Medicinal Natural Products. Paul M Dewick

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7

PEPTIDES, PROTEINS, AND OTHER AMINO ACID DERIVATIVES

The fundamental structures of peptides and proteins are considered, and their biosynthesis by ribosomal and nonribosomal (multi-enzyme) processes are discussed. Ribosomal peptide biosynthesis leads to peptide hormones, and major groups of these are described, including thyroid, hypothalamic, anterior pituitary, posterior pituitary, and pancreatic hormones, as well as interferons, opioid peptides, and enzymes. Nonribosomal peptide biosynthesis is responsible for the formation of peptide antibiotics, peptide toxins, and modified peptides such as the penicillins, cephalosporins, and other β-lactams. Also covered in this chapter are some other amino acid derivatives, such as cyanogenic glycosides, glucosinolates, and the cysteine sulphoxides characteristic of garlic. Monograph topics giving more detailed information on medicinal agents include thyroxine, calcitonin, thyrotrophin-releasing hormone, luteinizing hormone-releasing hormone, growth hormone-releasing hormone, somatostatin, corticotropin, growth hormone, prolactin, gonadotrophins, oxytocin, vasopressin, insulin, glucagon, interferons, pharmaceutically important enzymes, cycloserine, polymyxins, bacitracins, tyrothricin and gramicidins, capreomycin, vancomycin and teicoplanin, bleomycin, cyclosporins, streptogramins, dactinomycin, death cap, ricin, botulinum toxin, microcystins, snake venoms, penicillins, cephalosporins, cephamycins, carbacephems, clavulanic acid, carbapenems, monobactams, and garlic.

Although the participation of amino acids in the biosynthesis of some shikimate metabolites and particularly in the pathways leading to alkaloids has already been explored in Chapters 4 and 6, amino acids are also the building blocks for other important classes of natural products. The elaboration of shikimate metabolites and alkaloids utilized only a limited range of amino acid precursors. Peptides, proteins, and the other compounds considered in this chapter are synthesized from a very much wider range of amino acids. Peptides and proteins represent another grey area between primary metabolism and secondary metabolism, in that some materials are widely distributed in nature and found, with subtle variations, in many different organisms, whilst others are of very restricted occurrence.

PEPTIDES AND PROTEINS

Peptides and proteins are both polyamides composed of α-amino acids linked through their carboxyl and α-amino functions (Figure 7.1). In biochemistry, the amide linkage is traditionally referred to as a peptide bond. Whether the resultant polymer is classified as a peptide or a protein is not clearly defined; generally a chain length of more than 40 residues confers protein status, whilst the term polypeptide can be used to cover all chain lengths. Although superficially similar, peptides and proteins display a wide variety of biological functions and many have marked physiological properties. For example, they function as structural molecules in tissues, as enzymes, as antibodies, as

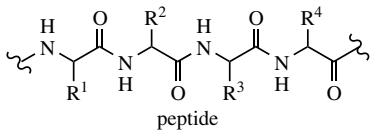
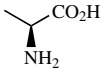
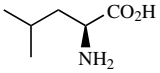
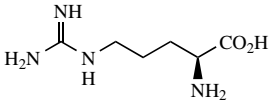
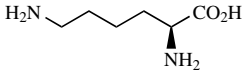
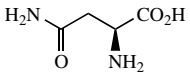
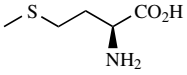
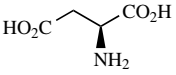
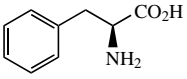
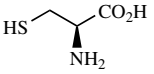
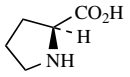
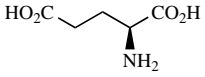
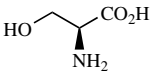
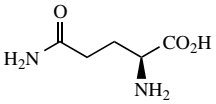
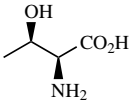
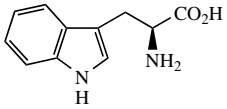
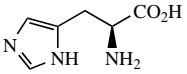
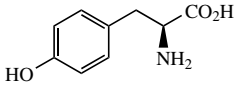
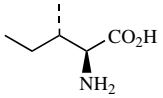
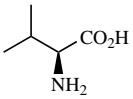


Figure 7.1

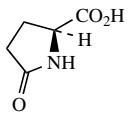
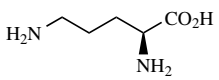
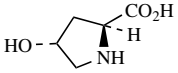
neurotransmitters, and acting as hormones can control many physiological processes, ranging from gastric acid secretion and carbohydrate metabolism to growth itself. The toxic components of snake and spider venoms are usually peptide in nature, as are some plant toxins. These different activities arise as a consequence of the sequence of amino acids in the peptide or protein (the primary structure), the three-dimensional structure which the molecule then adopts as a result of this sequence

Table 7.1 Amino acids: structures and standard abbreviations

Amino acids encoded by DNA

Alanine		Ala	A	Leucine		Leu	L
Arginine		Arg	R	Lysine		Lys	K
Asparagine		Asn	N	Methionine		Met	M
Aspartic acid		Asp	D	Phenylalanine		Phe	F
Cysteine		Cys	C	Proline		Pro	P
Glutamic acid		Glu	E	Serine		Ser	S
Glutamine		Gln	Q	Threonine		Thr	T
Glycine		Gly	G	Tryptophan		Trp	W
Histidine		His	H	Tyrosine		Tyr	Y
Isoleucine		Ile	I	Valine		Val	V

Some common amino acids not encoded by DNA

Pyroglutamic acid (5-oxoproline)		Glp oxoPro <Glu		Ornithine		Orn	
Hydroxyproline		HPro		Sarcosine (*N*-methylglycine)		Sar	

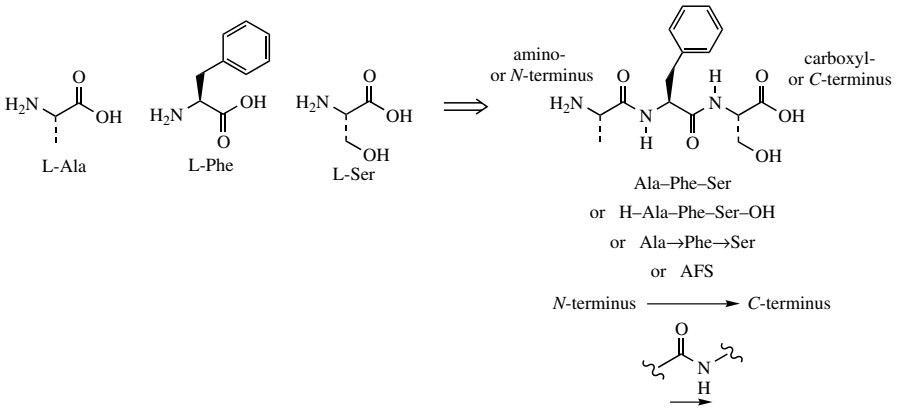


Figure 7.2

(the secondary and tertiary structures), and the specific nature of individual side-chains in the molecule. Many structures have additional modifications to the basic polyamide system shown in Figure 7.1, and these features may also contribute significantly to their biological activity.

The tripeptide formed from L-alanine, L-phenylalanine, and L-serine in Figure 7.2 by two condensation reactions is alanyl–phenylalanyl–serine, commonly represented as Ala–Phe–Ser, using the standard three-letter abbreviations for amino acids as shown in Table 7.1, which gives the structures of the 20 L-amino acids which are encoded by DNA. By convention, the left hand amino acid in this sequence is the one with a free amino group, the *N*-terminus, whilst the right hand amino acid has the free carboxyl, the *C*-terminus. Sometimes, the termini are emphasized by showing H– and –OH (Figure 7.2). In cyclic peptides, this convention can have no significance, so arrows are incorporated into the sequence to indicate peptide bonds in the direction CO→NH. As sequences become longer, one letter abbreviations for amino acids are commonly used instead of the three-letter abbreviations, thus Ala–Phe–Ser becomes AFS. The amino acid components of peptides and proteins predominantly have the L-configuration, but many peptides contain one or more D-amino acids in their structures. Abbreviations thus assume the L-configuration applies, and D-amino acids must be specifically noted, e.g. Ala–D-Phe–Ser. Some amino acids that are not encoded by DNA, but which are frequently encountered in peptides, are shown in Table 7.1, and these also have their appropriate abbreviations. Modified amino acids may be represented by an appropriate variation of the normal abbreviation, e.g. *N*-methyltyrosine as Tyr(Me). A frequently encountered modification is the conversion of the *C*-terminal carboxyl into an amide, and this is represented as Phe–NH_2 for example, which must not be interpreted as an indication of the *N*-terminus.

RIBOSOMAL PEPTIDE BIOSYNTHESIS

Protein biosynthesis takes place on the ribosomes, and a simplified representation of the process as characterized in *Escherichia coli* is shown in Figure 7.3. The messenger RNA (mRNA) contains a transcription of one of the genes of DNA, and carries the information necessary to direct the biosynthesis of a specific protein. The message is stored as a series of three-base sequences (codons) in its nucleotides, and is read (translated) in the 5′ to 3′ direction along the mRNA molecule. The mRNA is bound to the smaller 30S subunit of the bacterial ribosome. Initially, the amino acid is activated by an ATP-dependent process and it then binds via an ester linkage to an amino acid-specific transfer RNA (tRNA) molecule through a terminal adenosine group, giving an aminoacyl-tRNA (Figure 7.4). The aminoacyl-tRNA contains in its nucleotide sequence a combination of three bases (the anticodon) which allows binding via hydrogen bonding to the appropriate codon on mRNA. In prokaryotes, the first amino acid encoded in the sequence is *N*-formylmethionine, and the corresponding aminoacyl-tRNA is thus bound and

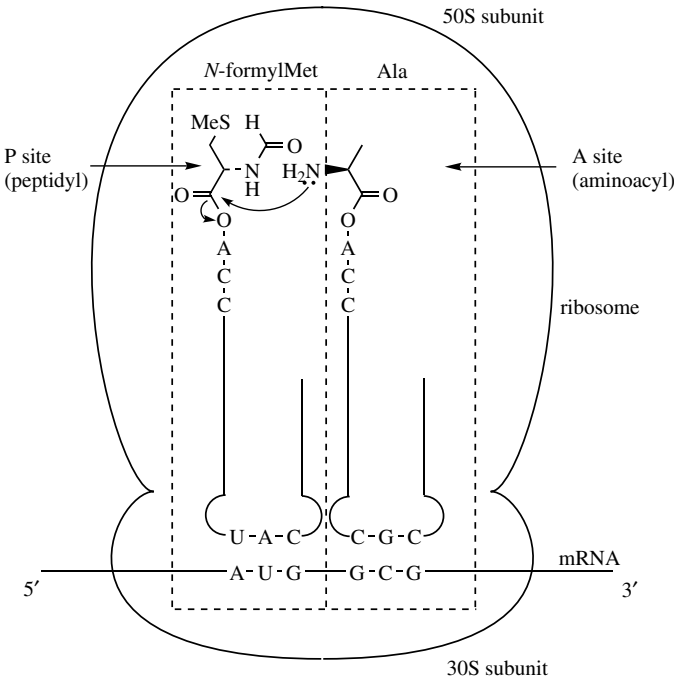


Figure 7.3

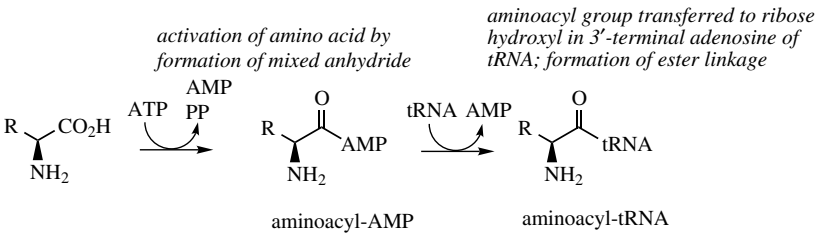


Figure 7.4

positioned at the P (for peptidyl) site on the ribosome (Figure 7.3). The next aminoacyl-tRNA (Figure 7.3 shows a tRNA specific for alanine) is also bound via a codon–anticodon interaction and is positioned at an adjacent A (for aminoacyl) site on the ribosome. This allows peptide bond formation to occur, the amino group of the amino acid in the A site attacking the activated ester in the P site. The peptide chain is thus initiated and has become attached to the tRNA located in the A site. The tRNA at the P site is no longer required and is released from the ribosome. Then the peptidyl-tRNA at the A site is translocated to the P site by the ribosome moving along the mRNA a codon at a time, exposing the A site for a new aminoacyl-tRNA appropriate for the particular codon, and a repeat of the elongation process occurs. The cycles of elongation and translocation continue until a termination codon is reached, and the peptide or protein is then hydrolysed and released from the ribosome. The individual steps of protein biosynthesis all seem susceptible to disruption by specific agents. Many of the antibiotics used clinically are active by their ability to inhibit protein biosynthesis in bacteria. They may interfere with the binding of the aminoacyl-tRNA to the A site (e.g. tetracyclines, see page 91), the formation of the peptide bond (e.g. chloramphenicol, see page 130), or the translocation step (e.g. erythromycin, see page 99).

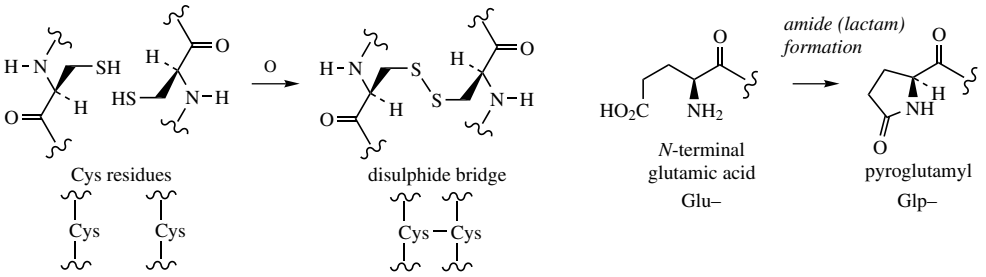


Figure 7.5

Glp–Gly–Pro–Trp–Leu–Glu–Glu–Glu–Glu–Glu–Ala–Tyr–Gly–Trp–Gly–Trp–Met–Asp–**Phe**–$\mathbf{NH_2}$

human gastrin

Figure 7.6

Many of the peptides and proteins synthesized on the ribosome then undergo enzymic post-translational modifications. In prokaryotes, the *N*-formyl group on the *N*-terminal methionine is removed, and in both prokaryotes and eukaryotes a number of amino acids from the *N*-terminus may be hydrolysed off, thus shortening the original chain. This type of chain shortening is a means by which a protein or peptide can be stored in an inactive form, and then transformed into an active form when required, e.g. the production of insulin from proinsulin (see page 416). **Glycoproteins** are produced by adding sugar residues via *O*-glycoside linkages to the hydroxyls of serine and threonine residues or via *N*-glycoside linkages to the amino of asparagine. **Phosphoproteins** have the hydroxyl groups of serine or threonine phosphorylated. Importantly, post-translational modifications allow those amino acids that are not encoded by DNA, yet are found in peptides and proteins, to be formed by the transformation of encoded ones. These include the hydroxylation of proline to hydroxyproline and of lysine to hydroxylysine, *N*-methylation of histidine, and the oxidation of the thiol groups of two cysteine residues to form a **disulphide bridge**, allowing cross-linking of polypeptide chains. This latter process (Figure 7.5) will form loops in a single polypeptide chain, or may join separate chains together as in insulin (see page 416). Many peptides contain a **pyroglutamic acid** residue (Glp) at the *N*-terminus, a consequence of intramolecular cyclization between the γ-carboxylic acid and the α-amino of an *N*-terminal glutamic acid (Figure 7.5). The *C*-terminal carboxylic acid may also frequently be converted into an amide. Both modifications are exemplified in the structure of **gastrin** (Figure 7.6), a peptide hormone that stimulates secretion of HCl in the stomach. Such terminal modifications comprise a means of protecting a peptide from degradation by exopeptidases, which remove amino acids from the ends of peptides, thus increasing its period of action.

With the rapid advances made in genetic engineering, it is now feasible to produce relatively large amounts of ribosome-constructed polypeptides by isolating or constructing DNA sequences encoding the particular product, and inserting these into suitable organisms, commonly *Escherichia coli*. Of course, such procedures will not duplicate any post-translational modifications, and these will have to be carried out on the initial polypeptide by chemical means, or by suitable enzymes if these are available. Not all of the transformations can be carried out both efficiently and selectively, thus restricting access to important polypeptides by this means. Small peptides for drug use are generally synthesized chemically, though larger peptides and proteins may be extracted from human and animal tissues or bacterial cultures. In the design of semi-synthetic or synthetic analogues for potential drug use, enzymic degradation can be reduced by the use of *N*-terminal pyroglutamic acid and *C*-terminal amide residues, the inclusion of D-amino acids, and the removal of specific residues. These ploys to change recognition by specific degradative

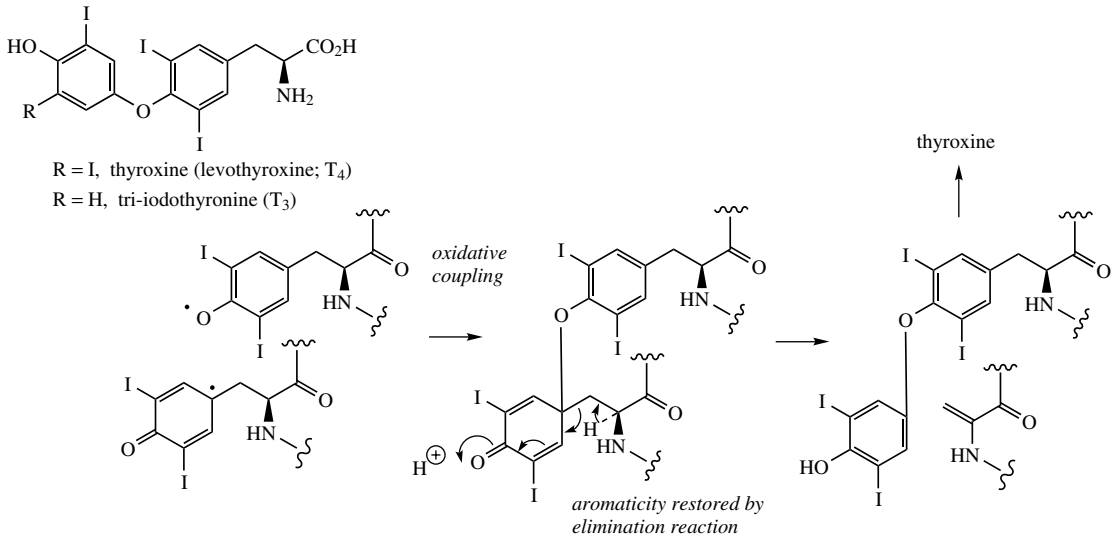


Figure 7.7

peptidases can increase the activity and lifetime of the peptide. Very few peptide drugs may be administered orally since they are rapidly inactivated or degraded by gastrointestinal enzymes, and they must therefore be given by injection.

PEPTIDE HORMONES

Hormones are mammalian metabolites released into the blood stream to elicit specific responses on a target tissue or organ. They are chemical messengers, and may be simple amino acid derivatives, e.g. adrenaline (see page 317), or polypeptides, e.g. insulin (see page 417), or they may be steroidal in nature, e.g. progesterone (see page 273). They may exert their effects in a variety of ways, e.g. by influencing the rate of synthesis of enzymes or proteins, by affecting the catalytic activity of an enzyme, or by altering the permeability of cell membranes. Hormones are not enzymes; they act by regulating existing processes. Frequently, this action depends on the involvement of a second messenger, such as cyclic AMP (cAMP).

Thyroid Hormones

Thyroid hormones are necessary for the development and function of cells throughout the body. The thyroid hormones **thyroxine*** and **tri-iodothyronine*** (Figure 7.7) are not peptides, but are actually simple derivatives of tyrosine. However, they are believed to be derived by degradation of a larger protein molecule. One

Thyroxine

The thyroid hormones thyroxine (T_4) and tri-iodothyronine (T_3) (Figure 7.7) are derivatives of tyrosine and are necessary for development and function of cells throughout the body. They increase protein synthesis in almost all types of body tissue and increase oxygen consumption dependent upon Na^+/K^+ ATPase (the Na pump). Excess thyroxine causes hyperthyroidism, with increased heart rate, blood pressure, overactivity, muscular weakness, and loss of weight. Too little thyroxine may lead to cretinism in children, with poor growth and mental deficiency, or myxoedema in adults, resulting in a slowing down of all body processes. Tri-iodothyronine is also

(*Continues*)

(*Continued*)

produced from thyroxine by mono-deiodination in tissues outside the thyroid, and is actually three to five times more active than thyroxine. **Levothyroxine** (**thyroxine**) and **liothyronine** (**triiodothyronine**) are both used to supplement thyroid hormone levels, and may be administered orally. These materials are readily produced by chemical synthesis.

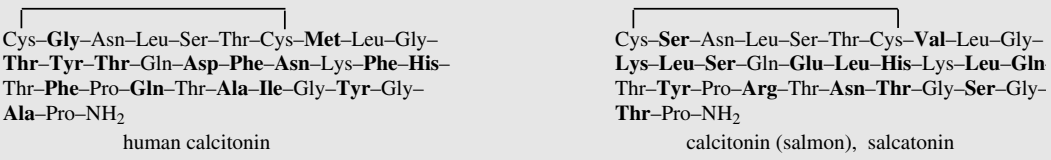


Figure 7.8

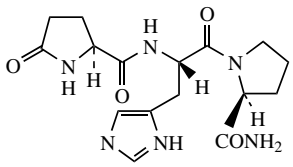
Calcitonin

Also produced in the thyroid gland, calcitonin is involved along with parathyroid hormone and 1α,25-dihydroxyvitamin D_3 (see page 259) in the regulation of bone turnover and maintenance of calcium balance. Under the influence of high blood calcium levels, calcitonin is released to lower these levels by inhibiting uptake of calcium from the gastrointestinal tract, and by promoting its storage in bone. It also suppresses loss of calcium from bone when levels are low. **Calcitonin** is used to treat weakening of bone tissue and hypercalcaemia. Human calcitonin (Figure 7.8) is a 32-residue peptide with a disulphide bridge, and synthetic material may be used. However, synthetic salmon calcitonin (**calcitonin** (**salmon**); **salcatonin**) (Figure 7.8) is found to have greater potency and longer duration than human calcitonin. Calcitonins from different sources have quite significant differences in their amino acid sequences; the salmon peptide shows 16 changes from the human peptide. Porcine calcitonin is also available.

hypothesis for their formation invokes tyrosine residues in the protein thyroglobulin, which are iodinated to mono- and di-iodotyrosine, with suitably placed residues reacting together by a phenolic oxidative coupling process (Figure 7.7). Re-aromatization results in cleavage of the side-chain of one residue, and thyroxine (or triiodothyronine) is released from the protein by proteolytic cleavage. Alternative mechanisms have been proposed, however. **Calcitonin*** (Figure 7.8) is also produced in the thyroid gland, and is involved in the regulation of bone turnover and maintenance of calcium balance. This is a relatively simple peptide structure containing a disulphide bridge.

Hypothalamic Hormones

Hypothalamic hormones (Figure 7.9) can modulate a wide variety of actions throughout the body, via the regulation of anterior pituitary hormone secretion. **Thyrotrophin-releasing hormone** (**TRH**)* is a tripeptide with an *N*-terminal pyroglutamyl residue, and a *C*-terminal prolineamide, whilst **luteinizing hormone-releasing hormone** (**LH-RH**)* is a straight-chain decapeptide that also has both *N*- and *C*-termini blocked, through pyroglutamyl and glycineamide respectively. **Somatostatin*** (**growth hormone-release inhibiting factor**; **GHRIH**) merely displays a disulphide bridge in its 14-amino acid chain. One of the largest of the hypothalamic hormones is **corticotropin-releasing hormone** (**CRH**) which contains 41 amino acids, with only the *C*-terminal blocked as an amide. This hormone controls release from the anterior pituitary of **corticotropin** (**ACTH**) (see page 414), which in turn is responsible for production of corticosteroids.



