CHAPTER TWENTY

Natural Products as Leads for New Pharmaceuticals

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1 INTRODUCTION

Of the 520 new pharmaceuticals approved between 1983 and 1994, 39% were derived from natural products, the proportion of antibacterials and anticancer agents of which was over 60% (1). Between 1990 and 2000, a total of 41 drugs derived from natural products were launched on the market by major pharmaceutical companies (Table 20.1), including azithromycin, orlistat, paclitaxel, sirolimus (rapamycin), Synercid, tacrolimus, and topotecan. In 2000, one-half of the top-selling pharmaceuticals were derived from natural products, having combined sales of more than US $40 billion. These included the biggest selling anticancer drug paclitaxel, the "statin" family of hypolipidemics, and the immunosuppressant cyclosporin. During 2001 we have seen the launch of caspofungin from Merck and galantamine from Johnson & Johnson, with rosuvastatin, telithromycin, daptomycin, and ecteinascidin-743 due to follow in 2002.

Despite the figures, the popularity of natural products, particularly those from higher plants as leads for new pharmaceuticals, tends to fluctuate. At the time of writing, several of the world's biggest pharmaceutical companies have reined back their natural product drug discovery programs and have placed great faith in combinatorial chemistry, coupled to very high throughput screening. Time will tell whether this is a wise stratagem, or whether the unique features of compounds that are themselves derived from living organisms will once again see renewed acceptance.

The abundance of plant and microbial secondary metabolites and their value in medicine are undisputed, but one question that is only partly answered concerns the reasons for this abundance of complex chemical substances. In the past, the production of what we would now call "bioactive" substances was a mystery. A modern view is that these compounds have a role in protecting the otherwise defenseless, stationary plant from attack by mammals, insects, fungi, bacteria, and viruses. Taking morphine as an example of a secondary metabolite whose value to the plant is not entirely obvious, 14 steps are required from available amino acids, including at least one step that is highly substrate specific (2). The presence of morphine in the tissues of *Papaver somniferum* must therefore confer a selectional advantage on the plant (3): genetic code is required for each,of the enzymes involved in the biosynthesis, valuable amino acids are utilized in forming the enzymes, and a relatively scarce nutrient (nitrogen) is locked up in the compounds produced. If the morphine did not continue to have value for the plant, mutants would have arisen with the advantage of not having a drain on their metabolic resources.

We can only guess at the ecological functions of morphine. Perhaps a mammalian herbivore that consumed too many poppies would become drowsy and itself fall prey to a carnivore. It may be significant that the cannabinoids, produced in greatest abundance in the nutritious growing tips of the plant, also induce mental effects that would compromise a herbivore's ability to escape a predator. Whatever their natural protective functions, natural products are a rich source of biologically active compounds that have arisen as the result of natural selection, over perhaps 300 million years. The challenge to the medicinal chemist is to exploit this unique chemical diversity. The following account illustrates how natural products have been used as what are called ***lead compounds,*** or templates for the development of important medicines.

Table 20.1 Drugs Derived from Natural Products (1990–2000)

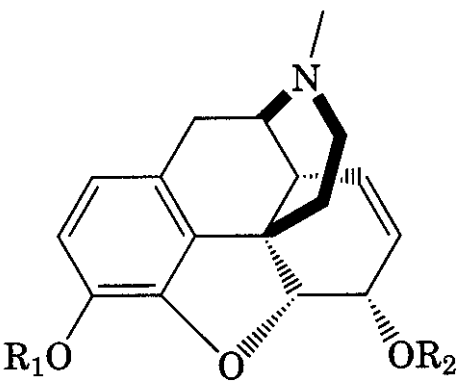
Name	Originator	Indication/Use
Acarbose	Bayer	Diabetes
Artemisinin	Kunming & Guilin	Malaria
Azithromycin	Pliva	Antibiotic
Carbenin	Sankyo	Antibiotic
Cefetamet pivoxil	Takeda	Antibiotic
Cefozopran	Takeda	Antibiotic
Cefpimizole	Ajinomoto	Antibiotic
Cefsulodin	Takeda	Antibiotic
Clarithromycin	Taisho	Antibiotic
Colforsin daropate	Nippon Kayaku	Asthma
Docetaxel	Aventis	Cancer
Dronabinol	Solvay	Alzheimer's disease
Galantamine	Intelligen	Alzheimer's disease, arthritis
Gusperimus	Nippon Kayaku	Arthritis
Irinotecan	Yakult Honsha	Cancer
Ivermectin	Merck & Co	Parasiticide
Lentinan	Ajinomoto	Cancer
LW-50020	Sankyo	Immunomodulation
Masoprocol	Access	Cancer
Mepartricin	SPA	Benign prostatic hyperplasia
Miglitol	Bayer	Diabetes
Mizoribine	Asahi Chemical	Arthritis
Mycophenolate mofetil	Hoffman-LaRoche	Arthritis
Orlistat	Hoffman-LaRoche	Obesity
Paclitaxel	Bristol-Myers Squibb	Cancer
Pentostatin	Warner-Lambert	Leukemia
Podophyllotoxin	Nycomed Pharma	Human papillomavirus
Policosanol	Dalmer	Hyperlipidaemia
Everolimus	Novartis	Immunomodulation
Sirolimus	American Home Products	Immunomodulation
Sizofilan	Taito	Cancer, hepatitis-B virus
Subreum	OM Pharma	Arthritis
Synercid	Novartis	Antibiotic
Tacrolimus	Fujisawa	Immunomodulation
Teicoplanin	Aventis	Antibiotic
Tirilazad mesylate	Pharmacia & Upjohn	Subarachnoid haemorrhage
Topotecan	GlaxoSmithKline	Diabetes
Ukrain	Nowicky Pharma	Cancer, HIV/AIDS
Vinorelbine	Pierre Fabre	Cancer
Voglibose	Takeda	Diabetes, obesity
Z-100	Zeria	Immunomodulation

2 DRUGS AFFECTING THE CENTRAL NERVOUS SYSTEM

2.1 Morphine Alkaloids

The history of the opium alkaloids is too well known to warrant repetition here, but the analgesics based on morphine (1) are too important to be left out of an account of natural products as leads. Thus we shall summarize the clinically more important developments that have occurred since the isolation of morphine in 1803. Codeine (2) continues to be used widely for the treatment of moderate pain and, although present in the opium poppy (*Papaver* somniferum), it is normally synthesized in higher yield from morphine (4).

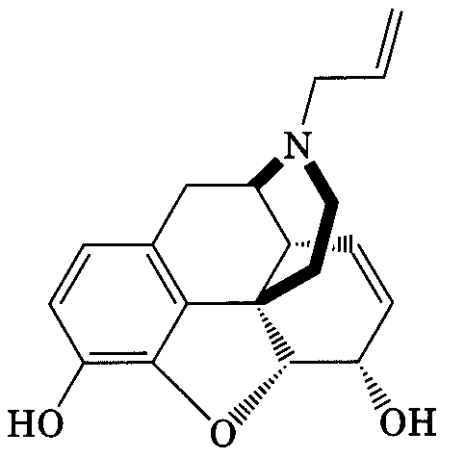
Other than codeine, the earliest significant semisynthetic derivative of morphine is the diacetate heroin (3), which is still widely used in terminal cancer where its addictiveness is ir-



(1) morphine $R_1 = R_2 = H$
(2) codeine $R_1 = CH_3$, $R_2 = H$
(3) heroin $R_1 = R_2 = COCH_3$

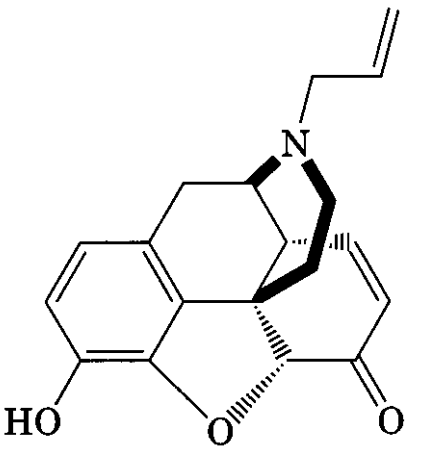
relevant. Acetylation masks the polar hydroxy groups, so that penetration into the central nervous system (CNS) is enhanced; hydrolysis then occurs to liberate the phenolic hydroxyl, giving an active analgesic, and ultimately regenerates morphine (5). Heroin was thus one of the first prodrugs.

Modifications to the C-ring of morphine are legion, but none of the derivatives is free from addictive liability, though many have been used clinically. N-Demethylation and realkylation yield more interesting analogs, notably N-allylnormorphine and nalorphine (4), which is a morphine antagonist (6). Further modification leads to naloxone (5), which unlike nalorphine has very little agonist activity (7) and has retained a place in therapy for treatment of opiate-induced respiratory depression. Naloxone will also precipitate withdrawal symptoms in opiate addicts, thereby facilitating diagnosis.



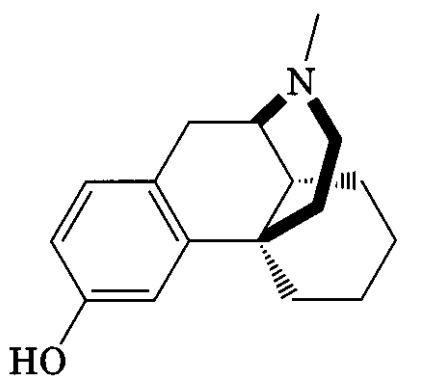
(4) nalorphine

Total synthesis of morphine is difficult, but analogs lacking the dihydrofuran ring are accessible (8) from 1-benzylisoquinolines, in analogy with the biosynthesis of morphine, to

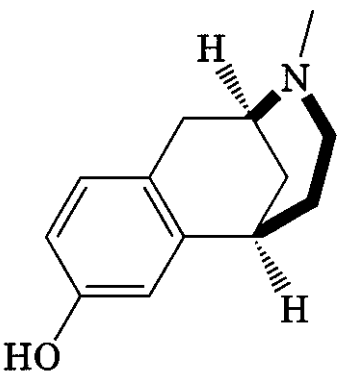


(5) naloxone

give the morphinans (6). The system may be simplified even further (9), to give the benzomorphans (7), although neither these nor the morphinans have provided the long-sought analgesic without addictive properties.

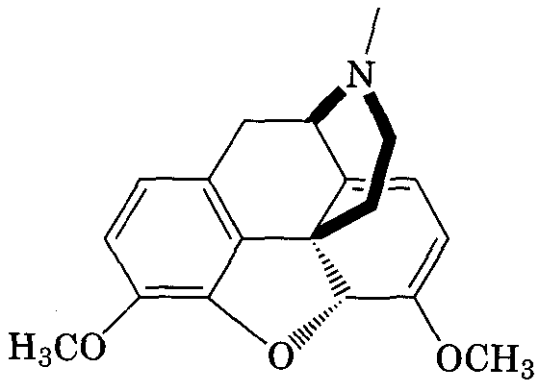


(6) morphinan

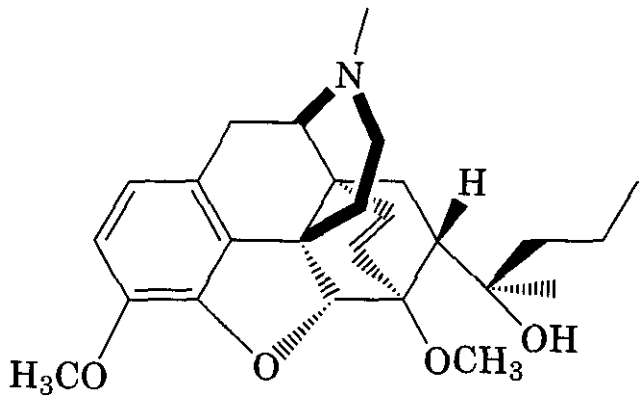


(7) benzomorphan

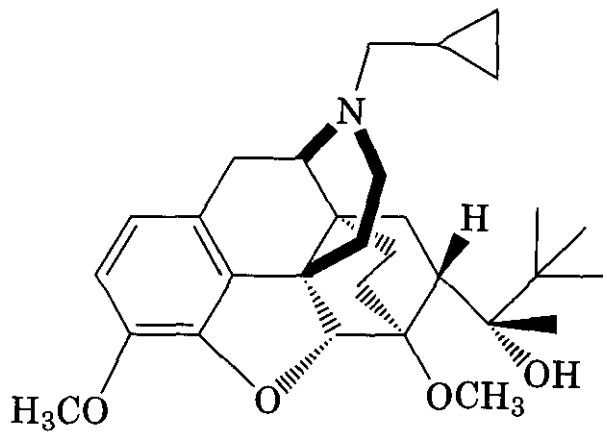
A semisynthetic route to morphine analogs was found (10) from thebaine (8) using Diels–Alder reactions in the C-ring. Adducts such as (9) have the distinction of enormous potency (11), sufficient to immobilize rhinoceroses at moderate dose levels! Unfortunately, the addictive liability runs parallel to the increase in analgesic potency, a tendency that was partly overcome (12) in the analog buprenorphine (10).



(8) thebaine



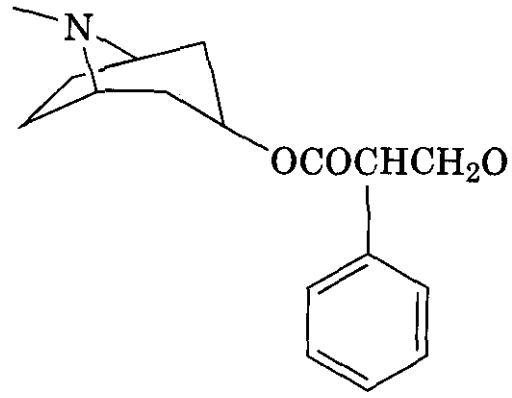
(9) etorphine



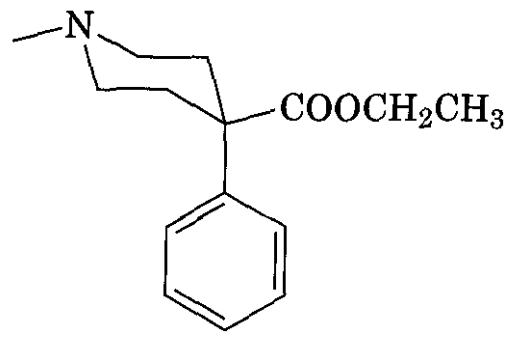
(10) buprenorphine

All this work was carried out in ignorance of the nature of the natural transmitter(s), which subsequently proved to be the peptides known as endorphins and their pentapeptide fragments, the enkephalins (13). It is perhaps significant that vastly improved understanding of the biochemical basis for analgesia and the characterization of a family of related receptors (14), known as δ, κ, and μ, have so far failed to yield any better drugs for the treatment of pain.

A series of analgesics that were discovered initially in an attempt to obtain smooth muscle relaxants based on another natural product, atropine (**11**), started with the observation that meperidine (pethidine) (12) unexpectedly produced a reaction in mice known as Straub tail, normally characteristic of the morphine series (15). Meperidine itself is still used widely in childbirth in the belief that there is a lower incidence of respiratory depression in the fetus. The realization that 4-phenylpiperidines, which are not obvious structural analogs of morphine, could give rise to useful analgesic effects, led to the synthesis of many thousands of derivatives (16), many with far greater potency than that of meperidine. Unfortunately, as potency increases so do addiction liability and respiratory depression.



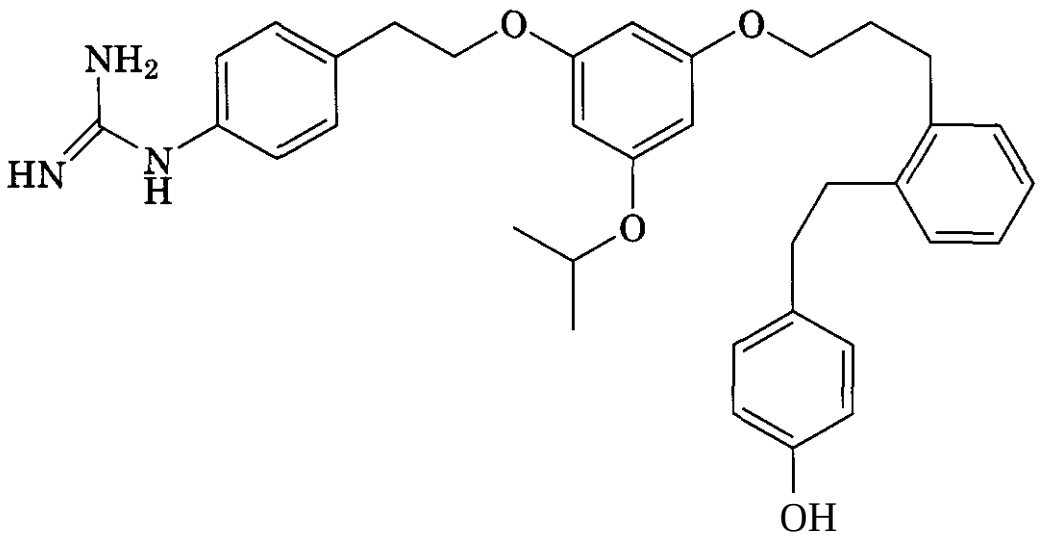
(11) atropine



(12) pethidine

2.2 Conotoxins

Elan Pharmaceuticals is developing SNX-111 (Ziconotide), the synthetic equivalent of ω-Conopeptide-MVIIA, found in the venom of the predatory marine snail *Conus* magus, for the treatment of severe pain and ischemia by the intrathecal or intravenous routes. The peptide has the structure H-^{1}Cys-Lys-Gly-Lys-Gly-Ala-Lys-^{8}Cys-Ser-Arg-Leu-Met-Try-Asp-^{15}Cys-^{16}Cys-Thr-Gly-Ser-^{20}Cys-Arg-Ser-Gly-Lys-^{25}Cys-NH_2cyclic(1–16),(8–20),(15–25)-tris(disulfide), which does not make it an



(13) conotoxin analog

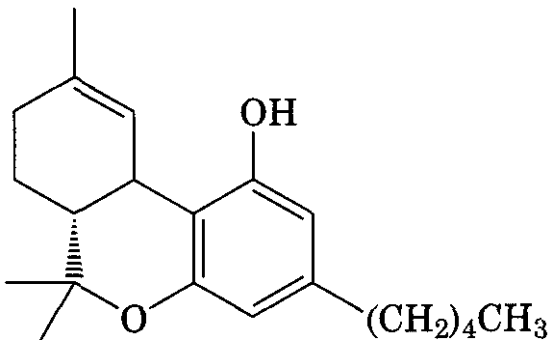
easy target for synthesis and gives it poor distribution properties in vivo (17).

SNX-111 blocks N-type calcium channels, which are located throughout the CNS on neuronal somata, dendrites, dendritic spines, and axon terminals, where they play a major role in the regulation of the neurotransmitters associated with pain transmission and stroke. The drive is to discover an orally active, selective, small-molecule modulator of N-type calcium channels to overcome the disadvantages of administration of SNX-111.

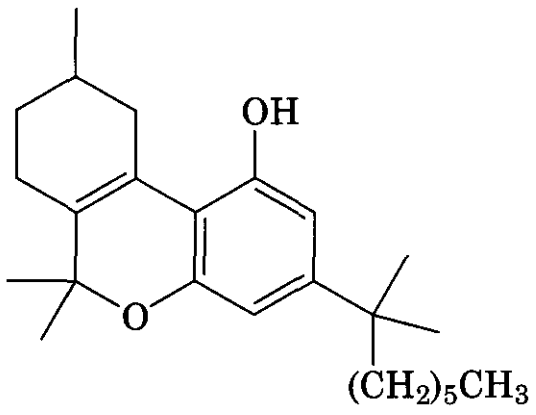
High-throughput screening campaigns have resulted in a number of leads being identified; whereas others have chosen to modify known drugs shown to block N-type channels. Workers at Parke-Davis, however, employed a ligand-based approach using the three-dimensional solution structure of the peptide (18). Compounds such as (13)were designed where key binding motifs are attached to an alkylphenyl ether scaffold. The compound had an IC_{50} value of **3.3** μM in a human N-type channel assay but showed no selectivity over the L-type channel. Structure-activity work on the conotoxins has shown that other regions of the peptide, absent in these synthetic ligands, are responsible for channel family selectivity (17, 18).

