Chapter 1

Pharmaceuticals, biologics and biopharmaceuticals

INTRODUCTION TO PHARMACEUTICAL PRODUCTS

Pharmaceutical substances form the backbone of modern medicinal therapy. Most traditional pharmaceuticals are low molecular mass organic chemicals (Table 1.1). Although some (e.g. aspirin) were originally isolated from biological sources, most are now manufactured by direct chemical synthesis. Two types of manufacturing companies thus comprise the 'traditional' pharmaceutical sector; the chemical synthesis plants, which manufacture the raw chemical ingredients in bulk quantities, and the finished product pharmaceutical facilities, which purchase these raw bulk ingredients, formulate them into final pharmaceutical products, and supply these products to the end-user.

In addition to chemical-based drugs, a range of pharmaceutical substances (e.g. hormones and blood products) are produced by or extracted from biological sources. Such products, some major examples of which are listed in Table 1.2, may thus be described as products of biotechnology. In some instances, categorizing pharmaceuticals as products of biotechnology or chemical synthesis becomes somewhat artificial, e.g. certain semi-synthetic antibiotics are produced by chemical modification of natural antibiotics produced by fermentation technology.

BIOPHARMACEUTICALS AND PHARMACEUTICAL BIOTECHNOLOGY

Terms such as 'biologic', 'biopharmaceutical' and 'products of pharmaceutical biotechnology' or 'biotechnology medicines' have now become an accepted part of the pharmaceutical literature. However, these terms are sometimes used interchangeably and can mean different things to different people.

While it might be assumed that 'biologic' refers to any pharmaceutical product produced by biotechnological endeavour, its definition is more limited. In pharmaceutical circles, 'biologic'

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Table 1.1. Some traditional pharmaceutical substances which are generally produced by direct chemical synthesis

Drug	Molecular formula	Molecular mass	Therapeutic indication
Acetaminophen (paracetamol)	$C_8H_9NO_2$	151.16	Analgesic
Ketamine	$C_{13}H_{16}ClNO$	237.74	Anaesthetic
Levamisole	$C_{11}H_{12}N_2S$	204.31	Anthelmintic
Diazoxide	$C_8H_7ClN_2O_2S$	230.7	Anti-hypertensive
Acyclovir	$C_8H_{11}N_5O_3$	225.2	Anti-viral agent
Zidovudine	$C_{10}H_{13}N_5O_4$	267.2	Anti-viral agent
Dexamethasone	$C_{22}H_{29}FO_5$	392.5	Anti-inflammatory and immunosuppressive agent
Misoprostol	$C_{22}H_{38}O_5$	382.5	Anti-ulcer agent
Cimetidine	$C_{10}H_{16}N_6$	252.3	Anti-ulcer agent

Table 1.2. Some pharmaceuticals which were traditionally obtained by direct extraction from biological source material. Many of the protein-based pharmaceuticals mentioned below are now also produced by genetic engineering

Substance	Medical application
Blood products (e.g. coagulation factors)	Treatment of blood disorders such as haemophilia A or B
Vaccines	Vaccination against various diseases
Antibodies	Passive immunization against various diseases
Insulin	Treatment of diabetes mellitus
Enzymes	Thrombolytic agents, digestive aids, debriding agents (i.e. cleansing of wounds)
Antibiotics	Treatment against various infectious agents
Plant extracts (e.g. alkaloids)	Various, including pain relief

generally refers to medicinal products derived from blood, as well as vaccines, toxins and allergen products. Thus, some traditional biotechnology-derived pharmaceutical products (e.g. hormones, antibiotics and plant metabolites) fall outside the strict definition.

The term 'biopharmaceutical' was first used in the 1980s and came to describe a class of therapeutic protein produced by modern biotechnological techniques, specifically via genetic engineering or (in the case of monoclonal antibodies) by hybridoma technology. This usage equated the term 'biopharmaceutical' with 'therapeutic protein synthesized in engineered (non-naturally occurring) biological systems'. More recently, however, nucleic acids used for purposes of gene therapy and antisense technology (Chapter 11) have come to the fore and they too are generally referred to as 'biopharmaceuticals'. Moreover, several recently approved proteins are used for *in vivo* diagnostic as opposed to therapeutic purposes. Throughout this book therefore, the term 'biopharmaceutical' refers to protein or nucleic acid based pharmaceutical substances used for therapeutic or *in vivo* diagnostic purposes, which are produced by means other than direct extraction from natural (non-engineered) biological sources (Tables 1.3 and 1.4).

As used herein, 'biotechnology medicines' or 'products of pharmaceutical biotechnology' are afforded a much broader definition. Unlike the term 'biopharmaceutical', the term

Table 1.3. A summary of the definition of the terms 'biologic', 'biopharmaceutical' and 'biotechnology medicine' as used throughout this book. Reprinted from European Journal of Pharmaceutical Sciences, vol 15, Walsh, Biopharmaceuticals and Biotechnology, p 135–138, ©2002, with permission from Elsevier Science

Biopharmaceutical	A protein or nucleic acid based pharmaceutical substance used for therapeutic or *in vivo* diagnostic purposes, which is produced by means other than direct extraction from a native (non-engineered) biological source
Biotechnology medicine/ product of pharmaceutical biotechnology	Any pharmaceutical product used for therapeutic or *in vivo* diagnostic purposes, which is produced in full or in part by biotechnological means
Biologic	A virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product or analogous product, or arsphenamine or its derivatives or any other trivalent organic arsenic compound applicable to the prevention, cure or treatment of disease or conditions of human beings

'biotechnology' has a much broader and long-established meaning. Essentially, it refers to the use of biological systems (e.g. cells or tissues) or biological molecules (e.g. enzymes or antibodies) for or in the manufacture of commercial products. Therefore, the term is equally applicable to long-established biological processes, such as brewing, and more modern processes, such as genetic engineering. As such, the term 'biotechnology medicine' is defined here as 'any pharmaceutical product used for a therapeutic or *in vivo* diagnostic purpose, which is produced in full or in part by either traditional or modern biotechnological means'. Such products encompass, for example, antibiotics extracted from fungi, therapeutic proteins extracted from native source material (e.g. insulin from pig pancreas) and products produced by genetic engineering (e.g. recombinant insulin) (Tables 1.3 and 1.4).

HISTORY OF THE PHARMACEUTICAL INDUSTRY

The pharmaceutical industry, as we now know it, is barely 60 years old. From very modest beginnings it has grown rapidly, reaching an estimated value of $100 billion by the mid-1980s. Its current value is likely double this figure or more. There are well in excess of 10 000 pharmaceutical companies in existence, although only about 100 of these can claim to be of true international significance. These companies manufacture in excess of 5000 individual pharmaceutical substances used routinely in medicine.

The first stages of development of the modern pharmaceutical industry can be traced back to the turn of the twentieth century. At that time (apart from folk cures), the medical community had at their disposal only four drugs that were effective in treating specific diseases:

- Digitalis, extracted from foxglove, was known to stimulate heart muscle and hence was used to treat various heart conditions.
- Quinine, obtained from the barks/roots of a plant (*Cinchona* sp.), was used to treat malaria.
- Pecacuanha (active ingredient is a mixture of alkaloids), used for treating dysentery, was obtained from the bark/roots of the plant species *Cephaelis*.
- Mercury, for the treatment of syphilis.

Table 1.4. The categorization of pharmaceutically significant biological molecules using the indicated definitions as listed in Table 1.3. Reproduced in modified form from European Journal of Pharmaceutical Sciences, vol 15, Walsh, Biopharmaceuticals and Biotechnology, p 135–138, ©2002, with permission from Elsevier Science

Pharmaceutical product	Biopharmaceutical?	Biotechnology medicine?	Biologic?
Recombinant protein	Yes	Yes	No
Monoclonal antibody	Yes	Yes	No
Proteins obtained by direct extraction from native source (e.g. blood derived clotting factors)	No	Yes	Some (e.g. blood factors and polyclonal antibodies)
Gene therapy products	Yes	Yes	No
Antisense oligonucleotides manufactured by direct chemical synthesis	Yes	No	No
Antisense oligonucleotides produced by enzymatic synthesis	Yes	Yes	No
Peptides manufactured by direct chemical synthesis	No	No	No
Peptides, if obtained by direct extraction from native producer source	No	Yes	No
Antibiotics obtained by direct extraction from native producer, or by semi-synthesis	No	Yes	No
Plant-based products obtained by direct extraction from a native producer, or by semi-synthesis (e.g. taxol)	No	Yes	No
Cell/tissue-based therapeutic agents	No	Yes	No

The lack of appropriate safe and effective medicines contributed in no small way to the low life expectancy characteristic of those times.

Developments in biology (particularly the growing realization of the microbiological basis of many diseases), as well as a developing appreciation of the principles of organic chemistry, helped underpin future innovation in the fledgling pharmaceutical industry. The successful synthesis of various artificial dyes, which proved to be therapeutically useful, led to the formation of pharmaceutical/chemical companies such as Bayer and Hoechst in the late 1800s, e.g. scientists at Bayer succeeded in synthesizing aspirin in 1895.

Despite these early advances, it was not until the 1930s that the pharmaceutical industry began to develop in earnest. The initial landmark discovery of this era was probably the discovery and chemical synthesis of the sulpha drugs. These are a group of related molecules derived from the red dye, *Prontosil rubrum*. These drugs proved effective in the treatment of a wide variety of bacterial infections (Figure 1.1). Although it was first used therapeutically in the early 1920s, large-scale industrial production of insulin also commenced in the 1930s.

The medical success of these drugs gave new emphasis to the pharmaceutical industry, which

was boosted further by the commencement of industrial-scale penicillin manufacture in the early 1940s. Around this time, many of the current leading pharmaceutical companies (or their forerunners) were founded. Examples include Ciba Geigy, Eli Lilly, Wellcome, Glaxo and Roche. Over the next two to three decades, these companies developed drugs such as tetracyclines, corticosteroids, oral contraceptives, antidepressants and many more. Most of these pharmaceutical substances are manufactured by direct chemical synthesis.

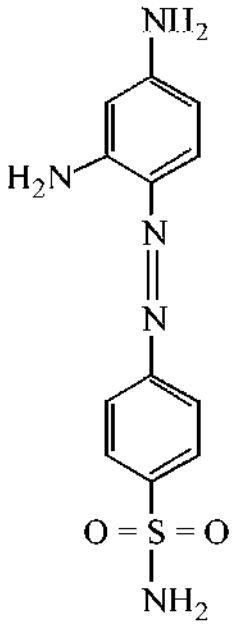
THE AGE OF BIOPHARMACEUTICALS

Biomedical research continues to broaden our understanding of the molecular mechanisms underlining both health and disease. Research undertaken since the 1950s has pinpointed a host of proteins produced naturally in the body which have obvious therapeutic applications. Examples include the interferons, and interleukins, which regulate the immune response; growth factors such as erythropoietin, which stimulates red blood cell production; and neurotrophic factors, which regulate the development and maintenance of neural tissue.

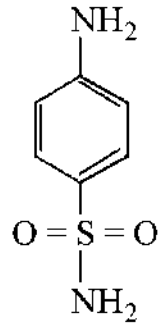
While the pharmaceutical potential of these regulatory molecules was generally appreciated, their widespread medical application was in most cases rendered impractical due to the tiny quantities in which they were naturally produced. The advent of recombinant DNA technology (genetic engineering) and monoclonal antibody technology (hybridoma technology) overcame many such difficulties, and marked the beginning of a new era of the pharmaceutical sciences.

Recombinant DNA technology has had a four-fold positive impact upon the production of pharmaceutically important proteins:

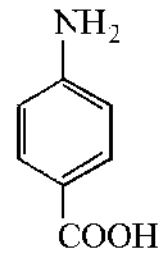
- *It overcomes the problem of source availability*. Many proteins of therapeutic potential are produced naturally in the body in minute quantities. Examples include interferons (Chapter 4), interleukins (Chapter 5) and colony stimulating factors (Chapter 6). This rendered impractical their direct extraction from native source material in quantities sufficient to meet likely clinical demand. Recombinant production (Chapter 3) allows the manufacture of any protein in whatever quantity it is required.
- *It overcomes problems of product safety*. Direct extraction of product from some native biological sources has, in the past, led to the unwitting transmission of disease. Examples include the transmission of blood-borne pathogens such as hepatitis B, C and HIV via infected blood products and the transmission of Creutzfeldt–Jakob disease to persons receiving human growth hormone preparations derived from human pituitaries.
- *It provides an alternative to direct extraction from inappropriate/dangerous source material*. A number of therapeutic proteins have traditionally been extracted from human urine. The fertility hormone FSH, for example, is obtained from the urine of post-menopausal women, while a related hormone, hCG, is extracted from the urine of pregnant women (Chapter 8). Urine is not considered a particularly desirable source of pharmaceutical products. While several products obtained from this source remain on the market, recombinant forms have now also been approved. Other potential biopharmaceuticals are produced naturally in downright dangerous sources. Ancrod, for example, is a protein displaying anti-coagulant activity (Chapter 9) and, hence, is of potential clinical use; however, it is produced naturally by the Malaysian pit viper. While retrieval by milking snake venom is possible, and indeed may be quite an exciting procedure, recombinant production in less dangerous organisms, such as *Escherichia coli* or *Saccharomyces cerevisiae*, would be considered preferable by most.
- *It facilitates the generation of engineered therapeutic proteins displaying some clinical*