Glp–His–Pro–NH_2

thyrotrophin-releasing hormone (TRH)
(protirelin)

Tyr–Ala–Asp–Ala–Ile–Phe–Thr–Asn–Ser–Tyr–Arg–Lys–Val–Leu–Gly–Gln–Leu–Ser–Ala–Arg–Lys–Leu–Leu–Gln–Asp–Ile–Met–Ser–Arg–NH_2

sermorelin

Ala–Gly–Cys–Lys–Asn–Phe–**Phe**–**Trp**
|
Cys–Ser–Thr–Phe–**Thr**–**Lys**

growth hormone-release inhibiting factor (GHRIH)
(somatostatin)

Ser–Gln–Glu–Pro–Pro–Ile–Ser–Leu–Asp–Leu–Thr–Phe–His–Leu–Leu–Arg–Glu–Val–Leu–Glu–Met–Thr–Lys–Ala–Asp–Gln–Leu–Ala–Gln–Gln–Ala–His–Ser–Asn–Arg–Lys–Leu–Leu–Asp–Ile–Ala–NH_2

corticotropin-releasing hormone (CRH)

Glp–His–Trp–Ser–Tyr–**Gly**–Leu–Arg–Pro–**Gly**–NH_2

luteinizing hormone-releasing factor (LH-RH)
(gonadorelin)

Glp–His–Trp–Ser–Tyr–**D-Trp**–Leu–Arg–Pro–Gly–NH_2

triptorelin

Glp–His–Trp–Ser–Tyr–**D-Ser(**^{t}**Bu)**–Leu–Arg–Pro–NHEt

buserelin

Glp–His–Trp–Ser–Tyr–**D-Ser(**^{t}**Bu)**–Leu–Arg–Pro–NH–NH–$CONH_2$

goserelin

Glp–His–Trp–Ser–Tyr–**D-Leu**–Leu–Arg–Pro–NHEt

leuprorelin

Glp–His–Trp–Ser–Tyr–**3-(2-naphthyl)-D-Ala**–Leu–Arg–Pro–Gly–NH_2

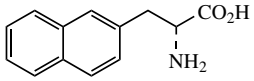
nafarelin

D-Phe–Cys–**Phe**–**D-Trp**
|
Thr(ol)–Cys–**Thr**–**Lys**

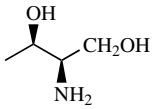
octreotide

3-(2-naphthyl)-D-Ala– Cys–**Tyr**–**D-Trp**
|
H_2N–Thr–Cys–**Val**–**Lys**

lanreotide



3-(2-naphthyl)-D-Ala



Thr(ol)

Figure 7.9

Thyrotrophin-Releasing Hormone

Thyrotrophin-releasing hormone (**TRH**) (Figure 7.9) acts directly on the anterior pituitary to stimulate release of thyroid-stimulating hormone (TSH), prolactin, and growth hormone. Many other hormones may be released by direct or indirect effects. Synthetic material (known as **protirelin**) is used to assess thyroid function and TSH reserves.

Luteinizing Hormone-Releasing Hormone

Luteinizing hormone-releasing hormone (**LH-RH**) (also gonadotrophin-releasing hormone, GnRH) (Figure 7.9) is the mediator of gonadotrophin secretion from the anterior pituitary, stimulating both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release (see page 415). These are both involved in controlling male and female reproduction, inducing the production of oestrogens and progestogens in the female, and of androgens in the male. LH is essential for causing ovulation, and for the development and maintenance of the corpus luteum in the ovary, whilst FSH is required for maturation of both ovarian follicles in women, and of the testes in men.

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Gonadorelin is synthetic LH-RH and is used for the assessment of pituitary function, and also for the treatment of infertility, particularly in women. Analogues of LH-RH, e.g. **triptorelin**, **buserelin**, **goserelin**, **leuprorelin**, and **nafarelin** (Figure 7.9), have been developed and find use to inhibit ovarian steroid secretion, and to deprive cancers such as prostate and breast cancers of essential steroid hormones. All of these analogues include a D-amino acid residue at position 6, and modifications to the terminal residue 10, including omission of this residue in some cases. Such changes increase activity and half-life compared with gonadorelin, e.g. leuprorelin is 50 times more potent, and half-life is increased from 4 minutes to 3–4 hours.

Growth Hormone-Releasing Hormone/Factor

Growth hormone-releasing hormone/factor (**GHRH/GHRF**) contains 40–44 amino acid residues and stimulates secretion of growth hormone (see page 414) from the anterior pituitary. Synthetic material containing the first 29-amino acid sequence has the full activity and potency of the natural material. This peptide (**sermorelin**) (Figure 7.9) is used as a diagnostic aid to test the secretion of growth hormone.

Somatostatin

Somatostatin (**growth hormone-release inhibiting factor**; **GHRIH**) (Figure 7.9) is a 14-amino acid peptide containing a disulphide bridge, but is derived from a larger precursor protein. It is found in the pancreas and gastrointestinal tract as well as in the hypothalamus. It inhibits the release of growth hormone (see page 414) and thyrotrophin from the anterior pituitary, and also secretions of hormones from other endocrine glands, e.g. insulin and glucagon. Receptors for somatostatin are also found in most carcinoid tumours. Somatostatin has a relatively short duration of action (half-life 2–3 minutes), and the synthetic analogue **octreotide** (Figure 7.9), which is much longer acting (half-life 60–90 minutes), is currently in drug use for the treatment of various endocrine and malignant disorders, especially treatment of neuroendocrine tumours and the pituitary-related growth condition acromegaly.

Octreotide contains only eight residues, but retains a crucial Phe–Trp–Lys–Thr sequence, even though the Trp now has the D-configuration. This prevents proteolysis between Trp and Lys, a major mechanism in somatostatin degradation. The *C*-terminus is no longer an amino acid, but an amino alcohol related to threonine. Radiolabelled octreotide has considerable potential for the visualization of neuroendocrine tumours. **Lanreotide** (Figure 7.9) is a recently introduced somatostatin analogue used in a similar way to octreotide. Lanreotide retains only a few of the molecular characteristics of octreotide, with the Phe–D-Trp–Lys–Thr sequence now modified to Tyr–D-Trp–Lys–Val and the *N*-terminal amino acid is the synthetic analogue 3-(2-napthyl)-D-alanine, also seen in the LH-RH analogue nafarelin.

Anterior Pituitary Hormones

The main hormones of the anterior pituitary are **corticotropin*** and **growth hormone***, which each consist of a single long polypeptide chain, and the **gonadotrophins***, which are glycoproteins containing two polypeptide chains. These hormones regulate release of glucocorticoids, human growth, and sexual development respectively. A further hormone, **prolactin***, which controls milk production in females, is structurally related to growth hormone.

Posterior Pituitary Hormones

The two main hormones of the posterior pituitary are **oxytocin***, which contracts the smooth muscle of the uterus, and **vasopressin***,

Corticotropin

Corticotropin (**corticotrophin; adrenocorticotrophin**; **ACTH**) is a straight-chain polypeptide with39 amino acid residues, and its function is to control the activity of the adrenal cortex, particularly the production of corticosteroids. Secretion of the hormone is controlled by corticotropin-releasing hormone (CRH) from the hypothalamus. ACTH was formerly used as an alternative to corticosteroid therapy in rheumatoid arthritis, but its value was limited by variable therapeutic response. ACTH may be used to test adrenocortical function. It has mainly been replaced for this purpose by the synthetic analogue**tetracosactide** (**tetracosactrin**) (Figure 7.10), which contains the first 24 amino acid residues of ACTH, and is preferred because of its shorter duration of action and lower allergenicity.

Ser–Tyr–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Val–Gly–Lys–Lys–Arg–Arg–Pro–Val–Lys–Val–Tyr–Pro

tetracosactide (tetracosactrin)

Figure 7.10

Growth Hormone

Growth Hormone (**GH**) (**human growth hormone** (**HGH**) or **somatotrophin**) is necessary for normal growth characteristics, especially the lengthening of bones during development. A lack of HGH in children results in dwarfism, whilst continued release can lead to gigantism, or acromegaly, in which only the bones of the hands, feet, and face continue to grow. Growth hormones from animal sources are very species specific, so it has not been possible to use animal hormones for drug use. HGH contains 191 amino acids with two disulphide bridges, one of which creates a large loop (bridging residues 53 and 165) and the other a very small loop (bridging residues 182 and 189) near the *C*-terminus. It is synthesized via a prohormone containing 26 extra amino acids. Production of material with the natural amino acid sequence has become possible as a result of recombinant DNA technology; this is termed **somatropin**. This drug is used to improve linear growth in patients whose short stature is known to be caused by a lack of pituitary growth hormone. There is also some abuse of the drug by athletes wishing to enhance performance. Although HGH increases skeletal mass and strength, its use can result in some abnormal bone growth patterns.

Prolactin

Prolactin has structural similarities to growth hormone, in that it is a single-chain polypeptide (198 amino acid residues) with three loops created by disulphide bonds. It is synthesized via a prohormone containing 29 extra amino acids. Growth hormone itself can bind to the prolactin receptor, but this is not significant under normal physiological conditions. Prolactin release is controlled by dopamine produced by the hypothalamus, and its main function is to control milk production. Prolactin has a synergistic action with oestrogen to promote mammary tissue proliferation during pregnancy, then at parturition, when oestrogen levels fall, prolactin levels rise and lactation is initiated. New nursing mothers have high levels of prolactin and this also inhibits gonadotrophin release and/or the response of the ovaries to these hormones. As a

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result, ovulation does not usually occur during breast feeding, preventing further conception. No prolactin derivatives are currently used in medicine, but the ergot alkaloid derivatives bromocriptine and cabergoline (see page 375) are dopamine agonists employed to inhibit prolactin release by pituitary tumours. They are not now recommended for routine suppression of lactation.

Gonadotrophins

Follicle-stimulating hormone (**FSH**) and **luteinizing hormone** (**LH**) are involved in controlling both male and female reproduction (see LH-RH, page 412). These are glycoproteins both composed of two polypeptide chains of 89 and 115 amino acid residues. The shorter chains are essentially identical, and differences in activity are caused by differences in the longer chain. Each chain has asparagine-linked oligosaccharide residues: the short chains each have one, the long chains two in the case of FSH or one for LH. FSH and LH together (**human menopausal gonadotrophins; menotrophin**) are purified from the urine of post-menopausal women and used in the treatment of female infertility. FSH alone is also available for this purpose, either natural material from urine termed **urofollitropin** (**urofollitrophin**), or the recombinant proteins **follitropin alfa** and **follitropin beta**. **Chorionic gonadotrophin** (**human chorionic gonadotrophin**; **HCG**) is a gonad-stimulating glycoprotein hormone which is obtained for drug use from the urine of pregnant women. It may be used to stimulate testosterone production in males with delayed puberty.

the antidiuretic hormone. These nonapeptides containing a disulphide bridge are structurally very similar, differing in only two amino acid residues (Figure 7.11). Structurally related peptides are classified as belonging to the vasopressin family when the amino acid residue at position 8 is basic, e.g. Arg or Lys, or to the oxytocin family when this amino acid is neutral.

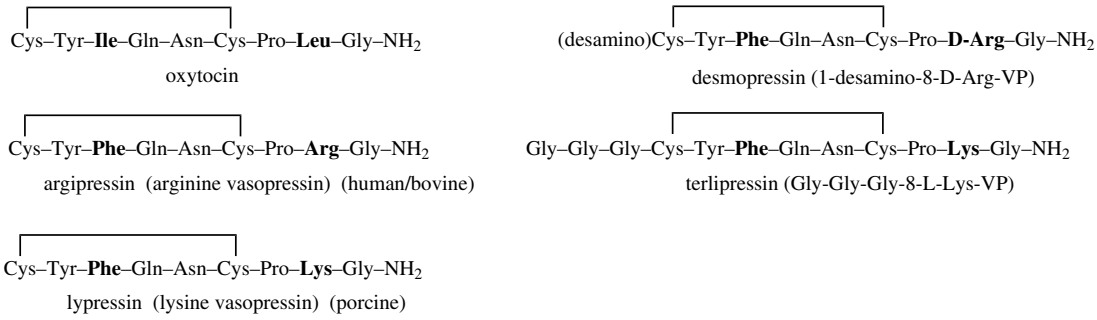

Figure 7.11

Oxytocin

Oxytocin (Figure 7.11) stimulates the pregnant uterus, causing contractions, and also brings about ejection of milk from the breasts. It thus plays a major role in the normal onset of labour at the end of pregnancy. Oxytocin for drug use is produced by synthesis, and is employed to induce or augment labour, as well as to minimize subsequent blood loss.

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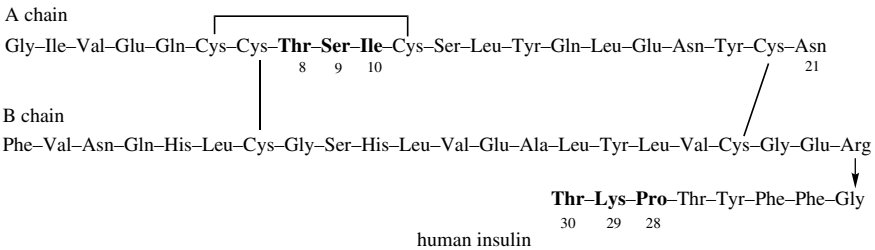
Vasopressin

Vasopressin (**antidiuretic hormone**, **ADH**) is a hormone that has an antidiuretic action on the kidney, regulating the reabsorption of water. A deficiency in this hormone leads to diabetes insipidus, where the patient suffers increased urine output and intense thirst, typically consuming enormous quantities of fluid. Vasopressin is used to treat this condition. At high dosage, vasopressin promotes contraction of arterioles and capillaries, and brings about an increase in blood pressure. The structure of human and bovine vasopressin (arginine vasopressin; **argipressin**) (Figure 7.11) differs from that of oxytocin only in two amino acid residues. Lysine vasopressin (**lypressin**) (Figure 7.11) from pigs differs from arginine vasopressin in the second amino acid from the *C*-terminus. Both bovine and porcine peptides have been used medicinally, but these have been replaced by synthetic materials. The 1-desamino-8-D-Arg-vasopressin analogue **desmopressin** (Figure 7.11) has a longer duration of action than vasopressin and may also be administered orally. In contrast to the natural hormone, desmopressin has no vasoconstrictor effect. **Terlipressin** (Figure 7.11) is a lypressin pro-drug in which the polypeptide chain has been extended by three glycine residues. Enzymic hydrolysis liberates lypressin. It is mainly used for control of oesophageal bleeding.

Pancreatic Hormones

The hormone **insulin*** (Figure 7.12) plays a key role in the regulation of carbohydrate, fat, and protein metabolism. In particular, it has a hypoglycaemic effect, lowering the levels of glucose in the blood. A deficiency in insulin synthesis leads to the condition diabetes, treatment of which requires daily injections of insulin. Insulin is composed of two straight chain polypeptides joined by disulphide bridges. This structure is known to arise from a straight chain polypeptide preproinsulin containing 100 amino acid residues. This loses a 16-residue portion of its chain, and forms proinsulin with disulphide bridges connecting the terminal portions of the chain in a loop (Figure 7.13). A central portion of the loop (the C chain) is then cleaved out, leaving the A chain (21 residues) bonded to the B chain (30 residues) by two disulphide bridges. This is the resultant insulin. Mammalian insulins (Figure 7.12) from different sources are very similar, showing variations in the sequence



human insulin

	A			B		
	8	9	10	28	29	30
insulin (human)	Thr	Ser	Ile	Pro	Lys	Thr
insulin (porcine)	Thr	Ser	Ile	Pro	Lys	**Ala**
insulin (bovine)	Ala	Ser	**Val**	Pro	Lys	**Ala**
insulin lispro	Thr	Ser	Ile	**Lys**	**Pro**	Thr
insulin aspart	Thr	Ser	Ile	**Asp**	Lys	Thr

His–Ser–Gln–Gly–Thr–Phe–Thr–Ser–Asp–Tyr–
Ser–Lys–Tyr–Leu–Asp–Ser–Arg–Arg–Ala–Gln–
Asp–Phe–Val–Gln–Trp–Leu–Met–Asn–Thr

glucagon

Figure 7.12

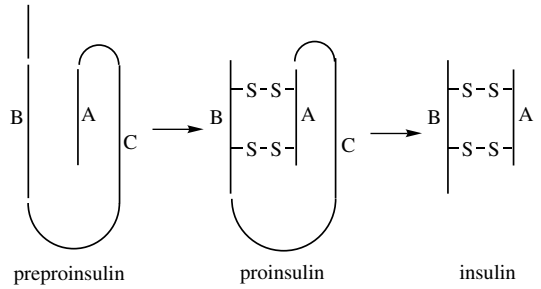


Figure 7.13

of amino acid residues 8–10 in chain A, and at amino acid 30 in chain B. **Glucagon*** (Figure 7.12) is a straight-chain polypeptide hormone containing 29 amino acids that is secreted by the pancreas when blood sugar levels are low, thus stimulating breakdown of glycogen in the liver. Unlike insulin, its structure is identical in all animals.

Interferons

The **interferons*** are a family of proteins secreted by animal cells in response to viral and parasitic infections, and are part of the host's defence mechanism. They display multiple activities, affecting the functioning of the immune system, cell proliferation, and cell differentiation, primarily by inducing the synthesis of other proteins. Accordingly, they have potential as antiviral, antiprotozoal, immunomodulatory, and cell growth regulatory agents.

Opioid Peptides

Although the pain-killing properties of morphine and related compounds have been known for a considerable time (see page 329), the existence of endogenous peptide ligands for the receptors to which these compounds bind is a more recent discovery. It is now appreciated that the body

Insulin

Insulin is a hormone produced by the pancreas that plays a key role in the regulation of carbohydrate, fat, and protein metabolism. In particular, it has a hypoglycaemic effect, lowering the levels of glucose in the blood. If a malfunctioning pancreas results in a deficiency in insulin synthesis or secretion, the condition known as diabetes mellitus ensues. This results in increased amounts of glucose in the blood and urine, diuresis, depletion of carbohydrate stores, and subsequent breakdown of fat and protein. Incomplete breakdown of fat leads to the accumulation of ketones in the blood, severe acidosis, coma, and death. Where the pancreas is still functioning, albeit less efficiently, the condition is known as type 2 diabetes (non-insulin-dependent diabetes, NIDDM) and can be controlled by a controlled diet or oral antidiabetic drugs. In type 1 diabetes (insulin-dependent diabetes, IDDM) pancreatic cells no longer function, and injections of insulin are necessary, one to four times daily, depending on the severity of the condition. These need to be combined with a controlled diet and regular monitoring of glucose levels, but do not cure the disease, so treatment is lifelong. Mammalian insulins from different sources are very similar and may be used to treat diabetes. **Porcine insulin** and **bovine insulin** (Figure 7.12) are extracted from the pancreas of pigs and cattle respectively. **Human insulin** (Figure 7.12) is produced by the use of recombinant DNA technology in *Escherichia coli* to obtain the two polypeptide chains, and linking these chemically to form the disulphide bridges (such material is coded 'crb'), or by modification of proinsulin produced in genetically modified *E. coli* (coded 'prb'). Human insulin may also be obtained from porcine insulin by semi-synthesis, replacing the terminal alanine in chain B with threonine by enzymic methods (coded 'emp'). Human insulin does not appear to be less immunogenic than animal insulin, but genetic engineering offers significant advantages over animal sources for obtaining highly purified material. Insulin may be provided in a rapid-acting soluble form, as suspensions of the zinc complex which have longer duration, or as suspensions with protamine

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(as **isophane insulin**). **Protamine** is a basic protein from the testes of fish of the salmon family, e.g. *Salmo* and *Onchorhynchus* species, which complexes with insulin, thereby reducing absorption and providing a longer period of action. Recently introduced recombinant human insulin analogues **insulin lispro** and **insulin aspart** have a faster onset and a shorter duration of action than soluble insulin. Insulin lispro has the reverse sequence for the 28 and 29 amino acids in the B chain, i.e. B28-Lys–B29-Pro, whilst insulin aspart has a single substitution of aspartic acid for proline at position 28 in the B chain (Figure 7.12). These changes in the primary structure affect the tendency of the molecule to associate into dimers and larger oligomers, thus increasing the availability of absorbable monomers. Insulin aspart is produced by expression in *Saccharomyces cerevisiae* (baker's yeast). **Insulin glargine** is a new ultra-long-acting analogue that differs from human insulin by replacing the terminal asparagine at position 21 in chain A with glycine, and also adding two arginines to the end of the B chain, i.e. positions B31 and B32. These changes result in enhanced basicity, causing precipitation at neutral pH post-injection, and consequently a delayed, very gradual and prolonged activity profile (up to 24–48 hour duration of action) and allowing once-daily dosing.

Glucagon

Glucagon (Figure 7.12) is a straight-chain polypeptide hormone containing 29 amino acids that is secreted by the pancreas when blood sugar levels are low, thus stimulating breakdown of glycogen in the liver. It may be isolated from animal pancreas, or be produced by recombinant DNA processes using *Saccharomyces cerevisiae*. It may be administered for the emergency treatment of diabetes patients suffering from hypoglycaemia as a result of building up a dangerously high insulin level. Normally, a patient would counter this by eating some glucose or sucrose, but hypoglycaemia can rapidly cause unconsciousness, requiring very prompt action.

produces a family of endogenous opioid peptides, which bind to a series of receptors in different locations. These peptides include **enkephalins**, **endorphins**, and **dynorphins**, and are produced primarily, but not exclusively, in the pituitary gland. The pentapeptides **Met-enkephalin** and **Leu-enkephalin** (Figure 7.14) were the first to be characterized. The largest peptide is **β-endorphin** (‘*end*ogenous m*orphine*’) (Figure 7.14), which is several times more potent than morphine in relieving pain. Although β-endorphin contains the sequence for Met-enkephalin, the latter peptide and Leu-enkephalin are derived from a larger peptide proenkephalin A, whilst β-endorphin itself is formed by cleavage of pro-opiomelanocortin. The proenkephalin A structure contains four

Interferons

Interferons were originally discovered as proteins that interfered with virus replication. When mice were injected with antibodies to interferons, they became markedly susceptible to virus-mediated disease, including virus-related tumour induction. Interferons can be detected at low levels in most human tissues, but amounts increase upon infection with viruses, bacteria, protozoa, and exposure to certain growth factors. Interferons were initially classified according to the cellular source, but recent nomenclature is based primarily on sequencing data. Thus leukocyte interferon (a mixture of proteins) is now known as interferon alfa, fibroblast interferon as interferon beta, and immune interferon as interferon gamma.

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These typically range in size from 165 to 172 amino acid residues. The interferon system can often impair several steps in viral replication, and can modulate the immune system, and affect cell growth and differentiation. They have potential in treating many human diseases, including leukaemias and solid tumours, and viral conditions such as chronic infection with hepatitis B and C. Much of their current drug use is still research based. **Interferon alfa** is a protein containing 166 amino acid residues with two disulphide bridges and is produced by recombinant DNA techniques using *Escherichia coli*, or by stimulation of specific human cell lines. Variants with minor differences in sequence may be obtained according to the gene used, and are designated as alfa-2a, alfa-2b, etc. Interferon alfa is employed as an antitumour drug against certain lymphomas and solid tumours, and in the management of chronic hepatitis. **Interferon beta** is of value in the treatment of multiple sclerosis patients, though not all patients respond. Variants are designated beta-1a (a glycoprotein with 165 amino acid residues and one disulphide bridge) or beta-1b (a non-glycosylated protein with 164 amino acid residues and one disulphide bridge). **Interferon gamma** produced by recombinant DNA methods contains an unbridged polypeptide chain, and is designated gamma-1a (146 residues) or gamma-1b (140 residues) according to sequence. The immune interferon interferon gamma-1b is used to reduce the incidence of serious infection in patients with chronic granulomatous disease.

Met-enkephalin sequences and one of Leu-enkephalin. The dynorphins, e.g. **dynorphin A** (Figure 7.14) are also produced by cleavage of a larger precursor, namely proenkephalin B (prodynorphin), and all contain the Leu-enkephalin sequence. Some 20 opioid ligands have now been characterized. When released, these endogenous opioids act upon specific receptors, inducing analgesia, and depressing respiratory function and several other processes. The individual peptides have relatively high specificity towards different receptors. It is known that morphine, β-endorphin, and Met-enkephalin are agonists for the same site. The opioid peptides are implicated in analgesia brought about by acupuncture, since opiate antagonists can reverse the effects. The hope of exploiting similar peptides as ideal, non-addictive analgesics has yet to be attained; repeated doses of endorphin or enkephalin produce addiction and withdrawal symptoms.

