnamic acid derivatives, lignans, and alkaloids. **Mevalonic acid** is itself formed from three molecules of acetyl-CoA, but the mevalonate pathway channels acetate into a different series of compounds than does the acetate pathway. **Deoxyxylulose phosphate** arises from a combination of two glycolytic pathway intermediates, namely pyruvic acid and glyceraldehyde 3-phosphate. The mevalonate and deoxyxylulose phosphate pathways are together

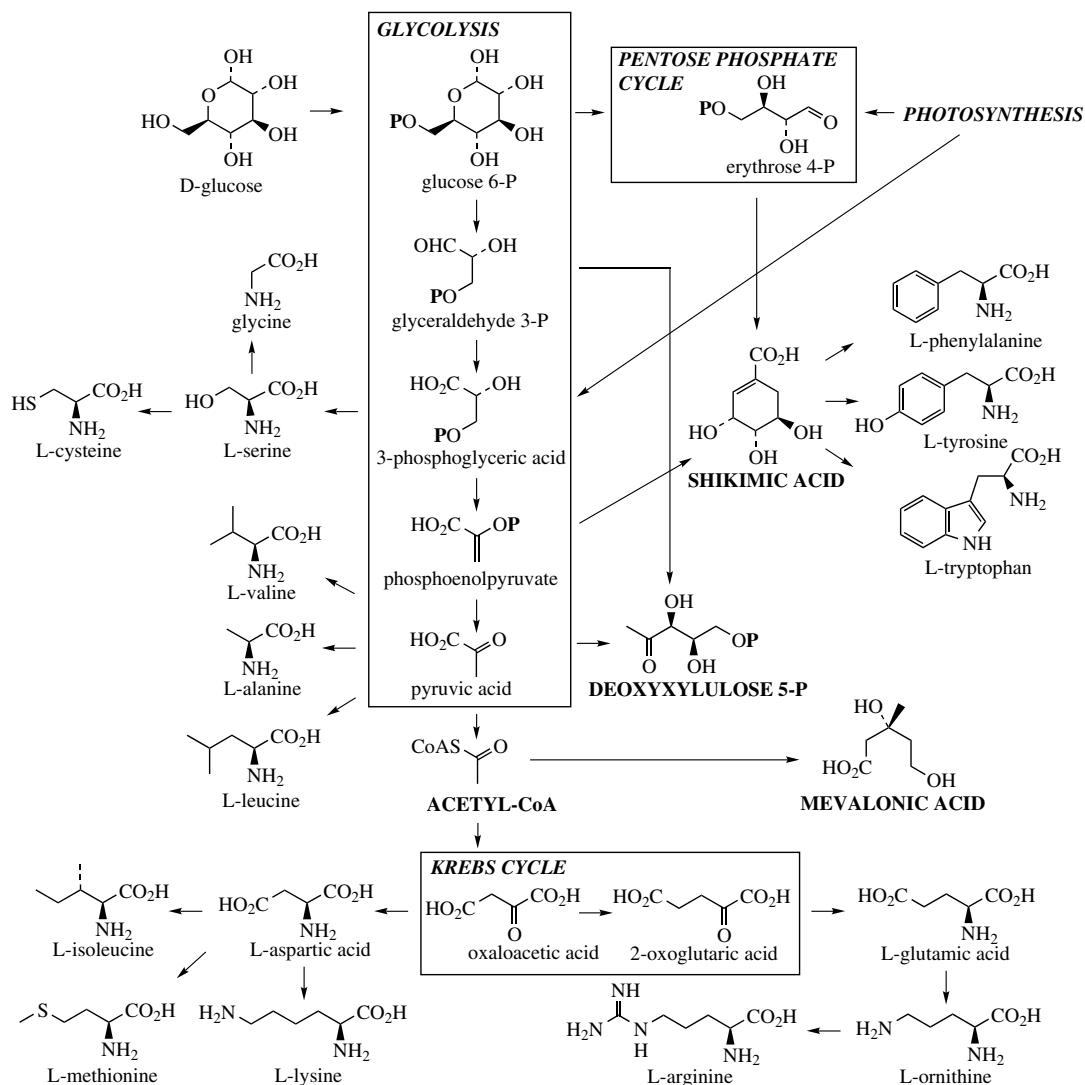


Figure 2.1

responsible for the biosynthesis of a vast array of terpenoid and steroid metabolites.

In addition to acetyl-CoA, shikimic acid, mevalonic acid, and deoxyxylulose phosphate, other building blocks based on amino acids are frequently employed in natural product synthesis. Peptides, proteins, alkaloids, and many antibiotics are derived from amino acids, and the origins of the most important amino acid components of these are briefly indicated in Figure 2.1. Intermediates from the glycolytic pathway and the Krebs cycle are used in constructing many of them, but the aromatic amino acids **phenylalanine**, **tyrosine**,

and **tryptophan** are themselves products from the shikimate pathway. **Ornithine**, a non-protein amino acid, along with its homologue **lysine**, are important alkaloid precursors having their origins in Krebs cycle intermediates.

Of special significance is the appreciation that secondary metabolites can be synthesized by combining several building blocks of the same type, or by using a mixture of different building blocks. This expands structural diversity, and consequently makes subdivisions based entirely on biosynthetic pathways rather more difficult. A typical natural product might be produced by combining elements

from the acetate, shikimate, and deoxyxylulose phosphate pathways. Many secondary metabolites also contain one or more sugar units in their structure, either simple primary metabolites such as glucose or ribose, or alternatively substantially modified and unusual sugars. To appreciate how a natural product is elaborated, it is of value to be able to dissect its structure into the basic building blocks from which it is made up, and to propose how these are mechanistically joined together. With a little experience and practice, this becomes a relatively simple process, and it allows the molecule to be rationalized, thus exposing logical relationships between apparently quite different structures. In this way, similarities become much more meaningful than differences, and an understanding of biosynthetic pathways allows rational connecting links to be established. This forms the basic approach in this book.

Relatively few building blocks are routinely employed, and the following list, though not comprehensive, includes those most frequently encountered in producing the carbon and nitrogen skeleton of a natural product. As we shall see, oxygen atoms can be introduced and removed by a variety of processes, and so are not considered in the initial analysis, except as a pointer to an acetate (see page 62) or shikimate (see page 123) origin. The structural features of these building blocks are shown in Figure 2.2.

- **C₁:** The simplest of the building blocks is composed of a single carbon atom, usually in the form of a methyl group, and most frequently it is attached to oxygen or nitrogen, but occasionally to carbon. It is derived from the *S*-methyl of **L-methionine**. The methylenedioxy group (OCH₂O) is also an example of a C₁ unit.
- **C₂:** A two-carbon unit may be supplied by **acetyl-CoA**. This could be a simple acetyl group, as in an ester, but more frequently it forms part of a long alkyl chain (as in a fatty acid) or may be part of an aromatic system (e.g. phenols). Of particular relevance is that in the latter examples, acetyl-CoA is first converted into the more reactive **malonyl-CoA** before its incorporation.
- **C₅:** The branched-chain C₅ 'isoprene' unit is a feature of compounds formed from **mevalonate** or **deoxyxylulose phosphate**. Mevalonate itself

is the product from three acetyl-CoA molecules, but only five of mevalonate's six carbons are used, the carboxyl group being lost. The alternative precursor deoxyxylulose phosphate, a straight-chain sugar derivative, undergoes a skeletal rearrangement to form the branched-chain isoprene unit.

- **C₆C₃:** This refers to a phenylpropyl unit and is obtained from the carbon skeleton of either **L-phenylalanine** or **L-tyrosine**, two of the shikimate-derived aromatic amino acids. This, of course, requires loss of the amino group. The C₃ side-chain may be saturated or unsaturated, and may be oxygenated. Sometimes the side-chain is cleaved, removing one or two carbons. Thus, **C₆C₂** and **C₆C₁** units represent modified shortened forms of the C₆C₃ system.
- **C₆C₂N:** Again, this building block is formed from either **L-phenylalanine** or **L-tyrosine**, **L-tyrosine** being by far the more common. In the elaboration of this unit, the carboxyl carbon of the amino acid is removed.
- **indole.C₂N:** The third of the aromatic amino acids is **L-tryptophan**. This indole-containing system can undergo decarboxylation in a similar way to **L-phenylalanine** and **L-tyrosine** so providing the remainder of the skeleton as an indole.C₂N unit.
- **C₄N:** The C₄N unit is usually found as a heterocyclic pyrrolidine system and is produced from the non-protein amino acid **L-ornithine**. In marked contrast to the C₆C₂N and indole.C₂N units described above, ornithine supplies not its α -amino nitrogen, but the δ -amino nitrogen. The carboxylic acid function and the α -amino nitrogen are both lost.
- **C₅N:** This is produced in exactly the same way as the C₄N unit, but using **L-lysine** as precursor. The ϵ -amino nitrogen is retained, and the unit tends to be found as a piperidine ring system.

These eight building blocks will form the basis of many of the natural product structures discussed in the following chapters. Simple examples of how compounds can be visualized as a combination of building blocks are shown in Figure 2.3. At this stage, it is inappropriate to justify why a particular combination of units is used, but this aspect should become clear as the pathways are described.

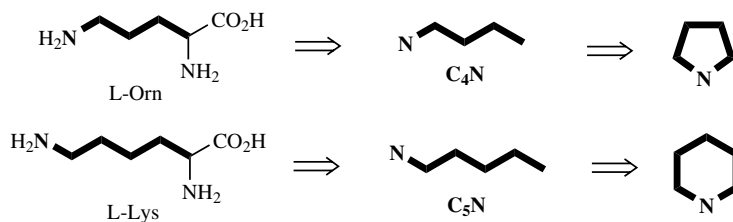
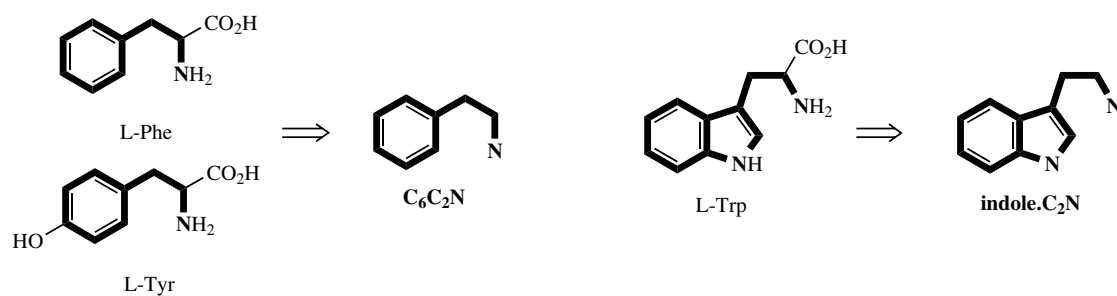
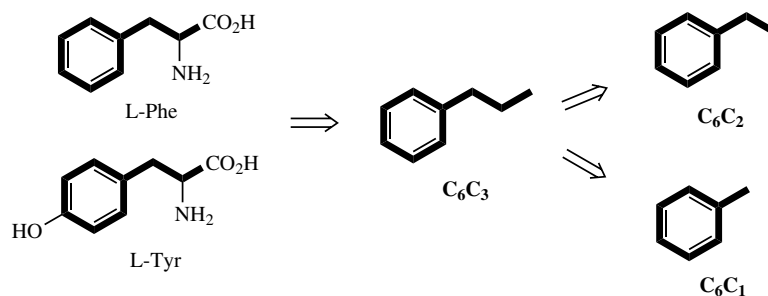
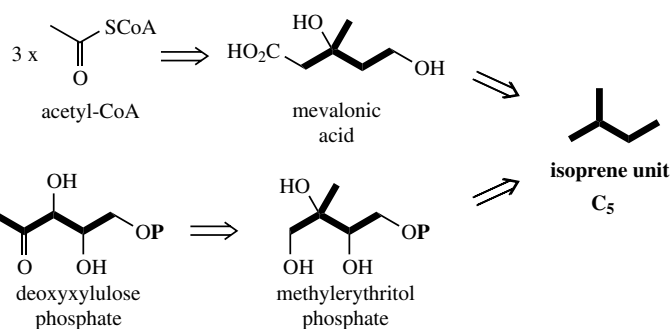
$$\begin{array}{ccc} \text{H}_3\text{C}-\text{S}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H} & \Rightarrow & -\text{X}-\text{CH}_3 \quad (\text{X} = \text{O}, \text{N}, \text{C}) \\ \text{L-Met} & & \text{C}_1 \end{array}$$


Figure 2.2

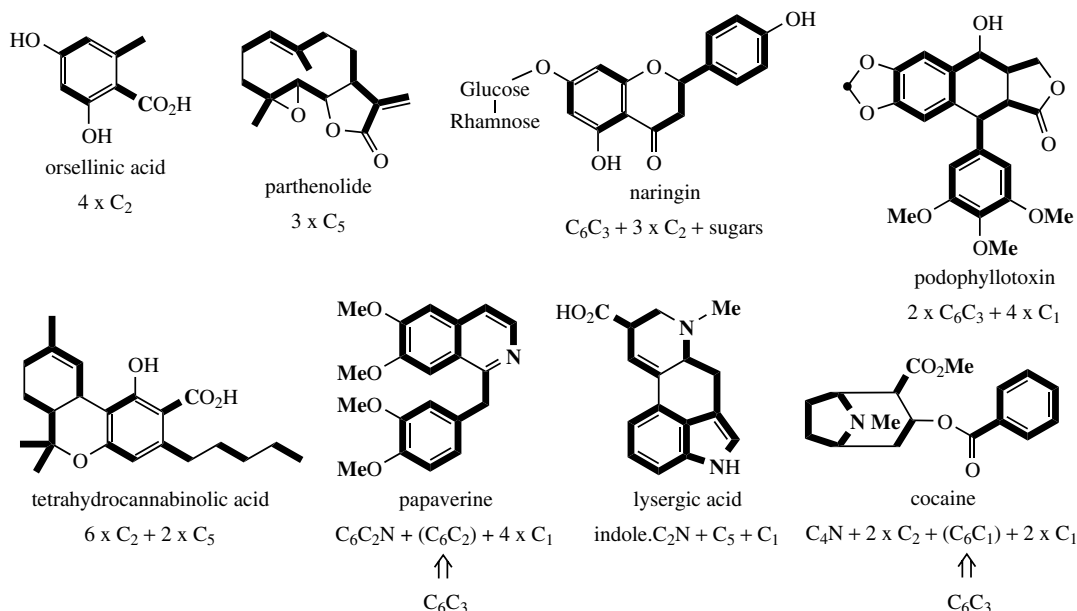


Figure 2.3

A word of warning is also necessary. Some natural products have been produced by processes in which a fundamental rearrangement of the carbon skeleton has occurred. This is especially common with structures derived from isoprene units, and it obviously disguises some of the original building blocks from immediate recognition. The same is true if one or more carbon atoms are removed by oxidation reactions.

THE CONSTRUCTION MECHANISMS

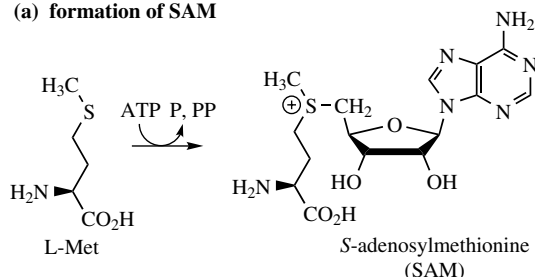
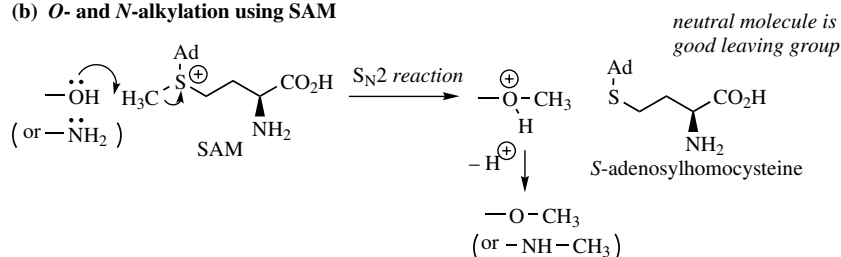
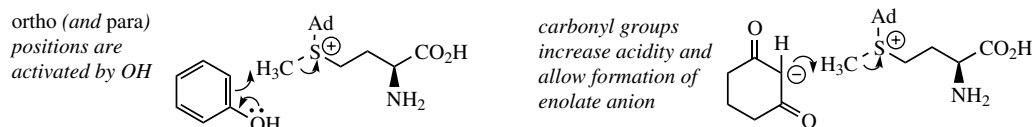
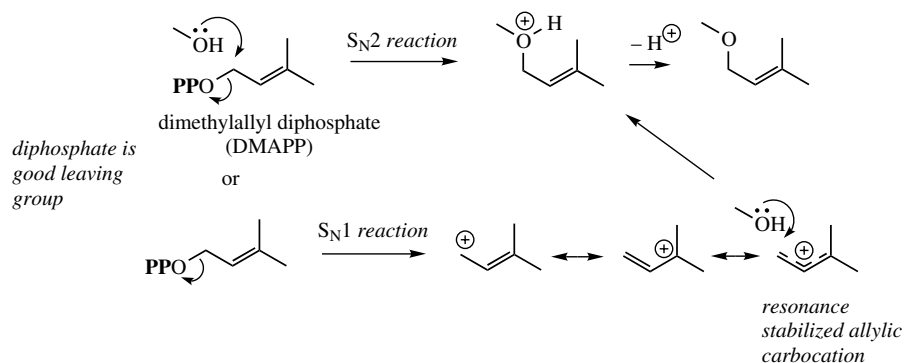
Natural product molecules are biosynthesized by a sequence of reactions which, with very few exceptions, are catalysed by enzymes. Enzymes are protein molecules which facilitate chemical modification of substrates by virtue of their specific binding properties conferred by the particular combination of functional groups in the constituent amino acids. In many cases, a suitable cofactor, e.g. NAD^+ , PLP, HSCoA (see below), as well as the substrate, may also be bound to participate in the transformation. Although enzymes catalyse some fairly elaborate and sometimes unexpected changes, it is generally possible to account for the reactions using sound chemical principles and mechanisms. As we explore the pathways to a wide variety of natural products, the reactions will generally be

discussed in terms of chemical analogies. Enzymes have the power to effect these transformations more efficiently and more rapidly than the chemical analogy, and also under very much milder conditions. Where relevant, they also carry out reactions in a stereospecific manner. Some of the important reactions frequently encountered are now described.

Alkylation Reactions: Nucleophilic Substitution

The C_1 methyl building unit is supplied from L-methionine and is introduced by a nucleophilic substitution reaction. In nature, the leaving group is enhanced by converting L-methionine into **S-adenosylmethionine (SAM)** [Figure 2.4(a)]. This gives a positively charged sulphur and facilitates the nucleophilic substitution (S_N2) type mechanism [Figure 2.4(b)]. Thus, *O*-methyl and *N*-methyl linkages may be obtained using hydroxyl and amino functions as nucleophiles. The generation of *C*-methyl linkages requires the participation of nucleophilic carbon. Positions *ortho* or *para* to a phenol group, or positions adjacent to one or more carbonyl groups, are thus candidates for *C*-methylation [Figure 2.4(c)].

A C_5 isoprene unit in the form of **dimethylallyl diphosphate (DMAPP)** may also act as an

Alkylation reactions: nucleophilic substitution**(a) formation of SAM****(b) O- and N-alkylation using SAM****(c) C-alkylation using SAM****(d) O-alkylation using DMAPP****Figure 2.4**

alkylating agent, and a similar $\text{S}_{\text{N}}2$ nucleophilic displacement can be proposed, the diphosphate making a good leaving group [Figure 2.4(d)]. In some cases, there is evidence that DMAPP may ionize first to the resonance-stabilized allylic carbocation and thus an $\text{S}_{\text{N}}1$ process operates instead. C-Alkylation at activated positions using DMAPP is analogous to the C-methylation process above.

Alkylation Reactions: Electrophilic Addition

As indicated above, the C_5 isoprene unit in the form of **dimethylallyl diphosphate (DMAPP)** can be used to alkylate a nucleophile. In the elaboration of terpenoids and steroids, two or more C_5 units are joined together, and the reactions

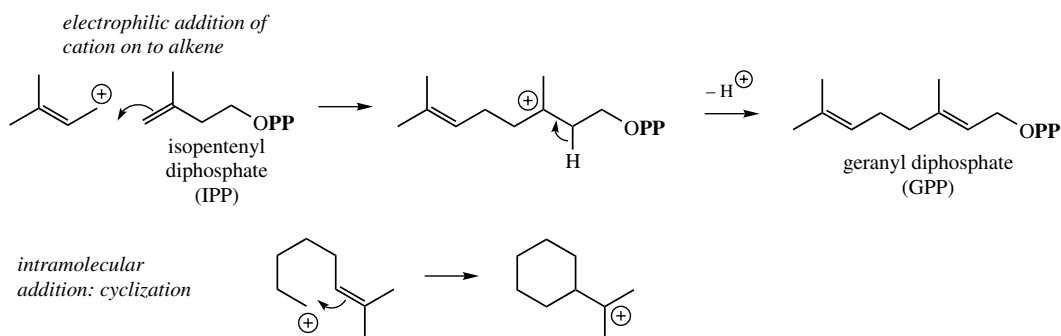
are rationalized in terms of carbocation chemistry, including electrophilic addition of carbocations on to alkenes. DMAPP may ionize to generate a resonance-stabilized allylic carbocation as shown in Figure 2.4(d), and this can then react with an alkene [e.g. **isopentenyl diphosphate (IPP)**] as depicted in Figure 2.5(a). The resultant carbocation may then lose a proton to give the uncharged product geranyl diphosphate (GPP). Where the alkene and carbocation functions reside in the same molecule, this type

of mechanism can be responsible for cyclization reactions [Figure 2.5(a)].

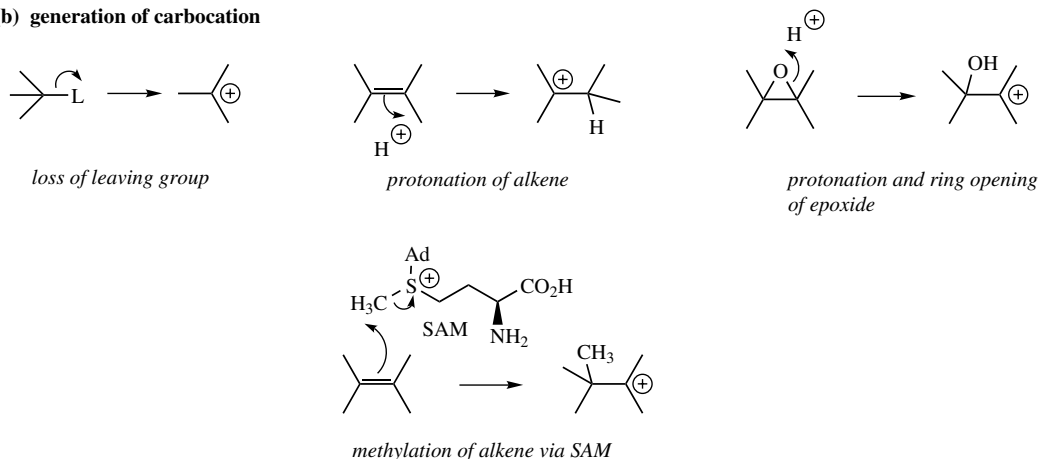
The initial carbocation may be generated by a number of mechanisms, important examples being loss of a leaving group, especially diphosphate (i.e. S_N1 type ionization), protonation of an alkene, and protonation/ring opening of epoxides [Figure 2.5(b)]. **S-Adenosylmethionine** may also alkylate alkenes by an electrophilic addition mechanism, adding a C_1 unit, and generating an intermediate carbocation.

Alkylation reactions: electrophilic addition

(a) inter- and intra-molecular additions



(b) generation of carbocation



(c) discharge of carbocation

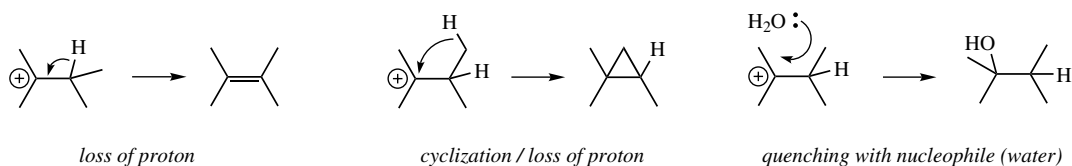


Figure 2.5

The final carbocation may be discharged by loss of a proton (giving an alkene or sometimes a cyclopropane ring) or by quenching with a suitable nucleophile, especially water [Figure 2.5(c)].

Wagner–Meerwein Rearrangements

A wide range of structures encountered in natural terpenoid and steroid derivatives can only be rationalized as originating from C_5 isoprene units if some fundamental rearrangement process has occurred during biosynthesis. These rearrangements have, in many cases, been confirmed experimentally, and are almost always consistent with the participation of carbocation intermediates. Rearrangements in chemical reactions involving carbocation intermediates, e.g. S_N1 and E1 reactions, are not uncommon, and typically consist of 1,2-shifts of hydride, methyl, or alkyl groups. Occasionally, 1,3- or longer shifts are encountered. These shifts, termed **Wagner–Meerwein rearrangements**, are readily rationalized in terms of generating a more stable carbocation, or relaxing ring strain (Figure 2.6). Thus, tertiary carbocations are favoured over secondary carbocations,

and the usual objective in these rearrangements is to achieve tertiary status at the positive centre. However, a tertiary to secondary transition might be favoured if the rearrangement allows a significant release of ring strain. These general concepts are occasionally ignored by nature, but it must be remembered that the reactions are enzyme-mediated and carbocations may not exist as discrete species in the transformations. An interesting feature of some biosynthetic pathways, e.g. that leading to steroids, is a remarkable series of concerted 1,2-migrations rationalized via carbocation chemistry, but entirely a consequence of the enzyme's participation (Figure 2.6).

Aldol and Claisen Reactions

The **aldol** and **Claisen** reactions both achieve carbon–carbon bond formation and in typical base-catalysed chemical reactions depend on the generation of a resonance-stabilized enolate anion from a suitable carbonyl system (Figure 2.7). Whether an aldol-type or Claisen-type product is formed depends on the nature of X and its potential as a leaving group. Thus, chemically, two molecules

Wagner–Meerwein rearrangements

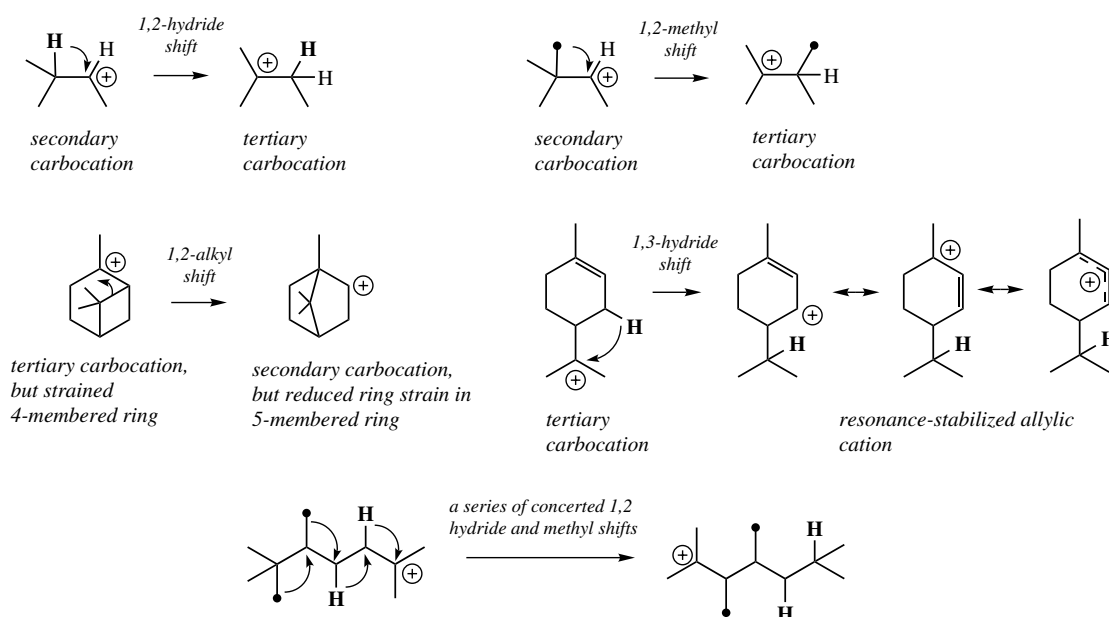


Figure 2.6

Aldol and Claisen reactions

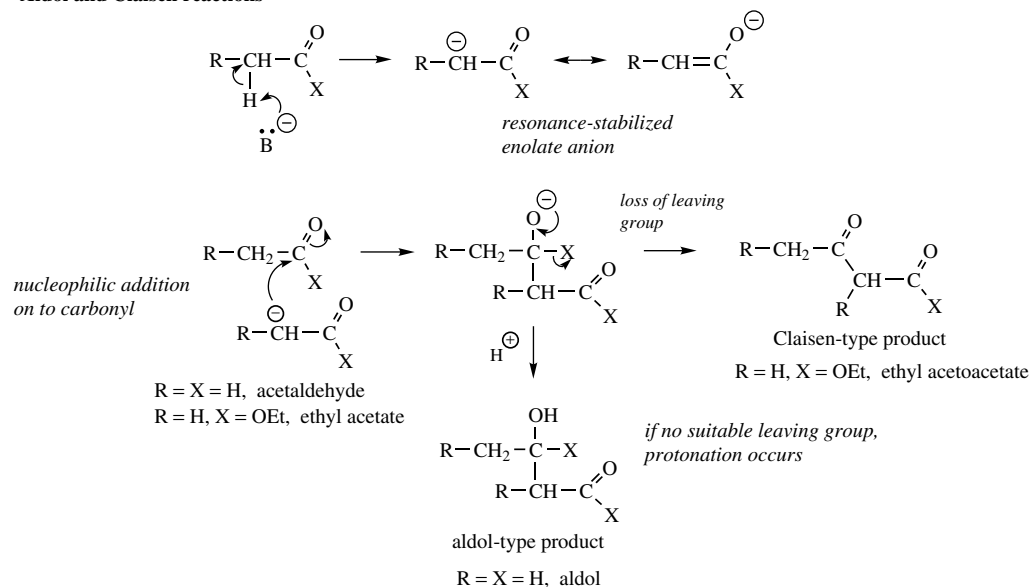


Figure 2.7

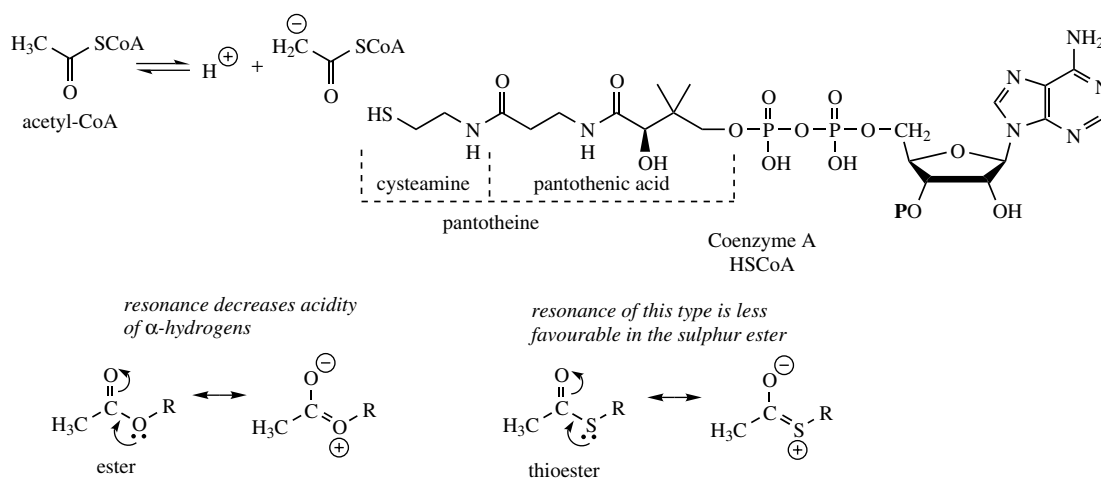


Figure 2.8

of acetaldehyde yield aldol, whilst two molecules of ethyl acetate can give ethyl acetoacetate. These processes are vitally important in biochemistry for the elaboration of both secondary and primary metabolites, but the enzyme catalysis obviates the need for strong bases, and probably means the enolate anion has little more than transitory existence. Nevertheless, the reactions do appear to parallel enolate anion chemistry, and are frequently responsible for joining together of C_2 acetate groups.

In most cases, the biological reactions involve coenzyme A esters, e.g. **acetyl-CoA** (Figure 2.8). This is a thioester of acetic acid, and it has significant advantages over oxygen esters, e.g. ethyl acetate, in that the α -methylene hydrogens are now more acidic, comparable in fact to those in the equivalent ketone, thus increasing the likelihood of generating the enolate anion. This is explained in terms of electron delocalization in the ester function (Figure 2.8). This type of delocalization is

more prominent in the oxygen ester than in the sulphur ester, due to oxygen's smaller size and thus closer proximity of the lone pair for overlap with carbon's orbitals. Furthermore, the thioester has a much more favourable leaving group than the oxygen ester, and the combined effect is to increase the reactivity for both the aldol and Claisen-type reactions.

Claisen reactions involving acetyl-CoA are made even more favourable by first converting acetyl-CoA into **malonyl-CoA** by a carboxylation reaction with CO_2 using ATP and the coenzyme biotin (Figure 2.9). ATP and CO_2 (as bicarbonate, HCO_3^-) form the mixed anhydride, which carboxylates the coenzyme in a biotin-enzyme complex. Fixation of carbon dioxide by biotin-enzyme complexes is not unique to acetyl-CoA, and another important example occurs in the generation of oxaloacetate from pyruvate in the synthesis of glucose from non-carbohydrate sources

(gluconeogenesis). The conversion of acetyl-CoA into malonyl-CoA means the α -hydrogens are now flanked by two carbonyl groups, and have increased acidity. Thus, a more favourable nucleophile is provided for the Claisen reaction. No acylated malonic acid derivatives are produced, and the carboxyl group introduced into malonyl-CoA is simultaneously lost by a decarboxylation reaction during the Claisen condensation (Figure 2.9). An alternative rationalization is that decarboxylation of the malonyl ester is used to generate the acetyl enolate anion without any requirement for a strong base. Thus, the product formed from acetyl-CoA as electrophile and malonyl-CoA as nucleophile is acetoacetyl-CoA, which is actually the same as in the condensation of two molecules of acetyl-CoA. Accordingly, the role of the carboxylation step is clear cut: the carboxyl activates the α -carbon to facilitate the Claisen condensation, and it is immediately removed on completion of this task.

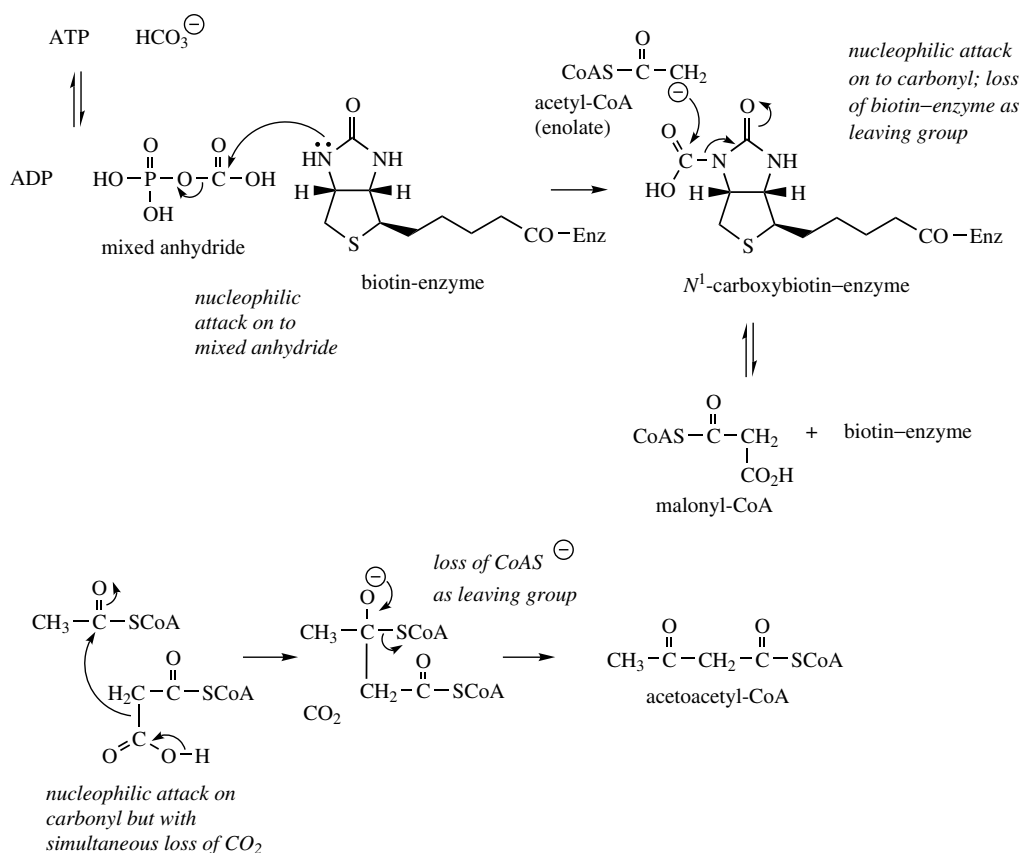


Figure 2.9

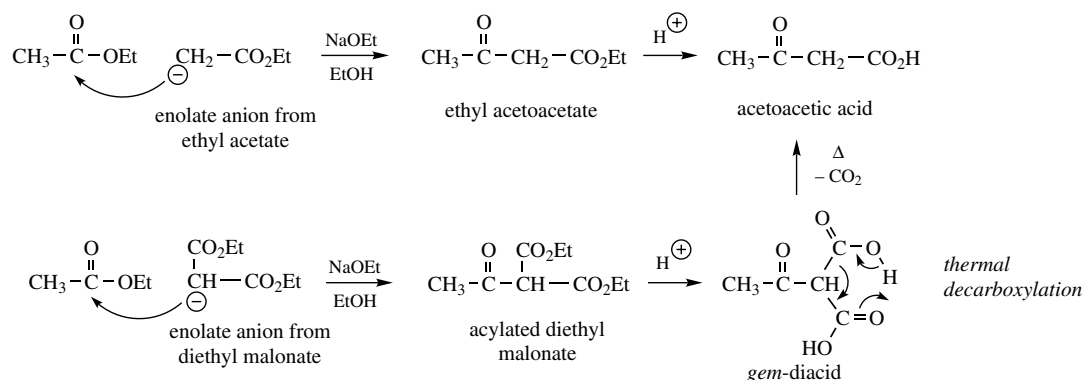


Figure 2.10

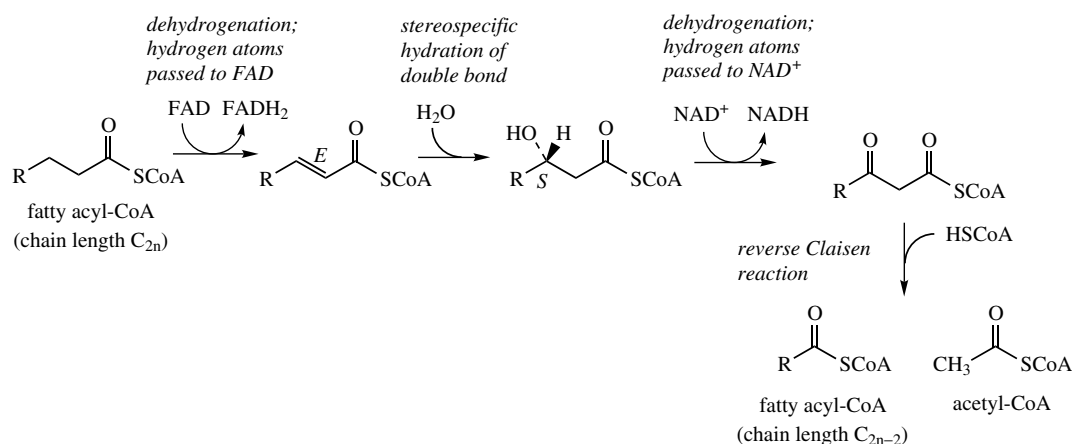
 β -Oxidation of fatty acids

Figure 2.11

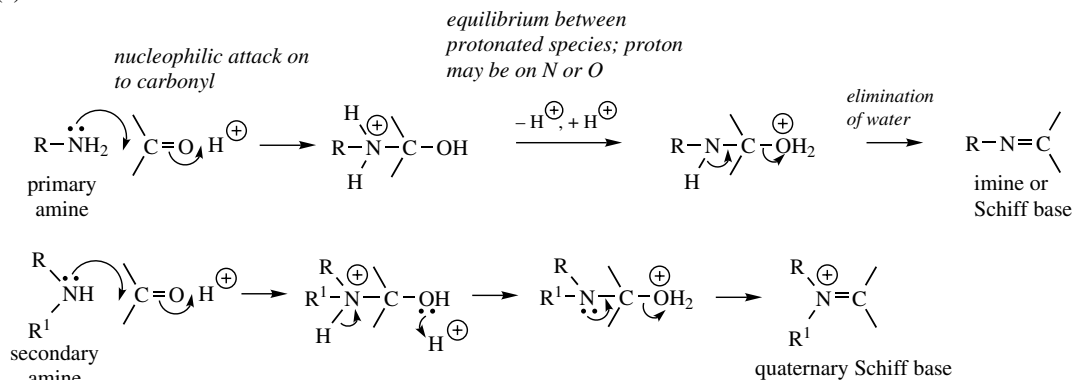
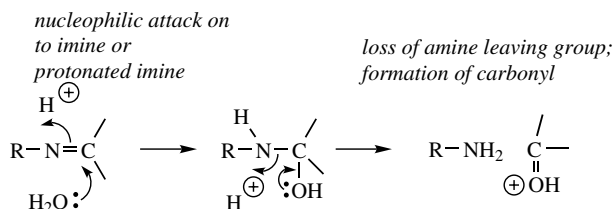
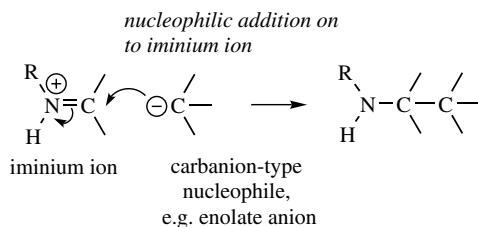
By analogy, the chemical Claisen condensation using the enolate anion from diethyl malonate in Figure 2.10 proceeds much more favourably than that using the enolate from ethyl acetate. The same acetoacetic acid product can be formed in the malonate condensation by hydrolysis of the acylated malonate intermediate and decarboxylation of the *gem*-diacid.

Both the **reverse aldol** and **reverse Claisen** reactions may be encountered in the modification of natural product molecules. Such reactions remove fragments from the basic skeleton already generated, but may extend the diversity of structures. The reverse Claisen reaction is a prominent feature of the **β -oxidation** sequence for the catabolic degradation of fatty acids (Figure 2.11),

in which a C_2 unit as acetyl-CoA is cleaved off from a fatty acid chain, leaving it two carbons shorter in length.

Schiff Base Formation and the Mannich Reaction

Formation of C–N bonds is frequently achieved by condensation reactions between amines and aldehydes or ketones. A typical nucleophilic addition is followed by elimination of water to give an imine or **Schiff base** [Figure 2.12(a)]. Of almost equal importance is the reversal of this process, i.e. the hydrolysis of imines to amines and aldehydes/ketones [Figure 2.12(b)]. The imine so produced, or more likely its protonated form the

(a) Schiff base formation**(b) Schiff base hydrolysis****(c) Mannich reaction****Figure 2.12**

iminium ion, can then act as an electrophile in a **Mannich reaction** [Figure 2.12(c)]. The nucleophile might be provided by an enolate anion, or in many examples by a suitably activated centre in an aromatic ring system. The Mannich reaction is encountered throughout alkaloid biosynthesis, and in its most general form involves combination of an amine (primary or secondary), an aldehyde or ketone, and a nucleophilic carbon. Secondary amines will react with the carbonyl compound to give an iminium ion (quaternary Schiff base) directly, and the additional protonation step is thus not necessary.

It should be appreciated that the Mannich-like addition reaction in Figure 2.12(c) is little different

from nucleophilic addition to a carbonyl group. Indeed, the imine/iminium ion is merely acting as the nitrogen analogue of a carbonyl/protonated carbonyl. To take this analogy further, protons on carbon adjacent to an imine group will be acidic, as are those α to a carbonyl group, and the isomerization to the enamine shown in Figure 2.13 is analogous to keto-enol tautomerism. Just as two carbonyl compounds can react via an aldol reaction, so can two imine systems, and this is indicated in Figure 2.13. Often aldehyde/ketone substrates in enzymic reactions become covalently bonded to the enzyme through imine linkages; in so doing they lose none of the carbonyl activation as a consequence of the new form of bonding.

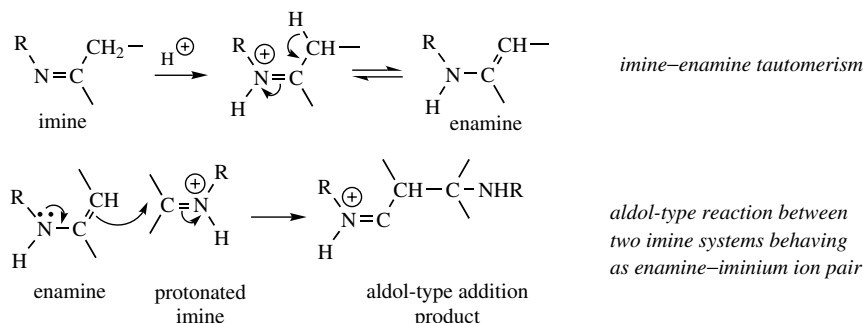


Figure 2.13

Transamination

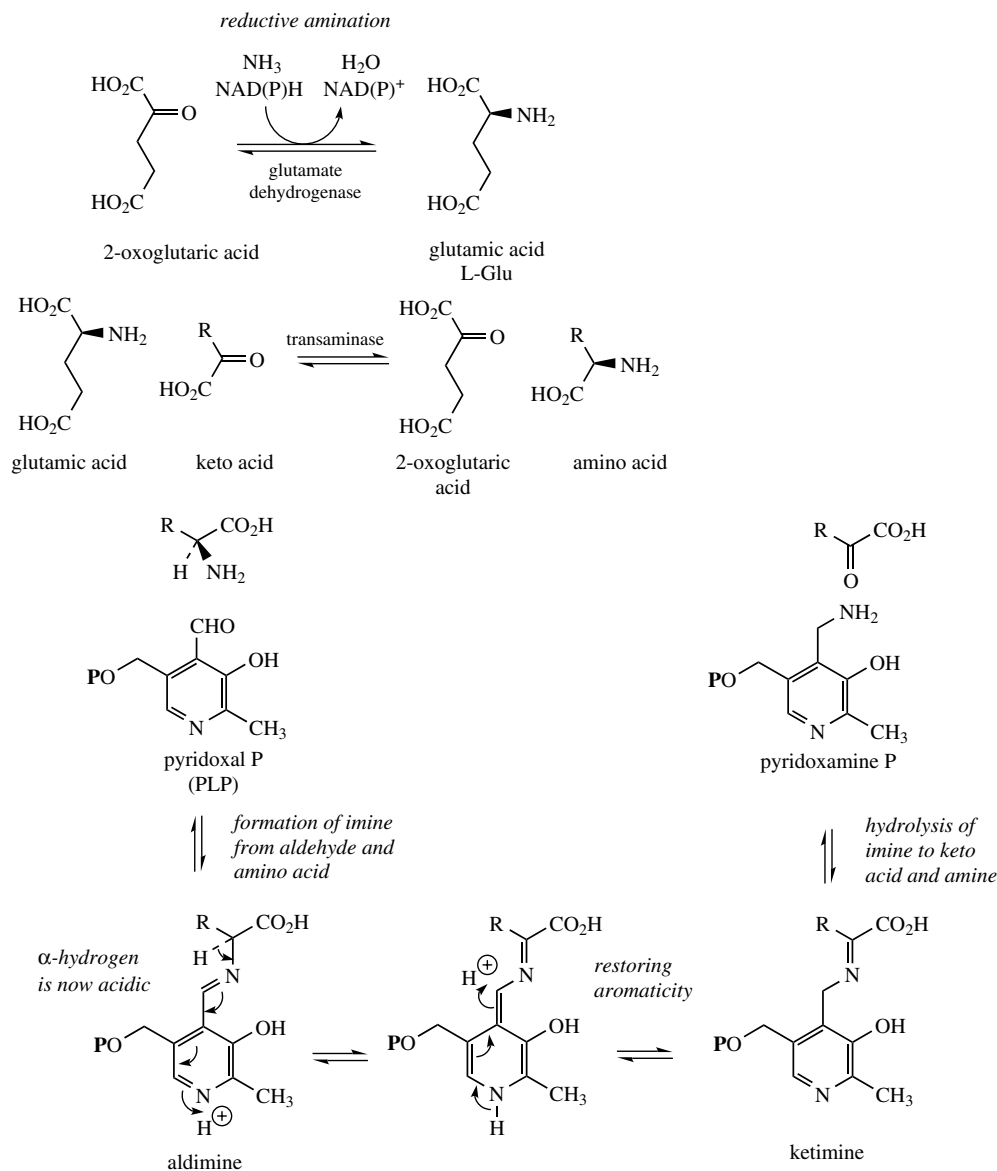
Transamination is the exchange of the amino group from an amino acid to a keto acid, and provides the most common process for the introduction of nitrogen into amino acids, and for the removal of nitrogen from them. The couple **glutamic acid/2-oxoglutaric acid** are the usual donor/acceptor molecules for the amino group. Reductive amination of the Krebs cycle intermediate 2-oxoglutaric acid to glutamic acid (Figure 2.14) is responsible for the initial incorporation of nitrogen, a reaction which involves imine formation and subsequent reduction. Transamination then allows the amino group to be transferred from glutamic acid to a suitable keto acid, or in the reverse mode from an amino acid to 2-oxoglutaric acid. This reaction is dependent on the coenzyme **pyridoxal phosphate (PLP)** and features a Schiff base/imine intermediate (aldimine) with the aldehyde group of PLP (Figure 2.14). The α -hydrogen of the original amino acid is now made considerably more acidic and is removed, leading to the ketimine by a reprotonation process which also restores the aromaticity in the pyridine ring. The keto acid is then liberated by hydrolysis of the Schiff base function, which generates pyridoxamine phosphate. The remainder of the sequence is now a reversal of this process, and transfers the amine function from pyridoxamine phosphate to another keto acid.

Decarboxylation Reactions

Many pathways to natural products involve steps which remove portions of the carbon skeleton. Although two or more carbon atoms may be

cleaved off via the reverse aldol or reverse Claisen reactions mentioned above, by far the most common degradative modification is loss of one carbon atom by a **decarboxylation** reaction. Decarboxylation is a particular feature of the biosynthetic utilization of amino acids, and it has already been indicated that several of the basic building blocks, e.g. C_6C_2N , indole, C_2N , are derived from an amino acid via loss of the carboxyl group. This decarboxylation of amino acids is also a **pyridoxal phosphate**-dependent reaction (compare transamination) and is represented as in Figure 2.15(a). This similarly depends on Schiff base formation and shares features of the transamination sequence of Figure 2.14. Decarboxylation is facilitated in the same way as loss of the α -hydrogen was facilitated for the transamination sequence. After protonation of the original α -carbon, the amine is released from the coenzyme by hydrolysis of the Schiff base function.

β -Keto acids are thermally labile and rapidly decarboxylated *in vitro* via a cyclic mechanism which proceeds through the enol form of the final ketone [Figure 2.15(b)]. Similar reactions are found in nature, though whether cyclic processes are necessary is not clear. *ortho*-Phenolic acids also decarboxylate readily *in vitro* and *in vivo*, and it is again possible to invoke a cyclic β -keto acid tautomer of the substrate. The corresponding decarboxylation of *para*-phenolic acids cannot have a cyclic transition state, but the carbonyl group in the proposed keto tautomer activates the system for decarboxylation. The acetate pathway frequently yields structures containing phenol and carboxylic acid functions, and decarboxylation reactions may thus feature as further modifications. Although the carboxyl group may originate by hydrolysis of the

Transamination**Figure 2.14**

thioester portion of the acetyl-CoA precursor, there are also occasions when a methyl group can be sequentially oxidized to a carboxyl, which then subsequently suffers decarboxylation.