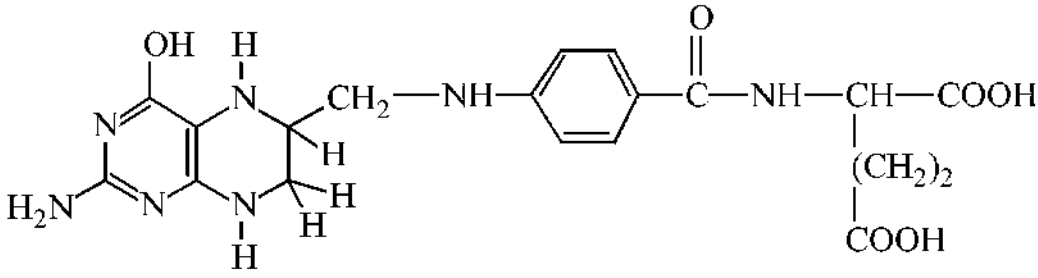
Prontosil rubrum
(a)



Sulphanilamide
(b)



PABA
(c)



Tetrahydrofolic acid
(d)

Table 1.5. Selected engineered biopharmaceutical types/products, which have now gained marketing approval. These and additional such products will be discussed in detail in subsequent chapters

Product description/type	Alteration introduced	Rationale
Faster-acting insulins (Chapter 8)	Modified amino acid sequence	Generation of faster-acting insulin
Slow-acting insulins (Chapter 8)	Modified amino acid sequence	Generation of slow-acting insulin
Modified tissue plasminogen activator (tPA: Chapter 9)	Removal of three of the five native domains of tPA	Generation of a faster-acting thrombolytic (clot degrading) agent
Modified blood factor VIII (Chapter 9)	Deletion of one domain of native factor VIII	Production of a lower molecular mass product
Chimaeric/humanized antibodies (Chapter 10)	Replacement of most/virtually all of the murine amino acid sequences with sequences found in human antibodies	Greatly reduced/eliminated immonogenicity. Ability to activate human effector functions
'Ontak', a fusion protein (Chapter 5)	Fusion protein consisting of the diphtheria toxin linked to interleukin-2	Targets toxin selectively to cells expressing an IL-2 receptor

advantage over the native protein product. Techniques such as site-directed mutagenesis facilitate the logical introduction of pre-defined changes in a protein's amino acid sequence. Such changes can be minimal, such as the insertion, deletion or alteration of a single amino acid residue, or can be more substantial, e.g. the alteration or deletion of an entire domain, or the generation of a novel hybrid protein. Such changes can be made for a number of reasons and several engineered products have now gained marketing approval. An overview summary of some engineered product types now on the market is provided in Table 1.5. These and other examples will be discussed in subsequent chapters.

Despite the undoubted advantages of recombinant production, it remains the case that many protein-based products extracted directly from native source material remain on the market. These products have proved safe and effective and selected examples are provided in Table 1.2. In certain circumstances, direct extraction of native source material can prove equally/more attractive than recombinant production. This may be for an economic reason, e.g. if the protein is produced in very large quantities by the native source and is easy to extract/purify, as is the case for human serum albumin (Chapter 9). Also, some blood factor preparations purified from

Figure 1.1. (*Opposite*) Sulpha drugs and their mode of action. The first sulpha drug to be used medically was the red dye prontosil rubrum (a). In the early 1930s, experiments illustrated that the administration of this dye to mice infected with haemolytic streptococci prevented the death of the mice. This drug, while effective *in vivo*, was devoid of *in vitro* antibacterial activity. It was first used clinically in 1935 under the name Streptozon. It was subsequently shown that prontosil rubrum was enzymatically reduced by the liver, forming sulphanilamide, the actual active antimicrobial agent (b). Sulphanilamide induces its effect by acting as an anti-metabolite with respect to *para*-aminobenzoic acid (PABA) (c). PABA is an essential component of tetrahydrofolic acid (THF) (d). THF serves as an essential co-factor for several cellular enzymes. Sulphanilamide (at sufficiently high concentrations) inhibits manufacture of THF by competing with PABA. This effectively inhibits essential THF-dependent enzyme reactions within the cell. Unlike humans, who can derive folates from their diets, most bacteria must synthesize it *de novo*, as they cannot absorb it intact from their surroundings

donor blood actually contain several different blood factors and hence can be used to treat several haemophilia patient types. Recombinant blood factor preparations, on the other hand, contain but a single blood factor and hence can be used to treat only one haemophilia type (Chapter 9).

The advent of genetic engineering and monoclonal antibody technology underpinned the establishment of literally hundreds of start-up biopharmaceutical (biotechnology) companies in the late 1970s and early 1980s. The bulk of these companies were founded in the USA, with smaller numbers of start-ups emanating from Europe and other world regions.

Many of these fledgling companies were founded by academics/technical experts who sought to take commercial advantage of developments in the biotechnological arena. These companies were largely financed by speculative monies attracted by the hype associated with the establishment of the modern biotech era. While most of these early companies displayed significant technical expertise, the vast majority lacked experience in the practicalities of the drug development process (Chapter 2). Most of the well-established large pharmaceutical companies, on the other hand, were slow to invest heavily in biotech research and development. However, as the actual and potential therapeutic significance of biopharmaceuticals became evident, many of these companies did diversify into this area. Most either purchased small established biopharmaceutical concerns or formed strategic alliances with them. An example was the long-term alliance formed by Genentech (see later) and the well-established pharmaceutical company, Eli Lilly. Genentech developed recombinant human insulin, which was then marketed by Eli Lilly under the trade name, Humulin. The merger of biotech capability with pharmaceutical experience helped accelerate development of the biopharmaceutical sector.

Many of the earlier biopharmaceutical companies no longer exist. The overall level of speculative finance available was not sufficient to sustain them all long-term (it can take 6–10 years and $200–500 million to develop a single drug; Chapter 2). Furthermore, the promise and hype of biotechnology sometimes exceeded its ability to actually deliver a final product. Some biopharmaceutical substances showed little efficacy in treating their target condition, and/or exhibited unacceptable side effects. Mergers and acquisitions also led to the disappearance of several biopharmaceutical concerns. Table 1.6 lists the major pharmaceutical concerns which now manufacture/market biopharmaceuticals approved for general medical use. Box 1.1 provides a profle of three well-established dedicated biopharmaceutical companies.

BIOPHARMACEUTICALS: CURRENT STATUS AND FUTURE PROSPECTS

By mid-2002, some 120 biopharmaceutical products had gained marketing approval in the USA and/or EU. Collectively, these represent a global biopharmaceutical market in the region of $15 billion (Table 1.7). A detailed list of the approved products is provided in Appendix 1. The products include a range of hormones, blood factors and thrombolytic agents, as well as vaccines and monoclonal antibodies (Table 1.8). All but one are protein-based therapeutic agents. The exception is Vitravene, an antisense oligonucleotide (Chapter 11), first approved in the USA in 1998. Many additional nucleic acid-based products for use in gene therapy or antisense technology (Chapter 11) are currently in clinical trials.

Many of the initial biopharmaceuticals approved were simple replacement proteins (e.g. blood factors and human insulin). The ability to logically alter the amino acid sequence of a protein, coupled to an increased understanding of the relationship between protein structure and function has facilitated the more recent introduction of several engineered therapeutic

Table 1.6. Pharmaceutical companies who manufacture and/or market biopharmaceutical products approved for general medical use in the USA and EU

Genetics Institute	Hoechst AG
Bayer	Aventis Pharmaceuticals
Novo Nordisk	Genzyme
Centeon	Schwartz Pharma
Genentech	Pharmacia and Upjohn
Centocor	Biotechnology General
Boehringer Mannheim	Serono
Galenus Mannheim	Organon
Eli Lilly	Amgen
Ortho Biotech	Dompe Biotec
Schering Plough	Immunex
Hoffman-la-Roche	Bedex Laboratories
Chiron	Merck
Biogen	SmithKline Beecham
Pasteur Mérieux MSD	Medeva Pharma
Immunomedics	Cytogen
Novartis	Med Immune
Abbott	Roche
Wyeth	Isis pharmaceuticals
Unigene	Sanofi-Synthelabo

proteins (Table 1.5). Thus far, the vast majority of approved recombinant proteins have been produced in *E. coli*, *S. cerevisiae* or in animal cell lines (most notably Chinese hamster ovary (CHO) cells or baby hamster kidney (BHK) cells). The rationale for choosing these production systems is discussed in Chapter 3.

While most biopharmaceuticals approved to date are intended for human use, a number of products destined for veterinary application have also come on the market. One early example of this is recombinant bovine growth hormone (somatotrophin), approved in the USA in the early 1990s and used to increase milk yields from dairy cattle. Additional examples of approved veterinary biopharmaceuticals include a range of recombinant vaccines and an interferon-based product (Table 1.9).

At least 500 potential biopharmaceuticals are currently being evaluated in clinical trials. Vaccines and monoclonal antibody-based products represent the two biggest product categories. Regulatory factors (e.g. hormones and cytokines), as well as gene therapy and antisense-based products, also represent significant groupings. While most protein-based products likely to gain marketing approval over the next 2–3 years will be produced in engineered *E. coli*, *S. cerevisiae* or animal cell lines, some products now in clinical trials are being produced in the milk of transgenic animals (Chapter 3). Additionally, plant-based transgenic expression systems may potentially come to the fore, particularly for the production of oral vaccines (Chapter 3).

Interestingly, the first generic biopharmaceuticals are already entering the market. Patent protection for many first-generation biopharmaceuticals (including recombinant human growth hormone, insulin, erythropoietin, α-interferon and granulocyte colony stimulating factor) has now come/is now coming to an end. Most of these drugs command an overall annual market value in excess of US$ 1 billion, rendering them attractive potential products for many biotechnology/pharmaceutical companies. Companies already producing, or about to produce,

Box 1.1. Amgen, Biogen and Genentech

Amgen, Biogen and Genentech represent three pioneering biopharmaceutical companies that remain in business. Founded in the 1980s as AMGen (Applied Molecular Genetics), Amgen now employs over 9000 people worldwide, making it one of the largest dedicated biotechnology companies in existence. Its headquarters are situated in Thousand Oaks, California, although it has research, manufacturing, distribution and sales facilities worldwide. Company activities focus upon developing novel (mainly protein) therapeutics for application in oncology, inflammation, bone disease, neurology, metabolism and nephrology. By mid-2002, six of its recombinant products had been approved for general medical use (the erythropoietin-based products, 'Aranesp' and 'Epogen' (Chapter 6), the colony stimulating factor-based products, 'Neupogen' and 'Neulasta' (Chapter 6) as well as the interleukin-1 receptor antagonist, 'Kineret' and the anti-rheumatoid arthritis fusion protein, Enbrel (Chapter 5)). Total product sales for 2001 reached US$ 3.5 billion and the company reinvested 25% of this in R&D. In July 2002, Amgen acquired Immunex Corporation, another dedicated biopharmaceutical company founded in Seattle in the early 1980s.

Biogen was founded in Geneva, Switzerland in 1978 by a group of leading molecular biologists. Currently, its international headquarters are located in Paris and it employs in excess of 2000 people worldwide. The company developed and directly markets the interferon-based product, 'Avonex' (Chapter 4), but also generates revenues from sales of other Biogen-discovered products which are licensed to various other pharmaceutical companies. These include Schering Plough's 'Intron A' (Chapter 4) as well as a number of hepatitis B-based vaccines sold by SmithKline Beecham (SKB) and Merck (Chapter 10). By 2001, worldwide sales of Biogen-discovered products had reached US$ 3 billion. Biogen reinvests ca. 33% of its revenues back into R&D and has ongoing collaborations with several other pharmaceutical and biotechnology companies.

Genentech was founded in 1976 by scientist Herbert Boyer and the venture capitalist, Robert Swanson. Headquartered in San Francisco, it employs almost 5000 staff worldwide and has 10 protein-based products on the market. These include human growth hormones ('Nutropin', Chapter 8), the antibody-based products 'Herceptin' and 'Rituxan' (Chapter 10) and the thrombolytic agents 'Activase' and 'TNKase' (Chapter 9). The company also has 20 or so products in clinical trials. In 2001, it generated some US$ 2.2 billion in revenues, 24% of which it reinvested in R&D.

generic biopharmaceuticals include Genemedix (UK), Sicor and Ivax (USA), Congene and Microbix (Canada) and BioGenerix (Germany); e.g. Genemedix secured approval for sale of a recombinant colony-stimulating factor in China in 2001 and is also commencing the manufacture of recombinant erythropoietin; Sicor currently markets human growth hormone and interferon-α in Eastern Europe and various developing nations. The widespread approval and marketing of generic biopharmaceuticals in regions such as the EU and USA is, however, unlikely to occur in the near future, mainly due to regulatory issues.

To date (mid-2002) no gene therapy based product has thus far been approved for general medical use (Chapter 11). Although gene therapy trials were initiated as far back as 1990, the

Table 1.7. Approximate annual market values of some leading approved biopharmaceutical products. Data gathered from various sources, including company home pages, annual reports and industry reports

Product and (Company)	Product description and (use)	Annual sales value (US$, billions)
Procrit (Amgen/Johnson & Johnson)	Erythropoietin (treatment of anaemia)	2.7
Epogen (Amgen)	Erythropoietin (treatment of anaemia)	2.0
Intron A (Schering Plough)	Interferon-α (treatment of leukaemia)	1.4
Neupogen (Amgen)	Colony stimulating factor (treatment of neutropenia)	1.2
Avonex (Biogen)	Interferon-β (treatment of multiple sclerosis)	0.8
Embrel (Immunex)	Monoclonal antibody (treatment of rheumatoid arthritis)	0.7
Betasteron (Chiron/Schering Plough)	Interferon-β (treatment of multiple sclerosis)	0.6
Cerezyme (Genzyme)	Glucocerebrosidase (treatment of Gaucher's disease)	0.5

Table 1.8. Summary categorization of biopharmaceuticals approved for general medical use in the EU and/or USA by August 2002. Refer to Appendix 1 for further details

Product type	Examples	Number approved	Refer to Chapter
Blood factors	Factors VIII and IX	7	9
Thrombolytic agents	Tissue plasminogen activator (tPA)	6	9
Hormones	Insulin, growth hormone, gonadotrophins	28	8
Haemapoietic growth factors	Erythropoietin, colony stimulating factors	7	6
Interferons	Interferons-α, -β, -γ	15	4
Interleukin-based products	Interleukin-2	3	5
Vaccines	Hepatitis B surface antigen	20	10
Monoclonal antibodies	Various	20	10
Additional products	Tumour necrosis factor, therapeutic enzymes	14	Various

results have been disappointing. Many technical difficulties remain, e.g. in relation to gene delivery and regulation of expression. Product effectiveness was not apparent in the majority of trials undertaken and safety concerns have been raised in several trials.