Enzymes

Enzymes are proteins that act as biological catalysts. They facilitate chemical modification of substrate molecules by virtue of their specific binding properties, which arise from particular combinations of functional groups in the constituent amino acids at the so-called active site. In many cases, an essential cofactor, e.g. NAD^+, PLP, or TPP, may also be bound to participate in the transformation. The involvement of enzymes in biochemical reactions has been a major theme throughout this book. The ability of enzymes to carry out quite complex chemical reactions, rapidly, at room temperature, and under essentially neutral conditions is viewed with envy by synthetic chemists, who are making rapid progress in harnessing this ability for their own uses. Several enzymes are currently of importance commercially, or for medical use, and

Tyr–Gly–Gly–Phe–Met–Thr–Ser–Glu–Lys–Ser–Gln–Thr–Pro–Leu–Val–Thr–Leu–Phe–Lys–Asn–Ala–Ile–Val–Lys–Asn–Ala–His–Lys–Lys–Gly–Gln

β-endorphin

Tyr–Gly–Gly–Phe–Met
Met-enkephalin

Tyr–Gly–Gly–Phe–Leu
Leu-enkephalin

Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Ile–Arg–Pro–Lys–Leu–Lys–Trp–Asp–Asn–Gln

dynorphin A

Figure 7.14

Table 7.2 Pharmaceutically important enzymes

Enzyme	Action	Source	Use
Hydrolytic enzymes			
Pancreatin	Hydrolysis of starch (amylase), fat (lipase), and protein (protease)	Porcine pancreas	Digestive aid
Papain	Hydrolysis of proteins	Papaya fruit (*Carica papaya*; Caricaceae)	Meat tenderizer; cleaning of contact lenses
Chymotrypsin	Hydrolysis of proteins	Bovine pancreas	Zonal lysis in cataract removal
Hyaluronidase	Hydrolysis of mucopolysaccharides	Mammalian testes	Renders tissues more permeable for subcutaneous or intramuscular injections
Pepsin	Hydrolysis of proteins	Porcine stomach	Digestive aid
Trypsin	Hydrolysis of proteins	Bovine pancreas	Wound and ulcer cleansing
Fibrinolytic enzymes			
Streptokinase	No enzymic activity, until it complexes with and activates plasminogen in blood plasma to produce the proteolytic enzyme plasmin, which hydrolyses fibrin clots	*Streptococcus haemolyticus*	Treatment of venous thrombosis and pulmonary embolism
Urokinase	A protease which activates plasminogen in blood plasma to form plasmin, which hydrolyses fibrin clots	Human urine, or human kidney tissue cultures	Treatment of venous thrombosis and pulmonary embolism; thrombolysis in the eye
Alteplase (recombinant tissue-type plasminogen activator; rt-PA)	A protease which binds to fibrin converting it to a potent plasminogen activator; only active at the surface of the blood clot	Recombinant genetic engineering: human gene expressed in Chinese hamster ovary cells	Treatment of acute myocardial infarction
Anistreplase (acylated plasminogen-streptokinase activator complex; APSAC)	An inactive acylated form of the plasminogen–strepto-kinase activator complex; the acyl group (*p*-anisoyl) is slowly hydrolysed in the blood to give the active agent	Semi-synthesis from urokinase	Treatment of acute myocardial infarction
Reteplase	A fibrinolytic protease; a genetically-engineered human tissue-type plasminogen activator differing from alteplase at four amino acid residues	Recombinant genetic engineering	Treatment of acute myocardial infarction

(*Continues*)

Table 7.2 (*Continued*)

Enzyme	Action	Source	Use
Others			
Asparaginase (crisantaspase)	Degradation of L-asparagine	*Erwinia chrysanthemi*	Treatment of acute lymphoblastic leukaemia; results in death of those tumour cells which require increased levels of exogenous L-asparagine; side-effects nausea and vomiting, allergic reactions and anaphylaxis
Streptodornase	Depolymerization of polymerized deoxyribonucleo-proteins	*Streptococcus haemolyticus*	In combination with streptokinase as desloughing agent to cleanse ulcers and promote healing

these are described in Table 7.2. Enzymes are typically larger than most of the polypeptides discussed above, and are thus extracted from natural sources. Recombinant DNA procedures are likely to make a very significant contribution in the future.

NONRIBOSOMAL PEPTIDE BIOSYNTHESIS

In marked contrast to the ribosomal biosynthesis of peptides and proteins, where a biological production line interprets the genetic code, many natural peptides are known to be synthesized by a more individualistic sequence of enzyme-controlled processes, in which each amino acid is added as a result of the specificity of the enzyme involved. The many stages of the whole process appear to be carried out by a multi-functional enzyme (nonribosomal peptide synthase, NRPS) with a modular arrangement comparable to that seen with type I polyketide synthases (see page 114). The linear sequence of modules in the enzyme usually corresponds to the generated amino acid sequence in the peptide product. The amino acids are first activated by conversion into AMP esters, which then bind to the enzyme through thioester linkages. The residues are held so as to allow a sequential series of peptide bond formations (Figure 7.15 gives a simplified representation), until the peptide is finally released from the enzyme. A typical module consists of an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, and a condensation (C) or elongation domain. The A domain activates a specific amino acid as an aminoacyl adenylate, which is then transferred to the PCP domain forming an aminoacyl thioester. Pantothenic acid (vitamin B_5, see page 31) bound to the enzyme as pantatheine is used to carry the growing peptide chain through its thiol group (Figure 7.15). The significance of this is that the long 'pantatheinyl arm' allows different active sites on the enzyme to be reached in the chain assembly process (compare biosynthesis of fatty acids, page 36, and polyketides, page 62). Nucleophilic attack by the amino group of the neighbouring aminoacyl thioester is catalysed by the C domain and results in amide (peptide) bond formation. Enzyme-controlled biosynthesis in this manner is a feature of many microbial peptides, especially those containing unusual amino acids not encoded by DNA and where post-translational modification is unlikely, and also for the cyclic structures which are frequently encountered. As well as activating the amino acids and catalysing formation of the peptide linkages, the enzyme may possess other domains that are responsible for epimerizing L-amino acids to D-amino acids, probably through

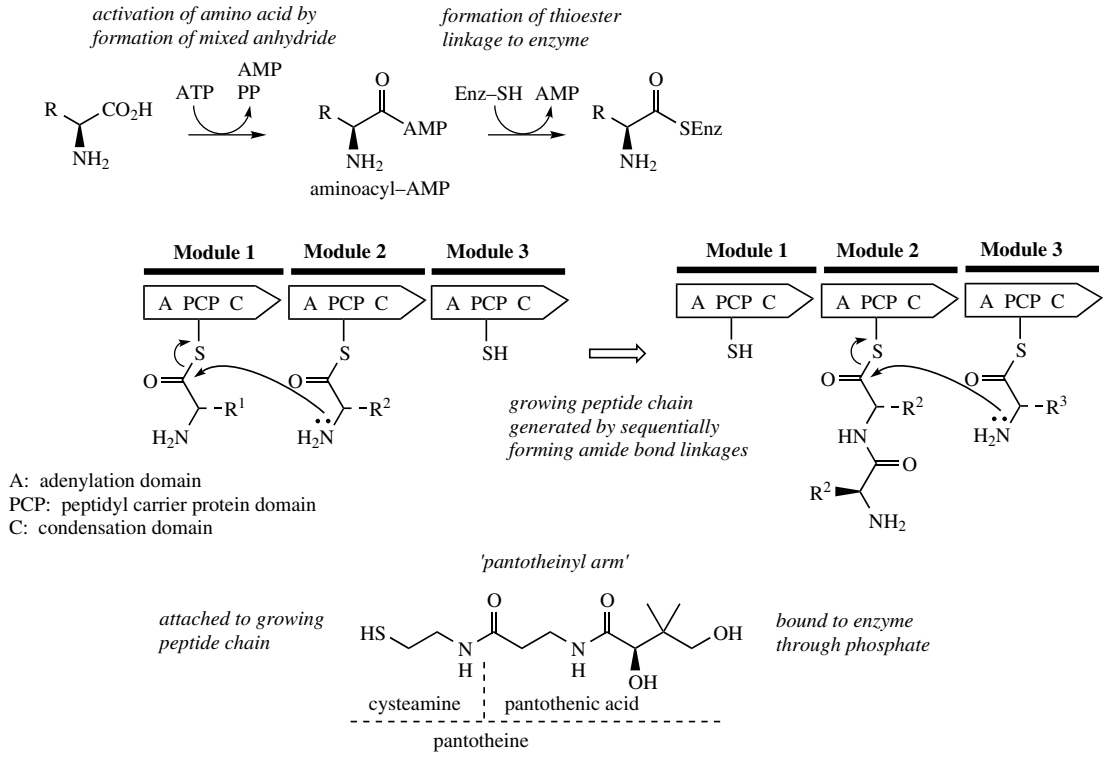


Figure 7.15

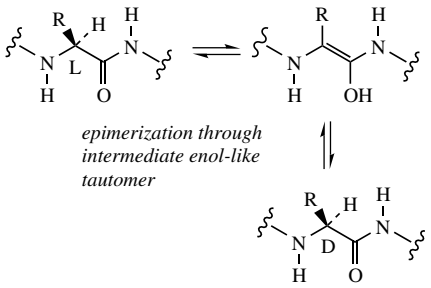


Figure 7.16

enol-like tautomers in the peptide (Figure 7.16). A terminal thioesterase domain is responsible for terminating chain extension and releasing the peptide from the enzyme. Many of the medicinally useful peptides have cyclic structures. Cyclization may result if the amino acids at the two termini of a linear peptide link up to form another peptide bond, but very often, ring formation can be the result of ester or amide linkages, which utilize side-chain functionalities in the constituent amino acids. As with polyketide synthases, genetic manipulation of nonribosomal peptide synthases allows production of peptide derivatives in which rational modifications may be programmed according to the genes encoded.

PEPTIDE ANTIBIOTICS

Cycloserine

D-**Cycloserine** (Figure 7.17) is produced by cultures of *Streptomyces orchidaceus*, or may be prepared synthetically, and is probably the simplest substance with useful antibiotic

(*Continues*)

activity. Cycloserine is water soluble and has a broad spectrum of antibacterial activity, but it is primarily employed for its activity against *Mycobacterium tuberculosis*. It behaves as a structural analogue of D-alanine, and inhibits the incorporation of D-alanine into bacterial cell walls. Since it can produce neurotoxicity in patients, it is reserved for infections resistant to first-line drugs.

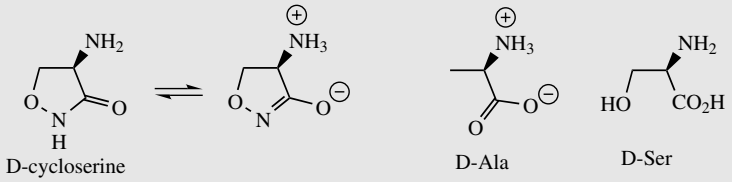
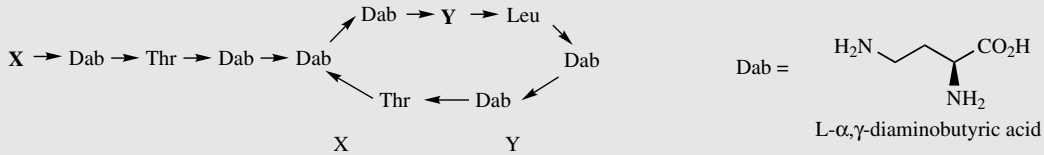


Figure 7.17

Polymyxins

The polymyxins are a group of cyclic polypeptide antibiotics produced by species of *Bacillus*. Polymyxins A–E were isolated from *Bacillus polymyxa*, though polymyxin B and polymyxin E were both subsequently shown to be mixtures of two components. A polypeptide mixture called colistin isolated from *Bacillus colistinus* was then found to be identical to polymyxin E. **Polymyxin B** and **colistin (polymyxin E)** are both used clinically. These antibiotic mixtures respectively contain principally polymyxin B_1 with small amounts of polymyxin B_2, or predominantly polymyxin E_1 (≡ colistin A) with small amounts of polymyxin E_2 (≡ colistin B) (Figure 7.18). These molecules contain ten amino acids, six of which are L-α,γ-diaminobutyric acid (L-Dab), with a fatty acid (6-methyloctanoic acid or 6-methylheptanoic acid) bonded to the *N*-terminus, and a cyclic peptide portion constructed via an amide bond between the carboxyl terminus and the γ-amino of one of the Dab residues. The γ-amino groups of the remaining Dab residues confer a strongly basic character to the antibiotics. This results in detergent-like properties and allows them to bind to and damage bacterial membranes. These peptides have been used for the treatment of infections with Gram-negative bacteria such as *Pseudomonas aeruginosa*, but are seldom used now because of



	X	Y
polymyxin B_1	6-methyloctanoic acid	D-Phe
polymyxin B_2	6-methylheptanoic acid	D-Phe
polymyxin E_1 (colistin A)	6-methyloctanoic acid	D-Leu
polymyxin E_2 (colistin B)	6-methylheptanoic acid	D-Leu

Figure 7.18

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neurotoxic and nephrotoxic effects. However, they are included in some topical preparations such as ointments, eye drops, and ear drops, frequently in combination with other antibiotics.

Bacitracins

Bacitracin is a mixture of at least nine peptides produced by cultures of *Bacillus subtilis*, with the principal component being bacitracin A (Figure 7.19). The structure contains a cyclic peptide portion, involving the carboxyl terminus and the ε-amino of lysine, and at the *N*-terminus an unusual thiazolinecarboxylic acid, which is a condensation product from isoleucine and cysteine residues (compare epothilones, page 105, and bleomycin, page 429). Bacitracin is active against a wide range of Gram-positive bacteria, and appears to affect biosynthesis of the bacterial cell wall by binding to and sequestering a polyprenyl diphosphate carrier of intermediates; this binding also requires a divalent metal ion, with zinc being especially active. It is rarely used systemically because some bacitracin components are nephrotoxic, but as zinc bacitracin, it is a component of ointment formulations for topical application. The vast majority of bacitracin manufactured is used at subtherapeutic doses as an animal feed additive, to increase feed efficiency, and at therapeutic dosage to control a variety of disorders in poultry and animals.

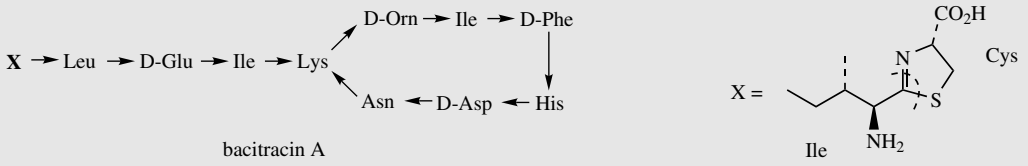

Figure 7.19

Tyrothricin and Gramicidins

Tyrothricin is a mixture of polypeptide antibiotics produced by cultures of *Bacillus brevis*. The mixture contains about 20–30% linear polypeptides called gramicidins (Figure 7.20), and 70–80% of cyclic structures called tyrocidines (Figure 7.20). Tyrothricin is active against many Gram-positive bacteria, with the linear gramicidins being more active than the cyclic tyrocidines. The two groups are readily separated by solvent fractionation, and the **gramicidin** fraction, also termed gramicidin D, a mixture of at least eight closely related compounds, is used principally in ophthalmic preparations. The gramicidins are neutral polypeptides having the *N*-terminal amino group formylated, and the carboxy group linked to ethanolamine. Most of the gramicidin mixture is composed of valine-gramicidin A (about 80%) (Figure 7.20). Apart from the glycine residue, these compounds have a sequence of alternating D- and L-amino acids. Gramicidins act by producing ion channels in bacterial membranes. The tyrocidines are too toxic for therapeutic use on their own, but the tyrothricin mixture is incorporated into lozenges for relief of throat infections.

Gramicidin S is a mixture of cyclic peptides obtained from another strain of *Bacillus brevis*. Its main component is gramicidin S_1 (Figure 7.20), a symmetrical

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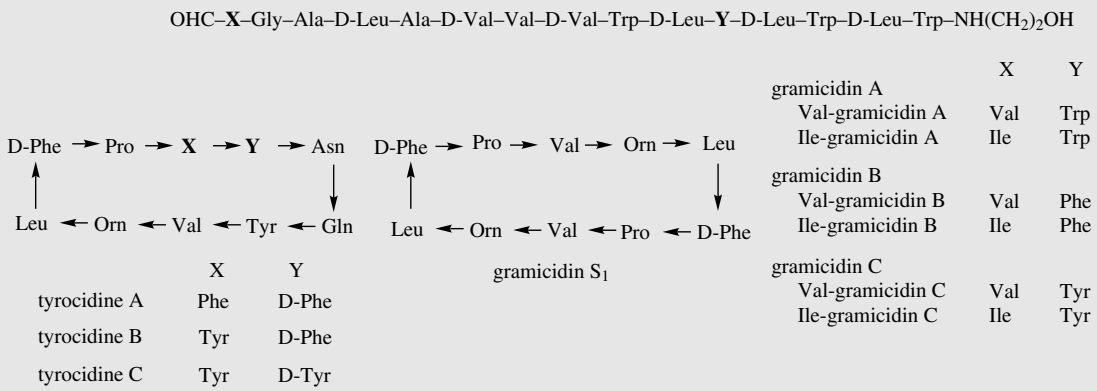

Figure 7.20

decapeptide known to be formed by the joining together of two separate chains. Gramicidin S is fairly toxic, and its use is restricted to topical preparations. Gramicidin S also acts on bacterial membranes, increasing permeability and loss of barrier function.

Capreomycin

Capreomycin is a mixture of cyclic polypeptides obtained from cultures of *Streptomyces capreolus*, and contains about 90% of capreomycins I, principally capreomycin IB (Figure 7.21). The capreomycin structures incorporate an L-capreomycidine moiety derived by cyclization of L-arginine, and three molecules of 2,3-diaminopropionic acid (Dap), which originate from serine via dehydroalanine. This antibiotic is given intramuscularly to treat tuberculosis patients who do not respond to first-line drugs, or where patients are sensitive to

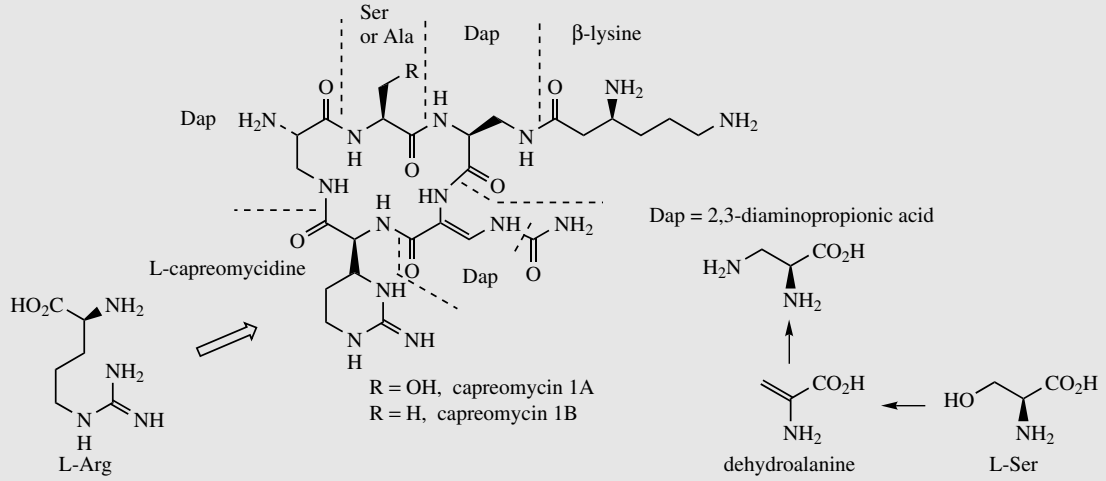

Figure 7.21

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streptomycin. It can cause irreversible hearing loss and impair kidney function. Capreomycin inhibits protein biosynthesis at the translocation step in sensitive bacteria.

Vancomycin and Teicoplanin

Vancomycin (Figure 7.22) is a glycopeptide antibiotic produced in cultures of *Amycolatopsis orientalis* (formerly *Streptomyces orientalis*), and has activity against Gram-positive bacteria, especially resistant strains of staphylococci, streptococci, and enterococci. It is an important agent in the control of methicillin-resistant *Staphylococcus aureus* (MRSA), with some strains now being sensitive only to vancomycin or teicoplanin (below). Vancomycin is not absorbed orally, and must be administered by intravenous injection. However, it can be given orally in the treatment of pseudomembranous colitis caused by *Clostridium difficile*, which may occur after administration of other antibiotics. Vancomycin acts by its ability to form a complex with terminal *N*-acyl–D-Ala–D-Ala residues of growing peptidoglycan chains (see Figure 7.36 and page 444), preventing their cross-linking to adjacent strands and thus inhibiting bacterial cell wall biosynthesis. The –D-Ala–D-Ala residues are accommodated in a 'carboxylate-binding pocket' in the vancomycin structure. By preventing peptidoglycan polymerization and cross-linking, it weakens the bacterial cell wall and ultimately causes cell lysis.

The novel feature of vancomycin, and several other related antibiotics, is the tricyclic structure generated by three phenolic oxidative coupling reactions. The β-hydroxychlorotyrosine and 4-hydroxyphenylglycine residues in vancomycin originate from L-tyrosine, but the 3,5-dihydroxyphenylglycine ring is actually acetate derived. These modified aromatic rings are presumably present in the heptapeptide before coupling occurs (Figure 7.22).

The teicoplanins (Figure 7.23) possess the same basic structure as vancomycin, but the *N*-terminal (4-hydroxyphenylglycine) and third (3,5-dihydroxyphenylglycine) amino acids are also aromatic, and this allows further phenolic oxidative coupling and generation of yet another ring system. **Teicoplanin** for drug use is a mixture of five teicoplanins produced by cultures of *Actinoplanes teichomyceticus*, which differ only in the nature and length of the fatty acid chain attached to the sugar residue. Teicoplanin has similar antibacterial activity to vancomycin, but has a longer duration of action, and may be administered by intramuscular as well as by intravenous injection. It is also used against Gram-positive pathogens resistant to established antibiotics.

Vancomycin, teicoplanin, and structurally related glycopeptides are often referred to as dalbaheptides (from *D-al*anyl–D-alanine-*b*inding *hepta*pept*ide*), reflecting their mechanism of action and their chemical nature. Unfortunately, with increasing use of vancomycin and teicoplanin, there have even been reports of these agents becoming ineffective because resistant bacterial strains have emerged, particularly in enterococci. In resistant strains, the terminal –D-Ala–D-Ala residues, to which the antibiotic normally binds, have become replaced by –D-Ala–D-lactate. The incorporation of D-lactate into the peptide intermediates results in loss of crucial hydrogen-bonding interactions and a thousand-fold lowering in binding efficiency.