Decarboxylation of **α -keto acids** is a feature of primary metabolism, e.g. pyruvic acid $\rightarrow$ acetaldehyde in glycolysis, and pyruvic acid $\rightarrow$ acetyl-CoA, an example of overall oxidative decarboxylation prior to entry of acetyl-CoA

into the Krebs cycle. Both types of reaction depend upon **thiamine diphosphate (TPP)**. TPP is a coenzyme containing a thiazole ring, which has an acidic hydrogen and is thus capable of yielding the carbanion. This acts as a nucleophile towards carbonyl groups. Decarboxylation of pyruvic acid to acetaldehyde is depicted as in Figure 2.15(c), which process also regenerates the carbanion. In the oxidation step of oxidative

Decarboxylation reactions

(a) amino acids

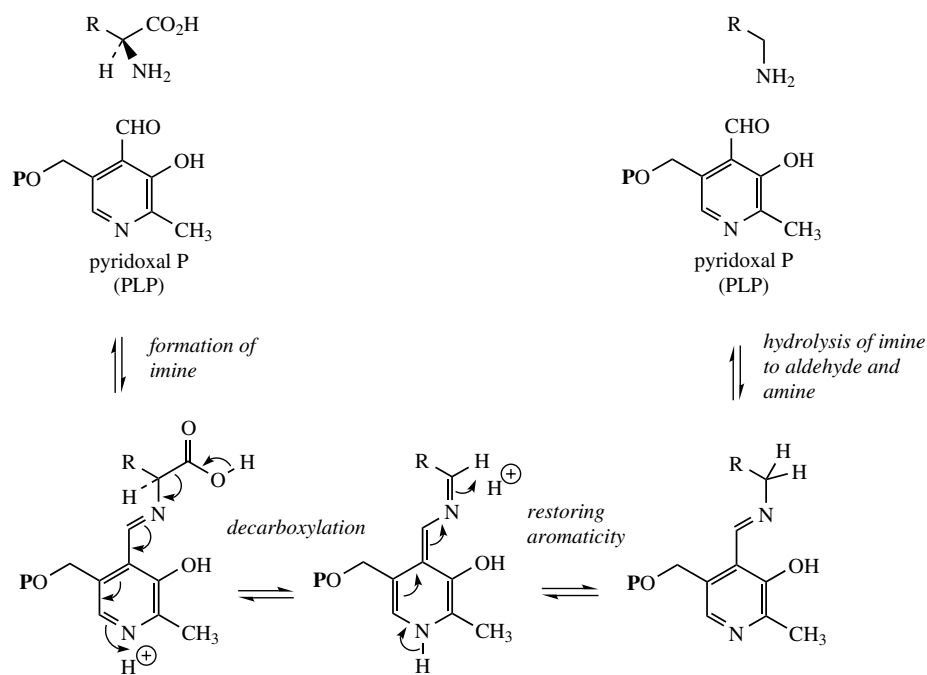
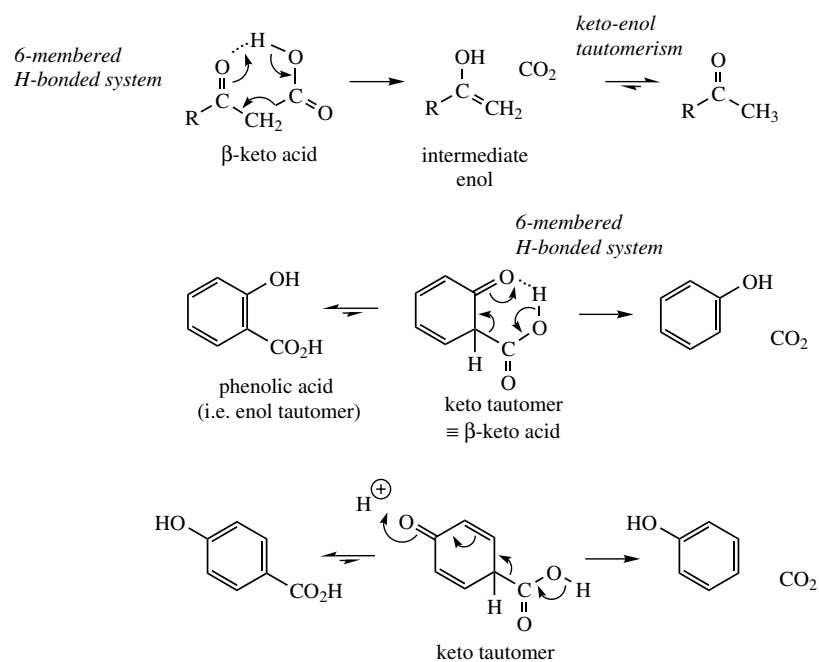
(b) β -keto acids

Figure 2.15

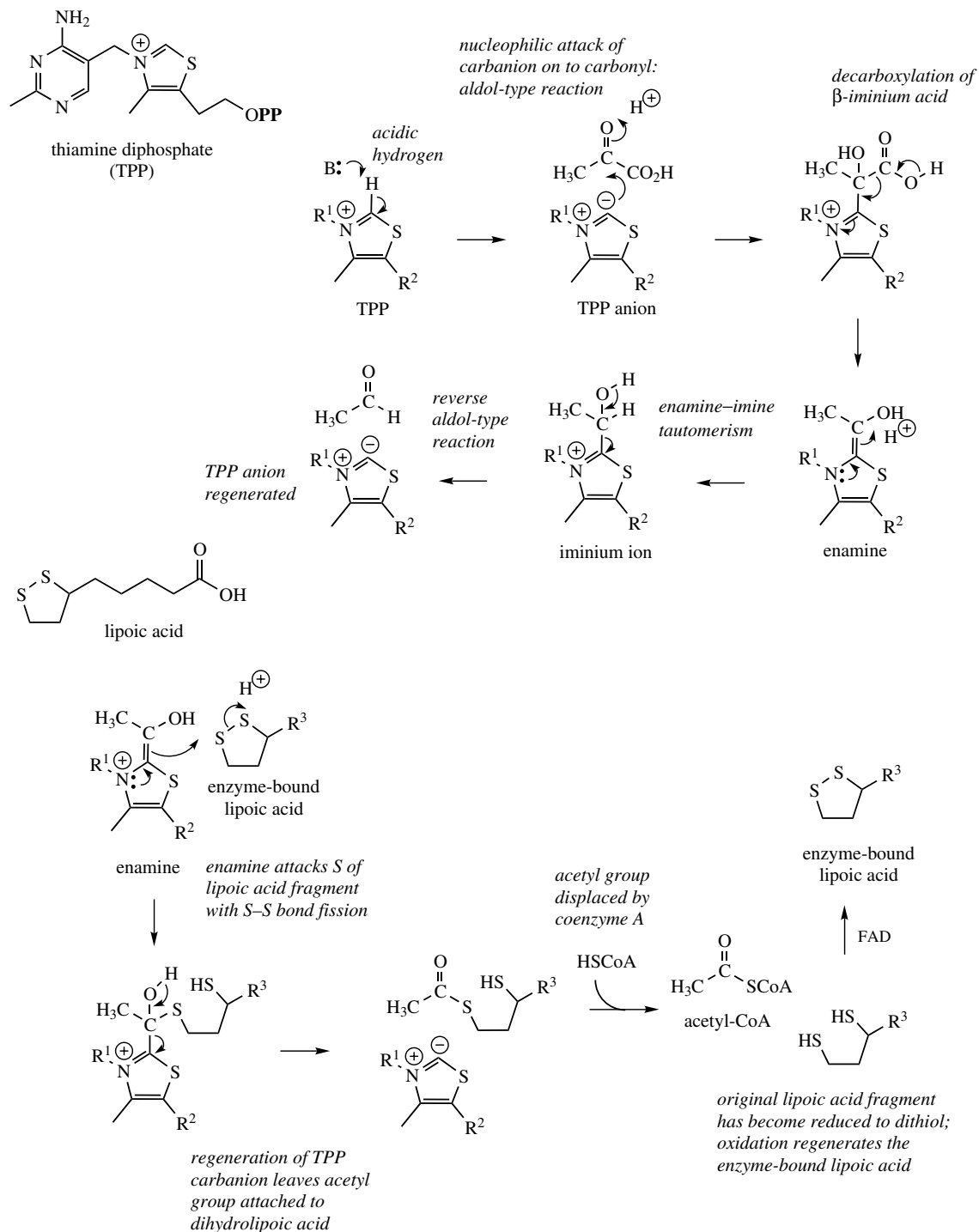
(c) α -keto acids

Figure 2.15 (continued)

decarboxylation, the enzyme-bound disulphide-containing coenzyme **lipoic acid** is also involved. The intermediate enamine in Figure 2.15(c), instead of accepting a proton, is used to attack a sulphur in the lipoic acid moiety with subsequent S—S bond fission, thereby effectively reducing the lipoic acid fragment. This allows regeneration of the TPP carbanion, and the acetyl group is bound to the dihydrolipoic acid. This acetyl group is then released as acetyl-CoA by displacement with the thiol coenzyme A. The bound dihydrolipoic acid fragment is then reoxidized to restore its function. An exactly equivalent reaction is encountered in the Krebs cycle in the conversion of 2-oxoglutaric acid into succinyl-CoA.

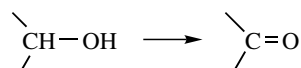
Oxidation and Reduction Reactions

Changes to the oxidation state of a molecule are frequently carried out as a secondary metabolite is synthesized or modified. The processes are not always completely understood, but the following general features are recognized. The processes may be classified according to the type of enzyme involved and their mechanism of action.

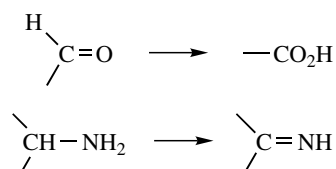
Dehydrogenases

Dehydrogenases remove two hydrogen atoms from the substrate, passing them to a suitable

coenzyme acceptor. The coenzyme system involved can generally be related to the functional group being oxidized in the substrate. Thus if the oxidation process is



then a pyridine nucleotide, **nicotinamide adenine dinucleotide (NAD⁺)** or **nicotinamide adenine dinucleotide phosphate (NADP⁺)**, tends to be utilized as hydrogen acceptor. One hydrogen from the substrate (that bonded to carbon) is transferred as hydride to the coenzyme, and the other, as a proton, is passed to the medium (Figure 2.16). NAD(P)⁺ may also be used in the oxidations



The reverse reaction, i.e. reduction, is also indicated in Figure 2.16, and may be compared with the chemical reduction process using complex metal hydrides, e.g. LiAlH₄ or NaBH₄, namely nucleophilic addition of hydride and subsequent protonation. The reduced forms NADH and NADPH are conveniently regarded as hydride-donating

Dehydrogenases: NAD⁺ and NADP⁺

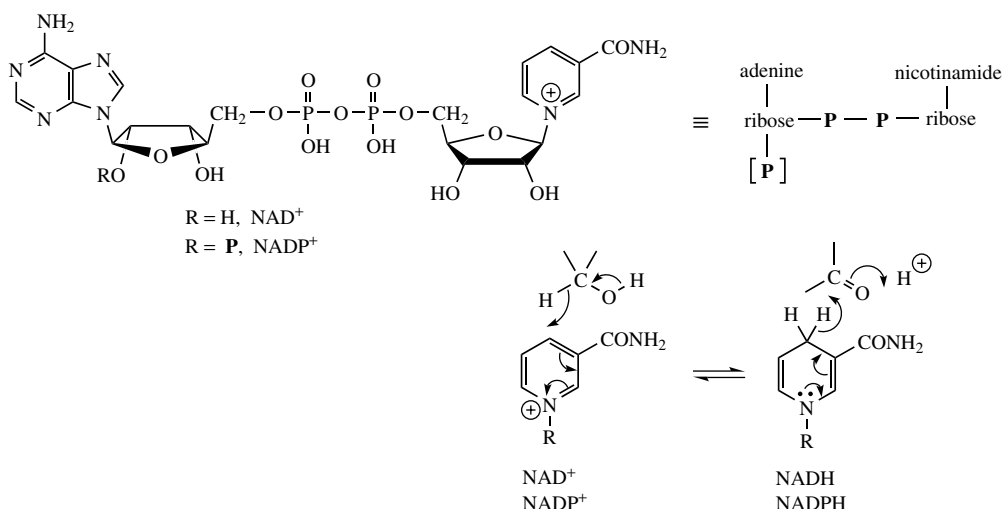
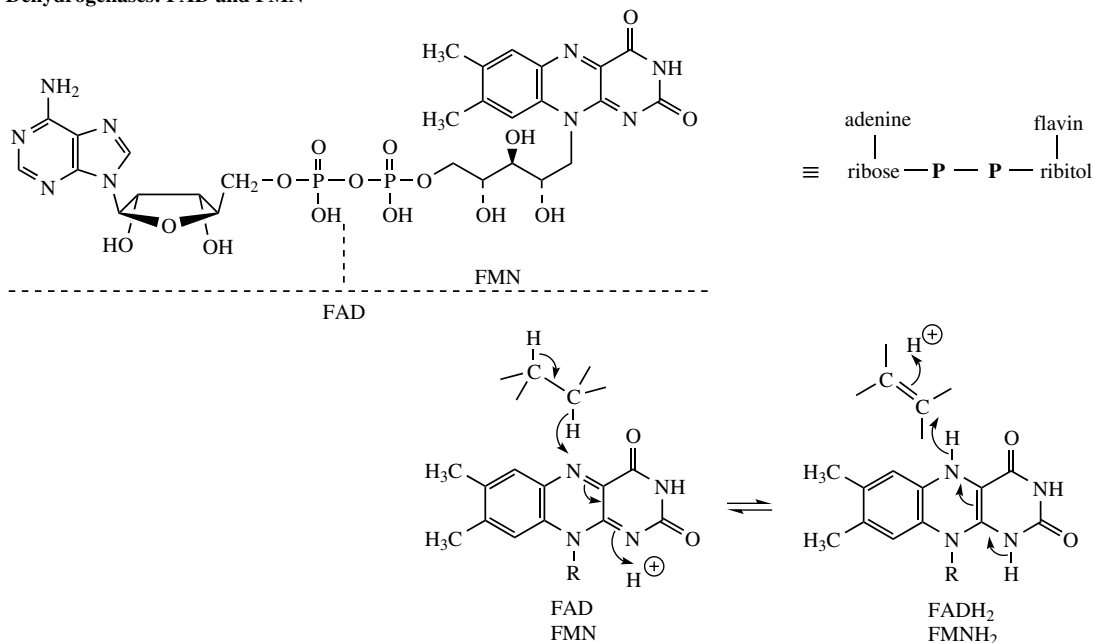
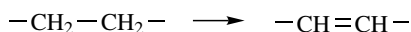


Figure 2.16

Dehydrogenases: FAD and FMN**Figure 2.17**

reducing agents. In practice, NADPH is generally employed in reductive processes, whilst NAD⁺ is used in oxidations.

Should the oxidative process be the conversion



the coenzyme used as acceptor is usually a flavin nucleotide, **flavin adenine dinucleotide (FAD)** or **flavin mononucleotide (FMN)**. These entities are bound to the enzyme in the form of a flavoprotein, and take up two hydrogen atoms, represented in Figure 2.17 as being derived by addition of hydride from the substrate and a proton from the medium. Alternative mechanisms have also been proposed, however. Reductive sequences involving flavoproteins may be represented as the reverse reaction in Figure 2.17. NADPH may also be employed as a coenzyme in the reduction of a carbon-carbon double bond.

These oxidation reactions employing pyridine nucleotides and flavoproteins are especially important in primary metabolism in liberating energy from fuel molecules in the form of ATP. The reduced coenzymes formed in the process are normally reoxidized via the electron transport chain

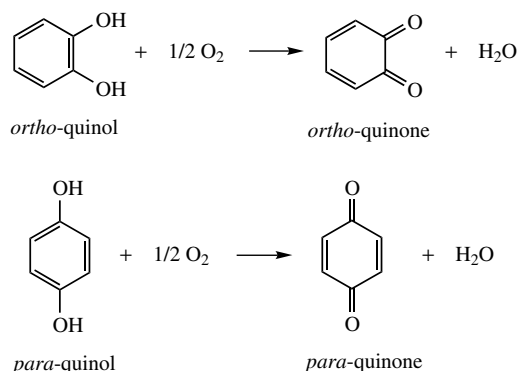
of oxidative phosphorylation, so that the hydrogen atoms eventually pass to oxygen giving water.

Oxidases

Oxidases also remove hydrogen from a substrate, but pass these atoms to molecular oxygen or to hydrogen peroxide, in both cases forming water. Oxidases using hydrogen peroxide are termed **peroxidases**. Mechanisms of action vary and need not be considered here. Important transformations in secondary metabolism include the oxidation of *ortho*- and *para*-quinols to quinones (Figure 2.18), and the peroxidase-induced phenolic oxidative coupling processes (see page 28).

Mono-oxygenases

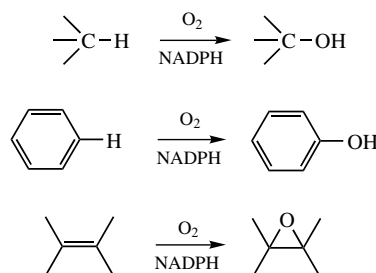
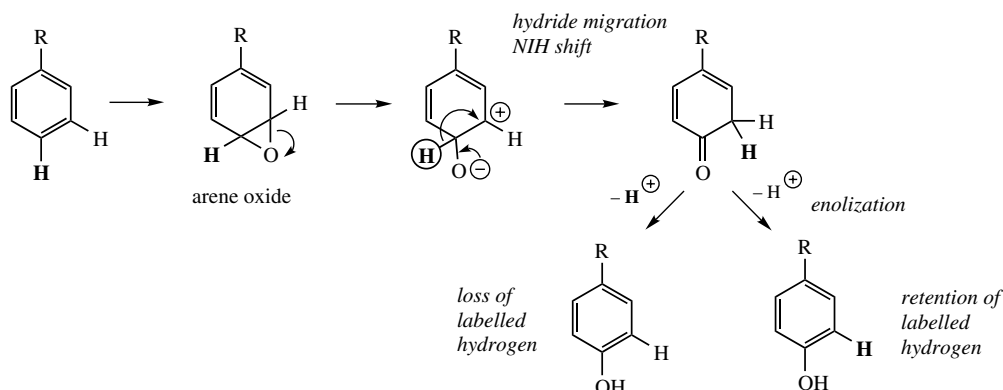
Oxygenases catalyse the direct addition of oxygen from molecular oxygen to the substrate. They are subdivided into mono- and di-oxygenases according to whether just one or both of the oxygen atoms are introduced into the substrate. With **mono-oxygenases**, the second oxygen atom from O₂ is reduced to water by an appropriate hydrogen

Oxidases**Figure 2.18**

donor, e.g. NADH, NADPH, or **ascorbic acid** (vitamin C). In this respect they may also be considered to behave as oxidases, and the term ‘mixed-function oxidase’ is also used for these enzymes. Especially important examples of these enzymes are the **cytochrome P-450-dependent mono-oxygenases**. These are frequently involved in biological hydroxylations, either in biosynthesis, or in the mammalian detoxification and metabolism of foreign compounds such as drugs, and such enzymes are thus termed ‘**hydroxylases**’. **Cytochrome P-450** is named after its intense absorption band at 450 nm when exposed to CO, which is a powerful inhibitor of these enzymes. It contains an iron–porphyrin complex (haem), which is bound to the enzyme, and a redox change involving the Fe atom allows binding and the

cleavage of an oxygen atom. Many such systems have been identified, capable of hydroxylating aliphatic or aromatic systems, as well as producing epoxides from alkenes (Figure 2.19). In most cases, NADPH features as hydrogen donor.

Aromatic hydroxylation catalysed by mono-oxygenases (including cytochrome P-450 systems) probably involves arene oxide (epoxide) intermediates (Figure 2.20). An interesting consequence of this mechanism is that when the epoxide opens up, the hydrogen atom originally attached to the position which becomes hydroxylated can migrate to the adjacent carbon on the ring. A high proportion of these hydrogen atoms is subsequently retained in the product, even though enolization allows some loss of this hydrogen. This migration is known as the **NIH shift**, having been originally observed at the National Institute of Health, Bethesda, MD, USA.

Mono-oxygenases**Figure 2.19****NIH shift****Figure 2.20**

The oxidative cyclization of an *ortho*-hydroxy-methoxy-substituted aromatic system giving a **methylenedioxy group** is also known to involve a cytochrome P-450-dependent mono-oxygenase. This enzyme hydroxylates the methyl to yield a formaldehyde hemiacetal intermediate, which can cyclize to the methylenedioxy bridge (the acetal of formaldehyde) by an ionic mechanism (Figure 2.21).

Dioxygenases

Dioxygenases introduce both atoms from molecular oxygen into the substrate, and are frequently involved in the cleavage of bonds, including

aromatic rings. Cyclic peroxides (dioxetanes) are likely to be intermediates (Figure 2.22). Oxidative cleavage of aromatic rings typically employs catechol (1,2-dihydroxy) or quinol (1,4-dihydroxy) substrates, and in the case of catechols, cleavage may be between or adjacent to the two hydroxyls, giving products containing aldehyde and/or carboxylic acid functionalities (Figure 2.22).

Some dioxygenases utilize two acceptor substrates and incorporate one oxygen atom into each. Thus, **2-oxoglutarate-dependent dioxygenases** hydroxylate one substrate, whilst also transforming 2-oxoglutarate into succinate with the release of CO_2 (Figure 2.23). 2-Oxoglutarate-dependent dioxygenases also require as cofactors

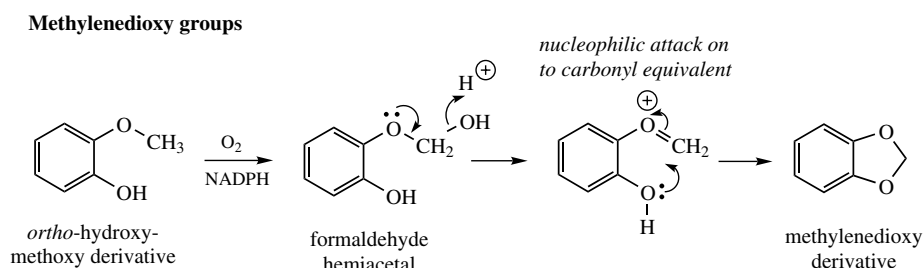


Figure 2.21

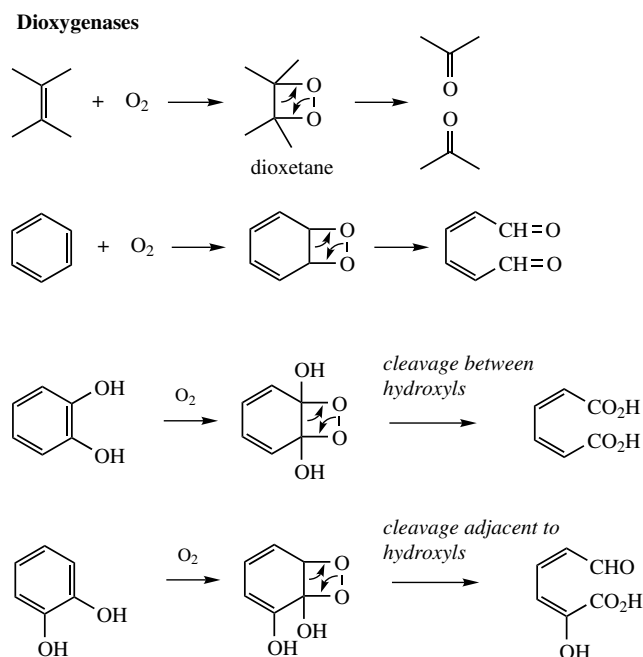
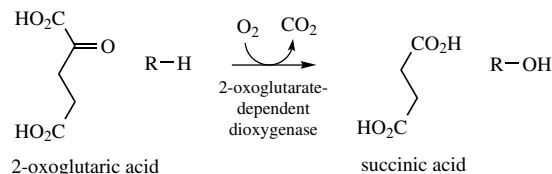
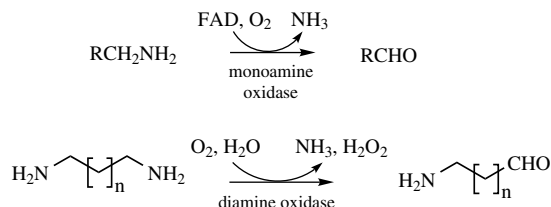


Figure 2.22

2-Oxoglutarate-dependent dioxygenases**Figure 2.23****Amine oxidases****Figure 2.24**

Fe^{2+} to generate an enzyme-bound iron–oxygen complex, and **ascorbic acid** (vitamin C) to subsequently reduce this complex.

Amine Oxidases

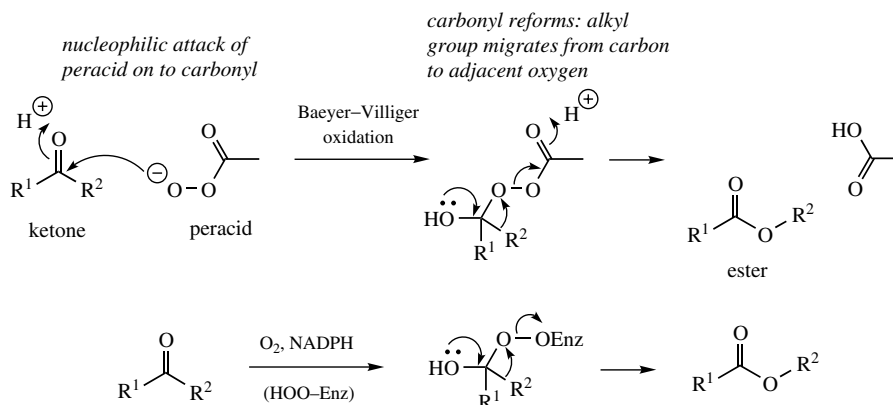
In addition to the oxidizing enzymes outlined above, those which transform an amine into an aldehyde, the **amine oxidases**, are frequently involved in metabolic pathways. These include **monoamine oxidases** and **diamine oxidases**. Monoamine oxidases utilize a flavin nucleotide, typically FAD, and molecular oxygen, and involve initial dehydrogenation to an imine, followed by hydrolysis to the aldehyde and ammonia (Figure 2.24). Diamine oxidases require a diamine substrate, and oxidize at one amino group using molecular oxygen to give the corresponding aldehyde. Hydrogen peroxide and ammonia are the other products formed. The aminoaldehyde so formed then has the potential to be transformed into a cyclic imine via Schiff base formation.

Baeyer–Villiger Oxidations

The chemical oxidation of ketones by peracids, the **Baeyer–Villiger oxidation**, yields an ester, and the process is known to involve migration of an alkyl group from the ketone (Figure 2.25). For comparable ketone $\rightarrow$ ester conversions known to occur in biochemistry, cytochrome-P-450- or FAD-dependent enzymes requiring NADPH and O_2 appear to be involved. This leads to formation of a peroxy–enzyme complex and a mechanism similar to that for the chemical Baeyer–Villiger oxidation may thus operate. The oxygen atom introduced thus originates from O_2 .

Phenolic Oxidative Coupling

Many natural products are produced by the coupling of two or more phenolic systems, in a process readily rationalized by means of free radical reactions. The reactions can be brought

Baeyer–Villiger oxidations**Figure 2.25**

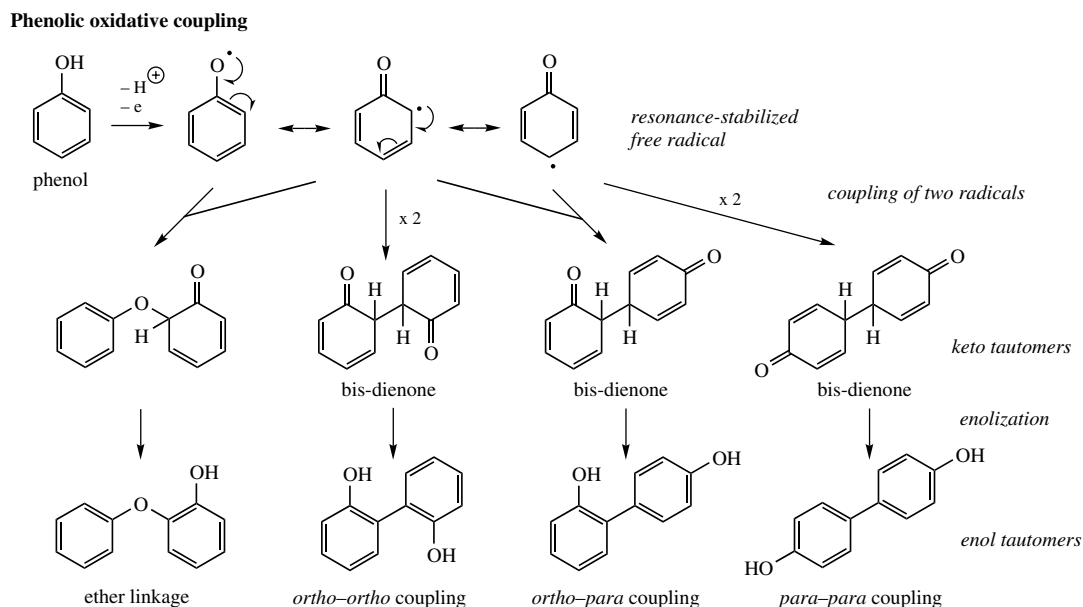


Figure 2.26

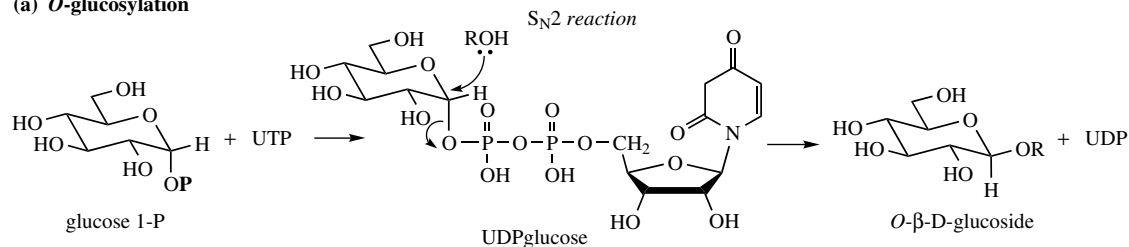
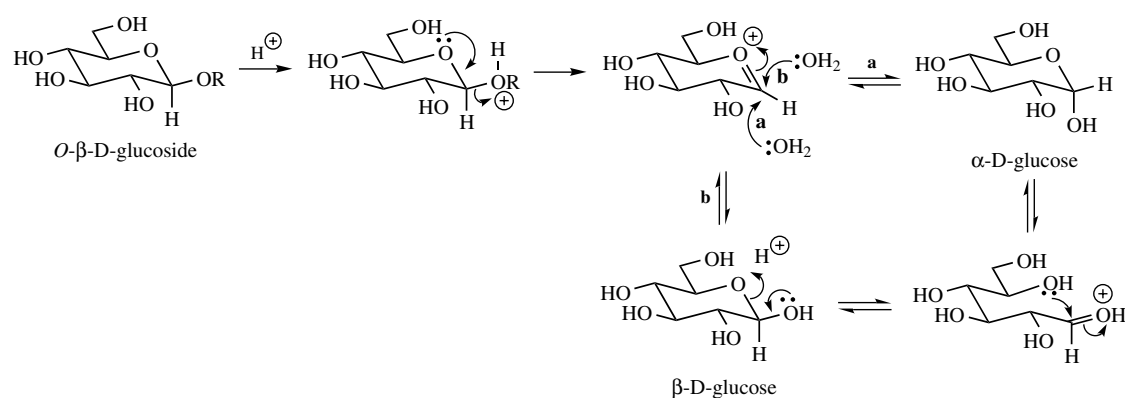
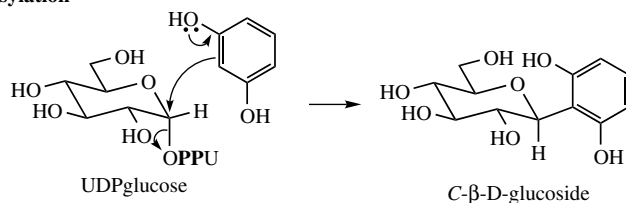
about by oxidase enzymes, including peroxidase and laccase systems, known to be radical generators. Other enzymes catalysing **phenolic oxidative coupling** have been characterized as cytochrome P-450-dependent proteins, requiring NADPH and O_2 cofactors, but no oxygen is incorporated into the substrate. A one-electron oxidation of a phenol gives the free radical, and the unpaired electron can then be delocalized via resonance forms in which the free electron is dispersed to positions *ortho* and *para* to the original oxygen function (Figure 2.26). Coupling of two of these mesomeric structures gives a range of dimeric systems as exemplified in Figure 2.26. The final products indicated are then derived by enolization, which restores aromaticity to the rings. Thus, carbon-carbon bonds involving positions *ortho* or *para* to the original phenols, or ether linkages, may be formed. The reactive dienone systems formed as intermediates may in some cases be attacked by other nucleophilic groupings, extending the range of structures ultimately derived from this basic reaction sequence.

Glycosylation Reactions

The widespread occurrence of **glycosides** and **polysaccharides** requires processes for attaching

sugar units to a suitable atom of an aglycone to give a glycoside, or to another sugar giving a polysaccharide. Linkages tend to be through oxygen, although they are not restricted to oxygen, since *S*-, *N*-, and *C*-glycosides are well known. The agent for glycosylation is a **uridine diphosphosugar**, e.g. **UDPglucose**. This is synthesized from glucose 1-phosphate and UTP, and then the glucosylation process can be envisaged as a simple S_N2 nucleophilic displacement reaction [Figure 2.27(a)]. Since UDPglucose has its leaving group in the α -configuration, the product has the β -configuration, as is most commonly found in natural glucosides. Note, however, that many important carbohydrates, e.g. sucrose and starch, possess α -linkages, and these appear to originate via double S_N2 processes (see page 470). Other UDPsugars, e.g. UDPgalactose or UDPxylose, are utilized in the synthesis of glycosides containing different sugar units.

The hydrolysis of glycosides is achieved by specific hydrolytic enzymes, e.g. β -glucosidase for β -glucosides and β -galactosidase for β -galactosides. These enzymes mimic the readily achieved acid-catalysed processes [Figure 2.27(b)]. Under acidic conditions, the α - and β -anomeric hemiacetal forms can also equilibrate via the open chain

Glycosylation reactions**(a) *O*-glucosylation****(b) hydrolysis of *O*-glucosides****(c) *C*-glucosylation****Figure 2.27**

sugar. Of particular importance is that although *O*-, *N*-, and *S*-glycosides may be hydrolysed by acid, *C*-glycosides are stable to acid. *C*-Glycosides are produced in a similar manner to the *C*-alkylation process described above, where

a suitable nucleophilic carbon is available, e.g. aromatic systems activated by phenol groups [Figure 2.27(c)]. The resultant *C*-glycoside thus contains a new carbon–carbon linkage, and cleavage would require oxidation, not hydrolysis.

SOME VITAMINS ASSOCIATED WITH THE CONSTRUCTION MECHANISMS***Vitamin B₁***

Vitamin B₁ (thiamine) (Figure 2.28) is a water-soluble vitamin with a pyrimidinylmethylthiazolium structure. It is widely available in the diet, with cereals, beans, nuts, eggs, yeast, and vegetables providing sources. Wheat germ and yeast have very high levels. Dietary deficiency leads to beriberi, characterized by neurological disorders, loss of appetite, fatigue,

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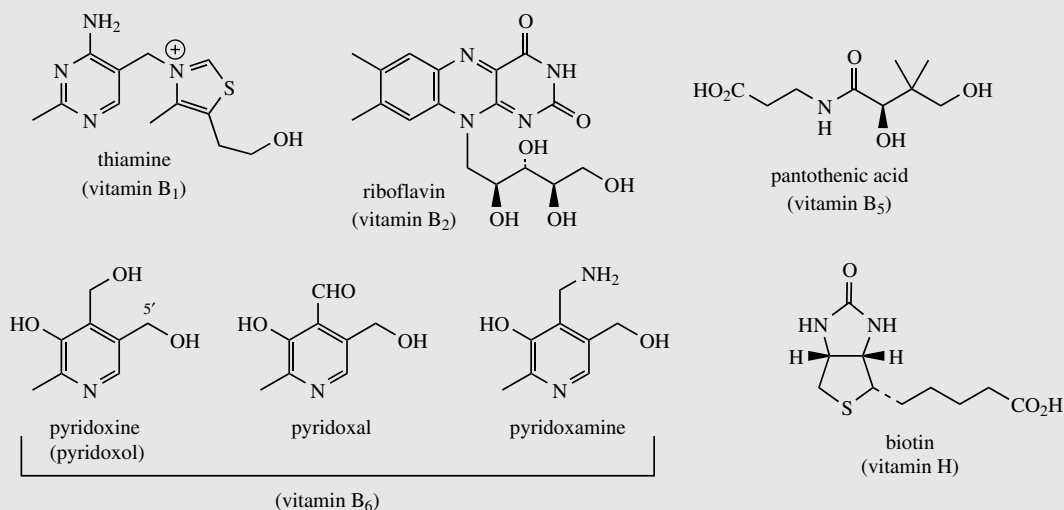


Figure 2.28

and muscular weakness. **Thiamine** is produced synthetically, and foods such as cereals are often enriched. The vitamin is stable in acid solution, but decomposes above pH 5, and is also partially decomposed during normal cooking. As thiamine diphosphate, vitamin B₁ is a coenzyme for pyruvate dehydrogenase which catalyses the oxidative decarboxylation of pyruvate to acetyl-CoA (see page 21), and also for transketolase which transfers a two-carbon fragment between carbohydrates in the pentose phosphate pathway (see page 446). Accordingly, this is a very important component in carbohydrate metabolism.

Vitamin B₂

Vitamin B₂ (riboflavin) (Figure 2.28) is a water-soluble vitamin having an isoalloxazine ring linked to D-ribitol. It is widely available in foods, including liver, kidney, dairy products, eggs, meat, and fresh vegetables. Yeast is a particularly rich source. It is stable in acid solution, not decomposed during cooking, but is sensitive to light. Riboflavin may be produced synthetically, or by fermentation using the yeastlike fungi *Eremothecium ashbyii* and *Ashbya gossypii*. Dietary deficiency is uncommon, but manifests itself by skin problems and eye disturbances. Riboflavin is a component of FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide), coenzymes which play a major role in oxidation–reduction reactions (see page 25). Many key enzymes containing riboflavin (flavoproteins) are involved in metabolic pathways. Since riboflavin contains ribitol and not ribose in its structure, FAD and FMN are not strictly nucleotides, though this nomenclature is commonly accepted and used.

Vitamin B₅

Vitamin B₅ (pantothenic acid) (Figure 2.28) is a very widely distributed water-soluble vitamin, though yeast, liver, and cereals provide rich sources. Even though animals must obtain the vitamin through the diet, pantothenic acid deficiency is rare, since most foods provide

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adequate quantities. Its importance in metabolism is as part of the structure of coenzyme A (see page 16), the carrier molecule essential for carbohydrate, fat, and protein metabolism. Pantothenic acid is specifically implicated in enzymes responsible for the biosynthesis of fatty acids (see page 36), polyketides (page 62) and some peptides (page 421).

Vitamin B₆

Vitamin B₆ covers the three pyridine derivatives pyridoxine (pyridoxol), pyridoxal, and pyridoxamine, and also their 5'-phosphates (Figure 2.28). These are water-soluble vitamins, pyridoxine predominating in plant materials, whilst pyridoxal and pyridoxamine are the main forms in animal tissues. Meat, salmon, nuts, potatoes, bananas, and cereals are good sources. A high proportion of the vitamin activity can be lost during cooking, but a normal diet provides an adequate supply. Vitamin B₆ deficiency is usually the result of malabsorption, or may be induced by some drug treatments where the drug may act as an antagonist or increase its renal excretion as a side-effect. Symptoms of deficiency are similar to those of niacin (vitamin B₃) and riboflavin (vitamin B₂) deficiencies, and include eye, mouth, and nose lesions, and neurological changes. Synthetic **pyridoxine** is used for supplementation. Pyridoxal 5'-phosphate is a coenzyme for a large number of enzymes, particularly those involved in amino acid metabolism, e.g. in transamination (see page 20) and decarboxylation (see page 20). The production of the neurotransmitter γ -aminobutyric acid (GABA) from glutamic acid is an important pyridoxal-dependent reaction.

Vitamin B₁₂

Vitamin B₁₂ (cobalamins) (Figure 2.29) are extremely complex structures based on a corrin ring, which, although similar to the porphyrin ring found in haem, chlorophyll, and cytochromes,

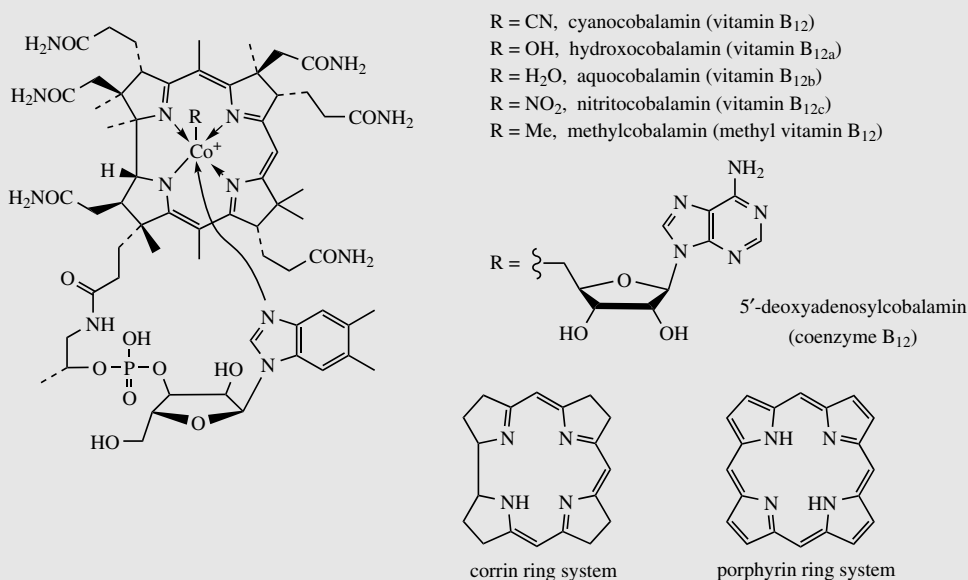


Figure 2.29

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has two of the pyrrole rings directly bonded. The central metal atom is cobalt; haem and cytochromes have iron, whilst chlorophyll has magnesium. Four of the six coordinations are provided by the corrin ring nitrogens, and a fifth by a dimethylbenzimidazole moiety. The sixth is variable, being cyano in **cyanocobalamin** (vitamin B₁₂), hydroxyl in **hydroxocobalamin** (vitamin B_{12a}), or other anions may feature. Cyanocobalamin is actually an artefact formed as a result of the use of cyanide in the purification procedures. The physiologically active coenzyme form of the vitamin is 5'-deoxyadenosylcobalamin (coenzyme B₁₂). Vitamin B₁₂ appears to be entirely of microbial origin, with intestinal flora contributing towards human dietary needs. The vitamin is then stored in the liver, and animal liver extract has been a traditional source. Commercial supplies are currently obtained by semi-synthesis from the total cobalamin extract of *Streptomyces griseus*, *Propionibacterium* species, or other bacterial cultures. This material can be converted into cyanocobalamin or hydroxocobalamin. The cobalamins are stable when protected against light. Foods with a high vitamin B₁₂ content include liver, kidney, meat, and seafood. Vegetables are a poor dietary source, and strict vegetarians may therefore risk deficiencies. Insufficient vitamin B₁₂ leads to pernicious anaemia, a disease that results in nervous disturbances and low production of red blood cells, though this is mostly due to lack of the gastric glycoprotein (intrinsic factor) which complexes with the vitamin to facilitate its absorption. Traditionally, daily consumption of raw liver was used to counteract the problem. Cyanocobalamin, or preferably hydroxocobalamin which has a longer lifetime in the body, may be administered orally or by injection to counteract deficiencies. Both agents are converted into coenzyme B₁₂ in the body. Coenzyme B₁₂ is a cofactor for a number of metabolic rearrangements, such as the conversion of methylmalonyl-CoA into succinyl-CoA in the oxidation of fatty acids with an odd number of carbon atoms, and for methylations, such as in the biosynthesis of methionine.

Vitamin H

Vitamin H (biotin) (Figure 2.28) is a water-soluble vitamin found in eggs, liver, kidney, yeast, cereals, and milk, and is also produced by intestinal microflora so that dietary deficiency is rare. Deficiency can be triggered by a diet rich in raw egg white, in which a protein, avidin, binds biotin so tightly so that it is effectively unavailable for metabolic use. This affinity disappears by cooking and hence denaturing the avidin. Biotin deficiency leads to dermatitis and hair loss. The vitamin functions as a carboxyl carrier, binding CO₂ via a carbamate link, then donating this in carboxylase reactions, e.g. carboxylation of acetyl-CoA to malonyl-CoA (see page 17), of propionyl-CoA to methylmalonyl-CoA (see page 92), and of pyruvate to oxaloacetate during gluconeogenesis.

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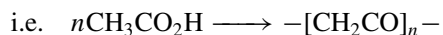
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3

THE ACETATE PATHWAY: FATTY ACIDS AND POLYKETIDES

Polyketides, metabolites built primarily from combinations of acetate units, are described. The biosynthesis of saturated and unsaturated fatty acids is covered, together with prostaglandins, thromboxanes, and leukotrienes. Cyclization of polyketides to give aromatic structures is then rationalized in terms of aldol and Claisen reactions. More complex structures formed via pathways involving alkylation reactions, phenolic oxidative coupling, oxidative cleavage of aromatic rings, and employing starter groups other than acetate are developed. The use of extender groups other than malonate gives rise to macrolides and polyethers, whilst further cyclization of polyketide structures may be achieved through Diels–Alder reactions. The application of genetic engineering to modify products from the acetate pathway is discussed. Monograph topics giving more detailed information on medicinal agents include fixed oils and fats, evening primrose oil, echinacea, prostaglandins and isoprostanes, thromboxanes, leukotrienes, senna, cascara, frangula and allied drugs, St John's wort, mycophenolic acid, khellin and cromoglicate, griseofulvin, poison ivy and poison oak, aflatoxins, cannabis, tetracyclines, anthracycline antibiotics, macrolide antibiotics, avermectins, polyene antifungals, tacrolimus and sirolimus, ansa macrolides, mevastatin and other statins.

Polyketides constitute a large class of natural products grouped together on purely biosynthetic grounds. Their diverse structures can be explained as being derived from poly- β -keto chains, formed by coupling of acetic acid (C_2) units via condensation reactions,



Included in such compounds are the fatty acids, polyacetylenes, prostaglandins, macrolide antibiotics and many aromatic compounds, e.g. anthraquinones and tetracyclines.

The formation of the poly- β -keto chain could be envisaged as a series of Claisen reactions, the reverse of which are involved in the β -oxidation sequence for the metabolism of fatty acids (see page 18). Thus, two molecules of acetyl-CoA could participate in a Claisen condensation giving acetoacetyl-CoA, and this reaction could be repeated to generate a poly- β -keto ester of appropriate chain length (Figure 3.1). However, a study of the enzymes involved in fatty acid biosynthesis showed this simple rationalization could not be correct, and a more complex series of

reactions was operating. It is now known that fatty acid biosynthesis involves initial carboxylation of acetyl-CoA to malonyl-CoA, a reaction involving ATP, CO_2 (as bicarbonate, HCO_3^-), and the coenzyme biotin as the carrier of CO_2 (see page 17).

The conversion of acetyl-CoA into malonyl-CoA increases the acidity of the α -hydrogens, and thus provides a better nucleophile for the Claisen condensation. In the biosynthetic sequence, no acylated malonic acid derivatives are produced, and no label from [^{14}C]bicarbonate is incorporated, so the carboxyl group introduced into malonyl-CoA is simultaneously lost by a decarboxylation reaction during the Claisen condensation (Figure 3.1). Accordingly, the carboxylation step helps to activate the α -carbon and facilitate Claisen condensation, and the carboxyl is immediately removed on completion of this task. An alternative rationalization is that decarboxylation of the malonyl ester is used to generate the acetyl enolate anion without any requirement for a strong base.

The pathways to fatty acids and aromatic polyketides branch early. The chain extension process of Figure 3.1 continues for aromatics,

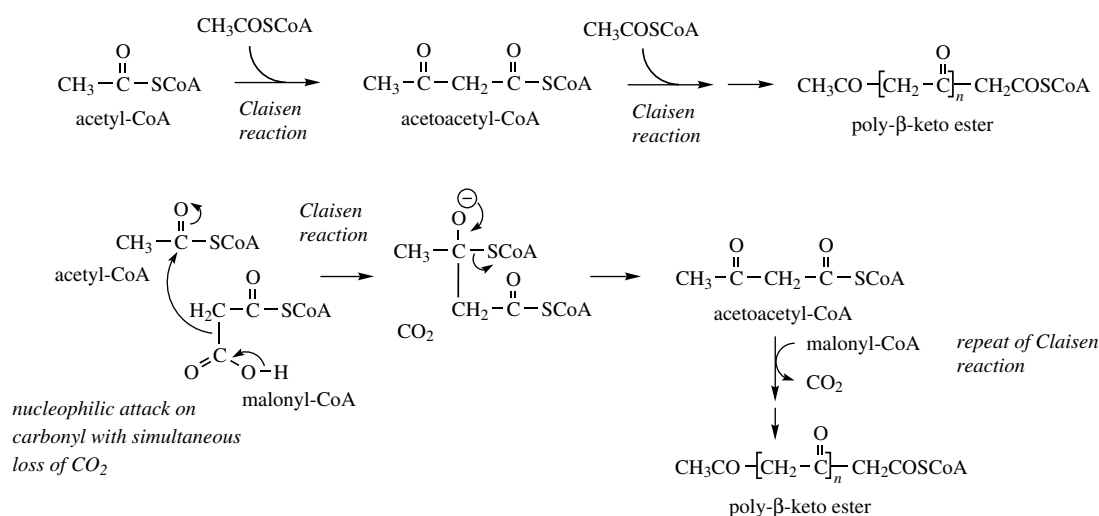


Figure 3.1

generating a highly reactive poly-β-keto chain, which has to be stabilized by association with groups on the enzyme surface until chain assembly is complete and cyclization reactions occur. However, for fatty acids, the carbonyl groups are reduced before attachment of the next malonate group. Partial reduction processes, leading to a mixture of methylenes, hydroxyls, and carbonyls, are characteristic of macrolides (see page 92).

SATURATED FATTY ACIDS

The processes of fatty acid biosynthesis are well studied and are known to be catalysed by the enzyme **fatty acid synthase**. In animals, this is a multifunctional protein containing all of the catalytic activities required, whilst bacteria and plants utilize an assembly of separable enzymes. Acetyl-CoA and malonyl-CoA themselves are not involved in the condensation step: they are converted into enzyme-bound thioesters, the malonyl ester by means of an acyl carrier protein (ACP) (Figure 3.2). The Claisen reaction follows giving acetoacetyl-ACP (β-keto acyl-ACP; R=H), which is reduced stereospecifically to the corresponding β-hydroxy ester, consuming NADPH in the reaction. Then follows elimination of water giving the *E* (*trans*) α,β-unsaturated ester. Reduction of the double bond again utilizes NADPH and generates a saturated acyl-ACP (fatty

acyl-ACP; R=H) which is two carbons longer than the starting material. This can feed back into the system, condensing again with malonyl-ACP, and going through successive reduction, dehydration, and reduction steps, gradually increasing the chain length by two carbons for each cycle, until the required chain length is obtained. At that point, the fatty acyl chain can be released as a fatty acyl-CoA or as the free acid. The chain length actually elaborated is probably controlled by the specificity of the thioesterase enzymes that subsequently catalyse release from the enzyme.