Only one antisense-based product has been approved to date (in 1998) and, although several such antisense agents continue to be clinically evaluated, it is unlikely that a large number of

Table 1.9. Some recombinant (r) biopharmaceuticals recently approved for veterinary application in the EU

Product	Company	Indication
Vibragen Omega (r feline interferon omega)	Virbac	Reduction of mortality/clinical symptoms associated with canine parvovirus
Fevaxyl Pentafel (combination vaccine containing r feline leukaemia viral antigen as one component)	Fort Dodge Laboratories	Immunization of cats against various feline pathogens
Porcilis porcoli (combination vaccine containing r *E. coli* adhesins)	Intervet	Active immunization of sows
Porcilis AR-T DF (combination vaccine containing a recombinant modified toxin from *Pasteurella multocida*)	Intervet	Reduction in clinical signs of progressive atrophic rhinitis in piglets
Porcilis pesti (combination vaccine containing r classical swine fever virus E_2 subunit antigen)	Intervet	Immunization of pigs against classical swine fever
Bayovac CSF E2 (combination vaccine containing r classical swine fever virus E_2 subunit antigen)	Intervet	Immunization of pigs against classical swine fever

such products will be approved over the next 3–4 years. Despite the disappointing results thus far generated by nucleic acid-based products, future technical advances will almost certainly ensure the approval of gene therapy- and antisense-based products in the intermediate future.

Technological developments in areas such as genomics, proteomics and high-throughput screening are also beginning to impact significantly upon the early stages of drug development (Chapter 2). For example, by linking changes in gene/protein expression to various disease states, these technologies will identify new drug targets for such diseases. Many/most such targets will themselves be proteins, and drugs will be designed/developed specifically to interact with them. They may be protein-based or (more often) low molecular mass ligands.

TRADITIONAL PHARMACEUTICALS OF BIOLOGICAL ORIGIN

The remaining chapters of this book are largely dedicated to describing the major biopharmaceuticals currently in use and those likely to gain approval for use in the not-too-distant future. Before undertaking this task, however, it would be useful to overview briefly some of the now-traditional pharmaceutical substances originally obtained from biological sources. This will provide a more comprehensive foundation for the study of biopharmaceuticals and facilitate a better overall appreciation of how biotechnology, in whatever guise, impacts upon the pharmaceutical industry.

As previously indicated, some of these biological substances are now synthesized chemically, although many continue to be extracted from their native biological source material. Animals, plants and microorganisms have all yielded therapeutically important compounds, as described below.

Table 1.10. Some pharmaceutical substances originally isolated from animal sources. While some are still produced by direct extraction from the native source, others are now also produced by direct chemical synthesis (e.g. peptides and some steroids), or by recombinant DNA technology (most of the polypeptide products). Abbreviations: hGH = human growth hormone; FSH = follicle stimulating hormone; hCG = human chorionic gonadotrophin; HSA = human serum albumin; HBsAg = hepatitis B surface antigen

Product	Indication	Original source
Insulin	Diabetes mellitus	Porcine/bovine pancreatic tissue
Glucagon	Used to reverse insulin-induced hypoglycaemia	Porcine/bovine pancreatic tissue
hGH	Treatment of short stature	Originally human pituitaries
FSH	Subfertility/infertility	Urine of post-menopausal women
hCG	Subfertility/infertility	Urine of pregnant women
Blood coagulation factors	Haemophilia and other related blood disorders	Human blood
HSA	Plasma volume expander	Human plasma/placenta
Polyclonal antibodies	Passive immunization	Serum of immunized animals/ humans
H Bs Ag	Vaccination against hepatitis B	Plasma of hepatitis B carriers
Urokinase	Thrombolytic agent	Human urine
Peptide hormones (e.g. gonadorelin, oxytocin, vasopressin)	Various	Mostly from pituitary gland
Trypsin	Debriding agent	Pancreas
Pancrelipase	Digestive enzymes	Pancreas
Glucocerebrosidase	Gaucher's disease	Placenta
Steroid (sex) hormones	Various, including subfertility	Gonads
Corticosteroids	Adrenal insufficiency, anti-inflammatory agents, immunosuppressants	Adrenal cortex
Prostaglandins	Various, including uterine stimulants, vasodilators and inhibition of gastric acid secretion	Manufactured in most tissues
Adrenaline	Management of anaphylaxis	Adrenal gland

Pharmaceuticals of animal origin

A wide range of pharmaceutical substances are derived from animal sources (Table 1.10). Many are protein-based and detailed description of products such as insulin and other polypeptide hormones, antibody preparations, vaccines, enzymes, etc., have been deferred to subsequent chapters. (Many of the therapeutic proteins are now also produced by recombinant DNA technology. Considerable overlap would have been generated had a product obtained by direct extraction from native sources been discussed here, with further discussion of a version of the same product produced by recombinant DNA technology at a later stage.) Non-proteinaceous pharmaceuticals originally derived from animal sources include steroid (sex) hormones, corticosteroids and prostaglandins. A limited discussion of these substances is presented below, as they will not be discussed in subsequent chapters. Most of these substances are now prepared synthetically.

The sex hormones

The male and female gonads, as well as the placenta of pregnant females and, to a lesser extent, the adrenal cortex, produce a range of steroid hormones which regulate the development and maintenance of reproductive and related functions. As such, these steroid sex hormones have found medical application in the treatment of various reproductive dysfunctions.

While these steroids directly regulate sexual function, their synthesis and release are, in turn, controlled by gonadotropins — polypeptide hormones produced by the pituitary gland. The biology and medical applications of the gonadotropins are outlined in Chapter 8. Sex hormones produced naturally may be classified into one of three groups:

- the androgens;
- the oestrogens;
- progesterone.

While these steroids can be extracted directly from human tissue, in most instances they can also be synthesized chemically. Direct chemical synthesis methodology has also facilitated the development of synthetic steroid analogues. Many such analogues exhibit therapeutic advantages over the native hormone, e.g. they may be more potent, be absorbed intact from the digestive tract, or exhibit a longer duration of action in the body. The majority of sex steroid hormones now used clinically are chemically synthesized.

The androgens The androgens are the main male sex hormones. They are produced by the Leydig cells of the testes, as well as in the adrenals. They are also produced by the female ovary. As in the case of all other steroid hormones, the androgens are synthesized in the body, using cholesterol as their ultimate biosynthetic precursor. The major androgen produced by the testes is testosterone, of which 4–10 mg is secreted daily into the bloodstream by healthy young men. Testosterone synthesis is stimulated by the gonadotrophin, luteinizing hormone.

Testosterone is transported in the blood bound to transport proteins, the most important of which are albumin (a non-specific carrier) and testosterone–oestradiol binding globulin (TEBG), a 40 kDa polypeptide which binds testosterone and oestrogens with high affinity.

Testosterone and other androgens induce their characteristic biological effects via binding to a specific intracellular receptor. These hormones promote:

- induction of sperm production;
- development/maintenance of male secondary sexual characteristics;
- general anabolic (growth-promoting) effects;
- regulation of gonadotrophin secretion.

The actions of androgens are often antagonized by oestrogens, and vice versa. This forms the basis of androgen administration in some forms of breast cancer, and oestrogen administration in the treatment of prostate cancer. Anti-androgenic compounds have also been synthesized. These antagonize androgen action due to their ability to compete with androgens for binding to the receptor.

Androgens are used medically as replacement therapy in male hypogonadal disorders (i.e. impaired functioning of the testes). They are administered to adolescent males displaying delayed puberty to promote an increase in the size of the scrotum and other sexual organs. Androgens are also sometimes administered to females, particularly in the management of some

Table 1.11. Major androgens/anabolic steroids used medically

Hormone	Description	Use
Danazol	Synthetic androgen. Suppresses gonadotrophin production. Exhibits some weak androgenic activity	Oral administration in the treatment of endometriosis, benign breast disorders, menorrhagia, premenstrual syndrome and hereditary angioedema
Methyltestosterone	Synthetic androgen, longer circulatory half-life than testosterone	Replacement therapy for male hypogonadal disorders. Breast cancer in females
Oxymetholone	Synthetic androgen	Treatment of anaemia
Stanozolol	Synthetic androgen	Treatment of some clinical presentations of Behçet's syndrome and management of hereditary angioedema
Testosterone	Main androgen produced by testes. Esterified forms display longer circulatory half lives	Treatment of male hypogonadism. Also sometimes used in treatment of post-menopausal breast carcinoma and osteoporosis

forms of breast cancer. The major androgens used clinically are listed in Table 1.11, and their chemical structures are outlined in Figure 1.2.

Oestrogens Oestrogens are produced mainly by the ovary in (non-pregnant) females. These molecules, which represent the major female sex steroid hormones, are also produced by the placenta of pregnant females. Testosterone represents the immediate biosynthetic precursor of oestrogens. Three main oestrogens have been extracted from ovarian tissue (oestrone, β-oestradiol and oestriol). β-oestradiol is the principal oestrogen produced by the ovary. It is 10 times more potent than oestrone and 25 times more potent than oestriol, and these latter two oestrogens are largely by-products of β-oestradiol metabolism.

Oestrogens induce their various biological effects by interacting with intracellular receptors. Their major biological activities include:

- stimulation of the growth and maintenance of the female reproductive system (their principal effect);
- influencing bone metabolism; as is evidenced from the high degree of bone decalcification (osteoporosis) occurring in post-menopausal women;
- influencing lipid metabolism.

Natural oestrogens generally only retain a significant proportion of their activity if administered intravenously. Several synthetic analogues have been developed which can be administered orally. Most of these substances also display more potent activity than native oestrogen. The most important synthetic oestrogen analogues include ethinyloestradiol and diethylstilboestrol (often simply termed stilboestrol). These are orally active and are approximately 10 and 5 times (respectively) more potent than oestrone.

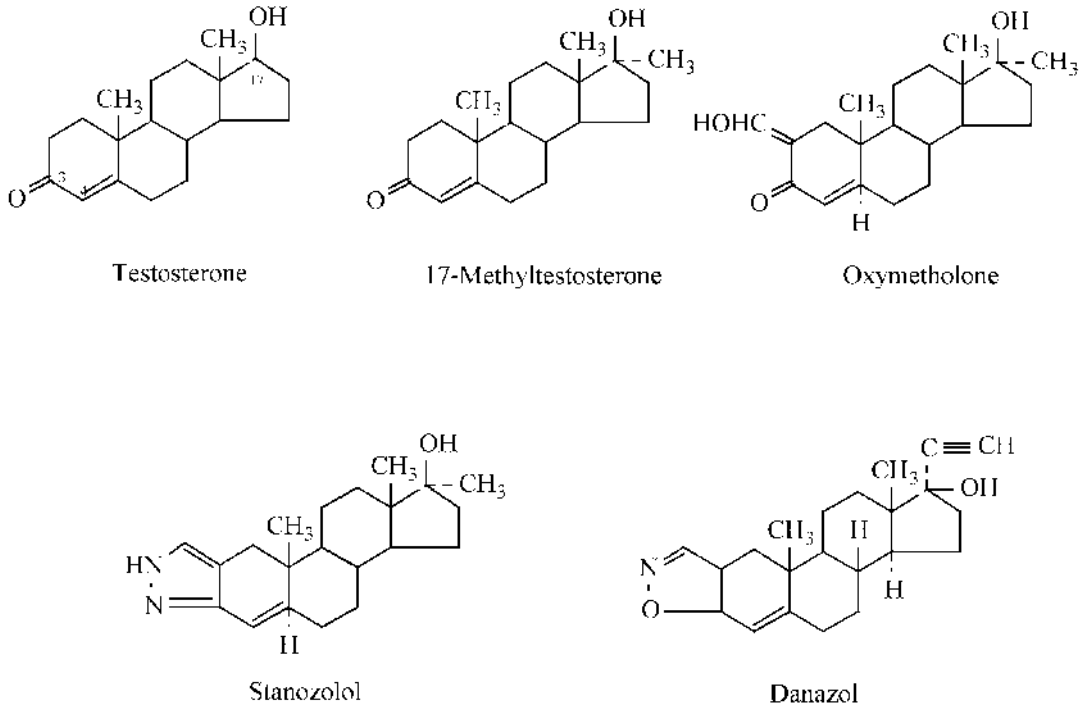


Figure 1.2. Chemical structure of the major synthetic and native androgens used clinically

Oestrogens are used to treat a number of medical conditions, including:

- replacement therapy for medical conditions underlined by insufficient endogenous oestrogen production;
- for alleviation of menopausal/post-menopausal disorders/symptoms;
- combating post-menopausal osteoporosis;
- treatment of some cancer forms, notably prostate and breast cancer;
- as an active ingredient in several oral contraceptive preparations.

The major oestrogen preparations used medically are outlined in Table 1.12, and their chemical structure is illustrated in Figure 1.3. The widest clinical application of oestrogens relate to their use as oral contraceptives. Most such contraceptive pills contain an oestrogen in combination with a progestin (discussed later).