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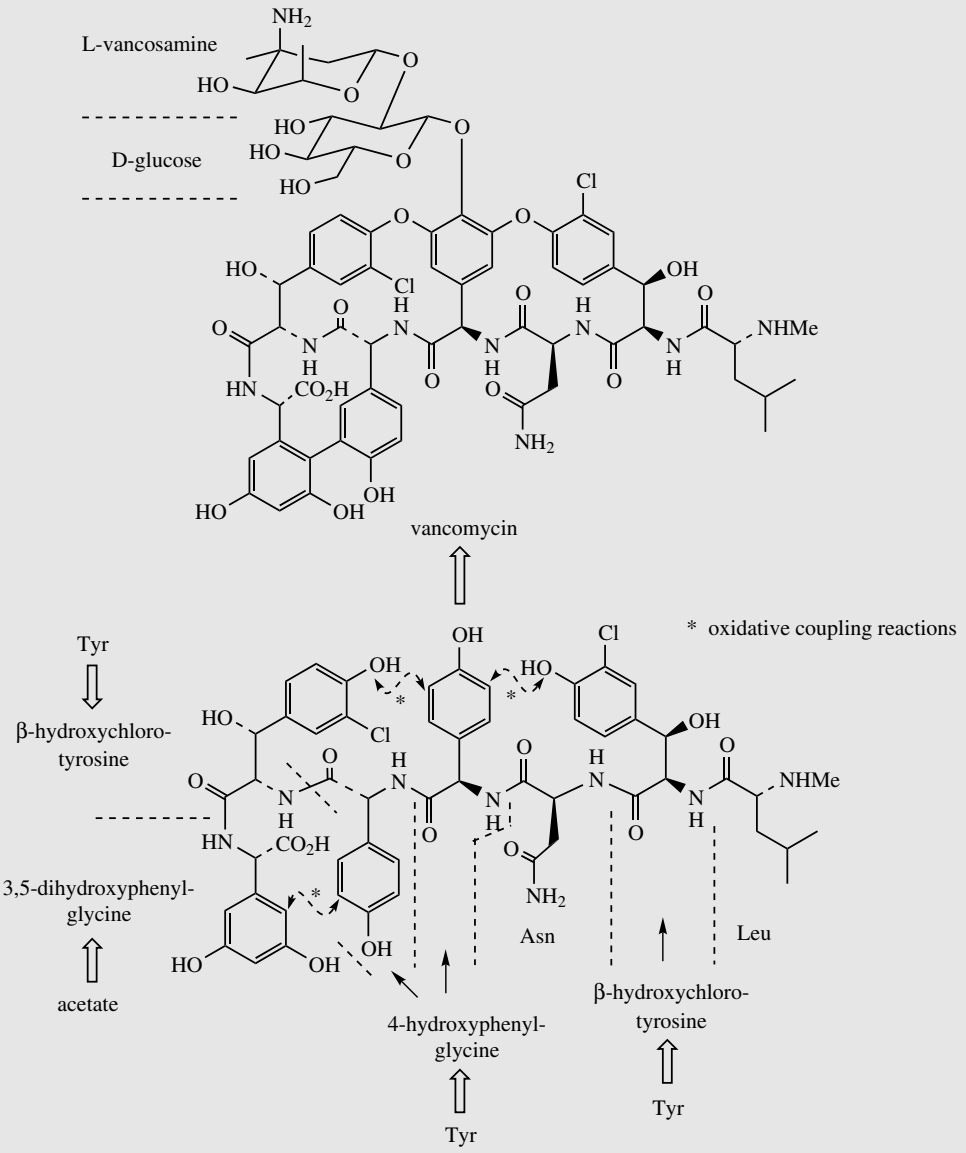

Figure 7.22

Bleomycin

Bleomycin is a mixture of glycopeptide antibiotics isolated from cultures of *Streptomyces verticillus*, used for its anticancer activity. The major component (55–70%) of the mixture

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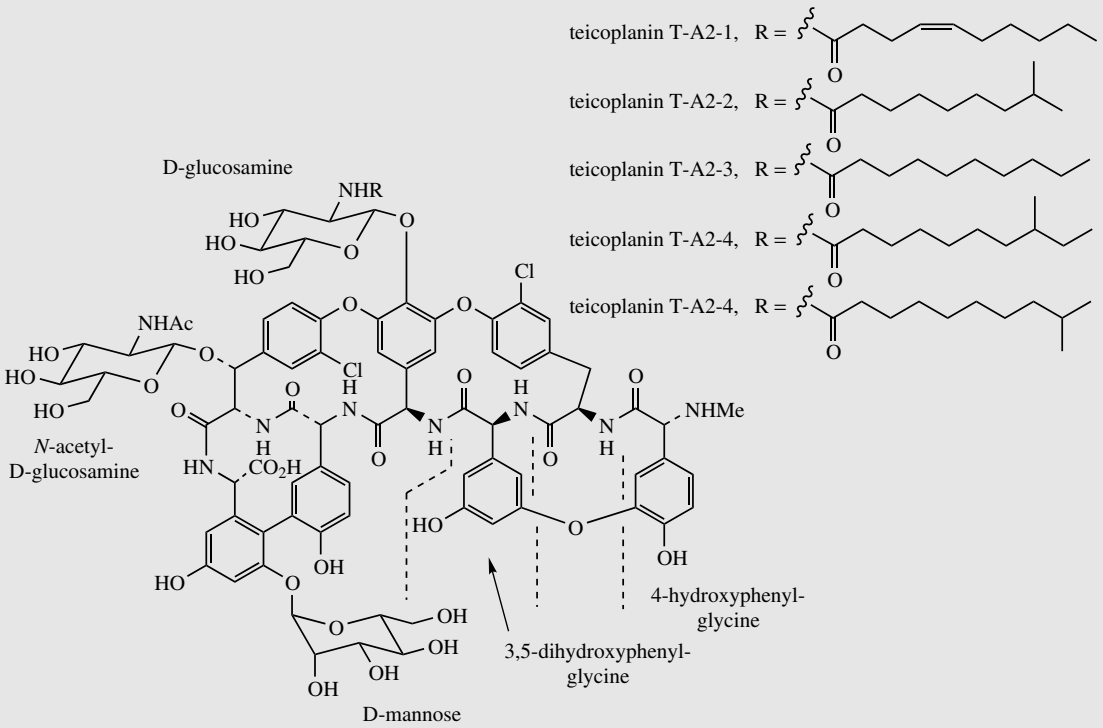

Figure 7.23

is bleomycin A_2 (Figure 7.24), with bleomycin B_2 constituting about 30%. The various bleomycins differ only in their terminal amine functions, the parent compound bleomycinic acid (Figure 7.24) being inactive. The molecules contain several unusual amino acids and sugars, an asparagine-derived pyrimidine ring, and a planar dithiazole ring system which has its origins in two cysteines (compare epothilones, page 105, and bacitracin, page 424). A C_2 unit supplied by malonyl-CoA also forms part of the main chain as a component of the amino acid 4-amino-3-hydroxy-2-methylvaleric acid, which in addition features a methionine-derived methyl group. Bleomycin is a DNA-cleaving drug, causing single and double-strand breaks in DNA. The dithiazole system is involved in binding to DNA, probably by intercalation, whilst other parts of the molecule near the *N*-terminus are involved in chelating a metal ion, usually Fe^{2+}, and oxygen, which are necessary for the DNA degradation reaction. More recently, bleomycin A_2 has been shown to cleave RNA as well as DNA.

Bleomycin is used alone, or in combination with other anticancer drugs, to treat squamous cell carcinomas of various organs, lymphomas, and some solid tumours. It is unusual amongst antitumour antibiotics in producing very little bone-marrow suppression, making it particularly useful in combination therapies with other drugs that do cause this response. However, there is some lung toxicity associated with bleomycin treatment. Various bleomycin analogues have been made by adding different precursor amines to the culture medium, or by semi-synthesis from bleomycinic acid. **Peplomycin** (Figure 7.24) is an example with some promise, in that it is more resistant to enzymes that cause *in vivo* hydrolysis of bleomycin at the *N*-terminal β-aminoalanine group.

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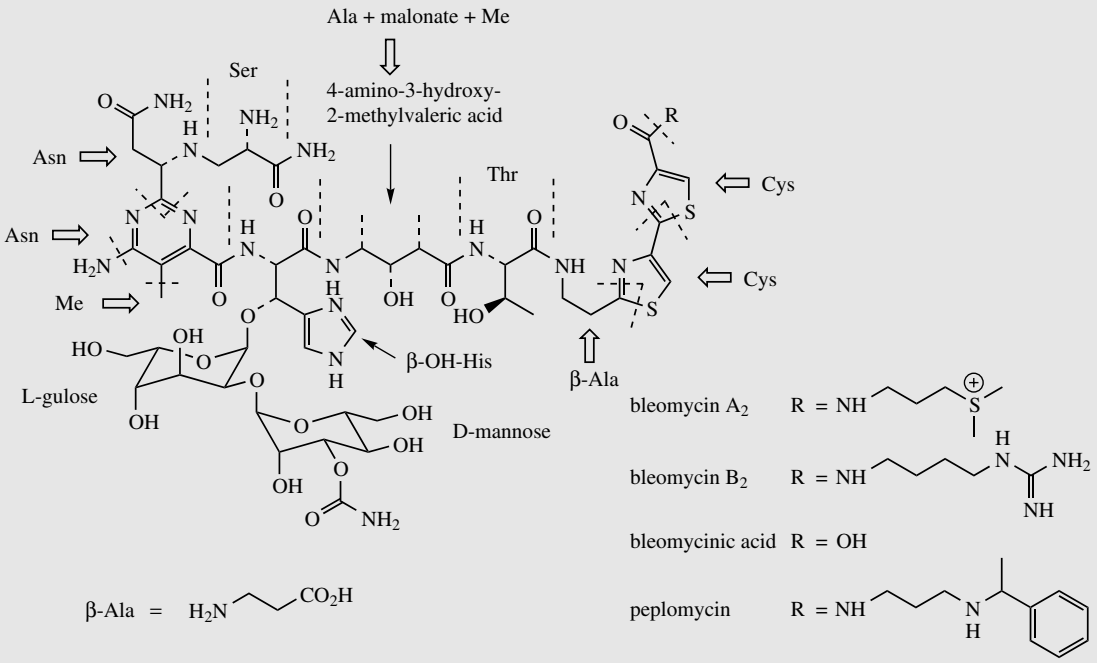

Figure 7.24

Cyclosporins

The cyclosporins are a group of cyclic peptides produced by fungi such as *Cylindrocarpon lucidum* and *Tolypocladium inflatum*. These agents showed a narrow range of antifungal activity, but high levels of immunosuppressive and anti-inflammatory activities. The main component from the culture extracts is cyclosporin A (**ciclosporin, cyclosporin**) (Figure 7.25), but some 25 naturally occurring cyclosporins have been characterized. Cyclosporin A contains several *N*-methylated amino acid residues, together with the less common L-α-aminobutyric acid and an *N*-methylated butenylmethylthreonine. This latter amino acid has been shown to originate via the acetate pathway, and it effectively comprises a C_8 polyketide chain, plus a methyl group from SAM. The sequence shown in Figure 7.26 is consistent with experimental data, and is analogous to other acetate-derived compounds (see Chapter 3). The assembly of the polypeptide chain is known to start from the D-alanine residue. Many of the other natural cyclosporin structures differ only with respect to a single amino acid (the α-aminobutyric acid residue) or the amount of *N*-methylation. Of all the natural analogues, and many synthetic ones produced, cyclosporin A is the most valuable for drug use. It is now widely exploited in organ and tissue transplant surgery, to prevent rejection following bone-marrow, kidney, liver, and heart transplants. It has revolutionized organ transplant surgery, substantially increasing survival rates in transplant patients. It may be administered orally or by intravenous injection, and the primary side-effect is nephrotoxicity, necessitating careful monitoring of kidney function. Cyclosporin A has the same mode of action as the macrolide FK-506 (tacrolimus) (see page 104) and is believed to inhibit T-cell activation in the immunosuppressive mechanism

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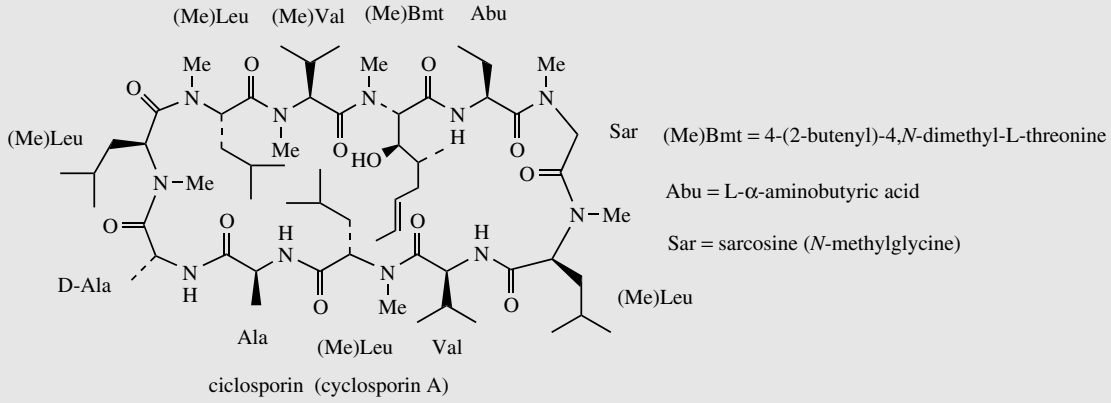

Figure 7.25

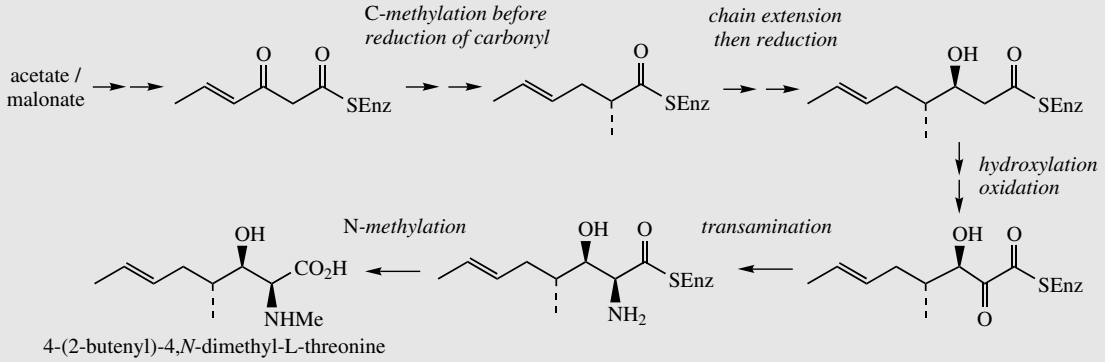

Figure 7.26

by first binding to a receptor protein, giving a complex that then inhibits a phosphatase enzyme called calcineurin. The resultant aberrant phosphorylation reactions prevent appropriate gene transcription and subsequent T-cell activation. Cyclosporin A also finds use in the specialist treatment of severe resistant psoriasis.

Streptogramins

The names streptogramin and virginiamycin have been applied to antibiotic mixtures isolated from strains of *Streptomyces virginiae*, and individual components have thus acquired multiple synonyms; as a family these antibiotics have now been termed streptogramin antibiotics. These compounds fall into two distinct groups, group A, containing a 23-membered unsaturated ring with peptide and lactone bonds, and group B, which are depsipeptides (essentially peptides cyclized via a lactone). These structures contain many nonprotein amino acids (Figure 7.27). Until recently, most commercial production of these antibiotics was directed towards animal feed additives, but the growing emergence of antibiotic-resistant

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bacterial strains has led to the drug use of some streptogramin antibiotics. Thus, **dalfopristin** and **quinupristin** (Figure 7.27) are water-soluble drugs that may be used in combination for treating infections caused by Gram-positive bacteria that have failed to respond to other antibiotics, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci and staphylococci, and drug-resistant *Streptococcus pneumoniae*. They may need to be combined also with other agents where mixed infections involve Gram-negative organisms. Dalfopristin is a semi-synthetic sulphonyl derivative of streptogramin A (also termed virginiamycin M1, mikamycin A, pristinamycin IIA, and other names), and quinupristin is a modified form of streptogramin B (also mikamycin B, pristinamycin IA, and other names). Members of the A group tend to be less powerful antibiotics than those of the B group, but together they act synergistically, providing greater activity than the combined activity expected from the separate components. The dalfopristin and quinupristin combination is supplied in a 70:30 ratio, which provides maximum synergy (a 100-fold increase in activity compared to the single agents), and also corresponds to the natural proportion of group A to group B antibiotics in the producer organism. The streptogramins bind to the peptidyl transferase domain of the 50S ribosomal subunit; the remarkable synergism arises because initial binding of the group A derivative causes a conformational change to the ribosome, increasing affinity for the group B derivative and formation of an extremely stable

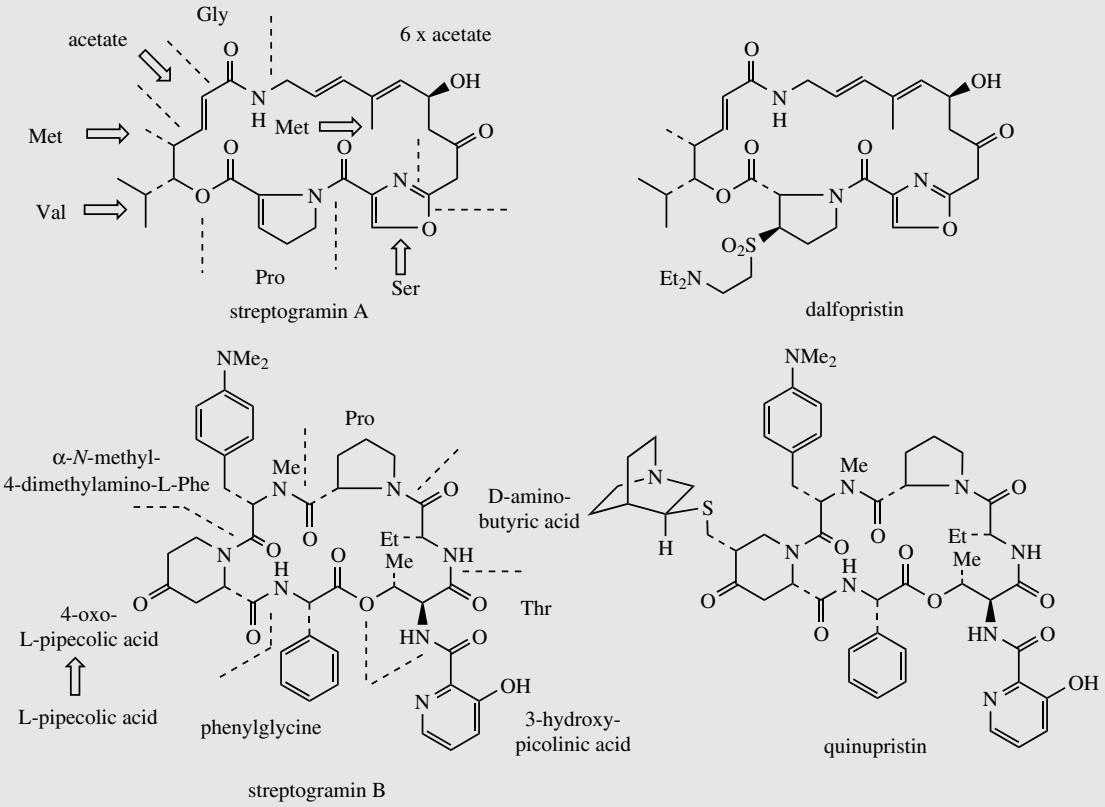

Figure 7.27

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ternary complex. This makes the streptogramin combination bactericidal, whereas the single agents provide only bacteriostatic activity.

Streptogramin A is known to be biosynthesized from four amino acids, namely valine, glycine, serine, and proline, a polyketide-like chain containing six acetate units, a further isolated acetate unit, and two methionine-derived methyl groups. The oxazole ring is formed from serine by incorporating the carboxyl terminus of the polyketide chain (compare the thiazole rings in bleomycin, page 429). Streptogramin B is formed by a typical nonribosomal peptide synthase, utilizing several rare modified amino acid precursors. All except one are modified before assembly; oxidation of the pipecolic acid residue (see page 310) to 4-oxopipecolic acid is carried out post-cyclization. The starter unit is 3-hydroxypicolinic acid, which arises via picolinic acid in the kynurenine pathway (see page 312); *p*-aminophenylalanine has been met previously in chloramphenicol biosynthesis (page 129).

Dactinomycin

Dactinomycin (**actinomycin D**) (Figure 7.28) is an antibiotic produced by *Streptomyces parvullus* (formerly *S. antibioticus*), which has antibacterial and antifungal activity, but whose high toxicity limits its use to anticancer therapy. Several related natural actinomycins are known, but only dactinomycin is used medicinally. Dactinomycin has a planar phenoxazinone dicarboxylic acid system in its structure, to which are attached two identical cyclic pentapeptides via amide bonds to threonine. The peptides are cyclized by lactone linkages utilizing the hydroxyl group of this threonine. The peptide portions of dactinomycin contain *N*-methylvaline and *N*-methylglycine (sarcosine) residues. In other actinomycins, the two peptides are not necessarily identical. The phenoxazinone ring system is known to be formed by fusing together two molecules of 3-hydroxy-4-methylanthranilic acid (Figure 7.28), which arises by *C*-methylation of 3-hydroxyanthranilic acid, a metabolite of tryptophan by the kynurenine pathway (see page 312). This planar phenoxazinone ring intercalates with double-stranded DNA inhibiting DNA-dependent RNA polymerases, but can also cause single-strand breaks in DNA. It is principally used to treat paediatric cancers, including Wilms' tumour of the kidney, but produces several serious and painful side-effects. However, as a selective inhibitor of DNA-dependent RNA synthesis (transcription), it has become an important research tool in molecular biology.

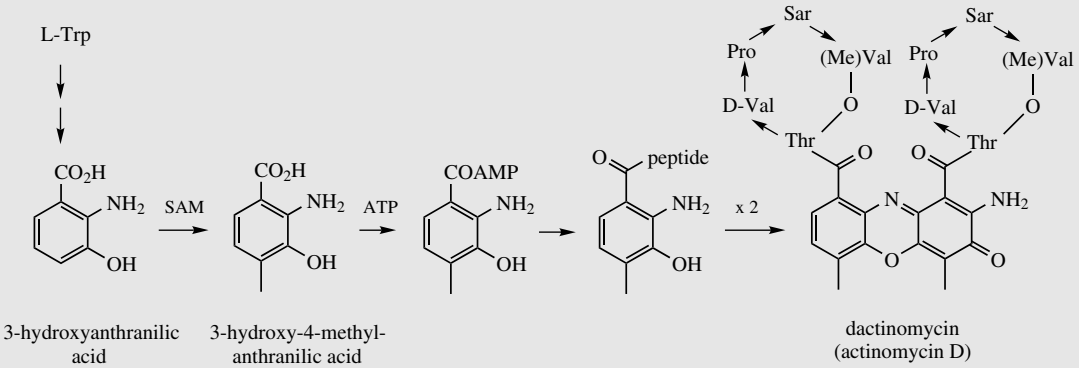

Figure 7.28

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PEPTIDE TOXINS

Death Cap (Amanita phalloides)

The death cap, *Amanita phalloides*, is a highly poisonous European fungus with a mushroom-like fruiting body. The death cap has a whitish-green cap, and white gills. It has a superficial similarity to the common mushroom, *Agaricus campestris*, and may sometimes be collected in error. Some 90% of human fatalities due to mushroom poisoning are attributed to the death cap. Identification of the death cap as a member of the genus *Amanita*, which includes other less poisonous species, is easily achieved by the presence of a volva at the base of the stem. This cuplike membranous structure is the remains of the universal veil in which the immature fruiting body was enclosed. Ingestion of the death cap produces vomiting and diarrhoea during the first 24 hours, followed after 3–5 days by coma and death. Some recoveries do occur, but the fatality rate is probably from 30 to 60%. There is no guaranteed treatment for death cap poisoning, though removal of material from the gastrointestinal tract, replacement of lost fluids, blood dialysis, and blood transfusions may be undertaken. The antihepatotoxic agent silybin (see page 153) has been used successfully. The toxic principles are cyclic polypeptides, which bring about major degeneration of the liver and kidneys. At least ten toxins have been identified, which may be subdivided into two groups, the phallotoxins and the amatoxins. The most extensively studied compounds are phalloin, a phallotoxin, and α-amanitin, an amatoxin (Figure 7.29). The phallotoxins are much less toxic than the amatoxins since after ingestion they are not well absorbed into the blood stream. When injected, they can cause severe damage to the membranes of liver cells, releasing potassium ions and enzymes. The amatoxins are extremely toxic when ingested, with a lethal dose of 5–7 mg for an adult human, and an average specimen of the death cap containing about 7 mg. The amatoxins cause lesions to the stomach and

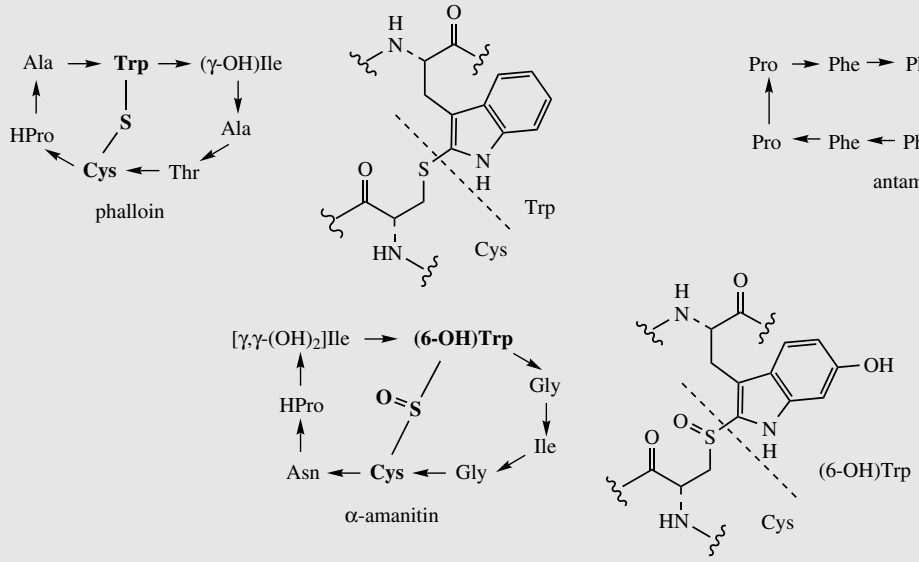

Figure 7.29

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intestine, and then irreversible loss of liver and kidney function. α-Amanitin has been shown to be a powerful inhibitor of RNA polymerase, blocking the elongation step. Structurally, the two groups of toxins appear similar. They both contain a γ-hydroxylated amino acid essential for toxic action, and have a sulphur bridge between a cysteine residue and the 2-position of a tryptophan or 6-hydroxytryptophan residue (Figure 7.29). In the case of the phallotoxins, this is a simple sulphide bridge, but in the amatoxins it is in the form of a sulphoxide. Interestingly, a rather more simple cyclic peptide antamanide (anti-amanita peptide) has also been isolated from *Amanita phalloides*. When pre-administered to laboratory animals, antamanide (Figure 7.29) provided prophylactic protection from the lethal affects of the phallotoxins.