2.3 Cannabinoids

The plant Cannabis sativa has been used by humans for thousands of years, both for the effects when ingested and for making rope from the fibers in the stem. The major constituent of pharmacological interest is Δ_9-tetrahydrocannabinol (**14**) (THC), which has a multiplicity of actions. In animals the effects include sedation and apparent hallucinations (19), which are similar to the major effects in the CNS in humans. There are also cardiovascular effects, notably tachycardia and postural hypotension, that can be separated from the CNS action, as in the synthetic analog $\Delta_{6a,10a}$-dimethylheptylTHC (**15**), which has minimal CNS activity **(20).**



(14) THC

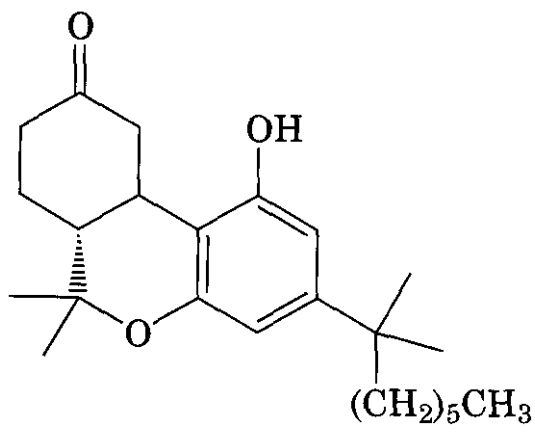


(15)

Given the widespread illicit use of *C.* sativa, it was perhaps inevitable that eventually one

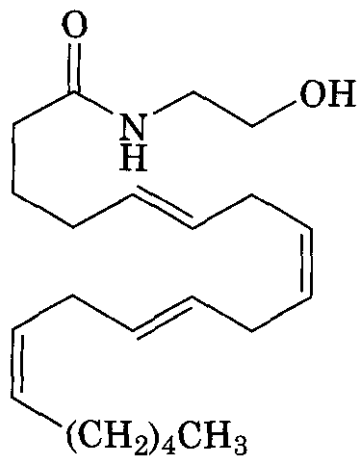
or two cancer patients receiving chemotherapy would dose themselves with their own sedative in the form of marijuana. An unexpected blessing from this uncontrolled combination was a reduction in the nausea experienced during chemotherapy. A variety of anticancer agents cause severe nausea and vomiting, including nitrogen mustard, adriamycin, 5-azacytidine, cyclophosphamide, and methotrexate: the unique situation arose in which the remedy was discovered by the patients themselves (21). Although smoking reefers gives rapid absorption and close control of the effects, smoking is itself carcinogenic and cannot be recommended to those who are unaccustomed to it; thus, when the physicians in charge were made aware of their patients' discovery, they devised a controlled clinical trial in which measured doses of THC were dissolved in sesame oil and administered in gelatin capsules. A placebo was similarly prepared for use in a randomized, double-blind, crossover experiment (21). The results left no doubt that a majority of patients benefited from THC pretreatment, even those who had previously been refractory to the effects of the standard antiemetics such as prochlorperazine. There remained the problem of tachycardia associated with THC treatment. The multiplicity of effects of THC have led to the synthesis of large numbers of analogs (22), particularly in the hope of finding non-morphine-like analgesics without addictiveness and without the other CNS effects of THC. The analog nabilone (16) had been shown to exert less effect than that of THC on the cardiovascular system, while retaining the mixture of CNS actions, including analgesic, antianxiety, and antipsychotic properties (23). When tested as an antiemetic, nabilone proved to be superior to THC (24) and has been used for this purpose for more than 30 years. The first 10 years of clinical experience was reviewed (25).

After the demonstration of THC binding sites in the CNS (26), a search for an endogenous ligand produced the long-chain ethanolamine derivative (17) of arachidonic acid, known as anandamide (27). Subsequently, the glycerol ester of arachidonic acid (18), known as 2-AG, was shown to be a more abundant endogenous ligand in the brain than anandamide (28). Further development has tended

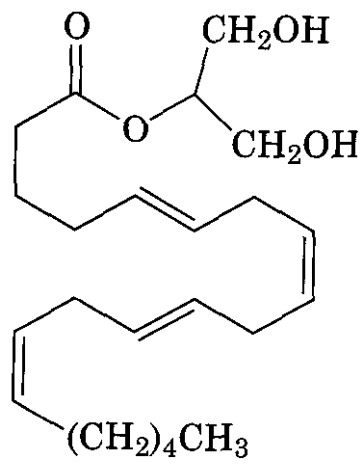


(16) nabilone

to concentrate on analogs of the natural ligands, notably the methyl derivative of anandamide (19), which is resistant to the amide hydrolase that terminates the action of anandamide itself and the dimethylheptyl analog (20) that is traceable to the earlier modifications to THC (29). Such analogs tend to have activity similar to that of THC.

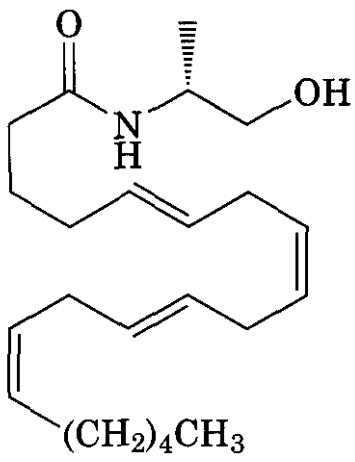


(17) anandamide

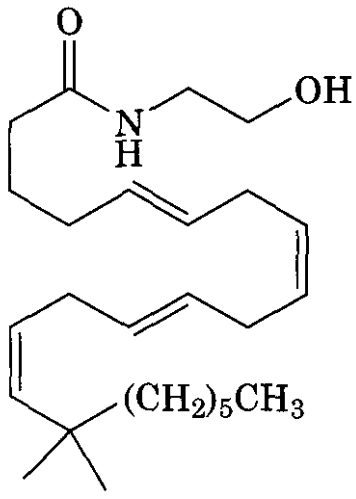


(18) 2-AG

An interesting twist in the tail is provided by the observation that anandamide is also a ligand for the so-called enigmatic vanilloid re-

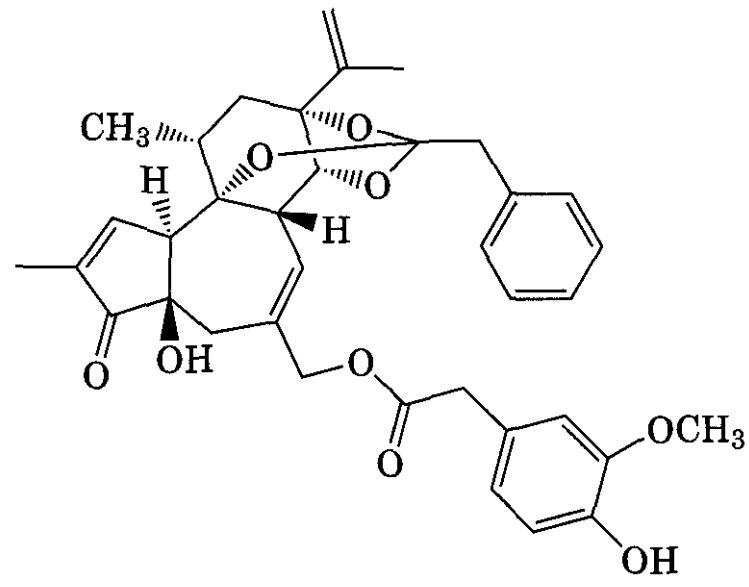


(19) R-methanandamide

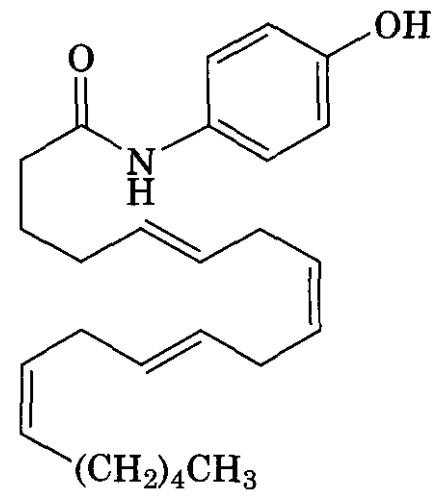


(20)

ceptors, previously characterized through their interactions with two other natural products, capsaicin (21) and resiniferatoxin (22) (30) and responsible for the "hot" sensation caused by compounds in, for example, chilies. A functional vanilloid receptor was cloned in 1997 and is activated by heat and acid as well as the chemical ligands (30). A combination of the anandamide structure with a vanilloid motif, as in AM404 (**23**), enhances the anandamide transport inhibitory properties (29). The situation is complex from the viewpoint of drug design, not least because there are two cannabinoid (CB) receptors, plus a hydrolase and a transport protein, interference with any or all of which might provide new drugs.

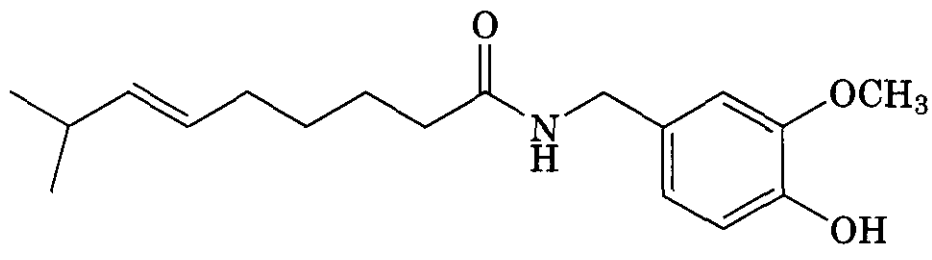


(22) resiniferatoxin



(23) AM404

The cannabinoid acids, which are devoid of psychotropic activity, are promising anti-inflammatory agents (31) and it is possible that the next useful therapeutic agent will come from this direction, rather than the sought-after analgesic.



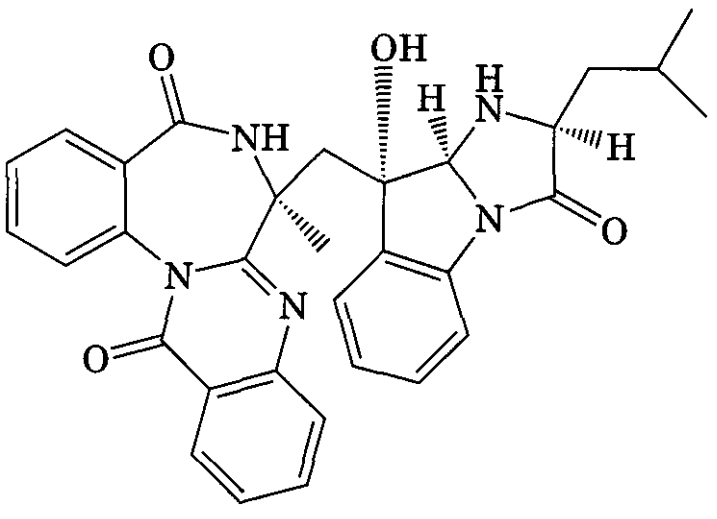
(21) capsaicin

2.4 Asperlicin

Cholecystokinin (CCK) is a peptide hormone, present in the gut and CNS; it is one of the most abundant peptides in the brain (32,331. The whole peptide is composed of 33 amino acids, but the C-terminal octapeptide H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 possesses the full range of activities, sufficient for it to be classed as a neurotransmitter (34). Specific, high-affinity binding sites have been found on mammalian CNS cell membranes and in other organs such as pancreas, gall bladder, and colon (35). The latter have been classed as CCK-A receptors, but the majority of CNS receptors were classed as CCK-B, based on affinity differences for various agonists and antagonists (36). To confuse the issue slightly, the gastrin receptor in the stomach is closely related to the CCK-B (now known as the CCK_2) receptor (37) and is stimulated by the C-terminal tetrapeptide of CCK: in the periphery, gastrin receptors are the same as CCK, receptors (38).

The effects of CCK on intestinal smooth muscle and pancreas are easy to demonstrate pharmacologically, unlike the role in the CNS, which is a matter for conjecture. It was assumed that the CNS activity must be significant, given the abundance of the peptide in the brain, and that the discovery of antagonists might lead to new drug treatments, as yet unspecified (39).

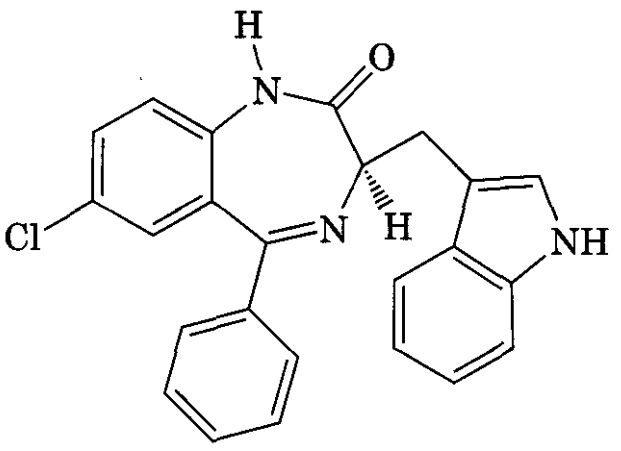
Fishing in microbial broths, using radioreceptors as bait, produced asperlicin (**24**), the first potent, competitive and selective CCK-A (CCK_1) antagonist, from a culture medium of *Aspergillus alliaceus* (40).



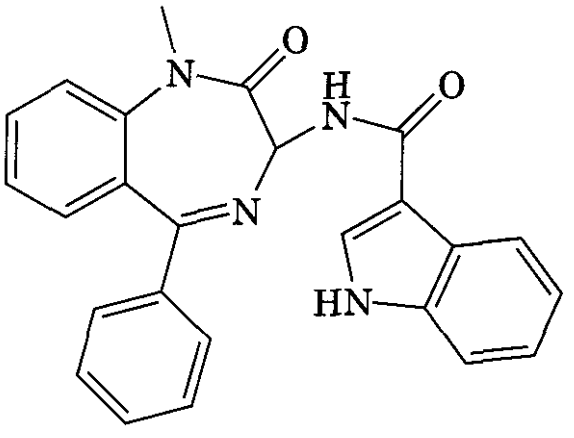
(24) asperlicin

Asperlicin is moderately potent, poorly soluble in water, and not bioavailable by the oral route (41). When discovered it was also, with morphine, one of the very few nonpeptides with affinity for a peptide receptor (peptoids are discounted in this assessment). It was an interesting target for synthetic modification, particularly viewed as a benzodiazepine derivative with potential CNS activity.

Based on the benzodiazepine nucleus, and an overt mimic of diazepam, one of the first successful synthetic analogs was L-364,286 (**25**), which had potency on CCK-A receptors similar to that of asperlicin. Better receptor affinity was achieved with 3-amide–substituted benzazepines: the 2-indolyl derivative L-364,718, also known as MK-329 (**26**), is five orders of magnitude more potent than asperlicin (42) at CCK-A receptors and is a valuable pharmacological tool.



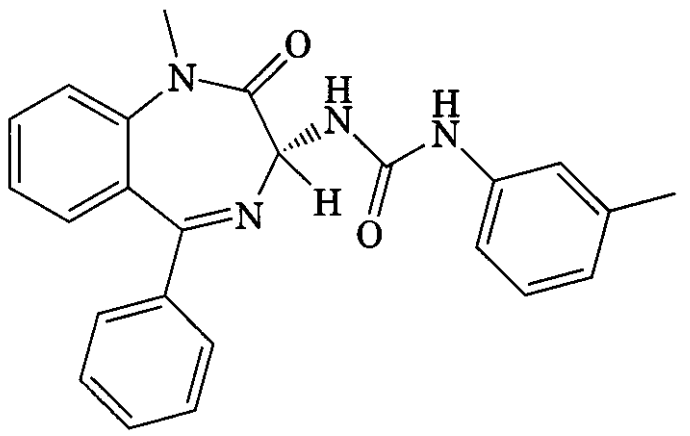
(25)



(26)

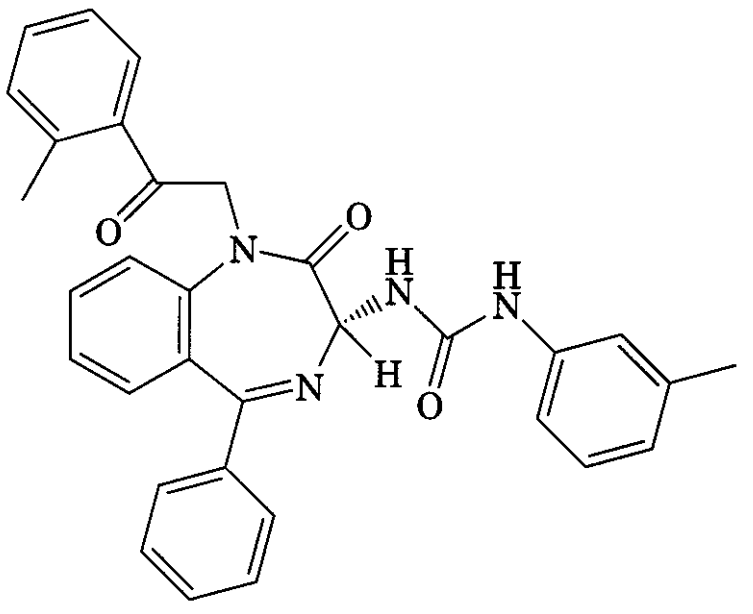
Modification of the 3-amide to give a urea linkage as in (27) led to a reduction in CCK-A receptor affinity. Importantly, discrimination between CCK-A and CCK-B receptors by (27)

is governed by the stereochemistry at C3, the (*S*)-enantiomer showing greater affinity for CCK-A receptors. The (*R*)-enantiomer, known as L-365,260, prefers CCK-B receptors, antagonizes gastrin-stimulated acid secretion in animal models, and, among other CNS effects, induces analgesia in primates and displays anxiolytic properties (32).



(27)

Further development in this series has very substantially improved receptor affinity: YM-022 (28) has IC_{50} 0.05 nM/kg (38). Clinical trials of compounds in this series have been disappointing because of poor bioavailability, but the general concept of finding a therapeutic agent through antagonism of CCK_2 receptors is still viable and it is reported that the number of patents in this area has increased in the last 5 years (43).

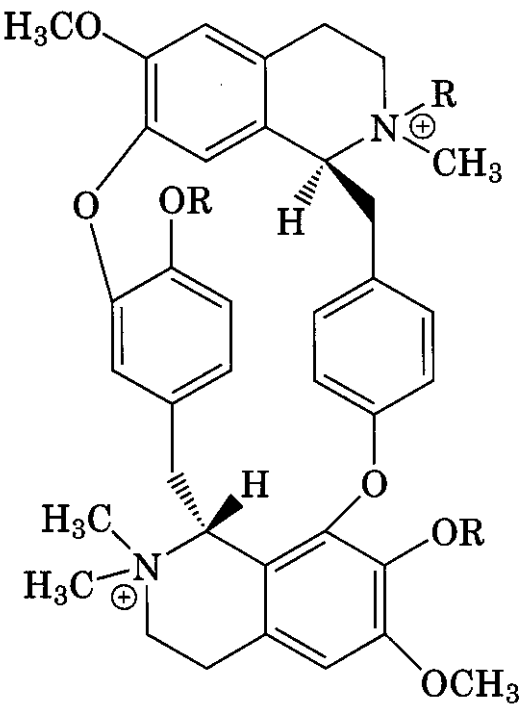


(28) YM-022

3 NEUROMUSCULAR BLOCKING DRUGS

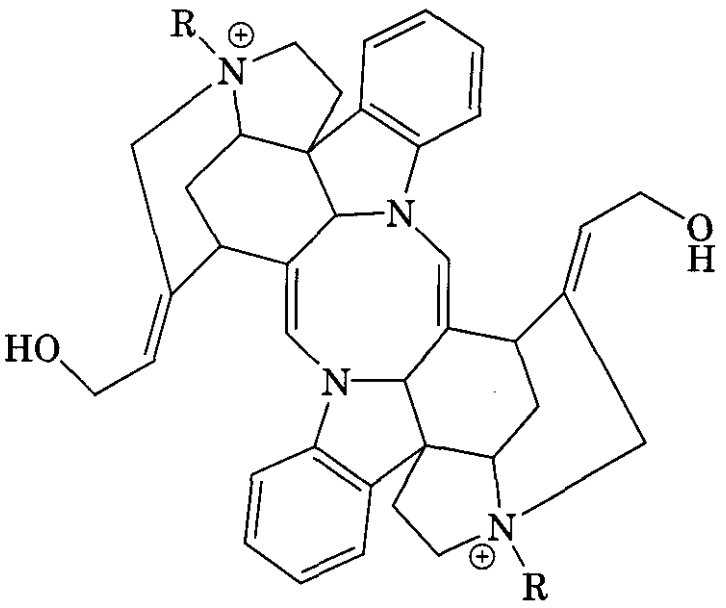
3.1 Curare, Decamethonium, and Atracurium

The development and use of muscle relaxants, to allow a reduction in the level of anesthesia during surgery, follows entirely from studies of South American arrow poisons (44) and particularly from the isolation by King (45) of pure D-tubocurarine (29) in the 1930s, from tube curare. Another of the South American blowpipe poisons, calabash curare, was used for similar purposes and developed (46, 47), to give alcuronium (30) from the alkaloid C-toxiferine 1 (31). Both types of curare paralyze skeletal muscle by a similar mechanism, antagonizing the effect of acetylcholine at the neuromuscular junction (48).



(29) tubocurarine R = H
(32) metocurine R = CH_3

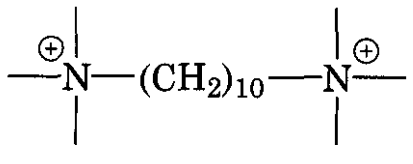
The muscle-paralyzing curare alkaloids are quaternary salts that are not absorbed when taken orally. For surgical procedures they must be administered by intravenous injection, which results in onset of paralysis in at most a few minutes: anesthesia is normally induced before administration of the muscle relaxant (44), which is followed by artificial respiration. Although the neuromuscular blocking agents are potentially lethal when administered alone, in the environment of an operating theater they are truly life-saving



(31) C-toxiferine **1** R = CH_3
(30) alcuronium R = $CH_2CH{=}CH_2$

drugs that have made a major impact on survival rates during surgery.