Several synthetic anti-oestrogens have also been developed. These non-steroidal agents, including clomiphene and tamoxifen (Figure 1.4) inhibit oestrogen activity by binding their intracellular receptors, but fail to elicit a subsequent cellular response. Such anti-oestrogens have also found clinical application. Many female patients with breast cancer improve when either endogenous oestrogen levels are reduced (e.g. by removal of the ovaries) or anti-oestrogenic compounds are administered. However, not all patients respond. Predictably, tumours exhibiting high levels of oestrogen receptors are the most responsive.

Table 1.12. Major oestrogens used medically

Hormone	Description	Use
Ethinyloestradiol	Synthetic oestrogen	Used for oestrogen replacement therapy in deficient states, both pre- and post-menopausal. Treatment of prostate cancer (male), breast cancer (post-menopausal women). Component of many oral contraceptives
Mestranol	Synthetic oestrogen	Treatment of menopausal, post-menopausal or menstrual disorders. Component of many oral contraceptives
Oestradiol	Natural oestrogen	Oestrogen replacement therapy in menopausal, post-menopausal or menstrual disorders. Management of breast cancer in post-menopausal women and prostate cancer in man
Oestrone	Natural oestrogen	Uses similar to oestradiol
Quinestrol	Synthetic oestrogen, with prolonged duration of action	Oestrogen deficiency
Stilboestrol	Synthetic oestrogen (non-steroidal)	Treatment of breast/prostate cancer. Management of menopausal/ post-menopausal disorders

Progesterone and progestogens Progesterone is the main hormone produced by the corpus luteum during the second half of the female menstrual cycle (Chapter 8). It acts upon the endometrial tissue (lining of the womb). Under its influence, the endometrium begins to produce and secrete mucus, which is an essential prerequisite for subsequent implantation of a fertilized egg. In pregnant females, progesterone is also synthesized by the placenta, and its continued production is essential for maintenance of the pregnant state. Administration of progesterone to a non-pregnant female prevents ovulation and, as such, the progestogens (discussed below) are used as contraceptive agents. Progesterone also stimulates breast growth, and is immunosuppressive at high doses.

Only minor quantities of intact biologically active progesterone are absorbed if the hormone is given orally. Progestogens are synthetic compounds which display actions similar to that of progesterone. Many progestogens are more potent than progesterone itself and can be absorbed intact when administered orally.

Progesterone, and particularly progestogens, are used for a number of therapeutic purposes, including:

- treatment of menstrual disorders;
- treatment of endometriosis (the presence of tissue similar to the endometrium at other sites in the pelvis);
- management of some breast and endometrial cancers;
- hormone replacement therapy, where they are used in combination with oestrogens;
- contraceptive agents, usually in combination with oestrogens.

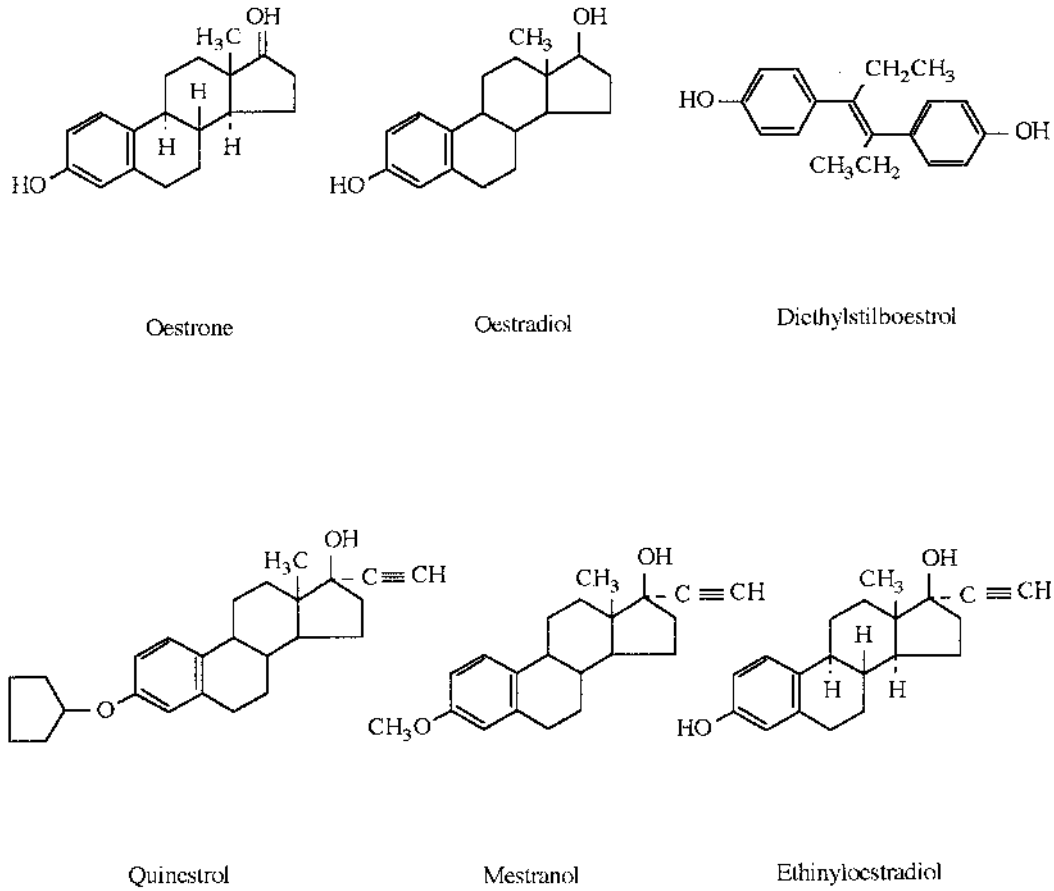


Figure 1.3. Chemical structure of the major synthetic and native oestrogens used clinically

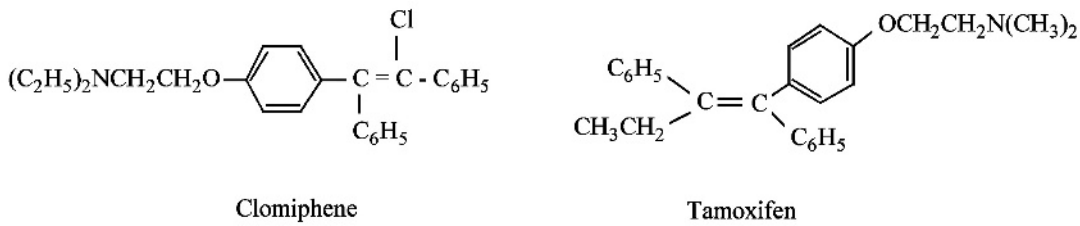


Figure 1.4. Structure of clomiphene and tamoxifen, two non-steroidal synthetic anti-oestrogens that have found medical application

Table 1.13. Progesterone and major progestogens used clinically

Hormone	Description	Use
Progesterone	Hormone produced naturally by corpus luteum, adrenals and placenta. Serum half-life is only a few minutes	Dysfunctional uterine bleeding. Sustaining pregnancy in threatening abortion
Chlormadinone	Synthetic progestogen	Menstrual disorders. Oral contraceptive
Ethynodiol diacetate	Synthetic progestogen	Component of many combined oral contraceptives. Progesterone replacement therapy
Medroxyprogesterone acetate	Synthetic progestogen	Treatment of menstrual disorders, endometriosis and hormone responsive cancer. Also used as long-acting contraceptive
Megestrol acetate	Synthetic progestogen	Treatment of endometrial carcinoma and some forms of breast cancer
Norethisterone	Synthetic progestogen	Abnormal uterine bleeding. Endometriosis, component of some oral contraceptives and in hormone replacement therapy

Table 1.13 lists some progestogens which are in common medical use, and their structures are presented in Figure 1.5.

A wide range of oestrogens and progesterone are used in the formulation of oral contraceptives. These contraceptive pills generally contain a combination of a single oestrogen and a single progestogen. They may also be administered in the form of long-acting injections.

These combined contraceptives seem to function by inducing feedback inhibition of gonadotrophin secretion which, in turn, inhibits the process of ovulation (Chapter 8). They also induce alterations in the endometrial tissue that may prevent implantation. Furthermore, the progestogen promotes thickening of the cervical mucus, which renders it less hospitable to sperm cells. This combination of effects is quite effective in preventing pregnancy.

Corticosteroids

The adrenal cortex produces in excess of 50 steroid hormones, which can be divided into 3 classes:

- glucocorticoids (principally cortisone and hydrocortisone, also known as cortisol);
- mineralocorticoids (principally deoxycorticosterone and aldosterone);
- sex corticoids (mainly androgens, as previously discussed).

Glucocorticoids and mineralocorticoids are uniquely produced by the adrenal cortex, and are collectively termed corticosteroids. Apart from aldosterone, glucocorticoid secretion is regulated by the pituitary hormone, corticotrophin. The principal corticosteroids synthesized in the body are illustrated in Figure 1.6. Glucocorticoids generally exhibit weak mineralocorticoid actions and vice versa.

The glucocorticoids induce a number of biological effects (Table 1.14), but their principal actions relate to modulation of glucose metabolism. The mineralocorticoids regulate water and

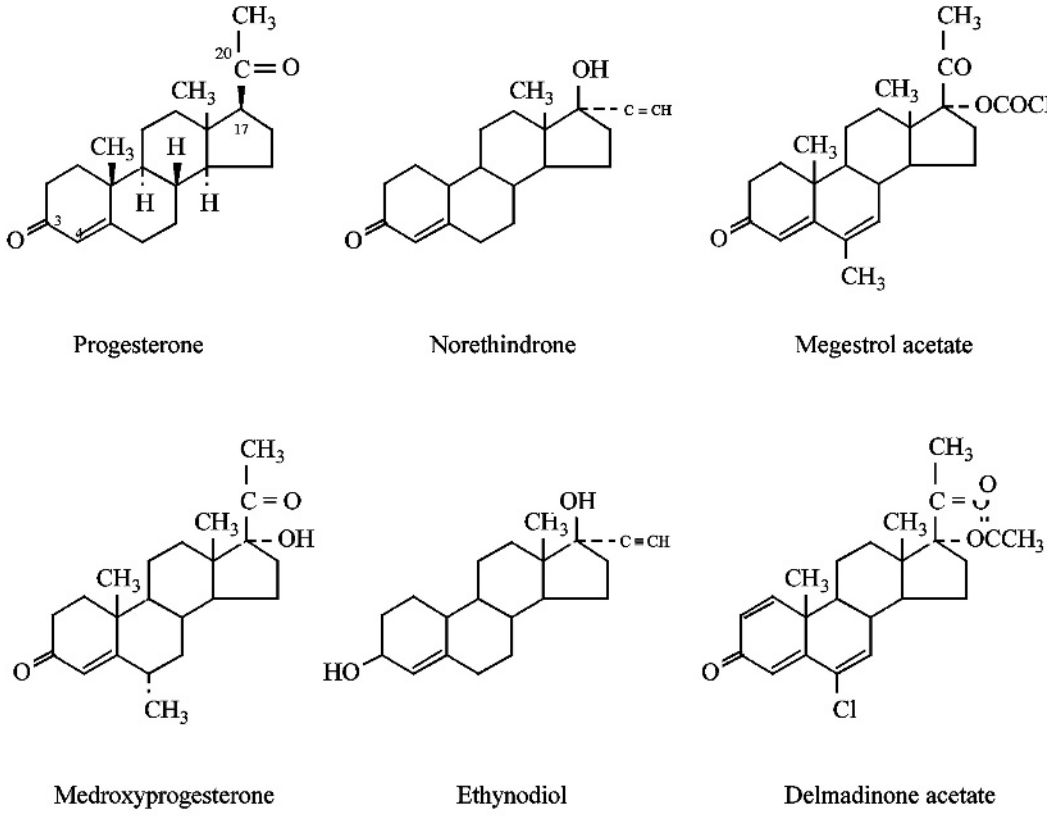


Figure 1.5. Chemical structure of progesterone and the major progestogens used clinically

electrolyte metabolism. They generally increase renal reabsorption of Na^+, and promote Na^+–K^+–H^+ exchange. This typically results in increased serum Na^+ concentrations and decreased serum K^+ concentrations. Elevated blood pressure is also usually induced.

Various synthetic corticosteroids have also been developed. Some display greater potency than the native steroids, while others exhibit glucocorticoid activity with little associated mineralocorticoid effects, or vice versa. The major glucocorticoids used clinically are synthetic. They are usually employed as:

- replacement therapy in cases of adrenal insufficiency;
- anti-inflammatory agents;
- immunosuppressive agents.

Examples of the corticosteroids used most commonly in the clinic are presented in Table 1.15 and Figure 1.7.