Ricin

The distinctive mottled-brown seeds of the castor oil plant *Ricinus communis* (Euphorbiaceae) are crushed to produce castor oil, which is predominantly composed of glycerides of ricinoleic acid (see page 47 and Table 3.2). The seed itself and the seed cake remaining after expression of the oil are highly toxic, due to the presence of polypeptide toxins, termed ricins. One or more forms of ricin are present according to the strain of plant. Seeds typically contain about 1mg g^{-1} ricin, representing about 5% of the protein content. The toxicity of ricins to mammals is so high that the ricin content of one seed (about 250 μg) is sufficient to kill an adult human, though, because of considerable variations in absorption and metabolism, consumption of a single seed might not be fatal, but would certainly lead to severe poisoning. The toxic symptoms include irritation and haemorrhage in the digestive tract, leading to vomiting and bloody diarrhoea, with subsequent convulsions and circulatory collapse. If death does not occur within 3–5 days, the patient may recover.

Ricin is probably the best studied of a group of polypeptide toxins, known as ribosome-inactivating proteins (the acronym RIP seems most appropriate!). These toxins are potent inhibitors of eukaryotic protein biosynthesis by virtue of their cleavage of an *N*-glycosidic bond at a specific nucleotide residue in the 28S molecule of ribosomal RNA, which is part of the larger (60S) subunit of the eukaryotic ribosome. RIPs fall into two types. Type I proteins comprise a single polypeptide chain, sometimes glycosylated. Type II proteins have two chains linked by a disulphide bond: an A chain is essentially equivalent to type I proteins, whilst the B chain functions as a lectin, which means it has high affinity for specific sugar groups, in this case sugars (especially galactose) in glycolipids or glycoproteins on a cell membrane. Thus the B chain binds to the cell membrane, and, in so doing, facilitates entry of the A chain into the cytosol, where it inactivates the 60S ribosomal subunit and rapidly stops protein biosynthesis. The A chain is thus the toxic principle, but it is nontoxic to intact cells, and requires the B chain for its action.

Ricin is a type II toxin. The A chain (ricin A) contains 267 amino acid residues, and the B chain (ricin B) 262 residues. Ricin A is exceptionally toxic, and it has been estimated that a single molecule is sufficient to kill an individual cell. This peptide can be prepared by genetic engineering using *Escherichia coli*. The potent action of this material on eukaryotic cells has been investigated in anticancer therapy. Ricin A has been coupled to monoclonal antibodies and successfully delivered specifically to the tumour cells. However, *in vitro* toxicity of ricin A-based immunotoxins is enhanced significantly if ricin B is also present.

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Similar toxic RIPs are found in other plants. Examples are trichosanthin, a type I toxin from the root tubers of *Trichosanthes kirilowii* (Cucurbitaceae), abrin, a type II toxin from the small brightly coloured red and black jequirity seeds (*Abrus precatorius*; Leguminosae/Fabaceae), and viscumin, a type II toxin from the leaf and stems of mistletoe (*Viscum album*, Loranthaceae).

Botulinum Toxin

The Gram-positive bacterium *Clostridium botulinum* produces one of the most toxic materials known to man, botulinum toxin. Poisoning by the neurotoxins from this source, known as botulism, is not uncommon, and is a life-threatening form of food poisoning. It has been estimated that as many as 50 million people could be killed by one gram of the toxin. *Clostridium botulinum* is an anaerobic organism that is significantly heat resistant, though botulinum toxin is easily destroyed by heat. Food poisoning is almost always associated with foods such as canned meats and fish that have been incompletely sterilized, allowing growth of the bacterium, after which the food is then consumed without further cooking. Botulinum toxin is an extremely potent neurotoxin that acts by blocking calcium-dependent acetylcholine release at the peripheral neuromuscular junctions. Poisoning results in paralysis and death from respiratory failure. Damage to the nervous system is usually preceded by vomiting, diarrhoea, and severe abdominal pains.

Seven different neurotoxins, types A–G, have been characterized, though only four of these, types A, B, E, and F, are clearly associated with human poisoning. A particular strain of the bacterium usually produces only one type of toxin. Each of these proteins is a single chain polypeptide (mass about 150 kDa) containing two subunits, a 'light' subunit with a mass of about 50 kDa, and a 'heavy' subunit with a mass of about 100 kDa. These subunits are linked by at least one disulphide bridge. The heavy subunit is responsible for toxin binding, whilst the light subunit possesses zinc metalloprotease activity, cleaving one of the proteins involved in the docking and release of synaptic vesicles. There is considerable structural similarity between botulinum toxins and tetanus toxin. **Botulism antitoxin** is available for the treatment of botulism food poisoning. This is a mixture of globulins raised against types A, B, and E toxins.

Botulinum toxin A complexed with haemagglutinin is currently employed medicinally to counter involuntary facial muscle spasms, e.g. around the eye. Very small (nanogram) amounts are injected locally and result in the destruction of the acetylcholine release mechanism at the neuromuscular junction. Since new nerve junctions will gradually be formed over two months or so, the result is not permanent, and the treatment will need to be repeated. It has also been found useful in easing muscle spasticity in children with cerebral palsy.

Microcystins

The microcystins are a group of some65 cyclic heptapeptides, produced by certain fresh water blue–green algae (cyanobacteria), including *Microcystis aeruginosa*, *M. viridis*, *Anabaena flos-aquae*, and others. These organisms form blooms on the surface of lakes and reservoirs during periods of calm hot weather, and can pollute drinking water for animals and humans. The microcystins cause acute hepatotoxicity and liver haemorrhage, and have been

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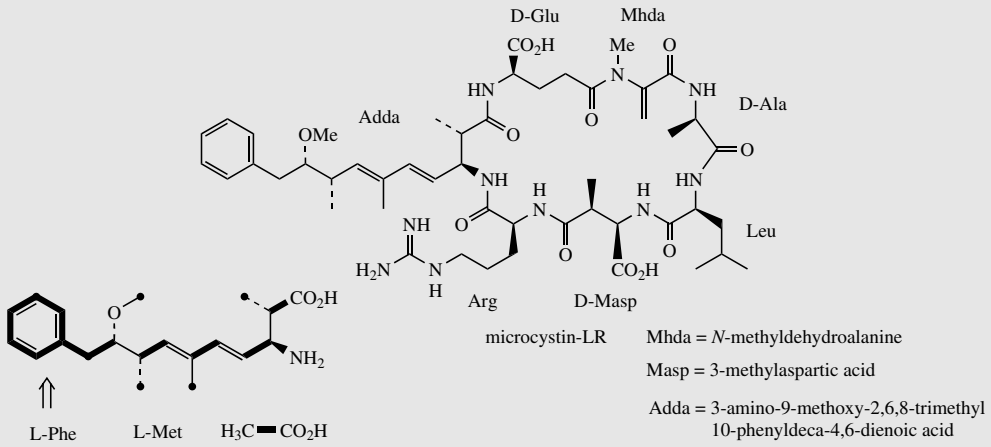

Figure 7.30

responsible for numerous animal deaths, though there is much less risk of human fatalities. They strongly inhibit protein phosphatases, and this activity may be associated with the hepatotoxicity and the promotion of liver tumours. The microcystins contain a mixture of D- and L-amino acids, and mainly differ in their combination of L-amino acids. The most frequently encountered compound, microcystin-LR (Figure 7.30) (LR = leucine–arginine) exemplifies the structures. Unusual amino acids are *N*-methyldehydroalanine (from *N*-methylserine), 3-methylaspartic acid (which originates from acetate and pyruvate), and the long chained aromatic-containing Adda. This amino acid is elaborated from phenylalanine, which supplies a C_6C_2 unit to act as a starter unit for a polyketide chain. Methyl substituents are provided by SAM (Figure 7.30).

Snake Venoms

It is estimated that some 1300 of the 3200 species of snake are venomous. Snake venoms are used to immobilize prey, and to facilitate its digestion. Most of the material is polypeptide in nature, and can include enzymes and polypeptide toxins. A number of enzymes have been identified in all venoms, and these include hyaluronidase (see Table 7.2), which facilitates the distribution of the other venom components through the tissues. Peptidases, phosphodiesterases, phospholipases, ribonuclease, and deoxyribonuclease are all hydrolytic enzymes designed to digest the tissue of the prey. Some enzymes induce direct toxic effects, for example L-amino acid oxidase liberates hydrogen peroxide, a powerful oxidizing agent. In some venoms, the enzyme acetylcholinesterase disturbs the normal physiological response of the prey by hydrolysing acetylcholine. Major groups of polypeptide toxins found in snake venoms may be classified as neurotoxins, cytotoxins (or cardiotoxins), dendrotoxins, proteinase inhibitors, or acetylcholinesterase inhibitors. α-Neurotoxins (curaremimetic neurotoxins) found in many mambas (*Dendroaspis*) and cobras (*Naja*) are capable of interacting with nicotinic acetylcholine receptors in the postsynaptic membranes of skeletal muscles, leading to paralysis, an action similar to that of curare (see page 324). κ-Neurotoxins, on the other hand, are selective for neuronal nicotinic acetylcholine receptors. Typically, the neurotoxins contain 60–74 amino acid residues with four or five

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disulphide bridges. Cytotoxins are present in cobra (*Naja*) and ringhal (*Hemachatus*) venoms and produce a rapid effect on the heart and circulation though the mode of action is not well established. Most have 60–62 amino acid residues. Dendrotoxins from mambas are characterized by their ability to facilitate the release of acetylcholine from nerve endings, and also act as highly potent and selective blockers of potassium channels. These contain about 60 amino acid residues, with three disulphide bridges. Anticholinesterase toxins have also been found in mamba venoms, typically with about 60 amino acids and four disulphide bridges.

MODIFIED PEPTIDES: PENICILLINS, CEPHALOSPORINS, AND OTHER β-LACTAMS

Penicillins

The **penicillins*** are the oldest of the clinical antibiotics, and are still the most widely used. The first of the many penicillins to be employed on a significant scale was **penicillin G** (**benzylpenicillin**) (Figure 7.31), obtained from the fungus *Penicillium chrysogenum* by fermentation in a medium containing corn-steep liquor. Penicillins contain a β-lactam-thiazolidine structure, which has its biosynthetic origins in a tripeptide, the components of which are L-aminoadipic acid, formed in β-lactam-producing organisms from lysine via piperideine-6-carboxylic acid (see page 311), together with L-cysteine, and L-valine (Figure 7.31). Non-ribosomal peptide assembly then leads to the tripeptide known as ACV, but, during the condensation, the configuration of the valine residue is also inverted to the D-form. (Caution: ACV is an acronym, and does not refer to the systematic abbreviation described on page 407; ACV is actually δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine). ACV is then cyclized to **isopenicillin N**, with a single enzyme catalysing formation of the bicyclic ring system of the penicillins. The reaction is oxidative and requires molecular oxygen, and there is evidence that the four-membered β-lactam ring is formed first. The mechanism shown in Figure 7.31 is given to simplify what is quite a complex reaction. **Penicillin G** differs from isopenicillin N by the nature of the side-chain attached to the 6-amino group. The α-aminoadipyl side-chain of isopenicillin N is removed and replaced by another according to its availability from the fermentation medium. Phenylethylamine in the corn-steep liquor medium was transformed by the fungus into phenylacetic acid, which then reacted as its coenzyme A ester to produce the new amide penicillin G. Other penicillins are accessible by

Penicillins

Commercial production of **benzylpenicillin** (**penicillin G**) (Figure 7.31) is by fermentation of selected high-yielding strains of *Penicillium chrysogenum* in the presence of phenylacetic acid. Although benzylpenicillin was the earliest commercially available member of the penicillin group of antibiotics, it still remains an important and useful drug for the treatment of many Gram-positive bacteria, including streptococcal, pneumococcal, gonococcal, and meningococcal infections. Benzylpenicillin is destroyed by gastric acid, and is thus not suitable for oral administration, and is best given as intramuscular or intravenous injection of the water-soluble sodium salt. Decomposition under acidic conditions leads to formation of penicillic acid and/or penicillenic acid, depending on pH (Figure 7.32). The β-lactam ring is opened by a mechanism in which the side-chain carbonyl participates, resulting in formation of an oxazolidine ring. Penicillic acid arises as the result of nucleophilic attack of the thiazolidine nitrogen on to the iminium function, followed by expulsion of the carboxylate leaving group.

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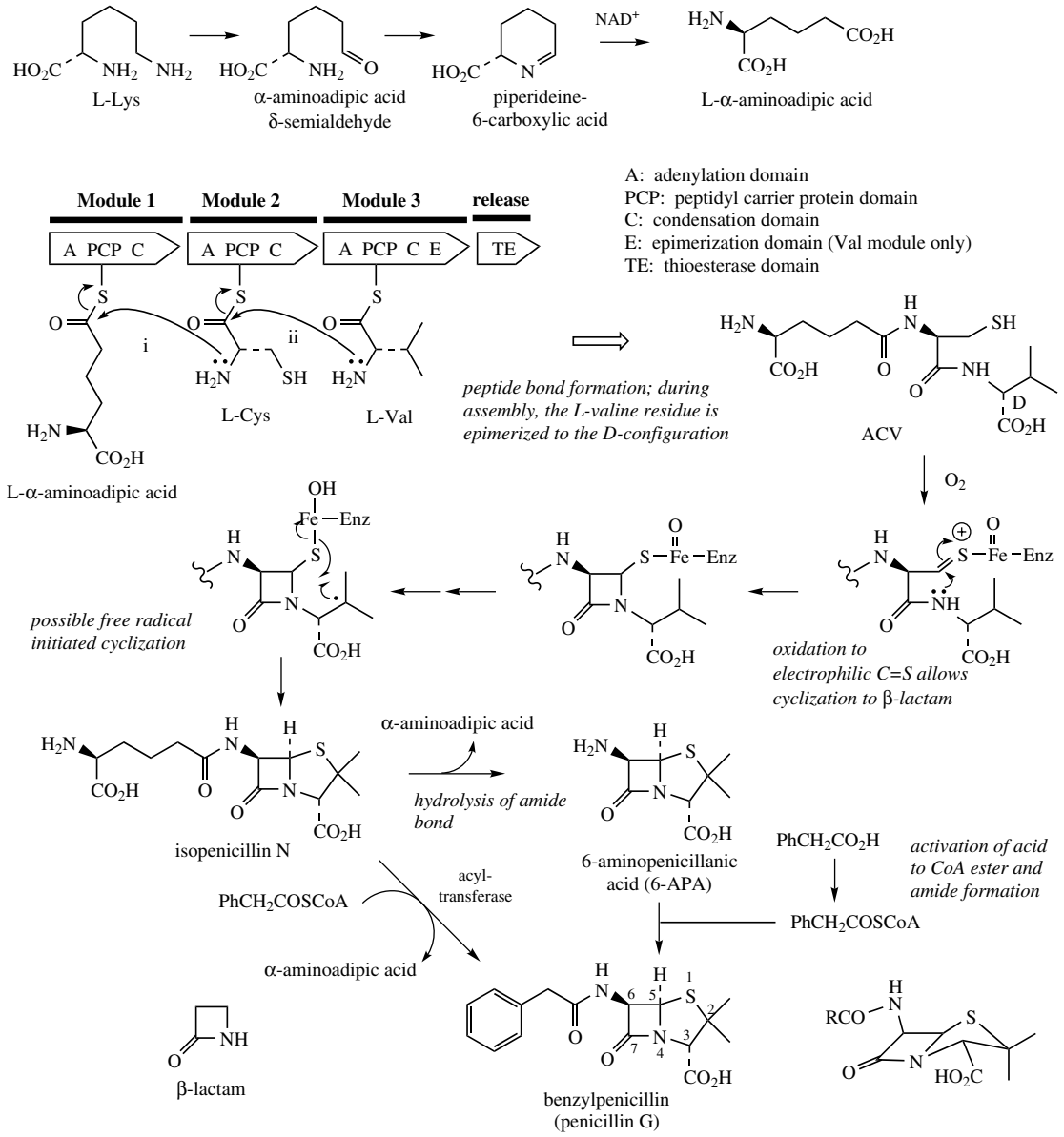

Figure 7.31

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Alternatively, elimination of thiol accounts for formation of penicillenic acid. At higher pHs, benzylpenicillin suffers simple β-lactam ring opening and gives penicilloic acid (Figure 7.32). The strained β-lactam (cyclic amide) ring is more susceptible to hydrolysis than the unstrained side-chain amide function, since the normal stabilizing effect of the lone pair from the adjacent nitrogen is not possible due to the geometric restrictions (Figure 7.33).

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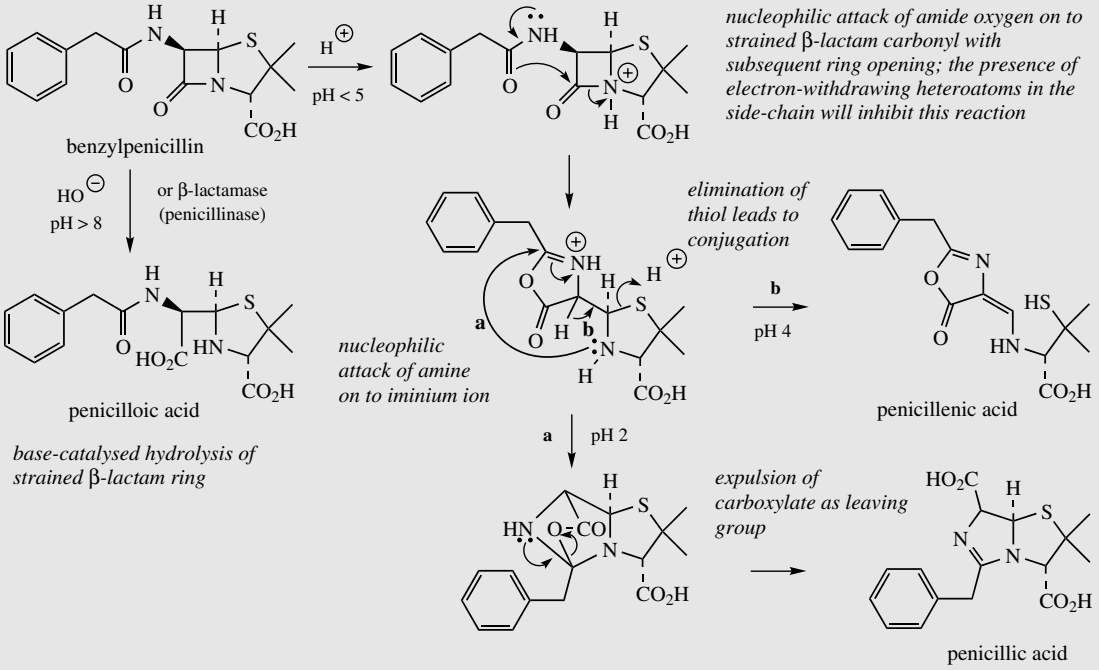
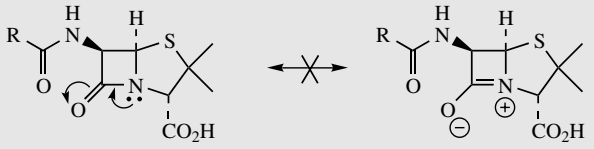


Figure 7.32



resonance involving the amide lone pair normally decreases carbonyl character and thus stabilizes the carbonyl against attack by nucleophilic reagents

this resonance form is sterically impossible; the β-lactam carbonyl function is thus reactive towards nucleophilic reagents

Figure 7.33

Supplementation of the fermentation medium with acids other than phenylacetic acid was used to provide structurally modified penicillins, though the scope was limited to series of monosubstituted acetic acids by the specificity of the fungal enzymes involved in the activation of the acids to their coenzyme A esters. The most important new penicillin produced was **phenoxymethylpenicillin** (**penicillin V**), a result of adding phenoxyacetic acid to the culture (Figure 7.34). This new penicillin had the great advantage of being acid resistant, the introduction of an electron-withdrawing heteroatom into the side-chain inhibiting participation of the side-chain carbonyl in the reaction shown in Figure 7.32. Thus, penicillin V is suitable for oral administration, and still has particular value for respiratory tract infections and tonsillitis.