The fatty acid synthase protein is known to contain an acyl carrier protein binding site, and also an active site cysteine residue in the β-ketoacyl synthase domain. Acetyl and malonyl groups are successively transferred from coenzyme A esters and attached to the thiol groups of Cys and ACP (Figure 3.3). The Claisen condensation occurs, and the processes of reduction, dehydration, and reduction then occur whilst the growing chain is attached to ACP. The ACP carries a phosphopantetheine group exactly analogous to that in coenzyme A, and this provides a long flexible arm, enabling the growing fatty acid chain to reach the active site of each enzyme in the complex, allowing the different chemical reactions to be performed without releasing intermediates from the enzyme (compare polyketide synthesis page 62 and peptide synthesis, page 421). Then the chain is transferred to the thiol of Cys, and the process can

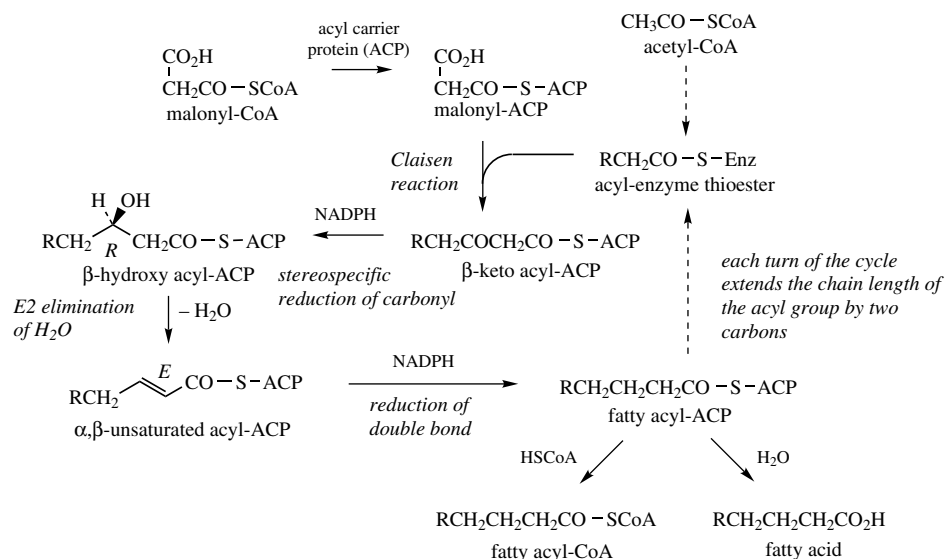


Figure 3.2

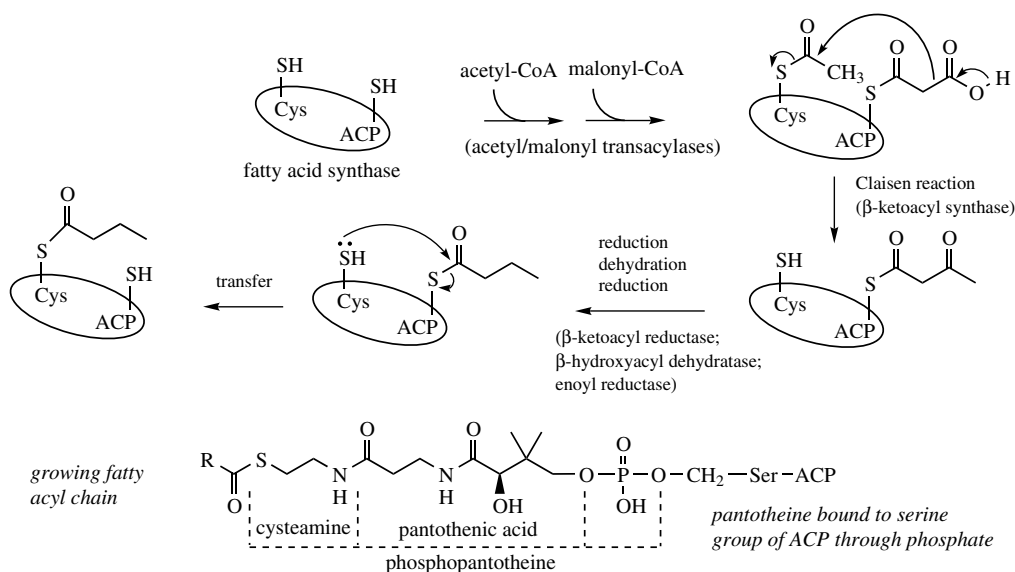


Figure 3.3

continue. Making the process even more efficient, animal fatty acid synthase is a dimeric protein containing two catalytic centres and is able to generate two growing chains. The monomeric subunits are also arranged head to tail so that the acyl group of one unit actually picks up a malonyl extender from the other unit (Figure 3.4). Note that the sequence of enzyme activities along the protein chain of the enzyme complex does

not correspond with the order in which they are employed.

Thus, combination of one acetate starter unit with seven malonates would give the C_{16} fatty acid, palmitic acid, and with eight malonates the C_{18} fatty acid, stearic acid. Note that the two carbons at the head of the chain (methyl end) are provided by acetate, not malonate, whilst the remainder are derived from malonate, which itself

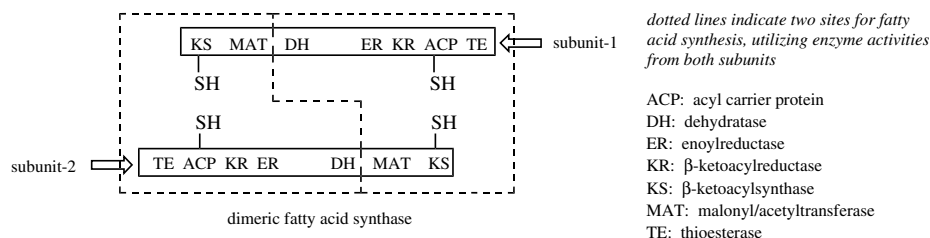
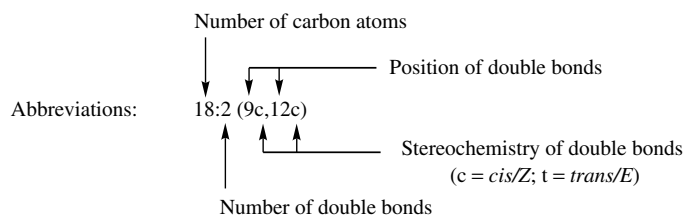


Figure 3.4

Table 3.1 Common naturally occurring fatty acids

<i>Saturated</i>					
butyric	$\text{CH}_3(\text{CH}_2)_2\text{CO}_2\text{H}$	4:0	stearic	$\text{CH}_3(\text{CH}_2)_{16}\text{CO}_2\text{H}$	18:0
caproic*	$\text{CH}_3(\text{CH}_2)_4\text{CO}_2\text{H}$	6:0	arachidic	$\text{CH}_3(\text{CH}_2)_{18}\text{CO}_2\text{H}$	20:0
caprylic*	$\text{CH}_3(\text{CH}_2)_6\text{CO}_2\text{H}$	8:0	behenic	$\text{CH}_3(\text{CH}_2)_{20}\text{CO}_2\text{H}$	22:0
capric*	$\text{CH}_3(\text{CH}_2)_8\text{CO}_2\text{H}$	10:0	lignoceric	$\text{CH}_3(\text{CH}_2)_{22}\text{CO}_2\text{H}$	24:0
lauric	$\text{CH}_3(\text{CH}_2)_{10}\text{CO}_2\text{H}$	12:0	cerotic	$\text{CH}_3(\text{CH}_2)_{24}\text{CO}_2\text{H}$	26:0
myristic	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}_2\text{H}$	14:0	montanic	$\text{CH}_3(\text{CH}_2)_{26}\text{CO}_2\text{H}$	28:0
palmitic	$\text{CH}_3(\text{CH}_2)_{14}\text{CO}_2\text{H}$	16:0	melissic	$\text{CH}_3(\text{CH}_2)_{28}\text{CO}_2\text{H}$	30:0
*To avoid confusion, systematic nomenclature (hexanoic, octanoic, decanoic) is recommended					
<i>Unsaturated</i>					
palmitoleic	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$	16:1 (9c)			
oleic	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$	18:1 (9c)			
cis-vaccenic	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_9\text{CO}_2\text{H}$	18:1 (11c)			
linoleic	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$	18:2 (9c,12c)			
α-linolenic	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$	18:3 (9c,12c,15c)			
γ-linolenic	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_4\text{CO}_2\text{H}$	18:3 (6c,9c,12c)			
gadoleic	$\text{CH}_3(\text{CH}_2)_9\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$	20:1 (9c)			
arachidonic	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_3\text{CO}_2\text{H}$	20:4 (5c,8c,11c,14c)			
eicosapentaenoic (EPA)	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_3\text{CO}_2\text{H}$	20:5 (5c,8c,11c,14c,17c)			
erucic	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}\text{CO}_2\text{H}$	22:1 (13c)			
docosapentaenoic (DPA)	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_5\text{CO}_2\text{H}$	22:5 (7c,10c,13c,16c,19c)			
docosahexaenoic (DHA)	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_2\text{CO}_2\text{H}$	22:6 (4c,7c,10c,13c,16c,19c)			
nervonic	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{13}\text{CO}_2\text{H}$	24:1 (15c)			
all double bonds are Z (cis)					



is produced by carboxylation of acetate. This means that all carbons in the fatty acid originate from acetate, but malonate will only provide the C_2 chain extension units and not the C_2 starter group. The linear combination of acetate C_2 units as in Figure 3.2 explains why the common fatty acids are straight chained and possess an even number

of carbon atoms. Natural fatty acids may contain from four to 30, or even more, carbon atoms, the most abundant being those with 16 or 18 carbons. Some naturally occurring fatty acids are shown in Table 3.1. The rarer fatty acids containing an odd number of carbon atoms typically originate from incorporation of a different starter unit, e.g.

propionic acid, or can arise by loss of one carbon from an even-numbered acid.

Fatty acids are mainly found in ester combination with glycerol in the form of triglycerides (Figure 3.5). These materials are called **fats** or **oils**, depending on whether they are solid or liquid at room temperature. If all three esterifying acids are the same, the triglyceride is termed simple, whereas a mixed triglyceride is produced if two or more of the fatty acids are different. Most natural fats and oils are composed largely of mixed triglycerides. In this case, isomers can exist, including potential optical isomers, since if the primary alcohols are esterified with different fatty acids the central carbon of glycerol will become chiral. In practice, only one of each pair of enantiomers is formed in nature. Triglycerides are produced from glycerol 3-phosphate by esterification with fatty

acyl-CoA residues, the phosphate being removed prior to the last esterification (Figure 3.5). The diacyl ester of glycerol 3-phosphate is also known as a **phosphatidic acid**, and is the basis of phospholipid structures. In these structures, the phosphate is also esterified with an alcohol, which is usually choline, ethanolamine, serine, or *myo*-inositol, e.g. **phosphatidyl choline** (Figure 3.6). **Phospholipids** are important structural components of cell membranes, and because of the polar and non-polar regions in their structure, they have detergent-like properties. They are also able to form liposomes, which have considerable potential as drug delivery systems. A particularly important natural phospholipid is **platelet-activating factor (PAF)** (Figure 3.6), which resembles a phosphatidylcholine, though this compound possesses an ether linkage to a long chain fatty alcohol,

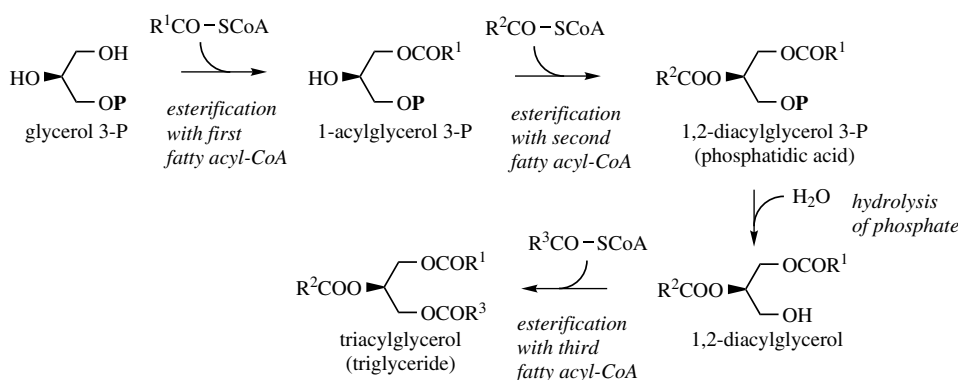


Figure 3.5

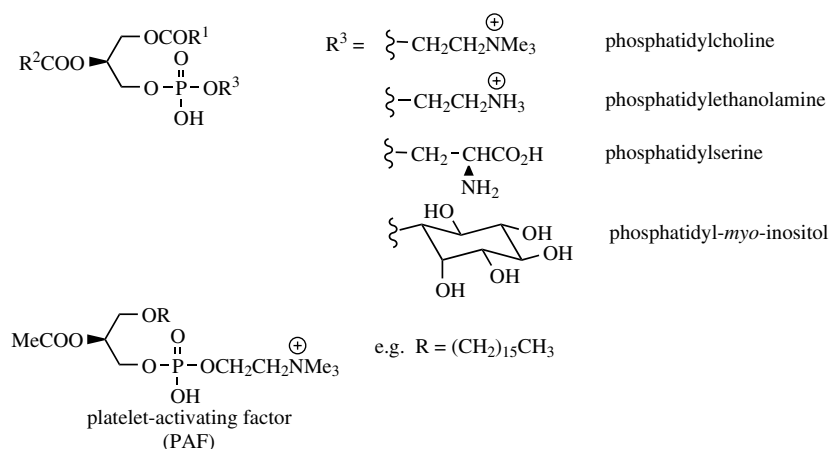


Figure 3.6

usually hexadecanol, rather than an ester linkage. The central hydroxyl of glycerol is esterified, but to acetic acid rather than to a long chain fatty acid. PAF functions at nanomolar concentrations, activates blood platelets and contributes to diverse biological effects, including thrombosis, inflammatory reactions, allergies, and tissue rejection. Long chain alcohols are reduction products from fatty acids and also feature in natural **waxes**. These are complex mixtures of esters of long chain fatty acids, usually C₂₀–C₂₄, with long chain monohydric alcohols or sterols.

UNSATURATED FATTY ACIDS

Animal fats contain a high proportion of glycerides of saturated fatty acids and tend to be solids, whilst those from plants and fish contain predominantly unsaturated fatty acid esters and tend to be liquids. Some of the common naturally occurring unsaturated fatty acids are also included in Table 3.1. A convenient shorthand representation for fatty acids indicating chain length with number, position and stereochemistry of double bonds is also presented in Table 3.1. A less systematic numbering starting from the methyl (the ω end) may also be encountered. Major groups of fatty acids are designated ω -3 (omega-3), ω -6 (omega-6), ω -9 (omega-9), etc (or sometimes n-3, n-6, n-9), if there is a double bond that number of carbons from the methyl terminus. This has some value in relating structures when an unsaturated fatty acid is biosynthetically elongated from the carboxyl end as during prostaglandin biosynthesis (see page 45). Double bonds at position 9 are common, but unsaturation can occur at other positions in the chain. Polyunsaturated fatty acids tend to have their double bonds in a non-conjugated array as a repeating unit $-(CH=CHCH_2)_n-$. In virtually all cases, the stereochemistry of the double bond is *Z* (*cis*), introducing a 'bend' into the alkyl chain. This interferes with the close association and aggregation of molecules that is possible in saturated structures, and helps to maintain the fluidity in oils and cellular membranes.

Fats and oils represent long term stores of energy for most organisms, being subjected to oxidative metabolism as required. Major oils which are produced commercially for use as foods, toiletries, medicinals, or pharmaceutical formulation

aids are listed in Table 3.2. Typical fatty acid analyses are shown, though it must be appreciated that these figures can vary quite widely. For instance, plant oils show significant variation according to the climatic conditions under which the plant was grown. In colder climates, a higher proportion of polyunsaturated fatty acids is produced, so that the plant can maintain the fluidity of its storage fats and membranes. The melting points of these materials depend on the relative proportions of the various fatty acids, reflecting primarily the chain length and the amount of unsaturation in the chain. Saturation, and increasing chain length in the fatty acids gives a more solid fat at room temperature. Thus, butterfat and cocoa butter (theobroma oil) contain a relatively high proportion of saturated fatty acids and are solids. Palm kernel and coconut oils are both semi-solids having a high concentration of the saturated C₁₂ acid **lauric acid**. A characteristic feature of olive oil is its very high **oleic acid** (18:1) content, whilst rapeseed oil possesses high concentrations of long chain C₂₀ and C₂₂ fatty acids, e.g. **erucic acid** (22:1). Typical fatty acids in fish oils have high unsaturation and also long chain lengths, e.g. **eicosapentaenoic acid (EPA)** (20:5) and **docosahexaenoic acid (DHA)** (22:6) in cod liver oil.

Unsaturated fatty acids can arise by more than one biosynthetic route, but in most organisms the common mechanism is by desaturation of the corresponding alkanoic acid, with further desaturation in subsequent steps. Most eukaryotic organisms possess a Δ^9 -desaturase that introduces a *cis* double bond into a saturated fatty acid, requiring O₂ and NADPH or NADH cofactors. The mechanism of desaturation does not involve any intermediates hydroxylated at C-9 or C-10, and the requirement for O₂ is as an acceptor at the end of an electron transport chain. A stearyl (C₁₈) thioester is the usual substrate giving an oleoyl derivative (Figure 3.7), coenzyme A esters being utilized by animal and fungal enzymes, and ACP esters by plant systems. The position of further desaturation then depends very much on the organism. Non-mammalian enzymes tend to introduce additional double bonds between the existing double bond and the methyl terminus, e.g. oleate $\rightarrow$ linoleate $\rightarrow$ α -linolenate. Animals always introduce new double bonds towards the carboxyl group. Thus **oleate** is desaturated to

Table 3.2 Fixed oils and fats

The term fat or oil has no precise significance, and merely describes whether the material is a solid (fat) or liquid (oil) at room temperature. Most commercial oils are obtained from plant sources, particularly seeds and fruits, and the oil is extracted by cold or hot expression, or less commonly by solvent extraction with hexane. The crude oil is then refined by filtration, steaming, neutralization to remove free acids, washing, and bleaching as appropriate. Many food oils are then partially hydrogenated to produce semi-solid fats. Animal fats and fish oils are usually extracted by steaming, the higher temperature deactivating enzymes that would otherwise begin to hydrolyse the glycerides.

Oils and fats feature as important food components and cooking oils, some 80% of commercial production being used as human food, whilst animal feeds account for another 6%. Most of the remaining production is used as the basis of soaps, detergents, and pharmaceutical creams and ointments. A number of oils are used as diluents (carrier or base oils) for the volatile oils employed in aromatherapy.

Oil	Source	Part used	Oil content [†] (%)	Typical fatty acid composition [†] (%)	Uses, notes
Almond	<i>Prunus amygdalus</i> var. <i>dulcis</i> , or var. <i>amara</i> (Rosaceae)	seed	40–55	oleic (62–86), linoleic (7–30), palmitic (4–9), stearic (1–2)	emollient base, toiletries, carrier oil (aromatherapy)
Arachis (groundnut, peanut)	<i>Arachis hypogaea</i> (Leguminosae/ Fabaceae)	seed	45–55	oleic (35–72), linoleic (13–43), palmitic (7–16), stearic (1–7), behenic (1–5), arachidic (1–3)	food oil, emollient base
Borage	<i>Borago officinalis</i> (Boraginaceae)	seed	28–35	linoleic (38), γ -linolenic (23–26), oleic (16), palmitic (11)	dietary supplement for γ -linolenic acid content (see page 46)
Butterfat	cow <i>Bos taurus</i> (Bovidae)	milk	2–5	palmitic (29), oleic (28), stearic (13), myristic (12), butyric (4), lauric (3), caproic (2), capric (2), palmitoleic (2)	food
Castor	<i>Ricinus communis</i> (Euphorbiaceae)	seed	35–55	ricinoleic (80–90), oleic (4–9), linoleic (2–7), palmitic (2–3), stearic (2–3)	emollient base, purgative, soap manufacture

Castor seeds contain the highly toxic,
but heat-labile protein ricin (see
page 434)

(Continued overleaf)

Table 3.2 (Continued)

Oil	Source	Part used	Oil content [†] (%)	Typical fatty acid composition [†] (%)	Uses, notes
Coconut	<i>Cocos nucifera</i> (Palmae/ Arecaceae)	seed kernel	65–68	lauric (43–53), myristic (15–21), palmitic (7–11), caprylic (5–10), capric (5–10), oleic (6–8), stearic (2–4)	soaps, shampoos Fractionated coconut oil containing only short to medium length fatty acids (mainly caprylic and capric) is a dietary supplement
Cod-liver	cod <i>Gadus morrhua</i> (Gadidae)	fresh liver	50	oleic (24), DHA (14), palmitic (11), EPA (6), palmitoleic (7), stearic (4), myristic (3)	dietary supplement due to presence of EPA and DHA, plus vitamins A (see page 230) and D (see page 259); halibut-liver oil from halibut <i>Hippoglossus vulgaris</i> (Pleuronectidae) has similar properties and is used in the same way
Cottonseed	<i>Gossypium</i> <i>hirsutum</i> (Malvaceae)	seed	15–36	linoleic (33–58), palmitic (17–29), oleic (13–44), stearic (1–4)	solvent for injections, soaps Cotton seeds also contain 1.1–1.3% gossypol (see page 200) and small amounts of cyclopropenoid fatty acids, e.g. sterculic and malvalic acids (see page 50)
Evening primrose	<i>Oenothera biennis</i> (Onagraceae)	seed	24	linoleic (65–80), γ -linolenic (7–14), oleic (9), palmitic (7)	dietary supplement for γ -linolenic acid content (see page 46)
Honesty	<i>Lunaria annua</i> (Cruciferae/ Brassicaceae)	seed	30–40	erucic (43), nervonic (25), oleic (24)	nervonic acid is being investigated for the treatment of multiple sclerosis; the disease is characterized by a deficiency in nervonic acid

Lard	pig <i>Sus scrofa</i> (Suidae)	abdominal fat		oleic (45), palmitic (25), stearic (12), linoleic (10), palmitoleic (3)	foods
Linseed (flaxseed)	<i>Linum usitatissimum</i> (Linaceae)	seed	35–44	α -linolenic (30–60), oleic (39), linoleic (15), palmitic (7), stearic (4)	liniments, dietary supplement for α -linolenic acid content Formerly the basis of paints, reacting with oxygen, polymerizing, and drying to a hard film food oil, dietary supplement, solvent for injections
Maize (corn)	<i>Zea mays</i> (Graminae/ Poaceae)	embryo	33–39	linoleic (34–62), oleic (19–50), palmitic (8–19), stearic (0–4)	
Olive	<i>Olea europaea</i> (Oleaceae)	fruits	15–40	oleic (56–85), palmitic (8–20), linoleic (4–20), stearic (1–4)	food oil, emollient base
Palm kernel	<i>Elaeis guineensis</i> (Palmae/ Arecaceae)	kernel	45–50	lauric (40–52), myristic (14–18), oleic (9–16), palmitoleic (6–10), caprylic (3–6), capric (3–5), stearic (1–4), linoleic (1–3)	soaps Fractionated palm oil is a solid obtained by fractionation and hydrogenation and is used as a suppository base
Rapeseed	<i>Brassica napus</i> (Cruciferae/ Brassicaceae)	seed	40–50	erucic (30–60), oleic (9–25), linoleic (11–25), gadoleic (5–15), α -linolenic (5–12), palmitic (0–5)	food oil, using varieties producing lower levels of erucic acid where the main components are now oleic (48–60%), linoleic (18–30%), α -linolenic (6–14%), and palmitic (3–6%) acids Erucic acid is used as a plasticizer in PVC clingfilm

(Continued overleaf)

Table 3.2 (Continued)

Oil	Source	Part used	Oil content [†] (%)	Typical fatty acid composition [†] (%)	Uses, notes
Sesame	<i>Sesamum indicum</i> (Pedaliaceae)	seed	44–54	oleic (35–50), linoleic (35–50), palmitic (7–12), stearic (4–6)	food oil, soaps, solvent for injections, carrier oil (aromatherapy)
Soya (soybean)	<i>Glycine max</i> (Leguminosae/ Fabaceae)	seed	18–20	linoleic (44–62%), oleic (19–30), palmitic (7–14), α -linolenic (4–11), stearic (1–5)	food oil, dietary supplement, carrier oil (aromatherapy)
Suet (mutton tallow)	sheep <i>Ovis aries</i> (Bovidae)	abdominal fat		stearic (32), oleic (31), palmitic (27), myristic (6)	Soya oil contains substantial amounts of the sterols sitosterol and stigmasterol (see page 256) foods
Suet (beef tallow)	cow <i>Bos taurus</i> (Bovidae)	abdominal fat		oleic (48), palmitic (27), palmitoleic (11), stearic (7), myristic (3)	foods
Sunflower	<i>Helianthus annuus</i> (Compositae/ Asteraceae)	seed	22–36	linoleic (50–70), oleic (20–40), palmitic (3–10), stearic (1–10)	food oil, carrier oil (aromatherapy)
Theobroma	<i>Theobroma cacao</i> (Sterculiaceae)	kernel	35–50	oleic (35), stearic (35), palmitic (26), linoleic (3)	suppository base, chocolate manufacture Theobroma oil (cocoa butter) is a solid

[†]The oil yields and fatty acid compositions given in the table are typical values, and can vary widely. The quality of an oil is determined principally by its fatty acid analysis. Structures of the fatty acids are shown in Table 3.1 (see page 38).

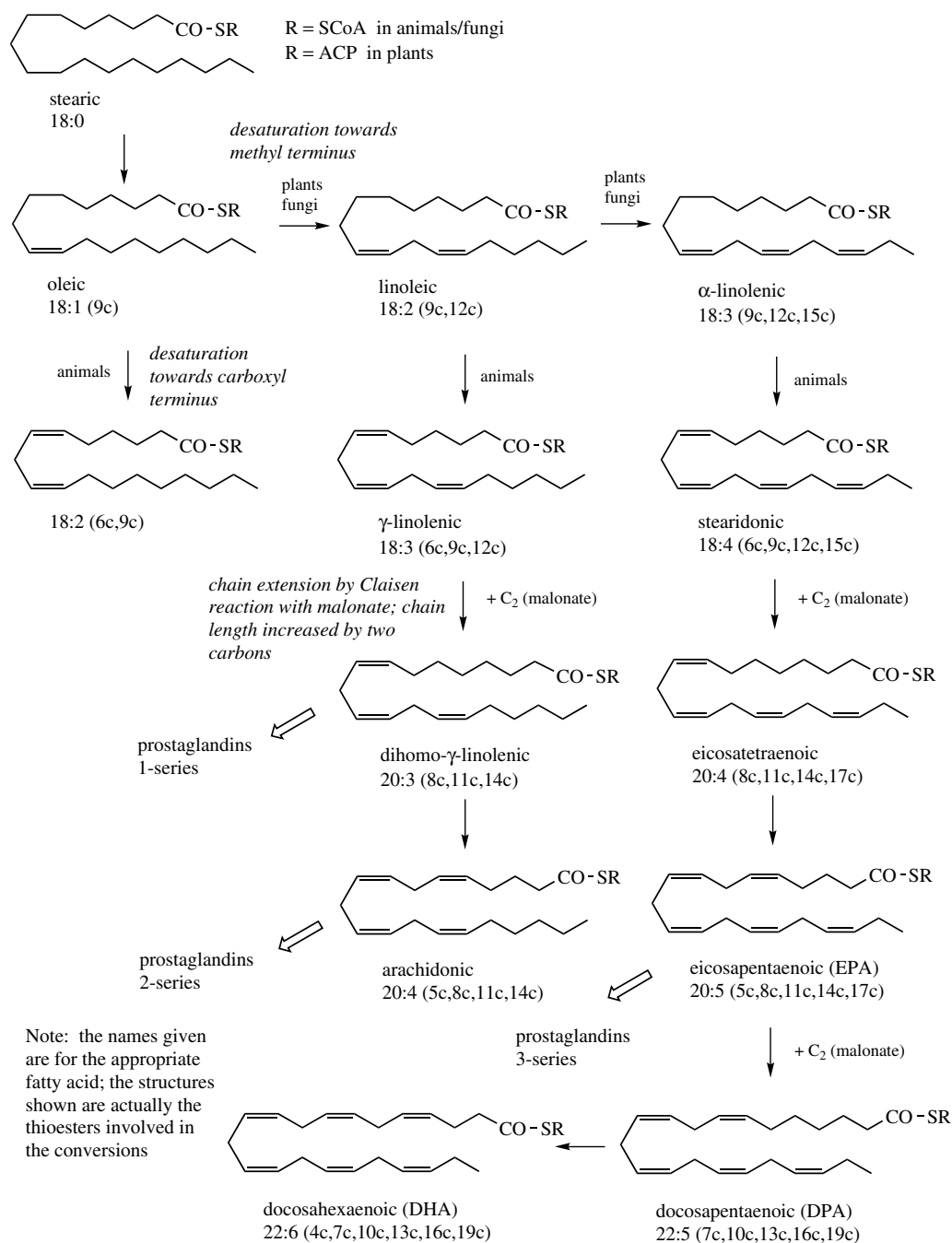


Figure 3.7

$\Delta^{6,9}$ -octadecadienoate rather than linoleate. However, animals need **linoleate** for the biosynthesis of **dihomo- γ -linolenate** ($\Delta^{8,11,14}$ -eicosatrienoate) and **arachidonate** ($\Delta^{5,8,11,14}$ -eicosatetraenoate), C₂₀ polyunsaturated fatty acid precursors of

prostaglandins in the 'one' and 'two' series respectively (see page 52). Accordingly, linoleic acid must be obtained from plant material in the diet, and it is desaturated towards the carboxyl to yield **γ -linolenate**, which is then used as the

substrate for further chain extension, adding a C₂ unit from malonate, and producing dihomog- γ -linolenate. Arachidonate derives from this by additional desaturation, again towards the carboxyl end of the chain (Figure 3.7). **α -Linolenate** is similarly a precursor on the way to $\Delta^{5,8,11,14,17}$ -**eicosapentaenoate (EPA)**, required for the synthesis of prostaglandins of the 'three' series, and it is also obtained from the diet. A similar chain extension process using further molecules of malonate is encountered in the sequence from α -linolenate in animal systems (Figure 3.7). Chain extension/dehydrogenations lead to formation of eicosapentaenoate (EPA) with further elaborations producing **docosapentaenoate (DPA)** and then **docosahexaenoate (DHA)**. DHA is a component of lipids in sperm, the retina, and the brain. It is thought to be important for brain development, and deficiency is associated with abnormalities in brain function. Linoleate and α -linolenate are referred to as '**essential fatty acids**' (EFAs) since they and their metabolites are required in the diet for normal good health. Some food sources such as the

oils present in fish are rich in the later metabolites derived from α -linolenic acid, e.g. EPA and DHA, and are also beneficial to health. Since these fatty acids all have a double bond three carbons from the methyl end of the chain, they are grouped together under the term ω -3 fatty acids (**omega-3 fatty acids**). Regular consumption of fish oils is claimed to reduce the risk of heart attacks and atherosclerosis.

Although most plant-derived oils contain high amounts of unsaturated fatty acid glycerides, including those of linoleic and α -linolenic acids, the conversion of linoleate into **γ -linolenate** can be blocked or inhibited in certain conditions in humans. This restricts synthesis of prostaglandins. In such cases, the use of food supplements, e.g. evening primrose oil* from *Oenothera biennis* (Onagraceae), which are rich in γ -linolenic esters, can be valuable and help in the disorder. Many plants in the Boraginaceae, e.g. borage (*Borago officinalis*), also accumulate significant amounts of γ -linolenic acid glycerides, as does evening primrose, indicating their unusual ability

Evening Primrose Oil

Evening primrose oil is extracted from the seeds of selected strains of the evening primrose (*Oenothera biennis*; Onagraceae), a biennial plant native to North America, which is now widely cultivated in temperate countries. The seeds contain about 24% fixed oil, which has a high content of glycerides of the unsaturated fatty acids linoleic acid (65–80%) and γ -linolenic acid (**gamolenic acid**) (7–14%). Because of this high γ -linolenic acid content, evening primrose oil is widely used as a dietary supplement, providing additional quantities of this essential fatty acid, which is a precursor in the biosynthesis of prostaglandins, which regulate many bodily functions (see page 54). Genetic and a number of other factors may inhibit the desaturation of linoleic acid into γ -linolenic acid. Ageing, diabetes, excessive alcohol intake, catecholamines, and zinc deficiency have all been linked to inhibition of the desaturase enzyme. The conversion may also be inhibited if there is a high proportion of fatty acids in the diet, which compete for the desaturase enzyme, including saturated and *trans*-unsaturated fatty acids. The latter group may be formed during the partial hydrogenation of polyunsaturated fatty acids which is commonly practised during food oil processing to produce semi-solid fats. Evening primrose oil appears to be valuable in the treatment of premenstrual tension, multiple sclerosis, breast pain (mastalgia), and perhaps also in eczema. There is potential for further applications, e.g. in diabetes, alcoholism, and cardiovascular disease. In evening primrose, γ -linolenic acid is usually present in the form of a dilinoleoylmono- γ -linolenylglycerol. This triglyceride is also being explored as a drug material for the treatment of diabetes-related neuropathy and retinopathy. γ -Linolenic acid is also found in the fixed oil of other plants, e.g. blackcurrant, comfrey, and borage, and in human milk. **Borage oil (starflower oil)** from the seeds of *Borago officinalis* (Boraginaceae) is used in the same way as evening primrose oil. It contains higher concentrations of γ -linolenic acid (23–26%), but rather less linoleic acid.

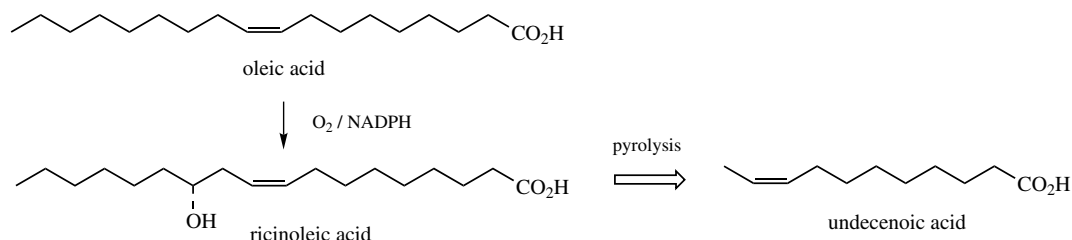


Figure 3.8

to desaturate linoleic esters towards the carboxyl terminus, rather than towards the methyl terminus as is more common in plants. Arachidonic acid itself has not been found in higher plants, but does occur in some algae, mosses, and ferns.

Ricinoleic acid (Figure 3.8) is the major fatty acid found in castor oil from seeds of the castor oil plant (*Ricinus communis*; Euphorbiaceae), and is the 12-hydroxy derivative of oleic acid. It is formed by direct hydroxylation of oleic acid (usually esterified as part of a phospholipid) by the action of an O_2 - and NADPH-dependent mixed function oxidase, but this is not of the cytochrome P-450 type. Castor oil has a long history of use as a domestic purgative, but it is now mainly employed as a cream base. **Undecenoic acid** (Δ^9 -undecenoic acid) can be obtained from ricinoleic acid by thermal degradation, and as the zinc salt or in ester form is used in fungistatic preparations.

Primary amides of unsaturated fatty acids have been characterized in humans and other mammals, and although their biological role is not fully understood, they may represent a group of important signalling molecules. **Oleamide**, the simple amide of oleic acid, has been shown to be a sleep-inducing lipid, and the amide of erucic acid, **erucamide**, stimulates the growth of blood vessels.

ACETYLENIC FATTY ACIDS

Many unsaturated compounds found in nature contain one or more acetylenic bonds, and these are predominantly produced by further desaturation of olefinic systems in fatty acid-derived molecules. They are surprisingly widespread in nature, and are found in many organisms, but are especially common in plants of the Compositae/Asteraceae, the Umbelliferae/Apiaceae, and fungi of the group

Basidiomycetes. These compounds tend to be highly unstable and some are even explosive if sufficient amounts are accumulated. Since only very small amounts are present in plants, this does not present any widespread hazard. Whilst fatty acids containing several double bonds usually have these in a non-conjugated array, molecules containing triple bonds tend to possess conjugated unsaturation. This gives the compounds intense and highly characteristic UV spectra which aids their detection and isolation.

The processes of desaturation are exemplified in Figure 3.9, in which oleic acid (probably as a thiol ester) features as a precursor of **crepenynic acid** and **dehydrocrepenynic acid**. The acetylenic bond is now indicated by *a* in the semi-systematic shorthand nomenclature. Chain shortening by β -oxidation (see page 18) is often a feature of these pathways, and formation of the C_{10} acetylenic acid **dehydromatricaria acid** proceeds through C_{18} intermediates, losing eight carbons, presumably via four β -oxidations. In the latter part of the pathway, the *Z*-double bond from oleic acid moves into conjugation with the polyacetylene chain via an allylic isomerization, giving the more favoured *E*-configuration. Some noteworthy acetylenic structures (though they are no longer acids and components of fats) are given in Figure 3.10. **Cicutoxin** from the water hemlock (*Cicuta virosa*; Umbelliferae/Apiaceae) and **oenanthotoxin** from the hemlock water dropwort (*Oenanthe crocata*; Umbelliferae/Apiaceae) are extremely toxic to mammals, causing persistent vomiting and convulsions, leading to respiratory paralysis. Ingestion of the roots of these plants may frequently lead to fatal poisoning. **Falcarinol** is a constituent of *Falcaria vulgaris* (Umbelliferae/Apiaceae), *Oenanthe crocata*, *Hedera helix* (Araliaceae), and several other plants, and is known to cause contact dermatitis in certain

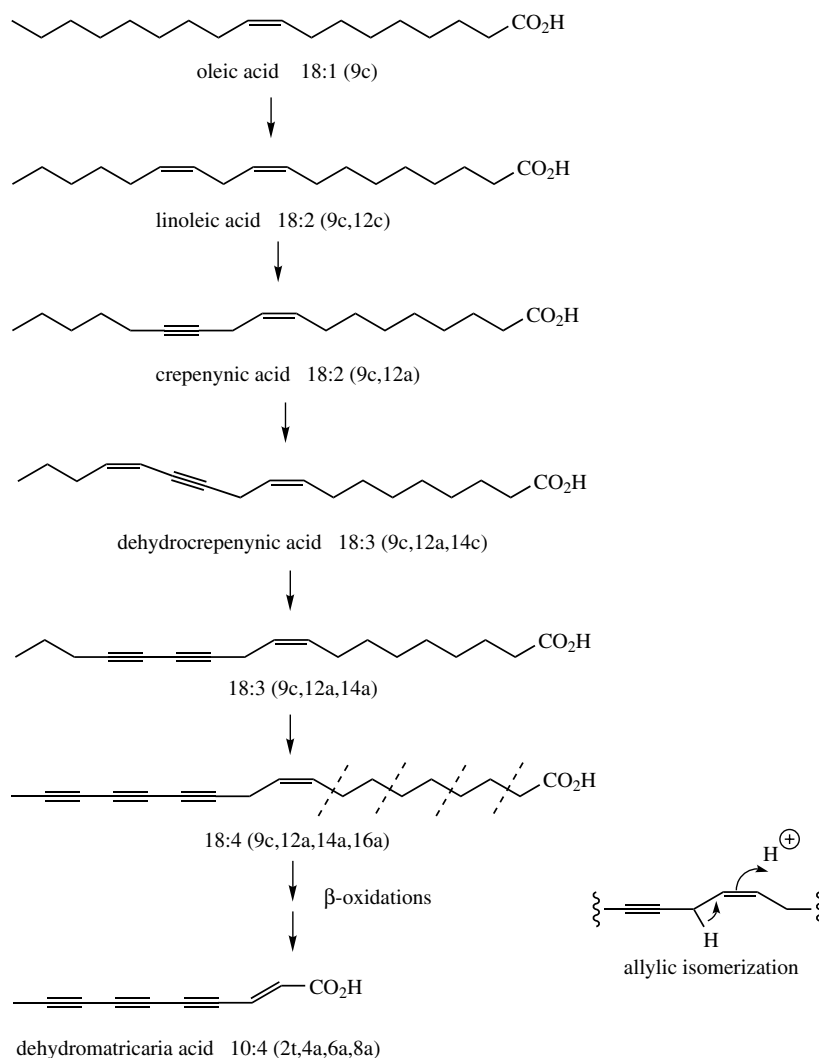


Figure 3.9

individuals when the plants are handled. Falcarinol (sometimes called panaxynol) and the structurally related **panaxytriol** are also characteristic polyacetylene components of ginseng (*Panax ginseng*; Araliaceae) (see page 222). **Wyerone** from the broad bean (*Vicia faba*; Leguminosae/Fabaceae) has antifungal properties, and its structure exemplifies how the original straight chain may be cross-linked to produce a ring system. The furan ring may originate from a conjugated diyne.

The herbal preparation echinacea* is derived from the roots of *Echinacea purpurea* (Compositae/Asteraceae) and is used for its immunostimulant properties, particularly as a prophylactic and

treatment for the common cold. At least some of its activity arises from a series of alkylamides, amides of polyunsaturated acids with isobutylamine. These acids are predominantly C_{11} and C_{12} diene-diyne (Figure 3.11).

BRANCHED-CHAIN FATTY ACIDS

Whilst straight-chain fatty acids are the most common, branched-chain acids have been found to occur in mammalian systems, e.g. in wool fat and butter fat. They are also characteristic fatty acid constituents of the lipid part of cell walls in some

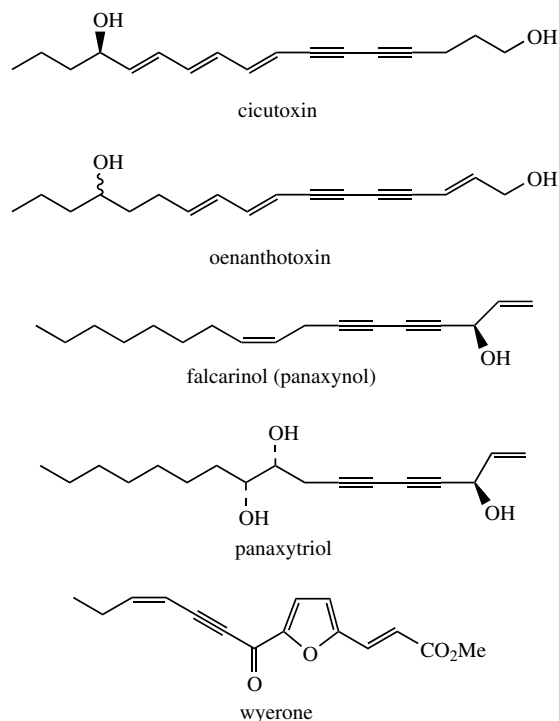


Figure 3.10

pathogenic bacteria. Several mechanisms appear to operate in their formation. Thus, the structure of **corynomycolic acid** from *Corynebacterium diphtheriae* can be rationalized from a combination of two palmitic acid units (Figure 3.12). Methyl side-chains can be introduced by using methylmalonyl-CoA instead of malonyl-CoA as the chain extending agent (Figure 3.13). Methylmalonyl-CoA arises by biotin-dependent carboxylation of propionyl-CoA in exactly the same way as malonyl-CoA was formed (see page 17). **2,4,6,8-Tetramethyldecanoic acid** found in the preen gland wax of the goose (*Anser anser*) is produced from an acetyl-CoA starter, and four methylmalonyl-CoA chain extender units. The incorporation of propionate as well as acetate is also a feature of many microbial antibiotic structures (see page 17). However, in other examples, methyl side-chains can be produced by a C-alkylation mechanism using S-adenosylmethionine (SAM). **Tuberculostearic acid** (Figure 3.14) found in *Mycobacterium tuberculosis*, the bacterium causing tuberculosis, is derived from oleic acid by alkylation on C-10,

Echinacea

Echinacea consists of the dried roots of *Echinacea purpurea*, *E. angustifolia*, or *E. pallida* (Compositae/Asteraceae), herbaceous perennial plants indigenous to North America, and widely cultivated for their large daisy-like flowers, which are usually purple or pink. Herbal preparations containing the dried root, or extracts derived from it, are promoted as immunostimulants, particularly as prophylactics and treatments for bacterial and viral infections, e.g. the common cold. Tests have validated stimulation of the immune response, though the origins of this activity cannot be ascribed to any specific substance. Activity has variously been assigned to lipophilic alkylamides, polar caffeic acid derivatives, high molecular weight polysaccharide material, or to a combination of these. Compounds in each group have been demonstrated to possess some pertinent activity, e.g. immunostimulatory, anti-inflammatory, antibacterial or antiviral effects. The alkylamides comprise a complex mixture of unsaturated fatty acids as amides with 2-methylpropanamine (isobutylamine) or 2-methylbutanamine, amines which are probably decarboxylation products from valine and isoleucine respectively. The acid portions are predominantly C₁₁ and C₁₂ diene-dynes or tetraenes (Figure 3.11). These compounds are found throughout the plant though relative proportions of individual components vary considerably. The root of *E. purpurea* contains at least 12 alkylamides (about 0.6%), of which C₁₂ diene-dynes predominate; levels of these compounds fall significantly during drying and storage. Caffeic acid derivatives present include caffeic acid (see page 132), chlorogenic acid (5-O-caffeoylquinic acid, see page 132), 2-O-caffeoyltartaric acid, and cichoric acid (2,3-di-O-caffeoyltartaric acid) (Figure 3.11). Cichoric acid is a major component (0.6–2.1%) in *E. purpurea*, but only minor in the other species.

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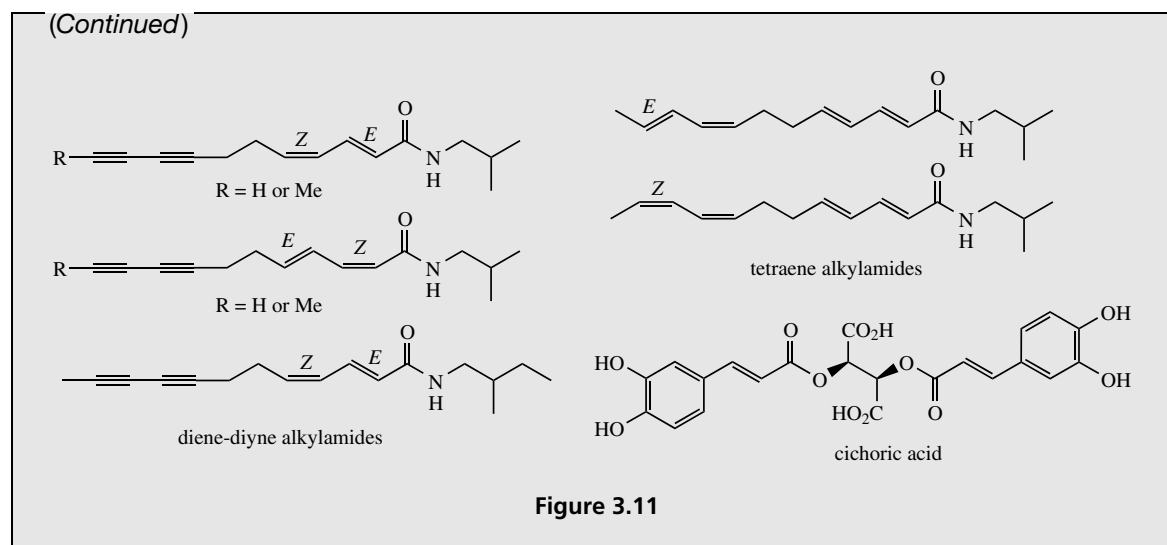


Figure 3.11

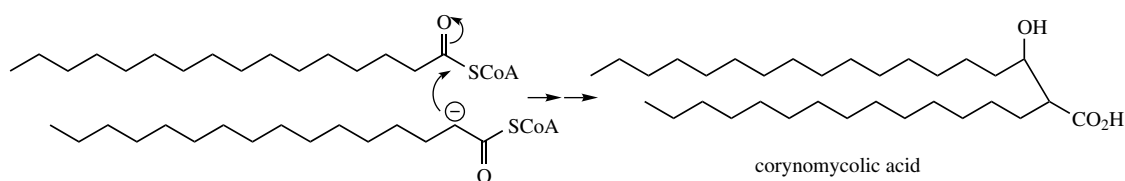


Figure 3.12

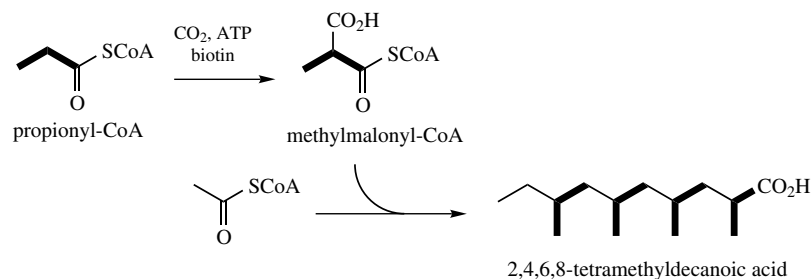


Figure 3.13

initiated by the double bond electrons. A postulated carbocation intermediate could then be discharged by accepting hydride from NADPH giving tuberculostearic acid. Alternatively, loss of a proton via cyclopropane ring formation could occur giving dihydrosterculic acid. This is known to be dehydrogenated to **sterculic acid**, an unusual fatty acid containing a highly strained cyclopropene ring. Sterculic acid is present in the seed oil from *Sterculia foetida* (Sterculiaceae) and with similar cyclopropene acids, e.g. malvalic acid, is present in edible cottonseed oil from *Gossypium* species

(Malvaceae). **Malvalic acid** is produced from sterculic acid by chain shortening from the carboxyl end (Figure 3.14). Sterculic acid is an inhibitor of the Δ^9 -desaturase which converts stearic acid into oleic acid and is potentially harmful to humans in that it can alter membrane permeability and inhibit reproduction.

Chaulmoogric and **hydnocarpic acids** (Figure 3.15) are cyclopentenyl fatty acids found in chaulmoogra oil expressed from seeds of *Hydnocarpus wightiana* (Flacourtiaceae). These acids are known to arise by malonate chain extension of the

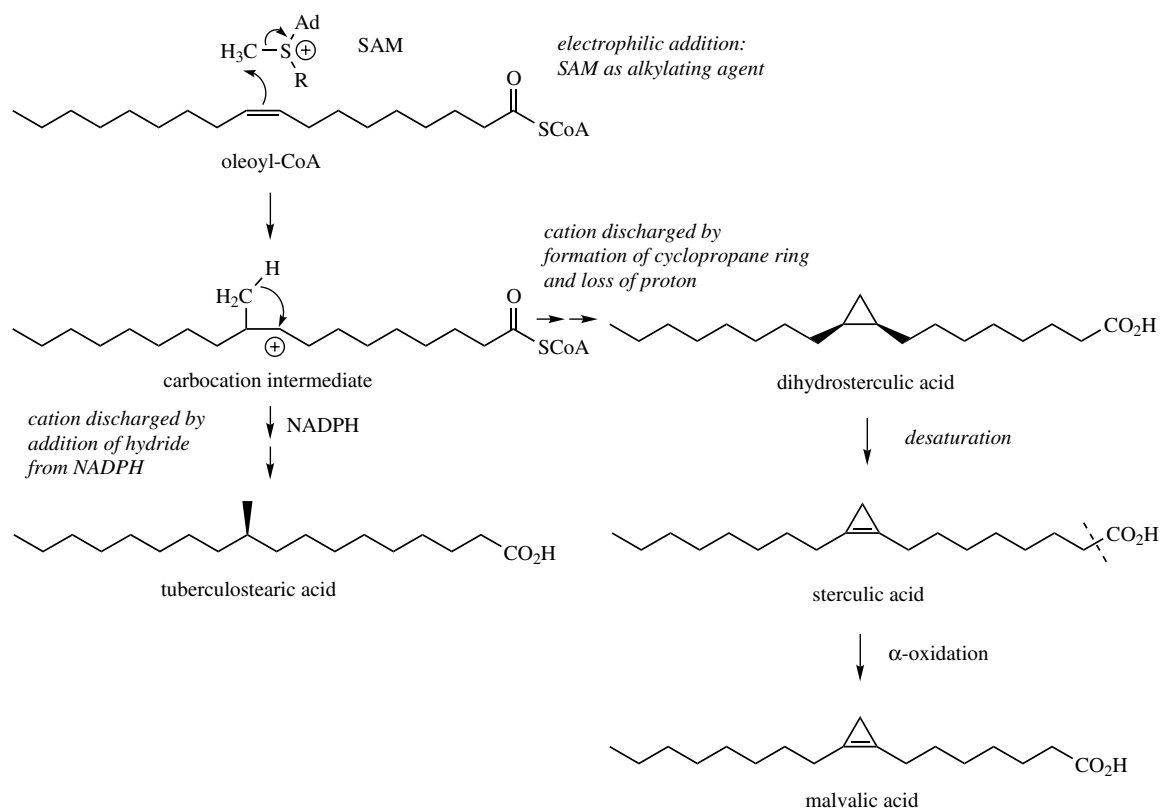


Figure 3.14

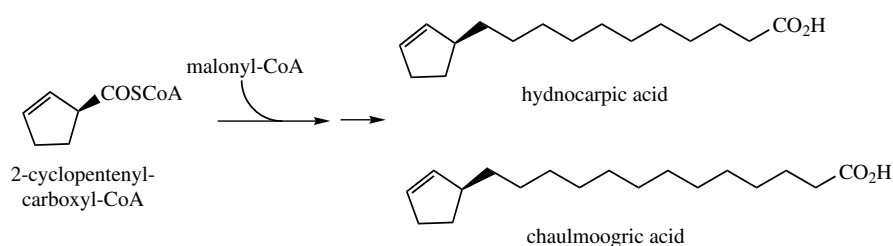


Figure 3.15

coenzyme A ester of 2-cyclopentenyl carboxylic acid as an alternative starter unit to acetate, demonstrating a further approach to unusual fatty acids. Chaulmoogra oil provided for many years the only treatment for the relief of leprosy, these two acids being strongly bactericidal towards the leprosy infective agent *Mycobacterium leprae*. Purified salts and esters of hydnocarpic and chaulmoogric acids were subsequently employed, until they were then themselves replaced by more effective synthetic agents.