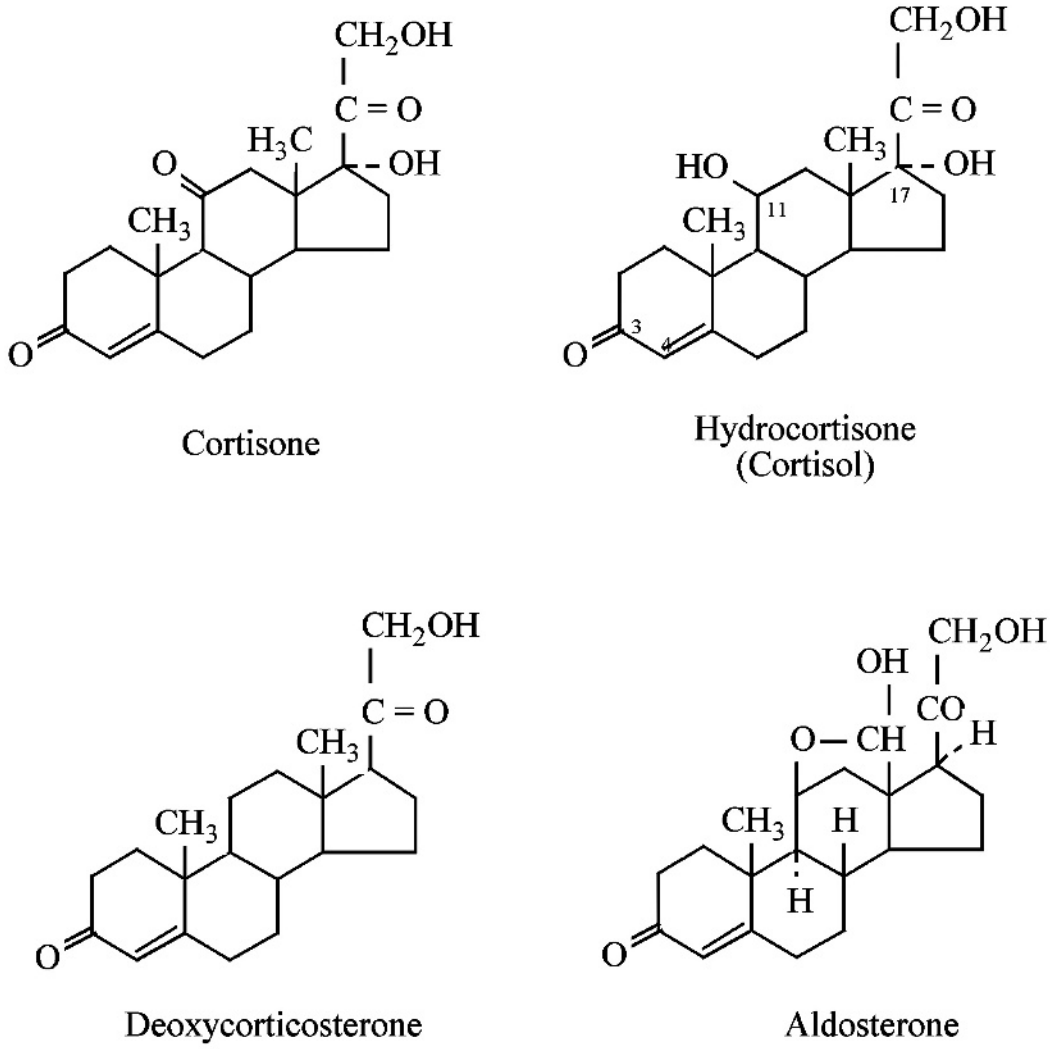


Figure 1.6. Corticosteroids produced naturally in the adrenal cortex

Catecholamines

The adrenal medulla synthesizes two catecholamine hormones, adrenaline (epinephrine) and noradrenaline (norepinephrine) (Figure 1.8). The ultimate biosynthetic precursor of both is the amino acid tyrosine. Subsequent to their synthesis, these hormones are stored in intracellular vesicles, and are released via exocytosis upon stimulation of the producer cells by neurons of the sympathetic nervous system. The catecholamine hormones induce their characteristic biological effects by binding to one of two classes of receptors, the α- and β-adrenergic receptors. These receptors respond differently (often oppositely) to the catecholamines.

Table 1.14. Some biological effects of glucocorticoids. Reproduced from Smith *et al.* (1983), *Principles of Biochemistry* (mammalian biochemistry), by kind permission of the publisher, McGraw Hill International, New York

System	Effect	Basis for change
General	Increase survival and resistance to stress	Composite of many effects (see below)
Intermediary metabolism		
Carbohydrate	Increase fasting levels of liver glycogen	Increase gluconeogenesis and glycogenesis
	Decrease insulin sensitivity	Inhibition of glucose uptake, increase gluconeogenesis
Lipid	Increase lipid mobilization from depots	Facilitate actions of lipolytic hormones
Proteins and nucleic acid	Increase urinary nitrogen during fasting	Anti-anabolic (catabolic) effect
Inflammatory and allergic phenomena and effects on leukocyte function	Anti-inflammatory and immunosuppressive	Impair cellular immunity
	Lymphocytopenia, eosinopenia, granulocytosis	Decrease influx of inflammatory cells into areas of inflammation
		Impair formation of prostaglandins
		Lymphoid sequestration in spleen, lymph node and other tissues
		Lymphocytolytic effect (not prominent in humans)
Haematologic system	Mild polycythaemia	Stimulation of erythropoiesis
Fluid and electrolytes	Promote water excretion	Increase glomerular filtration rate; inhibit vasopressin release
Cardiovascular	Increase cardiac output and blood pressure	
	Increase vascular reactivity	Not known
Bone and calcium	Lower serum Ca^{2+}	Inhibition of vitamin D function on intestinal Ca^{2+} absorption
	Osteoporosis	Inhibition of osteoblast function
Growth	Inhibit growth (pharmacologic doses)	Inhibition of cell division and DNA synthesis
Secretory actions	Tendency to increased peptic ulceration	Stimulate secretion of gastric acid and pepsinogen
Central nervous system	Changes in mood and behaviour	Unknown
Fibroblasts and connective tissue	Poor wound healing and thinning of bone (osteoporosis)	Impair fibroblast proliferation and collagen formation

Adrenaline and noradrenaline induce a wide variety of biological responses, including:

- stimulation of glycogenolysis and gluconeogenesis in the liver and skeletal muscle;
- lipolysis in adipose tissue;
- smooth muscle relaxation in the bronchi and skeletal blood vessels;
- increased speed and force of heart rate.

Table 1.15. Corticosteroids which find clinical use

Hormone	Description	Use
Betamethasone	Synthetic corticosteroid, displays glucocorticoid activity, lacks mineralocorticoid activity	Use mainly as anti-inflammatory and immunosuppressive effect
Deoxycortone acetate	Modified form of natural corticosteroid (deoxycortone). Is a mineralocorticoid, displays no significant glucocorticoid action	Used to treat Addison's disease and other adrenocortical deficiency states
Dexamethasone	Synthetic glucocorticoid, lacks mineralocorticoid activity	Used to treat range of inflammatory diseases. Used to treat some forms of asthma, also cerebral oedema and congenital adrenal hyperplasia
Fludrocortisone acetate	Synthetic corticosteroid with some glucocorticoid and potent mineralocorticoid activity	Administered orally to treat primary adrenal insufficiency
Hydrocortisone (cortisol)	Natural glucocorticoid	Used to treat all conditions for which corticosteroid therapy is indicated
Methylprednisolone	Synthetic glucocorticoid	Used as an anti-inflammatory agent and as an immunosuppressant

In general, the diverse effects induced by catecholamines serve to mobilize energy resources and prepare the body for immediate action — the 'fight or flight' response.

Catecholamines, as well as various catecholamine agonists and antagonists, can be synthesized chemically and many of these have found medical application.

The major clinical applications of adrenaline include:

- emergency management of anaphylaxis;
- emergency cardiopulmonary resuscitation;
- addition to some local anaesthetics (its vasoconstrictor properties help to prolong local action of the anaesthetic).

Noradrenaline is also sometimes added to local anaesthetics, again because of its vasoconstrictive properties. A prominent activity of this catecholamine is its ability to raise blood pressure. It is therefore used to restore blood pressure in acute hypotensive states.

Prostaglandins

The prostaglandins (PGs) are yet another group of biomolecules that have found clinical application. These molecules were named 'prostaglandins', as it was initially believed that their sole site of synthesis was the prostate gland. Subsequently, it has been shown that prostaglandins are but a sub-family of biologically active molecules, all of which are derived from arachidonic acid, a 20-carbon polyunsaturated fatty acid (Figure 1.9). These families of molecules, collectively known as ecosanoids, include the prostaglandins, thromboxanes and leukotrienes.

The principal naturally-occurring prostaglandins include prostaglandin E_1 (PGE_1), as well as PGE_2, PGE_3, $PGF_{1\alpha}$, $PGF_{2\alpha}$ and $PGF_{3\alpha}$ (Figure 1.10). All body tissues are capable of

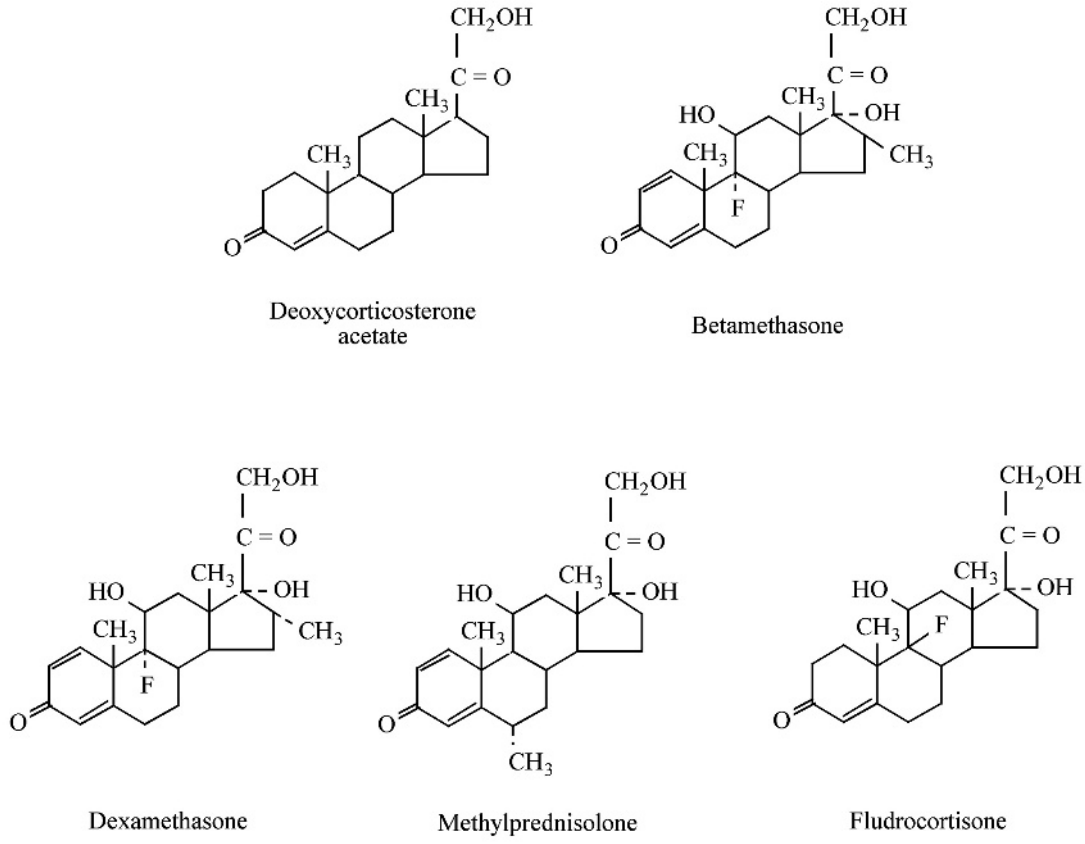


Figure 1.7. Structure of some of the major corticosteroids that have found routine therapeutic application

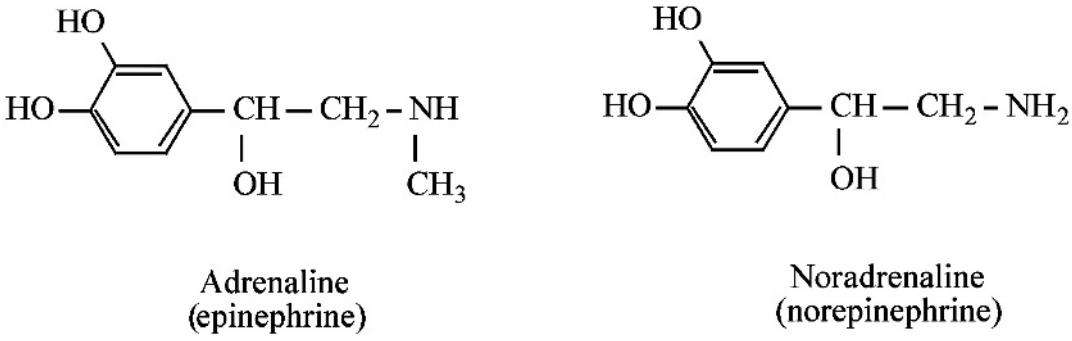


Figure 1.8. Structure of adrenaline and noradrenaline. Both are catecholamines (amide-containing derivatives of catechol, i.e. 1,2-dihydroxybenzene)

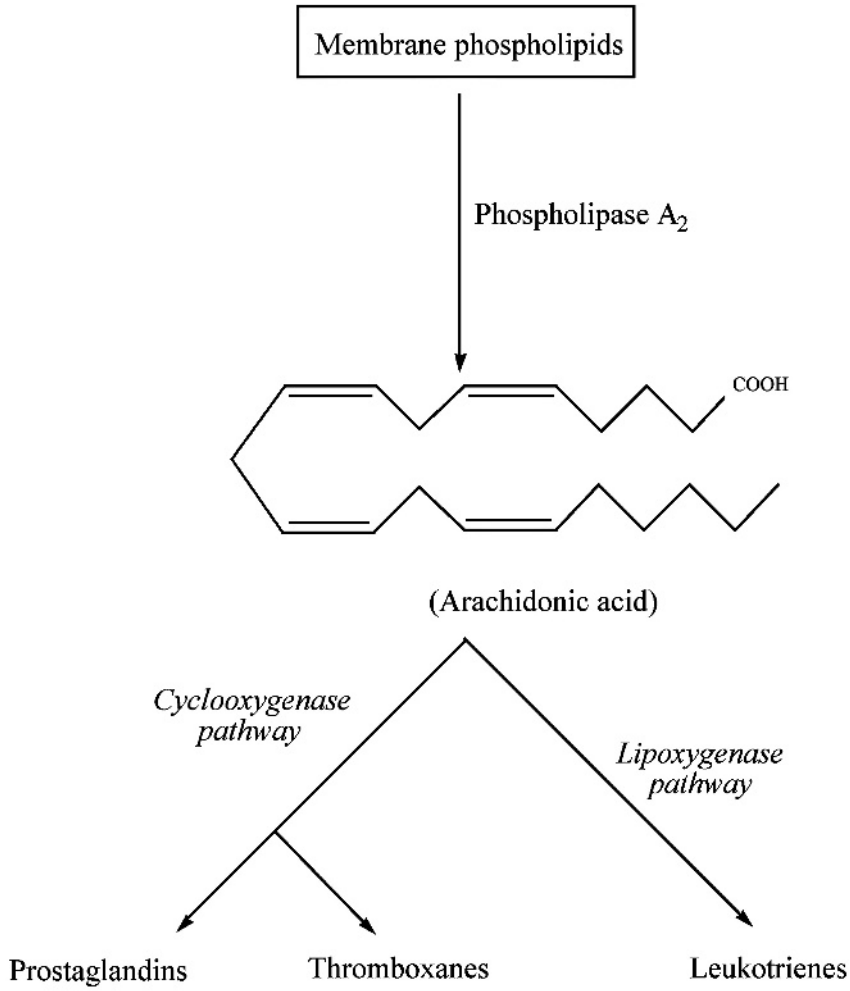
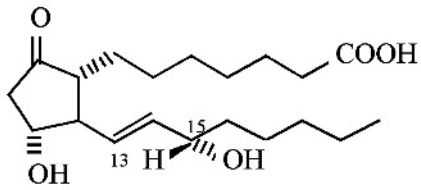