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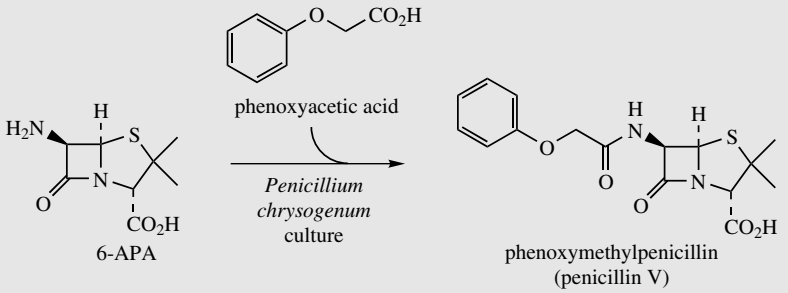

Figure 7.34

A much wider range of penicillins, many of which have become clinically useful, may be produced by semi-synthesis from 6-aminopenicillanic acid (6-APA). A multi-stage, but high-yielding, procedure has been developed to chemically hydrolyse a primary fermentation product like benzylpenicillin to 6-APA (Figure 7.35). This exploits the ability of the side-chain amide to adopt a resonance form, thus allowing conversion into an imidyl chloride, and then an imidyl ether, which is readily hydrolyzed. A new side-chain can then be added by simple esterification (Figure 7.35). Hydrolysis of penicillin G or penicillin V may also be accomplished enzymically in very high yield by using bacterial enzyme preparations from *Escherichia coli*, or species of *Fusarium* or *Erwinia*. Certain strains of *Penicillium chrysogenum* accumulate 6-aminopenicillanic acid, so that this compound may be produced by fermentation, though this is commercially less economic than the hydrolysis approach. Clinically useful penicillins produced by semi-synthesis or total synthesis are listed in Table 7.3. Penicillins with side-chains containing a basic amino group, e.g. **ampicillin** and **amoxicillin** (**amoxycillin**), are

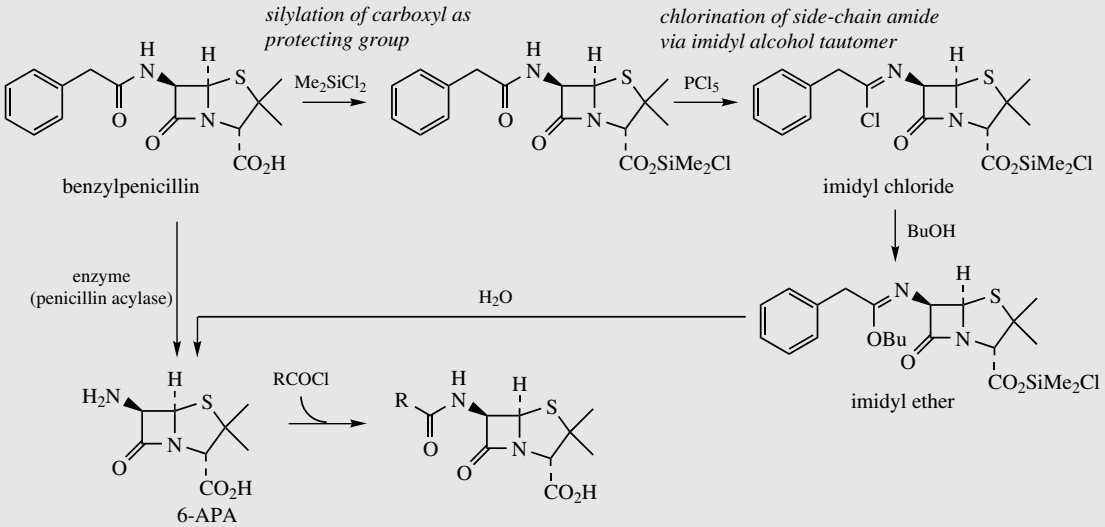

Figure 7.35

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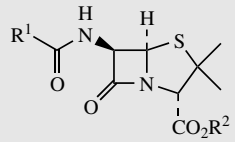
Table 7.3 Semi-synthetic and synthetic penicillins



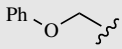
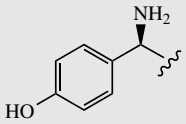
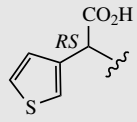
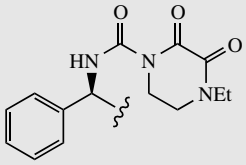
R^1	R^2	Name	Notes
	H	benzylpenicillin (penicillin G)	acid sensitive
	H	phenoxymethyl-penicillin (penicillin V)	acid resistant
	H	ampicillin	acid resistant, broad spectrum
	$-CH_2OCOCMe_3$	pivampicillin	pro-drug of ampicillin; better absorbed, and then hydrolysed by esterases
	*RS* $-CHMeOCO_2Et$	bacampicillin	pro-drug of ampicillin; better absorbed, and then hydrolysed by esterases
	H	amoxicillin (amoxycillin)	acid resistant, broad spectrum; better absorption than ampicillin
	H	carbenicillin	acid sensitive, broad spectrum; active against *Pseudomonas aeruginosa*
	H	ticarcillin	acid sensitive, broad spectrum; 2 × more active than carbenicillin; used in combination with β-lactamase inhibitor clavulanic acid
	H	piperacillin	broad spectrum, 8–10 × more active than carbenicillin against *P. aeruginosa*; used by injection for serious infections

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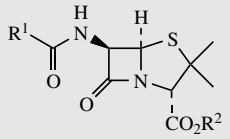
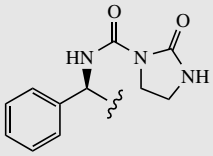
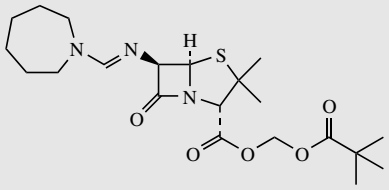
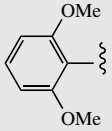
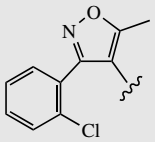
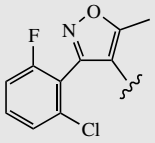
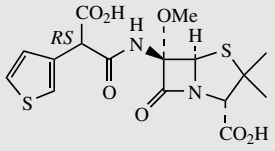
Table 7.3 (*continued*)



R^1	R^2	Name	Notes
	H	azlocillin	broad spectrum, more active than ticarcillin against *P. aeruginosa*; used by injection for serious infections
		pivmecillinam	orally active pro-drug of mecillinam; β-lactamase sensitive, active against Gram-negative organisms (except *P. aeruginosa*)
	H	methicillin	β-lactamase resistant, acid sensitive
	H	cloxacillin	β-lactamase and acid resistant
	H	flucloxacillin	β-lactamase and acid resistant
		temocillin	β-lactamase resistant; active against Gram-negative (except *P. aeruginosa*) but not Gram-positive organisms

also acid resistant, since this nitrogen is protonated in preference to the lactam nitrogen. In addition, these agents were found to have a broader spectrum of activity than previous materials, in particular, activity against some Gram-negative bacteria which were not affected by penicillins G and V. The polar side-chain improves water-solubility and cell penetration into these microorganisms. Amoxicillin shows better oral absorption properties

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than ampicillin, though this can also be achieved by using the pro-drugs **pivampicillin** and **bacampicillin**. These are acyloxymethyl esters through the thiazolidine carboxyl, and are hydrolysed to ampicillin by esterases in the gut. Broad spectrum activity is also found with penicillins containing a carboxyl group in the side-chain, e.g. **carbenicillin** and **ticarcillin**, and, although these compounds are still acid sensitive and are thus orally inactive, they demonstrate activity against pseudomonads, especially *Pseudomonas aeruginosa*. The activity of these agents is rather low, and it is necessary to inject very large doses. The acylureido penicillins **azlocillin** and **piperacillin** are much more active against *Pseudomonas aeruginosa*, and are also active against other Gram-negative bacteria such as *Klebsiella pneumoniae* and *Haemophilus influenzae*. **Pivmecillinam** is an acyloxymethyl ester pro-drug, and is hydrolysed to mecillinam after oral ingestion. It is unusual in being an amidino derivative instead of having an acyl side-chain; it has significant activity towards many Gram-negative bacteria.

Despite the dramatic successes achieved with the early use of penicillin antibiotics, it was soon realised that many bacteria previously susceptible to these agents were subsequently able to develop resistance. The principal mechanism of resistance lies in the ability of organisms to produce β-lactamase (penicillinase) enzymes capable of hydrolysing the β-lactam ring in the same manner as shown for the base-catalysed hydrolysis in Figure 7.32. Several distinct classes of bacterial β-lactamases are recognized, the main division being into serine enzymes and zinc enzymes. The former have an active site serine residue, which attacks the β-lactam carbonyl, forming an acyl–enzyme intermediate. On the basis of characteristic amino acid sequences they are then subdivided into three classes, A, C, and D. The zinc metallo-enzymes form class B, and appear to involve only non-covalently bound intermediates. Class A β-lactamases are the most common amongst pathogenic bacteria. Most staphylococci are now resistant to benzylpenicillin. The discovery of new penicillins that were not hydrolysed by bacterial β-lactamases was thus a major breakthrough. **Methicillin** (Table 7.3), though no longer used, was the first commercial β-lactamase-resistant penicillin, and the steric bulk of the side-chain appears to contribute to this valuable property, hindering the approach of β-lactamase enzymes. Methicillin is acid sensitive, since it lacks an electron-withdrawing side-chain, but other penicillins were developed that combined bulk and electron-withdrawing properties, and could thus be used orally. These include a group of isoxazole derivatives termed the oxacillins, of which **cloxacillin** and **flucloxacillin** are first-choice agents against penicillin-resistant *Staphylococcus aureus*. **Temocillin** also has excellent resistance to β-lactamases as well as high activity towards Gram-negative organisms. It differs from all the other penicillins described in possessing a 6α-methoxyl group (compare the cephamycins, page 450). Another way of overcoming the penicillin-degrading effects of β-lactamase is to combine a β-lactamase-sensitive agent, e.g. amoxicillin or ticarcillin, with clavulanic acid (see page 452), which is a specific inhibitor of β-lactamase. Other mechanisms of resistance which have been encountered include modification of the binding sites on penicillin-binding proteins (see below), thus reducing their affinity for the penicillin, and decreased cell permeability, leading to reduced uptake of the antibiotic. Strains of *Staphylococcus aureus* resistant to both methicillin and isoxazolylpenicillins, e.g. cloxacillin and flucloxacillin, are known to have modified and insensitive penicillin-binding proteins; such strains are termed methicillin-resistant *Staphylococcus aureus* (MRSA).

Penicillins and other β-lactam drugs exert their antibacterial effects by binding to proteins (penicillin-binding proteins) that are involved in the late stages of the biosynthesis of

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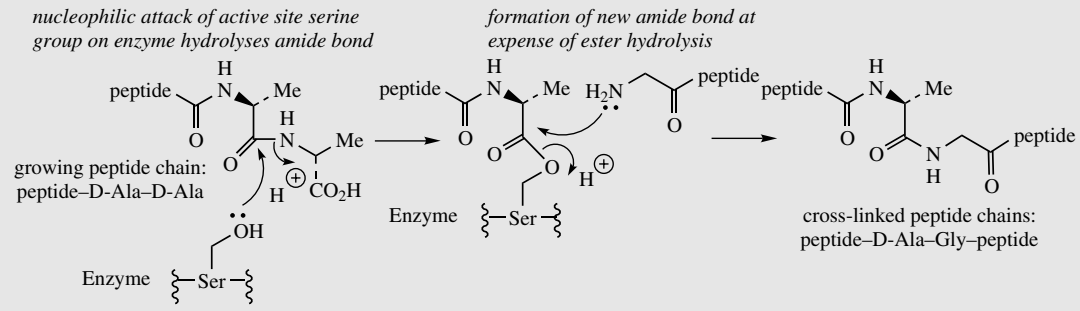
Cross-linking in peptidoglycan biosynthesis:



Enzyme inhibition by β-lactams:

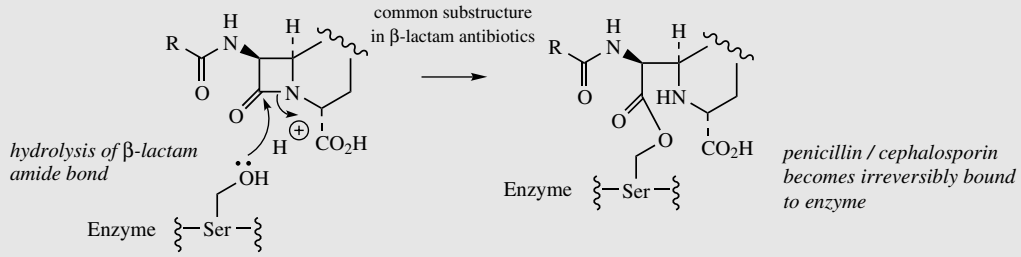


Figure 7.36

the bacterial cell wall. Cross-linking of the peptidoglycan chains which constitute the bacterial cell wall (see page 473) involves an acyl-D-Ala–D-Ala intermediate, which in its transition state conformation closely resembles the penicillin molecule (Figure 7.36). As a result, the penicillin occupies the active site of the enzyme, and becomes bound via an active site serine residue, this binding causing irreversible enzyme inhibition, and cessation of cell wall biosynthesis. Growing cells are then killed due to rupture of the cell membrane and loss of cellular contents. This binding reaction between penicillin-binding proteins and penicillins is chemically analogous to the action of β-lactamases, but in the latter case penicilloic acid is subsequently released from the β-lactamase, which can continue to function. The penicillins are very safe antibiotics for most individuals. The bacterial cell wall has no counterpart in mammalian cells, and the action is thus very specific. However, a significant proportion of patients can experience allergic responses ranging from a mild rash to fatal anaphylactic shock. Cleavage of the β-lactam ring through nucleophilic attack of an amino group in a protein is believed to lead to the formation of antigenic substances causing the allergic response.

supplying different acids. The new amide link may be achieved in two ways. Hydrolysis of isopenicillin N releases the amine **6-aminopenicillanic acid** (6-APA), which can then react with the coenzyme A ester. Alternatively, an acyltransferase enzyme converts isopenicillin N into penicillin G directly, without 6-aminopenicillanic acid actually being released from the enzyme.

Cephalosporins

The **cephalosporins***, e.g. **cephalosporin C** (Figure 7.37), are a penicillin-related group of antibiotics having a β-lactam-dihydrothiazine ring system, and are produced by species of *Cephalosporium*. The six-membered dihydrothiazine ring is produced from the five-membered

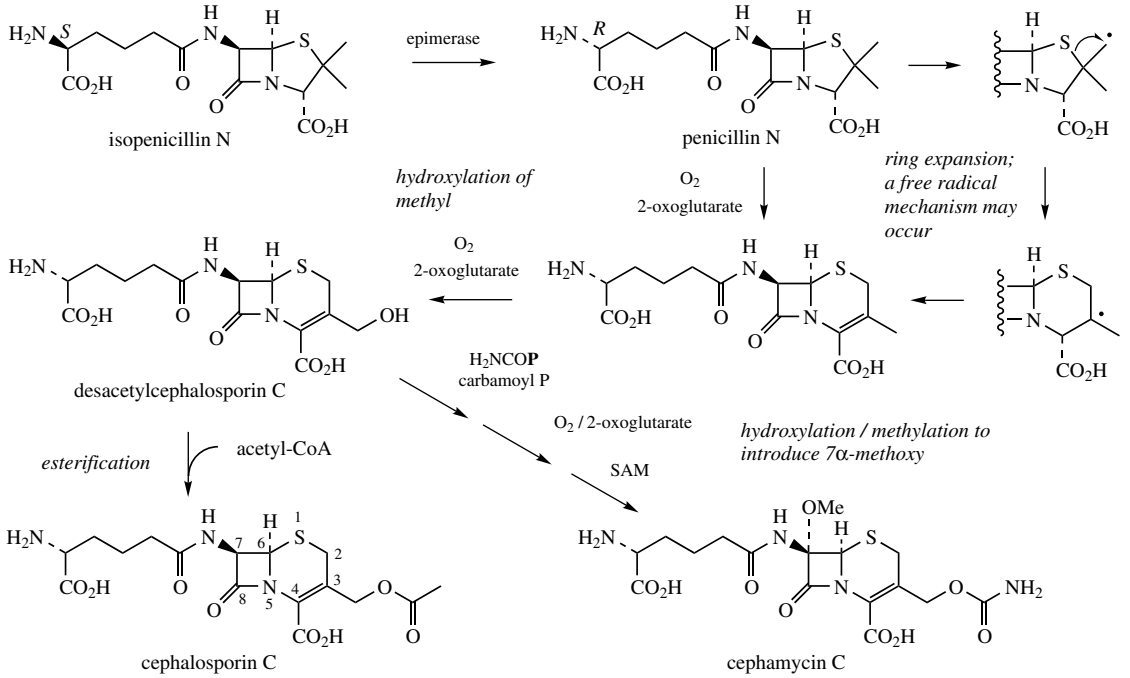


Figure 7.37

thiazolidine ring of the penicillin system by an oxidative process of ring expansion, incorporating one of the methyl groups. The pathway (Figure 7.37) diverges from that to penicillins at **isopenicillin N**, which is first epimerized in the α-aminoadipyl side-chain to give **penicillin N**. Ring expansion then occurs, incorporating one of the methyls into the heterocyclic ring, though the mechanism for this is not clearly defined. A free radical mechanism is suggested in Figure 7.37 to rationalize the transformation. Hydroxylation of the remaining methyl gives desacetylcephalosporin C. **Cephalosporin C** is the acetyl ester of this, whilst a further group of antibiotics termed the **cephamycins*** are characterized by a 7α-methoxy group, and are produced by hydroxylation/methylation, and, in the case of **cephamycin C**, introduction of a carbamate group from carbamoyl phosphate on to the hydroxymethyl function.

Cephalosporins

Cephalosporin C (Figure 7.37) is produced commercially by fermentation using cultures of a high-yielding strain of *Acremonium chrysogenum* (formerly *Cephalosporium acremonium*). Initial studies of the antibiotic compounds synthesized by *C. acremonium* identified penicillin N (originally called cephalosporin N) as the major component, with small amounts of cephalosporin C. In contrast to the penicillins, cephalosporin C was stable under acidic conditions and also was not attacked by penicillinase (β-lactamase). Antibacterial activity was rather low, however, and the antibiotic was poorly absorbed after oral administration. However, the structure offered considerable scope for side-chain modifications, more so than with the penicillins since it has two side-chains, and this has led to a wide variety of cephalosporin drugs, many of which are currently in clinical use. As with the penicillins, removal of the amide

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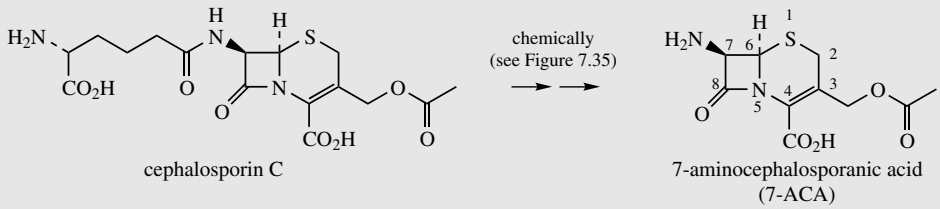

cephalosporin C

7-aminocephalosporanic acid
(7-ACA)

Figure 7.38

side-chain by the hydrolysis of cephalosporin C to 7-aminocephalosporanic acid (7-ACA) (Figure 7.38) was the key to semi-synthetic modifications, and this may be achieved chemically by the procedure used for the penicillins (compare Figure 7.35). Removal of this side-chain by suitable microorganisms or enzymes has proved elusive. The ester side-chain at C-3 may be hydrolysed enzymically by fermentation with a yeast, or, alternatively, the acetoxy group is easily displaced by nucleophilic reagents. It is also possible to convert readily available benzylpenicillin into the deacetoxy derivative of 7-ACA through a chemical ring expansion process and enzymic removal of the side-chain.

The semi-synthetic cephalosporins may be classified according to chemical structure, antibacterial spectrum, or β-lactamase resistance, but in practice, they tend to be classified by a more abitrary system, dividing them into 'generations' (Table 7.4). Note that all the cephalosporin antibiotics begin with the prefix *ceph-* or *cef-*, the latter spelling now being preferred, though both spellings are still encountered for some drugs. The classification into generations is based primarily on the antibacterial spectrum displayed by the drugs but it is also more or less related to the year of introduction. However, drugs in the second generation may have been introduced after the third generation of drugs had been established. There is no intention to suggest that third generation drugs automatically supersede second and first generation drugs, and, indeed, agents from all generations are still currently used. First generation cephalosporins, e.g. **cefalotin** (**cephalothin**), **cefalexin** (**cephalexin**), **cefradine** (**cephradine**), **cefadroxil**, and **cefazolin** (**cephazolin**) have good activity against Gram-positive bacteria but low activity against Gram-negative organisms. They have comparable activity to ampicillin, and are effective against penicillinase-producing *Staphylococcus*. However, another β-lactamase (cephalosporinase) developed that inactivated these agents. Cefalotin, the first modified cephalosporin to be marketed, is poorly absorbed from the gut, and is thus not orally active. However, cefalexin, cefradine, and cefadroxil may be administered orally, a property that appears to be related to the 3-methyl side-chain. Second generation cephalosporins show a broader spectrum of activity, and are more active against aerobic Gram-negative bacteria like *Haemophilus influenzae* and *Neisseria gonorrhoeae*. This group of antibiotics includes **cefaclor**, **cefuroxime**, and **cefamandole** (**cephamandole**), and in general displays better resistance to β-lactamases that inactivated first generation cephalosporins. The third generation of cephalosporin antibiotics, e.g. **cefotaxime**, **ceftazidime**, **ceftizoxime**, **cefodizime**, and **ceftriaxone**, have an extended Gram-negative spectrum, and are most active against enteric Gram-negative bacilli, but may be less active against some Gram-positive bacteria, especially *Staphylococcus aureus*. Many of the third generation cephalosporins are characterized by an aminothiazole ring on the amide side-chain, which appears to impart the high activity against Gram-negative bacteria. The *O*-substituted oxime group also improves potency, and confers resistance to β-lactamases. The oximes with *syn* stereochemistry as shown in Table 7.4 are considerably more active than the

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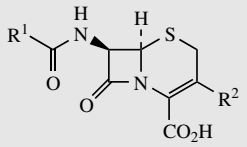
Table 7.4 Cephalosporin antibiotics



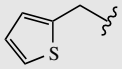
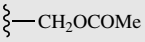
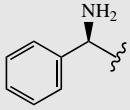
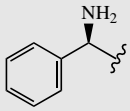
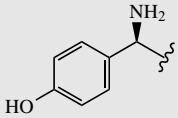
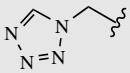
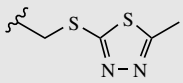
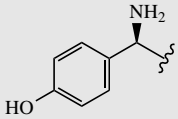
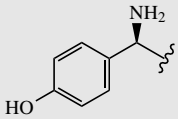
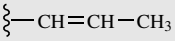
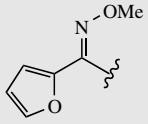
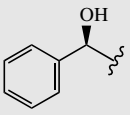
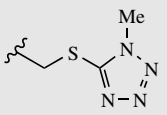
R^1	R^2	Name	Notes
First generation			
		cefalotin (cephalothin)	R^2 group unstable to mammalian esterases; generally superseded
		cefalexin (cephalexin)	orally active
		cefradine (cephradine)	orally active; generally superseded
		cefadroxil	orally active
		cefazolin (cephazolin)	generally superseded
Second generation			
		cefaclor	orally active
		cefprozil	orally active
		cefuroxime	high resistance to β-lactamases; resistant to mammalian esterases
		cefamandole (cephamandole)	high resistance to β-lactamases

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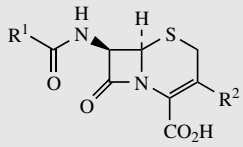
Table 7.4 (*continued*)



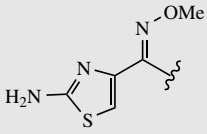
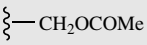
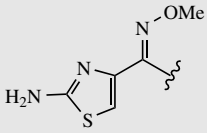
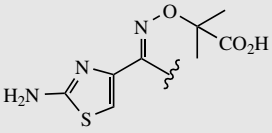
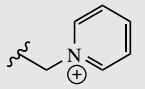
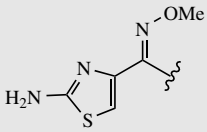
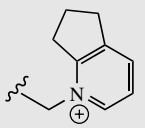
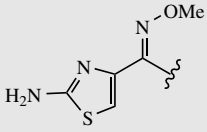
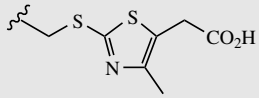
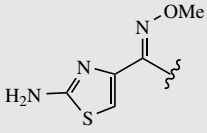
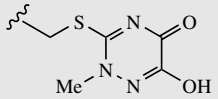
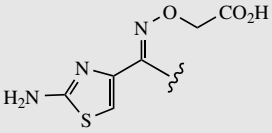
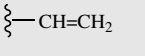
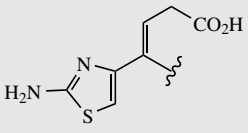
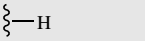
R^1	R^2	Name	Notes
Third generation			
		cefotaxime	unstable to mammalian esterases, but desacetyl metabolite still has considerable antimicrobial activity
		ceftizoxime	broad-spectrum Gram-negative activity; generally superseded
		ceftazidime	broad-spectrum Gram-negative activity; good activity towards *Pseudomonas*
		cefpirome	
		cefodizime	broad-spectrum Gram-negative activity
		ceftriaxone	broad-spectrum Gram-negative activity; longer half-life than other cephalosporins
		cefixime	2nd/3rd generation, orally active; long duration of action
		ceftibuten	2nd/3rd generation, orally active; generally superseded

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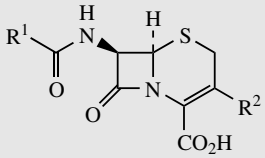
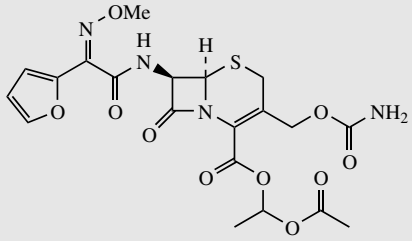
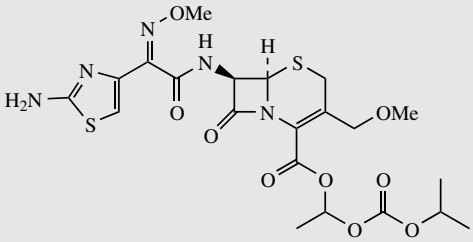
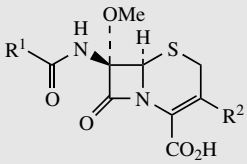
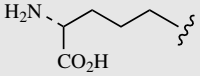
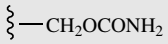
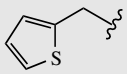
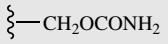
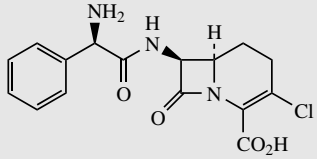
Table 7.4 (*continued*)



R^1	R^2	Name	Notes
Pro-drugs			
		cefuroxime-axetil	2nd generation, orally active; hydrolysed by esterases to liberate cefuroxime
		cefpodoxime -proxetil	2nd/3rd generation, orally active; hydrolysed by esterases to liberate cefpodoxime
			
R^1	**R^2**	**Name**	**Notes**
Cephamycins			
		cephamycin C	
		cefoxitin	stable to β-lactamases and mammalian esterases
Carbacephems			
		loracarbef	improved chemical stability, longer half-life, better oral bioavailability compared to cefaclor

(*Continues*)

(*Continued*)

anti isomers. A disadvantage of many of the current cephalosporin drugs is that they are not efficiently absorbed when administered orally. This is to some extent governed by the nature of the side-chain on C-3. The orally active **cefixime** and **ceftibuten** have spectra of activity between the second and third generations. Orally active pro-drugs such as **cefuroxime-axetil** and **cefpodoxime-proxetil** have been developed with an additional ester function on the C-4 carboxyl. These compounds are hydrolysed to the active agents by esterases.