PROSTAGLANDINS

The prostaglandins* are a group of modified C₂₀ fatty acids first isolated from human semen and initially assumed to be secreted by the prostate gland. They are now known to occur widely in animal tissues, but only in tiny amounts, and they have been found to exert a wide variety of pharmacological effects on humans and animals. They are active at very low, hormone-like concentrations and can regulate blood pressure, contractions of smooth

muscle, gastric secretion, and platelet aggregation. Their potential for drug use is extremely high, but it has proved difficult to separate the various biological activities into individual agents.

The basic prostaglandin skeleton is that of a cyclized C_{20} fatty acid containing a cyclopentane ring, a C_7 side-chain with the carboxyl function, and a C_8 side-chain with the methyl terminus. Prostaglandins are biosynthesized from three essential fatty acids, $\Delta^{8,11,14}$ -eicosatrienoic acid (**dihomo- γ -linolenic acid**), $\Delta^{5,8,11,14}$ -eicosatetraenoic acid (**arachidonic acid**), and $\Delta^{5,8,11,14,17}$ -eicosapentaenoic acid, which yield prostaglandins of the 1-, 2-, and 3-series, respectively (Figure 3.16) (see below for principles of nomenclature). The three precursors lead to products of similar structure, but with varying levels of unsaturation in the two side-chains. Some of the structures elaborated from arachidonic acid are shown in Figure 3.17. In the first reaction, arachidonic acid is converted into **prostaglandin G_2 (PGG_2)** by an oxygenase (**cyclooxygenase; COX**) enzyme, which incorporates two molecules of oxygen, liberating a compound with both cyclic and acyclic peroxide functions. In arachidonic acid the methylene group flanked by two double bonds is susceptible to oxidation, probably via a free radical process. This may lead to incorporation of oxygen giving the proposed free radical intermediate. Formation of PGG_2 is then depicted as a concerted cyclization reaction, initiated by the peroxide radical, in which a second oxygen molecule is incorporated. The

acyclic peroxide group in PGG_2 is then cleaved by a peroxidase to yield **prostaglandin H_2 (PGH_2)**, which occupies a central role and can be modified in several different ways. These modifications can be rationally accommodated by initial cleavage of the cyclic peroxide to the diradical; alternative ionic mechanisms may also be proposed. Quenching of the free radicals by abstraction of hydrogen atoms gives rise to **prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)**, whilst capture and loss of hydrogen atoms would provide either **prostaglandin E_2 (PGE_2)** or **prostaglandin D_2 (PGD_2)**. The bicyclic system in **prostaglandin I_2 (PGI_2 ; prostacyclin)** is envisaged as arising by involvement of a side-chain double bond, then loss of a hydrogen atom. Prostaglandin structures representative of the 1-series, e.g. **PGE_1** , or of the 3-series, e.g. **PGE_3** , can be formed in a similar way from the appropriate fatty acid precursor (Figure 3.16).

The basic skeleton of the prostaglandins is termed **prostanoid acid**, and derivatives of this system are collectively known as prostanoids. The term **eicosanoids** is also used to encompass prostaglandins, thromboxanes, and leukotrienes, which are all derived from C_{20} fatty acids (eicosanoic acids). Semi-systematic nomenclature of prostaglandins is based on the substitution pattern in the five-membered ring, denoted by a letter suffix (Figure 3.18), and the number of double bonds in the side-chains is given by a numerical subscript. Greek letters α and β are used to indicate the configuration at C-9, α indicating the substituent is below the plane (as

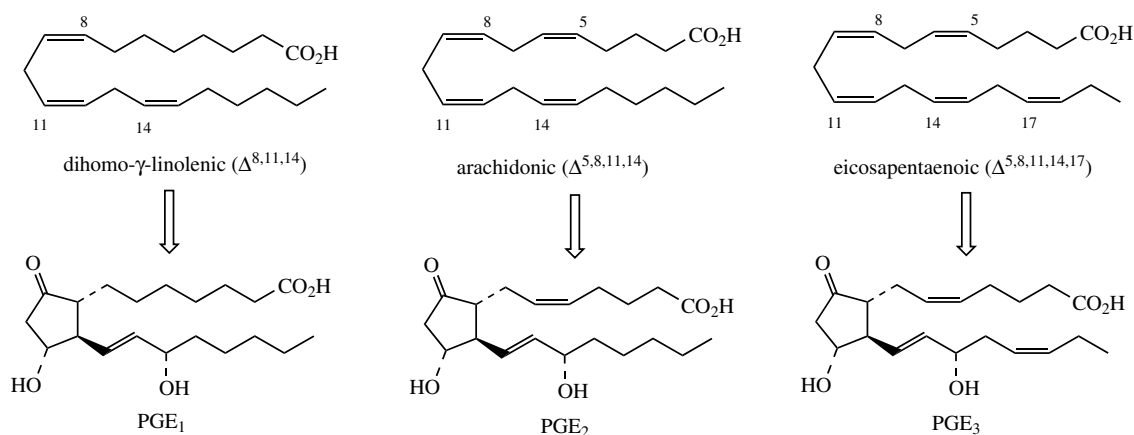


Figure 3.16

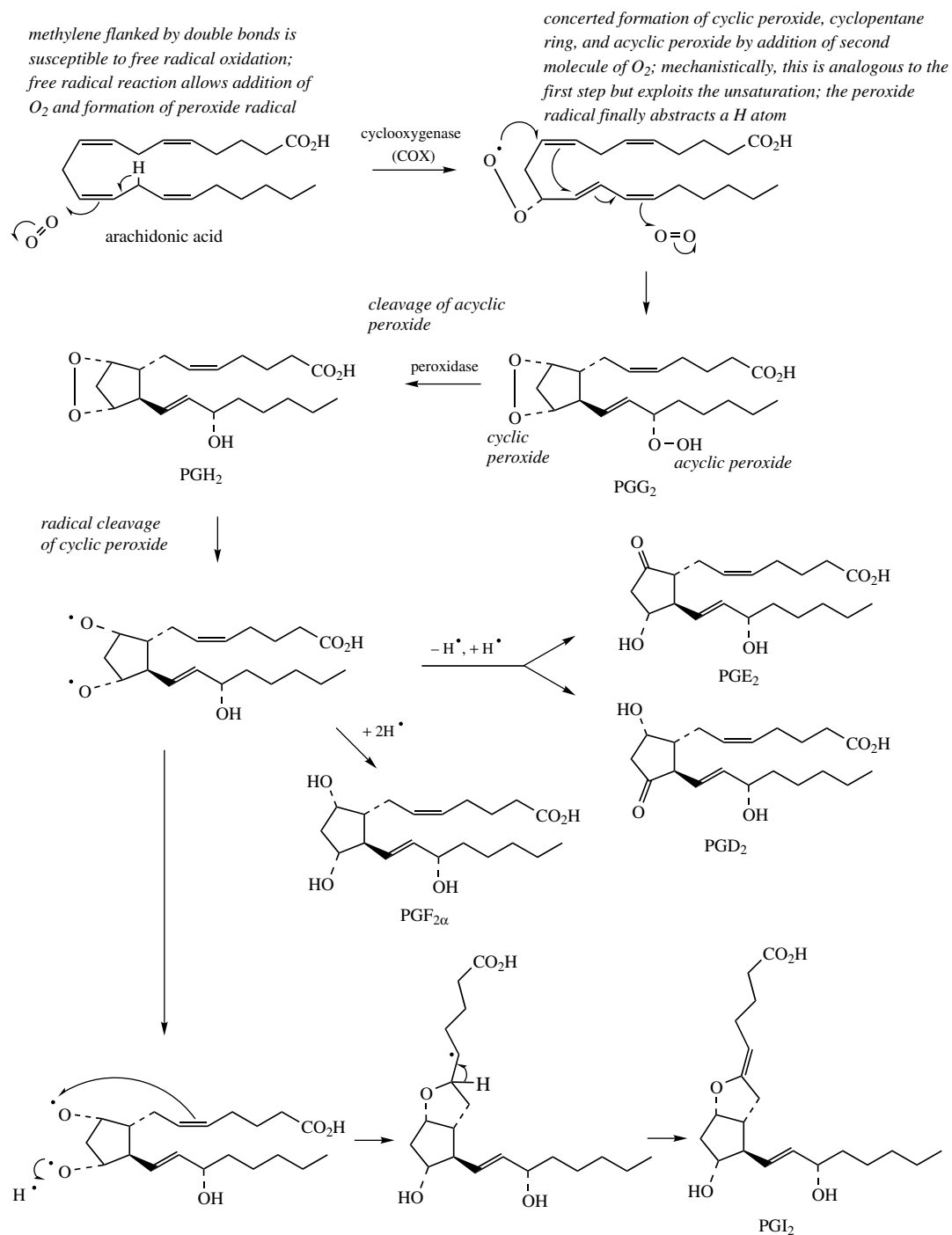


Figure 3.17

found in natural prostaglandins), and β indicating the substituent is above the plane (as in some synthetic analogues). 'Prostaglandin' is usually

abbreviated to PG. Prostaglandins A, B, and C are inactive degradation products from the natural prostaglandins.

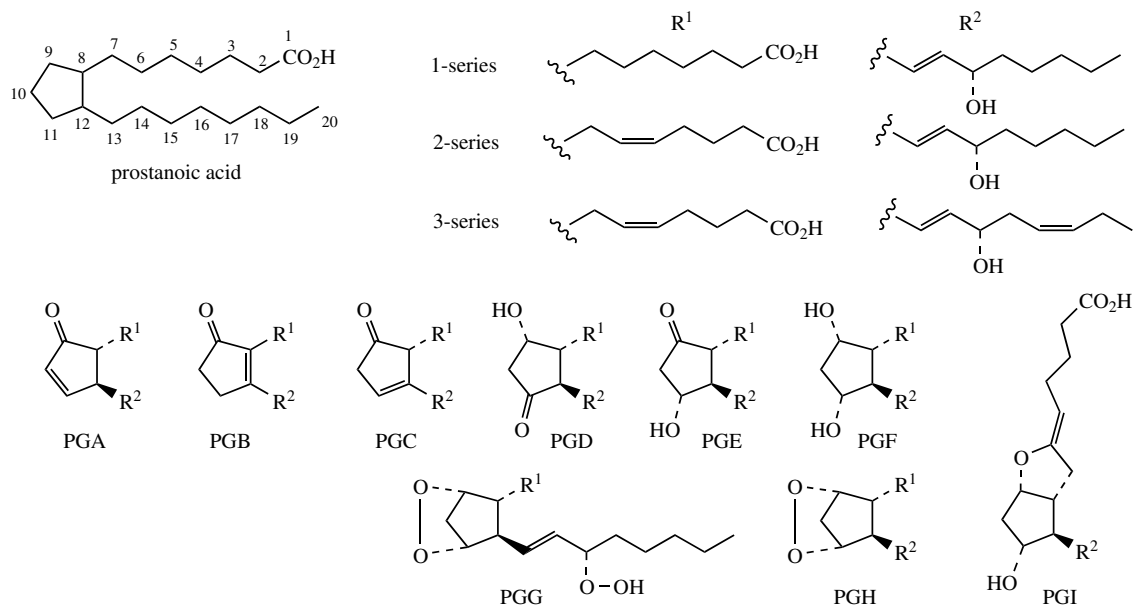


Figure 3.18

Prostaglandins

Prostaglandins occur in nearly all mammalian tissues, but only at very low concentrations. PGE₁ and PGF_{1α} were initially isolated from sheep seminal plasma, but these compounds and PGD₂, PGE₂, and PGF_{2α} are widely distributed. Animal sources cannot supply sufficient amounts for drug usage. The soft coral *Plexaura homomalla* (sea whip) from the Caribbean has been identified as having very high (2–3%) levels of prostaglandin esters, predominantly the C-15 epimer of PGA₂ (1–2%) with related structures. Prostaglandins of the A-, E-, and F-types are widely distributed in soft corals, especially *Plexaura*, but these are unlikely to provide a satisfactory and renewable natural source. Considerable effort has been exerted on the total synthesis of prostaglandins and their interconversions, and the high level of success achieved has opened up the availability of compounds for pharmacological testing and subsequent drug use. Synthetic analogues have also been developed to modify or optimize biological activity. The studies have demonstrated that biological activity is effectively confined to the natural enantiomers; the unnatural enantiomer of PGE₁ had only 0.1% of the activity of the natural isomer.

The prostaglandins display a wide range of pharmacological activities, including contraction and relaxation of smooth muscle of the uterus, the cardiovascular system, the intestinal tract, and of bronchial tissue. They may also inhibit gastric acid secretion, control blood pressure and suppress blood platelet aggregation. Some of these effects are consistent with the prostaglandins acting as second messengers, modulating transmission of hormone stimulation and thus metabolic response. Some prostaglandins in the A and J series have demonstrated potent antitumour properties. Since the prostaglandins control many important physiological processes in animal tissues, their drug potential is high, but the chances of precipitating unwanted side-effects are also high, and this has so far limited their therapeutic use.

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There is, however, much additional scope for controlling the production of natural prostaglandins in body tissues by means of specific inhibitors. Indeed it has been found that some established non-steroidal anti-inflammatory drugs (NSAIDs), e.g. aspirin, indometacin, and ibuprofen, inhibit early steps in the prostaglandin biosynthetic pathway that transform the unsaturated fatty acids into cyclic peroxides. Thus aspirin is known to irreversibly inactivate the cyclooxygenase activity (arachidonic acid $\rightarrow$ PGG₂), though not the peroxidase activity (PGG₂ $\rightarrow$ PGH₂), by selective acetylation of a serine residue of the enzyme; ibuprofen and indometacin compete with arachidonic acid at the active site and are reversible inhibitors of the cyclooxygenase. A recent discovery is that two forms of the cyclooxygenase enzyme exist, designated COX-1 and COX-2. COX-1 is expressed constitutively in most tissues and cells and is thought to control synthesis of those prostaglandins important for normal cellular functions such as gastrointestinal integrity and vascular homeostasis. COX-2 is not normally present, but is inducible in certain cells in response to inflammatory stimuli, resulting in enhanced prostaglandin release in the CNS and inflammatory cells with the characteristic inflammatory response. Current NSAIDs do not discriminate between the two COX enzymes, and so this leads to both therapeutic effects via inhibition of COX-2, and adverse effects such as gastrointestinal problems, ulcers, and bleeding via inhibition of COX-1. Because of differences in the nature of the active sites of the two enzymes, it has now been possible to develop agents that can inhibit COX-2 rather than COX-1 as potential new anti-inflammatory drugs. The first of these, meloxicam and rofecoxib, have recently been introduced for relief of pain and inflammation in osteoarthritis. The anti-inflammatory activity of corticosteroids correlates with their preventing the release of arachidonic acid from storage phospholipids, but expression of COX-2 is also inhibited by glucocorticoids.

The role of essential fatty acids (see page 46) such as linoleic and γ -linolenic acids, obtained from plant ingredients in the diet, can now be readily appreciated. Without a source of arachidonic acid, or compounds which can be converted into arachidonic acid, synthesis of prostaglandins would be compromised, and this would seriously affect many normal metabolic processes. A steady supply of prostaglandin precursors is required since prostaglandins are continuously being synthesized and then deactivated. Prostaglandins are rapidly degraded by processes which include oxidation of the 15-hydroxyl to a ketone, reduction of the 13,14-double bond, and oxidative degradation of both side-chains.

A major area of application of prostaglandins as drugs is in obstetrics, where they are used to induce abortions during the early to middle stages of pregnancy, or to induce labour at term. **PGE₂ (dinoprostone)** (Figure 3.19) is used in both capacities, whilst **PGF_{2 α} (dinoprost)** is now less commonly prescribed and restricted to abortions. PGF_{2 α} is rapidly metabolized in body tissues (half-life less than 10 minutes), and the modified version **15-methyl PGF_{2 α} (carboprost)** has been developed to reduce deactivation by blocking oxidation at position 15. Carboprost is produced by oxidizing the 15-hydroxyl in a suitably-protected PGF_{2 α} , then alkylating the 15-carbonyl with a Grignard reagent. Carboprost is effective at much reduced dosage compared with dinoprost, and is of value in augmenting labour at term, especially in cases where ergometrine (see page 375) or oxytocin (see page 415) are ineffective. **Gemeprost** is another unnatural structure and is used to soften and dilate the cervix in early abortions. These agents are usually administered vaginally.

PGE₁ (alprostadil) differs from PGE₂ by having unsaturation in only one side-chain. Though having effects on uterine muscle, it also has vasodilator properties, and these are exploited for maintaining new-born infants with congenital heart defects, facilitating blood oxygenation prior to corrective surgery. The very rapid metabolism of PGE₁ means this drug must be

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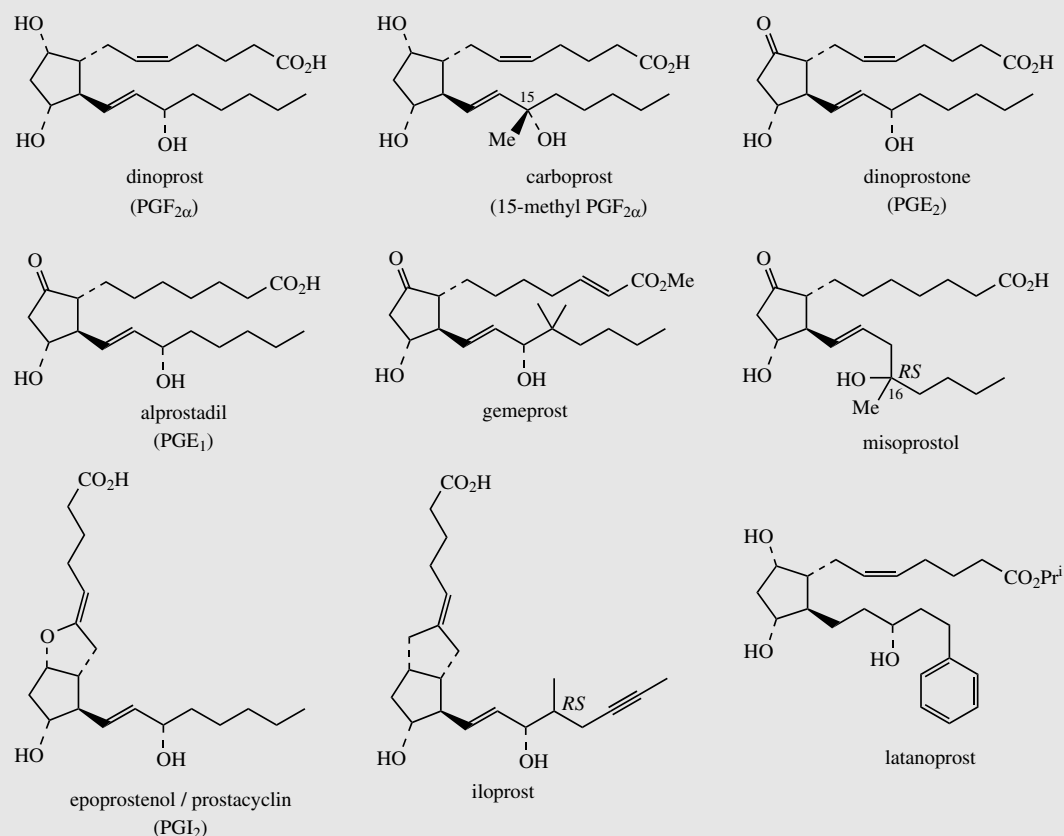


Figure 3.19

delivered by continuous intravenous infusion. Alprostadil is also of value in male impotence, self-injectable preparations being used to achieve erection of the penis. An interesting modification to the structure of PGE₁ is found in the analogue **misoprostol**. This compound has had the oxygenation removed from position 15, transferred to position 16, plus alkylation at position 16 to reduce metabolism (compare 15-methyl PGF_{2α} above). These modifications result in an orally active drug which inhibits gastric secretion effectively and can be used to promote healing of gastric and duodenal ulcers. In combination with non-specific NSAIDs, it can significantly lower the incidence of gastrointestinal side-effects such as ulceration and bleeding.

PGI₂ (epoprostenol, prostacyclin) reduces blood pressure and also inhibits platelet aggregation by reducing calcium concentrations. It is employed to inhibit blood clotting during renal dialysis, but its very low half-life (about 3 minutes) again necessitates continuous intravenous administration. The tetrahydrofuran ring is part of an enol ether and is readily opened by hydration, leading to 6-ketoprostaglandin F_{1α} (Figure 3.20). **Iloprost** (Figure 3.19) is a stable carbocyclic analogue of potential use in the treatment of thrombotic diseases.

Latanoprost (Figure 3.19) is a recently introduced prostaglandin analogue which increases the outflow of aqueous humour from the eye. It is thus used to reduce intraocular pressure in the treatment of the eye disease glaucoma.

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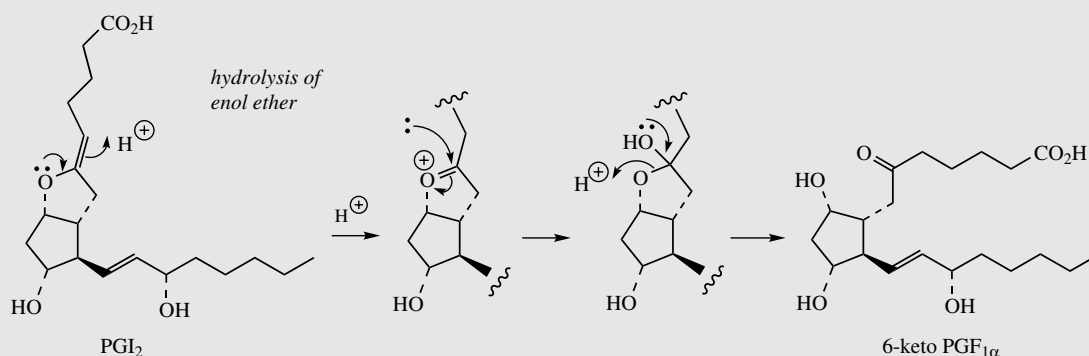


Figure 3.20

Isoprostanes

Isoprostanes represent a new class of prostaglandin-like compounds produced *in vivo* in humans and animals by non-enzymic free-radical-mediated oxidation of membrane-bound polyunsaturated fatty acids. An isomer of PGF_{2α} in which the two alkyl substituents on the five-membered ring were arranged *cis* rather than *trans* was detected in human urine and was the first of these compounds to be characterized. This compound was initially termed 8-*iso*-PGF_{2α}, or 8-*epi*-PGF_{2α}, though as many more variants in the isoprostane series were discovered it is now termed iPF_{2α}-III (Figure 3.21). The last figure refers to the compound being of type III, with eight types being differentiated by the nature of the non-carboxylic chain. Compounds may be formed from linolenic acid and γ -linolenic acid, as well as from arachidonic, eicosapentaenoic, and dihomo- γ -linolenic acids. Structural characteristics of the four classes of isoprostanes derived from arachidonic acid are shown in Figure 3.22; the letter code as in prostaglandin nomenclature is used to define the ring substitution pattern. The four types of isoprostane shown in Figure 3.22 can be viewed as arising by a free radical mechanism which resembles the enzyme-mediated formation of prostaglandins shown in Figure 3.17. The varying side-chain substituents arise by utilizing different double bonds from the several available in the cyclization mechanism, and incorporating an oxygen atom from molecular oxygen at different positions. Many variants are produced because chemical processes rather than enzyme-controlled processes are employed. Free-radical-derived isomers of leukotrienes and thromboxanes have also been reported.

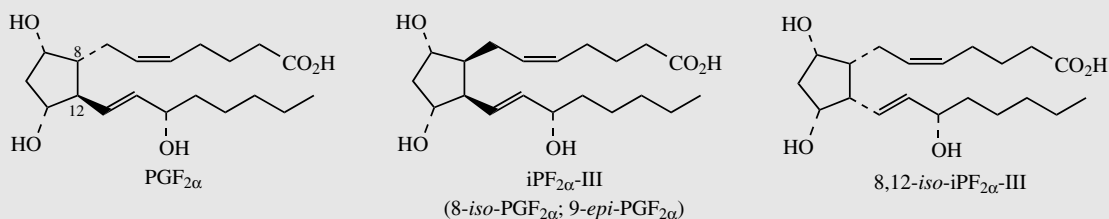
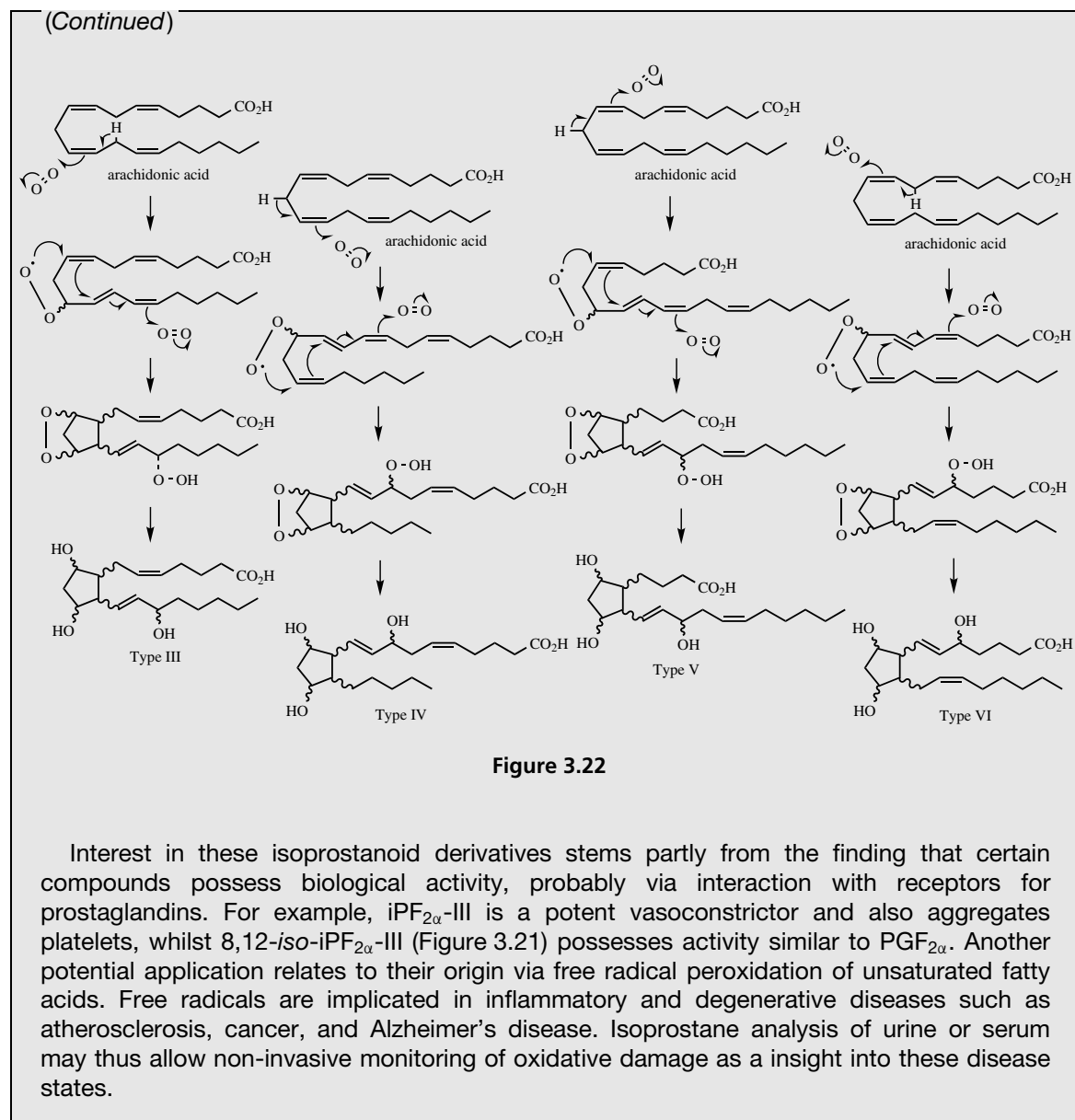


Figure 3.21

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THROMBOXANES

An intriguing side-branch from the prostaglandin pathway leads to thromboxanes* (Figure 3.23). The peroxide and cyclopentane ring functions of PGH_2 are cleaved and restructured to form **thromboxane A_2 (TXA_2)**, which contains a highly strained four-membered oxetane ring. TXA_2 is highly unstable, and reacts readily with nucleophiles. In an aqueous environment, it

reacts to yield the hemiacetal **thromboxane B_2 (TXB_2)**.

LEUKOTRIENES

Yet another variant for the metabolism of arachidonic acid is the formation of leukotrienes*, a series of fatty acid derivatives with a conjugated triene functionality, and first isolated from leukocytes. In a representative pathway (others have

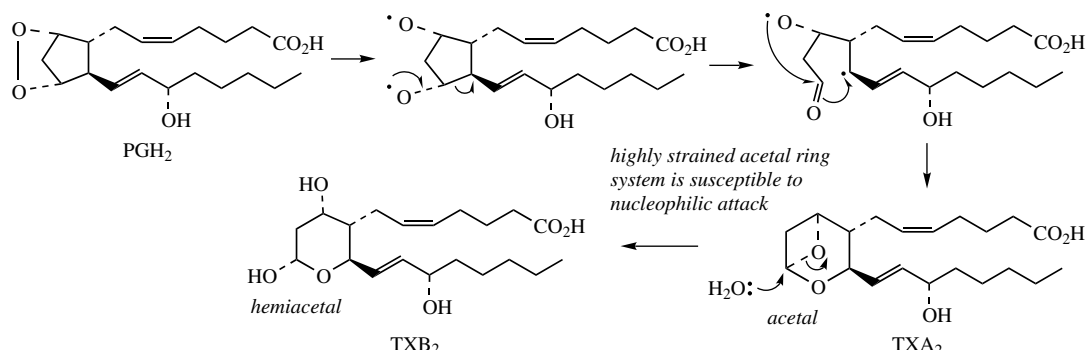


Figure 3.23

Thromboxanes

The thromboxanes were isolated from blood platelets, and whilst TXA₂ showed high biological activity TXB₂ was only weakly active. TXA₂ causes blood platelets to aggregate to form a clot or thrombus, by increasing cytoplasmic calcium concentrations and thus deforming the platelets which then fuse together. It has the opposite effect to PGI₂, and presumably the development of thrombosis reflects an imbalance in the two activities. Both compounds are produced from the same precursor, PGH₂, which is converted in the blood platelets to TXA₂, and in the blood vessel wall to PGI₂. Thromboxanes A₃ and B₃ have also been isolated from blood platelets, are structurally analogous to prostaglandins in the 3-series, and are derived from $\Delta^{5,8,11,14,17}$ -eicosapentaenoic acid. TXA₃ is not strongly aggregatory towards blood platelets. The highly unstable nature of the biologically active thromboxanes has made their synthesis difficult, and drug use of natural structures will probably be impracticable. It is likely that most efforts will be directed towards thromboxane antagonists to help reduce blood platelet aggregation in thrombosis patients. The value of aspirin in preventing cardiovascular disease is now known to be related to inhibition of thromboxane A₂ biosynthesis in platelets.

Leukotrienes

The leukotrienes are involved in allergic responses and inflammatory processes. An antigen-antibody reaction can result in the release of compounds such as histamine (see page 379) or materials termed slow reacting substance of anaphylaxis (SRSA). These substances are then mediators of hypersensitive reactions such as hay fever and asthma. Structural studies have identified SRSA as a mixture of LTC₄, LTD₄ and LTE₄. These cysteine-containing leukotrienes are powerful bronchoconstrictors and vasoconstrictors, and induce mucus secretion, the typical symptoms of asthma. LTE₄ is some 10–100-fold less active than LTD₄, so that degradation of the peptide side-chain represents a means of eliminating leukotriene function. LTB₄ appears to facilitate migration of leukocytes in inflammation, and is implicated in the pathology of psoriasis, inflammatory bowel disease, and arthritis. The biological effects of leukotrienes are being actively researched to define the cellular processes involved. This may lead to the development of agents to control allergic and inflammatory reactions. Drugs inhibiting the formation of LTC₄ and LTB₄ are in clinical trials, whilst montelukast and zafirlukast have been introduced as orally active leukotriene (LTD₄) receptor antagonists for the prophylaxis of asthma.

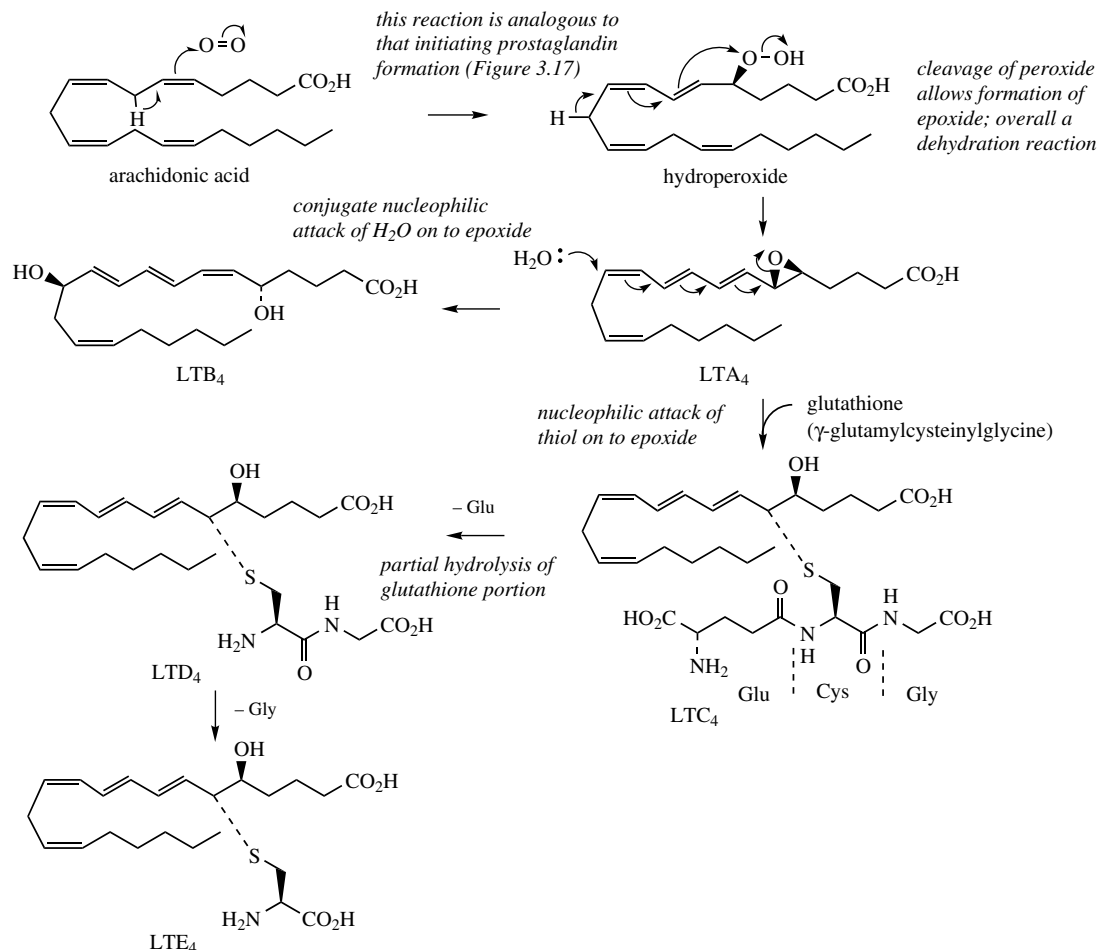


Figure 3.24

been characterized) (Figure 3.24), **arachidonic acid** is converted into a hydroperoxide, the point of oxygenation being C-5, rather than C-11 as in the prostaglandin pathway (Figure 3.17). This compound loses water via formation of an epoxide ring, giving **leukotriene A₄ (LTA₄)**. This unstable allylic epoxide may hydrolyse by conjugate addition giving **leukotriene B₄ (LTB₄)**, or alternatively the epoxide may be attacked directly by a nucleophile, in this case the sulphur atom of the tripeptide glutathione (γ-glutamylcysteinylglycine) (Figure 3.24). The adduct produced in the latter reaction is termed **leukotriene C₄ (LTC₄)**. Partial hydrolysis in the tripeptide fragment then leads to **leukotriene D₄ (LTD₄)** and **leukotriene E₄ (LTE₄)**. Analogues, e.g. LTA₃ and LTA₅, are

also known, and these are derived from Δ^{5,8,11}-**eicosatrienoic acid** and Δ^{5,8,11,14,17}-**eicosapentaenoic acid** respectively. The subscript numeral indicates the total number of double bonds in the leukotriene chain.

AROMATIC POLYKETIDES

For fatty acid biosynthesis, reduction after each condensation step affords a growing hydrocarbon chain. In the absence of this reduction process, the growing poly-β-keto chain needs to be stabilized on the enzyme surface until the chain length is complete, at which point cyclization or other reactions can occur. The poly-β-keto ester is very reactive, and there are various possibilities

for undergoing intramolecular Claisen or aldol reactions, dictated of course by the nature of the enzyme and how the substrate is folded. Methylenes flanked by two carbonyls are activated, allowing formation of carbanions/enolates and subsequent reaction with ketone or ester carbonyl groups, with a natural tendency to form strain-free six-membered rings.

Cyclization: Simple Phenols

The polyketo ester (Figure 3.25), formed from four acetate units (one acetate starter group and three

malonate chain extension units) is capable of being folded in at least two ways, A and B (Figure 3.25). For A, ionization of the α -methylene allows aldol addition on to the carbonyl six carbons distant along the chain, giving the tertiary alcohol. Dehydration occurs as in most chemical aldol reactions, giving the alkene, and enolization follows to attain the stability conferred by the aromatic ring. The thioester bond (to coenzyme A or ACP) is then hydrolysed to produce **orsellinic acid**. Alternatively, folding of the polyketo ester as in B allows a Claisen reaction to occur, which, although mechanistically analogous to the aldol reaction, is

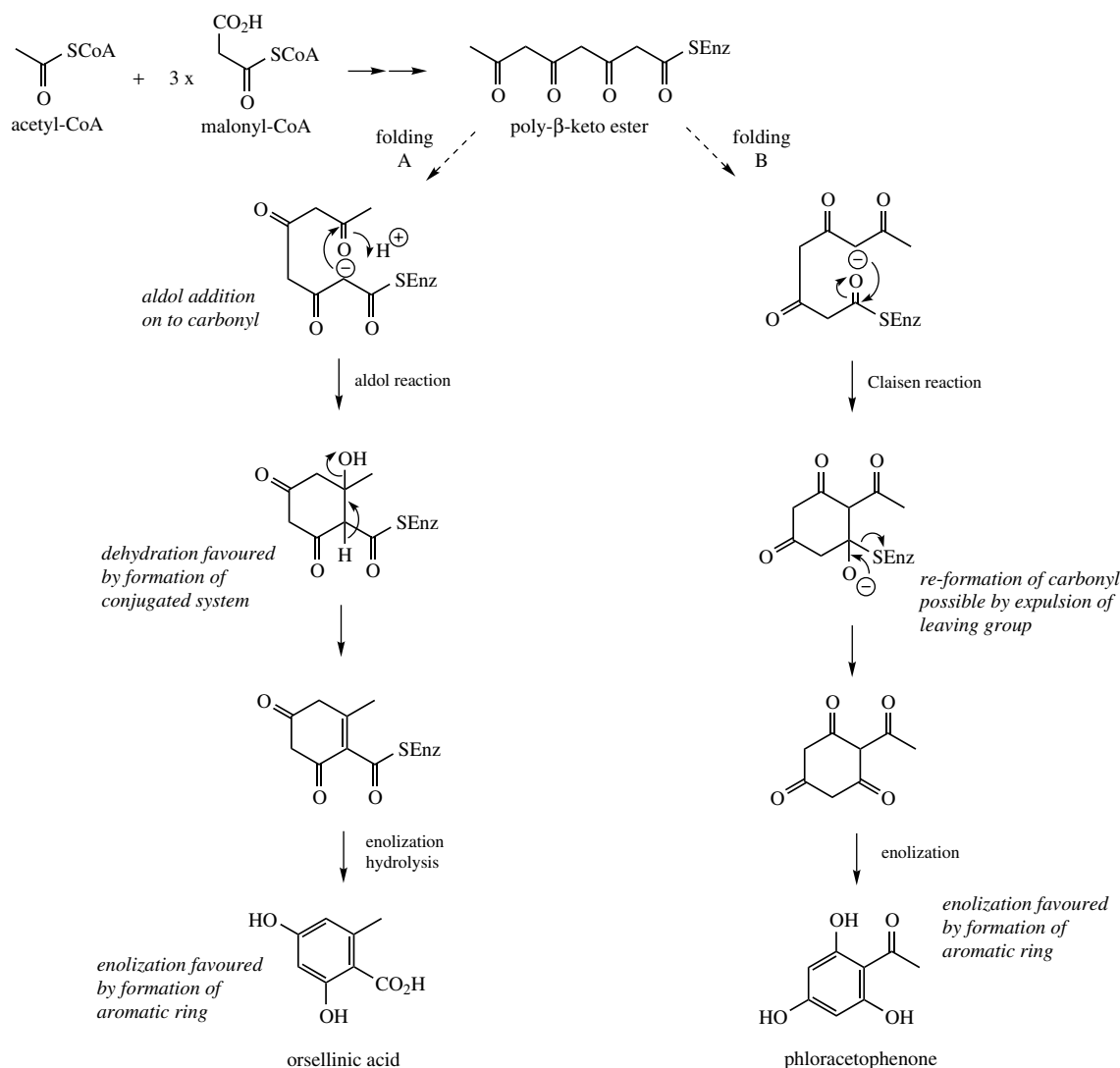


Figure 3.25

terminated by expulsion of the thiol leaving group, and direct release from the enzyme. Enolization of the cyclohexatriene produces **phloracetophenone**. As with fatty acid synthases, the whole sequence of reactions is carried out by an enzyme complex which converts acetyl-CoA and malonyl-CoA into the final product without giving any detectable free intermediates. These enzyme complexes combine **polyketide synthase** and **polyketide cyclase** activities and share many structural similarities with fatty acid synthases, including an acyl carrier protein with a phosphopantetheine group, a reactive cysteine residue, and an analogous β -ketoacyl synthase activity.

A distinctive feature of an aromatic ring system derived through the acetate pathway is that several of the carbonyl oxygens of the poly- β -keto system are retained in the final product. These end up on alternate carbons around the ring system. Of course, one or more might be used in forming a carbon-carbon bond, as in orsellinic acid. Nevertheless, this oxygenation on alternate carbon atoms, a *meta* oxygenation pattern, is usually easily recognizable, and points to the biosynthetic origin of the molecule. This *meta* oxygenation pattern contrasts to that seen on aromatic rings formed via the shikimate pathway (see Chapter 4).

6-methylsalicylic acid (Figure 3.26) is a metabolite of *Penicillium patulum*, and differs from orsellinic acid by the absence of a phenol group at position 4. It is also derived from acetyl-CoA

and three molecules of malonyl-CoA, and the 'missing' oxygen function is removed during the biosynthesis. Orsellinic acid is not itself deoxygenated to 6-methylsalicylic acid. The enzyme 6-methylsalicylic acid synthase requires NADPH as cofactor, and removes the oxygen function by reduction of a ketone to an alcohol, followed by a dehydration step (Figure 3.26). Whilst on paper this could be carried out on an eight-carbon intermediate involved in orsellinic acid biosynthesis (Figure 3.25), there is evidence that the reduction/dehydration actually occurs on a six-carbon intermediate as the chain is growing (compare fatty acid biosynthesis, page 36), prior to the final chain extension (Figure 3.26). Aldol condensation, enolization, and release from the enzyme then generate 6-methylsalicylic acid. Important evidence for reduction occurring at the C₆ stage as shown in Figure 3.26 comes from the formation of triacetic acid lactone if NADPH is omitted from the enzymic incubation.

The folding of a polyketide chain can be established by labelling studies, feeding carbon-labelled sodium acetate to the appropriate organism and establishing the position of labelling in the final product by chemical degradation and counting (for the radioactive isotope ¹⁴C), or by NMR spectrometry (for the stable isotope ¹³C). ¹³C NMR spectrometry is also valuable in establishing the location of intact C₂ units derived from feeding ¹³C₂-labelled acetate. This is exemplified in

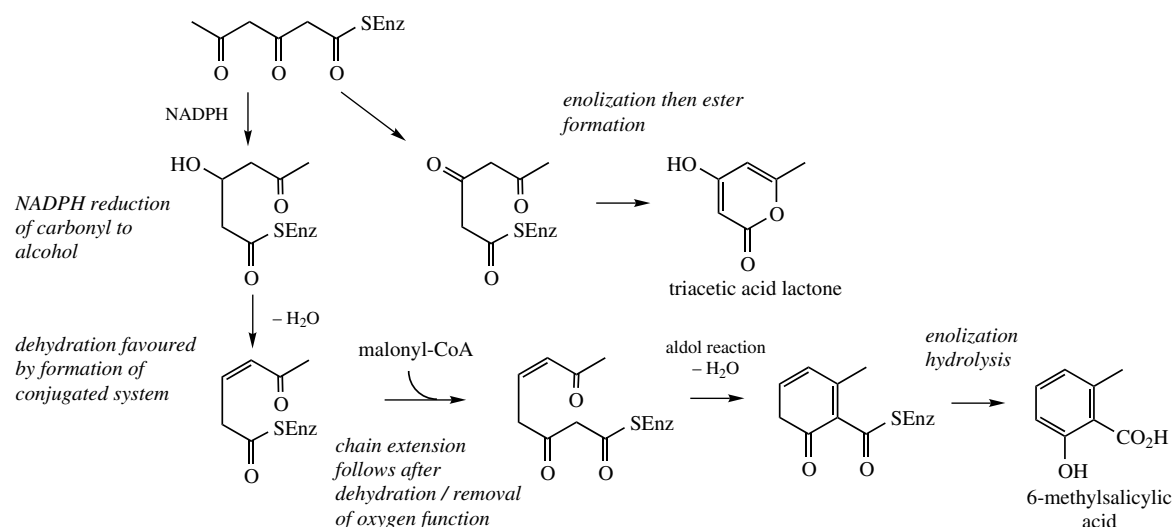


Figure 3.26

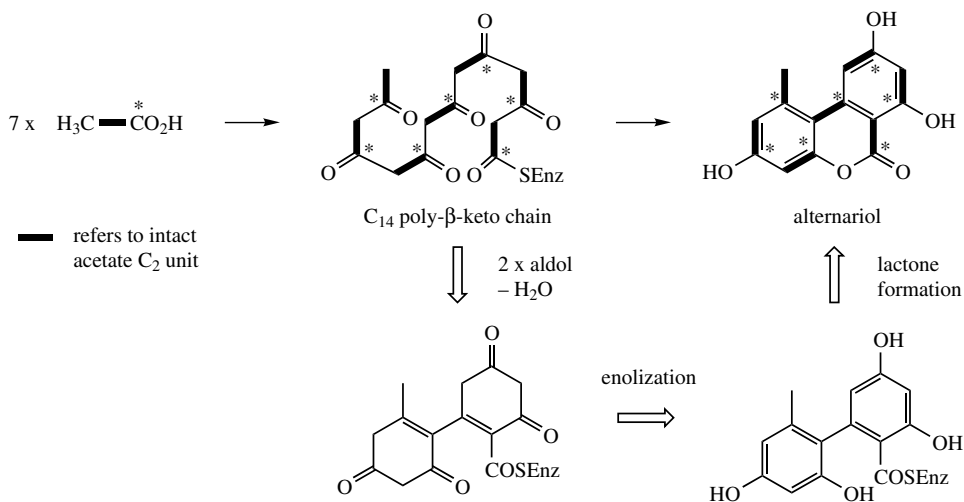


Figure 3.27

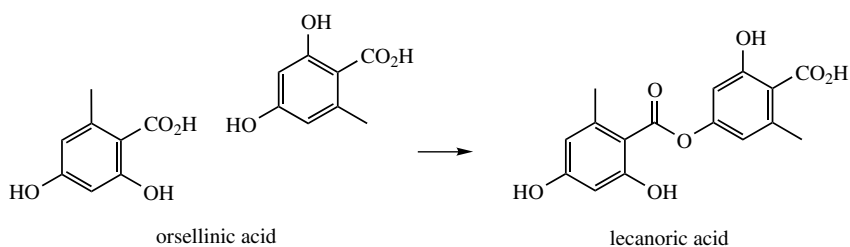


Figure 3.28

Figure 3.27, where **alternariol**, a metabolite from the mould *Alternaria tenuis*, can be established to be derived from a single C_{14} polyketide chain, folded as shown, and then cyclized. Whilst the precise sequence of reactions involved is not known, paper chemistry allows us to formulate the essential features. Two aldol condensations followed by enolization in both rings would give a biphenyl, and lactonization would then lead to alternariol. The oxygenation pattern in alternariol shows alternate oxygens on both aromatic rings, and an acetate origin is readily surmised, even though some oxygens have been used in ring formation processes. The lone methyl ‘start-of-chain’ is also usually very obvious in acetate-derived compounds, though the carboxyl ‘end-of-chain’ can often react with convenient hydroxyl functions, which may have arisen through enolization, and lactone or ester functions are thus reasonably common. For example, **lecanoric acid** is a **depside** (an ester

formed from two phenolic acids) found in lichens and produced from two orsellinic acid molecules (Figure 3.28).