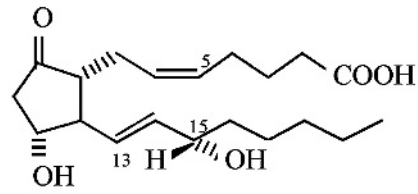
Figure 1.9. Overview of the biosynthesis of ecosanoids. The 20 carbon fatty acid arachidonic acid is released from cell membrane phospholipids by the actions of phospholipase A_2. Free arachidonic acid forms the precursor of prostaglandins and thromboxanes via the multi-enzyme cyclooxygenase pathway, while leukotrienes are formed via the lipoxygenase pathway

synthesizing endogenous prostaglandins and these molecules display an extremely wide array of biological effects, including:

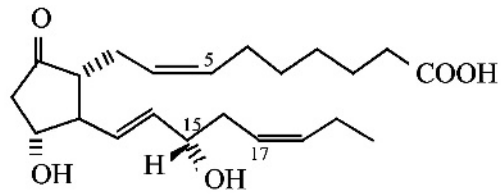
- effects upon smooth muscle;
- the induction of platelet aggregation (PGEs mostly);
- alteration of metabolism and function of various tissues/organs (e.g. adipose tissue, bone, kidney, neurons);
- mediation of inflammation;
- modulation of the immune response.



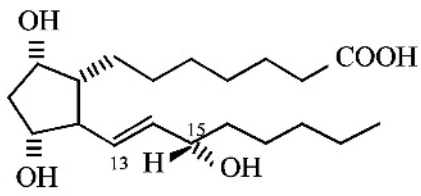
PGE_1



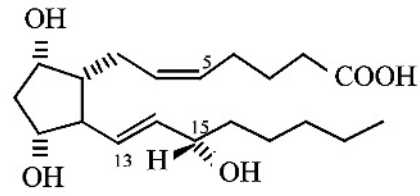
PGE_2



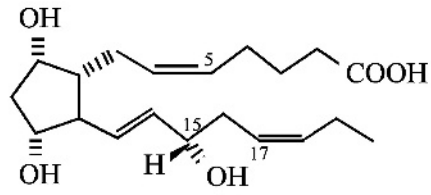
PGE_3



$PGF_{1\alpha}$



$PGF_{2\alpha}$



$PGF_{3\alpha}$

Figure 1.10. The major prostaglandins (PGs) synthesized naturally by the human body

Their actions on smooth muscle, in particular, can be complex. For example, both PGEs and PGFs induce contraction of uterine and gastrointestinal smooth muscle. On the other hand, PGEs stimulate dilation of vascular and bronchial smooth muscle, whereas PGFs induce constriction of these muscle types.

Although prostaglandins in some ways resemble hormones, they fail to fit the classical definition of a hormone in several respects, e.g. their synthesis is not localized to a specialized gland and they act primarily in a paracrine rather than true endocrine fashion. The diverse biological actions of the prostaglandins underpin their diverse clinical applications. Synthetic analogues have also been developed which display some clinical advantage over native PGs (e.g. greater stability, longer duration of action, more specific biological effects). The major clinical uses of native or synthetic prostaglandins can be grouped as follows:

- *Obstetrics and gynaecology*: several prostaglandin preparations (mainly E_2, $F_{2\alpha}$ and their analogues) are used to induce uterine contraction. The purpose can be either to induce labour as part of the normal childbirth procedure, or to terminate a pregnancy;
- *Induction of vasodilation and inhibition of platelet aggregation*: prostaglandin E_1 and its analogues are most commonly employed and are used to treat infants with congenital heart disease;
- *Inhibition of gastric acid secretion*: an effect promoted in particular by PGE_1 and its analogues.

In some circumstances, inhibition of endogenous prostaglandin synthesis can represent a desirable medical goal. This is often achieved by administration of drugs capable of inhibiting the cyclooxygenase system. Aspirin is a well known example of such a drug, and it is by this mechanism that it induces its analgesic and anti-inflammatory effects.

Pharmaceutical substances of plant origin

The vast bulk of early medicinal substances were plant-derived. An estimated 3 billion people worldwide continue to use traditional plant medicines as their primary form of healthcare. At least 25% of all prescription drugs sold in North America contain active substances which were originally isolated from plants (or are modified forms of chemicals originally isolated from plants). Many of these were discovered by a targeted knowledge-based ethnobotanical approach. Researchers simply recorded plant-based medical cures for specific diseases and then analysed the plants for their active ingredients. Plants produce a wide array of bioactive molecules via secondary metabolic pathways. Most of these probably evolved as chemical defence agents against infections or predators.

While some medicinal substances continue to be directly extracted from plant material, in many instances plant-derived drugs can now be manufactured, at least in part, by direct chemical synthesis. In addition, chemical modification of many of these plant 'lead' drugs have yielded a range of additional therapeutic substances.

The bulk of plant-derived medicines can be categorized into a number of chemical families, including alkaloids, flavonoids, terpenes and terpenoids, steroids (e.g. cardiac glycosides), as well as coumarins, quinines, salicylates and xanthines. A list of some better-known plant-derived drugs is presented in Table 1.16.

Table 1.16. Some drugs of plant origin

Drug	Chemical type	Indication	Plant producer
Aspirin	Salicylate	Analgesic, anti-inflammatory	*Salix alba* (white willow tree) and *Filipendula ulmaria* (meadowsweet)
Atropine	Alkaloid	Pupil dilator	*Atropa belladonna* (deadly nightshade)
Caffeine	Xanthine	Increases mental alertness	*Camellia sinensis*
Cocaine	Alkaloid	Ophthalmic anaesthetic	*Erythoxylum coca* (coca leaves)
Codeine	Alkaloid	Analgesic, cough suppressor	*Papaver somniferum* (opium poppy)
Dicoumarol	Coumarin	Anti-coagulant	*Melilotus officinalis*
Digoxin	Steroid	Increases heart muscle contraction	*Digitalis purpurea* (purple foxglove)
Digitoxin	Steroid	Increases heart muscle contraction	*Digitalis purpurea*
Ipecac	Alkaloid	Induces vomiting	*Psychotria ipecacuanha*
Morphine	Alkaloid	Analgesic	*Papaver somniferum* (opium poppy)
Pseudoephedrine	Alkaloid	Clears nasal congestion	*Ephedra sinica*
Quinine	Alkaloid	Malaria	*Cinchona pubescens* (fever tree)
Reserpine	Alkaloid	Antihypertensive (reduces blood pressure)	*Rauvolfia serpentina* (Indian snakeroot)
Scopalamine	Alkaloid	Motion sickness	*Datura stramonium* (Jimson weed)
Taxol	Terpenoid	Ovarian, breast cancer	*Taxus brevifolia* (western yew tree)
Theophylline	Xanthine	Anti-asthmatic, diuretic	*Camellia sinensis*
Vinblastine	Alkaloid	Hodgkin's disease	*Catharanthus roseus* (rosy periwinkle)
Vincristine	Alkaloid	Leukaemia	*Catharanthus roseus*

Alkaloids

Alkaloids may be classified as relatively low molecular mass bases containing one or more nitrogen atoms, often present in a ring system. They are found mainly in plants (from which over 6000 different alkaloids have been extracted). They are especially abundant in flowering plants, particularly lupins, poppies, tobacco and potatoes. They also are synthesized by some animals, insects, marine organisms and microorganisms. Most producers simultaneously synthesize several distinct alkaloids.

The solubility of these substances in organic solvents facilitates their initial extraction and purification from plant material using, for example, petroleum ether. Subsequent chromatographic fractionation facilitates separation of individual alkaloid components. Many alkaloids are poisonous (and have been used for this purpose), although at lower concentrations they may be useful therapeutic agents. Several of the best known alkaloid-based drugs are discussed below. The chemical structure of most of these is presented in Figure 1.11.

Atropine and scopalamine Atropine is found in the berries of the weeds deadly nightshade and black nightshade. It is also synthesized in the leaves and roots of *Hyoscyamus muticusi*. At high

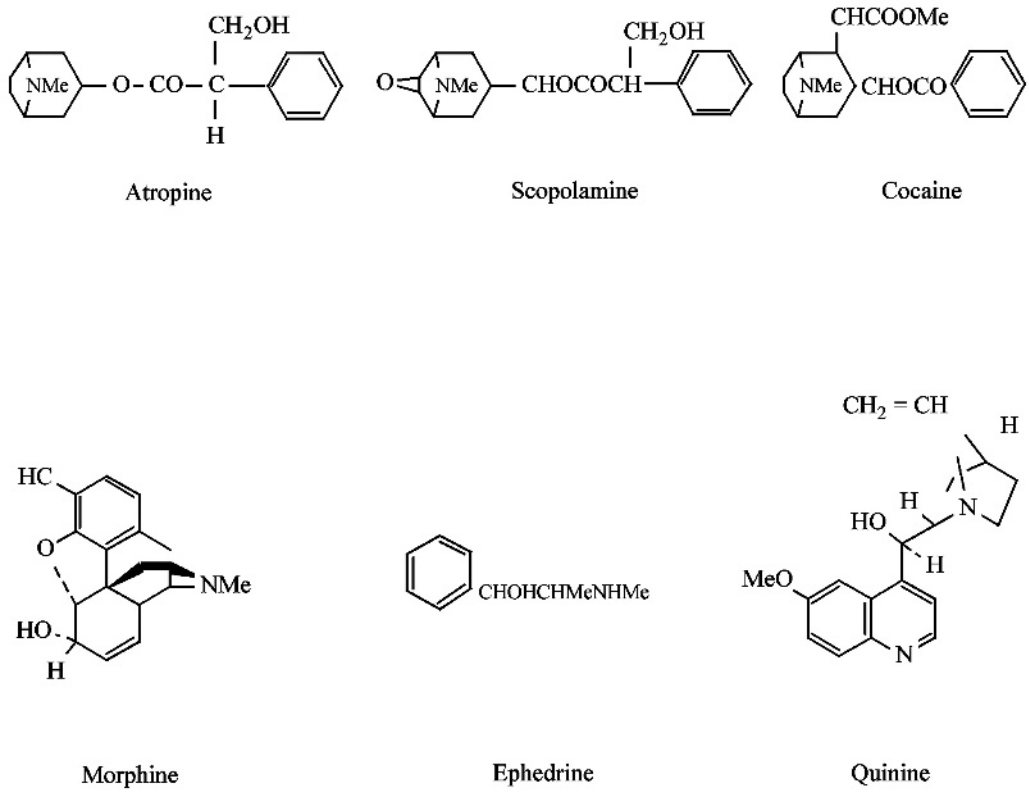


Figure 1.11. Chemical structure of some better-known alkaloids that have found medical application

concentrations it is poisonous but, when more dilute, displays a number of beneficial medical applications. Atropine sulphate solutions (1%, w/v) are used in ophthalmic procedures to dilate the pupil. It is also sometimes used as a pre-anaesthetic, as it inhibits secretion of saliva and mucus in the respiratory tract, which protects the patient from bronchoconstriction. Its ability to inhibit gastric secretion underlines its occasional use in the treatment of some stomach ulcers. Scopalamine, found in the leaves of the plant *Hyoscyamus niger*, shares some properties with atropine. Its major medical use is to treat motion sickness.

Morphine and cocaine Morphine is medically the most important alkaloid present in opium. Opium itself consists of the dried milky exudate extracted from unripe capsules of the opium poppy (*Papaver somniferum*), which is grown mainly in Asia, but also in some parts of India and China. Morphine is a powerful analgesic and has been used to treat severe pain. However, its addictive properties complicate its long-term medical use and it is also a drug of abuse. In addition to morphine, opium also contains codeine, which has similar, but weaker, actions.

Cocaine is extracted from coca leaves (*Erythoxylum coca*), which grows predominantly in South America and the Far East. Indigenous populations used coca leaves for medicinal and

recreational purposes (chewing them could numb the mouth, give a sense of vitality and reduce the sensation of hunger). Cocaine was first isolated in 1859 and (until its own addictive nature was discovered) was used to treat morphine addiction and as an ingredient in soft drinks. It also proved a powerful topical anaesthetic and coca leaf extract (and, subsequently, pure cocaine) was first introduced as an eye anaesthetic in Europe by Dr Carl Koller (who subsequently became known as Coca Koller). This represented its major medical application. While it is now rarely used medically, several derivatives (e.g. procaine, lignocaine, etc.) find widespread use as local anaesthetics.

Additional plant alkaloids Additional plant alkaloids of medical note include ephedrine, papaverine, quinine, vinblastine and vincristine.