Cephalosporin antibiotics are especially useful for treating infections in patients who are allergic to penicillins. Hypersensitivity to cephalosporins is much less common, and only about 5–10% of penicillin-sensitive patients will also be allergic to cephalosporins.

Cephamycins

The antibiotic cephamycin C (Figure 7.37) was isolated from *Streptomyces clavuligerus* and shown to have a 7α-methoxy group on the basic cephalosporin ring system. Although cephamycin C and other natural cephamycins have only weak antibacterial activity, they are resistant to β-lactamase hydrolysis, a property conferred by the increased steric crowding due to the additional methoxy group. Semi-synthetic analogues have been obtained either by modification of the side-chains of natural cephamycins, or by chemical introduction of the 7α-methoxyl. Currently, the only cephamycin in general use is **cefoxitin** (Table 7.4), which is active against bowel flora including *Bacterioides fragilis*, and is used for treatment of peritonitis.

Carbacephems

Although cephalosporin analogues where the sulphur heteroatom has been replaced with carbon are not known naturally (contrast carbapenems, natural penicillin analogues, page 451), synthetically produced carbacephems have shown good antibacterial activity with considerably improved chemical stability over cephalosporins. The first of these to be produced for drug use is **loracarbef** (Table 7.4), which has similar antibacterial activity to cefaclor, but considerably greater stability, a longer half-life, and better oral bioavailability.

Other β-Lactams

The fused β-lactam skeletons found in penicillins and cephalosporins are termed **penam** and **cephem** respectively (Figure 7.39). Other variants containing the basic β-lactam ring system are also found in nature. Of especial importance is the **clavam** (Figure 7.39) or oxapenam fused ring system typified by **clavulanic acid*** (Figure 7.40) from *Streptomyces clavuligerus*. The weak antibacterial activity of clavulanic acid is unimportant, for this compound is valuable as an efficient inhibitor of β-lactamases from both Gram-positive and Gram-negative bacteria. Despite the obvious structural similarity between clavulanic acid and the penicillins, they are not derived from common precursors, and there are some novel aspects associated with clavulanic acid biosynthesis. All

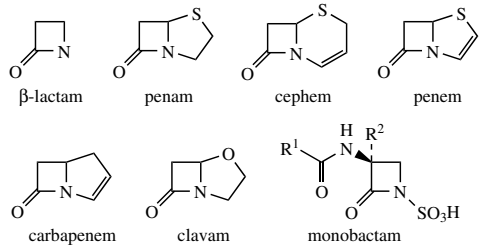


Figure 7.39

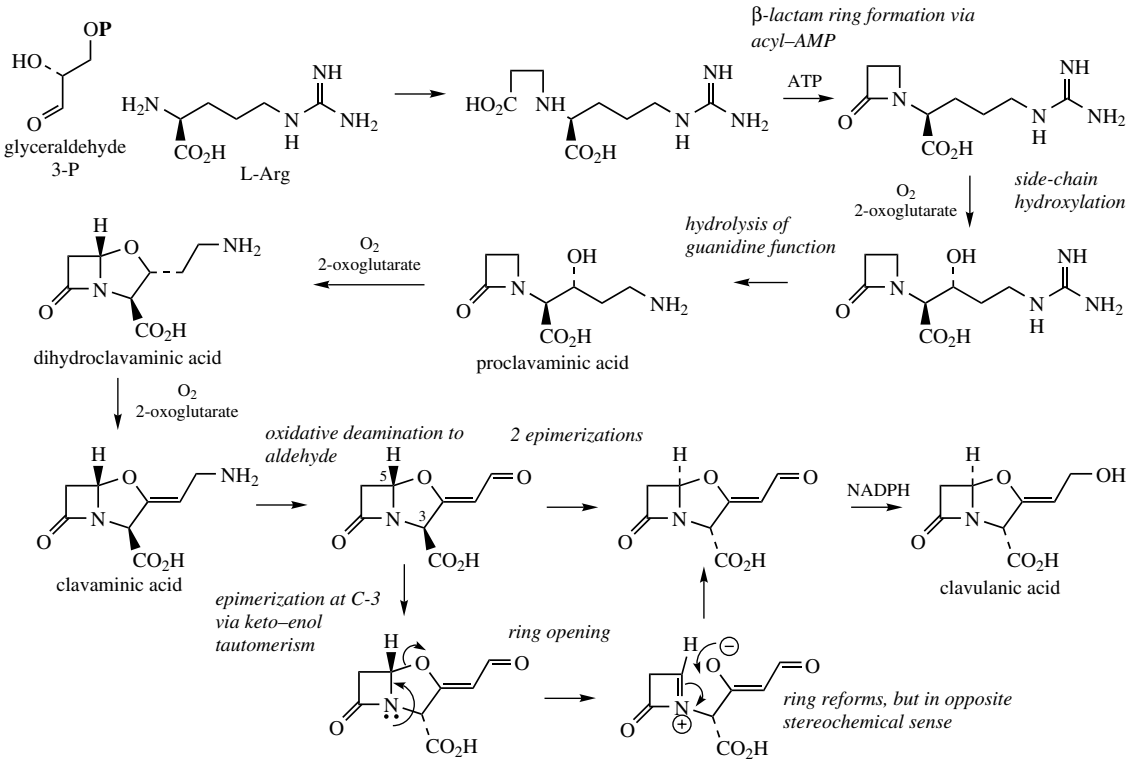


Figure 7.40

carbons are provided by two precursors, arginine and most likely glyceraldehyde 3-P. Glyceraldehyde 3-P supplies the β-lactam carbons whilst the α-amino group of arginine provides the β-lactam nitrogen. In contrast to the penicillins, the β-lactam ring is formed via an acyl-AMP activated intermediate. The sequence of reactions shown in Figure 7.40 leads to the monocyclic β-lactam **proclavaminic acid**, which is the substrate for oxidative cyclization to provide the oxazolidine ring then dehydrogenation to **clavaminic acid**. The three 2-oxoglutarate-dependent oxidations in this sequence are all catalysed by a single enzyme. The final transformation into **clavulanic acid** requires oxidative deamination of the terminal amine to an aldehyde and then reduction to an alcohol, but, more intriguingly, the two chiral centres C-3 and C-5 have to be epimerized. These reactions have yet to be fully clarified. The unsaturated aldehyde is easily susceptible to inversion of configuration at C-3 via keto–enol tautomerism, but a change of chirality at C-5 (which actually retains the C-5 hydrogen) must invoke opening of the oxazolidine ring (Figure 7.40).

Another variant on the penicillin penam ring system is found in a group of compounds termed **carbapenems*** (Figure 7.39), where the sulphur heteroatom has been replaced by carbon. This is exemplified by **thienamycin** (Figure 7.43), an antibiotic isolated from cultures of *Streptomyces cattleya*. The sulphur-containing side-chain is a feature of many of the natural examples, and this may feature sulphide or sulphone moieties, e.g. the **olivanic acids** (Figure 7.44) from *S. olivaceus*. The olivanic acids are potent β-lactamase inhibitors, especially towards the cephalosporinases, which are poorly inhibited by clavulanic acid. Only the broad outlines of the pathways to carbapenems are established (Figure 7.43). The fundamental ring system is derived from glutamic acid and acetate. It is suggested that an activated form of glutamic acid, e.g. γ-glutamyl phosphate, is chain

Clavulanic Acid

Clavulanic acid (Figure 7.38) is produced by cultures of *Streptomyces clavuligerus*, the same actinomycete that produces cephamycin C. Although it has only weak antibacterial activity, it is capable of reacting with a wide variety of β-lactamase enzymes, opening the β-lactam ring, in a process initially analogous to that seen with penicillins (Figure 7.41). However, binding to the enzyme is irreversible, and the β-lactamase is inactivated. It seems likely that the side-chain with its double bond may contribute to this effect, causing further ring opening to give intermediates which react at the active site of the enzyme (Figure 7.41). Thus, dihydroclavulanic acid is no longer an enzyme inhibitor. Clavulanic acid is usually combined with a standard penicillin, e.g. with amoxicillin (as **co-amoxyclav**) or with ticarcillin, to act as a suicide substrate and provide these agents with protection against β-lactamases, thus extending their effectivity against a wider range of organisms. Similar success has been achieved using the semi-synthetic penicillanic acid sulphone, **sulbactam** (Figure 7.42), which is also a potent inhibitor of β-lactamase by a similar double ring opening mechanism (Figure 7.42). Sulbactam was used in combination with ampicillin, but has been superseded by the related sulphone **tazobactam** (Figure 7.42), which is a β-lactamase inhibitor used in combination with piperacillin.

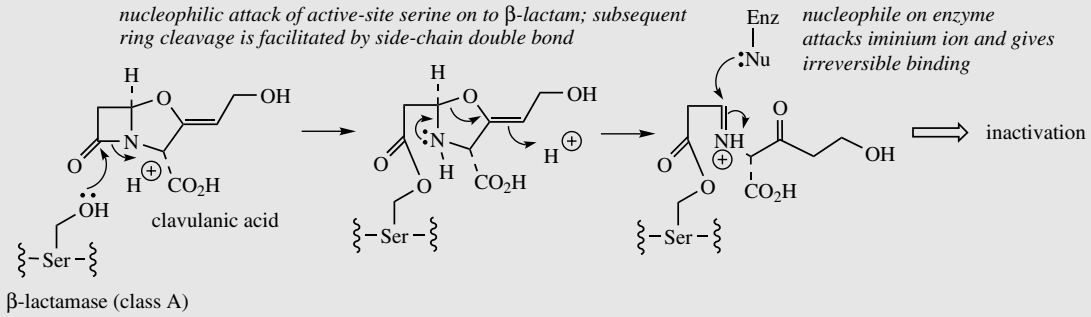


Figure 7.41

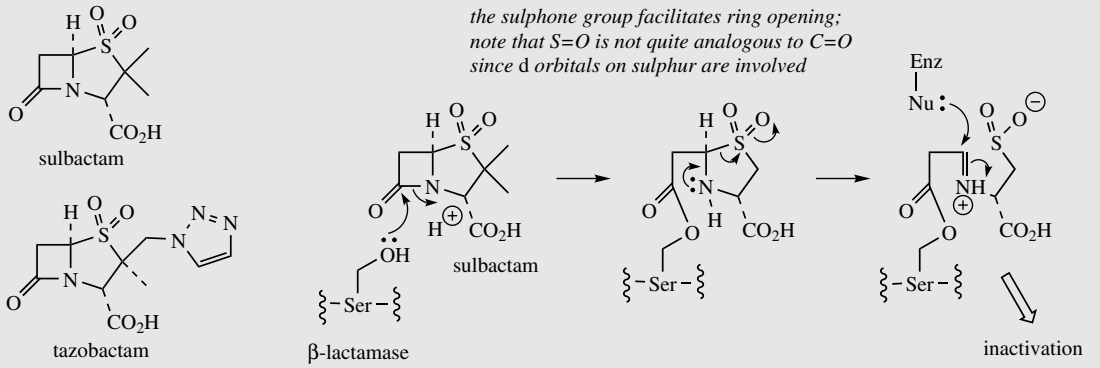


Figure 7.42

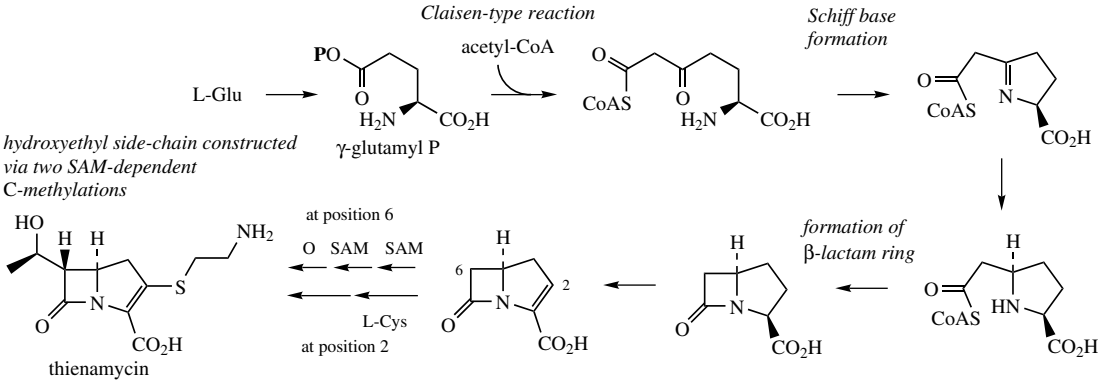


Figure 7.43

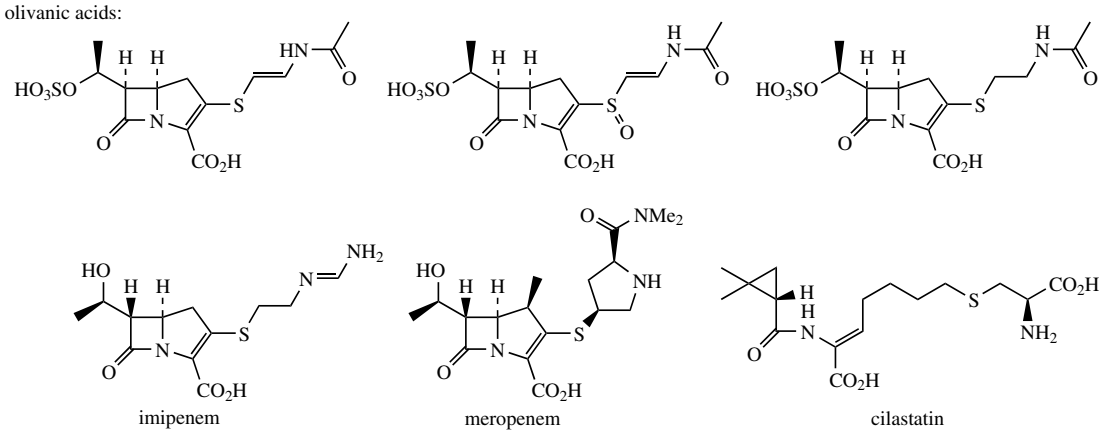


Figure 7.44

extended with acetyl-CoA, then cyclized to the imine (Figure 7.43). Reduction and β-lactam formation lead to the carbapenam, which yields the carbapenem by dehydrogenation. This material is modified to produce thienamycin by insertion of side-chains at C-2 and C-6; the detailed sequence has yet to be established. Both carbons of the two-carbon side-chain at C-2 are known to be derived from methionine, the result of a double methylation sequence reminiscent of the processes used for alkylating the side-chains of sterols (see page 254). The C-2 cysteaminyl side-chain is supplied by the amino acid cysteine.

The simple nonfused β-lactam ring is encountered in a number of natural structures, such as the **nocardicins**, e.g. nocardicin A (Figure 7.45), from *Nocardia uniformis*, and the so-called **monobactams*** (monocylic β-lactams) based on the structure shown in Figure 7.39, which was

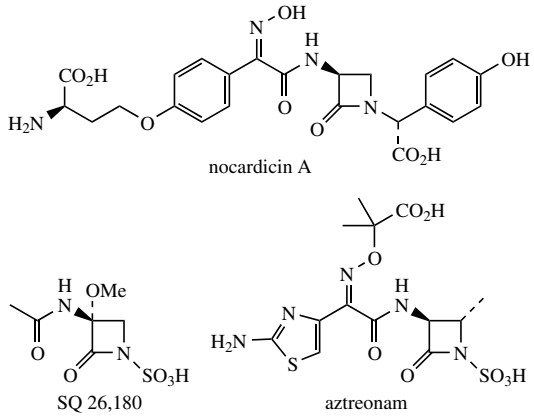


Figure 7.45

isolated from strains of bacteria. The simplest of these monobactams is the compound referred to by its research coding SQ 26,180 (Figure 7.45)

Carbapenems

Thienamycin (Figure 7.44) is produced by cultures of *Streptomyces cattleya*, but in insufficient amounts for commercial use. This compound is thus obtained by total synthesis. In addition, thienamycin is relatively unstable, its side-chain primary amino group reacting as a nucleophile with other species, including the β-lactam group in other molecules. For drug use, thienamycin is converted into its more stable *N*-formimidoyl derivative **imipenem** (Figure 7.44). Imipenem has a broad spectrum of activity which includes activity towards many aerobic and anaerobic Gram-positive and Gram-negative bacteria, it is resistant to hydrolysis by most classes of β-lactamases, and it also possesses β-lactamase inhibitory activity. However, it is partially inactivated by dehydropeptidase in the kidney, and is thus administered in combination with cilastatin (Figure 7.45), a specific inhibitor of this enzyme. The newer carbapenem **meropenem** (Figure 7.45) is stable to dihydropeptidase and can be administered as a single agent. It has good activity against all clinically significant aerobes and anaerobes, except MRSA (methicillin-resistant *S. aureus*) and *Enterococcus faecium*, and stability to serine-based β-lactamases.

discovered in *Chromobacterium violaceum*. Whilst many of the natural examples show a 3α-methoxyl (corresponding to the 7α-methoxyl in the cephamycins), the prominent feature in the monobactams is the *N*-sulphonic acid grouping. The fundamental precursor of the β-lactam ring in the monobactams is serine (Figure 7.46). In the simple structures, the β-lactam nitrogen presumably arises from ammonia, and cyclization occurs by displacement of the hydroxyl, in suitably activated form. The *N*-sulphonate function comes from sulphate. For the more complex structures such as the nocardicins, evidence points to a polypeptide origin with considerable parallels with penicillin and cephalosporin biosynthesis. The postulated D,L,D-tripeptide is formed from L-serine and two molecules of L-4-hydroxyphenylglycine, the latter having its origins in L-tyrosine (Figure 7.47). The inversion of configurations probably occurs during polypeptide formation (compare penicillins, page 438). The remaining portion in the carbon skeleton of nocardicin A is ether-linked to a 4-hydroxyphenylglycine unit, and actually derives from L-methionine. This probably involves an S_N2 displacement on SAM as with simple methylation reactions, though attack must be on the secondary rather than primary centre (Figure 7.47). Again, the configuration of the L-amino acid is inverted at a late stage, as with the isopenicillin N to penicillin N conversion in cephalosporin biosynthesis (see page 445).

Monobactams

The naturally occurring monobactams show relatively poor antibacterial activity, but alteration of the side-chain as with penicillins and cephalosporins has produced many potent new compounds. Unlike those structures, the nonfused β-lactam ring is readily accessible by synthesis, so all analogues are produced synthetically. The first of these to be used clinically is **aztreonam** (Figure 7.45), which combines the side-chain of the cephalosporin ceftazidime (Table 7.4) with the monobactam nucleus. Aztreonam is very active against Gram-negative bacteria, including *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Neisseria meningitidis*, but has little activity against Gram-positive organisms. It also displays a high degree of resistance to enzymatic hydrolysis by most of the common β-lactamases. Oral absorption is poor, and this drug is administered by injection.

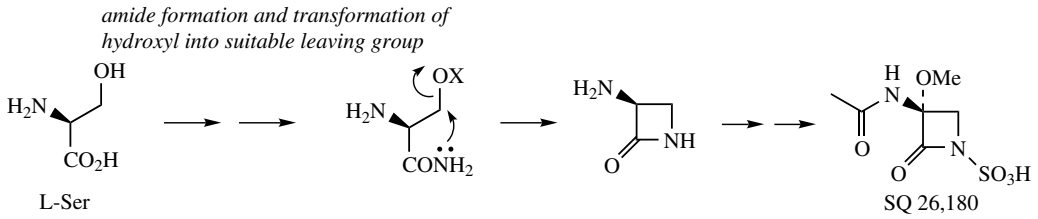


Figure 7.46

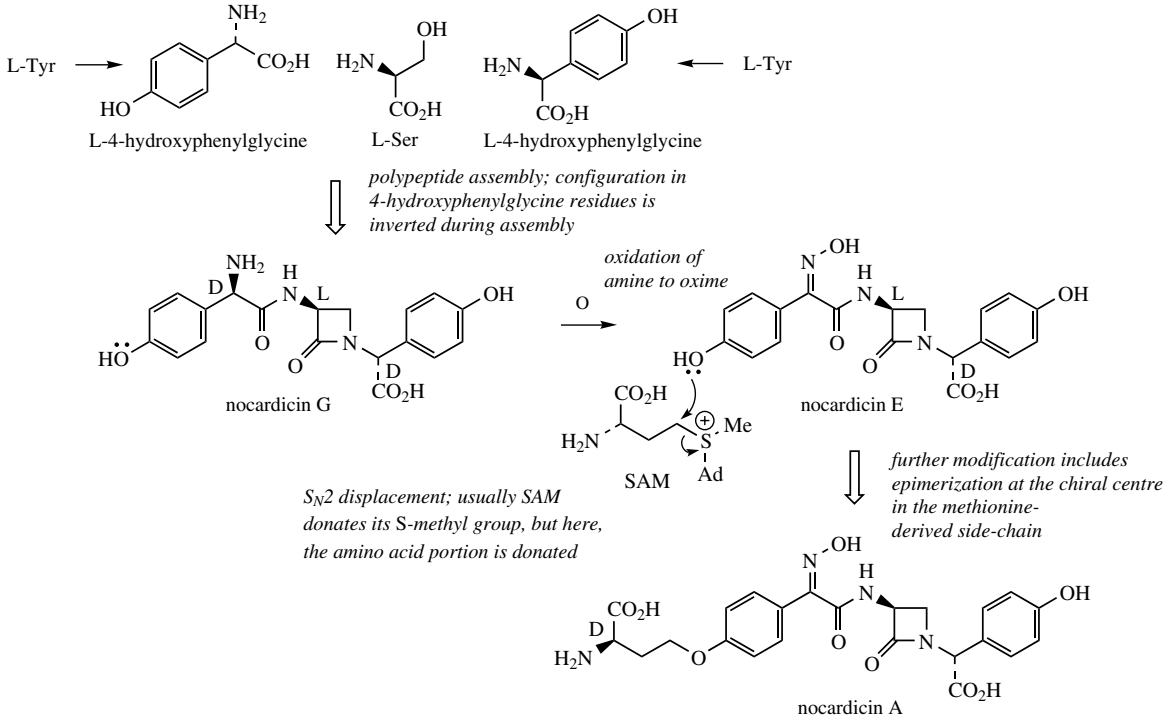


Figure 7.47

CYANOGENIC GLYCOSIDES

Cyanogenic glycosides are a group of mainly plant-derived materials, which liberate hydrocyanic acid (HCN) on hydrolysis, and are thus of concern as natural toxicants. The group is exemplified by **amygdalin** (Figure 7.48), a constituent in the kernels of bitter almonds (*Prunus amygdalus* var. *amara*; Rosaceae) and other *Prunus* species such as apricots, peaches, cherries, and plums. When plant tissue containing a cyanogenic glycoside is crushed, glycosidase enzymes also in the plant but usually located in different cells are brought into contact with the glycoside and begin to hydrolyse it. Thus, amygdalin is hydrolysed sequentially by β-glucosidase-type enzymes to **prunasin** and then **mandelonitrile**, the latter compound actually being the cyanohydrin of benzaldehyde (Figure 7.48). Mandelonitrile is then hydrolysed to its component parts, benzaldehyde and toxic HCN, by the action of a further enzyme. The kernels of bitter almonds, which contain amygdalin and the hydrolytic enzymes, are thus potentially toxic if ingested, whilst kernels of sweet almonds (*Prunus amygdalus* var. *dulcis*) are not toxic, containing the enzymes