Structural Modifications: Anthraquinones

A number of natural anthraquinone derivatives are also excellent examples of acetate-derived structures. **Endocrocin** (Figure 3.29) found in species of *Penicillium* and *Aspergillus* fungi is formed by folding a polyketide containing eight C_2 units to form the periphery of the carbon skeleton. Three aldol-type condensations would give a hypothetical intermediate 1, and, except for a crucial carbonyl oxygen in the centre ring, endocrocin results by enolization reactions, one of which involves the vinylogous enolization $-\text{CH}_2-\text{CH}=\text{CH}-\text{CO}- \longrightarrow -\text{CH}=\text{CH}-\text{CH}=\text{C}(\text{OH})-$. The additional carbonyl oxygen must be introduced at some stage

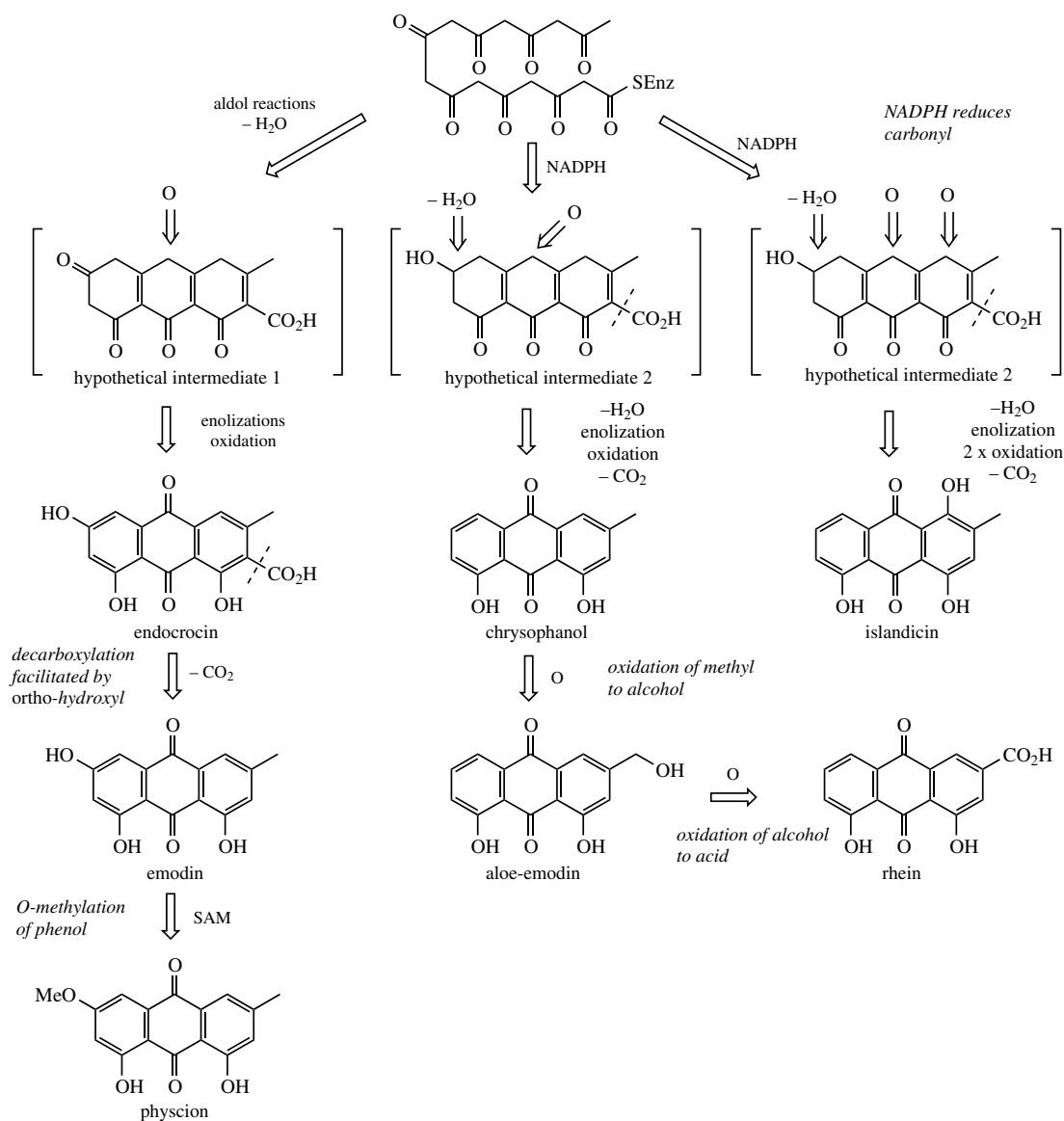


Figure 3.29

during the biosynthesis by an oxidative process, for which we have little information. **Emodin**, a metabolite of some *Penicillium* species, but also found in higher plants, e.g. *Rhamnus* and *Rumex* species, would appear to be formed from endocrocin by a simple decarboxylation reaction. This is facilitated by the adjacent phenol function (see page 20). *O*-Methylation of emodin would then lead to **physcion**. **Islandicin** is another anthraquinone pigment produced by *Penicillium islandicum*, and differs from emodin in two ways.

One hydroxyl is missing, and a new hydroxyl has been incorporated adjacent to the methyl. Without any evidence for the sequence of such reactions, the structure of intermediate 2 shows the result of three aldol condensations and reduction of a carbonyl. A dehydration reaction, two oxidations, and a decarboxylation are necessary to attain the islandicin structure. In **chrysophanol**, **aloe-emodin**, and **rhein**, the same oxygen function is lost by reduction as in islandicin, and decarboxylation also occurs. The three compounds

are interrelated by a sequential oxidation of the methyl in chrysophanol to a hydroxymethyl in aloë-emodin, and a carboxyl in rhein.

These structural modifications undergone by the basic polyketide are conveniently considered under two main headings, according to the timing of the steps in the synthetic sequence. Thus, 'missing' oxygen functions appear to be reduced out well before the folded and cyclized polyketide is detached from the enzyme, and are mediated by a reductase component of the enzyme complex during chain elongation *before the cyclization reaction*. On the other hand, reactions like the decarboxylation, *O*-methylation, and sequential oxidation of a methyl to a carboxyl are representative of transformations occurring *after the cyclization reaction*. It is often possible to demonstrate these later conversions by the isolation of enzymes catalysing the individual steps. Most of the secondary transformations are easily rationalized by careful consideration of the reactivity conferred on the molecule by the alternating and usually phenolic oxygenation pattern. These oxygens activate adjacent sites creating nucleophilic centres. Introduction of additional hydroxyl groups *ortho* or *para* to an existing phenol will be facilitated (see page 26), allowing the extra hydroxyl of islandicin to be inserted, for example. *Ortho*- or *para*-diphenols are themselves susceptible to further oxidation in certain circumstances, and may give rise to *o*- and *p*-quinones (see page 25). The quinone system in anthraquinones is built up by an oxidation of the central cyclohexadienone ring, again at a nucleophilic centre activated by the enone system. Methyls on an aromatic ring are also activated towards oxidation, facilitating the chrysophanol $\rightarrow$ aloë-emodin oxidation, for example. Decarboxylation, e.g. endocrocin $\rightarrow$ emodin, is readily achieved in the presence of an *ortho* phenol function, though a *para* phenol can also facilitate this (see page 20).

It is now appreciated that the assembly of the anthraquinone skeleton (and related polycyclic structures) is achieved in a step-wise sequence. After the polyketide chain is folded, the ring at the centre of the fold is formed first, followed in turn by the next two rings. The pathway outlined for the biosynthesis of endocrocin and emodin is shown in Figure 3.30. Mechanistically, there is little difference between this and

the speculative pathway of Figure 3.29, but the sequence of reactions is altered. Decarboxylation appears to take place before aromatization of the last-formed ring system, and tetrahydroanthracene intermediates such as atrochrysone carboxylic acid and atrochrysone are involved. These dehydrate to the anthrones **endocrocin anthrone** and **emodin anthrone**, respectively, prior to introduction of the extra carbonyl oxygen as a last transformation in the production of anthraquinones. This oxygen is derived from O_2 .

Note that many other natural anthraquinone structures are not formed via the acetate pathway, but by a more elaborate sequence involving shikimate and an isoprene unit (see page 158). Such structures do not contain the characteristic *meta* oxygenation pattern, and often have oxygenation in only one aromatic ring (see page 164).

Emodin, physcion, chrysophanol, aloë-emodin, and rhein form the basis of a range of purgative anthraquinone derivatives found in long-established laxatives such as Senna*, Cascara*, Frangula*, Rhubarb*, and Aloes*. The free anthraquinones themselves have little therapeutic activity and need to be in the form of water-soluble glycosides to exert their action. Although simple anthraquinone *O*-glycosides are present in the drugs, the major purgative action arises from compounds such as **cascarosides**, e.g. cascaroside A (Figure 3.33), which are both *O*- and *C*-glycosides, and **sennosides**, e.g. sennoside A (Figure 3.33), which are dianthrone *O*-glycosides. These types of derivative are likely to be produced from intermediate anthrone structures. This could act as substrate for both *O*- and *C*-glucosylation, employing the glucose donor UDPglucose (see page 29), and would generate a cascaroside structure (Figure 3.31). Alternatively, a one-electron oxidation allows oxidative coupling (see page 28) of two anthrone systems to give a dianthrone (Figure 3.32). This can be formulated as direct oxidation at the benzylic $-CH_2-$, or via the anthranol, which is the phenolic tautomer of the anthrone (Figure 3.32). Glycosylation of the dianthrone system would then give a sennoside-like product. However, further oxidative steps can create a dehydrodianthrone, and then allow coupling of the aromatic rings through **protohypericin** to give a naphthodianthrone, e.g. **hypericin** (Figure 3.32). The reactions of Figure 3.32 can be achieved

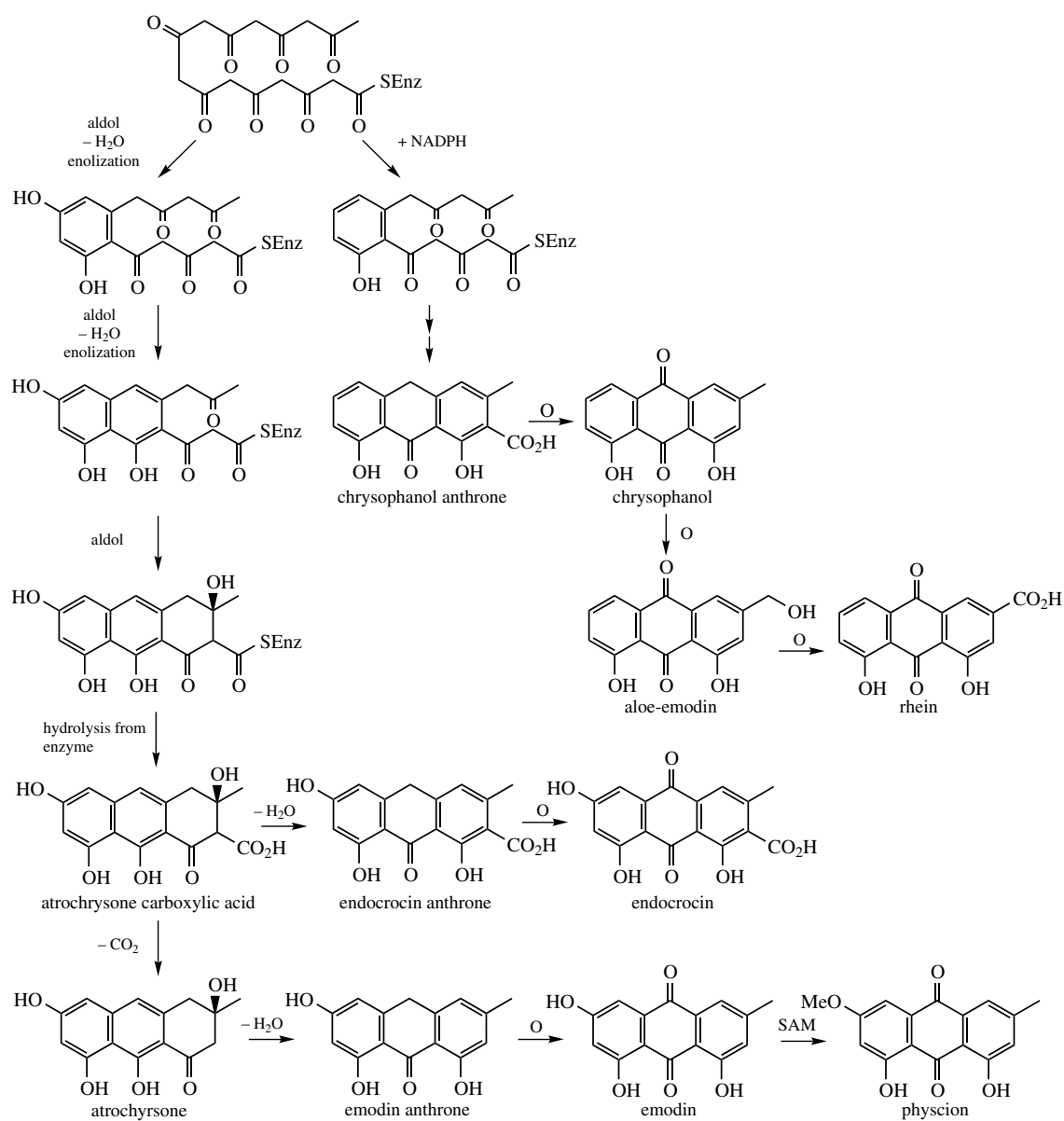


Figure 3.30

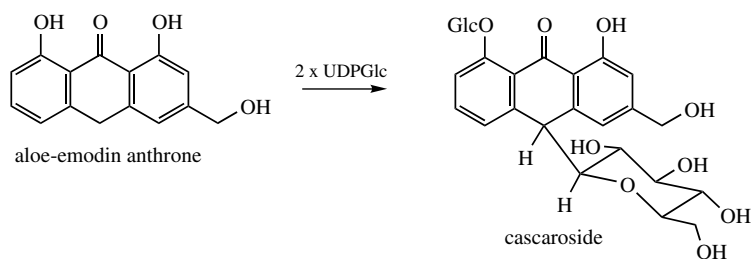


Figure 3.31

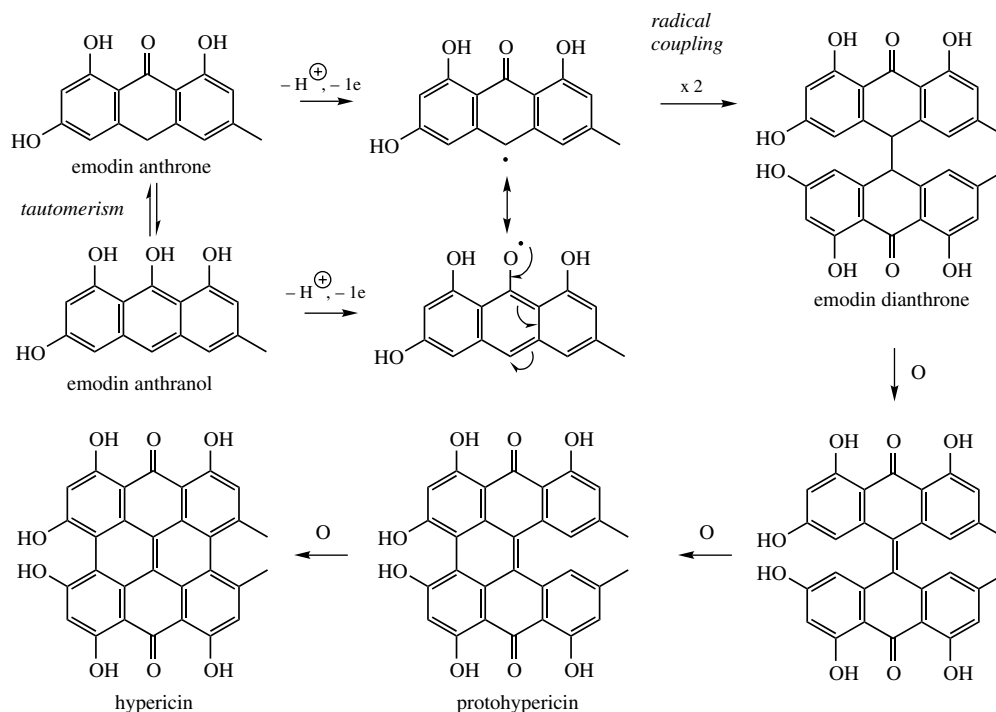


Figure 3.32

chemically by passing air into an alkaline solution of **emodin anthrone**. **Hypericin** is found in cultures of *Dermocybe* fungi, and is also a constituent of St John's Wort, *Hypericum perforatum* (Guttiferae/Hypericaceae), which is a popular herbal medicine in the treatment of depression. The naphthodianthrone has no purgative action, but

hypericin can act as a photosensitizing agent in a similar manner to furocoumarins (see page 146). Thus ingestion of hypericin results in an increased absorption of UV light and can lead to dermatitis and burning. Hypericin is also being investigated for its antiviral activities, in particular for its potential activity against HIV.

Senna

Senna leaf and fruit are obtained from *Cassia angustifolia* (Leguminosae/Fabaceae), known as Tinnevely senna, or less commonly from *Cassia senna* (syn *C. acutifolia*), which is described as Alexandrian senna. The plants are low, branching shrubs, *C. angustifolia* being cultivated in India and Pakistan, and *C. senna* being produced in the Sudan, much of it from wild plants. Tinnevely senna is cultivated in wetter conditions than Alexandrian senna, which gives more luxuriant growth. Early harvests provide leaf material whilst later on, both leaf and fruit (senna pods) are obtained, a mixture which is separated by sieving (Alexandrian) or hand picking after drying (Tinnevely). There are no significant differences in the chemical constituents of the two sennas, or between leaf and fruit drug. However, amounts of the active constituents do vary, and appear to be a consequence of cultivation conditions and the time of harvesting of the plant material.

The active constituents in both senna leaf and fruit are dianthrone glycosides, principally sennosides A and B (Figure 3.33). These compounds are both di-O-glucosides of rhein

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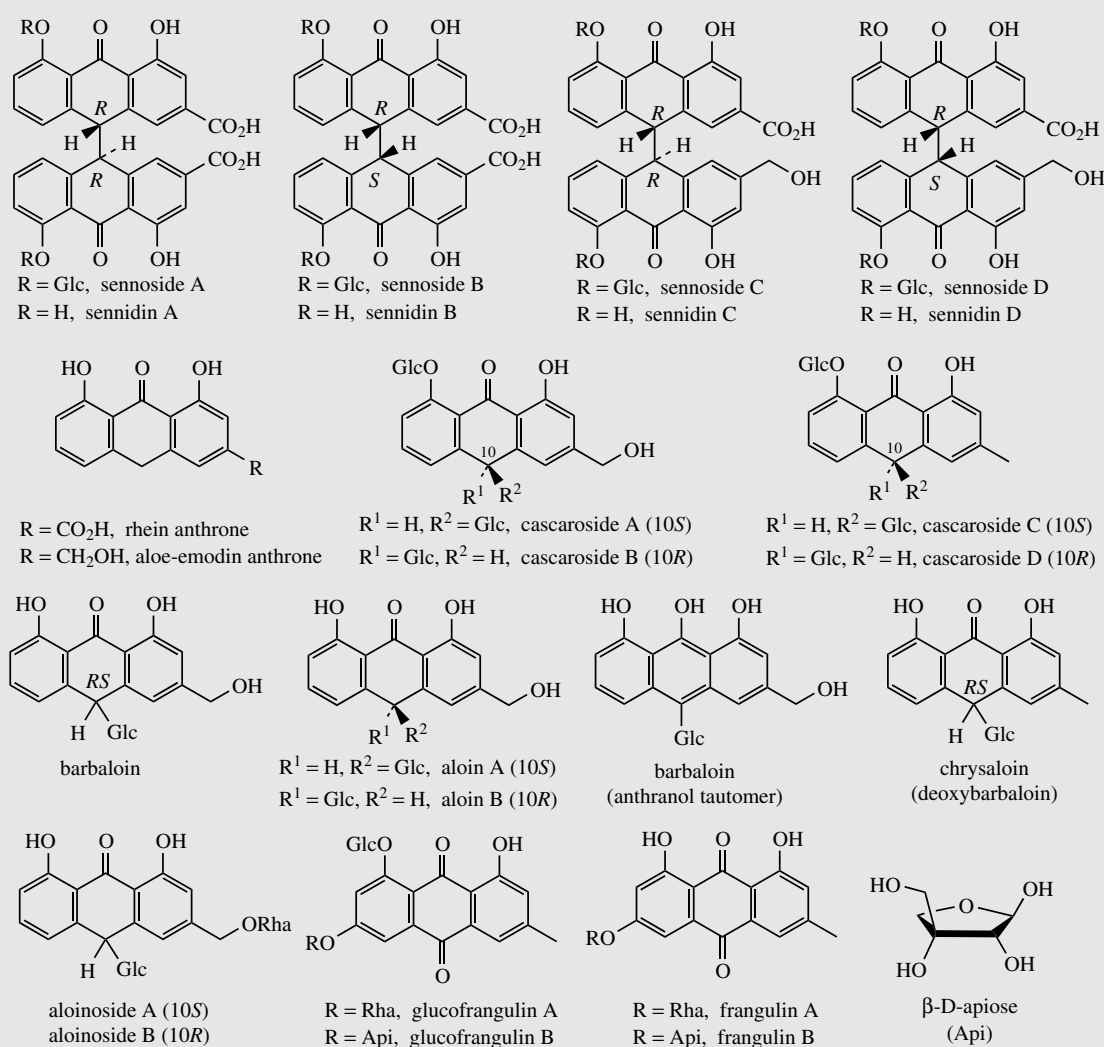


Figure 3.33

dianthrone (sennidins A and B), and liberate these aglycones on acid hydrolysis, or the anthraquinone rhein (Figure 3.30) on oxidative hydrolysis (e.g. aq HNO_3 or $\text{H}_2\text{O}_2/\text{HCl}$). Sennidins A and B are optical isomers: sennidin A is dextrorotatory (+) whilst sennidin B is the optically inactive *meso* form. Minor constituents include sennosides C and D (Figure 3.33), which are also a pair of optical isomers, di-O-glucosides of heterodianthrone sennidins C and D. Sennidin C is dextrorotatory, whilst sennidin D is optically inactive, approximating to a *meso* form in that the modest change in substituent does not noticeably affect the optical rotation. Oxidative hydrolysis of sennosides C and D would produce the anthraquinones rhein and aloe-emodin (Figure 3.30). Traces of other anthraquinone glycoside derivatives are also present in the plant material. Much of the sennoside content of the dried leaf appears to be formed by enzymic oxidation of anthrone glycosides during the drying process. Fresh leaves

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and fruits also seem to contain primary glycosides which are more potent than sennosides A and B, and which appear to be partially hydrolysed to sennosides A and B (the secondary glycosides) by enzymic activity during collection and drying. The primary glycosides contain additional glucose residues.

Senna leaf suitable for medicinal use should contain not less than 2.5% dianthrone glycosides calculated in terms of sennoside B. The sennoside content of Tinnevely fruits is between 1.2 and 2.5%, that of Alexandrian fruits being 2.5–4.5%. Senna preparations, in the form of powdered leaf, powdered fruit, or extracts, are typically standardized to a given sennoside content. Non-standardized preparations have unpredictable action and should be avoided. Senna is a stimulant laxative and acts on the wall of the large intestine, increasing peristaltic movement. After oral administration, the sennosides are transformed by intestinal flora into rhein anthrone (Figure 3.33), which appears to be the ultimate purgative principle. The glycoside residues in the active constituents are necessary for water-solubility and subsequent transportation to the site of action. Although purgative action is provided by the aglycones, including anthraquinones, these materials are conjugated and excreted in the urine after oral administration rather than being transported to the colon. Senna is a purgative drug suitable for either habitual constipation, or for occasional use, and is widely prescribed.

Cascara

Cascara is the dried bark of the cascara buckthorn *Rhamnus purshianus* (Rhamnaceae), a small tree native to the forests of the Pacific coast of North America. Most of the drug material is gathered from wild trees in Oregon, Washington, and British Columbia. Trees are felled and the bark is stripped from the trunk and branches, then dried. The fresh bark is unsuitable for drug use, causing griping and nausea, and thus the bark is stored for at least a year before being processed. During this time, enzymic hydrolysis and oxidation modify the anthraquinone-based constituents and thus the cathartic activity. Cascara preparations are mainly formulated from extracts of the bark.

Cascara bark contains about 6–9% of anthracene derivatives, 80–90% of which are anthrone C-glycosides. The major constituents are cascariosides A and B (Figure 3.33), which contain both O- and C-glucoside linkages, and represent a pair of optical isomers differing only in the stereochemistry of the C-glucoside bond. These have a substitution pattern analogous to aloe-emodin (Figure 3.30) and oxidative hydrolysis (e.g. aq HNO₃ or H₂O₂/HCl) liberates aloe-emodin. Acid hydrolysis does not cleave the C-glucose linkage, and instead generates barbaloin (Figure 3.33), a mixture of two diastereoisomeric forms, which have been named aloin A and aloin B. It is likely that during any chemical manipulation, the two forms may interconvert via the anthranol tautomer (Figure 3.33). Similar components in the bark, though usually present in smaller amounts than cascariosides A and B, are cascariosides C and D (Figure 3.33). These are also a pair of diastereoisomers, and have a substitution pattern analogous to chrysophanol (Figure 3.30). Hydrolysis of the O-glucose linkage yields chrysaloin, sometimes referred to as deoxybarbaloin. Barbaloin and chrysaloin are also found in the bark, and are thought to be breakdown products formed by enzymic hydrolysis of the cascariosides. Other compounds identified in the bark include simple anthraquinones and their O-glycosides, and some dianthrone derivatives.

The principal purgative activity originates from the cascariosides, the C-glycosides barbaloin and chrysaloin being less active when taken orally. As with the sennosides, the actual purgative

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agent is produced by the action of intestinal flora, and the cascariosides are transformed into aloe-emodin anthrone (Figure 3.33). Cascara has a similar pharmacological action to senna, i.e. it stimulates peristalsis of the large intestine, and has found major use in the correction of habitual constipation. It has a stronger effect than senna, however, and its routine usage is not now recommended.

Frangula

The bark of the alder buckthorn, *Rhamnus frangula* (Rhamnaceae) is used in a similar way to cascara, and is preferred to cascara in many European countries, though not in the UK. *Rhamnus frangula* is a small tree of European origin, and supplies of the bark come from South-Eastern Europe. The bark is also stored for a year before use. **Frangula** bark contains about 6% anthracene derivatives, mainly anthraquinone O-glycosides. These are derivatives of emodin (Figure 3.30) and comprise glucofrangulins A and B, and frangulins A and B (Figure 3.33). Free aglycones emodin, physcion and chrysophanol are also present.

Allied Drugs

Aloes and rhubarb have found considerable use as purgatives in the past, but they both have a rather drastic action and their use for this purpose has largely been abandoned.

Aloes consists of the dried juice from the leaves of various *Aloe* species (Liliaceae/Aloeaceae), including *A. ferox* (Cape aloes), *A. barbadensis* (Curacao aloes), and *A. perryi* (Socotrine aloes). The dark brown-black solid extract is extremely bitter, and contains 10–30% anthracene derivatives, the main component of which is barbaloin (Figure 3.33). Aloinosides A and B (Figure 3.33) are present in some varieties. Large amounts of resinous material form the bulk of the extract. Aloes is still used as a pharmaceutical aid in Compound Benzoin Tincture. The fresh mucilaginous gel obtained from *Aloe* species, particularly *Aloe vera* (= *A. barbadensis*), is held to assist wound healing, and is also widely used in skin cosmetics for its moisturizing and emollient properties. This material, mainly carbohydrate in nature (pectins and glucomannans), does not contain anthraquinone derivatives.

Rhubarb consists of the dried rhizome and root of *Rheum officinale*, *R. palmatum*, and other *Rheum* species (Polygonaceae). This contains 3–7.5% anthracene derivatives, mainly in the form of mono- and di-O-glucosides of rhein, physcion, and chrysophanol. Aglycones, especially rhein, are also present, and dianthrone derivatives have also been characterized. A high proportion of tannin-like materials gives rhubarb astringent as well as purgative properties. The common rhubarb cultivated for culinary use is *Rheum rhaponticum*, a species containing similar anthraquinone derivatives to the drug material, but which was not officially acceptable. In common with other *Rheum* species, this plant is considered poisonous due to the high concentration of oxalic acid present in the leaf (though not in the stem, which is edible). Toxic effects result from hypocalcaemia caused by removal of calcium from the bloodstream by formation of the insoluble calcium oxalate.

Dantron (danthron; 1,8-dihydroxyanthraquinone) (Figure 3.34) is known as a natural product, but for drug use is produced synthetically. It is prescribed to relieve constipation in geriatric and terminally ill patients. **Dithranol** (1,8-dihydroxyanthrone) is used as topical agent to treat troublesome cases of psoriasis. **Diacetylrhein** is marketed in some countries for the treatment of osteoarthritis.

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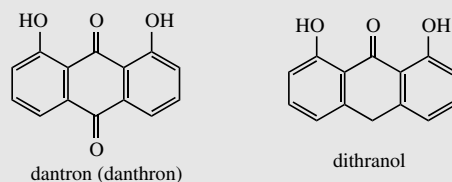


Figure 3.34

Hypericum/St John's Wort

The dried flowering tops of St John's Wort (*Hypericum perforatum*; Guttiferae/Hypericaceae) have been used as a herbal remedy for many years, an extract in vegetable oil being used for its antiseptic and wound healing properties. St John's Wort is now a major crop marketed as an antidepressant, that is claimed to be as effective in its action as the widely prescribed antidepressants of the selective serotonin re-uptake inhibitor (SSRI) class such as fluoxetine (Prozac®), and with fewer side-effects. There is considerable clinical evidence that extracts of St John's Wort are effective in treating mild to moderate depression and improving mood. However, to avoid potentially dangerous side-effects, St John's Wort should not be used at the same time as prescription antidepressants. St John's Wort is a small to medium height herbaceous perennial plant with numerous yellow flowers characteristic of this genus. It is widespread throughout Europe, where it is generally considered a weed, and has also become naturalized in North America. The tops, including flowers at varying stages of development, which contain considerable amounts of the active principles, are harvested and dried in late summer.

The dried herb contains significant amounts of phenolic derivatives, including 4–5% of flavonoids, though the antidepressant activity is considered to derive principally from naphthodianthrone structures such as hypericin (about 0.1%) and pseudohypericin (about 0.2%), and a prenylated phloroglucinol derivative hyperforin (Figure 3.35). The fresh plant also contains significant levels of protohypericin and protopseudohypericin, which are converted into hypericin and pseudohypericin during drying and processing, as a result of irradiation with visible light. Hyperforin is a major lipophilic constituent in the leaves and flowers (2–3%),

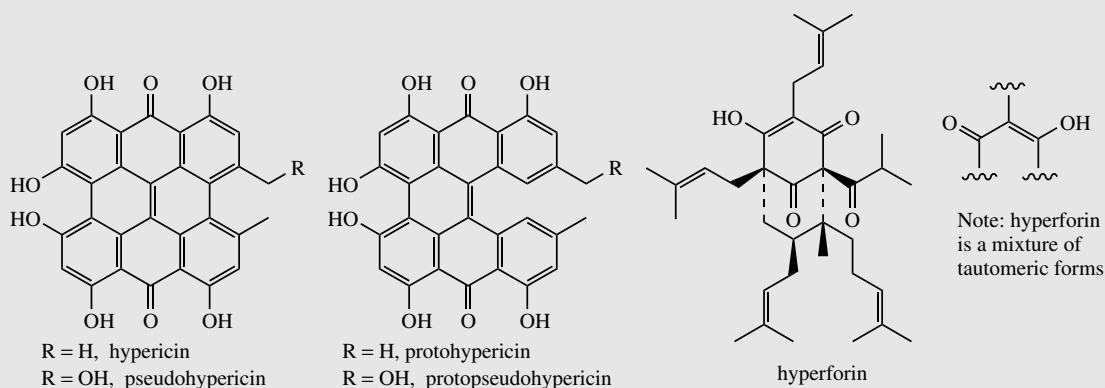


Figure 3.35

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and is now thought to be the major contributor to the antidepressant activity, as well as to the antibacterial properties of the oil extract. Studies show clinical effects of St John's Wort on depression correlate well with hyperforin content. Standardized aqueous ethanolic extracts containing 0.15% hypericin and 5% hyperforin are usually employed. The aqueous solubility of hypericin and pseudohypericin is markedly increased by the presence of flavonoid derivatives in the crude extract, particularly procyanidin B₂, a dimer of epicatechin (see page 151). *Hypericum* extracts have been demonstrated to increase levels of serotonin, noradrenaline, and dopamine, which may be responsible for the antidepressant activity.

Hypericin also possesses extremely high toxicity towards certain viruses, a property that requires light and may arise via photo-excitation of the polycyclic quinone system. It is currently under investigation as an antiviral agent against HIV and hepatitis C. Antiviral activity appears to arise from an inhibition of various protein kinases, including those of the protein kinase C family. Hypericin and pseudohypericin are potent photosensitizers initiating photochemical reactions, and are held responsible for hypericium, a photodermatitis seen in cattle after consuming *Hypericum* plants present in pasture. Patients using St John's Wort as an antidepressant should also be warned to avoid over-exposure to sunlight. There is also considerable evidence that St John's Wort interacts with a number of prescription drugs including the anticoagulant warfarin, the cardiac glycoside digoxin, the bronchodilator theophylline, the HIV protease inhibitor indinavir, the immunosuppressive drug cyclosporin, and oral contraceptives. In some cases, it is known to promote the cytochrome P-450-dependent metabolism of the co-administered drugs.

C-Alkylation Reactions

A common feature of many natural products containing phenolic rings is the introduction of alkyl groups at nucleophilic sites. Obviously, the phenol groups themselves are nucleophilic, and with a suitable alkylating agent, *O*-alkyl derivatives may be formed (see page 12), e.g. the *O*-methylation of emodin to physcion (Figure 3.30). However, a phenol group also activates the ring carbons at the *ortho* and *para* positions, so that these positions similarly become susceptible to alkylation, leading to *C*-alkyl derivatives. The *meta* oxygenation pattern, which is a characteristic feature of acetate-derived phenolics, has the effect of increasing this nucleophilicity considerably, and the process of *C*-alkylation is very much facilitated (see page 12). Suitable natural alkylating agents are *S*-adenosylmethionine (SAM), and dimethylallyl diphosphate (DMAPP). Other polyprenyl diphosphate esters may also be encountered in biological alkylation reactions (e.g. see vitamin K, page 159). A minor inconsistency has been discovered, in

that, while *C*-alkylation with dimethylallyl and higher diphosphates is mediated *after* the initial polyketide cyclization product is liberated from the enzyme, there are several examples where *C*-methylation undoubtedly occurs *before* release of any aromatic compound from the enzyme. **5-methylorsellinic acid** (Figure 3.36) is a simple *C*-methylated analogue of orsellinic acid found in *Aspergillus flaviceps*, and the extra methyl is derived from SAM. However, orsellinic acid is not a precursor of 5-methylorsellinic acid and it is proposed that the poly- β -keto ester is therefore methylated as part of the series of reactions catalysed by the synthase complex (Figure 3.36). Similarly, 5-methylorsellinic acid, but not orsellinic acid is a precursor of **mycophenolic acid*** in *Penicillium brevicompactum* (Figure 3.36). However, *C*-alkylation by farnesyl diphosphate (see page 191) proceeds *after* the aromatization step, and a phthalide intermediate is the substrate involved. The phthalide is a lactone derived from 5-methylorsellinic acid by hydroxylation of its starter methyl group and reaction with the end-

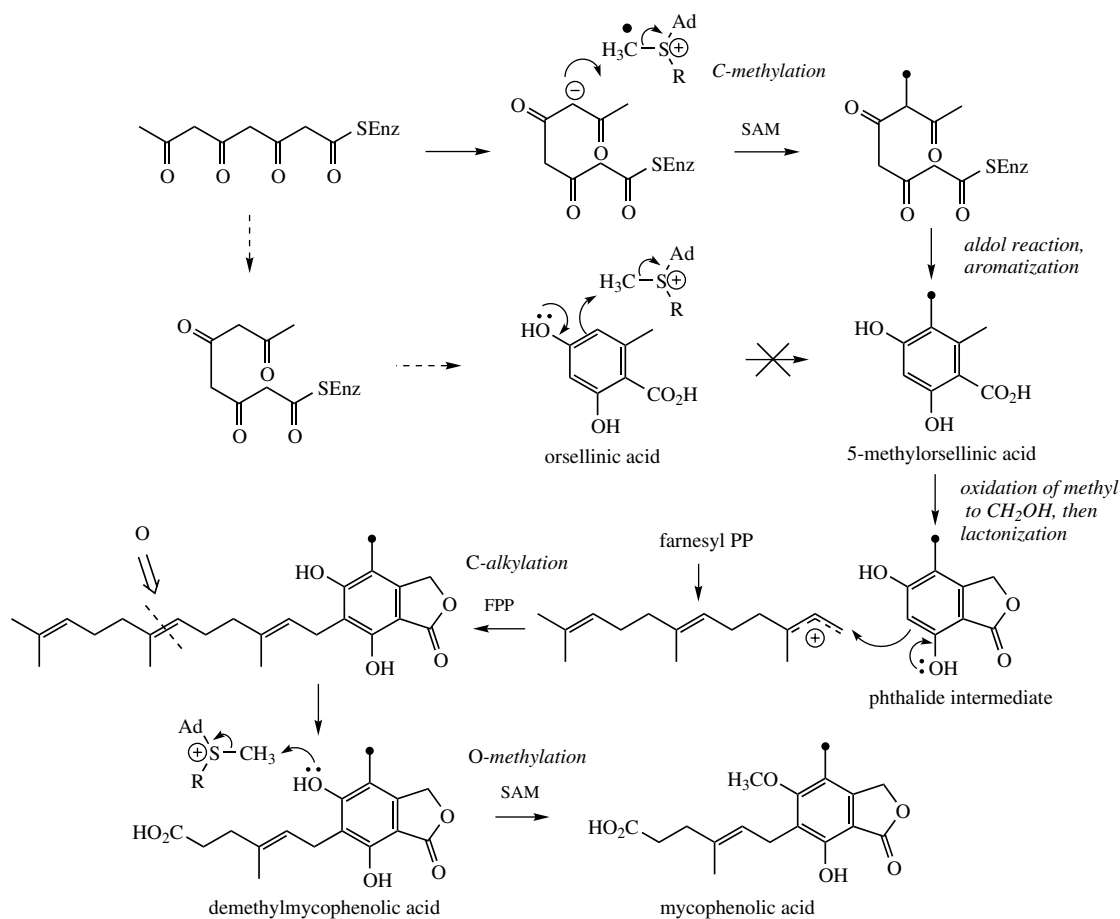


Figure 3.36

Mycophenolic Acid

Mycophenolic acid (Figure 3.36) is produced by fermentation cultures of the fungus *Penicillium brevicompactum*. It has been known for many years to have antibacterial, antifungal, antiviral, and antitumour properties. It has recently been introduced into medicine as an immunosuppressant drug, to reduce the incidence of rejection of transplanted organs, particularly kidney and heart transplants. It is formulated as the *N*-morpholinoethyl ester **mycophenolate mofetil** (Figure 3.37), which is metabolized after ingestion to mycophenolic

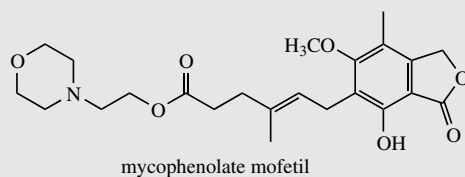


Figure 3.37

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acid, and is usually administered in combination with cyclosporin (see page 429). The drug is a specific inhibitor of mammalian inosine monophosphate dehydrogenase and has an antiproliferative activity on cells due to inhibition of guanosine nucleotide biosynthesis. This enzyme catalyses the NAD^+ -dependent oxidation of inosine monophosphate (IMP) to xanthosine monophosphate (XMP), a key transformation in the synthesis of guanosine triphosphate (GTP) (see also caffeine biosynthesis, page 394). Rapidly growing cells have increased levels of the enzyme, so this forms an attractive target for anticancer, antiviral, and immunosuppressive therapy.

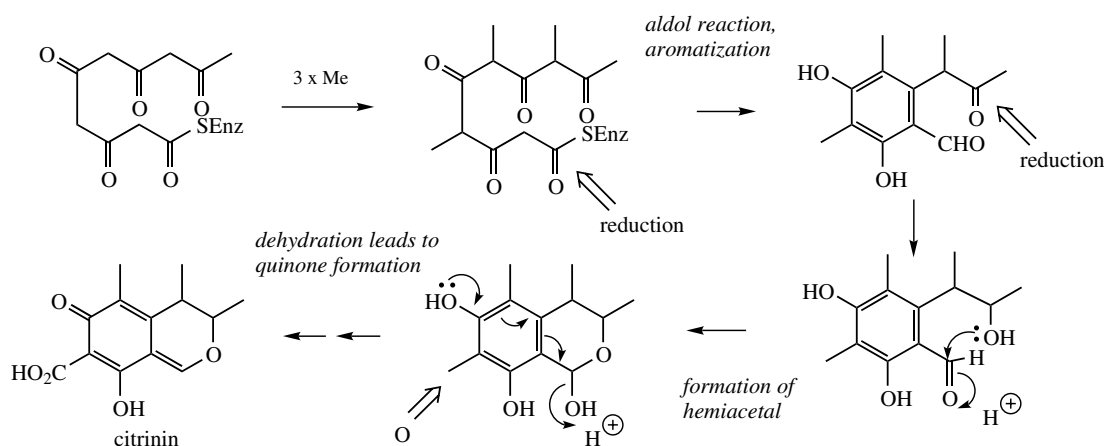


Figure 3.38

of-chain carboxyl. The chain length of the farnesyl alkyl group is then shortened by oxidation of a double bond giving demethylmycophenolic acid, which is then *O*-methylated, again involving SAM, to produce mycophenolic acid. Note that the *O*-methylation step only occurs after the *C*-alkylations, so that the full activating benefit of two *meta* positioned phenols can be utilized for the *C*-alkylation.

Three *C*-methyl substituents are inserted into the acetate-derived skeleton of **citrinin** (Figure 3.38), an antimicrobial metabolite from *Penicillium citrinum* and several *Aspergillus* species, which also displays potentially dangerous carcinogenic and nephrotoxic (kidney-damaging) activity. One of these introduced methyls has undergone oxidation to a carboxyl, adding to the difficulties in immediately recognizing the biosynthetic origins of this compound which contains a quinonemethide system rather than the

simpler aromatic ring. The methyls are probably introduced into the polyketide prior to release of the first aromatic intermediate, which could well be an aldehyde rather than the corresponding acid if a reductase component also forms part of the synthase complex. The hemiacetal can be produced after reduction of the side-chain carbonyl, and then in the later stages, oxidation of one methyl to a carboxyl will follow. The quinonemethide system in citrinin is simply the result of a dehydration reaction on the hemiacetal (Figure 3.38).

Khellin* and **visnagin** (Figure 3.39) are furochromones found in the fruits of *Ammi visnaga* (Umbelliferae/Apiaceae), and the active principles of a crude plant drug which has a long history of use as an antiasthmatic agent. Figure 3.39 presents the sequence of steps utilized in the biosynthesis of these compounds, fully consistent with the biosynthetic rationale developed above. The two carbons C-2' and C-3' forming part of the furan

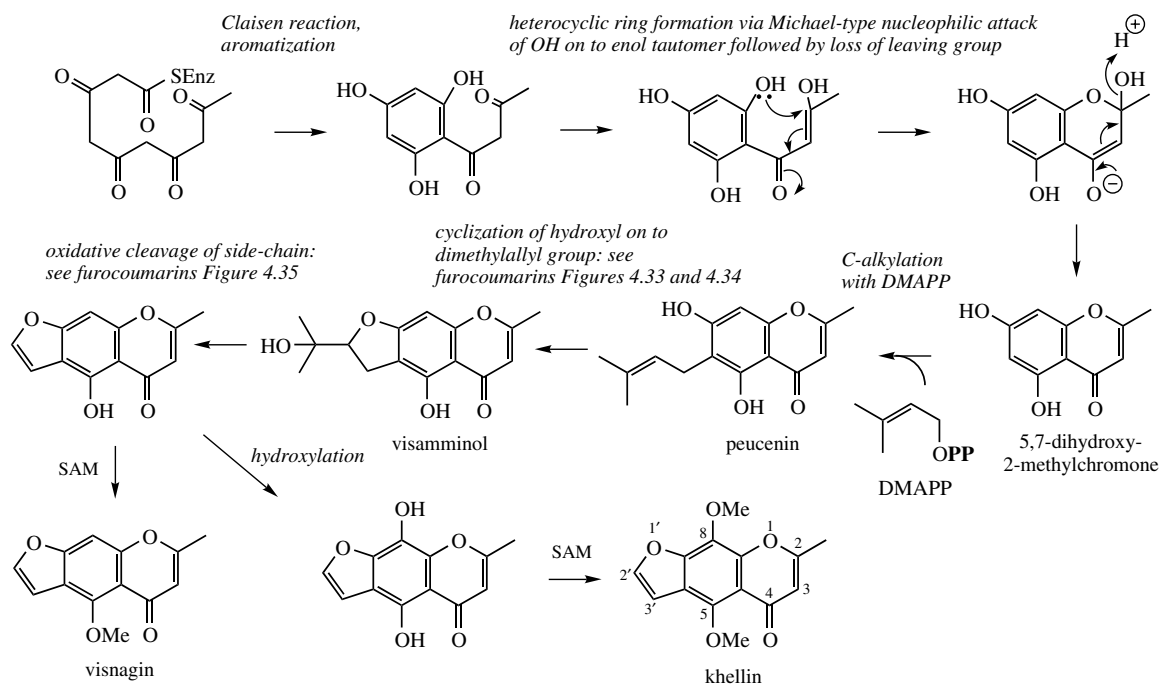


Figure 3.39

Khellin and Cromoglicate

The dried ripe fruits of *Ammi visnaga* (Umbelliferae/Apiaceae) have a long history of use in the Middle East as an antispasmodic and for the treatment of angina pectoris. The drug contains small amounts of coumarin derivatives, e.g. visnadin (Figure 3.40) (compare *Ammi majus*, a rich source of furocoumarins, page 146), but the major constituents (2–4%) are

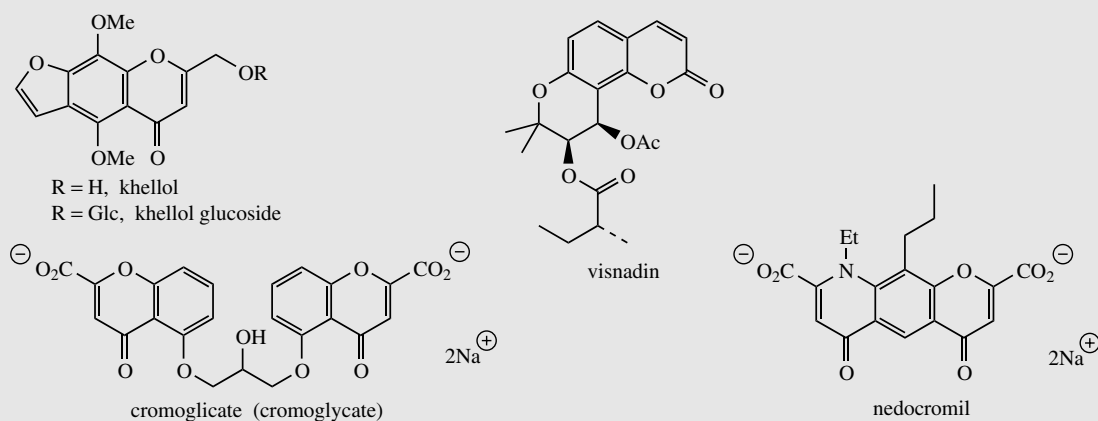


Figure 3.40

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furochromones, including khellin and visnagin (Figure 3.34), and khellol and khellol glucoside (Figure 3.40). Both khellin and visnadin are coronary vasodilators and spasmolytic agents, with visnadin actually being the more potent agent. Khellin has been used in the treatment of angina pectoris and bronchial asthma. The synthetic analogue **cromoglicate (cromoglycate)** (Figure 3.40) is a most effective and widely used agent for the treatment and prophylaxis of asthma, hay fever, and allergic rhinitis. Cromoglicate contains two chromone systems containing polar carboxylic acid functions, joined by a glycerol linker. The mode of action is not fully established. It was believed to prevent the release of bronchospasm mediators by stabilizing mast cell membranes, but an alternative suggestion is that it may act by inhibiting the effect of sensory nerve activation, thus interfering with bronchoconstriction. It is poorly absorbed orally and is thus administered as inhalation or nasal spray. Eyedrops for relief of allergic conjunctivitis are also available. The more potent **nedocromil** (Figure 3.40) has also been introduced.

ring originate by metabolism of a five-carbon dimethylallyl substituent attached to C-6 (for a full discussion, see furocoumarins, page 145). The 8-methoxy group in khellin is absent from visnagin, so must be introduced late in the sequence. The key intermediate is thus 5,7-dihydroxy-2-methylchromone. On inspection, this has the alternate acetate-derived oxygenation pattern and a methyl chain starter, so is formed from a poly- β -keto chain through Claisen condensation then heterocyclic ring formation by an overall dehydration reaction. After formation of the furan ring via the C-dimethylallyl derivative peucenin and then visamminol, **visnagin** can be obtained by O-methylation. Alternatively, further hydroxylation *para* to the free phenol, followed by two methylations, yields **khellin**. The antiasthmatic properties of khellin have been exploited by developing the more polar, water-soluble derivative **cromoglicate***.

Phenolic Oxidative Coupling

C-Methylation also features in the biosynthesis of **usnic acid** (Figure 3.41), an antibacterial metabolite found in many lichens, e.g. *Usnea* and *Cladonia* species, which are symbiotic combinations of alga and fungus. However, the principal structural modification encountered involves phenolic oxidative coupling (see page 28). Two molecules of **methylphloracetophenone** are incorporated, and these are known to derive from a pre-aromatization methylation reaction and not by

methylation of phloracetophenone (Figure 3.41). The two molecules are joined together by an oxidative coupling mechanism which can be rationalized via the one-electron oxidation of a phenol group in methylphloracetophenone giving free radical A, for which resonance forms B and C can be written. Coupling of B and C occurs. Only the left-hand ring can subsequently be restored to aromaticity by keto-enol tautomerism, this state being denied to the right-hand ring because coupling occurred on to the methyl-containing position *para* to the original phenol. Instead, a heterocyclic ring is formed by attack of the phenol on to the enone system (see khellin, above). The outcome of this reaction is enzyme controlled, since two equivalent phenol groups are present as potential nucleophiles, and two equivalent enone systems are also available. Therefore, four different products could be formed, but only one is actually produced. Loss of water then leads to usnic acid.

Phenolic oxidative coupling is widely encountered in natural product biosynthesis, and many other examples are described in subsequent sections. A further acetate-derived metabolite formed as a result of oxidative coupling is the antifungal agent **griseofulvin*** (Figure 3.42) synthesized by cultures of *Penicillium griseofulvin*. The sequence of events leading to griseofulvin has now been established in detail, and the pathway also includes O-methylation steps and the introduction of a halogen (chlorine) atom at one of the nucleophilic sites, which is represented as involving the electrophile Cl^+ (Figure 3.42).

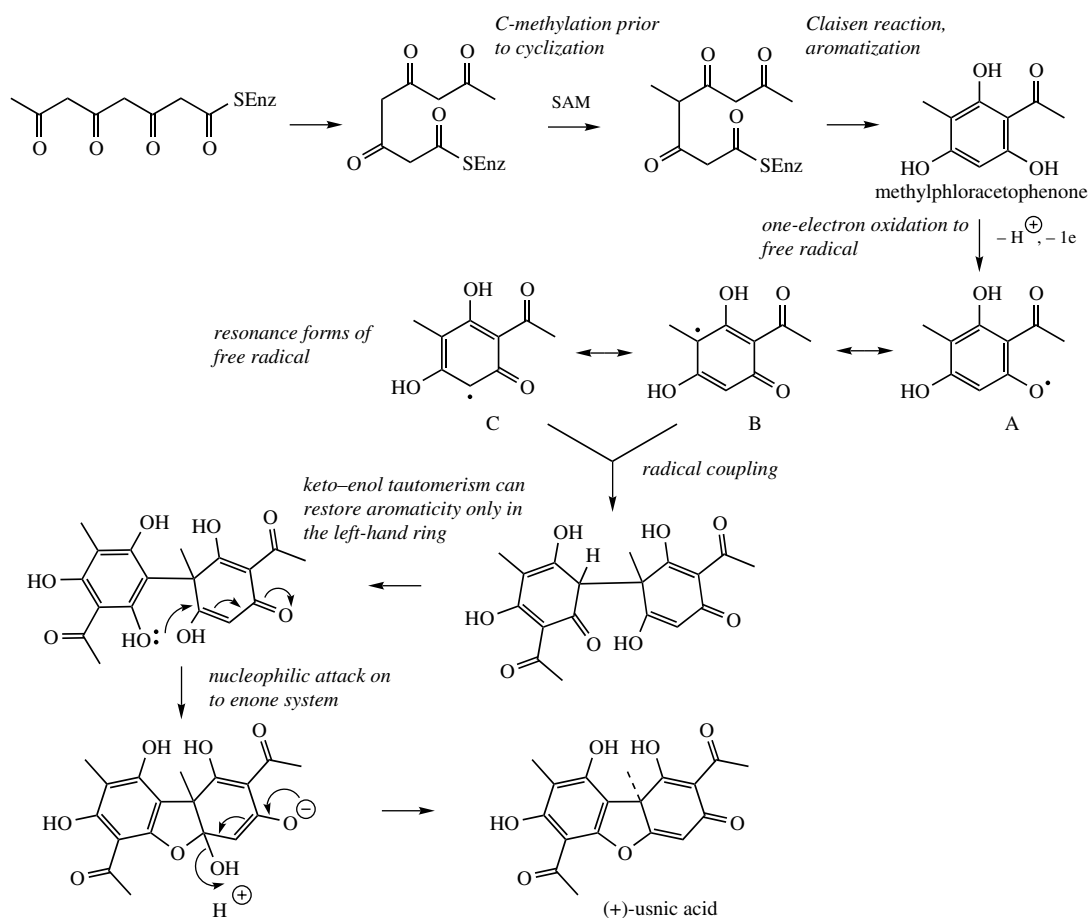


Figure 3.41

Griseofulvin

Griseofulvin is an antifungal agent produced by cultures of *Penicillium griseofulvum* and a number of other *Penicillium* species, including *P. janczewski*, *P. nigrum*, and *P. patulum*. Griseofulvin is the drug of choice for widespread or intractable dermatophyte infections, but is ineffective when applied topically. However, it is well absorbed from the gut and selectively concentrated into keratin, so may be used orally to control dermatophytes such as *Epidermophyton*, *Microsporium*, and *Trichophyton*. Treatment for some conditions, e.g. infections in fingernails, may have to be continued for several months, but the drug is generally free of side-effects. The antifungal action appears to be through disruption of the mitotic spindle, thus inhibiting fungal mitosis.