At the time of King's work in the 1930s there were no spectroscopic aids to structure elucidation, and it is not surprising that he made a small error in the structure assigned to D-tubocurarine, believing it to have two quaternary nitrogens, a mistake that was not corrected (49) until 1970. The methylation product of D-tubocurarine, known as **metocurine** (32) is a more potent muscle relaxant. It was known for a long time as **dimethyltubocurarine** because of the error in the structure allocated to compound (29). King's error, in assigning a bisquaternary structure to a molecule with one quaternary and one protonated tertiary nitrogen, led to a large number of highly active synthetic bisquaternaries. The simplest of these was decamethonium (**33**), which was nothing more than two **trimethylammonium** end groups connected with a decamethylene chain. **As** one of a series with different chain lengths (50), decamethonium became the prototype for many more complex structures with 10 atoms between the quaternary centers, which appeared to be optimal for

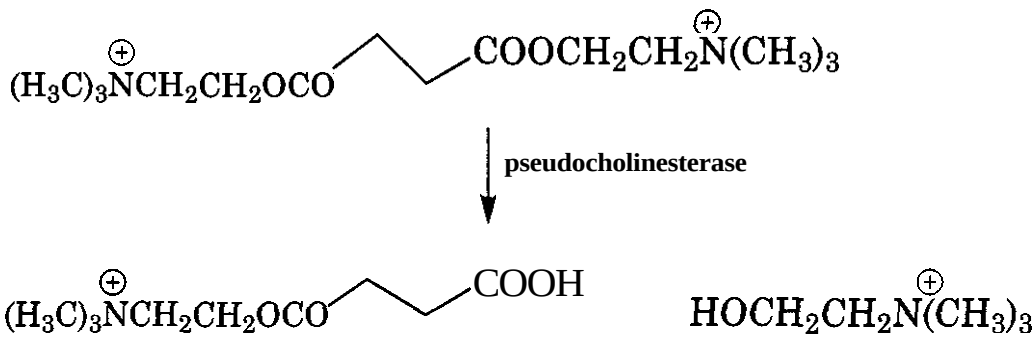


(33) decamethonium

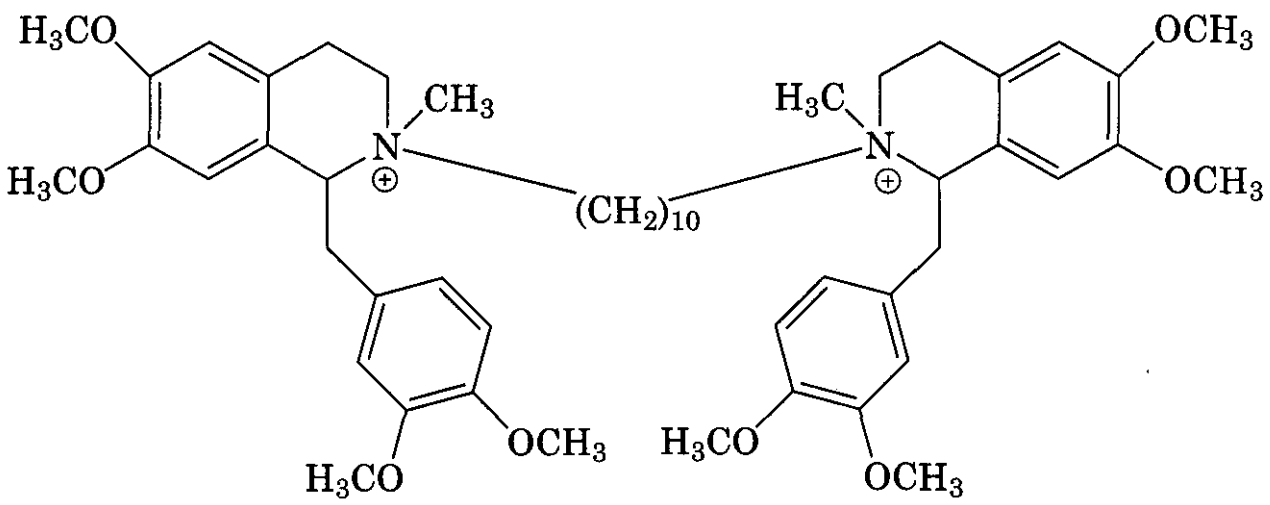
binding to the acetylcholine receptor at the neuromuscular junction.

Unlike tubocurarine, decamethonium depolarizes the muscle endplate, rendering the membrane insensitive to acetylcholine (48). The action of tubocurarine is competitive and can be overcome with increased concentrations of acetylcholine, brought about by administration of an anticholinesterase: the latter is thus an antidote to tubocurarine, but not to decamethonium. Despite the lack of an antidote, decamethonium was used very widely for over two decades. One of its disadvantages is an overlong duration of action, during which time the patient has to be maintained on artificial respiration, because the muscle of the diaphragm is also susceptible to the actions of the drug. An early and highly successful attempt (51) to shorten the action of decamethonium gave suxamethonium (**34**), a diester formed between succinic acid and two molecules of choline, which hydrolyzes rapidly in the presence of pseudocholinesterase.

Tubocurarine suffers from cardiovascular side effects induced by direct interactions with ganglionic acetylcholine receptors and from stimulation of histamine release, so analogs have been well worth pursuing. The **macrocyclic** structure of tubocurarine is a difficult synthetic target, but fortunately ring-opened analogs, such as laudexium (**35**), have high potency and relatively few side effects (52). The main problem with (35) is the duration of action, which at about 40 min is too long for many operations. Two approaches have been used to shorten the duration of action. The concept of pH-controlled Hofmann elimination was employed successfully (53) in the design of atracurium (**36**), which in clinical use **(54)** has the big advantage that the drug disappears at a constant rate, irrespective of liver or kidney function. Some ester hydrolysis contributes to the destruction of atracurium in *vivo*, as might be expected. **A** slightly later development (55) centered on an empirical search for structures that would undergo ester hydrolysis more rapidly, resulting in **mivacurium** (**37**), which has a slightly shorter duration of action than that of atracurium, the latter being about 15–20 min.



decomposition of suxamethonium (34)



(35) laudexium

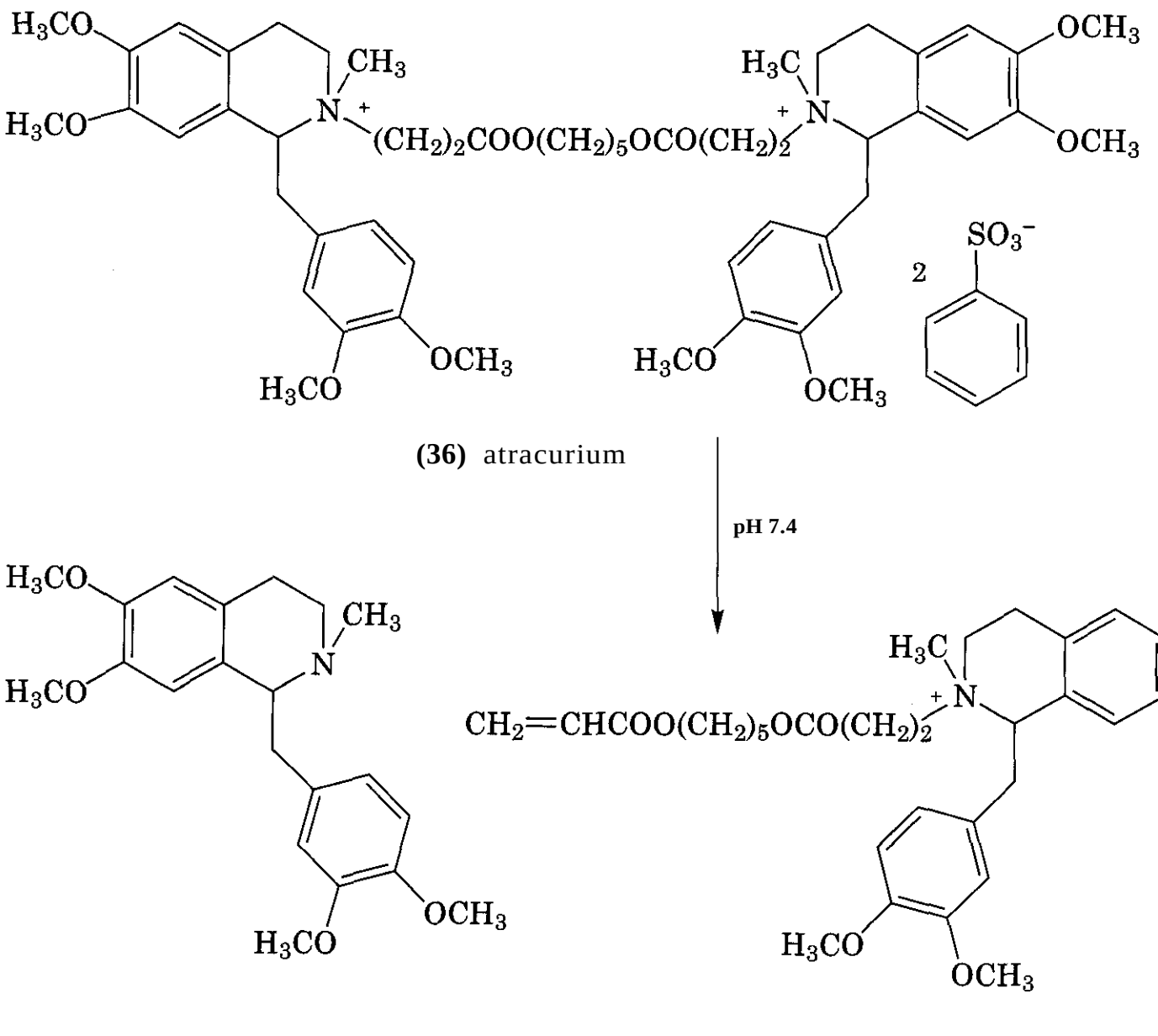
4 ANTICANCER DRUGS

4.1 Catharanthus (Vinca) Alkaloids

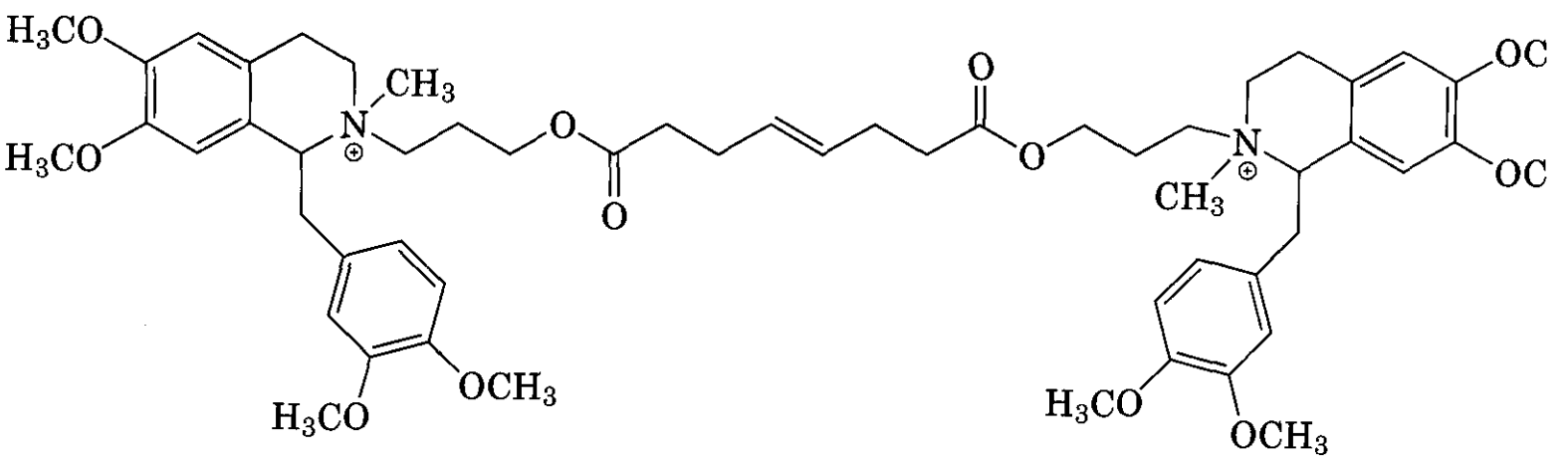
In 1949 Canadian researchers at the University of Western Ontario began investigating the medicinal properties of the rosy periwinkle (Catharanthus *roseus*), a plant that had been used for many years to treat diabetes mellitus in the West Indies. Despite finding that the plant extract when given orally had no effect on blood sugar levels in rats or rabbits, the researchers noted that when given intravenously, the extract caused the animals to succumb to bacterial infection and die. This curious observation prompted further studies, which showed that the plant extract reduced levels of white blood cells, causing granulocytopenia and bone marrow damage, toxic effects that are encountered with many antitumor drugs (56). These findings led the Canadian group to isolate an alkaloid fraction with potent cytotoxic activity. The active principle was eventually purified and became known as vinblastine (38), a dimeric indole-dihydroindole alkaloid.

Concurrently, researchers at the Lilly Research Laboratories had been investigating extracts of *C. roseus* and they too had detected cytotoxic activity, specifically against acute lymphocytic leukemia (57, 58). The U.S. group isolated several alkaloids, including vinblastine and another closely related alkaloid, vincristine (39).

Although many other alkaloids have been isolated from *C. roseus*, only vinblastine and vincristine have been developed for clinical use. The antiproliferative activity of the two compounds is related to their specific interaction with tubulin, thus preventing assembly of tubulin into microtubules and arresting cell division (59). However, despite this apparent identical mechanism of action and their clear chemical similarities, vinblastine and vincristine display very different clinical effects. Vinblastine, for example, is used to treat Hodgkin's disease and metastatic testicular tumors, whereas vincristine is used mainly in combination with other anticancer drugs for the treatment of acute lymphocytic leukemia in children. Toxicity profiles are also different, in that vinblastine causes bone-marrow depression, whereas peripheral neuropathy often proves to be dose-limiting in vincristine therapy.

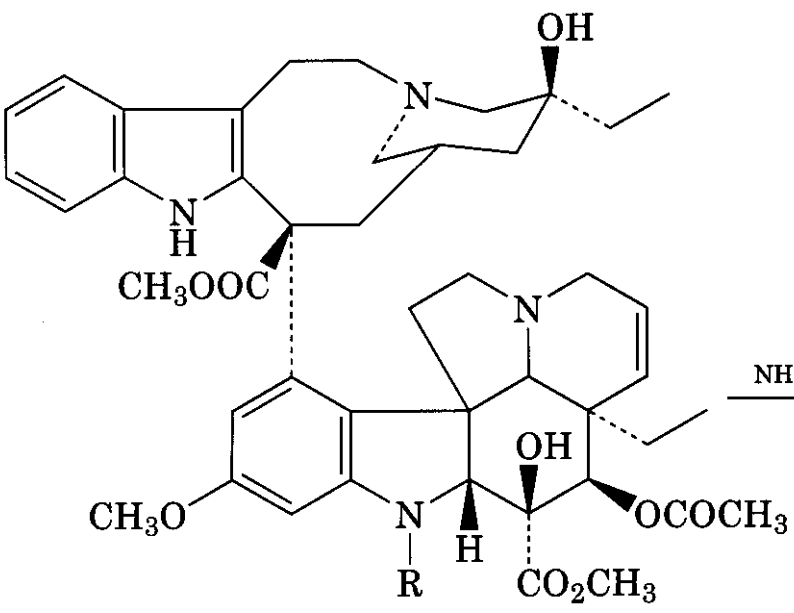


(36) atracurium

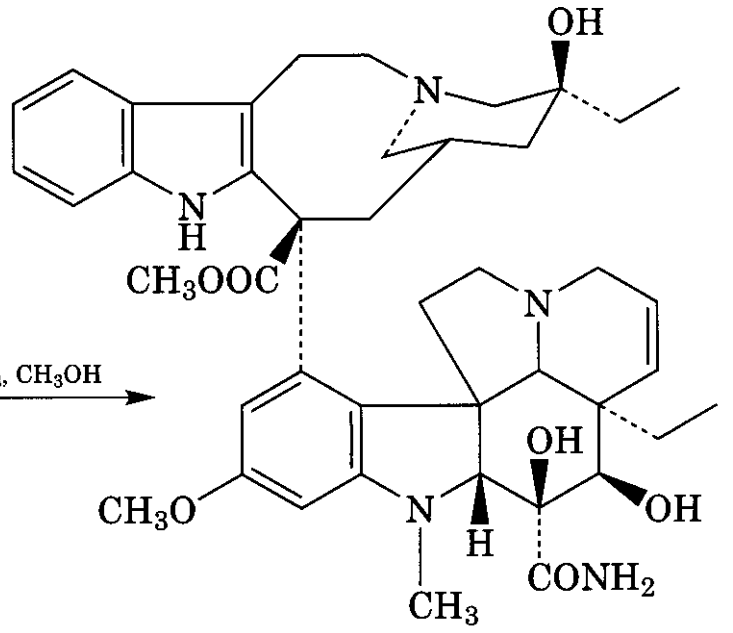


(37) mivacurium

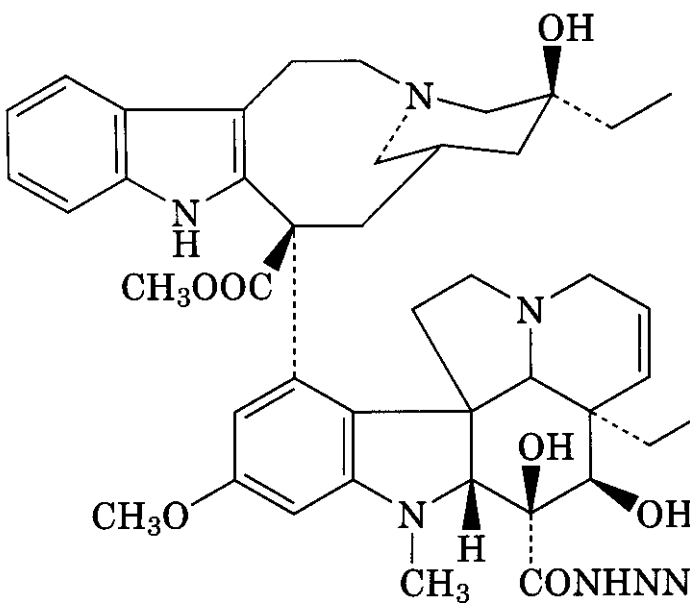
Lilly introduced vinblastine and vincristine into the clinic in 1960 and 1963, respectively, but this did not preclude the search for improved derivatives. **A** chemical modification program aimed at improving antitumor activity and reducing toxicity was initiated in 1972 (60). Concern about the neurotoxicity displayed by vincristine, its chemical instability, and low natural abundance (0.03 **g/kg** dried plant material) led to vinblastine's being chosen as a template for semisynthetic modification. Selective ammonolysis of the ester function at **C-3** and hydrolysis of the adjacent acetyl group yielded the desacetyl vinblastine amide, vindesine (40). Better yields of vindesine were obtained from the hydrazide (41) on treatment with nitrous acid and reacting the resultant azide (42) with ammonia. The azide (42) proved to be a useful intermediate for the preparation of a range of substituted amides, although vindesine proved to be the derivative of choice, with significant differ-



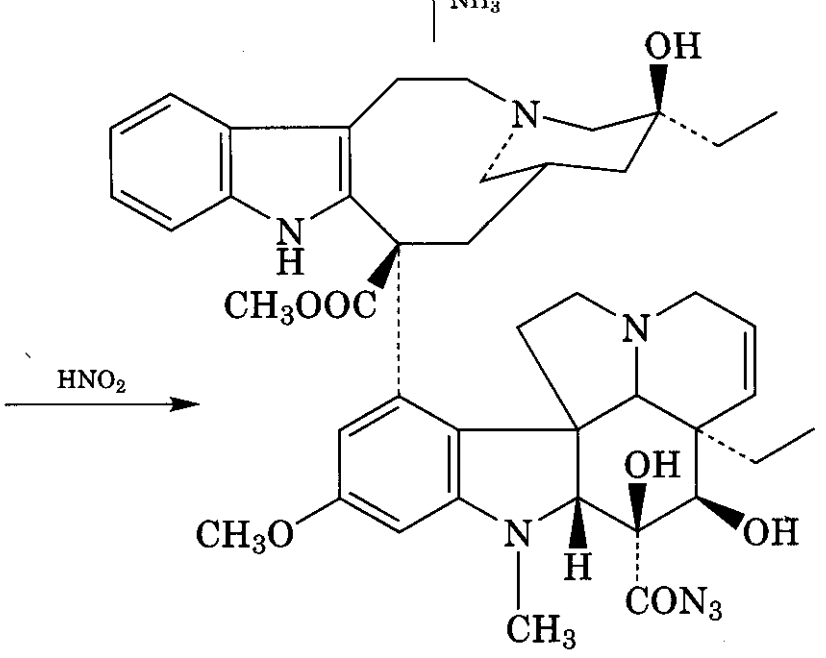
(38) R = CH_3
(39) R = CHO



(40)



(41)



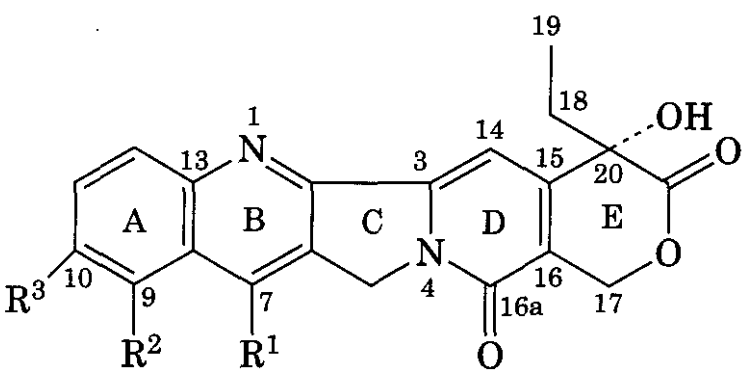
(42)

ences in the spectrum of antitumor activity and toxicity compared to that of the naturally occurring alkaloids. Phase I clinical trials commenced in 1977 and vindesine has been used for the treatment of non-small cell lung cancer, lymphoblastic leukemia, and non-Hodgkin's lymphomas. In combination with cisplatin, vindesine ranks among the foremost treatments for non-small cell lung cancer with respect to response rate and survival (61). Back in the 1950s, the U.S. researchers could not have guessed that 30 years on, the demand for Catharanthus alkaloids would necessitate the processing of around 8000 kg of plant material per year (62)!

4.2 Camptothecin

Camptothecin (43) was first isolated by Monroe Wall and Mansukh Wani in 1966, after ethanolic extracts of *Camptotheca acuminata*, a tree native to China, showed unusual and potent antitumor activity (63). Starting with 19 kg of dried wood and bark, Wall and Wani painstakingly purified the principal active component with a combination of hot solvent extraction, an 11-stage Craig countercurrent partition process, silica gel chromatography, and crystallization. Camptothecin was characterized as a novel pentacyclic alkaloid, present as just 0.01% w/w of the stem bark of C. *acumi-*

nata. Of particular note was the unusual activity that camptothecin displayed in L1210 and P388 mouse leukemia life-prolongation assays. The compound also inhibited the growth of solid tumors in vivo and the water-soluble sodium salt was progressed to phase II clinical trials before being withdrawn because of severe bladder toxicity.