Ephedrine is the main alkaloid produced in the roots of *Ephedra sinica*, preparations of which have found medical application in China for at least 5000 years. It was first purified from its natural source in 1887, and its chemical synthesis was achieved in 1927. It was initially used in cardiovascular medicine, but subsequently found wider application in the treatment of mild hayfever and asthma. It is also used as a nasal decongestant and cough suppressant.

Papaverine is an opium alkaloid initially isolated in the mid-1800s. It relaxes smooth muscle and is a potent vasodilator. As such it is used to dilate pulmonary and other arteries. It is therefore sometimes of use in the treatment of angina pectoris (usually caused by partial blockage of the coronary artery), heart attacks and bronchial spasms.

Quinine is an alkaloid produced by various *Cinchona* species (e.g. *Cinchona pubescens* or fever tree), which are mainly native to South America. The bark of these trees were initially used to treat malaria. Quinine itself was subsequently isolated in 1820 and found to be toxic not only to the protozoan *Plasmodium* (which causes malaria) but also to several other protozoan species.

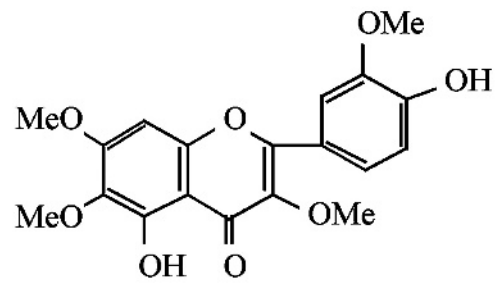
The Madagascar periwinkle plant (*Vinca rosea*) produces in the region of 60 alkaloids, including vinblastine and vincristine. These two closely related alkaloids (which are obtained in yields of 3 and 2 g/tonne of leaves, respectively) are important anti-tumour agents. They are used mainly to treat Hodgkin's disease and certain forms of leukaemia. They exert their effect by binding to tubulin, thus inhibiting microtubule formation during cell division.

Ergot alkaloids Ergot is a parasitic fungus which grows upon a variety of grains. It synthesizes over 30 distinct alkaloids, of which ergometrine is the most medically significant. This alkaloid stimulates contractions of the uterus when administered intravenously. It is sometimes used to assist labour and to minimize bleeding following delivery.

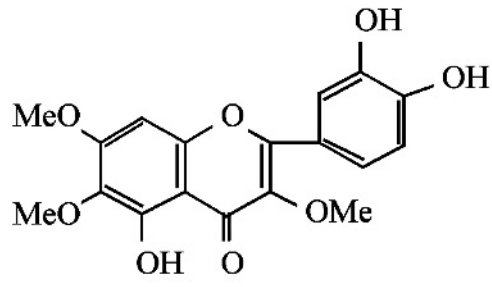
Another ergot derivative is the well known hallucinogenic drug, D-lysergic diethylamide (LSD). Other fungi also produce hallucinogenic/toxic alkaloids. These include the hallucinogenic psilocybin (produced by a number of fungi, including *Psilocybe mexicana*). These are often consumed by Mexican Indians during religious ceremonies.

Flavonoids, xanthines and terpenoids

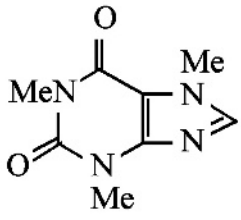
Flavonoids are 15-carbon polyphenols produced by all vascular plants. Well over 4000 distinct flavonoids have now been identified, a limited number of which display therapeutic activity. These substances are generally degraded if taken orally, and are administered medically via the intravenous route. This is probably just as well (the average Western daily diet contains approximately 1 g of flavonoids). A number of flavonoids appear to display anti-viral activity and, hence, are of pharmaceutical interest (Figure 1.12).



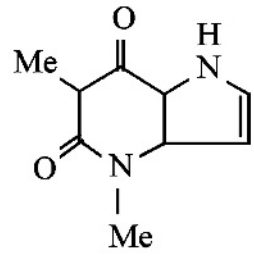
Chrysosplenol B



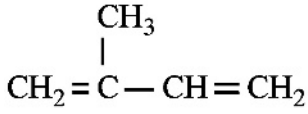
Axillarin



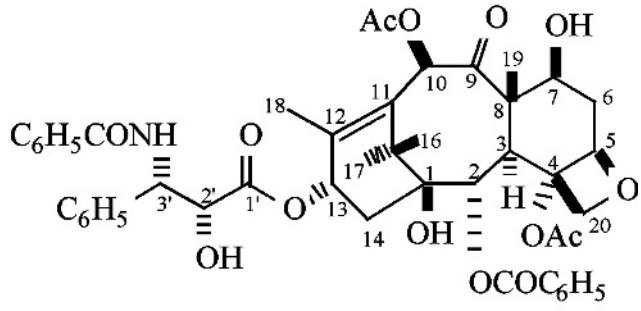
Caffeine



Theophylline



Isoprene



Taxol

Figure 1.12. Some flavonoids, xanthines and terpenoids. Chrysoplenol B and axillarin are two flavonoids exhibiting anti-viral activity against Rhinovirus (causative agent of the common cold). Examples of xanthines include caffeine and theophylline. Terpenes are polymers of the 5-carbon compound isoprene. Perhaps the best-known such substance discovered in recent years is the diterpenoid taxol, which is used as an anti-tumour agent

The principal xanthines of medical interest include caffeine, theophylline and aminophylline. Caffeine is synthesized by several plants and was originally isolated from tea in 1838. It is a methylxanthine (Figure 1.12) which stimulates the central nervous system, increasing mental alertness. It also acts as a diuretic and stimulates gastric acid secretion. It is absorbed upon oral administration and is frequently included in drugs containing an analgesic, such as aspirin or paracetamol.

Theophilline is also a minor constituent of tea, but is prepared by direct chemical synthesis for medical use. It functions to relax smooth muscle and, therefore, can be used as a bronchodilator in the treatment of asthma and bronchitis. Aminophylline is a derivative of theophilline (theophylline ethylenediamine), which is often used in place of theophilline due to its greater aqueous solubility.

Terpenes are polymers of the 5-carbon compound isoprene (Figure 1.12) and, as such, generally display properties similar to those of hydrocarbons. Terpenoids are substituted terpenes (i.e. contain additional chemical groups, such as an alcohol, phenols, aldehydes, ketones, etc.). Only a few such substances could be regarded as true drugs. Terpenes, such as limonene, menthol and camphor, form components of various essential oils with pseudo-pharmaceutical uses. A number of these molecules, however, exhibit anti-tumour activity, of which taxol is by far the most important.

The diterpenoid taxol (Figure 1.12) was first isolated from the pacific yew tree (*Taxus brevifolia*) in the late 1960s. Its complete structure was elucidated by 1971. Difficulties associated with the subsequent development of taxol as a useful drug mirror those encountered during the development of many plant-derived metabolites as drug products. Its low solubility made taxol difficult to formulate into a stable product, and its low natural abundance required large-scale extraction from its native source.

Despite such difficulties, encouraging *in vitro* bioassay results against transformed cell lines fuelled pre-clinical studies aimed at assessing taxol as an anti-cancer agent. Initial clinical trials in humans commenced in 1983 using a product formulated as an emulsion in a modified castor oil. Initial difficulties associated with allergic reactions against the oil were largely overcome by modifying the treatment regimen used. Large-scale clinical trials proved the efficacy of taxol as an anti-cancer agent, and it was approved for use in the treatment of ovarian cancer by the US Food and Drug Administration (FDA) in 1992.

Direct extraction from the bark of *T. brevifolia* yielded virtually all of the taxol used clinically up to almost the mid-1990s. The yield of active principle was in the range 0.007–0.014%. Huge quantities of bark were thus required to sustain taxol production (almost 30 000 kg bark were extracted in 1989 to meet requirements during large-scale clinical trials). A major (late) intermediate in the biosynthesis of taxol is 10-decacetylbaccatin (10-DAB). This can be obtained from the leaves (needles) of many species of yew, and at concentrations in excess of 0.1%. Chemical methods have been developed allowing synthesis of taxol from 10-DAB, and much of the taxol now used therapeutically is produced in this way. Semi-synthesis of taxol also facilitates generation of taxol analogues, some of which have also generated clinical interest. Although semi-synthesis of taxol is relatively straightforward, its total *de novo* synthesis is extremely complex. The cost of achieving *de novo* synthesis ensures that this approach will not be adopted for commercial production of this drug.

An alternative route of taxol production under investigation entails the use of plant cell culture techniques. Plant cell culture is considered to be an economically viable production route for plant-derived drugs, if the drug commands a market value in excess of $1000–2000/kg. While many commonly used plant-derived metabolites fall into this category, plant cell culture has not

generally been adopted for their industrial production. In many cases, this is because the plant cell lines fail to produce the desired drug, or produce it in minute quantities. Several cultures of *T. brevifolia*, however, have been shown to produce taxol. Interestingly, a fungus, *Taxomyces andreanae* (isolated growing on *T. brevifolia*) also produces taxol, although at very low levels. Genetic manipulation of this fungus may, however, yet yield mutants capable of synthesizing taxol in quantities rendering production by this means economically viable.

Betulinic acid is a five-ringed triterpene which has recently generated interest as an anti-cancer agent. It is produced in relatively substantial quantities in the bark of the white birch tree, from which it can easily be isolated. Initial studies indicate that betulinic acid is capable of selectively destroying melanoma cells by inducing apoptosis. Over the past number of years, the incidence of melanoma has increased at a faster rate than any other cancer. In the region of 7000 patients die annually from this condition in the USA alone. Although early surgery produces a 10-year survival rate of greater than 90%, treatment of late (metastatic) melanoma is more problematic. The current most effective drug (dacarbazine) is only effective in 25% of cases. A more effective drug would be a valuable therapeutic tool in combating advanced cases of this cancer.

Cardiac glycosides and coumarins

Cardiac glycosides are steroids to which a carbohydrate component is attached. Although produced by a variety of plants, the major cardiac glycosides that have found medical use have been isolated from species of *Digitalis* (foxgloves). 'Digitalis' in pharmaceutical circles has also come to mean a crude extract of dried foxglove leaves. This contains two glycoside components — digoxin and digitoxin — which increase heart muscle contraction. These drugs are in widespread use in the treatment of heart failure; both can be administered either orally or by injection. Digoxin induces an immediate but short-lived effect, whereas digitoxin is slower-acting but its effects are prolonged.

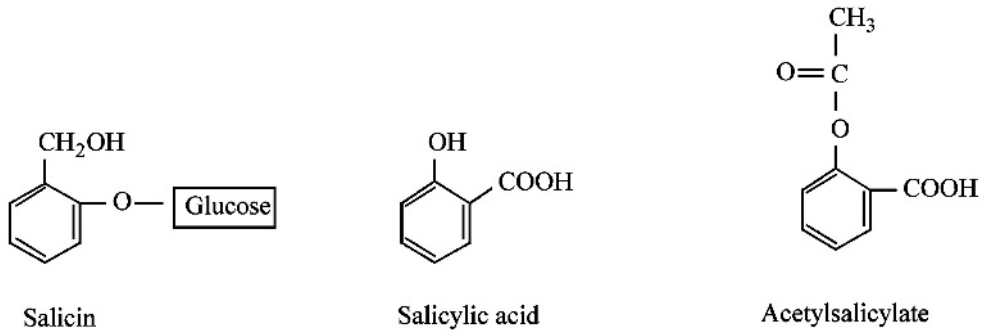
Coumarins are also synthesized by a variety of plant species. Medically, the most significant coumarins are dicoumarol and its derivative, warfarin. Dicoumarol was initially discovered as the active substance in mouldy sweet clover hay, which could induce haemorrhagic disease in cattle. Dicoumarol and warfarin are now used clinically as anticoagulants, as discussed in Chapter 9.

Aspirin

Few drugs have gained such widespread use as aspirin. The story of aspirin begins in the annals of folk medicine, where willow bark and certain flowers (e.g. *Filipendula ulmaria*) were used to relieve rheumatic and other pain. The bark of the white and black willow was subsequently found to contain salicin (Figure 1.13), which is metabolized to salicylic acid when ingested by humans. The flowers of *F. ulmaria* (meadowsweet) were also found to contain salicylic acid, which possesses anti-pyretic, anti-inflammatory and analgesic properties. Although it was an effective pain reliever, it irritates the stomach lining, and it was not until its modification to acetylsalicylate by Bayer chemists that it found widespread medical application (Figure 1.13). Bayer patented its acetylsalicylate drug under the trade name 'Aspirin' in 1900.

Pharmaceutical substances of microbial origin

Microorganisms produce a wide variety of secondary metabolites, many of which display actual or potential therapeutic application. Antibiotics are by far the most numerous such substances



Salicin Salicylic acid Acetylsalicylate

Figure 1.13. Chemical structure of salicin, salicylic acid and acetylsalicylate

and this family of pharmaceuticals arguably has had the greatest single positive impact upon human healthcare in history. A detailed discussion of antibiotics goes far beyond the scope of this text and, as such, only a brief overview is presented here. The interested reader is referred to the further reading section at the end of this chapter.

Antibiotics are generally defined as low molecular mass microbial secondary metabolites which, at low concentrations, inhibit the growth of other microorganisms. To date, well in excess of 10 000 antibiotic substances have been isolated and characterized. Overall, antibiotics are a chemically heterogeneous group of molecules, although (as described later) many can be classified into different families based upon similarity of chemical structure.

While chemically heterogeneous, it is interesting to note that in excess of half the antibiotic substances described to date are produced by a single bacterial order, the Actinomycetales. Within this order, the genus *Streptomyces* are particularly prolific producers of antibiotic substances. Although several fungal genera are known to produce antibiotics, only two (*Aspergillus* and *Penicillium*) do so to a significant extent. Indeed, the first antibiotic to be used medically — penicillin — was extracted from *Penicillium notatum*.