Initial inspection of the structure of griseofulvin shows the alternate oxygenation pattern, and also a methyl group which identifies the start of the polyketide chain. Cyclization of the C₁₄ poly-β-keto chain folded as shown allows both

Claisen (left-hand ring) and aldol (right-hand ring) reactions to occur giving a benzophenone intermediate. Two selective methylations lead to griseophenone C, which is the substrate for chlorination to griseophenone B; both these

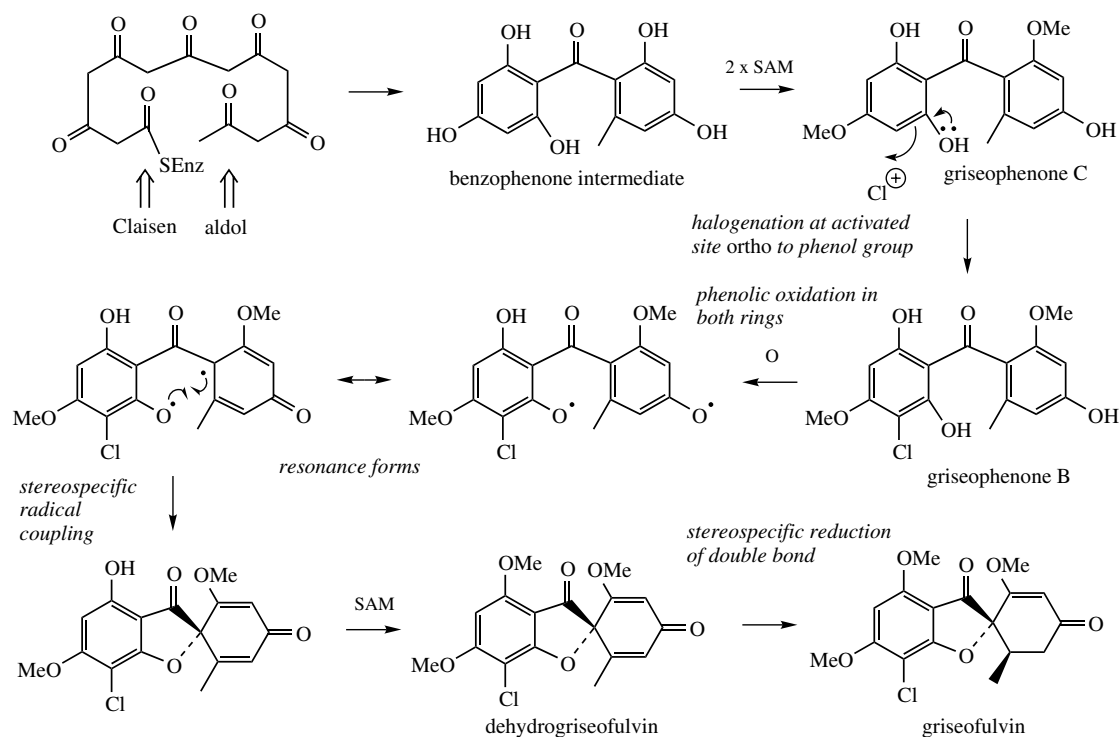


Figure 3.42

compounds appear as minor metabolites in *P. griseofulvin* cultures. One-electron oxidations on a phenolic group in each ring give a diradical and its mesomer, the latter allowing radical coupling to the basic grisan skeleton. **Griseofulvin** is then the result of methylation of the remaining phenol group and stereospecific reduction of the double bond in dehydrogriseofulvin.

Oxidative Cleavage of Aromatic Rings

Perhaps the most drastic modification which can happen to an aromatic ring is ring cleavage brought about by oxidative enzymes called dioxygenases (see page 27). These enzymes typically use catechol (1,2-dihydroxy) or quinol (1,4-dihydroxy) substrates, require molecular oxygen and Fe^{2+} cofactors, and incorporate both the oxygen atoms into the ring-cleaved product. In the case of catechols, cleavage may be between or adjacent to the two hydroxyls, giving products containing aldehyde and/or carboxylic acid functionalities (Figure 3.43). These groups are then able to react with other substituents in the molecule creating

compounds in which the characteristic acetate-derived features are probably no longer apparent. Shikimate-derived aromatic rings can suffer similar oxidative cleavage reactions.

Patulin is an excellent example of an acetate-derived structure synthesized from an aromatic substrate via oxidative cleavage and subsequent modifications (Figure 3.44). Patulin is a potent carcinogen produced by *Penicillium patulum*, a common contaminant on apples. If mould-infected apples find their way into food products, e.g.

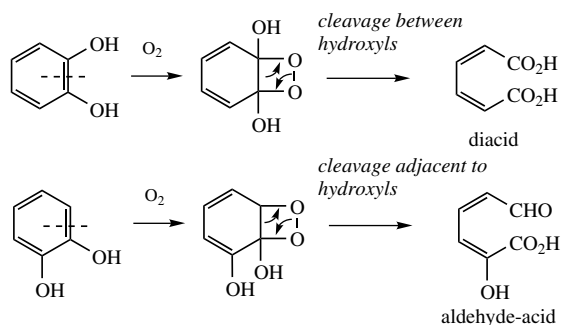


Figure 3.43

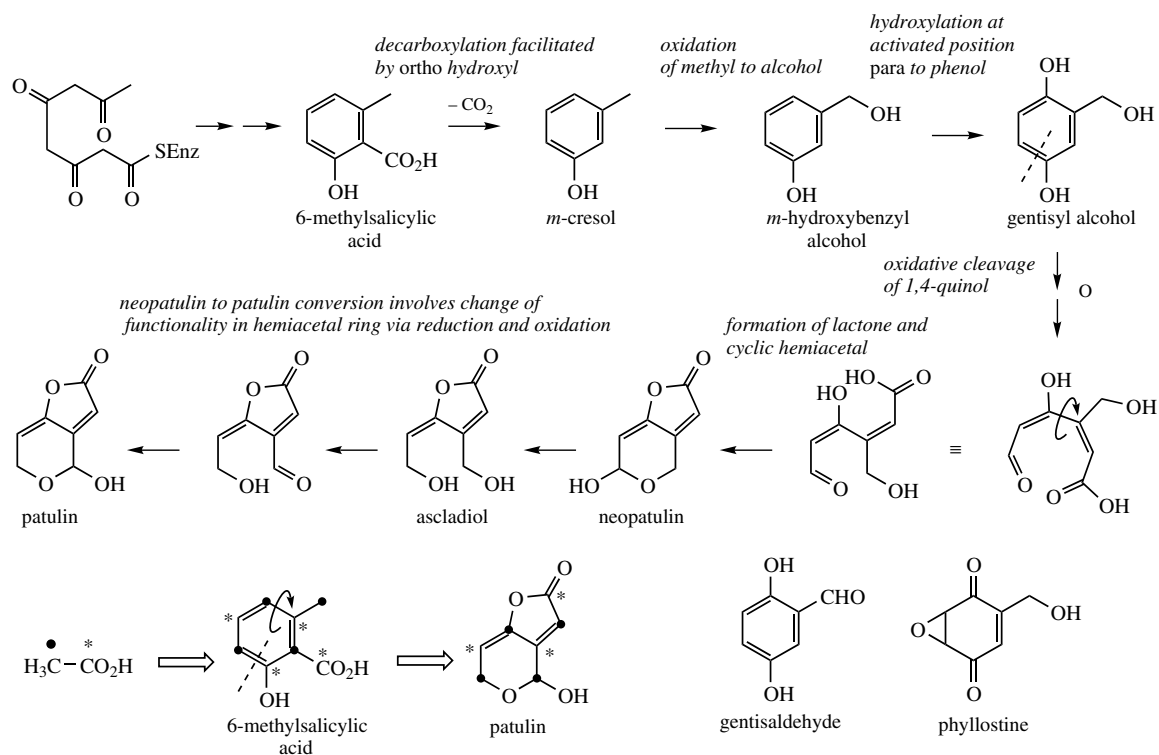


Figure 3.44

apple juice, fruit pies, etc., then these products may contain unacceptable and dangerous levels of patulin. Such food materials are routinely screened for patulin content, with a tolerance level set at $50 \mu\text{g kg}^{-1}$. Patulin is derived from acetate via **6-methylsalicylic acid** (Figure 3.26). Decarboxylation and hydroxylation reactions then lead to **gentisyl alcohol** (Figure 3.44), which may suffer oxidative cleavage as shown. Cleavage of the aromatic ring would generate aldehyde and carboxylic acid functions. By rotating the molecule around the carbon-carbon single bond as shown, it is easy to see that **neopatulin** can result by formation of hemiacetal and lactone groups. The reversal of functionality in the hemiacetal ring to produce **patulin** is achieved by reduction and oxidation reactions involving aldehyde and alcohol components of the hemiacetal. The sequence shown in Figure 3.44 has been deliberately simplified to rationalize the oxidative cleavage. The true sequence involves gentisaldehyde and the epoxyquinone phyllostine as intermediates between gentisyl alcohol and neopatulin.

Penicillic acid (Figure 3.45), another microbially produced food contaminant with carcinogenic properties, is synthesized by cultures of *Penicillium cyclopium* and *P. baarnense*, and also features oxidative ring fission of an aromatic compound. This time **orsellinic acid** (Figure 3.25) is a precursor, and ring fission appears to proceed via a quinone, which is the result of decarboxylation, oxidation, and methylation reactions. Figure 3.45 also represents an over-simplistic rationalization of the ring fission process.

Starter Groups Other Than Acetate

In the examples so far discussed, the basic carbon skeleton has been derived from an acetate starter group, with malonate acting as the chain extender. The molecule has then, in some cases, been made more elaborate by the inclusion of other carbon atoms, principally via alkylation reactions. However, the range of natural product structures that are at least partly derived from acetate is increased enormously by altering the nature of the

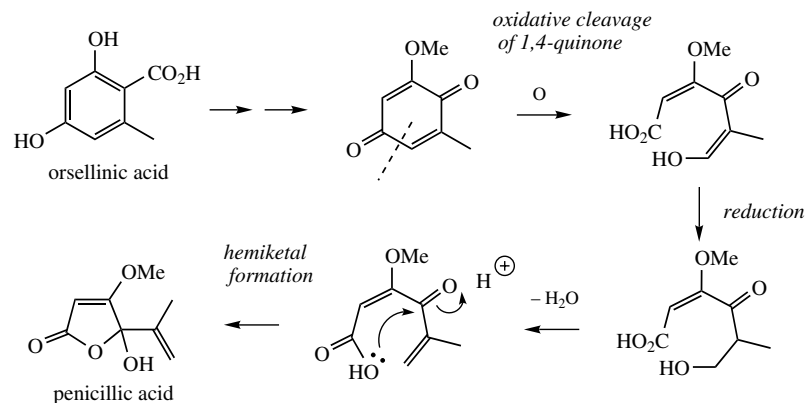


Figure 3.45

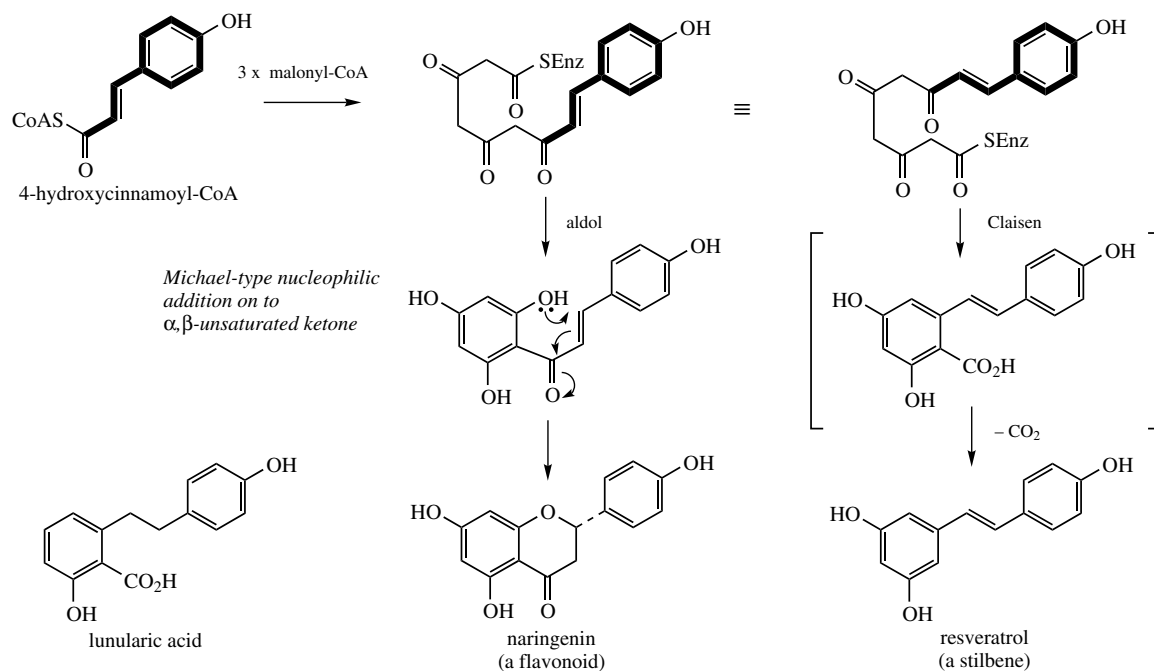


Figure 3.46

starter group from acetate to a different carboxylate system, as its coenzyme A ester, with malonyl-CoA again providing the chain extender. There is less detailed knowledge here about the precise nature of how substrates are bound to the enzyme, and whether coenzyme A esters are initially transformed into thio esters of the ACP type.

Flavonoids and **stilbenes** are simple examples of molecules in which a suitable cinnamoyl-CoA C_6C_3 precursor from the shikimate pathway (see

page 130) has acted as a starter group. Thus, if **4-hydroxycinnamoyl-CoA** (Figure 3.46) is chain extended with three malonyl-CoA units, the poly-β-keto chain can then be folded in two ways, allowing aldol or Claisen-type cyclizations to occur, respectively. The six-membered heterocyclic ring characteristic of most flavonoids, e.g. **naringenin**, is formed by nucleophilic attack of a phenol group from the acetate-derived ring on to the α,β -unsaturated ketone. Stilbenes, such as

resveratrol, incorporate the carbonyl carbon of the cinnamoyl unit into the aromatic ring, and typically lose the end-of-chain carboxyl by a decarboxylation reaction. Although some related structures, e.g. **lunularic acid** from the liverwort *Lunularia cruciata*, still contain this carboxyl, in general it is lost in a pre-cyclization modification, and intermediates of the type shown in brackets are not produced. Flavonoids and stilbenes are discussed in more detail in Chapter 4 (see page 149).

Anthranilic acid (2-aminobenzoic acid) (see page 126) is another shikimate-derived compound which, as its CoA ester anthraniloyl-CoA, can act as a starter unit for malonate chain extension. Aromatization of the acetate-derived portion then leads to **quinoline** or **acridine** alkaloids, according to the number of acetate units incorporated (Figure 3.47). These products are similarly discussed elsewhere, under alkaloids (Chapter 6, page 376).

Fatty acyl-CoA esters are similarly capable of participating as starter groups. Fatty acid

biosynthesis and aromatic polyketide biosynthesis are distinguished by the sequential reductions as the chain length increases in the former, and by the stabilization of a reactive poly- β -keto chain in the latter, with little or no reduction involved. It is thus interesting to see natural product structures containing both types of acetate-malonate-derived chains. In plants of the Anacardiaceae, e.g. poison ivy* (*Rhus radicans*) and poison oak* (*Rhus toxicodendron*), contact allergens called **urushiols** are encountered, which derive from just such a pathway. Thus, **palmitoleoyl-CoA** (Δ^9 -hexadecenoyl-CoA) can act as starter group for extension by three malonyl-CoA units, with a reduction step during chain extension (Figure 3.48). Aldol cyclization then gives **anacardic acid**, which is likely to be the precursor of **urushiol** by decarboxylation/hydroxylation. It is likely that different fatty acyl-CoAs can participate in this sequence, since urushiols from poison ivy can contain up to three double bonds in the C_{15} side-chain, whilst those from poison oak also have variable unsaturation

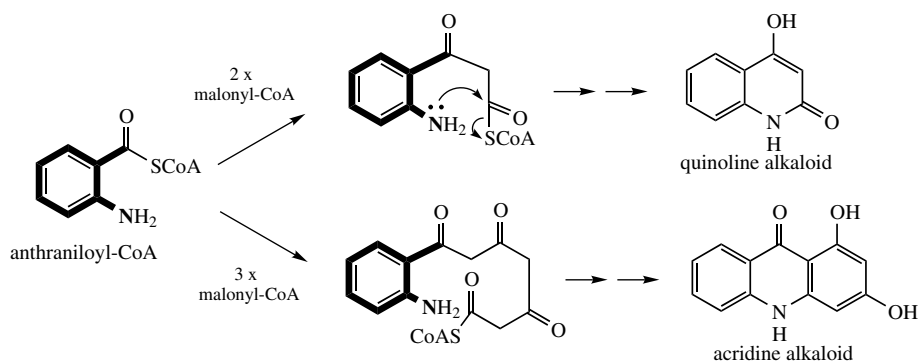


Figure 3.47

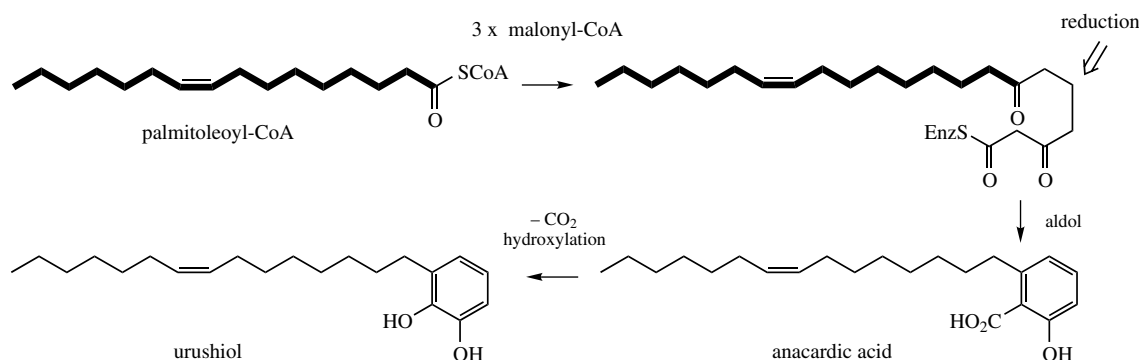


Figure 3.48

Poison Ivy and Poison Oak

Poison ivy (*Rhus radicans* or *Toxicodendron radicans*; Anacardiaceae) is a woody vine with three-lobed leaves that is common in the USA. The plant may be climbing, shrubby, or may trail over the ground. It presents a considerable hazard to humans should the sap, which exudes from damaged leaves or stems, come into contact with the skin. The sap sensitizes most individuals, producing delayed contact dermatitis after a subsequent encounter. This results in watery blisters that break open, the fluid quickly infecting other parts of the skin. The allergens may be transmitted from one person to another on the hands, on clothing, or by animals. The active principles are urushiols, a mixture of alkenyl polyphenols. In poison ivy, these are mainly pentadecylcatechols with varying degrees of unsaturation (Δ^8 , $\Delta^{8,11}$, $\Delta^{8,11,14}$) in the side-chain. Small amounts of C₁₇ side-chain analogues are present. These catechols become oxidized to an *ortho*-quinone, which is then attacked by nucleophilic groups in proteins to yield an antigenic complex.

Poison oak (*Rhus toxicodendron* or *Toxicodendron toxicaria*; Anacardiaceae) is nearly always found as a low-growing shrub, and has lobed leaflets similar to those of oak. It is also common throughout North America. There appears considerable confusion over nomenclature, and *Rhus radicans* may also be termed poison oak, and *R. toxicodendron* oakleaf poison ivy. Poison oak contains similar urushiol structures in its sap as poison ivy, though heptadecylcatechols (i.e. C₁₇ side-chains) predominate over pentadecylcatechols (C₁₅ side-chains).

Related species of *Rhus*, e.g. *R. diversiloba* (Pacific poison oak) and *R. vernix* (poison sumach, poison alder, poison dogwood) are also allergenic with similar active constituents. The allergen-containing species of *Rhus* have been reclassified under the genus *Toxicodendron*, though this nomenclature is not commonly employed. Dilute purified extracts containing urushiols may be employed to stimulate antibody production and thus build up immunity to the allergens.

in a C₁₇ side-chain. Large quantities of anacardic acids containing C₁₅ side-chains with one, two, and three double bonds are also found in the shells of cashew nuts (*Anacardium occidentale*; Anacardiaceae).

A saturated C₆ **hexanoate** starter unit is used in the formation of the **aflatoxins***, a group of highly toxic metabolites produced by *Aspergillus flavus*, and probably responsible for the high incidence of liver cancer in some parts of Africa. These compounds were first detected following the deaths of young turkeys fed on mould-contaminated peanuts (*Arachis hypogaea*; Leguminosae/Fabaceae). Peanuts still remain one of the crops most likely to represent a potential risk to human health because of contamination with fungal toxins. These and other food materials must be routinely screened to ensure levels of aflatoxins do not exceed certain set limits. The aflatoxin structures contain a bisfuran unit fused to an aromatic

ring, e.g. **aflatoxin B₁** and **aflatoxin G₁**, and their remarkably complex biosynthetic origin begins with a poly- β -keto chain derived from a hexanoyl-CoA starter and seven malonyl-CoA extender units (Figure 3.49). This gives an anthraquinone **norsolorinic acid** by now-familiar condensation reactions, but the folding of the chain is rather different from that seen with simpler anthraquinones (see page 64). The six-carbon side-chain of norsolorinic acid is cyclized to give, in several steps, the ketal **averufin**. **Versiconal acetate** is another known intermediate, and its formation involves a Baeyer–Villiger oxidation (see page 28), resulting principally in transfer of a two-carbon fragment (the terminal ethyl of hexanoate) to become an ester function. These two carbons can then be lost in formation of **versicolorin B**, now containing the tetrahydrobisfuran moiety, oxidized in **versicolorin A** to a dihydrobisfuran system. **Sterigmatocystin** is derived from versicolorin A by

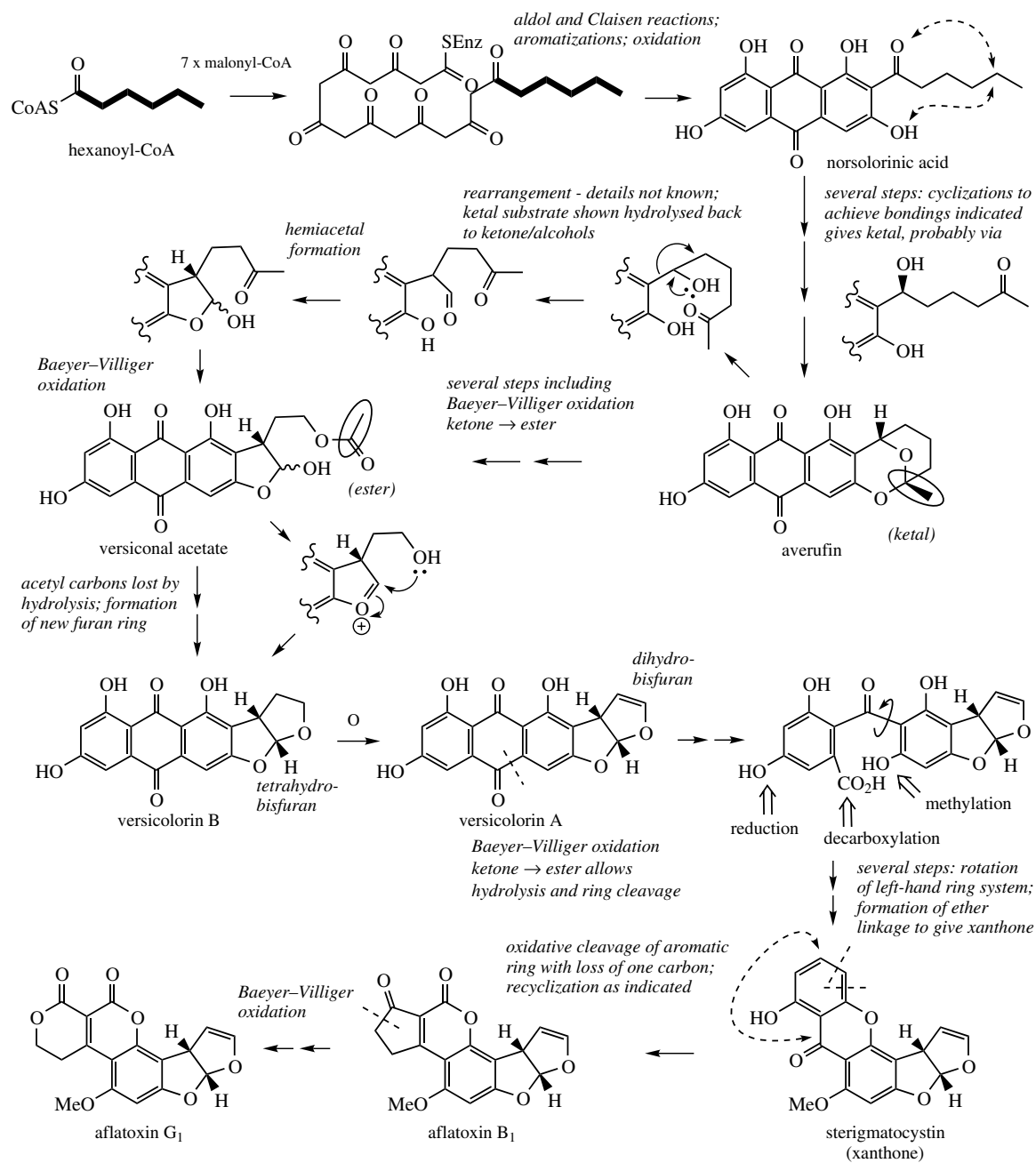


Figure 3.49

oxidative cleavage of the anthraquinone system involving a second Baeyer–Villiger oxidation, and recyclization through phenol groups to give a xanthone skeleton. Rotation of an intermediate leads to the angular product as opposed to a linear product. One phenol group is methylated,

and, quite unusually, another phenol group is lost (contrast loss of oxygen functions via reduction/dehydration prior to cyclization, see page 62). **Aflatoxin B₁** formation requires oxidative cleavage of an aromatic ring in sterigmatocystin, loss of one carbon and recyclization exploiting the

Aflatoxins

Aflatoxins are potent mycotoxins produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Four main naturally occurring aflatoxins, aflatoxins B₁, B₂, G₁, and G₂ (Figure 3.50), are recognized, but these can be metabolized by microorganisms and animals to other aflatoxin structures, which are also toxic. Aflatoxin B₁ is the most commonly encountered member of the group, and is also the most acutely toxic and carcinogenic example. Aflatoxin B₂ is a dihydro derivative of aflatoxin B₁, whilst aflatoxins G₁ and G₂ are an analogous pair with a six-membered lactone rather than a five-membered cyclopentenone ring. These toxins are most commonly associated with peanuts (groundnuts), maize, rice, pistachio nuts, and Brazil nuts, though other crops can be affected, and, although found world-wide, they are particularly prevalent in tropical and subtropical regions. Aflatoxin M₁ (Figure 3.50) is a hydroxy derivative of aflatoxin B₁ and equally toxic. It may occur in cow's milk as a result of mammalian metabolism of aflatoxin B₁ originally contaminating the animal's food. Because these compounds fluoresce strongly under UV light, they are relatively easily detected and monitored.

The aflatoxins primarily affect the liver, causing enlargement, fat deposition, and necrosis, at the same time causing cells of the bile duct to proliferate, with death resulting from irreversible loss of liver function. In the case of aflatoxin B₁, this appears to be initiated by cytochrome P-450-dependent metabolism in the body to the epoxide (Figure 3.50). The epoxide intercalates with DNA, and in so doing becomes orientated towards nucleophilic attack from guanine residues. This leads to inhibition of DNA replication and of RNA synthesis, and initiates mutagenic activity. Aflatoxins are also known to cause hepatic carcinomas, this varying with the species of animal. The above normal incidence of liver cancer in parts of Africa and Asia has been suggested to be linked to the increased amounts of aflatoxins found in foodstuffs, and a tolerance level of 30 ppb has been recommended. Acute hepatitis may result from food containing aflatoxin B₁ at levels of the order of 0.1 ppm, and levels of more than 1 ppm are frequently encountered.

The biosynthesis of aflatoxins proceeds through intermediates sterigmatocystin and versicolorin (see Figure 3.49). Toxins related to these structures but differing in aromatic substituents are also produced by various fungi. The sterigmatocystins are synthesized by species of *Aspergillus* and *Bipolaris*, and contain a reduced bifuran fused to a xanthone, whilst the versicolorins from *Aspergillus versicolor* contain the same type of reduced bisfuran system but fused to an anthraquinone. Like the aflatoxins, the sterigmatocystins are acutely toxic and carcinogenic. The versicolorins are less toxic though still carcinogenic.

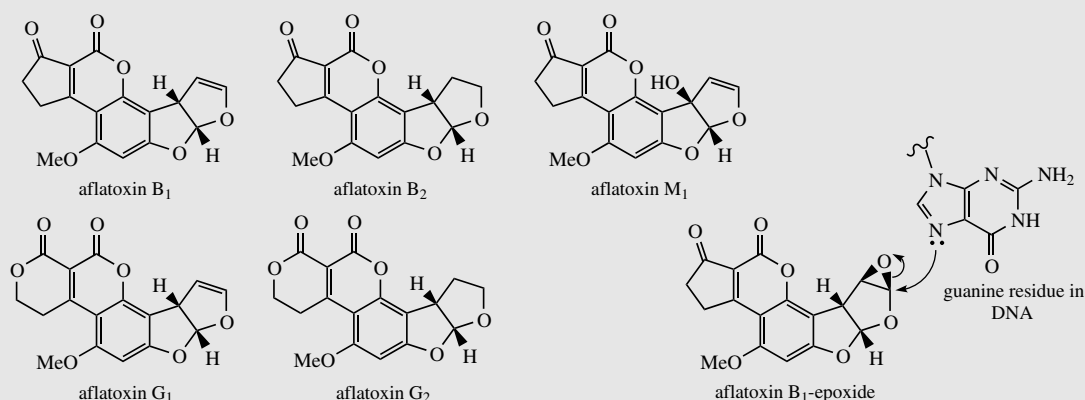


Figure 3.50

carbonyl functionality. **Aflatoxin G₁** is derived by further modification of aflatoxin B₁, cleaving the cyclopentenone ring and forming a lactone, perhaps via a further Baeyer–Villiger reaction.

Hexanoate is also likely to feature as a starter unit in the formation of the **cannabinoids**, a group of **terpenophenolics** found in Indian hemp (*Cannabis sativa*; Cannabaceae). This plant, and preparations from it, known under a variety of names including hashish, marihuana, pot, bang, charas, and dagga, have been used for centuries for the pleasurable sensations and mild euphoria experienced after its consumption, usually by smoking.

The principal psychoactive component is **tetrahydrocannabinol (THC)** (Figure 3.51), whilst structurally similar compounds such as **cannabinol (CBN)** and **cannabidiol (CBD)**, present in similar or larger amounts, are effectively inactive. In recent years, the beneficial effects of cannabis*, and especially THC, in alleviating nausea and vomiting in cancer patients undergoing chemotherapy, and in the treatment of glaucoma and multiple sclerosis, has led to a study of cannabinoid analogues for potentially useful medicinal activity. All the cannabinoid structures contain a monoterpene C₁₀ unit attached to a phenolic ring having a C₅ alkyl chain. The aromatic ring/C₅ chain

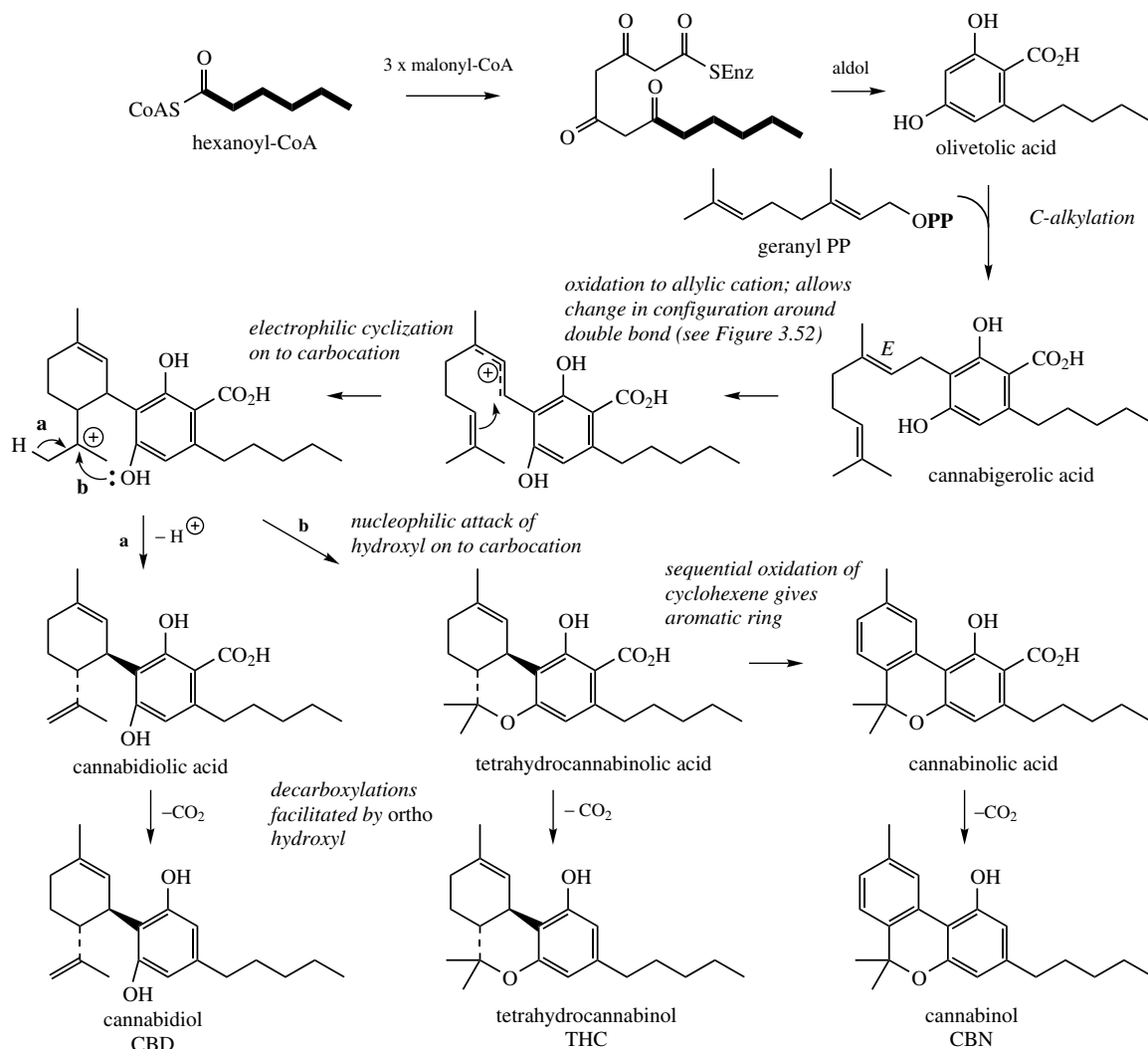


Figure 3.51

is likely to originate from hexanoate and malonate, cyclization to a polyketide giving **olivetolic acid**, from which **cannabigerolic acid** can be obtained by C-alkylation with the monoterpene unit geranyl diphosphate (Figure 3.51). Cyclization in the monoterpene unit necessitates a change in configuration of the double bond, and this may be rationalized as involving the allylic cation, which will then also allow electrophilic cyclization to proceed (for further detail see Figure 3.52,

and compare terpenoid cyclization mechanisms, page 173). **Cannabidiolic acid** is the result of proton loss, whilst **tetrahydrocannabinolic acid** is the product from heterocyclic ring formation. **CBD** and **THC** are then the respective decarboxylation products from these two compounds. The aromatic terpenoid derived ring in **cannabinolic acid** and **cannabinol** can arise via a dehydrogenation process (compare thymol, page 186).

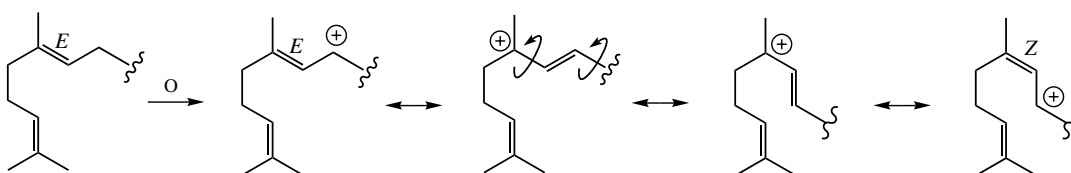


Figure 3.52

Cannabis

Indian hemp, *Cannabis sativa* (Cannabaceae) is an annual herb indigenous to Central and Western Asia, cultivated widely in India and many tropical and temperate regions for its fibre (hemp) and seed (for seed oil). The plant is also grown for its narcotic and mild intoxicant properties, and in most countries of the world its possession and consumption is illegal. Over many years, cannabis plants have been selected for either fibre production or drug use, the former resulting in tall plants with little pharmacological activity, whilst the latter tend to be short, bushy plants. Individual plants are almost always male or female, though the sex is not distinguishable until maturity and flowering. Seeds will produce plants of both sexes in roughly equal proportions. The active principles are secreted as a resin by glandular hairs, which are more numerous in the upper parts of female plants, and resin is produced from the time flowers first appear until the seeds reach maturity. However, all parts of the plant, both male and female, contain cannabinoids. In a typical plant, the concentration of cannabinoids increases in the following order: large leaves, small leaves, flowers, and bracts (which surround the ovaries), with stems containing very little. Material for drug use (ganja) is obtained by collecting the flowering tops (with little leaf) from female plants, though lower quality material (bhang) consisting of leaf from both female and male plants may be employed. By rubbing the flowering tops, the resin secreted by the glandular hairs can be released and subsequently scraped off to provide cannabis resin (charas) as an amorphous brown solid or semi-solid. A potent form of cannabis, called cannabis oil, is produced by alcoholic extraction of cannabis resin. A wide variety of names are used for cannabis products according to their nature and the geographical area. In addition to the Indian words above, the names hashish (Arabia), marihuana (Europe, USA), kief and dagga (Africa) are frequently used. The term 'assassin' is a corruption of 'hashishin', a group of 13th century murderous Persians who were said to have been rewarded for their activities with hashish. The names grass, dope, pot, hash, weed, and wacky backy are more likely to be in current usage.

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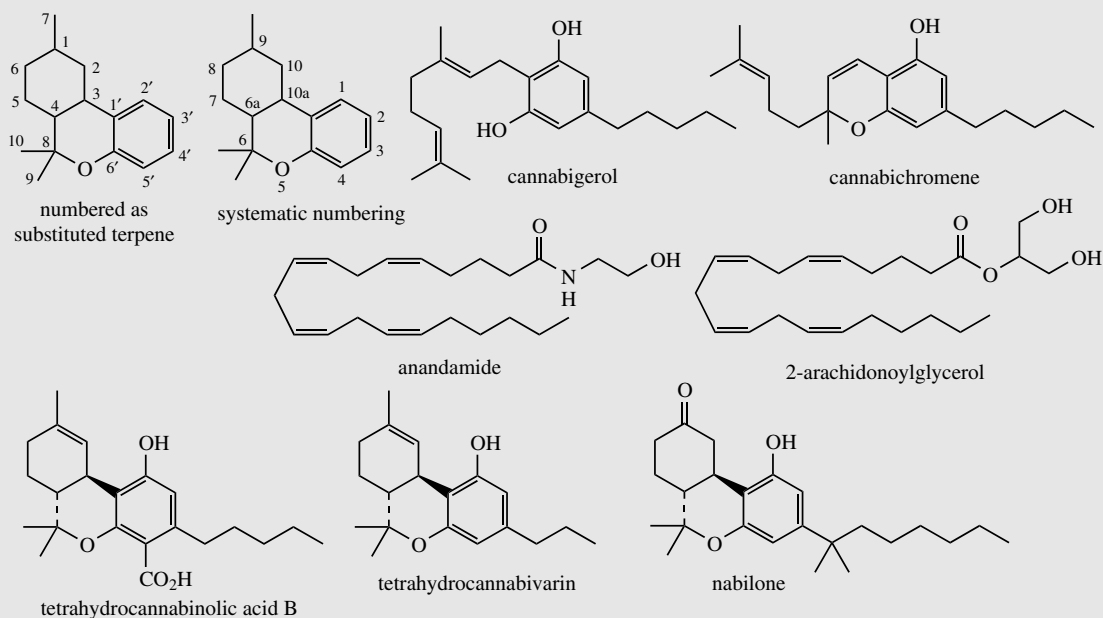


Figure 3.53

The quantity of resin produced by the flowering tops of high quality Indian cannabis is about 15–20%. The amount produced by various plants is dependent on several features, however, and this will markedly alter biological properties. Thus, in general, plants grown in a tropical climate produce more resin than those grown in a temperate climate. The tall fibre-producing plants are typically low resin producers, even in tropical zones. However, the most important factor is the genetic strain of the plant, and the resin produced may contain high levels of psychoactive compounds, or mainly inactive constituents. The quality of any cannabis drug is potentially highly variable.

The major constituents in cannabis are termed cannabinoids, a group of more than 60 structurally related terpenophenolics. The principal psychoactive agent is tetrahydrocannabinol (THC) (Figure 3.51). This is variously referred to as Δ^1 -THC or Δ^9 -THC according to whether the numbering is based on the terpene portion, or as a systematic dibenzopyran (Figure 3.53). Both systems are currently in use. Also found, often in rather similar amounts, are cannabinol (CBN) and cannabidiol (CBD) (Figure 3.51), which have negligible psychoactive properties. These compounds predominate in the inactive resins. Many other cannabinoid structures have been characterized, including cannabigerol and cannabichromene (Figure 3.53). A range of cannabinoid acids, e.g. cannabidiolic acid, tetrahydrocannabinolic acid, and tetrahydrocannabinolic acid B (Figure 3.53) are also present, as are some analogues of the other compounds mentioned, where a propyl side-chain replaces the pentyl group, e.g. tetrahydrocannabivarin (Figure 3.53). The latter compounds presumably arise from the use of butyrate rather than hexanoate as starter unit in the biosynthetic sequence.

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The THC content of high quality cannabis might be in the range 0.5–1% for large leaves, 1–3% for small leaves, 3–7% for flowering tops, 5–10% for bracts, 14–25% for resin, and up to 60% in cannabis oil. Higher amounts of THC are produced in selected strains known as skunk cannabis, so named because of their powerful smell; flowering tops from skunk varieties might contain 10–15% THC. The THC content in cannabis products tends to deteriorate on storage, an effect accelerated by heat and light. Cannabis leaf and resin stored under ordinary conditions rapidly lose their activity and can be essentially inactive after about 2 years. A major change which occurs is oxidation in the cyclohexene ring resulting in conversion of THC into CBN. THC is more potent when smoked than when taken orally, its volatility allowing rapid absorption and immediate effects, so smoking has become the normal means of using cannabis. Any cannabinoid acids will almost certainly be decarboxylated upon heating, and thus the smoking process will also effectively increase somewhat the levels of active cannabinoids available, e.g. THC acid → THC (Figure 3.51). The smoking of cannabis produces a mild euphoria similar to alcohol intoxication, inducing relaxation, contentment, and a sense of well-being, with some changes in perception of sound and colour. However, this is accompanied by a reduced ability to concentrate and do complicated tasks, and a loss of short-term memory. Users claim cannabis is much preferable to alcohol or tobacco, insisting it does not cause dependence, withdrawal symptoms, or lead to the use of other drugs, and they campaign vociferously for its legalization. However, psychological dependence does occur, and cannabis can lead to hallucinations, depression, anxiety, and panic, with the additional risk of bronchitis and lung cancer if the product is smoked.

Cannabis has been used medicinally, especially as a mild analgesic and tranquilizer, but more effective and reliable agents replaced it, and even controlled prescribing was discontinued. In recent times, cannabis has been shown to have valuable anti-emetic properties, which help to reduce the side-effects of nausea and vomiting caused by cancer chemotherapeutic agents. This activity stems from THC, and has resulted in some use of **THC (dronabinol)** and the prescribing of cannabis for a small number of patients. A synthetic THC analogue, **nabilone** (Figure 3.53), has been developed as an anti-emetic drug for reducing cytotoxic-induced vomiting. Some of the psychoactive properties of THC, e.g. euphoria, mild hallucinations, and visual disturbances, may be experienced as side-effects of nabilone treatment. Cannabis has also been shown to possess properties which may be of value in other medical conditions. There is now ample evidence that cannabis can give relief to patients suffering from chronic pain, multiple sclerosis, glaucoma, asthma, migraine, epilepsy, and other conditions. Many sufferers who cannot seem to benefit from any of the current range of drugs are obtaining relief from their symptoms by using cannabis, but are breaking the law to obtain this medication. Current thinking is that cannabis offers a number of beneficial pharmacological responses and that there should be legal prescribing of cannabinoids or derivatives. Clinical trials have already confirmed the value of cannabis and/or THC taken orally for the relief of chronic pain and the painful spasms characteristic of multiple sclerosis, and in reducing intraocular pressure in glaucoma sufferers. In general, cannabis is only able to alleviate the symptoms of these diseases, and does not provide a cure. The non-psychoactive CBD has been shown to have anti-inflammatory properties potentially useful in arthritis treatment.

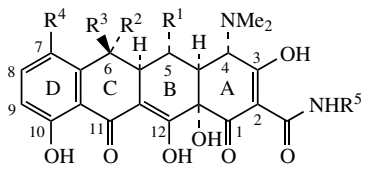
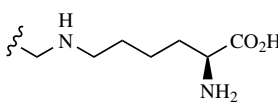
Recently, the ethanolamide of arachidonic acid (anandamide; ananda is the Sanskrit word for bliss) (Figure 3.53) has been isolated from animal brain tissue, and has been shown

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to mimic several of the pharmacological properties of THC. This appears to be a natural ligand which interacts with central receptors (CB1) to which cannabinoids also bind. Two other polyunsaturated fatty acid ethanolamides, namely dihomono- γ -linolenoyl- (20:3) and adrenoyl- (22:4) ethanolamides have also been isolated from mammalian brain, and shown to have THC-like properties. Another type of cannabinoid receptor (CB2), expressed mainly in the immune system, has been identified; its natural ligand is 2-arachidonoylglycerol (Figure 3.53). Since this compound also interacts with the anandamide receptor, and levels of 2-arachidonoylglycerol in the brain are some 800 times higher than those of anandamide, it is now thought to be the physiological ligand for both receptors, rather than anandamide. The identification of these endogenous materials may open up other ways of exploiting some of the desirable pharmacological features of cannabis.

Table 3.3 Tetracyclines

						
	R ¹	R ²	R ³	R ⁴	R ⁵	
tetracycline	H	Me	OH	H	H	natural
chlortetracycline	H	Me	OH	Cl	H	
oxytetracycline	OH	Me	OH	H	H	
demeclocycline	H	H	OH	H	H	
methacycline	OH	=CH ₂	H	H	H	semi-synthetic
doxycycline	OH	Me	H	H	H	
minocycline	H	H	H	NMe ₂	H	
lymecycline	H	Me	OH	H		

The **tetracyclines*** (Table 3.3) are a group of broad spectrum, orally active antibiotics produced by species of *Streptomyces*, and several natural and semi-synthetic members are used clinically. They contain a linear tetracyclic skeleton of polyketide origin in which the starter group is **malonamyl-CoA** (Figure 3.54), i.e. the coenzyme A ester of malonate semi-amide. Thus, in contrast to most acetate-derived compounds, malonate supplies all carbon atoms of the tetracycline skeleton, the starter group as well as the chain extenders. The main features of the pathway (Figure 3.54) were deduced from extensive studies of mutant strains of *Streptomyces aureofaciens* with genetic blocks

causing accumulation of mutant metabolites or production of abnormal tetracyclines. This organism typically produces **chlortetracycline**, whilst the parent compound **tetracycline** (Table 3.3) is in fact an aberrant product synthesized in mutants blocked in the chlorination step. The use of mutants with genetic blocks has also enabled the shikimate pathway (Chapter 4) to be delineated. In that case, since a primary metabolic pathway was affected, mutants tended to accumulate intermediates and could not grow unless later components of the pathway were supplied. With the tetracyclines, a secondary metabolic pathway is involved, and the relatively broad specificity of some of the

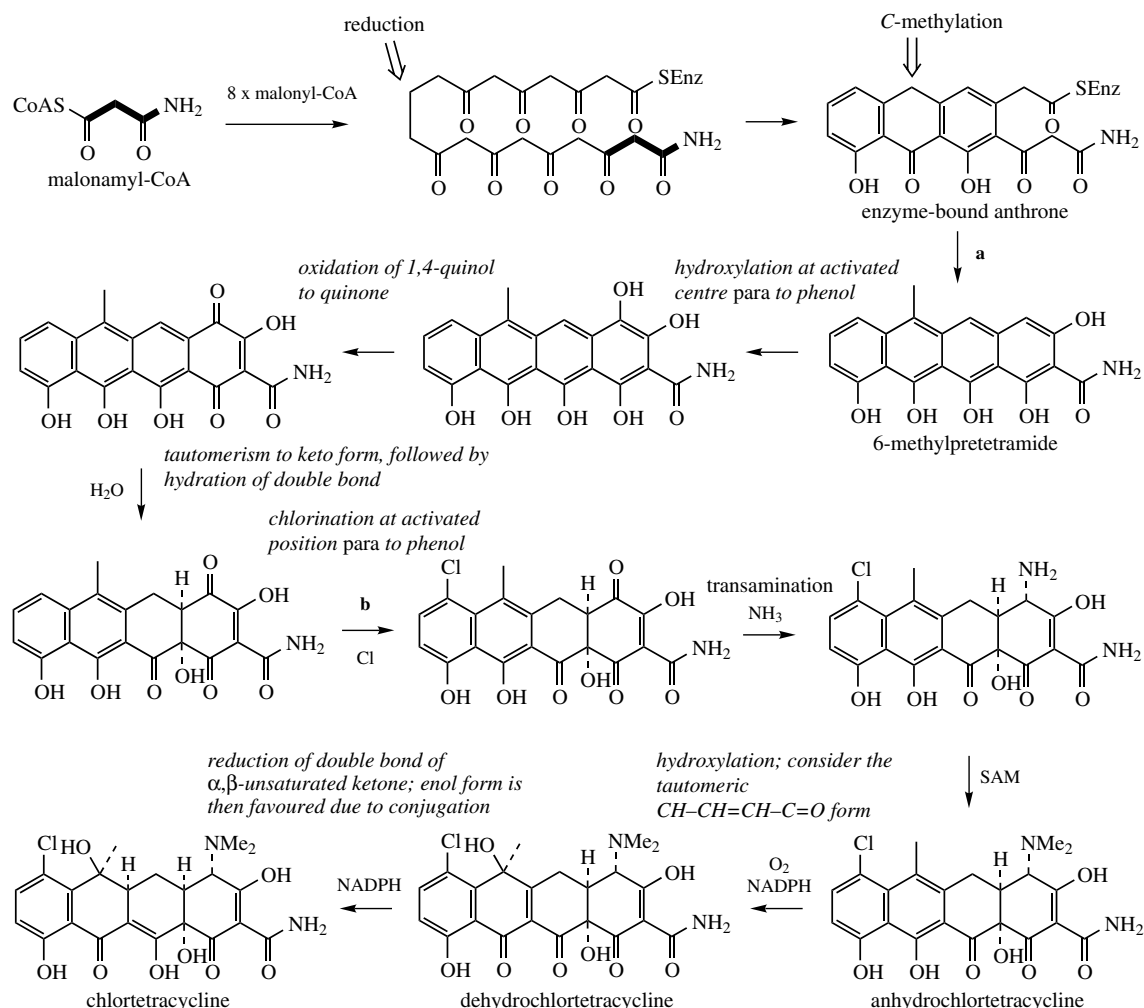


Figure 3.54

Tetracyclines

The tetracyclines (Table 3.3) are a group of broad spectrum, orally active antibiotics produced by cultures of *Streptomyces* species. **Chlortetracycline** isolated from *Streptomyces aureofaciens* was the first of the group to be discovered, closely followed by **oxytetracycline** from cultures of *S. rimosus*. **Tetracycline** was found as a minor antibiotic in *S. aureofaciens*, but may be produced in quantity by utilizing a mutant strain blocked in the chlorination step *b* (Figure 3.54). Similarly, the early C-6 methylation step (included in *a*) can also be blocked, and such mutants accumulate 6-demethyltetracyclines, e.g. **demeclocycline** (**demethylchlorotetracycline**). These reactions can also be inhibited in the normal strain of *S. aureofaciens* by supplying cultures with either aminopterin (which inhibits C-6 methylation) or mercaptothiazole (which inhibits C-7 chlorination). Oxytetracycline from *S. rimosus* lacks

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the chlorine substituent, but has an additional 5 α -hydroxyl group, probably introduced at a late stage. Only minor alterations can be made to the basic tetracycline structure to modify the antibiotic activity, and these are at positions 5, 6, and 7. Other functionalities in the molecule are all essential to retain activity. Semi-synthetic tetracyclines used clinically include **methacycline**, obtained by a dehydration reaction from oxytetracycline, and **doxycycline**, via reduction of the 6-methylene in methacycline. **Minocycline** contains a 7-dimethylamino group and is produced by a sequence involving aromatic nitration. **Lymecycline** is an example of an antibiotic developed by chemical modification of the primary amide function at C-2.