(43) Camptothecin: $R^1 = R^2 = R^3 = H$

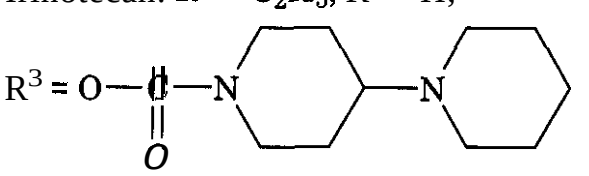
(44) 10-hydroxycamptothecin: $R^1 = R^2 = H$, $R^3 = OH$

(48) 7-ethyl-10-hydroxycamptothecin: $R^1 = C_2H_5$, $R^2 = H$, $R^3 = OH$

(46) Topotecan: $R^1 = H$, $R^2 = CH_2—N(CH_3)_2$, $R^3 = OH$

Interest in camptothecin gained new impetus in 1985, when it was discovered that the compound exerts its antitumor activity through a novel mechanism of action (64). Camptothecin binds to the covalent complex formed by topoisomerase I and DNA, which initiates DNA replication and thus stabilizes the enzyme–DNA complex and prevents cell proliferation. The elucidation of the mechanism of action provided a means of evaluating camptothecin analogs as topoisomerase inhibitors in vitro and efforts then focused on synthesizing water-soluble analogs with broad-spectrum antitumor activities. The α-hydroxy lactone (ring E) and, in particular, the 20(*S*)-form proved essential for maintaining biological activity, but the 10-hydroxy analog (44) showed greater activity than that of (43) (65). Wall and Wani successfully deployed the Friedlander reaction between substituted 2-aminobenzaldehydes and the tricyclic intermediate (**45**), to synthesize a variety of ring-A–substituted analogs. These studies may have prompted SmithKline Beecham (now GlaxoSmithKline) to synthesize the water-soluble 10-hydroxycamptothecin analog topotecan (46) that was first approved in 1996 for the treatment of recurrent ovarian cancer and, 2 years later, for small cell lung cancer (66). Irinotecan (**47**), developed by Daiichi and Yakult Honsha in Japan and marketed by Pharmacia, was also approved in 1996 for the treatment of advanced colorectal cancer. Irinotecan is inactive as a topoisomerase I inhibitor, but acts as a prodrug of the active 7-ethyl-10-hydroxycamptothecin (**48**) (67).

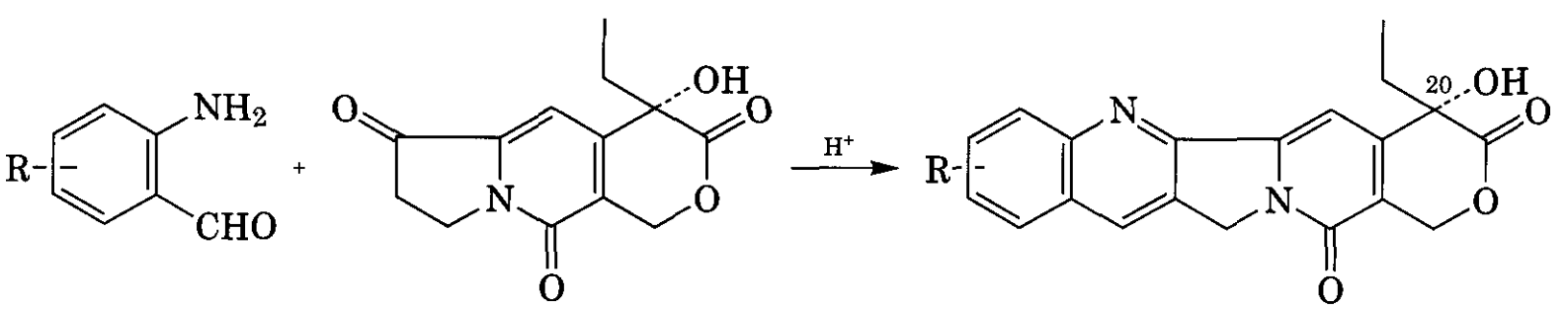
(47) Irinotecan: $R^1 = C_2H_5$, $R^2 = H$,



4.3 Paclitaxel and Docetaxel

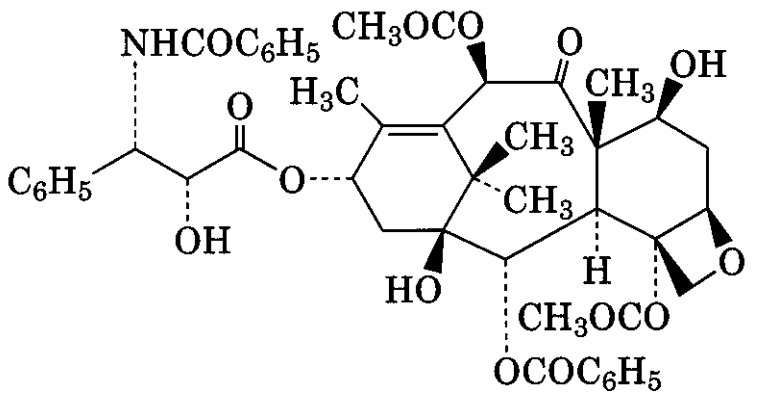
Regarded as the tree of death by the Greeks and used to prepare arrow poison by the Celts, the yew tree has been associated with death and poisoning for centuries (68, 69). The English yew, *Taxus baccata*, was used to make funeral wreaths and it was believed that one could die by merely standing beneath the boughs of the tree.

Yew certainly contains highly toxic metabolites and their potency and fast duration of action has often made extracts of yew the poison of choice for numerous murders and suicide attempts. It is thus ironic that extracts from the Pacific yew, T. *brevifolia*, after being



(45)

tested in the National Cancer Institute's (NCI) screening program during the 1960s, yielded what was described (70) as the most exciting anticancer compound discovered in the previous 20 years; that is, paclitaxel (49) (originally given the name taxol by Wall and Wani).



(49)

The initial isolation and characterization of paclitaxel proved particularly difficult because of (1) its very low natural abundance in T. *breuifolia* bark (although this was the best known source, the isolated yield was only 0.02% w/w, equivalent to 650 mg per tree), (2) the poor analytical data obtained from the purified compound, and (3) the failure of paclitaxel to give crystals that were suitable for X-ray analysis (71). The structure of paclitaxel was published in 1971 (72), but further biological testing continued to be troubled by difficulties. The compound showed only modest in *vivo* activity in various leukemia assays, which was no better than that displayed by a number of other new compounds at the time. In addition to the limited supplies of paclitaxel (the complexity of the molecule precluded chemical synthesis), the compound was very poorly soluble in water, which made formulation difficult. However, various new assays were developed in the 1970s, including the murine B16 melanoma model, in which paclitaxel showed very good activity, and another boost came when Horwitz et al. (73) discovered that the compound prevented cell division by a unique mode of action. In contrast to the antimitotic vinblastine and podophyllotoxin analogs (q.v.), which prevent microtubule assembly, paclitaxel inhibits cell division by promoting assembly of stable microtubule bundles, which leads to cell death.

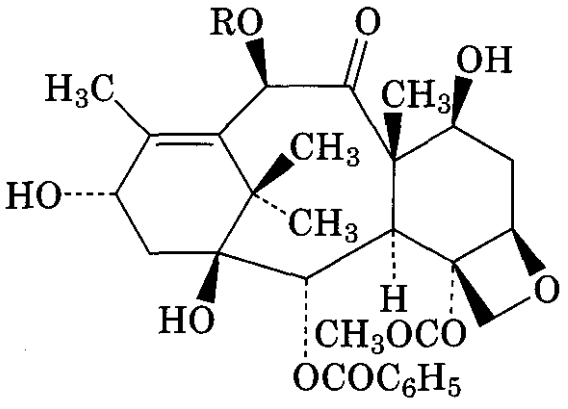
Phase I clinical trials were initiated in 1983, but these were to proceed at a slow and tortuous pace and proved all but disastrous when the high levels of oil-based adjuvant used to formulate paclitaxel caused severe allergic reactions in many volunteers. Undaunted by the formulation problem and spurred on by paclitaxel's novel mechanism of action, clinicians were able eventually to minimize the allergic events and demonstrate useful activity. Phase II clinical trials began in 1985 despite continuing supply problems, and 4 years later the program received a significant boost when McGuire et al. (74) reported good responses from patients suffering from refractory ovarian cancer, a disease that kills some 12,500 women a year in the United States alone.

In many ways, the development of paclitaxel mirrored that of the camptothecin analogs, both being dogged for many years by supply issues, poor pharmacokinetics, and toxicity, but the subsequent uncovering of novel mechanisms of action fueled renewed efforts to develop these leads into important new anticancer agents (75).

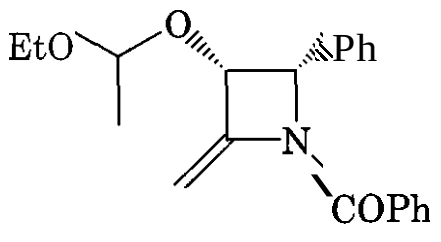
In 1991 Bristol-Myers Squibb in conjunction with the NCI agreed to manage the supplies of paclitaxel and were granted a licence to further develop the compound. The following year the U.S. Federal Drug Administration approved paclitaxel for the treatment of ovarian cancer in patients unresponsive to standard treatments, and in December 1993 approval was given for the treatment of metastatic breast cancer.

The sourcing of paclitaxel from T. *brevifolia* was a major problem (76) because to treat just the groups of patients suffering ovarian cancer in the United States would require about 25 kg of compound per year, necessitating the felling of some 38,000 trees (70)! Although the Pacific yew is not a rare tree, it is extremely slow growing and such harvesting could not be sustained indefinitely. It has been estimated that there were enough trees available to maintain a supply of paclitaxel for only 2–7 years (77). The isolation of paclitaxel from other Taxus species has been investigated at length and reasonable quantities have been obtained from the needles of several species including T. *baccata*. Using the needles has

alleviated the supply problem because they can be harvested without damaging the tree. However, the needles contain much higher quantities of several biosynthetic precursors of paclitaxel and two of these, baccatin III (50) and 10-desacetylbaccatin III (51) have been used to prepare paclitaxel semisynthetically. One approach, developed by Potier et al. (78), involved acylation of the sterically hindered C-13 position of baccatin III with cinnamic acid and subsequent double-bond functionalization through hydroxyamination, to give paclitaxel together with various regio- and stereoisomers. A better approach involved protection of 10-desacetylbaccatin III as the triethylsilyl ether, followed by direct acylation with the phenylisoserine derivative (**52**), giving paclitaxel in 38% overall yield (79). Further improvements were made using less sterically demanding acylating reagents; for example, acylation with the β-lactam (53) gave paclitaxel in up to 90% yield (80) and this may be the preferred method for commercial production in the future.

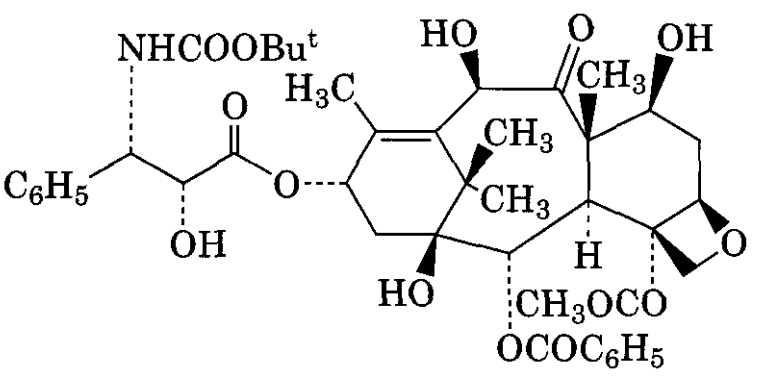


(50) R = $COCH_3$
(51) R = H



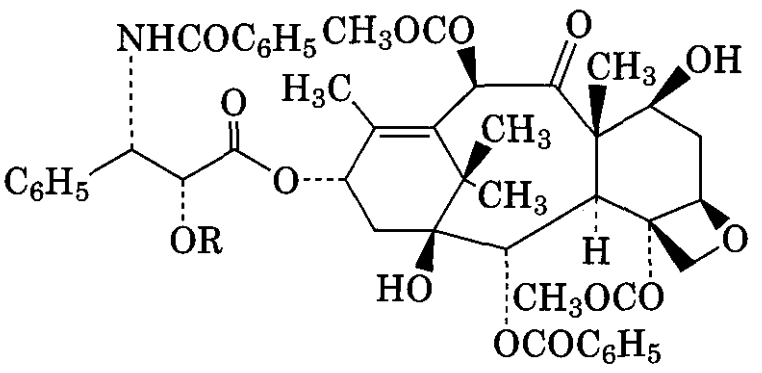
(53)

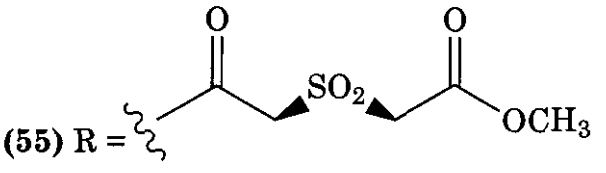
These semisynthetic approaches also provide access to analogs with potential advantages over paclitaxel itself. Structure-activity studies have shown that, although the oxetane ring appears to be essential for activity, wide variation in the nature and stereochemistry of the C-13 ester side-chain can be tolerated. Thus, the N-t-(butoxycarbonyl)derivative, docetaxel (**54**), which appears to be more potent than paclitaxel (81) and has better solubility characteristics, has been developed and launched by Aventis for the treatment of ovarian, breast, and lung cancers.



(54)

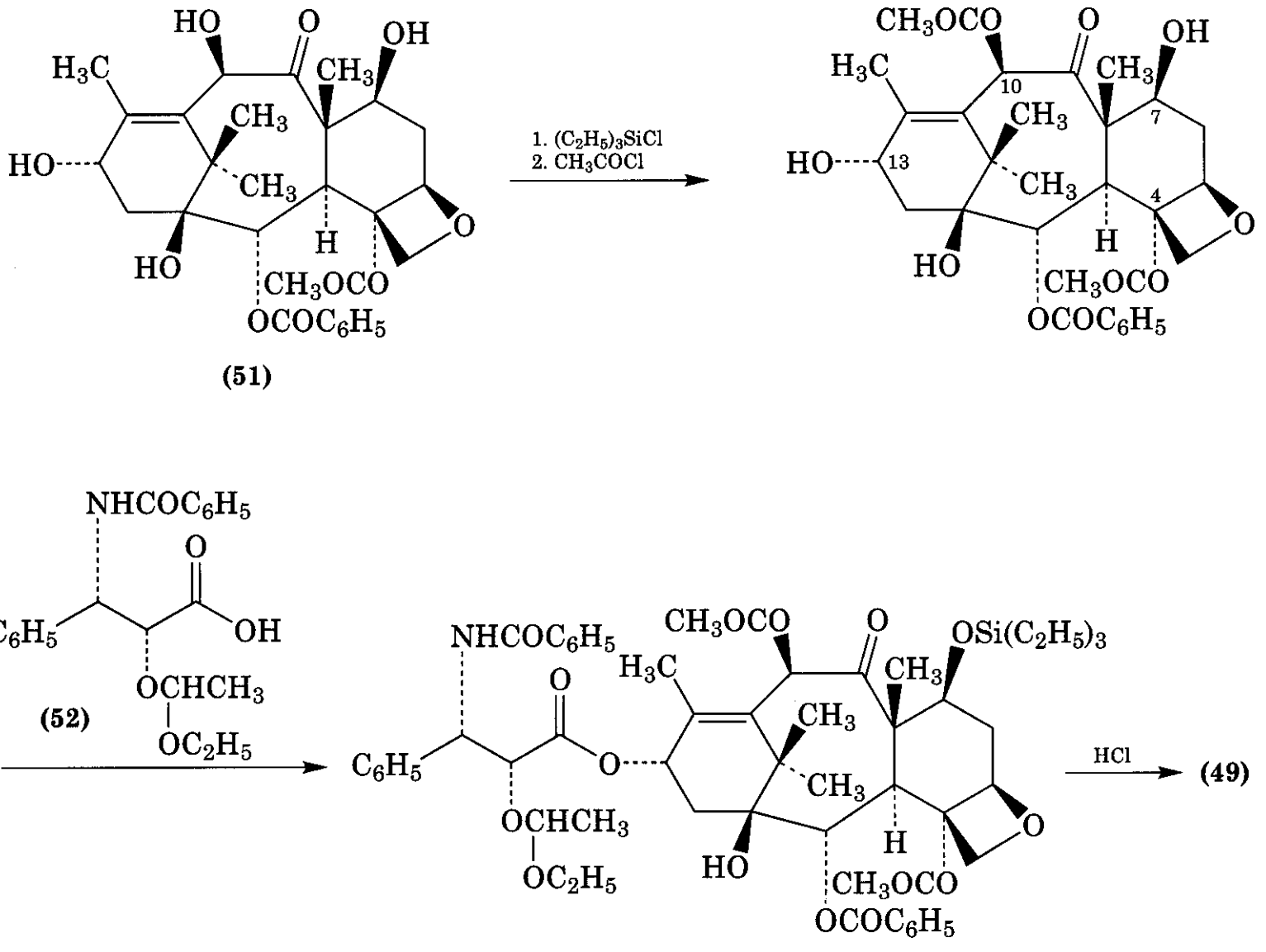
Various "protaxols," designed to release paclitaxel *in situ* under physiological conditions, have been prepared by acylating the C-2' hydroxyl group. Nicolaou et al. (82) reported the synthesis of the sulfone (**55**), which is soluble and stable in aqueous media, but is able to release paclitaxel rapidly in human blood plasma.





(55) R =

Plant tissue culture (70), microbial fermentation (83), and total synthesis (84, 85) provide other possibilities for the production of paclitaxel and its derivatives, although it is far from certain whether any of them will be commercially viable.



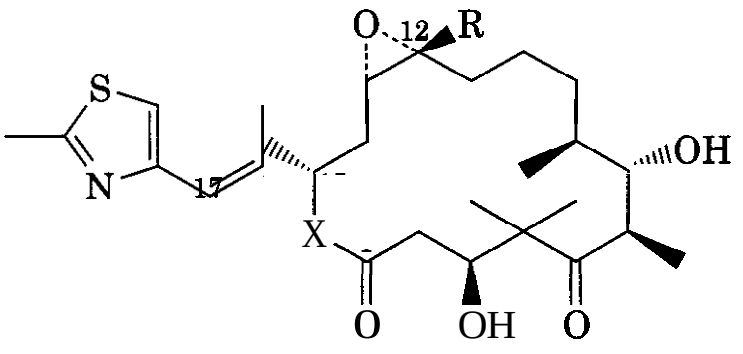
(51)

(52)

(49)

4.4 Epothilones

Epothilones A (56) and B (**57**), 16-membered macrocyclic polyketide lactones, were first isolated from the cellulose-degrading myxobacterium Sorangium *cellulosum* by Hoefle, Reichenbach, and coworkers (86) as narrow-spectrum antifungal and cytotoxic metabolites. The compounds were then tested by the National Cancer Institute in the United States and found to be highly active against breast and colon cancer cell lines (87). Subsequently,



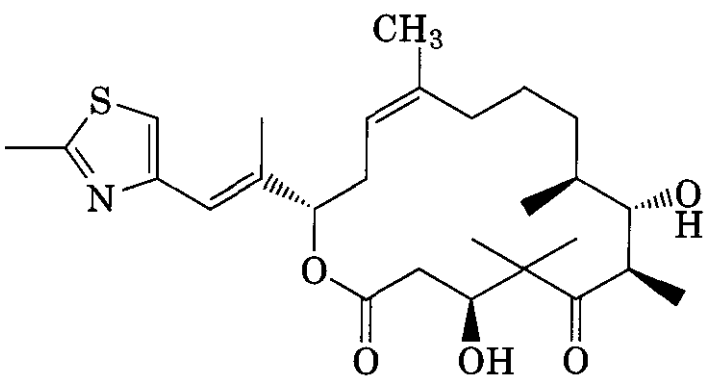
(56) epothilone A: X = O, R = H
(57) epothilone B: X = O, R = CH_3
(59) BMS 247550: X = NH, R = CH_3

Bollag et al. (88) at the Merck Research Laboratories discovered that the epothilones stabilize microtubule assembly and thus inhibit cell division by the same mechanism as that of paclitaxel (see above). This observation, together with their less complex chemical structure, increased water solubility, more rapid action in vitro, and effectiveness against multidrug-resistant tumor cells, has prompted significant interest in the epothilones as anticancer agents.

On learning the absolute stereochemistry of (56) and (**57**), three academic research groups embarked on the total synthesis of the epothilones. Nicolaou, Danishefsky, and Schinzer independently adopted successful, elegant synthetic approaches involving olefin metathesis, macrolactonization, Suzuki coupling, or ester–enolate–aldehyde condensation (89). Within 3 years of the disclosure of their absolute stereochemistry, 17 different total syntheses of the natural products were reported. These syntheses paved the way for the generation of a large number of epothilone

analogs for biological evaluation, including the use of solid-phase combinatorial approaches.

The academic groups focused on modifications around the core macrocyclic lactone, establishing important structure–activity relationships, but not improving on the in vitro biological activity of the most active natural product, epothilone B (**57**). In vivo biological data were comparatively scarce and, although one group reported that epothilones B (57) and D (58) showed activity in murine tumor models, researchers at Bristol-Myers Squibb have shown that (58) lacks in vivo activity as a result of rapid metabolic inactivation (90). It was postulated that esterase-mediated hydrolysis of the macrocyclic lactone formed an inactive ring-opened species and, therefore, efforts were focused on replacing the lactone with a more stable macrocyclic lactam moiety. Several macrocyclic lactam derivatives were synthesized from (**57**) and (**58**). Of note was the preparation of BMS-247550 (**59**) in a three-step synthesis from epothilone B (**57**), utilizing a novel Pd(0)-catalyzed ring-opening reaction followed by reduction and macrolactamization. BMS-247550 (**59**), which is in phase I clinical trials, retains its activity against human cancer cells that are naturally insensitive to paclitaxel or that have developed resistance to paclitaxel, both in vitro and in vivo (91).