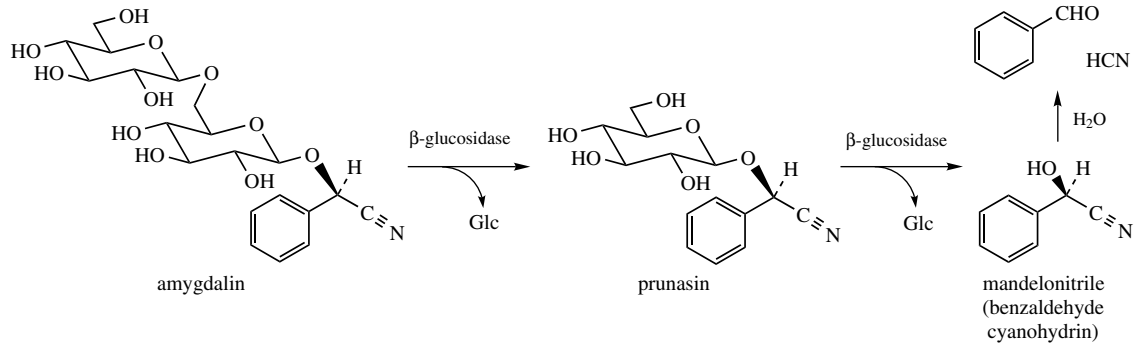


Figure 7.48

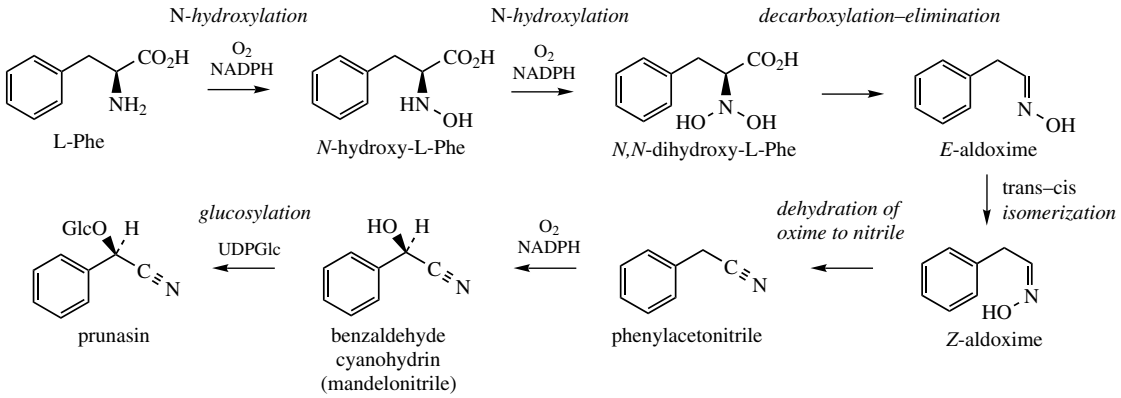


Figure 7.49

but no cyanogenic glycoside. Amygdalin itself is not especially toxic to animals; toxicity depends on the co-ingestion of the hydrolytic enzymes. Although formed by the hydrolysis of amygdalin, prunasin is also a natural cyanogenic glycoside and may be found in seeds of black cherry (*Prunus serotina*), and in the seeds and leaves of cherry laurel (*Prunus laurocerasus*). The food plant cassava (or tapioca) (*Manihot esculenta*; Euphorbiaceae) also produces the cyanogenic glycosides **linamarin** and **lotaustralin** (see Figure 7.50), and preparation of the starchy tuberous roots involves prolonged hydrolysis and boiling to release and drive off the HCN before they are suitable for consumption.

Cyanogenic glycosides are produced from a range of amino acids by a common pathway (Figure 7.49). The amino acid precursor of prunasin is phenylalanine, which is *N*-hydroxylated and then converted into the aldehyde oxime (aldoxime) by a sequence that involves further *N*-hydroxylation and subsequent decarboxylation–elimination, with all of these reactions catalysed by a single cytochrome P-450-dependent enzyme. The nitrile is formed by dehydration of the oxime, but this reaction actually proceeds on the *Z*-aldoxime produced by isomerization of the first formed *E*-aldoxime. (*R*)-Mandelonitrile is then the result of a stereoselective cytochrome P-450-dependent hydroxylation reaction. Finally, glycosylation occurs, the sugar unit usually being glucose as in the case of prunasin and amygdalin. The stereoselectivity of the nitrile hydroxylation step varies depending on the plant system, so that epimeric cyanohydrins are found in nature, though not in the same plant. Thus, the (*S*)-enantiomer of prunasin (called sambunigrin) is found in the leaves of elder (*Sambucus nigra*; Caprifoliaceae).

The main amino acids utilized in the biosynthesis of cyanogenic glycosides are phenylalanine (e.g. prunasin, sambunigrin, and amygdalin), tyrosine (e.g. **dhurrin** from sorghum (*Sorghum bicolor*; Graminae/Poaceae)), valine (e.g. linamarin from flax (*Linum usitatissimum*; Linaceae)), isoleucine (e.g. lotaustralin, also from flax), and leucine (e.g. **heterodendrin** from *Acacia* species (Leguminosae/Fabaceae)) (Figure 7.50). Although cyanogenic glycosides are widespread, they are particularly found in the families Rosaceae, Leguminosae/Fabaceae, Graminae/Poaceae, Araceae, Compositae/Asteraceae, Euphorbiaceae, and Passifloraceae. It is highly likely that plants synthesize these compounds as protecting agents against herbivores. Some insects also accumulate cyanogenic glycosides in their bodies, again as a protective device. Whilst many insects obtain these compounds by feeding on suitable plant sources, it is remarkable that others are known to synthesize cyanogenic glycosides themselves from amino acid precursors. There is hope that cyanogenesis may provide a means of destroying cancer cells. By targeting cancer cells with linamarase via a retrovirus and then supplying linamarin, it has been possible to selectively generate toxic HCN in cancer cells.

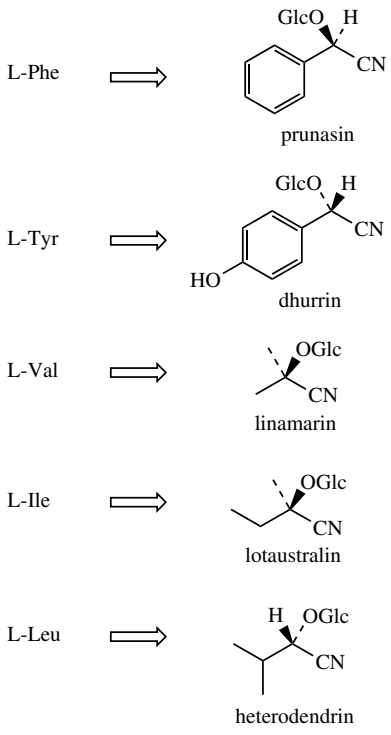


Figure 7.50

GLUCOSINOLATES

Glucosinolates have several features in common with the cyanogenic glycosides. They too are glycosides which are enzymically hydrolysed in damaged plant tissues giving rise to potentially toxic materials, and they share the early stages of the cyanogenic glycoside biosynthetic pathway for their formation in plants. A typical structure is **sinalbin** (Figure 7.51), found in seeds of white mustard (*Sinapis alba*; Cruciferae/Brassicaceae). Addition of water to the crushed or powdered seeds results in hydrolysis of the glucoside bond via the enzyme myrosinase (a thioglucosidase) to give a thiohydroximate sulphonate (Figure 7.51). This compound usually yields the isothiocyanate **acrinylisothiocyanate** by a Lössen-type rearrangement as shown, prompted by the sulphate leaving group. Under certain conditions, dependent on pH, or the presence of metal ions or other enzymes, related compounds such as thiocyanates (RSCN) or nitriles (RCN) may be formed from glucosinolates. Acrinylisothiocyanate is a pungent-tasting material (mustard oil) typical of many plants in the Cruciferae/Brassicaceae used as vegetables (e.g. cabbage, radish) and condiments (e.g. mustard, horseradish). Black mustard (*Brassica nigra*) contains **sinigrin** (Figure 7.51) in its seeds, which by a similar sequence is hydrolysed to **allylisothiocyanate**. Allylisothiocyanate is considerable more volatile than acrinylisothiocyanate, so that condiment mustard prepared from black mustard has a pungent aroma as well as taste.

The biosynthesis of **sinalbin** from tyrosine is indicated in Figure 7.52. The aldoxime is produced from the amino acid by the early part of the cyanogenic glycoside pathway shown in Figure 7.49. This aldoxime incorporates sulphur from methionine (or cysteine) to give the thiohydroximic acid, perhaps by attack of a thiolate ion on to the imine system, and this compound is then *S*-glucosylated using UDPglucose. In nature, sulphate groups are provided by PAPS (3′-phosphoadenosine-5′-phosphosulphate), and sulphation features as the last step in the pathway. Similarly, phenylalanine is the precursor of **benzylglucosinolate**

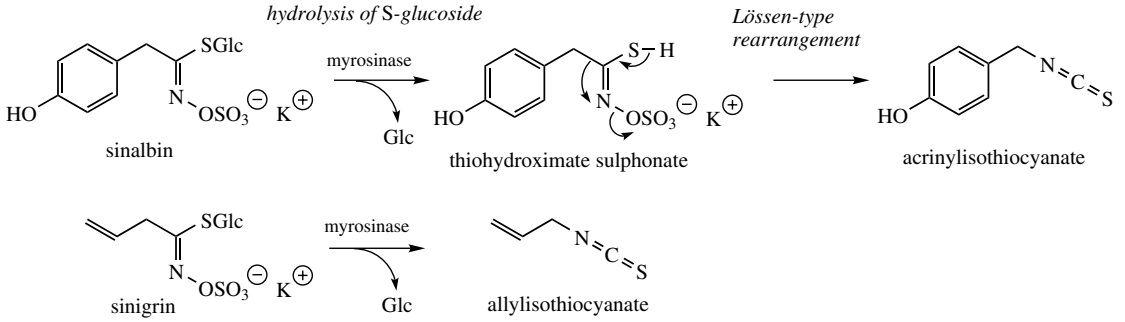


Figure 7.51

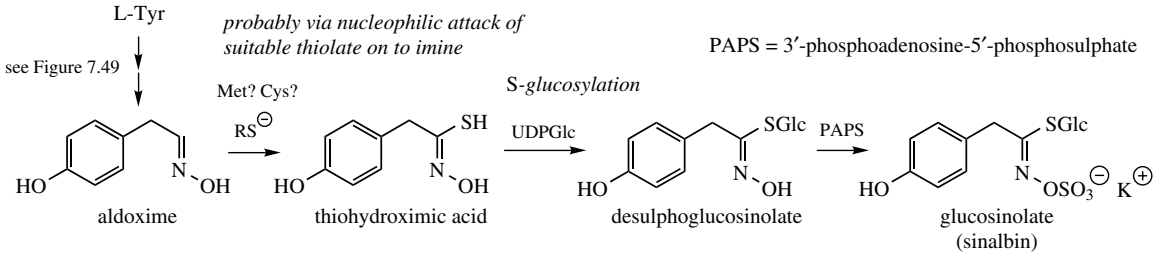


Figure 7.52

(Figure 7.53) in nasturtium (*Tropaeolum majus*; Tropaeolaceae), and tryptophan yields **glucobrassicin** in horseradish (*Armoracia rusticana*; Cruciferae/Brassicaceae). Interestingly, chain extension of methionine to **homomethionine** and of phenylalanine to **homophenylalanine** is involved in the formation of **sinigrin** in *Brassica nigra* and **gluconasturtiin** in rapeseed (*Brassica napus*) respectively (Figure 7.54). Two carbons derived from acetate are incorporated into the side-chain in each case, together with loss of the original carboxyl, and this allows biosynthesis of the appropriate glucosinolates. For elaboration of the allyl side-chain in the biosynthesis of sinigrin, loss of methanethiol occurs as a late step (Figure 7.54).

Glucosinolates are found in many plants of the Cruciferae/Brassicaceae, Capparidaceae, Euphorbiacae, Phytolaccaceae, Resedaceae, and Tropaeolaceae, contributing to the pungent properties of their crushed tissues. They are often at their highest concentrations in seeds rather than leaf tissue. These compounds and their degradation products presumably deter some predators, but may actually attract others, e.g. caterpillars on cabbages and

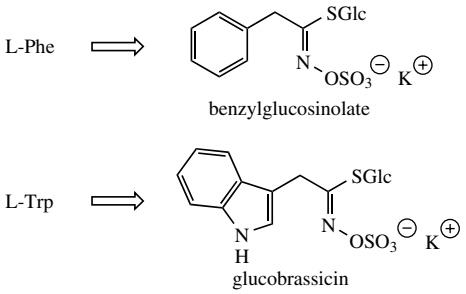


Figure 7.53

similar crops. There is evidence that consumption of the hydrolysis products from glucosinolates in food crops may induce goitre, an enlargement of the thyroid gland. Thus, **progoitrin** in oil seed rape (*Brassica napus*; Cruciferae/Brassicaceae) on hydrolysis yields the oxazolidine-2-thione **goitrin** (Figure 7.55), which is a potent goitrogen, inhibiting iodine incorporation and thyroxine formation (see page 410). The goitrogenic effects of glucosinolates cannot be alleviated merely by the administration of iodine. This severely limits economic utilization for animal foodstuffs of

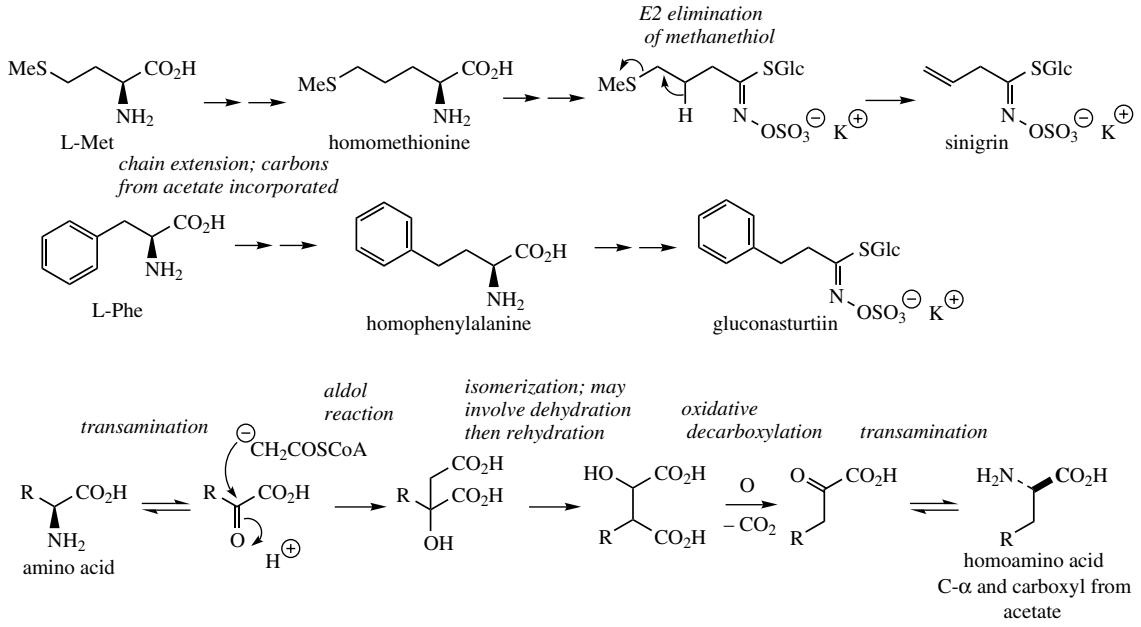


Figure 7.54

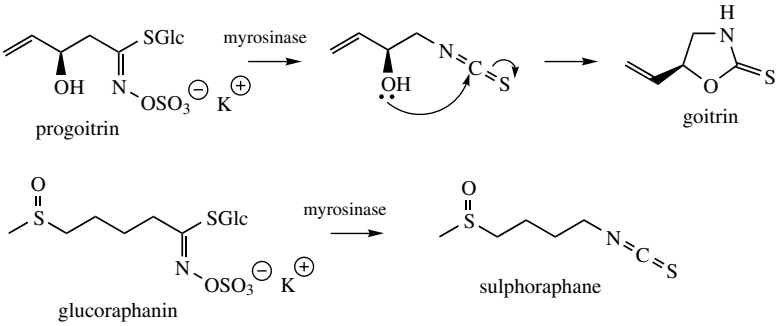


Figure 7.55

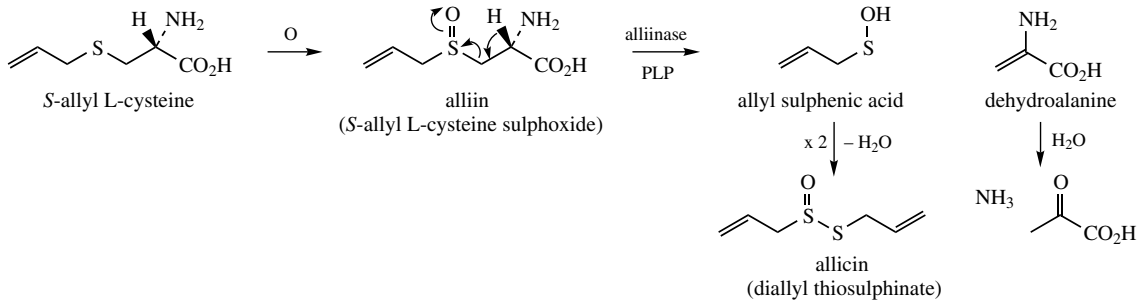


Figure 7.56

the rapeseed meal remaining after oil expression unless strains with very low levels of the glucosinolate are employed. On the other hand, **glucoraphanin**, the glucosinolate precursor of **sulphoraphane** (Figure 7.55) from broccoli (*Brassica oleracea italica*; Cruciferae/Brassicaceae), has been shown to have beneficial medicinal properties, in that it induces carcinogen-detoxifying enzyme systems and accelerates the removal of xenobiotics. Young sprouted seedlings contain some 10–100 times as much glucoraphanin as the mature plant, but, nevertheless, broccoli may be regarded as a valuable dietary vegetable.

CYSTEINE SULPHOXIDES

The major flavour component of garlic* (*Allium sativum*; Liliaceae/Alliaceae) is a thiosulphinate called **allicin** (Figure 7.56). This compound is formed when garlic tissue is damaged as a hydrolysis product of *S*-allyl cysteine sulphoxide (**alliin**) brought about by the pyridoxal phosphate-dependent enzyme alliinase (Figure 7.56). Under these conditions, alliin is cleaved by an elimination reaction, and two molecules of the sulphenic acid then form allicin. Pyruvic acid and ammonia are the other hydrolysis products. Allicin has considerable antibacterial and antifungal properties. There is widespread use of garlic, either fresh, dried, or as garlic oil, as a beneficial agent to reduce cholesterol levels and reduce the risk of heart attacks. *S*-Alkyl cysteine sulphoxides are characteristic components of the onion (*Allium*) genus. All *Allium* species contain *S*-methyl cysteine sulphoxide, though the *S*-propyl analogue (Figure 7.57) predominates in chives (*A. schoenoprasum*), the *S*-1-propenyl derivative in onions (*A. cepa*) and the *S*-allyl compound alliin in garlic. Propenyl sulphenic acid derived by hydrolysis of *S*-1-propenyl cysteine sulphoxide provides a common kitchen hazard, rearranging to the lachrymatory factor of onions (Figure 7.58).

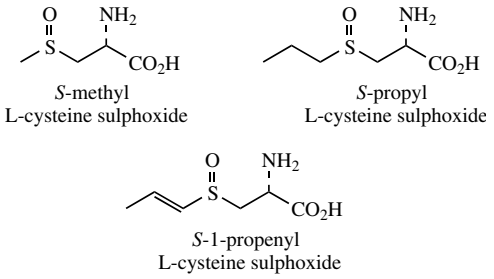


Figure 7.57

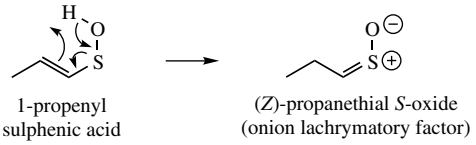


Figure 7.58

Garlic

Garlic (*Allium sativum*; Liliaceae/Alliaceae) has a long history of culinary and medicinal use. The compound bulb is composed of several smaller sections termed cloves. Allicin (Figure 7.56) is considered to be the most important of the biologically active components in the crushed bulb. It is not present in garlic, but is rapidly produced when the precursor alliin is cleaved by the action of the enzyme alliinase upon crushing the tissue. Both alliin and alliinase are stable when dry, and dried garlic still has the potential for releasing allicin when subsequently moistened. However, allicin itself is very unstable to heat or organic solvents degrading to many other compounds, including diallyl sulphides (mono-, di-, and oligo-sulphides), vinyldithiins, and ajoenes (Figure 7.59). Processed garlic preparations typically contain a range of different sulphur compounds. Garlic preparations used medicinally include steam-distilled oils, garlic macerated in vegetable oils (e.g. soybean oil), dried garlic powder, and gel-suspensions of garlic powder. Analyses indicate wide variations in the nature and amounts of constituents in the various preparations. Thus, freshly crushed garlic cloves typically contain allicin (about 0.4%) and other thiosulphinates (about 0.1%, chiefly allyl methyl thiosulphinate). The

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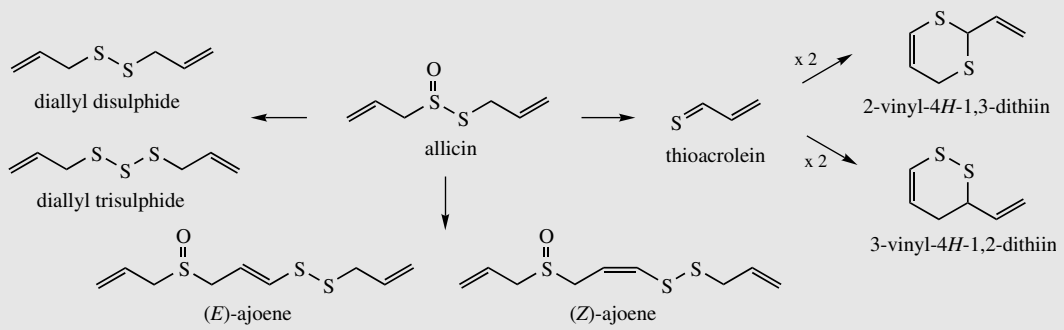

Figure 7.59

garlic powder usually produces less of these materials, though high quality samples may be similar to the fresh samples. Oil-macerated powders appear to lose up to 80% of their sulphur compounds, and vinyldithiins and ajoenes predominate. Steam-distilled garlic oil has dialk(en)yl sulphides (i.e. diallyl sulphide, allyl methyl sulphide, etc) as the major sulphur components (0.1–0.5%). Bad breath and perspiration odours which often follow the ingestion of garlic, either medicinally or culinary, are due to allyl methyl sulphide and disulphide, diallyl sulphide and disulphide, and 2-propenethiol.

Garlic is used for a variety of reasons, and some of the attributes associated with it, e.g. for cancer prevention, or to reduce heart attacks, may not be substantiated. Other properties such as antimicrobial activity, effects on lipid metabolism, and platelet aggregation inhibitory action have been demonstrated. Ajoene has been shown to be a potent antithrombotic agent through inhibition of platelet aggregation.

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