Having both amino and phenolic functions, tetracyclines are amphoteric compounds, and are more stable in acid than under alkaline conditions. They are thus suitable for oral administration, and are absorbed satisfactorily. However, because of the sequence of phenol and carbonyl substituents in the structures, they act as chelators and complex with metal ions, especially calcium, aluminium, iron, and magnesium. Accordingly, they should not be administered with foods such as milk and dairy products (which have a high calcium content), aluminium- and magnesium-based antacid preparations, iron supplements, etc, otherwise erratic and unsatisfactory absorption will occur. A useful feature of doxycycline and minocycline is that their absorptions are much less affected by metal ions. Chelation of tetracyclines with calcium also precludes their use in children developing their adult teeth, and in pregnant women, since the tetracyclines become deposited in the growing teeth and bone. In children, this would cause unsightly and permanent staining of teeth with the chelated yellow tetracycline.

Although the tetracycline antibiotics have a broad spectrum of activity spanning Gram-negative and Gram-positive bacteria, their value has decreased as bacterial resistance has developed in pathogens such as *Pneumococcus*, *Staphylococcus*, *Streptococcus*, and *E. coli*. These organisms appear to have evolved mechanisms of resistance involving decreased cell permeability; a membrane-embedded transport protein exports the tetracycline out of the cell before it can exert its effect. Nevertheless, tetracyclines are the antibiotics of choice for infections caused by *Chlamydia*, *Mycoplasma*, *Brucella*, and *Rickettsia*, and are valuable in chronic bronchitis due to activity against *Haemophilus influenzae*. They are also used systemically to treat severe cases of acne, helping to reduce the frequency of lesions by their effect on skin flora. There is little significant difference in the antimicrobial properties of the various agents, except for **minocycline**, which has a broader spectrum of activity, and being active against *Neisseria meningitidis* is useful for prophylaxis of meningitis. The individual tetracyclines do have varying bioavailabilities, however, which may influence the choice of agent. **Tetracycline** and **oxytetracycline** are probably the most commonly prescribed agents. Tetracyclines are formulated for oral application or injection, as ear and eye drops, and for topical use on the skin. **Doxycycline** also finds use as a prophylactic against malaria in areas where there is widespread resistance to chloroquine and mefloquine (see page 363).

Their antimicrobial activity arises by inhibition of protein synthesis. This is achieved by interfering with the binding of aminoacyl-tRNA to acceptor sites on the ribosome by disrupting the codon-anticodon interaction (see page 407). Evidence points to a single strong binding site on the smaller 30S subunit of the ribosome. Although tetracyclines can also bind to mammalian ribosomes, there appears to be preferential penetration into bacterial cells, and there are few major side-effects from using these antibiotics.

A series of tetracycline derivatives has recently been isolated from species of *Dactylosporangium*. These compounds, the dactylocyclines (Figure 3.55), are glycosides and have the opposite configuration at C-6 to the natural tetracyclines. Importantly, these compounds are active towards tetracycline-resistant bacteria.

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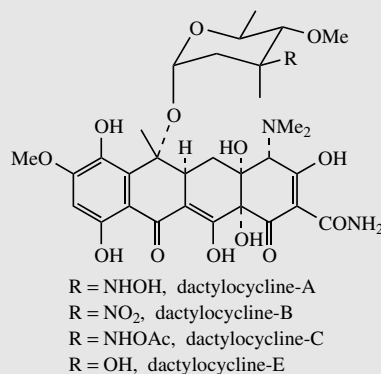


Figure 3.55

enzymes concerned allows many of the later steps to proceed even if one step, e.g. the chlorination, is not achievable. This has also proved valuable for production of some of the clinical tetracycline antibiotics.

One of the early intermediates in the pathway to chlortetracycline is 6-methylpretetramide (Figure 3.54). This arises from the poly- β -keto ester via an enzyme-bound anthrone (compare Figure 3.30). Reduction of one carbonyl will occur during chain extension, whilst the methylation must be a later modification. Hydroxylation in ring A followed by oxidation gives a quinone, the substrate for hydration at the A/B ring fusion. The product now features the keto tautomer in ring B, since its aromaticity has been destroyed. Chlorination of ring D at the nucleophilic site *para* to the phenol follows, and an amine group is then introduced stereospecifically into ring A by a transamination reaction. This amino function is then di-*N*-methylated using SAM as the methylating agent yielding **anhydrochlortetracycline**. In the last two steps, C-6 is hydroxylated via an O₂-, NADPH-, and flavin-dependent oxygenase giving the enone **dehydrochlortetracycline**, and NADPH reduction of the C-5a/11a double bond generates **chlortetracycline**.

A number of **anthracycline antibiotics***, e.g. **doxorubicin** (Figure 3.56) from *Streptomyces peuceticus* and **daunorubicin** from *S. coeruleorubicus*, have structurally similar tetracyclic skeletons and would appear to be related to the tetracyclines. There are similarities in that the molecules are essentially acetate derived, but for

the anthracyclines the starter group is **propionate** rather than malonamide, and labelling studies have demonstrated a rather different folding of the poly- β -keto chain (Figure 3.56). As a result, the end-of-chain carboxyl is ultimately lost through decarboxylation. This carboxyl is actually retained for a considerable portion of the pathway, and is even protected against decarboxylation by methylation to the ester, until no longer required. Most of the modifications which occur during the biosynthetic pathway are easily predictable. Thus, the anthraquinone portion is likely to be formed first, then the fourth ring can be elaborated by a aldol reaction (Figure 3.56). A feature of note in molecules such as doxorubicin and daunorubicin is the amino sugar L-daunosamine which originates from TDPglucose (thymidine diphosphoglucose; compare UDPglucose, page 29) and is introduced in the latter stages of the sequence. Hydroxylation of daunorubicin to doxorubicin is the very last step. Doxorubicin and daunorubicin are used as antitumour drugs rather than antimicrobial agents. They act primarily at the DNA level and so also have cytotoxic properties. Doxorubicin in particular is a highly successful and widely used antitumour agent, employed in the treatment of leukaemias, lymphomas, and a variety of solid tumours.

MACROLIDES AND POLYETHERS

Extender Groups other than Malonate

The use of propionate as a starter group as in the formation of the anthracyclines is perhaps

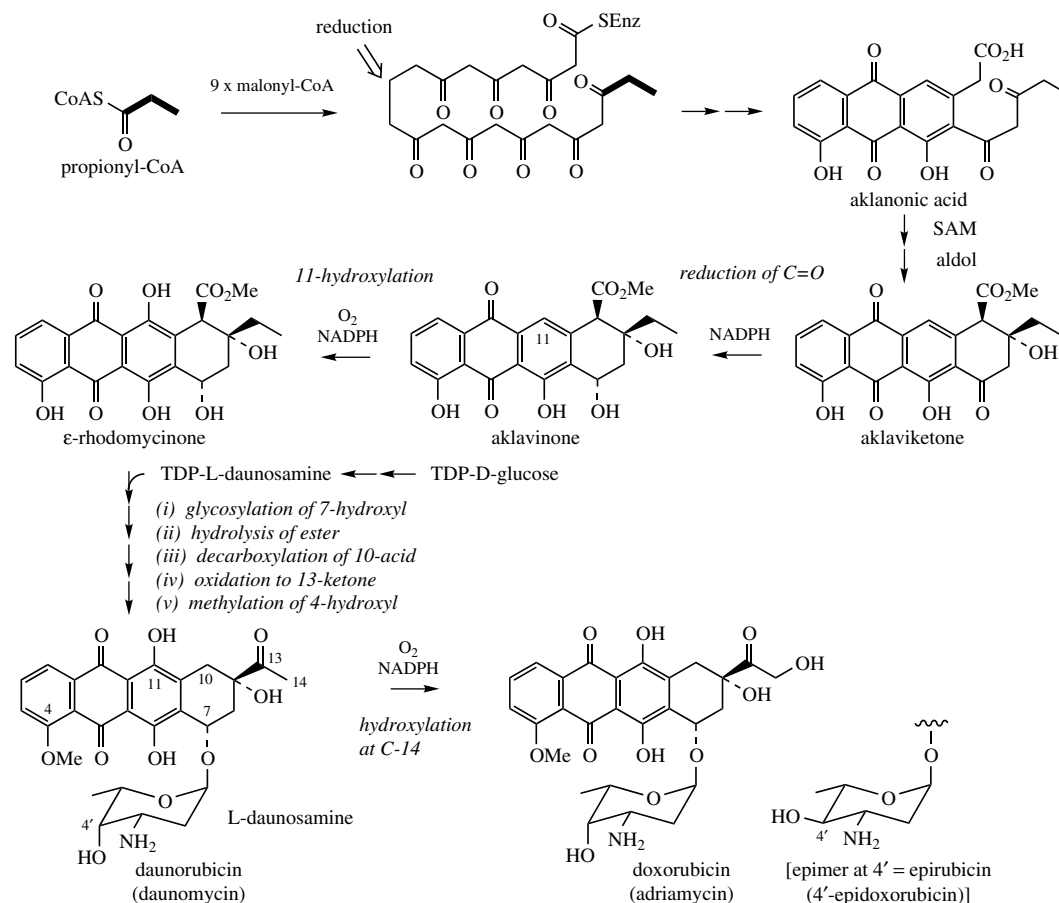


Figure 3.56

Anthracycline Antibiotics

Doxorubicin (adriamycin) (Figure 3.56) is produced by cultures of *Streptomyces peucetius* var *caesius* and is one of the most successful and widely used antitumour drugs. The organism is a variant of *S. peucetius*, a producer of daunorubicin (see below), in which mutagen treatment resulted in expression of a latent hydroxylase enzyme and thus synthesis of doxorubicin by 14-hydroxylation of daunorubicin. Doxorubicin has one of the largest spectra of antitumour activity shown by antitumour drugs and is used to treat acute leukaemias, lymphomas, and a variety of solid tumours. It is administered by intravenous injection and largely excreted in the bile. It inhibits the synthesis of RNA copies of DNA by intercalation of the planar molecule between base pairs on the DNA helix. The sugar unit provides further binding strength and also plays a major role in sequence-recognition for the binding. Doxorubicin also exerts some of its cytotoxic effects by inhibition of the enzyme topoisomerase II, which is responsible for cleaving and resealing of double-stranded DNA during replication (see page 137). Common toxic effects include nausea and vomiting, bone marrow suppression, hair loss, and local tissue necrosis, with cardiotoxicity at higher dosage.

Daunorubicin (Figure 3.56) is produced by *Streptomyces coeruleorubidus* and *S. peucetius*,

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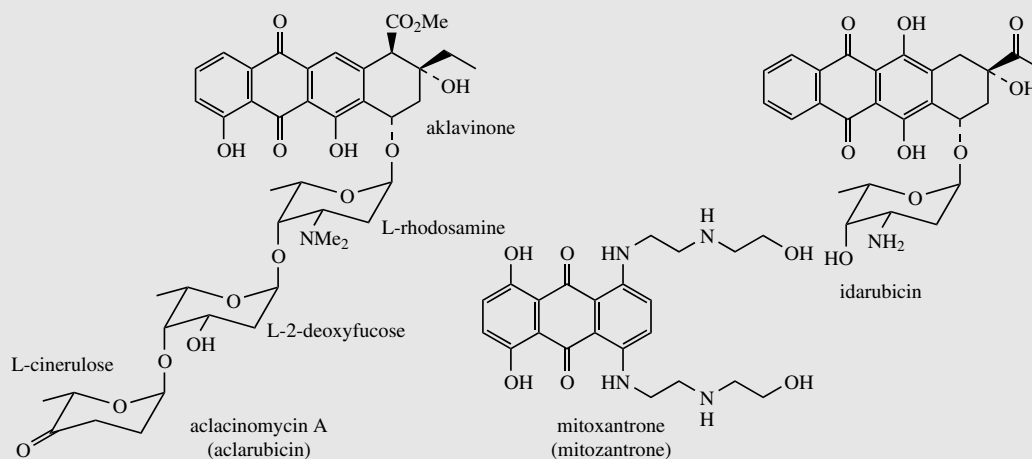


Figure 3.57

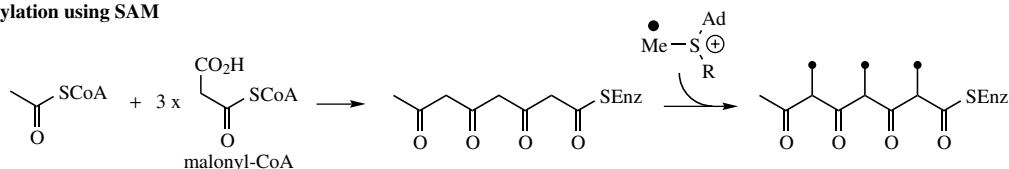
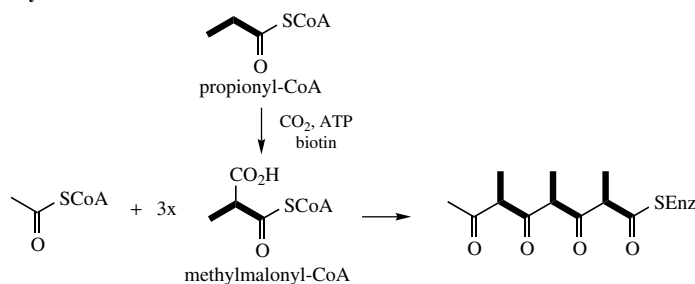
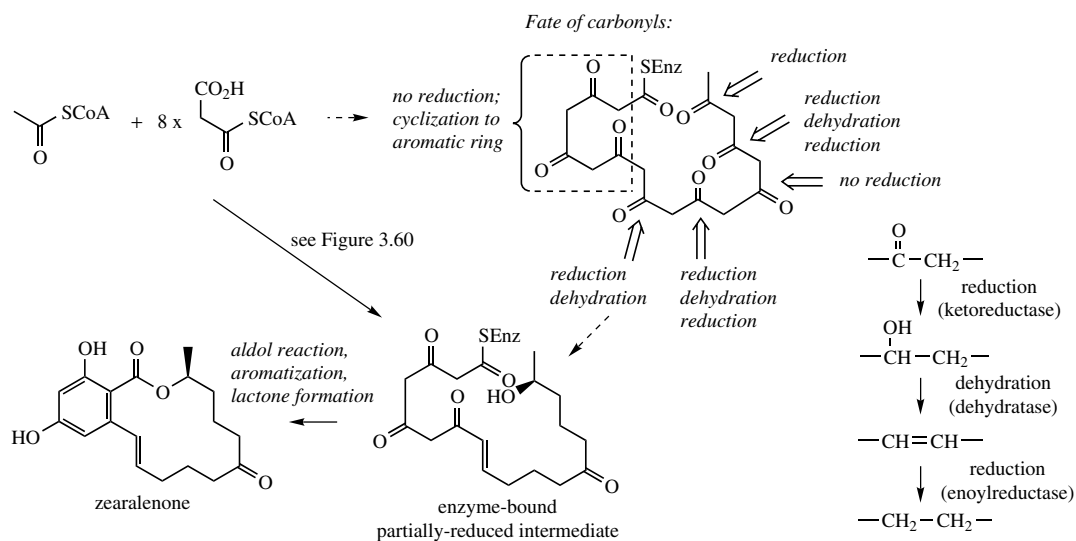
and, though similar to doxorubicin in its biological and chemical properties, it is no longer used therapeutically to any extent. It has a much less favourable therapeutic index than doxorubicin, and the markedly different effectiveness as an antitumour drug is not fully understood, though differences in metabolic degradation may be responsible. **Epirubicin** (Figure 3.56), the 4'-epimer of doxorubicin, is particularly effective in the treatment of breast cancer, producing lower side-effects than doxorubicin. The antileukaemics **aclarubicin** from *Streptomyces galilaeus*, a complex glycoside of aklavinone (Figure 3.56), and the semi-synthetic **idarubicin** are shown in Figure 3.57. These compounds are structurally related to doxorubicin but can show increased activity with less cardiotoxicity. The principal disadvantage of all of these agents is their severe cardiotoxicity which arises through inhibition of cardiac Na^+, K^+ -ATPase.

Mitoxantrone (mitozantrone) (Figure 3.57) is a synthetic analogue of the anthracyclines in which the non-aromatic ring and the aminosugar have both been replaced with aminoalkyl side-chains. This agent has reduced toxicity compared with doxorubicin, and is effective in the treatment of solid tumours and leukaemias.

less common than incorporating it as a chain extender via methylmalonyl-CoA. We have already encountered this process in the formation of some branched-chain fatty acids with methyl substituents on the basic chain (see page 49). Of course, methyl groups can also be added to a fatty acid chain via SAM (see page 49), and there are also many examples for the methylation of poly- β -keto chains, several of which have already been discussed. Accordingly, methylation using SAM, and incorporation of propionate via methylmalonyl-CoA, provide two different ways of synthesizing a methylated polyketide (Figure 3.58). The former process is the more common in fungi, whilst Actinomycetes (e.g. *Streptomyces*) tend to

employ propionate by the latter route. The incorporation of propionate by methylmalonate extender units can frequently be interrupted and normal malonate extenders are added, thus giving an irregular sequence of methyl side-chains.

The **macrolide antibiotics*** provide us with excellent examples of natural products conforming to the acetate pathway, but composed principally of propionate units, or mixtures of propionate and acetate units. The macrolides are a large family of compounds, many with antibiotic activity, characterized by a macrocyclic lactone ring, typically 12, 14, or 16 membered, reflecting the number of units utilized. **Zearalenone** (Figure 3.59), a toxin produced by the

Methylation using SAM**Incorporation of propionate via methylmalonyl-CoA****Figure 3.58****Figure 3.59**

fungus *Gibberella zeae* and several *Fusarium* species, has a relatively simple structure which is derived entirely from acetate-malonate units. It could be envisaged as a cyclization product from a poly- β -keto ester, requiring a variety of reduction processes and formation of an aromatic ring by aldol condensation near the carboxyl terminus (Figure 3.59). However, the poly- β -keto ester shown in Figure 3.59 would not be produced, since its reactivity might tend to favour formation of a polycyclic aromatic system (compare anthraquinones, page 63, and

tetracyclines, page 89). Instead, appropriate reductions, dehydrations, etc., involving the β -carbonyl group are achieved during the chain extension process as in the fatty acid pathway (see page 36), and *before* further malonyl-CoA extender units are added (Figure 3.60). In contrast to fatty acid biosynthesis, where there is total reduction of each carbonyl group before further chain extension, macrolide biosynthesis frequently involves partial reduction, with the enzymic machinery being accurately controlled to leave the units at the right oxidation level before further chain extension

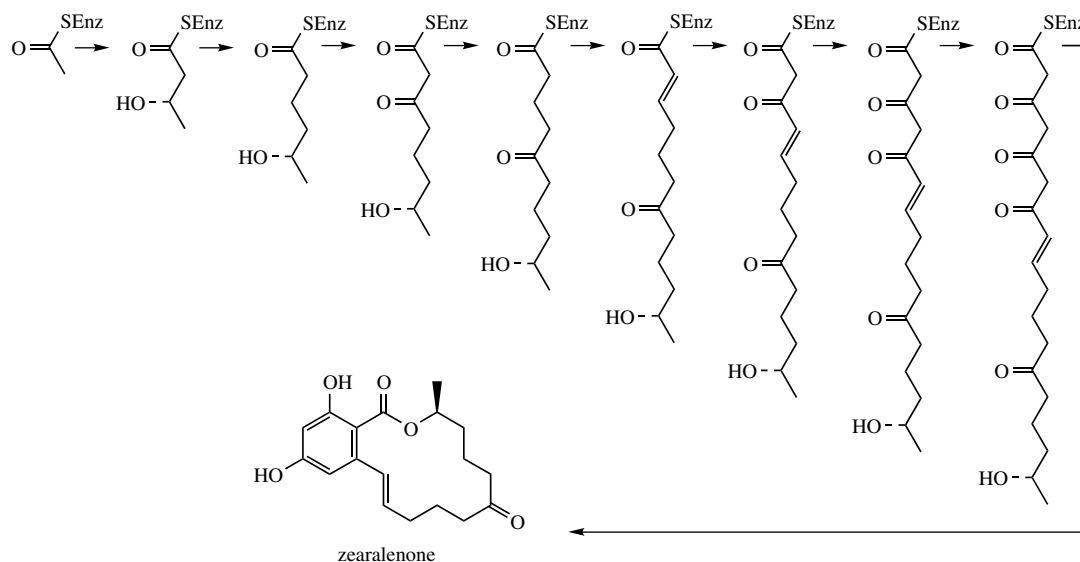


Figure 3.60

occurs. This then provides an enzyme-bound intermediate, which leads on to the final product (Figure 3.59). As a result, zearalenone is a remarkable example of an acetate-derived metabolite containing all types of oxidation level seen during the fatty acid extension cycle, i.e. carbonyl, secondary alcohol (eventually forming part of the lactone), alkene, and methylene, as well as having a portion which has cyclized to an aromatic ring because no reduction processes occurred in that fragment of the chain. There is now extensive genetic evidence from a variety of **polyketide synthase** systems to show that macrolide assembly is accomplished on a biological production line of multifunctional proteins organised as discrete modules, in which the developing polyketide chain attached to an acyl carrier protein is modified according to the appropriate enzyme activities encoded genetically, and is then passed on to another ACP prior to the next condensation and modification (see page 115 for more details).

Erythromycin A (Figure 3.61) from *Saccharopolyspora erythraea* is a valuable antibacterial drug and contains a 14-membered macrocycle composed entirely of propionate units, both as starter and extension units, the latter via methylmalonyl-CoA. In common with many antibacterial macrolides, sugar units, including amino sugars, are attached through glycoside linkages. These unusual 6-deoxy sugars are frequently

restricted to this group of natural products. In erythromycin A, the sugars are L-cladinose and D-desosamine. Chain extension and appropriate reduction processes lead to an enzyme-bound polyketide in which one carbonyl group has suffered total reduction, four have been reduced to alcohols, whilst one carbonyl is not reduced, and remains throughout the sequence. These processes ultimately lead to release of the modified polyketide as the macrolide ester **deoxyerythronolide**, a demonstrated intermediate in the pathway to erythromycins (Figure 3.61; see also page 115). The stereochemistry in the chain is controlled by the condensation and reduction steps during chain extension, but a reassuring feature is that there appears to be a considerable degree of stereochemical uniformity throughout the known macrolide antibiotics. In the later stages of the biosynthesis of erythromycin, hydroxylations at carbons 6 and 12, and addition of sugar units, are achieved.

A combination of propionate and acetate units is used to produce the 14-membered macrocyclic ring of **oleandomycin** (Figure 3.62) from *Streptomyces antibioticus*, but otherwise many of the structural features and the stereochemistry of oleandomycin resemble those of erythromycin A. One acetate provides the starter unit, whilst seven propionates, via methylmalonyl-CoA, supply the extension units (Figure 3.62). One methyl group derived

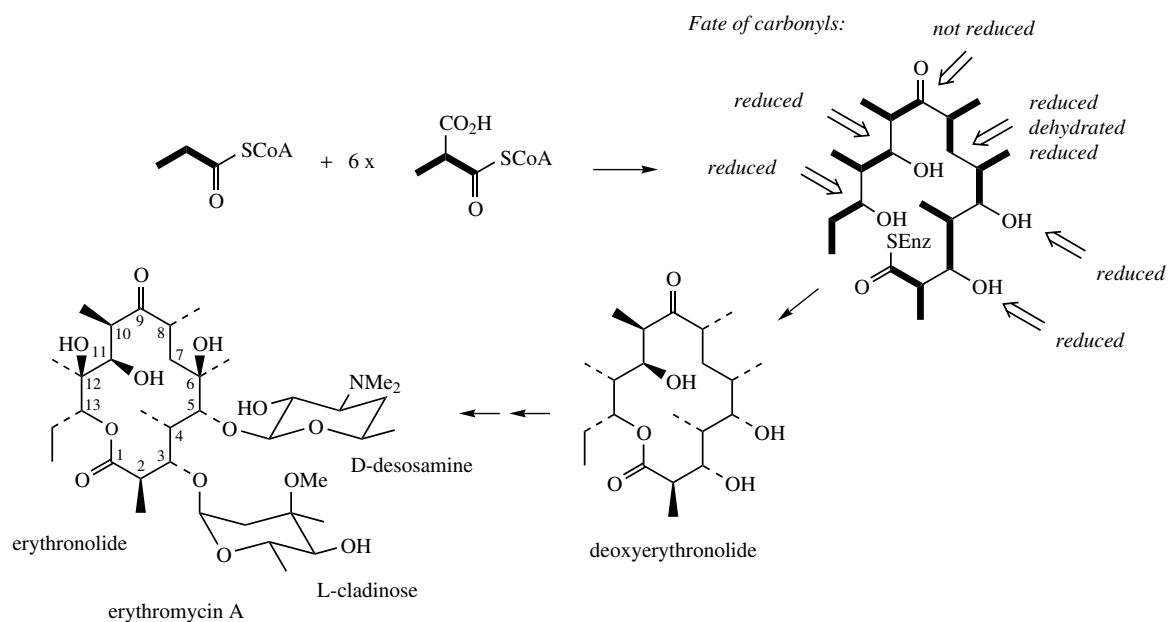


Figure 3.61

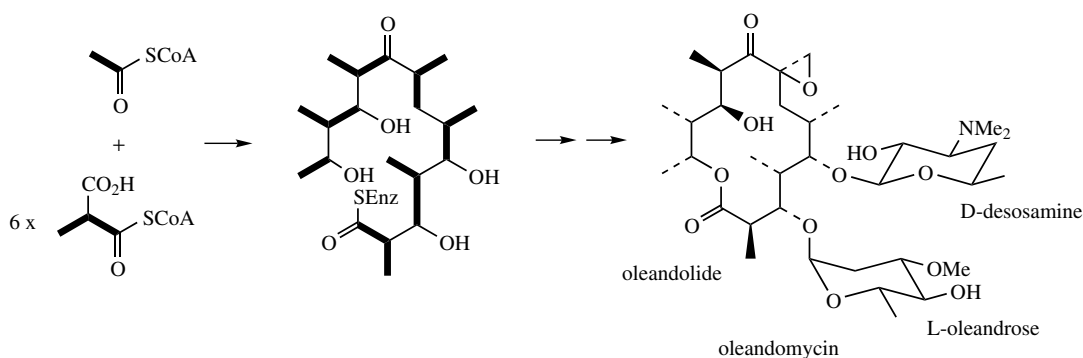


Figure 3.62

from propionate has been modified to give an epoxide function. The sugar units in oleandomycin are L-oleandrose and D-desosamine. **Spiramycin I** (Figure 3.63) from *Streptomyces ambofaciens* has a 16-membered lactone ring, and is built up from a combination of six acetate units (one as starter), one propionate extender, together with a further variant, butyrate as chain extender. Butyrate will be incorporated via ethylmalonyl-CoA and yield an extension unit having an ethyl side-chain. This is outlined in Figure 3.63. In due course, this ethyl group is oxidized generating an aldehyde. Spiramycin I also contains a conjugated diene,

the result of carbonyl reductions being followed by dehydration during chain assembly. **Tylosin** (Figure 3.64) from *Streptomyces fradiae* has many structural resemblances to the spiramycins, but can be analysed as a propionate starter with chain extension from two malonyl-CoA, four methylmalonyl-CoA, and one ethylmalonyl-CoA.

The **avermectins*** (Figure 3.67) have no antibacterial activity, but possess anthelmintic, insecticidal, and acaricidal properties, and these are exploited in human and veterinary medicine. The avermectins are also 16-membered macrolides, but their structures are made up from a much longer

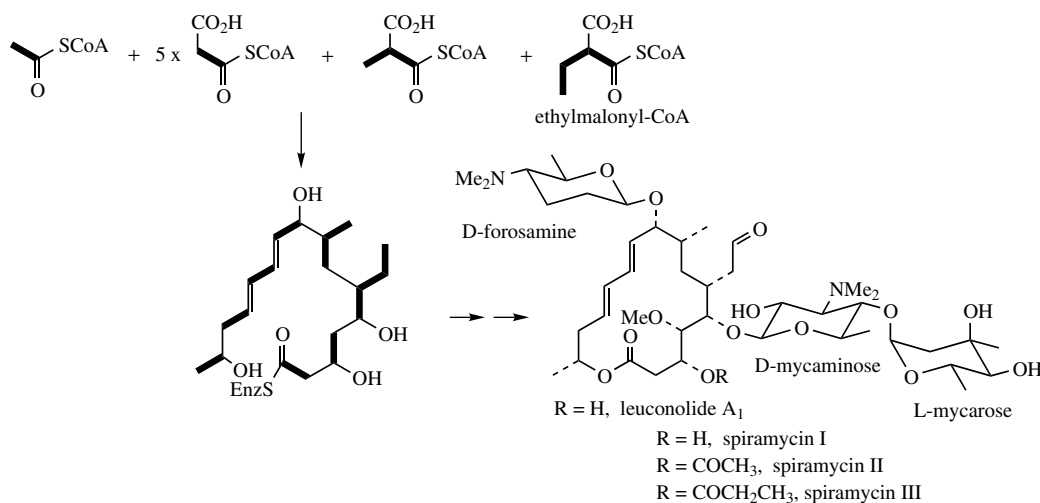


Figure 3.63

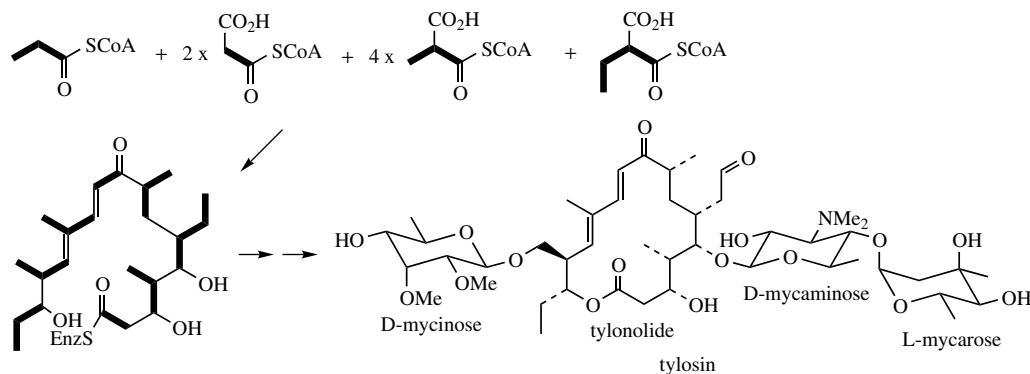


Figure 3.64

Macrolide Antibiotics

The macrolide antibiotics are macrocyclic lactones with a ring size typically 12–16 atoms, and with extensive branching through methyl substituents. Two or more sugar units are attached through glycoside linkages, and these sugars tend to be unusual 6-deoxy structures often restricted to this class of compounds. Examples include *L*-cladinose, *L*-mycarose, *D*-mycinose, and *L*-oleandrose. At least one sugar is an amino sugar, e.g. *D*-desosamine, *D*-forosamine, and *D*-mycaminose. These antibiotics have a narrow spectrum of antibacterial activity, principally against Gram-positive microorganisms. Their antibacterial spectrum resembles, but is not identical to, that of the penicillins, so they provide a valuable alternative for patients allergic to the penicillins. Erythromycin is the principal macrolide antibacterial currently used in medicine.

The erythromycins (Figure 3.65) are macrolide antibiotics produced by cultures of *Saccharopolyspora erythraea* (formerly *Streptomyces erythreus*). The commercial product

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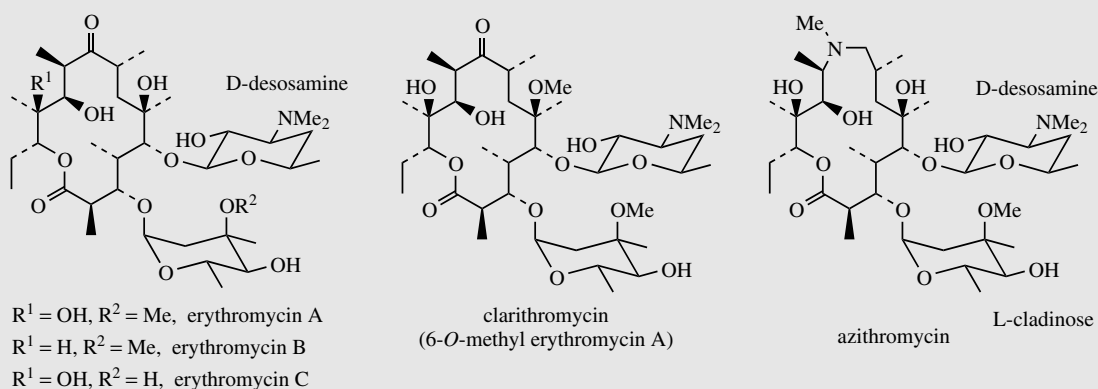


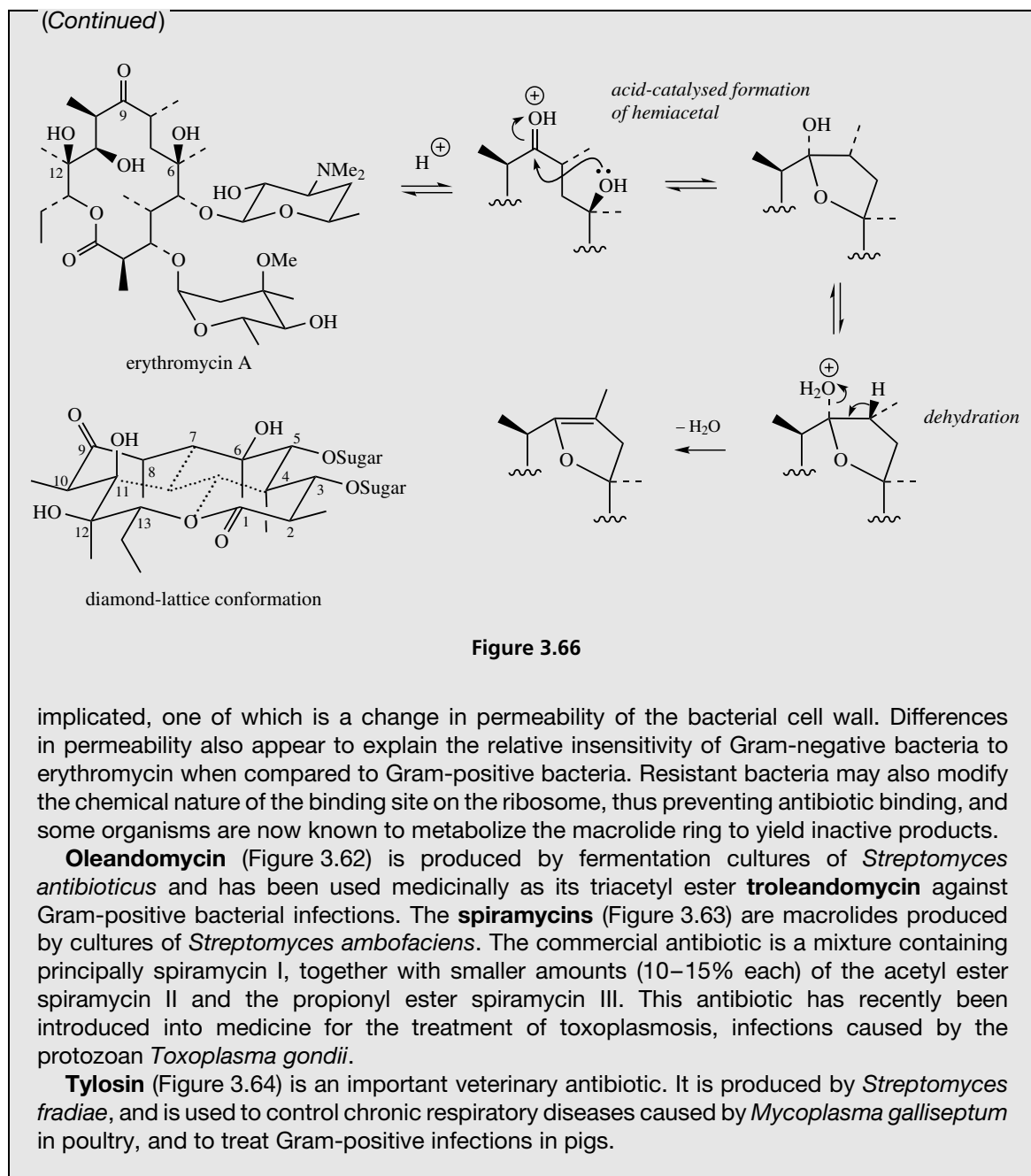
Figure 3.65

erythromycin is a mixture containing principally erythromycin A, plus small amounts of erythromycins B and C (Figure 3.65). Erythromycin activity is predominantly against Gram-positive bacteria, and the antibiotic is prescribed for penicillin-allergic patients. It is also used against penicillin-resistant *Staphylococcus* strains, in the treatment of respiratory tract infections, and systemically for skin conditions such as acne. It is the antibiotic of choice for infections of *Legionella pneumophila*, the cause of legionnaire's disease. Erythromycin exerts its antibacterial action by inhibiting protein biosynthesis in sensitive organisms. It binds reversibly to the larger 50S subunit of bacterial ribosomes and blocks the translocation step in which the growing peptidyl-tRNA moves from the aminoacyl acceptor site to the peptidyl donor site on the ribosome (see page 408). The antibiotic is a relatively safe drug with few serious side-effects. Nausea and vomiting may occur, and if high doses are prescribed, a temporary loss of hearing might be experienced. Hepatotoxicity may also occur at high dosage.

Erythromycin is unstable under acidic conditions, undergoing degradation to inactive compounds by a process initiated by the 6-hydroxyl attacking the 9-carbonyl to form a hemiketal. Dehydration then follows (Figure 3.66). The 14-membered ring in erythromycin A adopts a modified version of the diamond lattice chairlike conformation shown in Figure 3.66. Studies have indicated that carbon 6 is displaced from this conformation to reduce the 1,3-diaxial interactions at C-4 and C-6, and the two relatively large sugar units attached to the hydroxyls at C-3 and C-5 also distort the ring system further. The distortion of the chairlike conformation brings the 6-hydroxyl sufficiently close to react with the 9-carbonyl. A similar reaction may occur between the C-12 hydroxyl and the 9-carbonyl. Thus, to protect oral preparations of erythromycin against gastric acid, they are formulated as enteric-coated tablets, or as insoluble esters (e.g. ethyl succinate esters), which are then hydrolysed in the intestine. Esterification typically involves the hydroxyl of the amino sugar desosamine. To reduce this acid instability, semi-synthetic analogues of erythromycin have also been developed. **Clarithromycin** (Figure 3.65) is a 6-O-methyl derivative of erythromycin A; this modification blocks hemiketal formation as in Figure 3.66. **Azithromycin** (Figure 3.65) is a ring-expanded aza-macrolide in which the carbonyl function has been reduced. In both analogues, the changes enhance activity compared with that of erythromycin.

Bacterial resistance to erythromycin has become significant and has limited its therapeutic use against many strains of *Staphylococcus*. Several mechanisms of resistance have been

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polyketide chain, which is also used to form oxygen heterocycles fused to the macrolide. **Avermectin B_{1a}** exemplifies a typical structure and the basic carbon skeleton required to produce this can be postulated as in Figure 3.67. The starter unit in this case would be 2-methylbutyryl-CoA, which is derived from the amino acid L-isoleucine (compare necic acids, page 305, and tiglic acid, page 197).

Both malonyl-CoA and methylmalonyl-CoA are then utilized as extender units. The heterocyclic rings are easily accounted for: the spiro system is merely a ketal, though the tetrahydrofuran ring requires further hydroxylations of the basic skeleton for its construction. Avermectins are usually isolated as a mixture in which the main *a* component has a 2-methylpropyl group

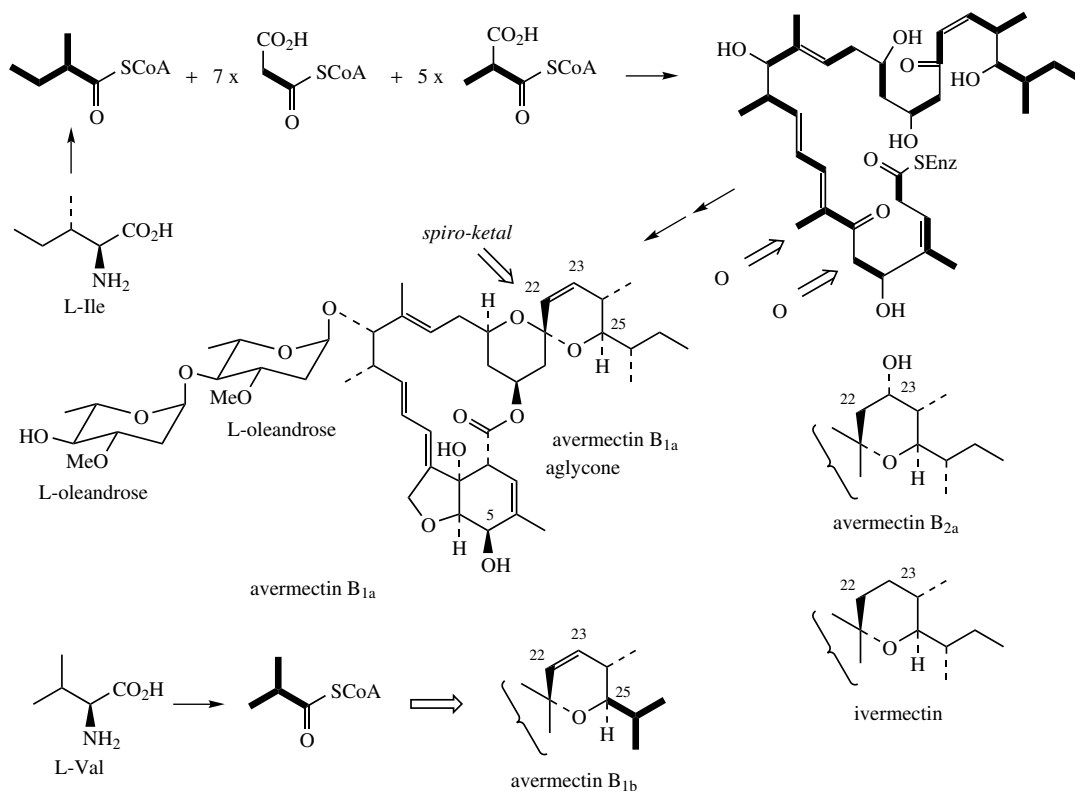


Figure 3.67

Avermectins

The avermectins (Figure 3.67) are a group of macrolides with strong anthelmintic, insecticidal, and acaricidal properties, but with low toxicity to animals and humans. They are produced by cultures of *Streptomyces avermectilis*. Some eight closely related structures have been identified, with avermectins B_{1a} and B_{2a} being the most active antiparasitic compounds. **Abamectin** (a mixture of about 85% avermectin B_{1a} and about 15% avermectin B_{1b}) is used on agricultural crops to control mites and insects. **Ivermectin** (Figure 3.67) is a semi-synthetic 22,23-dihydro derivative of avermectin B_{1a} and was first used in veterinary practice against insects, ticks, mites, and roundworms. Although it is a broad spectrum nematocide against roundworms, it is inactive against tapeworms and flatworms, or against bacteria and fungi. It is an extremely potent agent, and is effective at very low dosages. It has now been introduced for use against filarial and several other worm parasites in humans. Avermectins act by blocking neuromuscular transmission in sensitive organisms by acting on GABA (γ -aminobutyric acid) receptors.

(derived from isoleucine) at C-25, whilst the minor *b* component has an isopropyl group instead, e.g. **avermectin B_{1b}**. In this case, the starter group is 2-methylpropionyl-CoA, derived from the amino acid L-valine. The A-series of avermectins are the 5-methoxy analogues of the B-series.

Even larger macrolides are encountered in the **polyene macrolides***, most of which have antifungal properties, but not antibacterial activity. The macrolide ring size ranges from 26 to 38 atoms, and this also accommodates a conjugated polyene of up to seven *E* double bonds. Relatively

few methyl groups are attached to the ring, and thus malonyl-CoA is utilized more frequently than methylmalonyl-CoA as chain extender. Typical examples are **amphotericin B** (Figure 3.68) from *Streptomyces nodosus* and **nystatin A₁** from *Streptomyces noursei*. These have very similar structures and are derived from the same basic precursors (Figure 3.68). The ring size is contracted due to cross-linking by formation of a hemiketal. They have slightly different hydroxylation patterns, part

of which is introduced by hydroxylation, and the two areas of conjugation in nystatin A₁ are extended into a heptaene system in amphotericin B. Both compounds are glycosylated with the amino sugar D-mycosamine, and both are carboxylic acids, a result of oxidation of a propionate-derived methyl group.

An unusual and clinically significant macrolide isolated from *Streptomyces tsukubaensis* is **FK-506 (tacrolimus)*** (Figure 3.69), which contains a

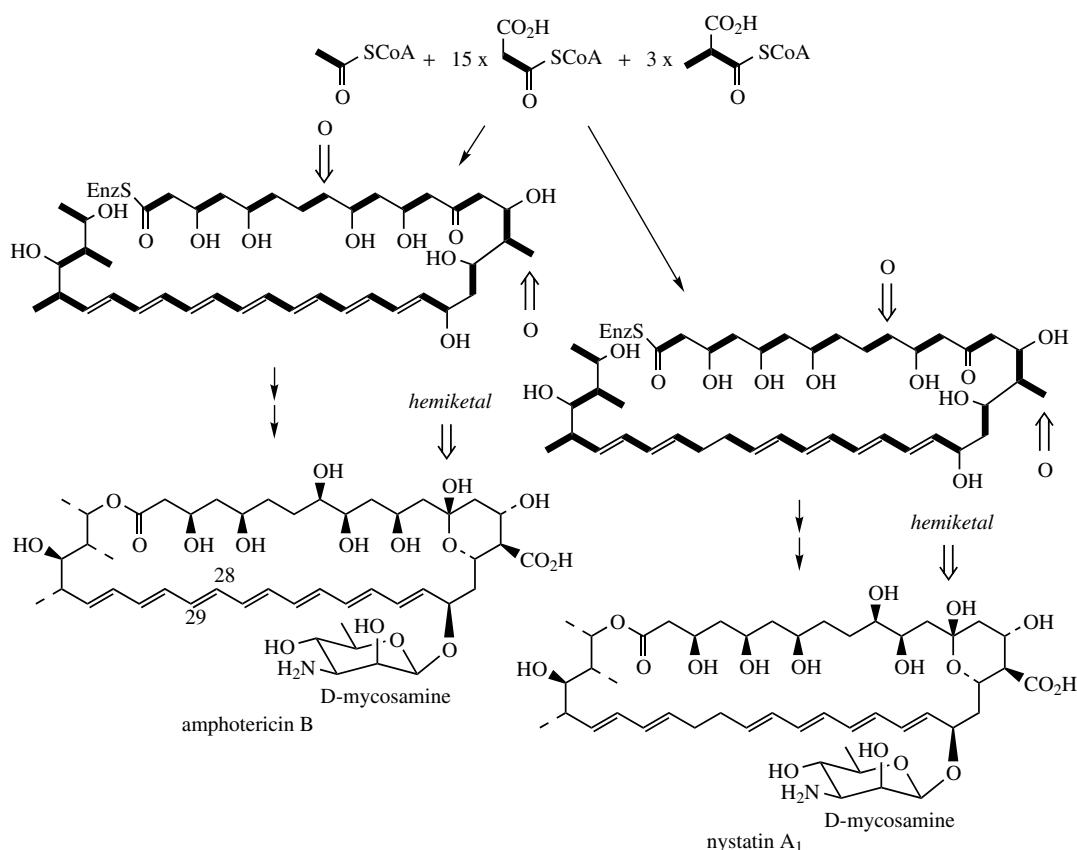


Figure 3.68

Polyene Antifungals

The polyene antifungals are a group of macrocyclic lactones with a very large 26–38-membered ring. They are characterized by the presence of a series of conjugated *E* double bonds and are classified according to the longest conjugated chain present. Medically important ones include the heptaene amphotericin B, and the tetraene nystatin. There are relatively few methyl branches in the macrocyclic chain. The polyenes have no antibacterial

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activity but are useful antifungal agents. Their activity is a result of binding to sterols in the eukaryotic cell membrane, which action explains the lack of antibacterial activity because bacterial cells do not contain sterol components. Fungal cells are also attacked rather than mammalian cells, since the antibiotics bind much more strongly to ergosterol, the major fungal sterol (see page 253), than to cholesterol, the main animal sterol component (see page 236). This binding modifies the cell wall permeability and leads to formation of transmembrane pores allowing K^+ ions, sugars, and proteins to be lost from the microorganism. Though binding to cholesterol is less than to ergosterol, it is responsible for the observed toxic side-effects of these agents on humans. The polyenes are relatively unstable, undergoing light-catalysed decomposition, and are effectively insoluble in water. This insolubility actually protects the antibiotic from gastric decomposition, allowing oral treatment of infections in the intestinal tract.

Amphotericin is an antifungal polyene produced by cultures of *Streptomyces nodosus* and contains principally the heptaene amphotericin B (Figure 3.68) together with structurally related compounds, e.g. the tetraene amphotericin A (about 10%), which is the 28,29-dihydro analogue of amphotericin B. Amphotericin A is much less active than amphotericin B. Amphotericin is active against most fungi and yeasts, but it is not absorbed from the gut, so oral administration is restricted to the treatment of intestinal candidiasis. It is administered intravenously for treating potentially life-threatening systemic fungal infections. However, it then becomes highly protein bound resulting in poor penetration and slow elimination from the body. After parenteral administration, toxic side-effects, including nephrotoxicity, are relatively common. Close supervision and monitoring of the patient is thus necessary, especially since the treatment may need to be prolonged. A liposome-encapsulated formulation of amphotericin has been shown to be much less toxic and may prove a significant advance. *Candida* infections in the mouth or on the skin may be treated with appropriate formulations.

Nystatin is a mixture of tetraene antifungals produced by cultures of *Streptomyces noursei*. The principal component is nystatin A_1 (Figure 3.68), but the commercial material also contains nystatin A_2 and A_3 , which have additional glycoside residues. Nystatin is too toxic for intravenous use, but has value for oral treatment of intestinal candidiasis, as lozenges for oral infections, and as creams for topical control of *Candida* species.

23-membered macrolactone that also incorporates an *N*-heterocyclic ring. This compound is known to be derived from acetate and propionate, the fragments of which can readily be identified in the main chain. The starter unit is cyclohexanecarboxylic acid, a reduction product from shikimate, and the piperidine ring and adjacent carbonyl are incorporated as pipecolic acid (see page 310) via an amide linkage on to the end of the growing chain. An unusual pentanoic acid unit is also incorporated to provide the propenyl side-chain. FK-506 is a particularly effective immunosuppressant, and is proving valuable in organ transplant surgery. Although **rapamycin (sirolimus)*** contains a very large 31-membered macrocycle, several portions of the structure are identical to those of FK-506. Cyclohexanecarboxylic acid and pipecolic acid are

again utilized in its formation, whilst the rest of the skeleton is supplied by simple acetate and propionate residues (Figure 3.69).