Sir Alexander Fleming first noted the ability of the mould *P. notatum* to produce an antibiotic substance (which he called penicillin) in 1928. However, he also noted that when penicillin was added to blood *in vitro*, it lost most of its antibiotic action, and Fleming consequently lost interest in his discovery. In the late 1930s, Howard Florey, Ernst Chain and Norman Heatley began to work on penicillin. They purified it and, unlike Fleming, studied its effect on live animals. They found that administration of penicillin to mice after their injection with lethal doses of streptococci protected the mice from an otherwise certain death.

The Second World War conferred urgency to their research and they began large-scale culture of *Penicillium* in their laboratory (mostly in bedpans). However, low levels of antibiotic production rendered large-scale medical trials difficult. The first human to be treated was Albert Alexander, a policeman. Although dying from a severe bacterial infection, he responded immediately to penicillin treatment. Supplies were so scarce that medical staff collected his urine to extract traces of excreted penicillin. He was almost cured 4 days after commencement of treatment, but supplies ran out. He relapsed and died. The first serious clinical trials in 1940–1941 proved so encouraging that massive effort was expended (particularly in the USA) to develop large-scale penicillin production systems. This succeeded and, before the end of the war,

Table 1.17. Major families of antibiotics

β-Lactams
Tetracyclines
Aminoglycoside antibiotics
Macrolides
Ansamycins
Peptide/glycopeptide antibiotics
Miscellaneous antibiotics

it was in plentiful supply. At this stage, scientists also initiated screening programmes to identify additional antibiotics. Streptomycin was an early success in this regard. It was first isolated in 1944 from a strain of *Streptomyces griseus*.

Today there are in excess of 100 antibiotics available on the market. Due to the availability of such a wide range, many pharmaceutical companies cut back or abandoned ongoing antibiotic screening programmes during the 1980s. However, the emergence of microbial strains resistant to most antibiotics threatens medical reversion effectively to a pre-antibiotic era. These developments have rejuvenated interest in discovering new antibiotics and many pharmaceutical companies have recommenced antibiotic screening programmes.

When grouped on the basis of similarities in their chemical structure, most antibiotics fall into the categories listed in Table 1.17. β-Lactams, which include penicillins and cephalosporins, exhibit a characteristic β-lactam core ring structure (a four-atom cyclic amide) (Figure 1.14). They induce their bacteriocidal activity by inhibiting the synthesis of peptidoglycan, an essential component of the bacterial cell wall.

Penicillins refer to a family of both natural and semi-synthetic antibiotics. Although all exhibit a 6-aminopenicillanic acid core ring structure (Figure 1.15), they differ in the structure of their side-chains. Naturally produced penicillins include penicillins G and V. Semi-synthetic penicillins can be manufactured by enzymatic removal of a natural penicillin side-chain (using

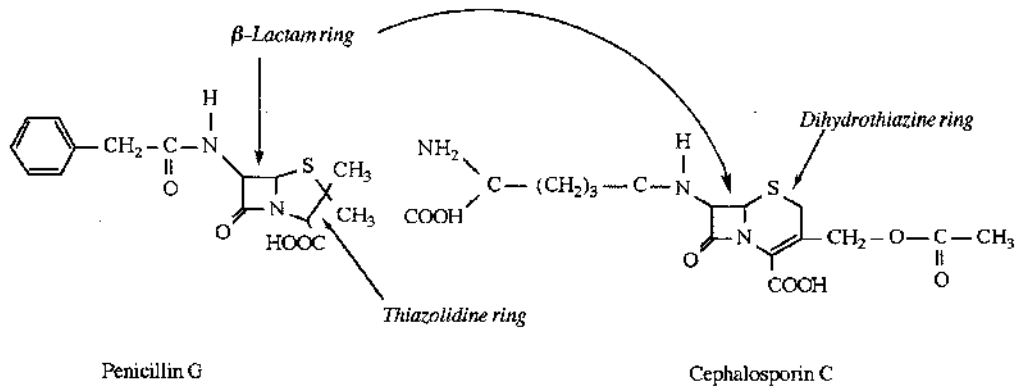
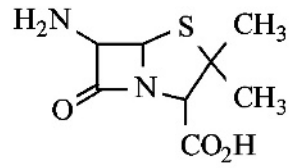
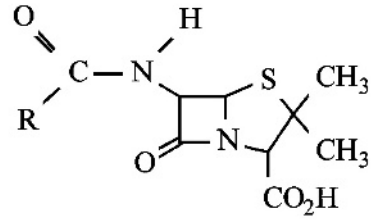


Figure 1.14. Structure of penicillin G and cephalosporin C, two of the best-known β-lactam antibiotics. Both exhibit the 4-atom β-lactam ring

(a)



(b)



(c)

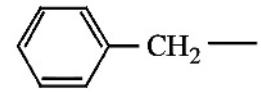
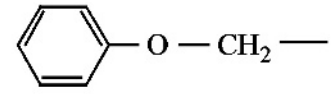
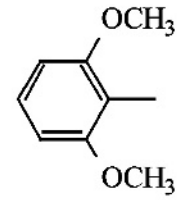
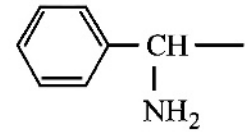
NAME	SUBSTITUENT (R)
PENICILLIN G (NATURAL)	
PENICILLIN V (NATURAL)	
METHICILLIN (SEMISYNTHETIC)	
AMPICILLIN (SEMISYNTHETIC)	

Figure 1.15. Structure of 6-aminopenicillanic acid (a); generalized penicillin structure (b) and side-groups present in two natural penicillins and two semisynthetic penicillins (c)

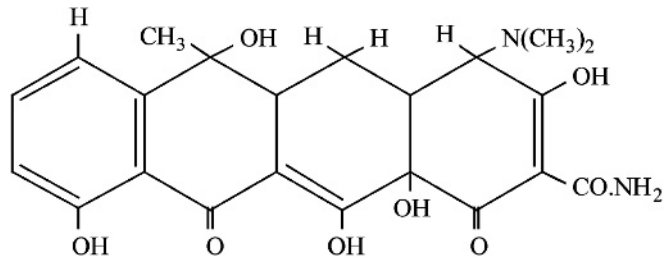


Figure 1.16. Chemical structure of the antibiotic tetracycline. Other members of the tetracycline family (see also Table 1.18) also display this characteristic 4-ring structure

penicillin acylase). Downward adjustment of the reaction pH to 4.3 results in precipitation of the resultant 6-aminopenicillanic acid ring, which can then be easily harvested. Novel side-chains can subsequently be attached, yielding semi-synthetic penicillins. Examples of the latter include phenethicillin, propicillin and oxacillin. Some semi-synthetic penicillins are effective against bacterial pathogens that have become resistant to natural penicillins. Others are acid-stable, allowing their oral administration.

Cephalosporins display an antibiotic mechanism of action identical to that of the penicillins. Cephalosporin C (Figure 1.14) is the prototypic natural cephalosporin and is produced by the fungus *Cephalosporium acremonium*. Most other members of this family are semi-synthetic derivatives of cephalosporin C. Chemical modification normally targets side-chains at position 3 (the acetoxymethyl group) or 7 (derived from D-α-aminoadipic acid).

Tetracyclines are a family of antibiotics which display a characteristic 4-fused-core ring structure (Figure 1.16). They exhibit broad antimicrobial activity and induce their effect by inhibiting protein synthesis in sensitive microorganisms. Chlortetracycline was the first member of this family to be discovered (in 1948). Penicillin G and streptomycin were the only antibiotics in use at that time, and chlortetracycline was the first antibiotic employed therapeutically that retained its antimicrobial properties upon oral administration. Since then, a number of additional tetracyclines have been discovered (all produced by various strains of *Streptomyces*), and a variety of semi-synthetic derivatives have also been prepared (Table 1.18).

Tetracyclines gained widespread medical use due to their broad spectrum of activity, which includes not only Gram-negative and Gram-positive bacteria, but also mycoplasmas, rickettsias, chlamydias and spirochaetes. However, adverse effects (e.g. staining of teeth and gastro-intestinal disturbances), along with the emergence of resistant strains, now somewhat limits their therapeutic applications.

Table 1.18. Natural and semi-synthetic tetracyclines which have gained medical application

Natural	Semi-synthetic
Chlortetracycline	Methacycline
Oxytetracycline	Doxycycline
Tetracycline	Minocycline
Demeclocycline	

Table 1.19. Some aminoglycoside antibiotics which have gained significant therapeutic application. Producer microorganisms are listed in brackets. In addition to naturally produced aminoglycosides, a number of semi-synthetic derivatives have also found medical application. Examples include amikacin, a semi-synthetic derivative of kanamycin and netilmicin, an N-ethyl derivative of sissomicin

Streptomycin (*Streptomyces griseus*)
Tobramycin (*Streptomyces tenebrarius*)
Framycetin (*Streptomyces* spp.)
Neomycin (*Streptomyces* spp.)
Kanamycin (*Streptomyces* spp.)
Paromycin (*Streptomyces* spp.)
Gentamicin (*Micromonospora purpurea*)
Sissomicin (*Micromonospora* spp.)

The aminoglycosides are a closely related family of antibiotics produced almost exclusively by members of the genus *Streptomyces* and *Micromonospora* (Table 1.19). Most are polycationic compounds, composed of a cyclic amino alcohol to which amino sugars are attached. They all induce their bacteriocidal effect by inhibiting protein synthesis (apparently by binding to the 30 S and, to some extent, the 50 S, ribosomal subunits). Most are orally inactive, generally necessitating their parenteral administration.

The aminoglycosides are most active against Gram-negative rods. Streptomycin was the first aminoglycoside to be used clinically. Another notable member of this family, gentamicin, was first purified from a culture of *Micromonospora purpurea* in 1963. Its activity against *Pseudomonas aeruginosa* and *Serratia marcescens* renders it useful in the treatment of these (often life-threatening) infections.

The macrolides and ansamycins

The macrolides are a large group of antibiotics. They are characterized by a core ring structure containing 12 or more carbon atoms (closed by a lactone group), to which one or more sugars are attached. The core ring of most anti-bacterial macrolides consists of 14 or 16 carbon atoms, while that of the larger anti-fungal and anti-protozoal macrolides contain up to 30 carbons. This family of antibiotics are produced predominantly by various species of *Streptomyces*. Antibacterial macrolides induce their effects by inhibiting bacterial synthesis (anti-fungal/protozoal macrolides appear to function by interfering with sterols, thus compromising membrane structure). The only member of this family that enjoys widespread therapeutic use is erythromycin, which was discovered in 1952.

Ansamycins, like the macrolides, are synthesized by condensation of a number of acetate and propionate units. These antibiotics, which are produced by several genera of the Actinomycetales, display a characteristic core aromatic ring structure. Amongst the best-known family members are the rifamycins, which are particularly active against Gram-positive bacteria and mycobacteria. They have been used, for example, in the treatment of *Mycobacterium tuberculosis*.

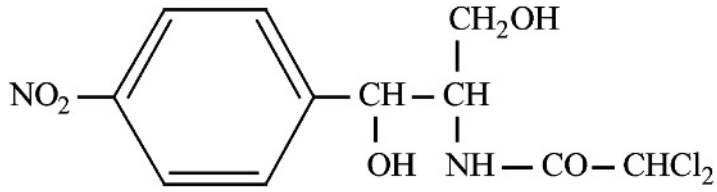


Figure 1.17. Chemical structure of chloramphenicol, the first broad-spectrum antibiotic to gain clinical use

Peptide and other antibiotics

Peptide antibiotics consist of a chain of amino acids which often have cyclized, forming a ring-like structure. The first such antibiotics isolated were bacitracin and gramicidin, although neither are used clinically due to their toxicity. While a number of microbes produce peptide antibiotics, relatively few such antibiotics are applied therapeutically. Polymyxins are the most common exception. Vancomycin, a glycopeptide, has also gained therapeutic application. It functions by interfering with bacterial cell wall synthesis, and is particularly active against Gram-positive cocci.

A variety of additional antibiotics are known that, based on their chemical structure, do not fit into any specific antibiotic family. Perhaps the most prominent such antibiotic is chloramphenicol (Figure 1.17). Chloramphenicol was first isolated from a culture of *Streptomyces venezuelae* in 1947, but it is now obtained by direct chemical synthesis. It was the first truly broad-spectrum antibiotic to be discovered, and was found effective against Gram-negative and Gram-positive bacteria, rickettsias and chlamydias. It retains activity when administered orally and functions by inhibiting protein synthesis. However, due to its adverse effects upon bone marrow function, clinical application of chloramphenicol is undertaken with caution. Its main use has been to combat *Salmonella typhi*, *Haemophilus influenzae* (especially in cases of meningitis) and *Bacteroides fragilis* (an anaerobe which can cause cerebral abscess formation). Some semi-synthetic derivatives of chloramphenicol (e.g. thiamphenicol) have also been developed for clinical use.

CONCLUSION

Most major life form families (microorganisms, plants and animals) have each yielded a host of valuable therapeutic substances. Many pharmaceutical companies and other institutions continue to screen plants and microbes in the hope of discovering yet more such therapeutic agents. However, in recent years, more and more emphasis is being placed upon developing the 'body's own drugs' as commercially produced pharmaceutical substances. Most such drugs are protein-based, and these biopharmaceuticals represent an exciting new family of pharmaceutical products. The number of such drugs gaining approval for general medical use continues to grow, as does their range of therapeutic applications. A fuller discussion of these biopharmaceuticals forms the basis of the remaining chapters of this text. In addition, the reader's attention is drawn to Appendix 2 of this book, which contains a list of Internet sites of relevance to the biopharmaceutical sector. Much additional valuable information may be downloaded from these sites.

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