(58) epothilone D

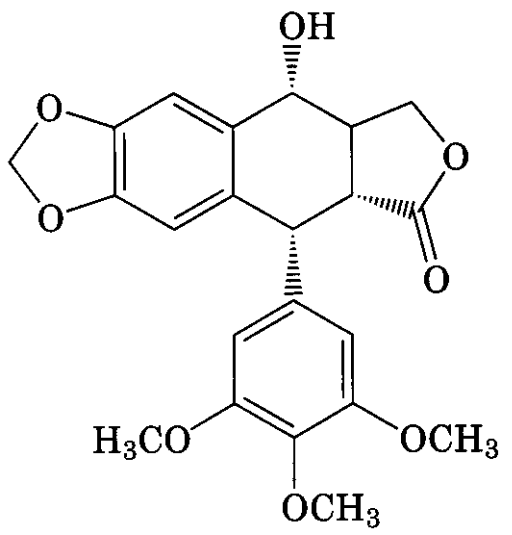
4.5 Podophyllotoxin, Etoposide, and Teniposide

The development of the natural constituents of Podophyllum Resin into effective semisynthetic and, ultimately, totally synthetic compounds for the treatment of various kinds of cancer provides one of the most sustained and intriguing stories of drug discovery (92, 93). The story has all the classic ingredients, starting with observation and reasoning, extending through chance into new areas, and characterized throughout by persistence and determination, particularly when biological activity had to be traced to very minor constituents in the crude plant extract.

Podophyllum *peltatum* (may apple, or American mandrake) and P. *emodi* are. respectively, American and Himalayan plants, widely separated geographically but used in both places as cathartics in folk medicine (94). An alcoholic extract of the rhizome known as podophyllin was included in many pharmacopoeias for its gastrointestinal effects; it was included in the U.S.P., for example, from 1820 to 1942. At about this time the beneficial effect of podophyllin, applied topically to benign tumors known as condylomata acuminata, was demonstrated clinically (95). This usage was not inspirational, given that there are records of topical application in the treatment of cancer by the Penobscot Indians of Maine and, subsequently, by various medical practitioners in the United States from the 19th century (96). The crude resinous podophyllin is an irritant and unpleasant mixture unsuited to systemic administration.

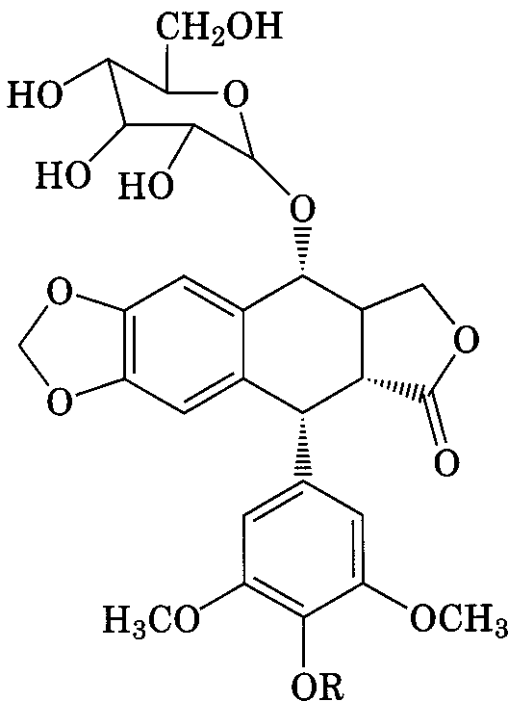
The first chemical constituent was isolated from podophyllin in 1880 and named podophyllotoxin (97). A structure was proposed in 1932 and after some fine-tuning (98) was shown to be the lignan (60). As might be expected, the crude resin contains a variety of chemical types, including the flavonols quercetin and kaempferol (99). Although these other constituents undoubtedly have biological activity, it is the lignans that have received most attention and to which we shall devote the remainder of this section.

Chemists at Sandoz in the early 1950s reasoned that crude podophyllin might contain lignan glycosides with anticancer activity, which might be more water soluble and less toxic than podophyllotoxin (92). The reasoning for the latter is not entirely clear, but in the event they proved to be correct in both respects. Careful isolation gave podophyllotoxin β-D-glucopyranoside (61) its 4'-desmethyl analog (62) and some less important lignans lacking the B-ring hydroxy group (100–102). Unfortunately, the sugar deriva-



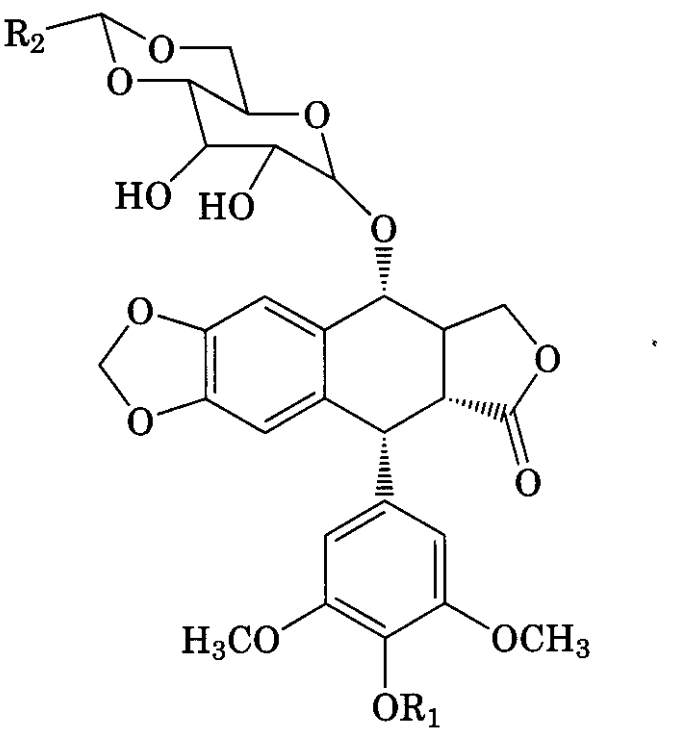
(60) podophyllotoxin

tives were less active as inhibitors of cell proliferation than were the aglycones, as well as less toxic; however, as expected, they were much more water soluble (92). While continuing work to isolate more natural lignans, a substantial program of structural modification of the known compounds was undertaken, with a view to protecting the glucosides from hydrolytic enzymes and also to improve cellular uptake. Most of these changes were ineffective: the per-acylated derivatives, for example, were insoluble in water and had inferior cytostatic effects (103).



(61) R = CH_3
(62) R = H

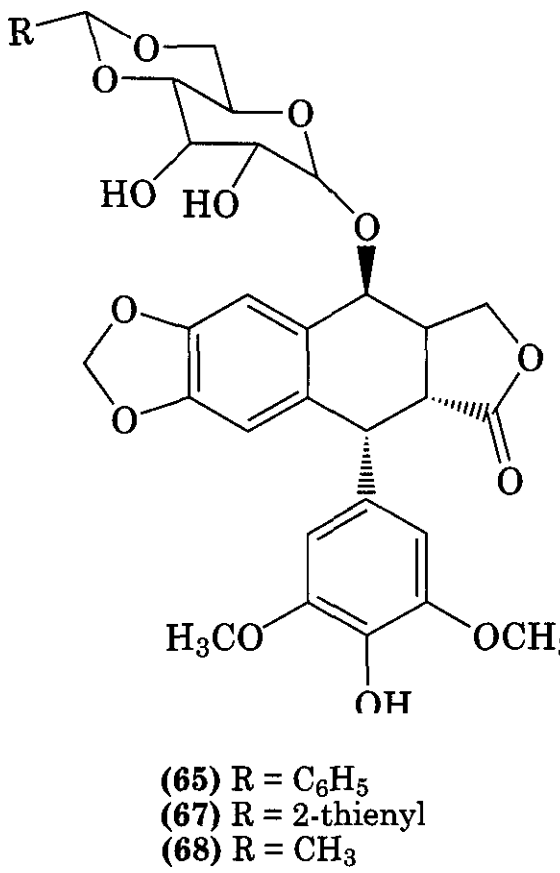
Condensation of the glucosides with a variety of aldehydes was more useful, in that not all the hydroxy groups were blocked. Despite this, water solubility was a problem with the podophyllotoxin derivatives (63). Gastrointestinal absorption was greatly improved, however, as was chemical stability (104), and positive effects were observed in a few cancer patients with the benzylidene derivative (**64**). It was at this point that luck played a hand, backed up by a good deal of determination. A crude podophyllin fraction, which was simpler and cheaper to prepare than pure podophyllin glucoside, was also treated with benzaldehyde to give a mixture of benzylidene derivatives, about 80% of which was compound (64). The crude product was found to be more potent than compound (64) and subsequently to possess a different mode of action from that of the lead compounds: rather than arresting cells in metaphase, cells were prevented from entering mitosis altogether (105). The crude mixture was marketed for cancer treatment as Proresid.



(63) R_1 = H,CH_3 R_2 = **various alkyl, aryl**
(64) R_1 = CH_3 R_2 = C_6H_5

Improved biological assay methods (106) indicated the presence of an unknown, highly active constituent of Proresid. For example, Proresid prolonged the life of mice inoculated with L1210 leukemia cells (93), an effect that was not observed with the known major constituent. In the early 1960s chromatographic and spectroscopic techniques were not as highly developed as they are now and more

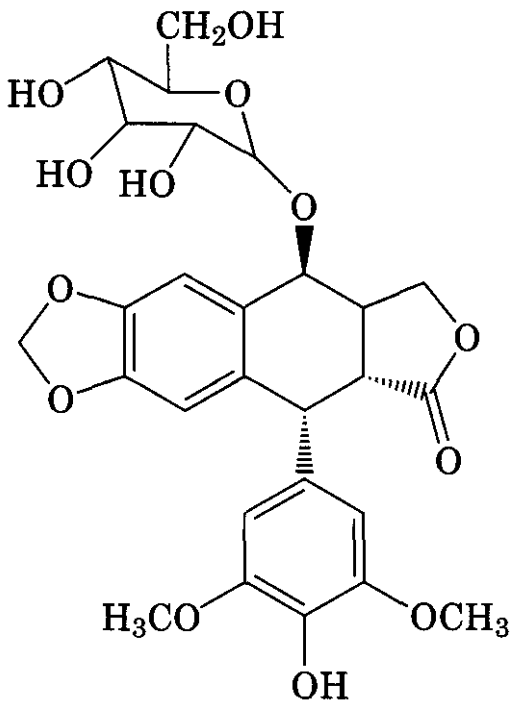
than 2 years' work was required to isolate and identify the unknown component of the mixture, which proved to be the 4′-desmethoxy-1-epi analog (65) of the podophyllotoxin glucoside adduct (92). Present only in very small amounts in the derivatized extract, it was necessary to devise a synthesis from readily available materials. It was fortunate that the desired 1β configuration was readily secured from 1α-hydroxy-4′-desmethylpodophyllotoxin, itself obtained by selective demethylation of podophyllotoxin: the remainder of the synthesis would now be considered fairly routine (107).



(65) R = C_6H_5
(67) R = 2-thienyl
(68) R = CH_3

Given a large supply of the key intermediate (66), it was straightforward to prepare a number of aldehyde derivatives, resulting in analogs with up to a 1000-fold increase in potency (108). The selected adducts were those prepared from thiophen-2-aldehyde, giving teniposide (67), and from acetaldehyde, giving etoposide (68). Both drugs are of value, etoposide in the treatment of small-cell lung cancer and testicular cancer, teniposide in the treatment of lymphomas and leukemias. The thiophene derivative is also of use in the treatment of brain tumors (93).

The natural products, podophyllotoxin and its congeners, are "spindle poisons" that inhibit cell proliferation by binding to tubulin



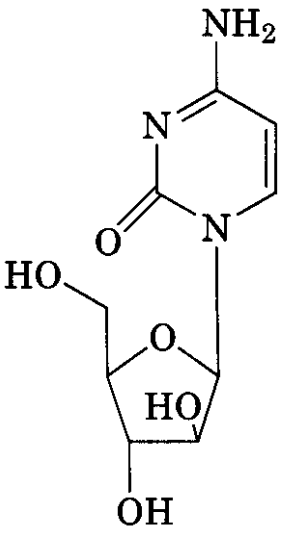
(66)

and preventing formation of microtubules (105). Presumably this effect is sufficient to account for the success of podophyllin in the treatment of condylomata acuminata, although the crude extract contains many other candidates for a contribution to the biological activity. As has been described, a very minor component of the natural mixture, missing the 4' hydroxy group, having the 1β- instead of the la-hydroxy configuration and with this hydroxy group conjugated with β-D-glucose, must be treated with an aldehyde to produce the highly active and most important derivatives. These derivatives do not bind to tubulin, but have been shown to be inhibitors of topoisomerase II, which may account for most of the observed biological effects, including DNA strand breaks, that lead to anticancer activity (109).

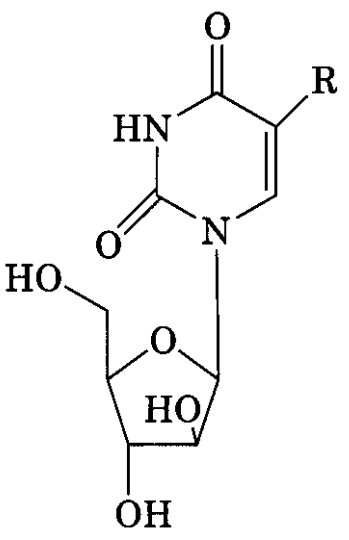
4.6 Marine Sources

Cytosine arabinoside (69), a synthetic analog of the C-nucleosides spongouridine (70) and spongothymidine (71) from the sea sponge Cryptotheca cripta, was the first and, so far, the only marine-derived compound used routinely as an anticancer agent (110). However, a number of chemically diverse natural products from marine sources have been progressed to clinical trials. The three most advanced compounds are in phase II trials; ecteinascidin-743 (72), a tetrahydroisoquino-

line alkaloid isolated from the mangrove ascidian *Ecteinascidia turbinata,* bryostatin-1 (**73**), a macrolide isolated from the bryozoan *Bugula neritina,* and dolastatin-10 (**74**), a linear peptide from the sea mollusk *Dolabella auricularia.* Ecteinascidin-743 (72) was first isolated by Rinehart's group at the University of Illinois (111) and has been licensed to PharmaMar (Zeltia), which plans, together with Ortho Biotech, to launch the compound in late 2002 initially for the treatment of soft tissue sarcoma (112).

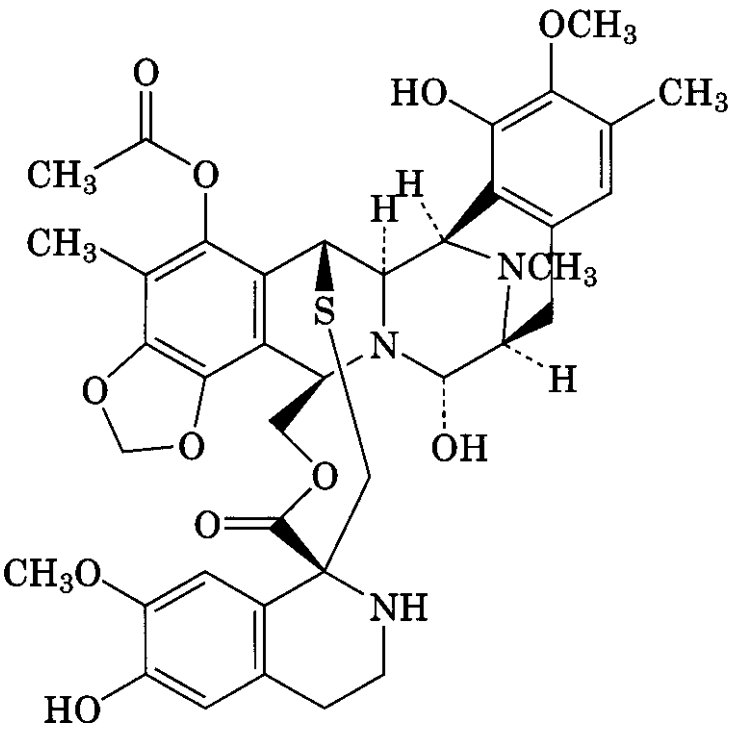


(69) Cytosine arabinoside



(70) Spongouridine: R = H
(71) Spongothymidine: R = CH_3

Yields of marine-derived natural products are invariably low and supply problems have delayed their development as useful pharmaceutical agents. For example, over 3000 kg of the sea squirt *E. turbinata* is required to produce 3 g of ecteinascidin-743, sufficient for just one cycle of treatment (113) and 1000 kg of *B. neritina* yields 1.5 g of bryostatin-1 (114). Fortunately, supplies of ecteinascidin-743 (72) and also bryostatin-1 (73) have been met by aquaculture techniques (115), and more viable synthetic routes are now available for (73) (116) and dolastatin-10 (**74**) (117).

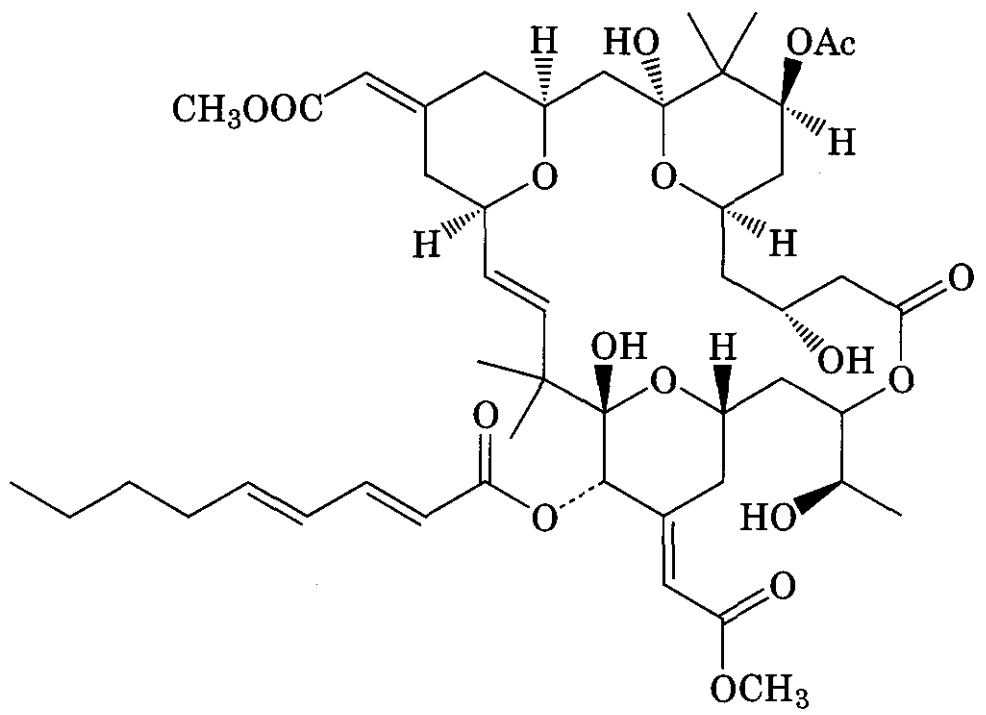


(72) Ecteinascidin 743

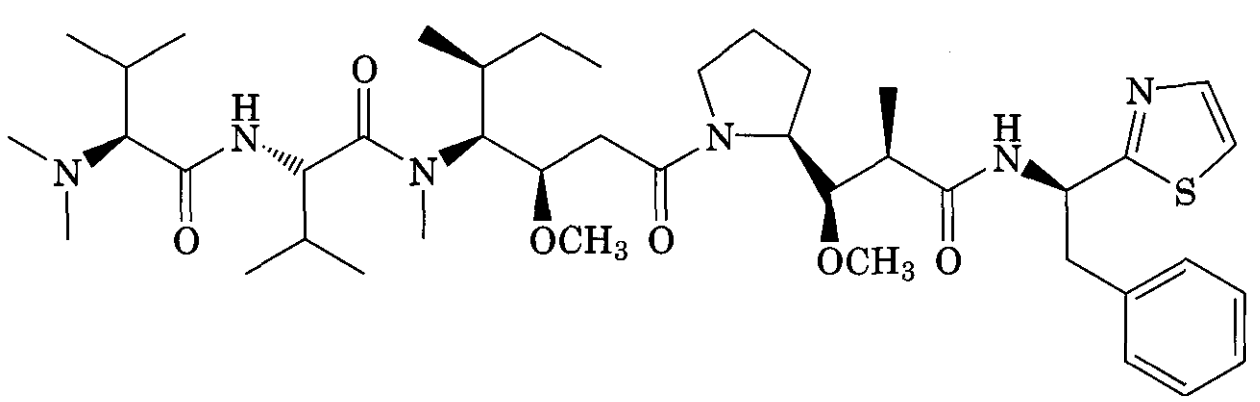
5 ANTIBIOTICS

5.1 β-Lactams

In 1929 Alexander Fleming published the results of his chance finding that a Penicillium mold caused lysis of staphylococcal colonies on an agar plate (118). He also showed that the culture filtrate, named penicillin, possessed activity against important pathogens including Gram-positive bacteria and Gram-negative cocci. However, it was not until 1940 that the true therapeutic efficacy of penicillin was revealed, when Chain et al. (119) successfully tested the material in mice that had been previously infected with a lethal dose of streptococci. Several years later the precise chemical structure of the main active component, benzylpenicillin (**75**), was determined and efforts to synthesize the compound were initiated (120). Benzylpencillin proved to be an elusive target because of the instability of the β-lactam ring: it was unstable under acid conditions and was deactivated by β-lactamase enzymes produced by various Gram-positive and Gram-negative bacteria.