Attracting considerable interest at the present time are the **epothilones** (Figure 3.70), a group of macrolides produced by cultures of the bacterium *Sorangium cellulosum*. These compounds employ an unusual starter unit containing a thiazole ring, which is almost certainly constructed from the amino acid cysteine and an acetate unit (see also thiazole rings in bleomycin, page 429). The macrolide ring also contains an extra methyl group at C-4, the result of methylation after or during polyketide chain assembly. The other interesting feature is that this bacterium produces epothilone A and epothilone B in the ratio of about 2:1. These compounds differ in the nature

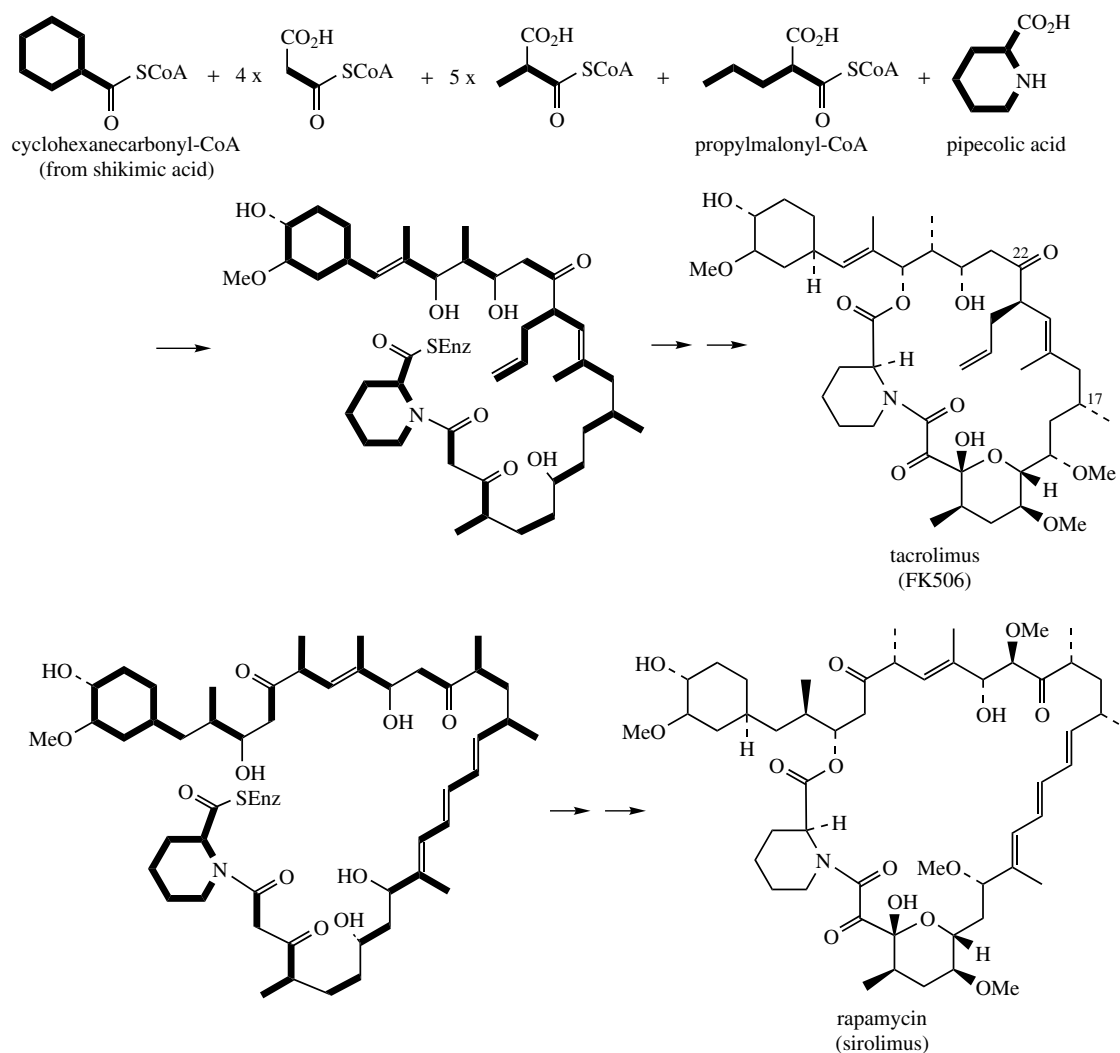


Figure 3.69

Tacrolimus and Sirolimus

Tacrolimus (FK-506) (Figure 3.69) is a macrolide immunosuppressant isolated from cultures of *Streptomyces tsukubaensis*. It is used in liver and kidney transplant surgery. Despite the significant structural differences between tacrolimus and the cyclic peptide cyclosporin A (ciclosporin; see page 429), these two agents have a similar mode of action. They both inhibit T-cell activation in the immunosuppressive mechanism by binding first to a receptor protein giving a complex, which then inhibits a phosphatase enzyme called calcineurin. The resultant aberrant phosphorylation reactions prevent appropriate gene transcription and subsequent T-cell activation. Structural similarities between the region C-17 to C-22 and fragments of the cyclosporin A peptide chain have been postulated to account for this binding. Tacrolimus is

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up to 100 times more potent than cyclosporin A, but produces similar side-effects including neurotoxicity and nephrotoxicity.

Rapamycin (sirolimus) (Figure 3.69) is produced by cultures of *Streptomyces hygroscopicus* and is also being investigated as an immunosuppressant drug. Although tacrolimus and rapamycin possess a common structural unit, and both inhibit T-cell activation, they appear to achieve this by somewhat different mechanisms. The first-formed rapamycin–receptor protein binds not to calcineurin, but to a different protein. Rapamycin suppresses lymphocyte production. Rapamycin also possesses pronounced antifungal activity, but is not active against bacteria.

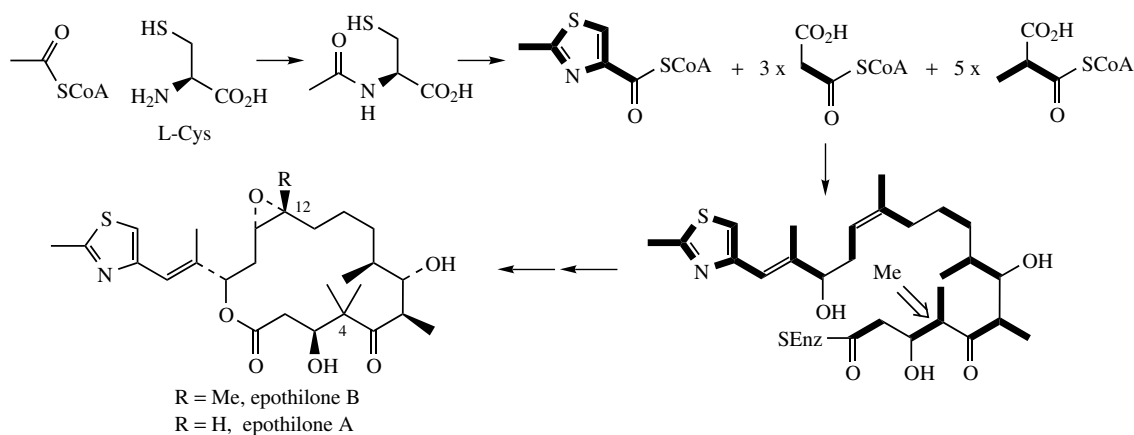


Figure 3.70

of the substituent at C-12, which is hydrogen in epothilone A but a methyl group in epothilone B. Genetic evidence shows that the polyketide synthase enzyme can accept either malonyl-CoA or methylmalonyl-CoA extender units for this position. Thus, epothilone B is constructed from three malonate and five methylmalonate extender units as shown in Figure 3.70, whilst epothilone A requires four units of each type. The epothilones display marked antitumour properties with a mode of action paralleling that of the highly successful anticancer drug taxol (see page 205). However, the epothilones have a much higher potency (2000–5000 times) and are active against cell lines which are resistant to taxol and other drugs. There appears to be considerable potential for developing the epothilones or analogues into valuable anti-cancer drugs.

A further group of macrolides in which non-adjacent positions on an aromatic ring are bridged by the long aliphatic chain is termed **ansa**

macrolides*. These are actually lactams rather than lactones, and the nitrogen atom originates from **3-amino-5-hydroxybenzoic acid**, which acts as the starter unit for chain extension with malonyl-CoA or methylmalonyl-CoA. 3-Amino-5-hydroxybenzoic acid (Figure 3.71) is a simple phenolic acid derivative produced by an unusual variant of the shikimate pathway (see Chapter 4), in which aminoDAHP is formed in the initial step, and then the pathway continues with amino analogues. This proceeds through to aminodehydroshikimic acid which yields 3-amino-5-hydroxybenzoic acid on dehydration. In the biosynthesis of **rifamycin B** (Figure 3.71) in *Amycolatopsis mediterranei*, this starter unit, plus two malonyl-CoA and eight methylmalonyl-CoA extenders, are employed to fabricate **proansamycin X** as the first product released from the enzyme. The enzyme-bound intermediate shown in Figure 3.71 is not strictly correct, in that the naphthoquinone ring system is now

Ansa Macrolides

Ansamycins are a class of macrocyclic compounds in which non-adjacent positions on an aromatic ring system are spanned by the long aliphatic bridge (Latin: *ansa* = handle). The aromatic portion may be a substituted naphthalene or naphthaquinone, or alternatively a substituted benzene ring. The macrocycle in the ansamycins is closed by an amide rather than an ester linkage, i.e. ansamycins are lactams. The only ansamycins currently used therapeutically are semi-synthetic naphthalene-based macrocycles produced from rifamycin B.

The rifamycins are ansamycin antibiotics produced by cultures of *Amycolatopsis mediterranei* (formerly *Nocardia mediterranei* or *Streptomyces mediterranei*). The crude antibiotic mixture was found to contain five closely related substances rifamycins A–E, but if the organism was cultured in the presence of sodium diethyl barbiturate (barbitone or barbitol), the product was almost entirely rifamycin B (Figure 3.71). Rifamycin B has essentially no antibacterial activity, but on standing in aqueous solution in the presence of air, it is readily transformed by oxidation and intramolecular nucleophilic addition into rifamycin O, which

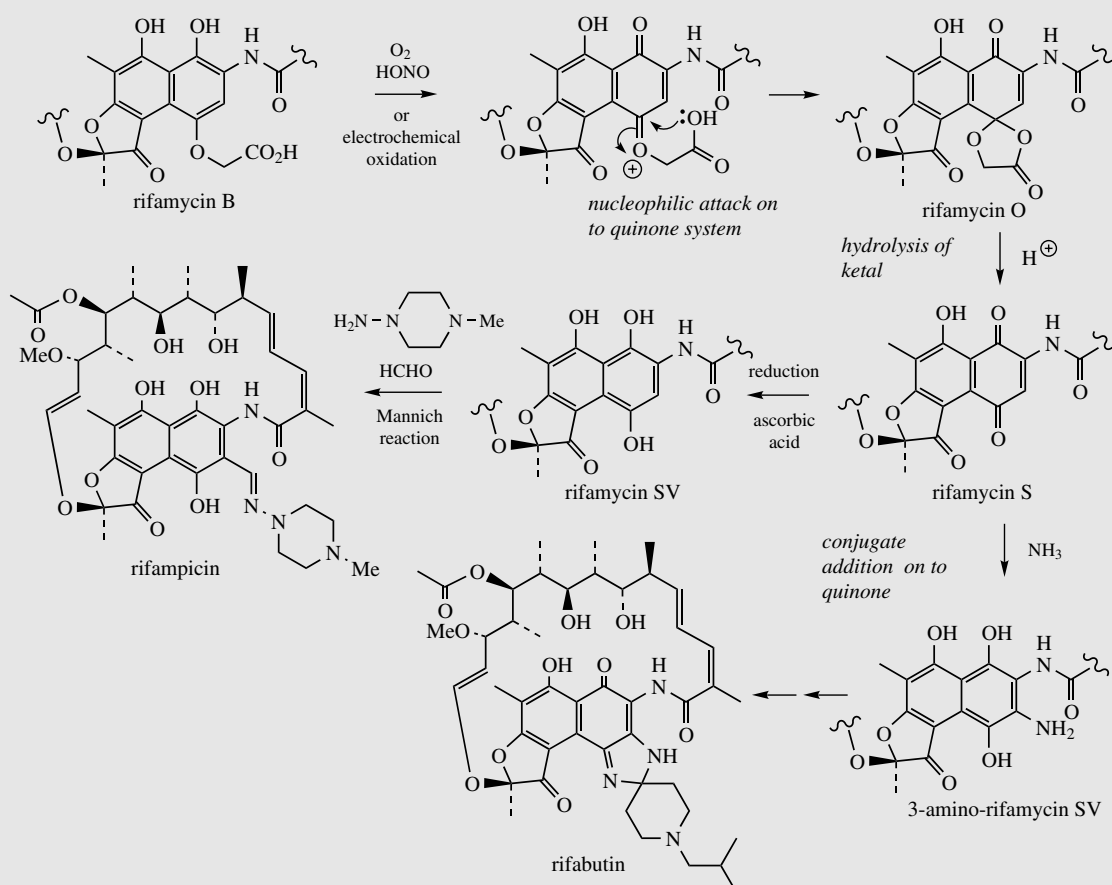


Figure 3.73

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under acidic conditions then hydrolyses and gives rifamycin S, a highly active antibacterial agent (Figure 3.73). Chemical reduction of rifamycin S using ascorbic acid (vitamin C) converts the quinone into a quinol and provides a further antibacterial, rifamycin SV. Rifamycins O, S, and SV can all be obtained by fermentation using appropriate strains of *A. mediterranei*. Rifamycin SV is actually the immediate biosynthetic precursor of rifamycin B under normal conditions, so this conversion can be genetically blocked and lead to accumulation of rifamycin SV. Several other rifamycin analogues have also been characterized. Rifamycin O is usually produced by chemical or electrochemical oxidation of rifamycin B, and converted into rifamycin SV as in Figure 3.73.

The most useful rifamycin employed clinically is **rifampicin** (Figure 3.73), a semi-synthetic derivative produced from rifamycin SV via a Mannich reaction (see page 18) using formaldehyde and *N*-amino-*N'*-methylpiperazine. Rifampicin has a wide antibacterial spectrum, with high activity towards Gram-positive bacteria and a lower activity towards Gram-negative organisms. Its most valuable activity is towards *Mycobacterium tuberculosis* and rifampicin is a key agent in the treatment of tuberculosis, usually in combination with at least one other drug to reduce the chances for development of resistant bacterial strains. It is also useful in control of meningococcal meningitis and leprosy. Rifampicin's antibacterial activity arises from inhibition of RNA synthesis by binding to DNA-dependent RNA polymerase. RNA polymerase from mammalian cells does not contain the peptide sequence to which rifampicin binds, so RNA synthesis is not affected. In contrast to the natural rifamycins which tend to have poor absorption properties, rifampicin is absorbed satisfactorily after oral administration, and is also relatively free of toxic side-effects. The most serious side-effect is disturbance of liver function. A trivial, but to the patient potentially worrying, side-effect is discoloration of body fluids, including urine, saliva, sweat, and tears, to a red–orange colour, a consequence of the naphthalene/naphthoquinone chromophore in the rifamycins. **Rifamycin**, the sodium salt of rifamycin SV (Figure 3.73), has also been used clinically in the treatment of Gram-positive infections, and particularly against tuberculosis. **Rifabutin** (Figure 3.73) is a newly introduced derivative, synthesized via 3-amino-rifamycin SV, which also has good activity against the *Mycobacterium avium* complex frequently encountered in patients with AIDS.

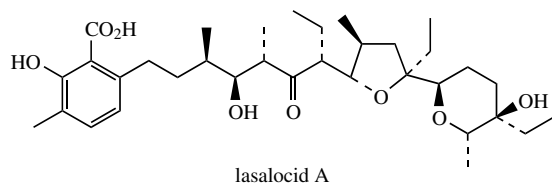


Figure 3.74

and controlling coccidia and also having the ability to improve the efficiency of food conversion in ruminants. The polyether antibiotics are characterized by the presence of a number of tetrahydrofuran and/or tetrahydropyran rings along the basic chain. The polyether acts as an ionophore, increasing influx of sodium ions into the parasite, causing a resultant and fatal

increase in osmotic pressure. Current thinking is that these ring systems arise via a cascade cyclization mechanism, probably involving epoxide intermediates. Thus, in the biosynthesis of **monensin A** (Figure 3.75), chain assembly from acetate, malonate, methylmalonate, and ethylmalonate precursors could produce the triene shown. If the triepoxide is then formed, a concerted stereospecific cyclization sequence initiated by a hydroxyl and involving carbonyls and epoxides could proceed as indicated.

Even more remarkable polyether structures are found in some toxins produced by marine dinoflagellates, which are in turn taken up by shellfish and pass on their toxicity to the shellfish. **Okadaic acid** (Figure 3.76) and related polyether structures from *Dinophysis* species are responsible for

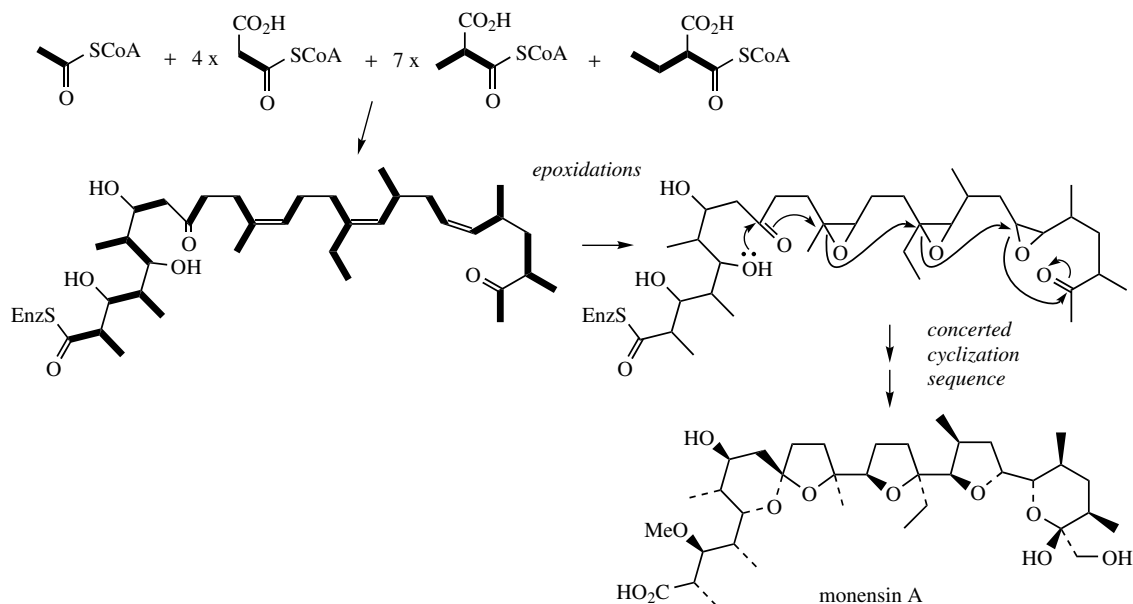


Figure 3.75

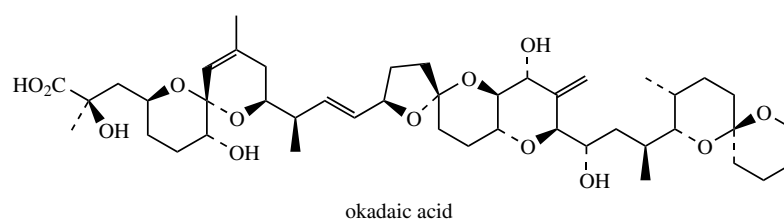


Figure 3.76

diarrhoeic shellfish poisoning in mussels, causing severe diarrhoea in consumers of contaminated shellfish in many parts of the world. **Brevetoxin A** (Figure 3.77) is an example of the toxins associated with 'red tide' blooms of dinoflagellates, which affect fishing and also tourism especially in Florida and the Gulf of Mexico. The red tide toxins are derived from *Gymnodinium breve* and are the causative agents of neurotoxic shellfish poisoning, leading to neurological disorders as well as gastrointestinal troubles. The toxins are known to bind to sodium channels, keeping them in an open state. Fatalities among marine life, e.g. fish, dolphins, whales, and in humans, are associated with these toxins synthesized by organisms at the base of the marine food chain. These compounds are postulated to be produced from a polyunsaturated fatty acid by epoxidation of the double bonds, and then a

concerted sequence of epoxide ring openings leads to the extended polyether structure (Figure 3.77). The carbon skeleton does not conform to a simple polyketide chain, and biosynthetic studies have shown that fragments from the citric acid cycle and a four-carbon starter unit from mevalonate are also involved, and that some of the methyls originate from methionine. **Ciguatoxin** (Figure 3.78) is one of the most complex examples of a polyether structure found in nature. This is found in the moray eel (*Gymnothorax javanicus*) and in a variety of coral reef fish, such as red snapper (*Lutjanus bohar*). Ciguatoxin is remarkably toxic even at microgram levels, causing widespread food poisoning (ciguatera) in tropical and subtropical regions, characterized by vomiting, diarrhoea, and neurological problems. Most sufferers slowly recover, and few cases are fatal, due principally to the very low

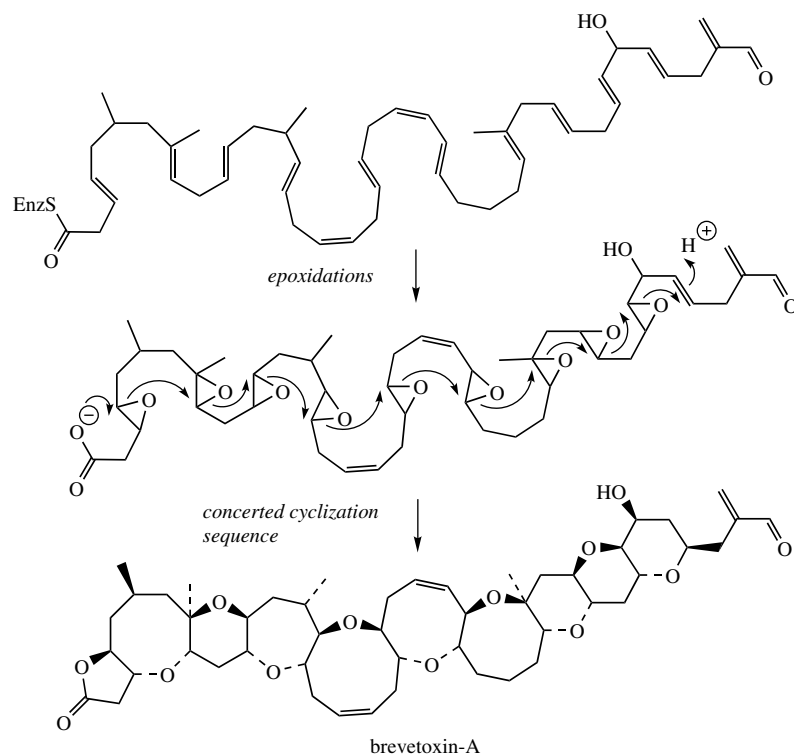


Figure 3.77

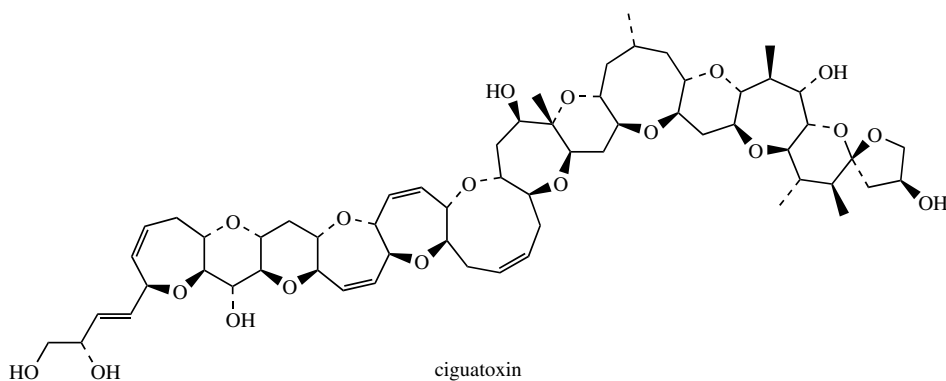


Figure 3.78

levels of toxin actually present in the fish. A dinoflagellate *Gambierdiscus toxicus* is ultimately responsible for polyether production, synthesizing a less toxic analogue, which is passed through the food chain and eventually modified into the very toxic ciguatoxin by the fish.

The **zaragozic acids** (**squalestatins**) are not macrolides, but they are primarily acetate derived, and the central ring system is suggested to be

formed by an epoxide-initiated process resembling the polyether derivatives just described. Thus, **zaragozic acid A** (Figure 3.79) is known to be constructed from two acetate-derived chains and a C₄ unit such as the Krebs cycle intermediate oxaloacetate (see Figure 2.1). One chain has a benzoyl-CoA starter (from the shikimate pathway, see page 141), and both contain two methionine-derived side-chain substituents (Figure 3.79). The

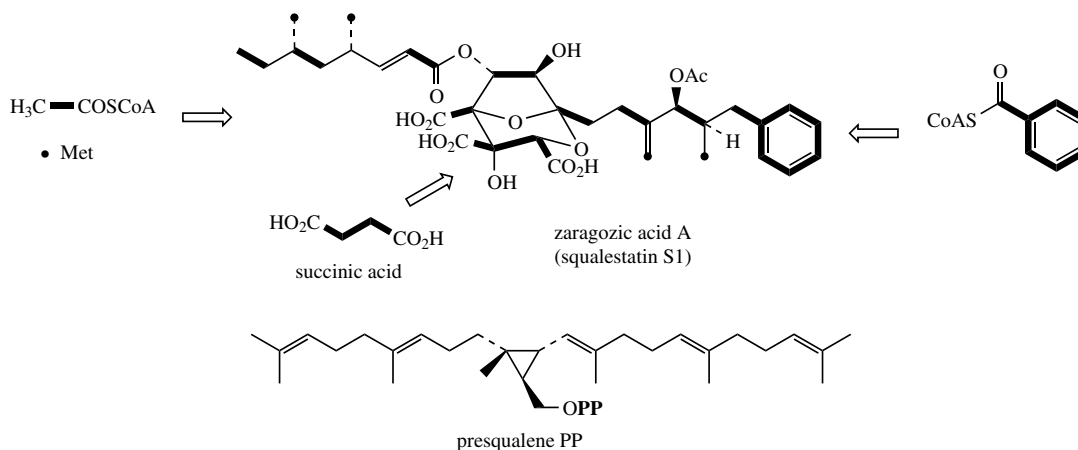


Figure 3.79

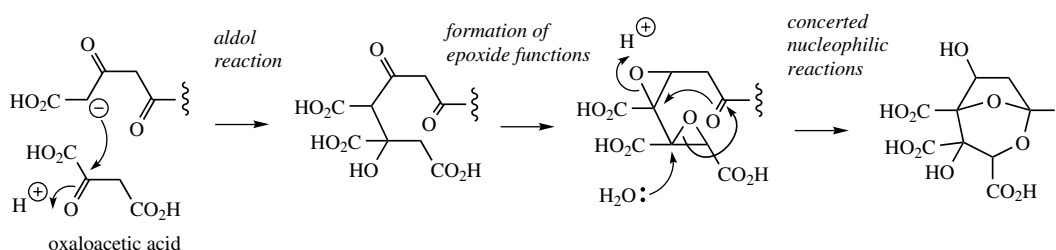


Figure 3.80

heterocyclic ring system can be envisaged as arising via nucleophilic attack on to oxaloacetic acid, formation of a diepoxide, then a concerted sequence of reactions as indicated (Figure 3.80). The zaragozic acids are produced by a number of fungi, including *Sporomiella intermedia* and *Leptodontium elatius*, and are attracting considerable interest since they are capable of reducing blood cholesterol levels in animals by acting as potent inhibitors of the enzyme squalene synthase (see page 212). This is achieved by mimicking the steroid precursor presqualene PP (Figure 3.79) and irreversibly inactivating the enzyme. They thus have considerable medical potential for reducing the incidence of coronary-related deaths (compare the statins, below).

the acetate pathway, but experimental evidence supports cyclization processes different from the aldol and Claisen reactions seen in the biosynthesis of aromatic compounds. They can, however, be rationalized in terms of an enzymic Diels–Alder reaction, represented as the electrocyclic sequence shown in Figure 3.81. Thus, **lovastatin** can be formulated as arising from two polyketide chains with C-methylation as outlined in Figure 3.82, with relatively few of the oxygen functions being retained in the final product. Accordingly, it is possible that lovastatin is formed by cyclization of the trienoic acid (Figure 3.82), which is likely to arise by a variant of the macrolide

CYCLIZATION THROUGH DIELS–ALDER REACTIONS

A number of cyclic structures, typically containing cyclohexane rings, are known to be formed via

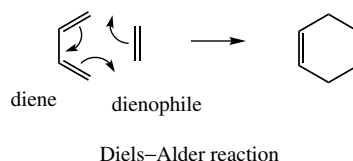


Figure 3.81

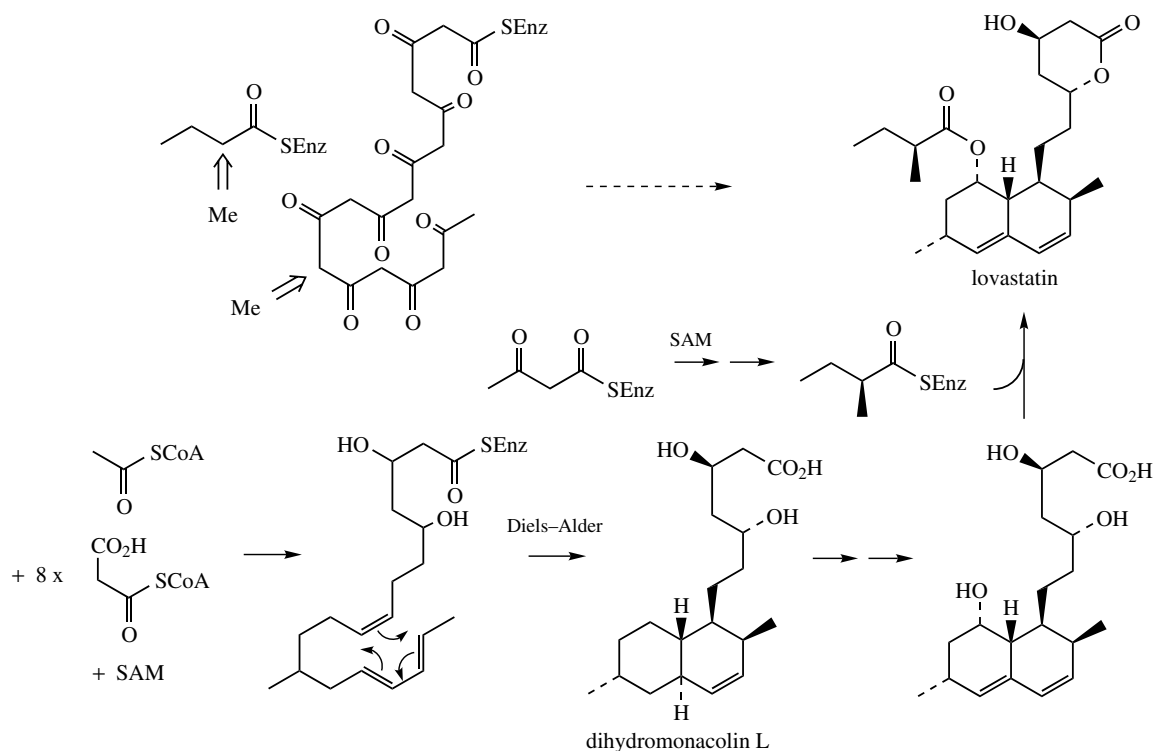


Figure 3.82

Mevastatin and other Statins

Mevastatin (formerly compactin) (Figure 3.83) is produced by cultures of *Penicillium citrinum* and *P. brevicompactum*, and was shown to be a reversible competitive inhibitor of HMG-CoA reductase, dramatically lowering sterol biosynthesis in mammalian cell cultures and animals, and reducing total and low density lipoprotein cholesterol levels (see page 236). Mevastatin in its ring-opened form (Figure 3.84) mimics the half-reduced substrate mevaldate hemithioacetal during the two-stage reduction of HMG-CoA to mevalonate (see page 170), and the affinity of this agent towards HMG-CoA reductase is 10 000-fold more than the normal substrate. High blood cholesterol levels contribute to the incidence of coronary heart disease (see page 236), so mevastatin, or analogues, are of potential value in treating high risk coronary patients, and some agents are already in use. Although lowering of cholesterol levels reduces the risk of heart attacks, there is evidence that the beneficial effects of statins may extend beyond simply cholesterol reduction.

Lovastatin (formerly called mevinolin or monacolin K) (Figure 3.83) is produced by *Monascus ruber* and *Aspergillus terreus* and is slightly more active than mevastatin, but has been superseded by more active agents. **Simvastatin** is obtained from lovastatin by ester hydrolysis and then re-esterification, and is two to three times as potent as lovastatin. **Pravastatin** is prepared from mevastatin by microbiological hydroxylation using *Streptomyces carbophilus* and is consequently more hydrophilic than the other drugs, with an activity similar to lovastatin. Lovastatin and simvastatin are both lactones, and are inactive until metabolized

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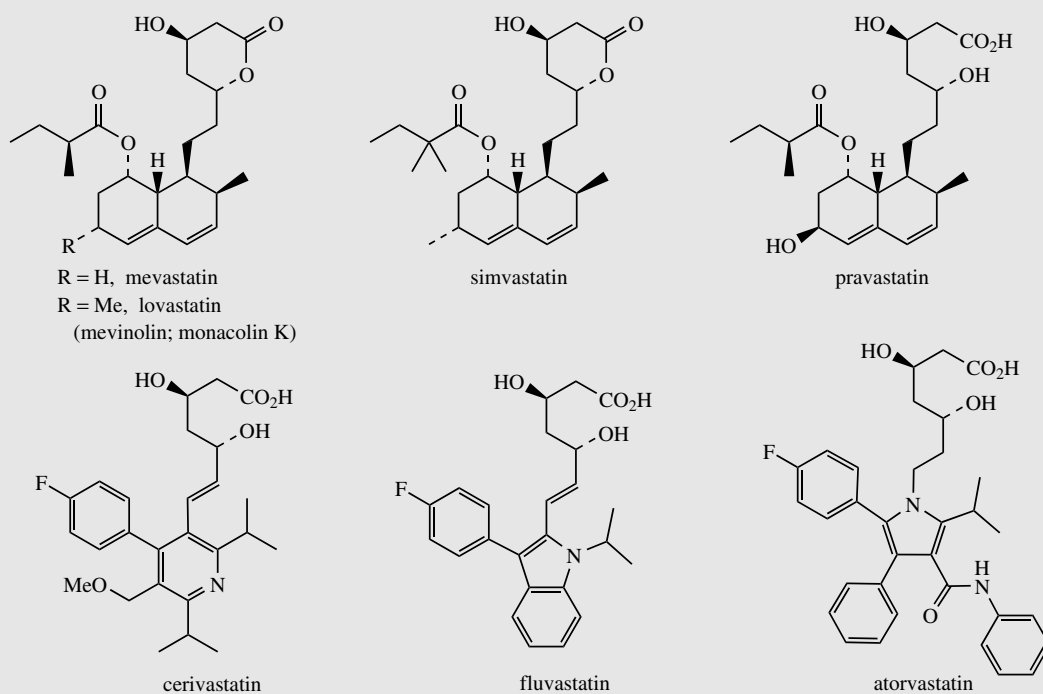


Figure 3.83

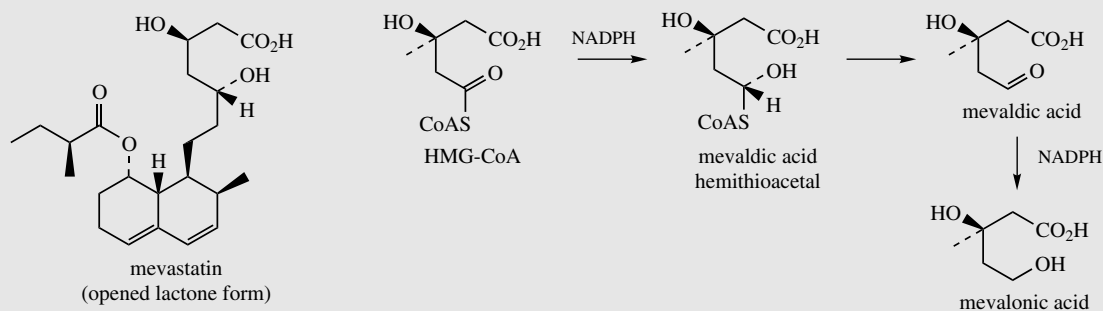


Figure 3.84

in the liver to the open-ring hydroxy acids typified by pravastatin. Other agents currently in use are synthetic, though they feature the same dihydroxycarboxylic acid side-chain as in pravastatin. **Atorvastatin**, **cerivastatin**, and **fluvastatin** have all been introduced recently.

biosynthetic processes, though *C*-methylation must occur during chain assembly whilst activating carbonyl groups are available. The Diels–Alder reaction can then account for formation of the decalin system and further reactions will allow the other functional groups in lovastatin to be

produced. The ester side-chain is derived as a separate unit from two acetates with a methyl from methionine, again with *C*-methylation preceding reduction processes. Lovastatin was isolated from cultures of *Aspergillus terreus* and was found to be a potent inhibitor of hydroxymethylglutaryl-CoA

(HMG-CoA) reductase, a rate-limiting enzyme in the mevalonate pathway (see page 169). Analogues of lovastatin (statins*) (Figure 3.83) find drug use as HMG-CoA reductase inhibitors, thus lowering blood cholesterol levels in patients.

GENETIC MANIPULATION OF THE ACETATE PATHWAY

With only a few exceptions, the transformations in any particular biosynthetic pathway are catalysed by enzymes. These proteins facilitate the chemical modification of substrates by virtue of binding properties conferred by a particular combination of functional groups in the constituent amino acids. As a result, enzymes tend to demonstrate quite remarkable specificity towards their substrates, and usually catalyse only a single transformation. This specificity means enzymes do not accept alternative substrates, or, if they do, they convert a limited range of structurally similar substrates and usually much less efficiently. Any particular organism thus synthesizes a range of secondary metabolites dictated largely by its enzyme complement and the supply of substrate molecules. Occasionally, where enzymes do possess broader substrate specificities, it is possible to manipulate an organism's secondary metabolite pattern by supplying an alternative, but acceptable, substrate. A good example of this approach is in the directed biosynthesis of modified penicillins by the use of phenylacetic acid analogues in cultures of *Penicillium chrysogenum* (see page 437), but its scope is generally very limited. It has also been possible, particularly with microorganisms, to select natural mutants, or to generate mutants artificially, where the new strain synthesizes modified or substantially different products. For example, mutant strains of *Streptomyces aureofaciens* synthesize tetracycline or demeclocycline rather than chlortetracycline (see page 90). Such mutants are usually deficient in a single enzyme and are thus unable to carry out a single transformation, but the broader specificity of later enzymes in the sequence means subsequent modifications may still occur. However, as exemplified throughout this book, the vast bulk of modified natural products of medicinal importance are currently obtained by chemical synthesis or semi-synthesis.

Rapid advances in genetic engineering have now opened up tremendous scope for manipulating the processes of biosynthesis by providing an organism with, or depriving it of, specific enzymes. The genes encoding a particular protein (see page 407) can now be identified, synthesized, and inserted into a suitable organism for expression; to avoid complications with the normal biosynthetic machinery, this is usually different from the source organism. Specific genes can be damaged or deleted to prevent a particular enzyme being expressed. Genes from different organisms can be combined and expressed together so that an organism synthesizes abnormal combinations of enzyme activities, allowing production of modified products. Although the general approaches for genetic manipulation are essentially the same for all types of organism and/or natural product, it has proved possible to make best progress using the simpler organisms, especially bacteria, and in particular there have been some substantial achievements in the area of acetate-derived structures. Accordingly, some results from this group of compounds are used to exemplify how genetic manipulation may provide an extra dimension in the search for new medicinal agents. However, it is important that an organism is not viewed merely as a sackful of freely diffusible and always available enzymes; biosynthetic pathways are under sophisticated controls in which there may be restricted availability or localization of enzymes and/or substrates (see the different localizations of the mevalonate and deoxyxylulose phosphate pathways to terpenoids in plants, page 172). Enzymes involved in the biosynthesis of many important secondary metabolites are often grouped together as enzyme complexes, or may form part of a multifunctional protein.

A detailed study of amino acid sequences and mechanistic similarities in various **polyketide synthase** (PKS) enzymes has led to two main types being distinguished. **Type I enzymes** consist of one or more large multifunctional proteins that possess a distinct active site for every enzyme-catalysed step. On the other hand, **Type II enzymes** are multienzyme complexes that carry out a single set of repeating activities. Like fatty acid synthases, PKSs catalyse the condensation of coenzyme A esters of simple carboxylic acids. However, the variability at each step in

the biosynthetic pathway gives rise to much more structural diversity than encountered with fatty acids. The usual starter units employed are acetyl-CoA or propionyl-CoA, whilst malonyl-CoA or methylmalonyl-CoA are the main extender units. At each cycle of chain extension, Type I PKSs may retain the β -ketone, or modify it to a hydroxyl, methenyl, or methylene, according to the presence of ketoreductase, dehydratase, or enoylreductase activities (see page 95). The enzyme activities for each extension cycle with its subsequent modification is considered a 'module'. The linear sequence of modules in the enzyme corresponds to the generated sequence of extender units in the polyketide product. The β -ketone groups are predominantly left intact by Type II PKSs, and the highly reactive polyketide backbone undergoes further enzyme-catalysed intramolecular cyclization reactions, which are responsible for generating a range of aromatic structures (see page 61).

6-Deoxyerythronolide B synthase (DEBS) is a modular Type I PKS involved in **erythromycin** biosynthesis (see page 96) and its structure and function are illustrated in Figure 3.85. The enzyme contains three subunits (DEBS-1, 2, and 3), each encoded by a gene (*eryA*-I, II, and III). It has a linear organization of six modules, each of which

contains the activities needed for one cycle of chain extension. A minimal module contains a β -ketoacyl synthase (KS), an acyltransferase (AT), and an acyl carrier protein (ACP), that together would catalyse a two-carbon chain extension. The specificity of the AT for either malonyl-CoA or an alkyl-malonyl-CoA determines which two-carbon chain extender is used. The starter unit used is similarly determined by the specificity of the AT in a loading domain in the first module. After each condensation reaction, the oxidation state of the β -carbon is determined by the presence of a β -ketoacyl reductase (KR), a KR + a dehydratase (DH), or a KR + DH + an enoylreductase (ER) in the appropriate module. The sequence is finally terminated by a thioesterase (TE) activity which releases the polyketide from the enzyme and allows cyclization. Thus in DEBS, module 3 lacks any β -carbon modifying domains, modules 1, 2, 5, and 6 contain KR domains and are responsible for hydroxy substituents, whereas module 4 contains the complete KR, DH, and ER set, and results in complete reduction to a methylene. Overall, the AT specificity and the catalytic domains on each module determine the structure and stereochemistry of each two-carbon extension unit, the order of the modules specifies the sequence of the units,

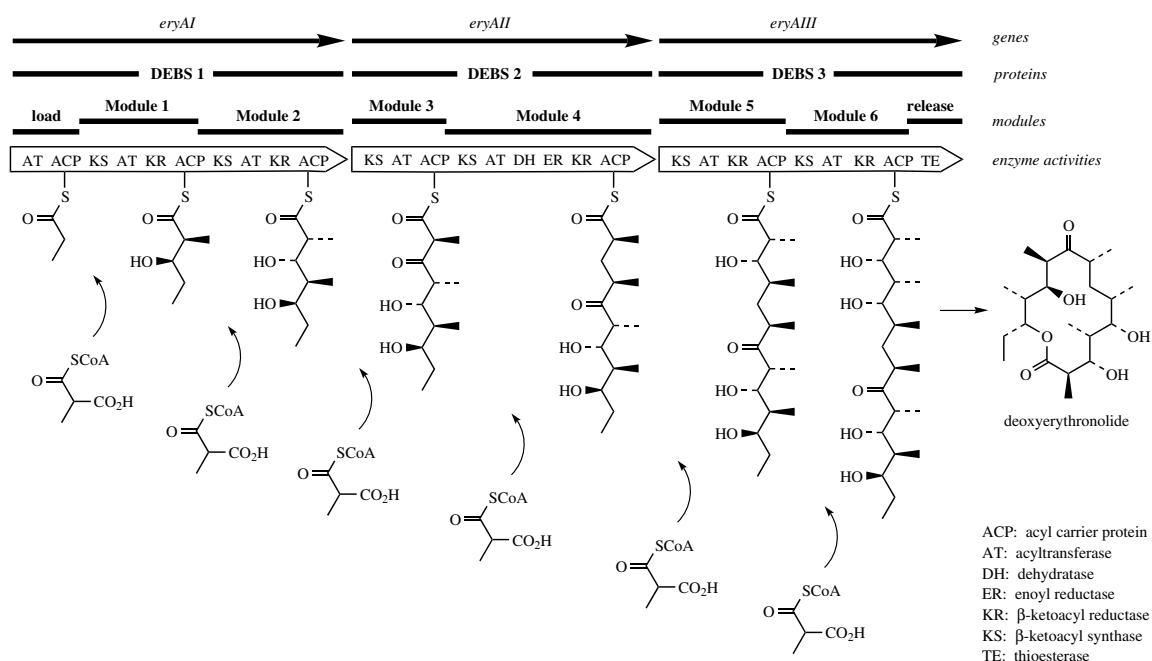


Figure 3.85

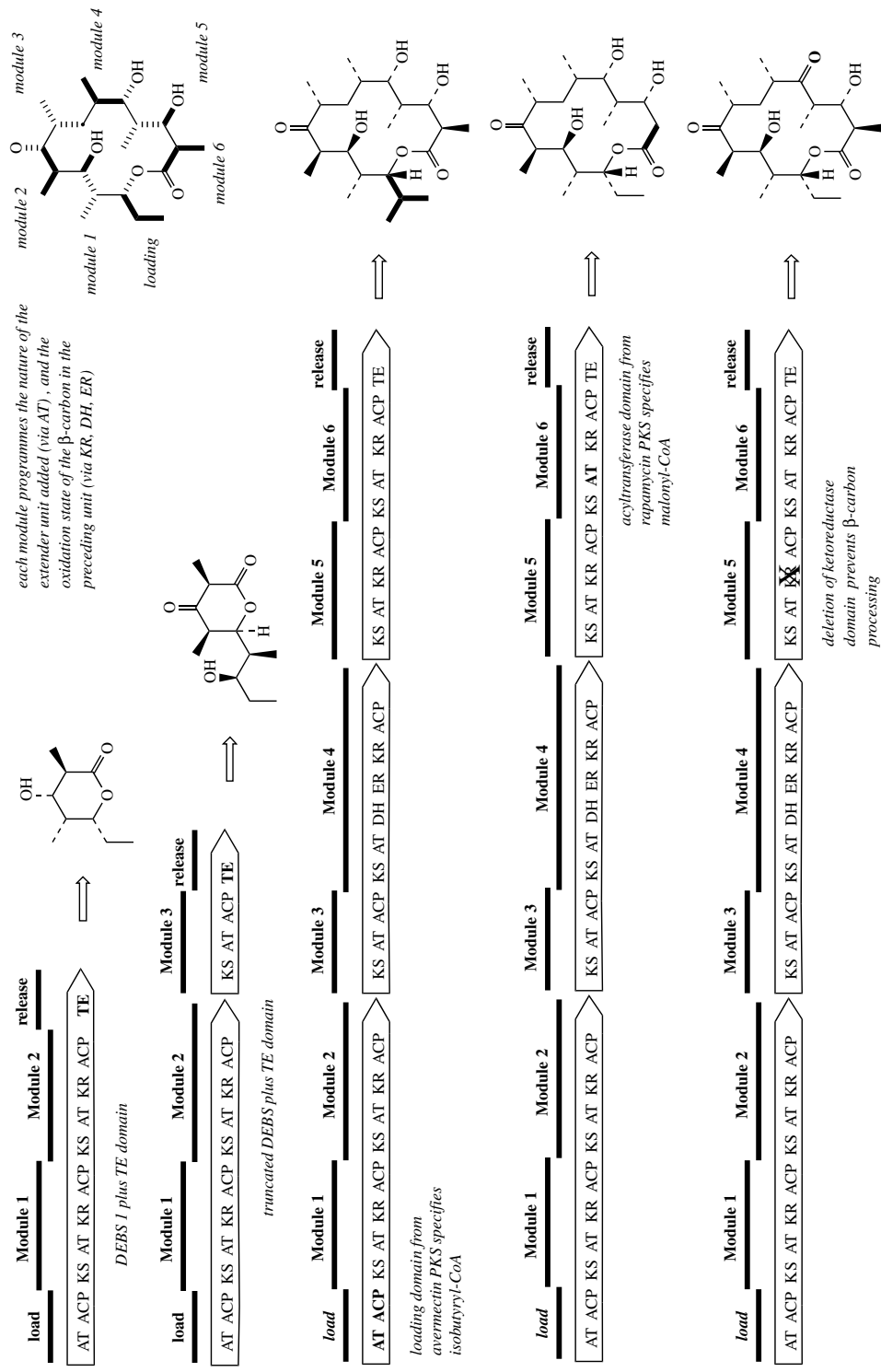


Figure 3.86

and the number of modules determines the size of the polyketide chain. The vast structural diversity of natural polyketides arises from combinatorial possibilities of arranging modules containing the various catalytic domains, the sequence and number of modules, and the post-PKS enzymes which subsequently modify the first-formed product, e.g. 6-deoxyerythronolide B $\rightarrow$ erythromycin (see page 96). Genetic engineering now offers vast opportunities for rational modification of the resultant polyketide structure.

A few representative examples of successful experiments leading to engineered polyketides are shown in Figure 3.86. Reducing the size of the gene sequence so that it encodes fewer modules results in the formation of smaller polyketides, characterized by the corresponding loss of extender units; in these examples the gene encoding the chain terminating thioesterase also has to be attached to complete the biosynthetic sequence. Replacing the loading domain of DEBS with that from another PKS, e.g. that producing avermectin (see page 97), alters the specificity of the enzyme for the starter unit. The loading module of the avermectin-producing PKS actually has a much broader specificity than that for DEBS; Figure 3.86 shows the utilization of isobutyryl-CoA as features in the natural biosynthesis of avermectin B_{1b}. Other examples include the replacement of an AT domain (in DEBS specifying a methylmalonyl extender) with a malonyl-specific AT domain from the rapamycin-producing PKS (see page 103), and deletion of a KR domain, thus stopping any β -carbon processing for that module with consequent retention of a carbonyl group. Not all experiments in gene modification are successful, and even when they are yields can be disappointingly lower than in the natural system. There is always a fundamental requirement that enzymes catalysing steps after the point of modification need to have sufficiently broad substrate specificities to accept and process the abnormal compounds being synthesized; this becomes more unlikely where two or more genetic changes have been made. Nevertheless, multiple modifications have been successful, and it has also been possible to exploit changes in a combinatorial fashion using different expression vectors for the individual subunits, thus creating a library of polyketides, which may then be screened for potential biological activity.

Non-ribosomal peptide synthases (see page 421) are also modular and lend themselves to similar genetic manipulation as the Type I PKSs. The production of modified aromatic polyketides by genetically engineered Type II PKSs is not quite so 'obvious' as with the modular Type I enzymes, but significant progress has been made in many systems. Each Type II PKS contains a minimal set of three protein subunits, two β -ketoacyl synthase (KS) subunits and an ACP to which the growing chain is attached. Additional subunits, including KRs, cyclases (CYC), and aromatases (ARO), are responsible for modification of the nascent chain to form the final cyclized structure. Novel polyketides have been generated by manipulating Type II PKSs, exchanging KS, CYC, and ARO subunits among different systems. However, because of the highly reactive nature of poly- β -keto chains, the cyclizations that occur with the modified gene product frequently vary from those in the original compound. Compared with Type I PKSs, the formation of new products with predictable molecular structure has proven less controllable.

The polyketide synthases responsible for chain extension of cinnamoyl-CoA starter units leading to flavonoids and stilbenes, and of anthraniloyl-CoA leading to quinoline and acridine alkaloids (see page 377) do not fall into either of the above categories and have now been termed **Type III PKSs**. These enzymes differ from the other examples in that they are homodimeric proteins, they utilize coenzyme A esters rather than acyl carrier proteins, and they employ a single active site to perform a series of decarboxylation, condensation, cyclization, and aromatization reactions.

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