(73) Bryostatin 1

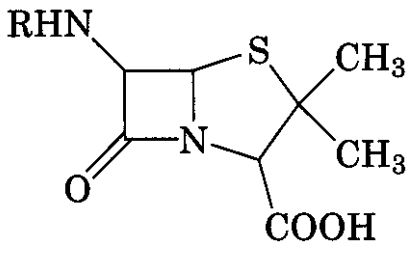


(74) Dolastatin 10

The discovery that the fused β-lactam nucleus, 6-aminopenicillanic acid (6-APA) (**76**), could be obtained from cultures of Penicillium chrysogenum led to the preparation of new, semisynthetic derivatives with improved stability to gastric acid and p-lactamases, and with activity against a wider range of pathogenic organisms (121). Sheehan (122) showed that compound (76) would react readily with acid chlorides to form new penicillin derivatives with novel substituents at the 6-position. Methicillin (**77**), with a sterically demanding 2,6-dimethoxybenzamide side-chain, was the first semisynthetic penicillin to show resistance to staphylococcal p-lactamases, although the compound was still acid labile. Ampicillin (**78**) has an a-aminophenylatamido side-chain and displays good activity against Gram-negative organisms, it is stable to acid and thus can be administered orally, although it is susceptible to degradation by p-lactamases. Amoxycillin (79) differs from ampicillin by the addition of a single hydroxy group, but the compound is better absorbed by the gastrointestinal tract.

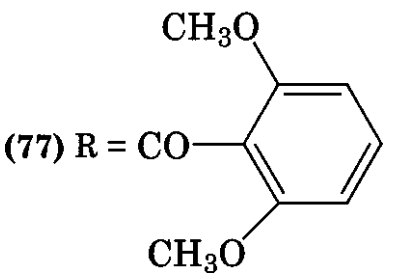
Clavulanic acid (**80**), isolated from *Streptomyces clavuligerus*, is similar in structure to the penicillins, except oxygen replaces sulfur in the five-membered ring (123). Clavulanic acid has weak antibacterial activity, but is a potent inhibitor of p-lactamases (124). A mixture of clavulanic acid and the β-lactamase–sensitive amoxycillin was introduced in 1981 as Augmentin and has proved to be an effective combination to combat β-lactamase–producing bacteria (125). In 2001, 20 years after its launch, Augmentin is the best-selling antibacterial worldwide.

The clinical introduction of the penicillin group of antibiotics prompted an intensive search for novel antibiotic-producing organisms and Selman Waksman demonstrated the value of actinomycetes in this role, discovering the aminoglycoside streptomycin (**81**) from Streptomyces griseus in 1943 (126). Pharma-

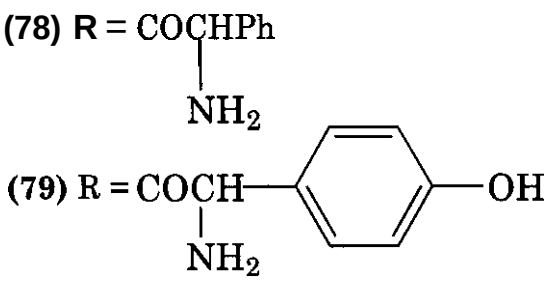


(75) R = $COCH_2Ph$

(76) R = H

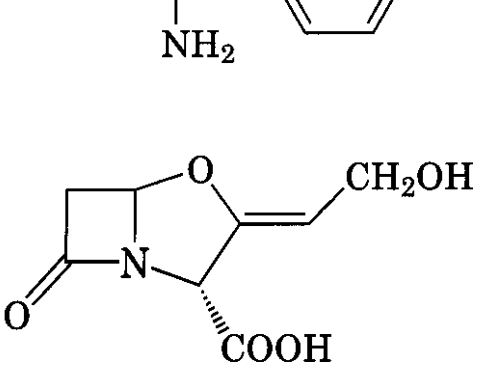


(77) R = CO

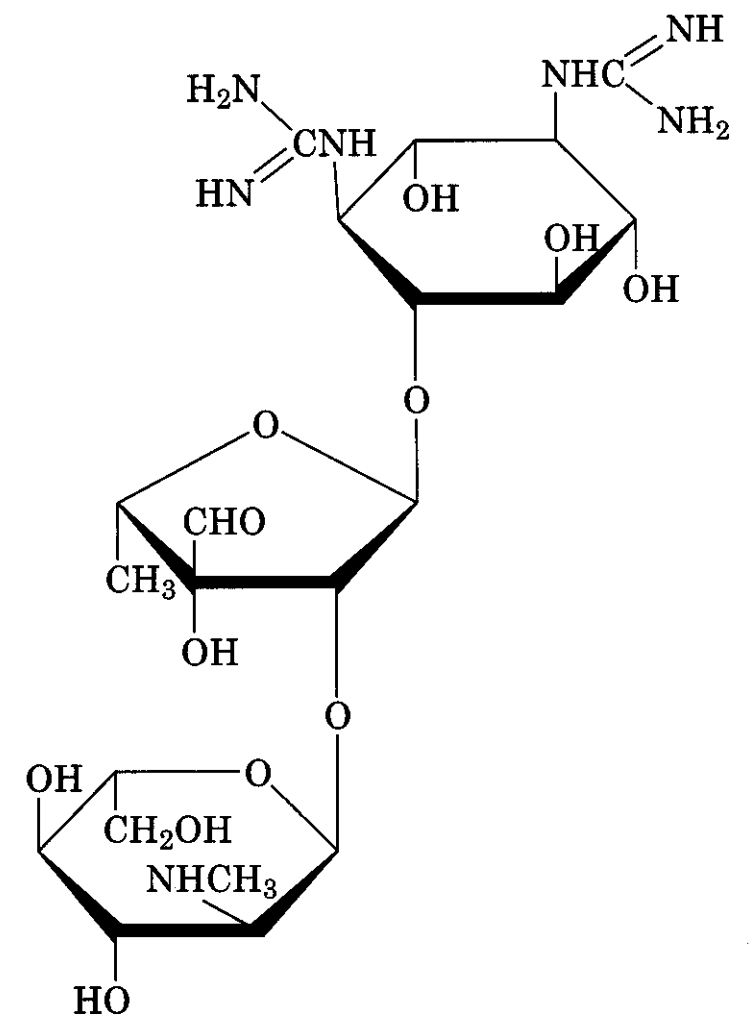


(78) R = COCHPh

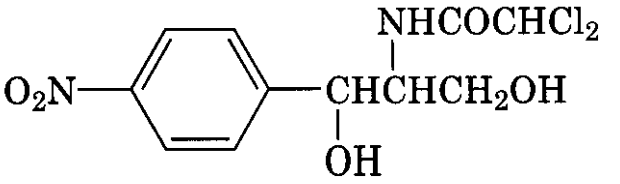
(79) R = COCH



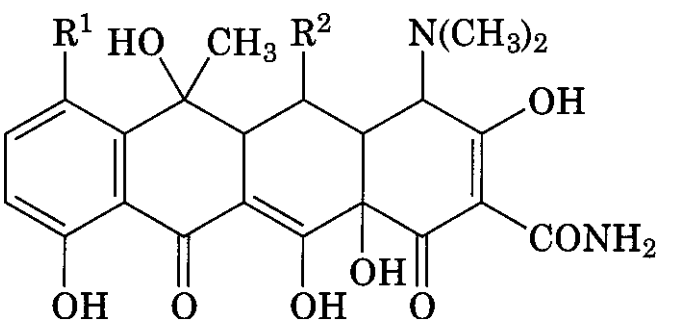
(80)



(81)



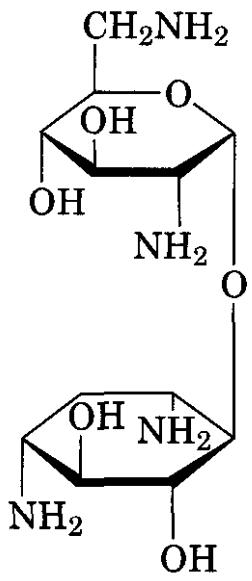
(82)



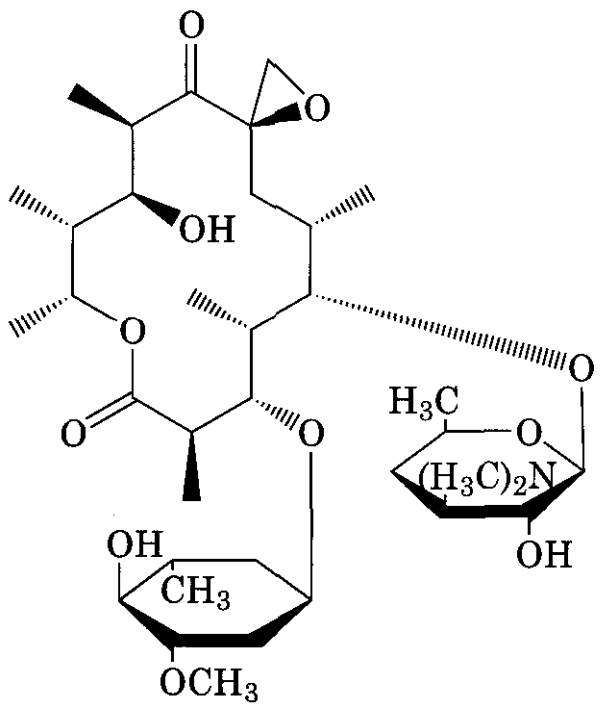
(83) R^1 = -Cl, R^2 = -H
(85) R^1 = -H, R^2 = -OH

ceutical companies also embarked on large programs of screening soil samples for antibiotic-producing microorganisms (127). Chloramphenicol (**82**) was isolated from *Streptomyces venezuelae* in 1948 and other clinically important antibiotics followed: chlortetracycline (**83**), neomycin (**84**), oxytetracyclin (**85**), erythromycin (**86**), oleandomycin (**87**), kanamycin (**88**), and rifamycin (89).

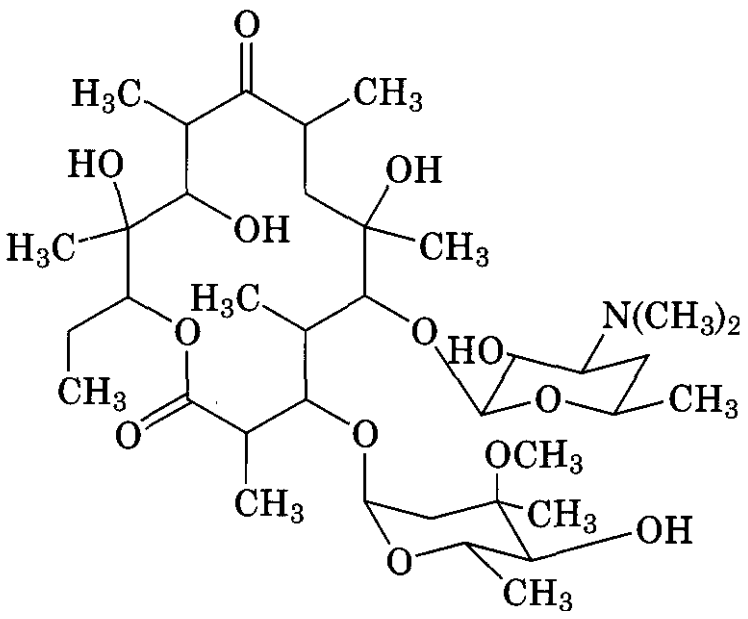
In 1948 Giuseppe Brotzu isolated the fungus ***Cephalosporium acremonium*** from ***a*** water sample collected off the coast of Sardinia. The culture showed significant antimicrobial activity, but Brotzu could not interest the Italian authorities in his discovery. He then turned to a friend in England for help, who arranged for Howard Florey at Oxford to receive a sample of the producing culture. Eventually, an antibacterial substance was isolated and named cephalosporin C (90) (128). The compound, which had a structure similar to that of the penicillins, except it had a dihydrothiazine ring fused to the β-lactam core,



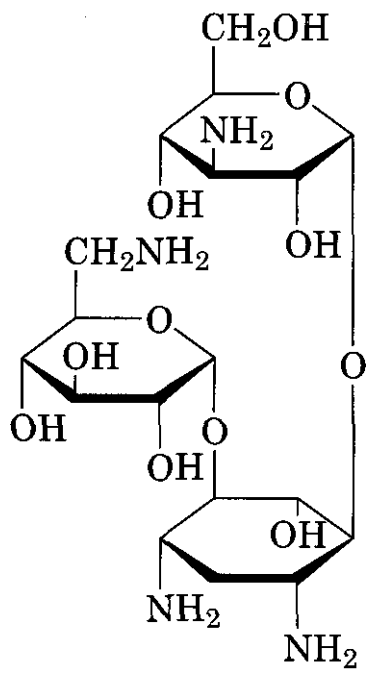
(84)



(87)



(86)

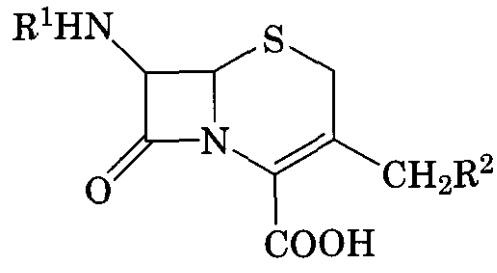


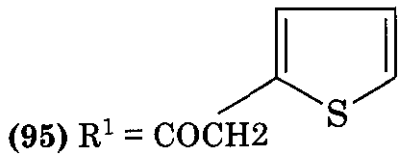
(88)

showed good resistance to β-lactamases and was less toxic than benzylpenicillin. However, plans to market the compound were terminated with the introduction of methicillin (see above).

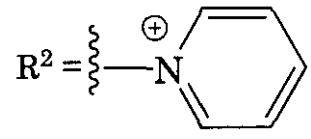
The discovery that the basic structural building block of cephalosporin C, that is, 7-aminocephalosporanic acid (7-ACA) (**91**), could be synthesized led to the preparation of numerous cephalosporin derivatives in a similar way to the synthesis of penicillins from 6-aminopenicillanic acid (129, 130). Modification of the substituent at the 7-position, while retaining the 3-acetoxymethyl group, gave cephalothin (**92**), cephacetrile (**93**), and cephapirin (**94**), so-called first-generation cephalosporins with good activity against Gram-positive bacteria, although the acetyl ester was susceptible to degradation by esterases and thus limited the duration of action. Replacement of the acetoxy group by other substituents rendered the products less prone to esterase attack. For example, the pyridinium derivative, cephaloridine (**95**), has a longer duration of action than that of cephalothin.

The first orally active cephalosporin was cephaloglycin (**96**), which possessed a phenylglycine substituent in the C-7 side-chain, although the labile 3-acetoxymethyl group was retained. Replacing the acetoxy group with a proton or chlorine, for example, cephalexin

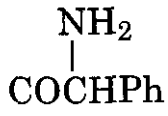




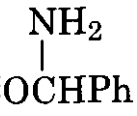
(95) R^1 = COCH2



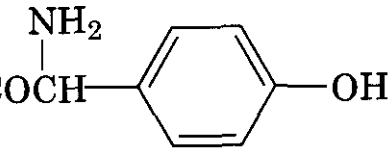
R^2 =

(96) 

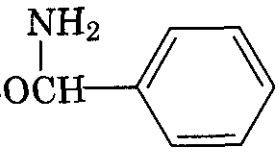
R^2 = $OCOCH_3$

(97) R^1 = 

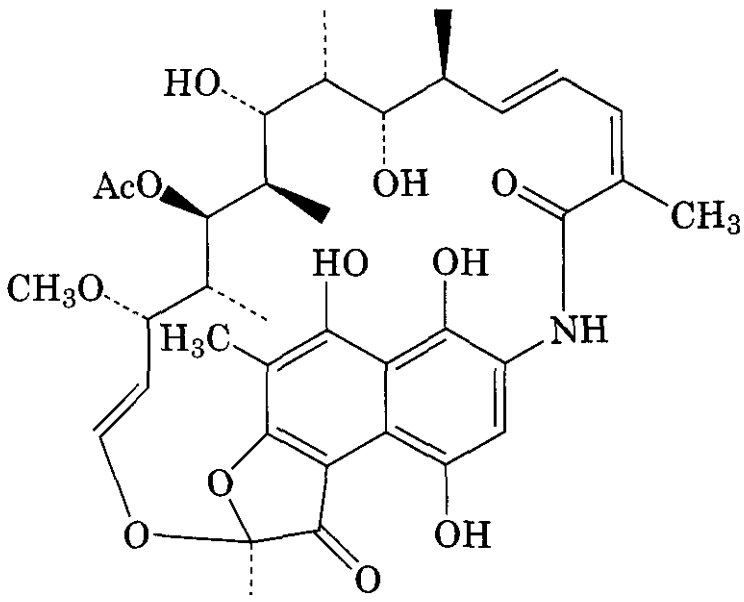
R^2 = H

(98) R^1 = 

R^2 = H

(99) R^1 = 

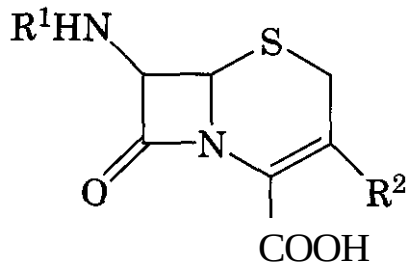
R^2 = H

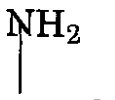


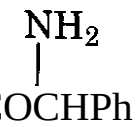
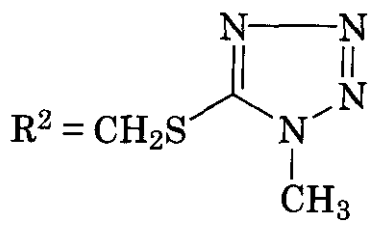
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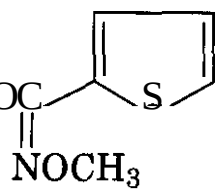
(**97**), cefadroxil (**98**), cephradine (**99**), and cefaclor (**100**), extended the duration of action of these orally active products. Cefaclor has been classified as a second-generation cephalosporin because it has a wider spectrum of activity, which includes Gram-negative bacteria such as *Haemophilus influenzae.* Cephamandole **(101)** and cefuroxime (**102**) are parenterally administered **cephalosporins** with similar activities against clinically important Gram-negative bacteria and are also resistant to many types of β-lactamases.

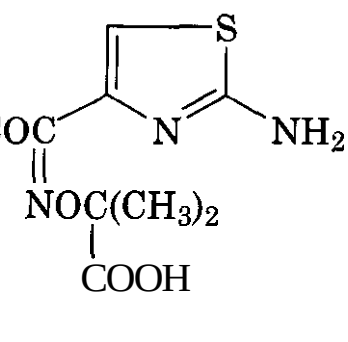
The newer third-generation cephalosporins, including ceftazidime (**103**), ceftizoxime (**104**), and **ceftriaxone** (**105**), which all contain an α-aminothiazolyl group in the C-7 sidechain, have been developed for treating specific pathogens such as Pseudomonas *aeruginosa.* Thienamycin (**106**), isolated from Streptomyces *cattleya* in **1976,** represented a new class of β-lactam antibiotics produced by bacteria where the sulfur of the penicillin nucleus was replaced by a methylene group **(131). An** N-formylimidoyl derivative, **imipenem** (**107**), was the first example from this

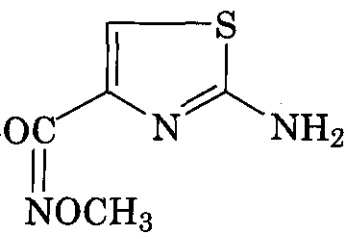


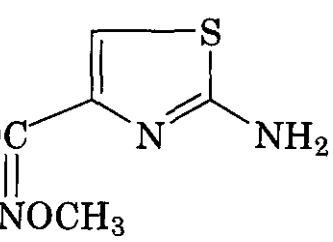
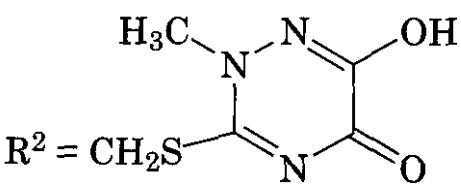
(100) R^1 =  R^2 = Cl

(101) R^1 =  R^2 = 

(102) R^1 =  $R^2 = CH_2OCONH_2$

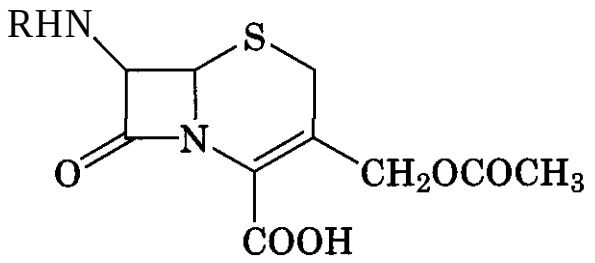
(103) R^1 =  R^2 =

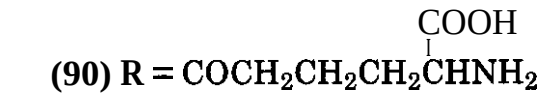
(104) R^1 =  $R^2 = H$

(105) R^1 =  R^2 = 

new class of carbapenem antibiotics to become available for clinical use (132). Imipenem has a very broad spectrum of activity against most Gram-positive and Gram-negative aerobic and anaerobic bacteria.

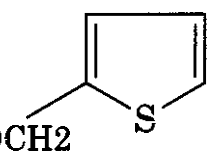
Screening bacteria such as Pseudomonas acidophila and Chromobacterium uiolacium for production of β-lactam antibiotics resulted in the discovery of naturally occurring monobactams, which had moderate antimicrobial activity (133–135). Side-chain variations, as developed for the penicillins and cephalosporins, led to compounds with improved activity against both Gram-positive and Gram-negative bacteria. A derivative containing the a-aminothiazoyl group, a side-chain component common to the third-generation cephalosporins (see above), showed specific activity against Gram-negative aerobic bacteria, including Pseudomonas spp., and was stable to most types of β-lactamases. The compound aztreonam (108) became the first





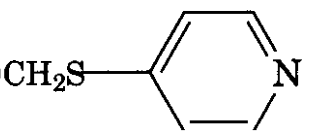
(90) R = $COCH_2CH_2CH_2CHNH_2$ (COOH)

(91) R = H

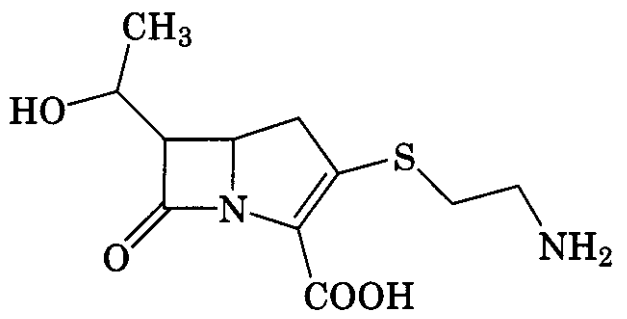


(92) R = COCH2

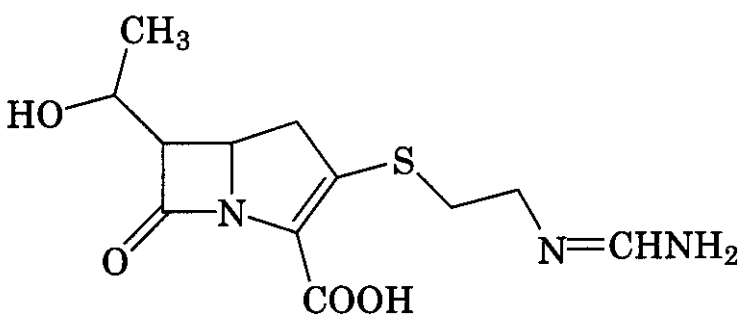
(93) R = $COCH_2CN$



(94) R = $COCH_2S$



(106)

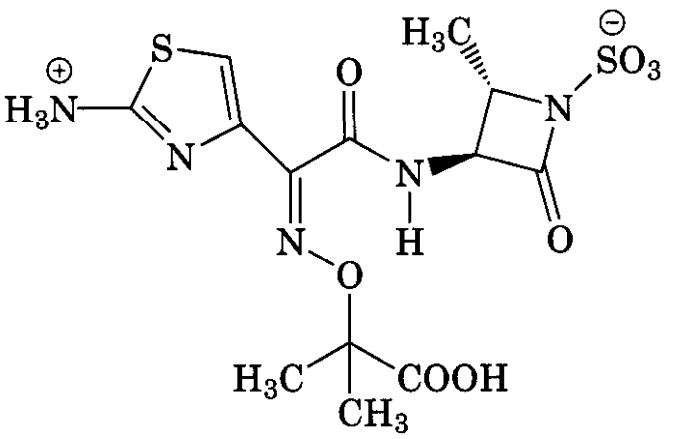


(107)

commercially available monobactam and showed a mode of action similar to that of the other β-lactam antibiotics by blocking bacterial cell wall synthesis (136).

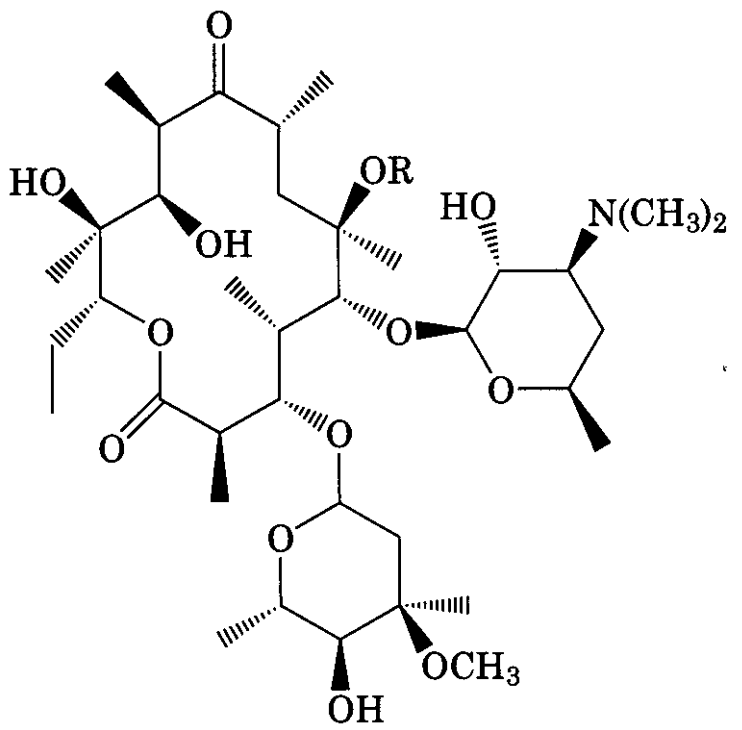
5.2 Erythromycin Macrolides

Erythromycin (109) was isolated, in 1952, from a strain of Saccharopolyspora erythraea



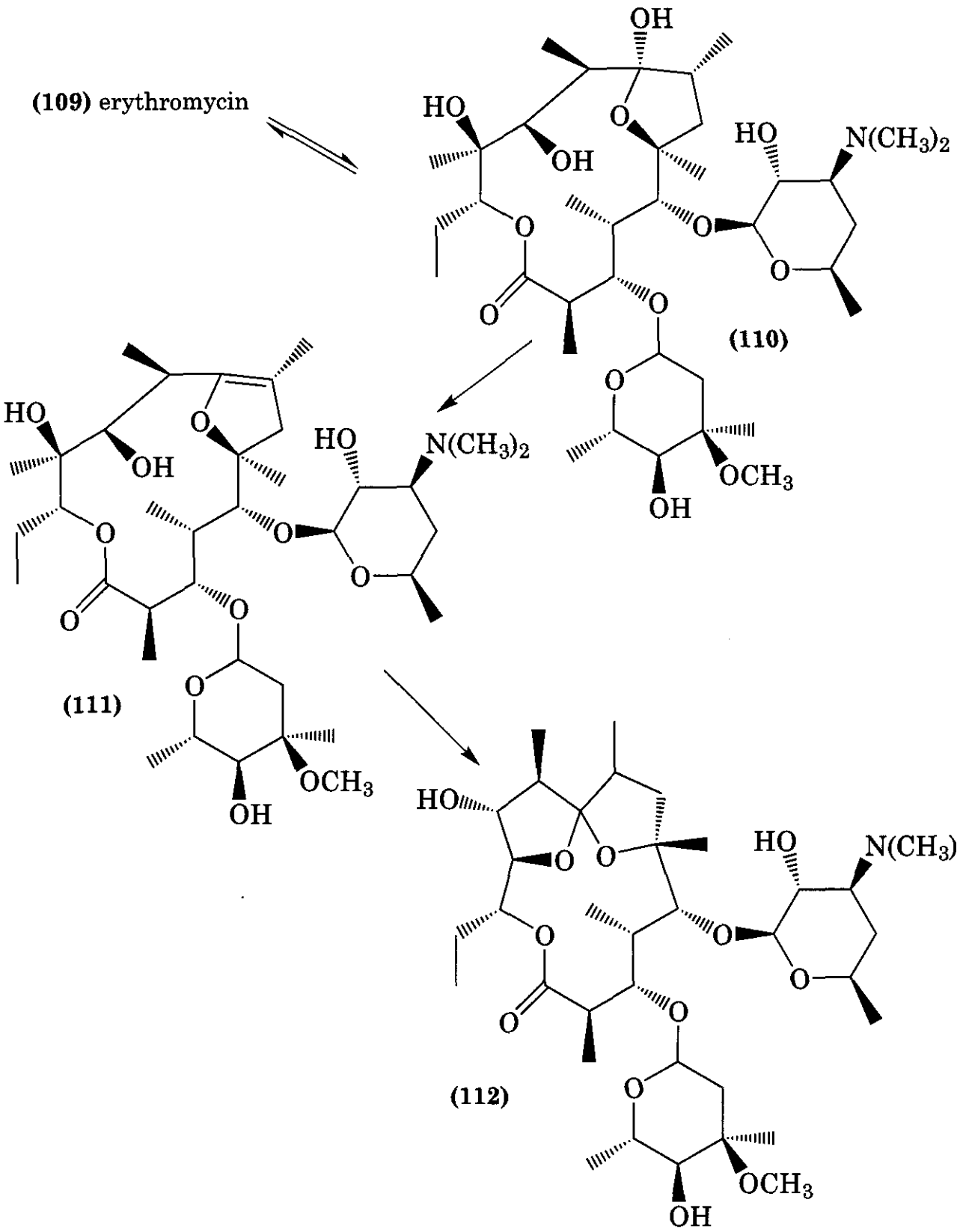
(108)

(formerly Streptomyces erythreus). As a broad-spectrum antibiotic erythromycin has proved invaluable for the treatment of bacterial infections in patients with β-lactam hypersensitivity and is also the drug of choice in the treatment of infections caused by species of Legionella, *Mycoplasma,* Campylobacter, and Bordetella (137).



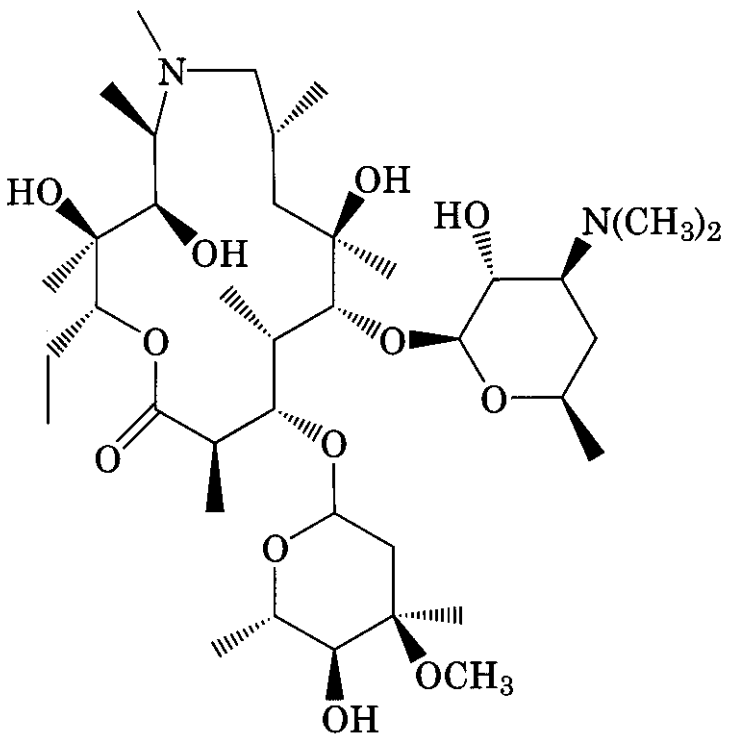
(109) Erythromycin A, R = H
(114) Clarithromycin, R = CH_3

Although safe and effective, erythromycin is not a perfect antibacterial. The presence of hydroxy groups suitably disposed with respect to the keto function at C-9 leads to the formation of a tautomeric mixture of hemiketals (138). The 6,9-hemiketal (110) may be dehydrated in stomach acid to give the inactive Δ_8 analog (111), which may undergo further ring closure to give the 9,12-tetrahydrofuran (112) that is also inactive (139). The A, derivative



(111) may be responsible for some gastrointestinal disturbance (140). To avoid these problems by increasing the stability to acid, the 2'-stearate, estolate, and ethylsuccinate esters have been prepared (141), but even when the tablets are enteric-coated the bioavailability is erratic and relatively frequent dosing is required (137).

An understanding of the acid-catalyzed decomposition of erythromycin has led to a variety of semisynthetic derivatives with improved oral bioavailability (142). Reductive amination of the 9-keto function gives erythromycylamine, which reads with (2-methoxyethoxy)acetaldehyde (143) to give dithromycin. Beckmann rearrangement of the 9-oxime followed by reduction and methylation (144) gives azithromycin (113), which shows good activity against Gram-negative bacteria, including *Haemophilus influenzae.* An alternative for prevention of cyclization between the 9-keto and 6-hydroxy is to mask the 6-hydroxy group. If the 6-hydroxy is methylated (145), the result is clarithromycin (114), which like (113), has an improved pharmacokinetic profile compared with that of the parent molecule.

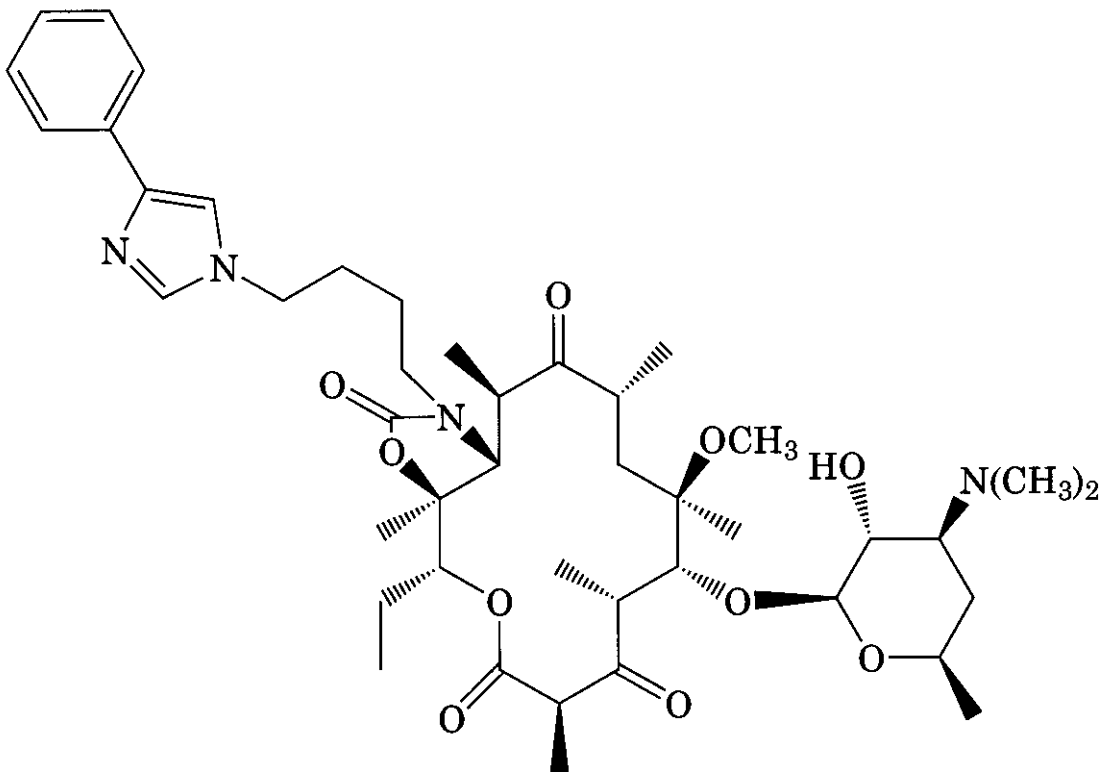


(113) Azithromycin

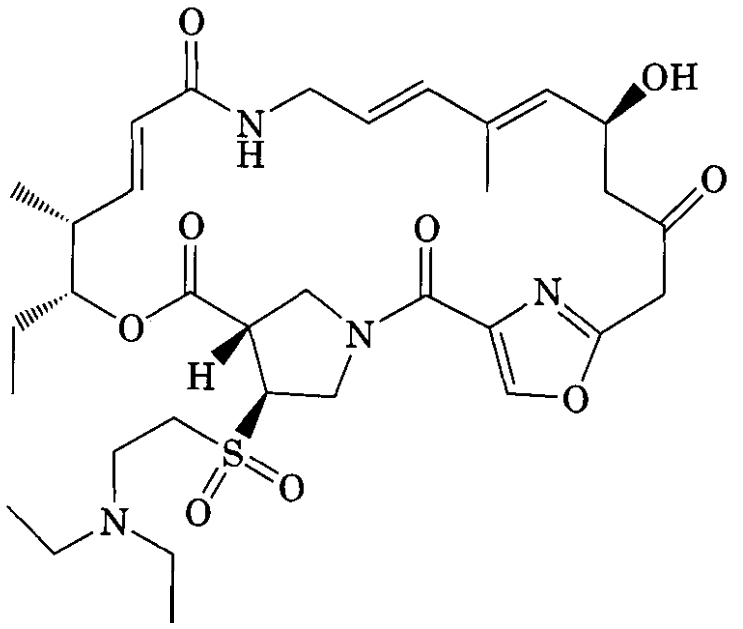
Both azithromycin and clarithromycin have been used for various bacterial infections for a number of years. Within the last decade, resistance has emerged to a range of antibacterials, including the macrolides, arising from methylation of an adenine in the 23S ribosomal RNA target site, which prevents binding (146). The invention of the ketolides [e.g., telithromycin (**115**)] overcomes MLS_B resistance by removing the L-cladinose moiety at position 3: the exposed hydroxyl is also oxidised to a ketone (147). The loss of potency that would ensue is compensated by two further modifications, which improve binding, formation of a carbamate at positions 11/12, and extension with a heterocycle-substituted side-chain. In ABT 773 a similar side-chain is placed at position 6, with comparable results (147).

5.3 Streptogramins

The streptogramins are produced by *Streptomyces* species and have been classified into two groups: Group A are polyunsaturated macrocyclic lactones and Group B are cyclic hexadepsipeptides. Both groups bind bacterial ribosomes and inhibit protein synthesis at the elongation step and they act synergistically against many Gram-positive microorganisms. However, the naturally occurring streptogramins are poorly soluble in water and this, until recently, has limited their use to treat bacterial infections. New, water-soluble derivatives have been developed and the semisynthetic dalfopristin (116) and quinupristin (117) mixture (Synercid) has been approved for the treatment of Gram-positive infections, including multidrug-resistant strains of *Enterococcus faecium, Staphylococcus aureus,* and *S. pneumoniae* (148).



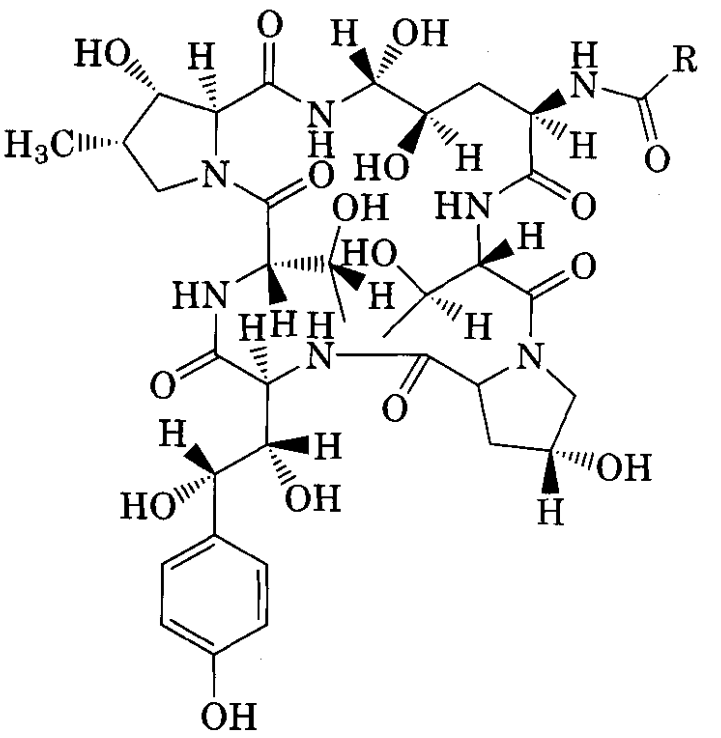
(115) Telithromycin



(116) Dalfopristin

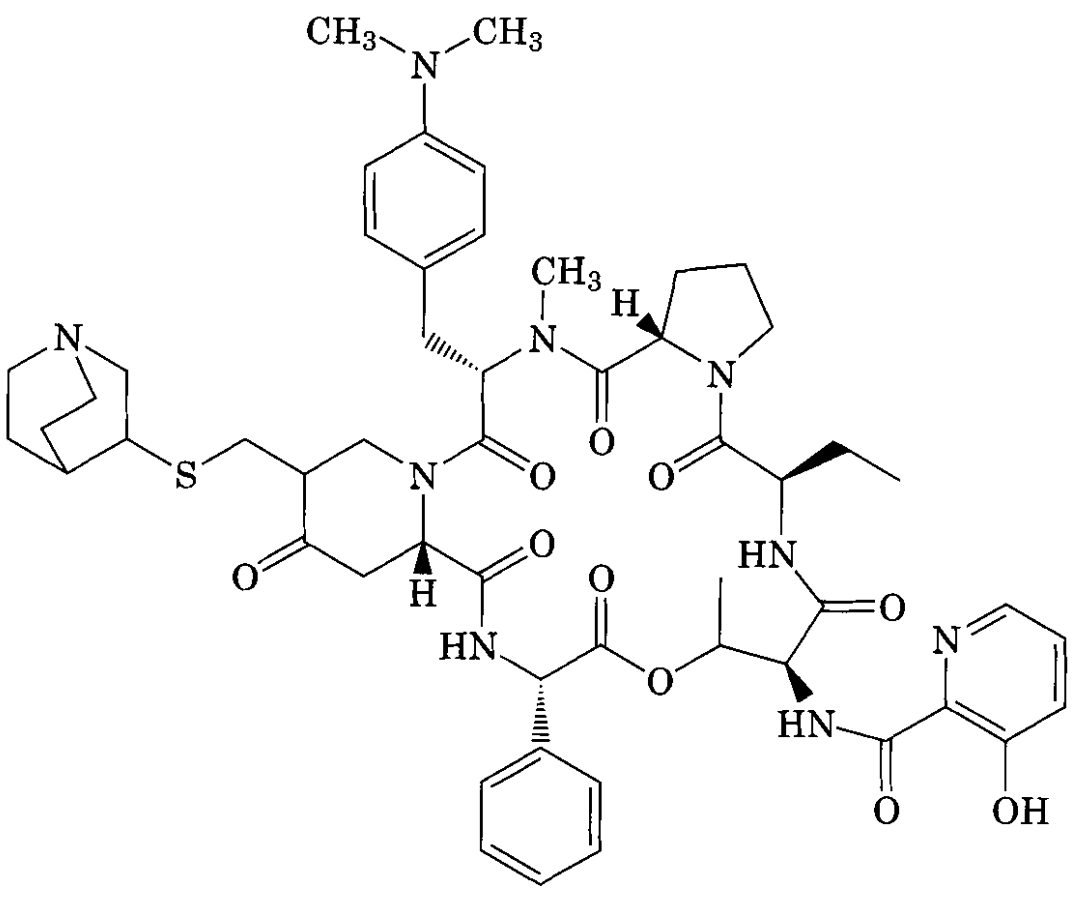
5.4 Echinocandins

The fungal metabolite echinocandin B (118)is one of the lipopeptides, in which a cyclic hexapeptide is combined with a long-chain fatty acid. Echinocandin B inhibits β-1,3-glucan synthesis and as a result has anti-Candida and anti-Pneumocystis *carinii* activity (149). As a group, the echinocandins are not orally bioavailable, are haemolytic, and are not very water soluble (150), despite the hydrogen-bonding ability of the polyhydroxylated hexapeptide.



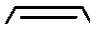
(118) echinocandin **B, R** = linoleyl

Synthesis of the cyclic hexapeptide is unattractive for the purpose of securing analogs with improved biological activity because of the unusual nature of the amino acids used



(117) Quinupristin

and the complex stereochemistry generated by the high degree of hydroxylation. However, echinocandin B can be produced efficiently by fermentation of a culture of *Aspergillus nidulans* and then deacylated by fermentation with *Actinoplanes utahensis* (151). The free amino group thus exposed can be derivatized with a number of active esters. Synthesis of the amide from 4-octylbenzoic acid gives cilofungin (**119**), which has specifically high potency against *Candida albicans* and some other Candida species (151).



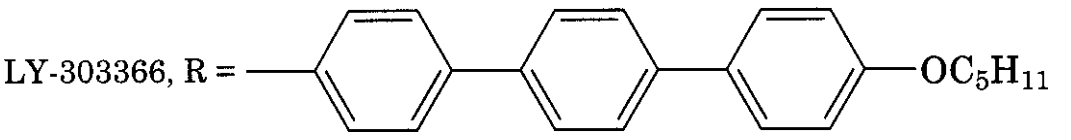
(119) cilofungin, **R** –

For systemic use cilofungin had to be given intravenously and unfortunately ran into problems associated with the cosolvent (PEG) (152). **A** better derivative, LY-303366 (**120**) is now in clinical trial and has the major advantage of oral bioavailability (153). Many other antifungal peptides are under investigation (152). The member of this series that is furthest advanced is caspofungin (MK-991, L-743,872) (**121**), following its approval by the FDA, early in 2001, for the treatment of aspergillosis. The two analogs, LY-303366 and caspofungin, have been compared against clinical fungal isolates *in vitro* (154) and the latter has been evaluated in immunosuppressed mice (155).

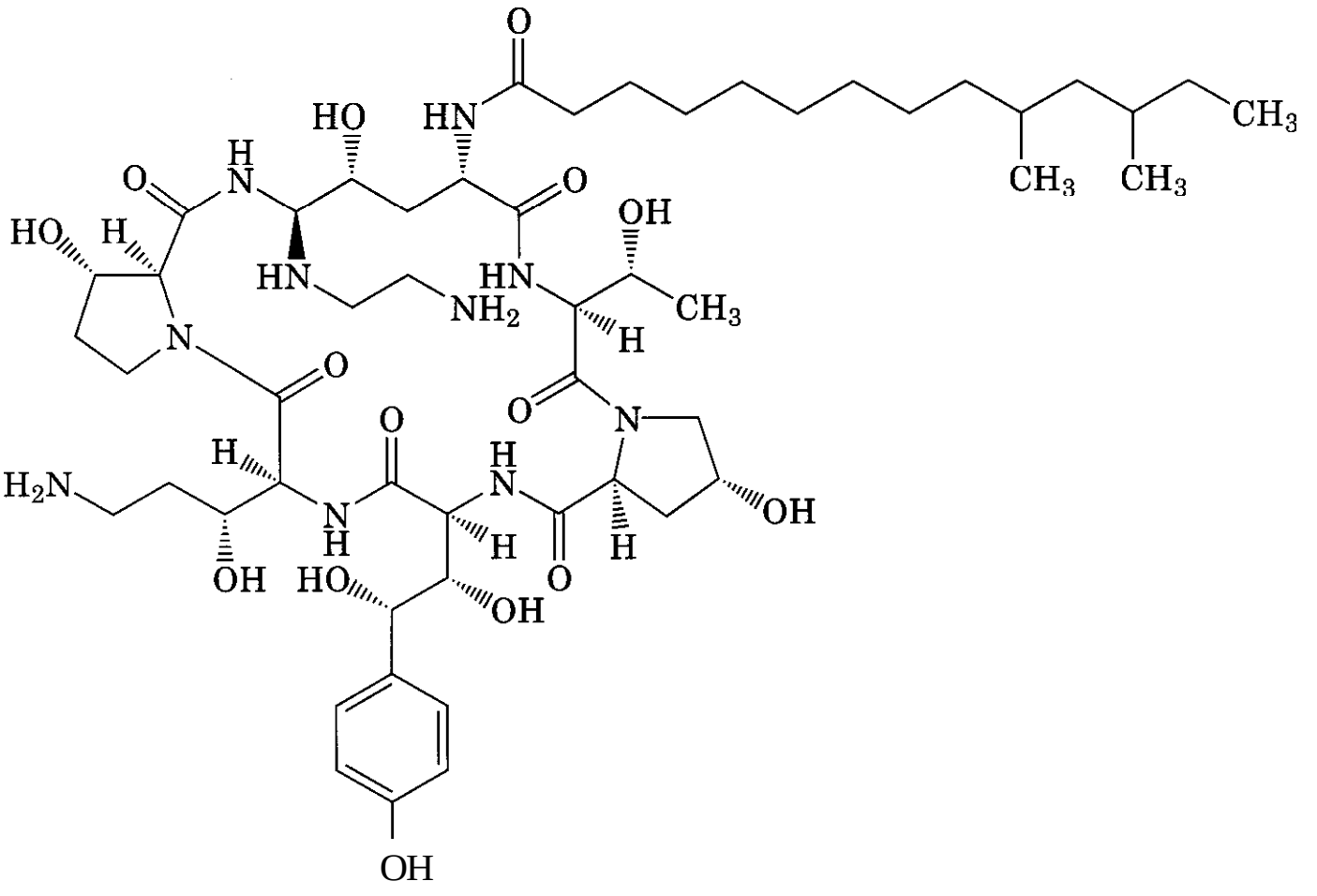
6 CARDIOVASCULAR DRUGS

6.1 Lovastatin, Simvastatin, and Pravastatin

One of the most significant natural product discoveries in the last 25 years has been a fungal secondary metabolite called lovastatin (122). Heralded as a major breakthrough in

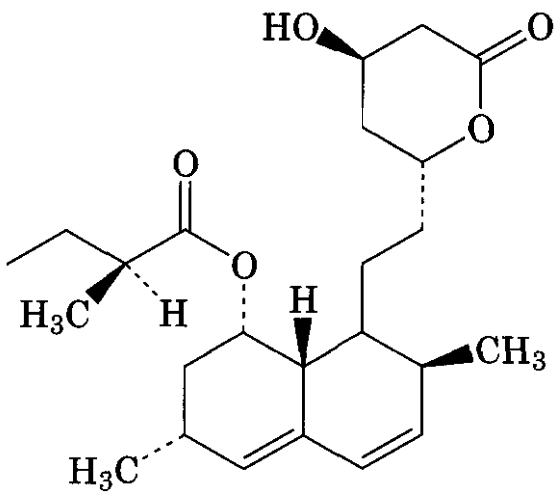


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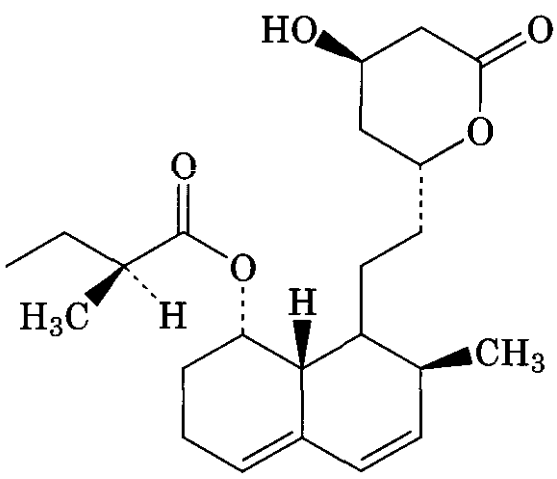
(121) caspofungin

the treatment of coronary heart disease (156), lovastatin was introduced onto the market by Merck in 1987 for the treatment of hypercholesterolemia, a condition marked by elevated levels of cholesterol in the blood.



(122)

Lovastatin works by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key rate-limitingenzyme in the cholesterol biosynthetic pathway. However, the first specific inhibitors of this enzyme were discovered several years earlier by Endo et al. at Sankyo (157). The compounds, which are structurally related to lovastatin, were isolated from Penicillium *citrinum* and shown to block cholesterol synthesis in rats and lower cholesterol levels in the blood. Development of the most active compound, designated ML-236B (**123**), is believed to have been curtailed because of toxicity problems (158).

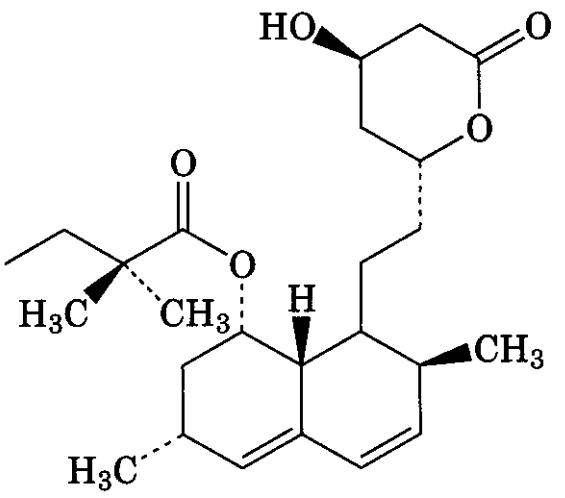


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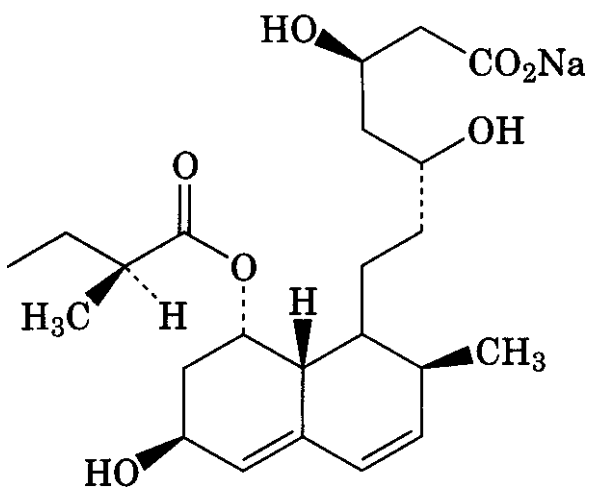
Brown et al. at Beechams also reported the isolation of (**123**), but as a metabolite from Penicillium brevicompactum (159). The group, naming the compound compactin, reported its antifungal activity but failed to reveal its mode of action as an inhibitor of HMG-CoA reductase. The search for naturally occurring inhibitors of HMG-CoA reductase gained pace and after spending several years developing appropriate screens, Merck found during only the second week of testing a culture of Aspergillus terreus that displayed interesting inhibitory activity (160). In February 1979 the active component, lovastatin (mevinolin), was isolated and characterized (161), and in November the following year Merck was granted patent protection in the United States. Although lovastatin proved to be identical to monocolin K, a metabolite isolated earlier from Monasus ruber (162), the chemical structure of the latter compound had not been reported, whereas Merck filed for patent protection giving complete structural details for lovastatin.

The discovery of compactin and lovastatin prompted efforts to develop derivatives with improved biological properties (163, 164). Modification of the methylbutyryl side chain of lovastatin led to a series of new ester derivatives with varying potency and, in particular, introduction of an additional methyl group a to the carbonyl gave a compound with 2.5 times the intrinsic enzyme activity of lovastatin (165). The new derivative, named simvastatin (**124**), was the second HMG-CoA reductase inhibitor to be marketed by Merck. Both lovastatin and simvastatin are prodrugs and are hydrolyzed to their active open-chain dihydroxy acid forms in the liver (166). A third compound, pravastatin (**125**), launched by Sankyo and Squibb in 1989, is the open hydroxyacid form of compactin that was first identified as a urinary metabolite in dogs. Pravastatin is produced by microbial biotransformation of compactin.

The HMG-CoA reductase inhibitors described above bind to two active sites on the enzyme: the hydroxymethylglutaryl binding domain and an adjacent hydrophobic pocket to which the decalin moiety binds (167). The recognition that the ring-opened hydroxy acids resemble mevalonic acid and that the decalin moiety could be replaced by 4-fluorophenyl–substituted heterocycles led to the launch of several new products including fluvastatin

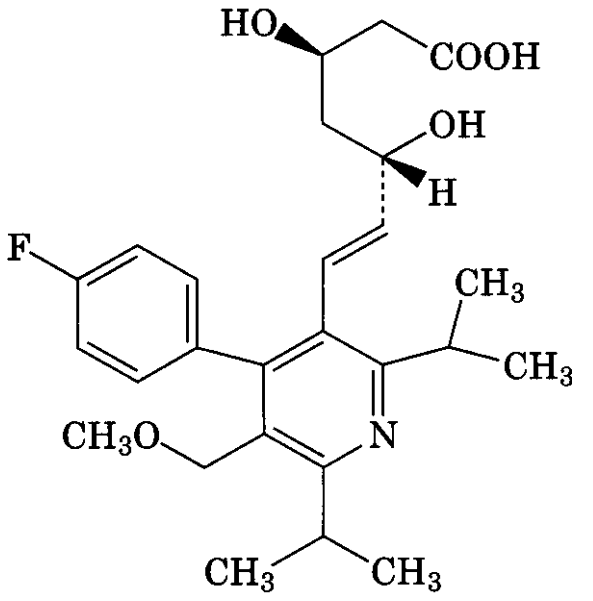


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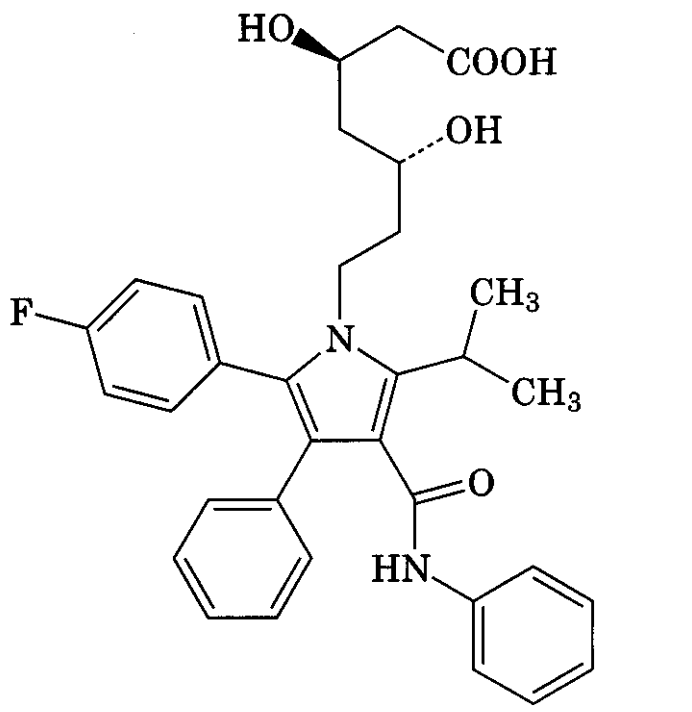


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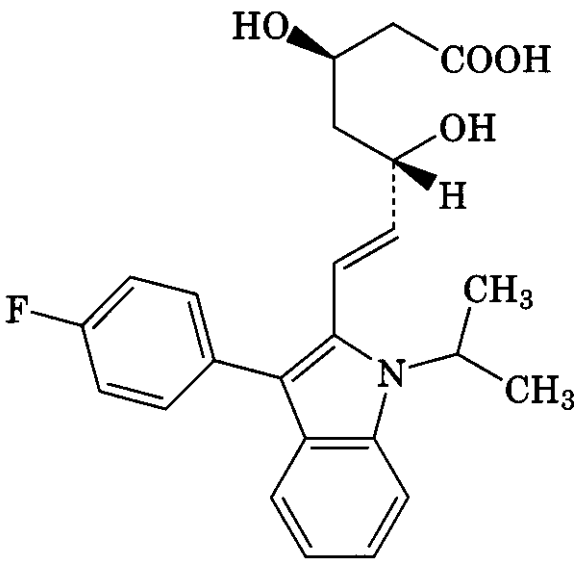
(126), the ill-fated cerivastatin (127), and the so-called turbostatin atorvastatin (128). Although cerivastatin was withdrawn from the market in 2001 because of fatal adverse drug–drug interactions, the "statins" remain one of the fastest growing segments of the pharmaceutical industry. The latest member of this group of cholesterol-lowering drugs, Astra-



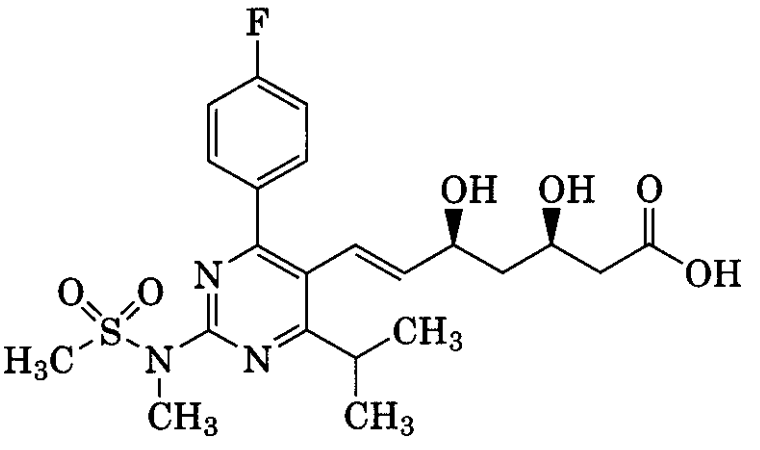
(127) Cerivastatin



(128) Atorvastatin



(126) Fluvastatin



(129) Rosuvastatin

Zeneca's rosuvastatin (**129**), is due to be launched in 2002 and is forecast to achieve sales of US $2.8 billion by 2005 (168).

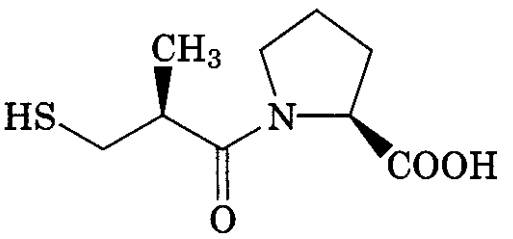
6.2 Teprotide and Captopril

While studying the physiological effects of snake poisoning, Ferreira (169) discovered that specific components in the venom of the pit viper Bothrops *jararaca* inhibited degradation of the peptide bradykinin and potentiated its hypotensive action. The "potentiating factors" proved to be a family of peptides that worked by inhibiting the dipeptidyl carboxypeptidase, angiotensin-converting enzyme (ACE) (170,171). In addition to catalyzing the degradation of bradykinin, ACE also catalyzes the conversion of human prohormone, angiotensin 1, to the potent vasoconstrictor octapeptide, angiotensin II. However, the significance of ACE in the pathogenesis of hypertension was not fully appreciated until the 1970s after Ondetti et al. (172) had first isolated and then synthesized the naturally occurring nonapeptide, teprotide (130). The compound proved to be a specific potent inhibitor of ACE and showed excellent antihypertensive properties in clinical trials, although its use was limited by the lack of oral activity.

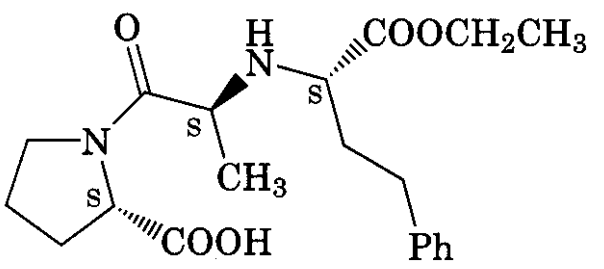
Pyr–Trp–Pro–Arg–Pro–Gln–Ile–Pro–Pro

(130)

The discovery of teprotide led to a search for new, specific, orally active ACE inhibitors. Ondetti et al. (172) proposed a hypothetical model of the active site of ACE, based on analogy with pancreatic carboxypeptidase A, and used it to predict and design compounds that would occupy the carboxy-terminal binding site of the enzyme. Carboxyalkanoyl and mercaptoalkanoyl derivatives of proline were found to act as potent, specific inhibitors of ACE and 2-D-methyl-3-mercaptopropanoyl-L-proline (**131**) (captopril) was developed and launched in 1981 as an orally active treatment for patients with severe or advanced hypertension. Captopril, modeled on the biologically active peptides found in the venom of the pit viper, made an important contribution to the understanding of hypertension and paved the way for other ACE inhibitors, such as enalapril (**132**) and lisinopril, which have had a major impact on the treatment of cardiovascular disease (173).



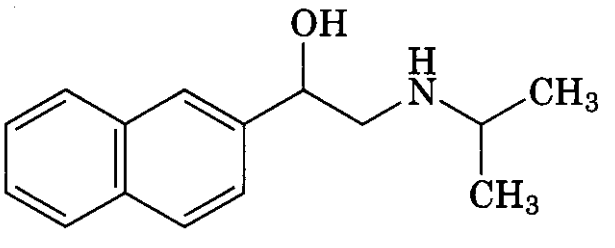
(131)



(132)

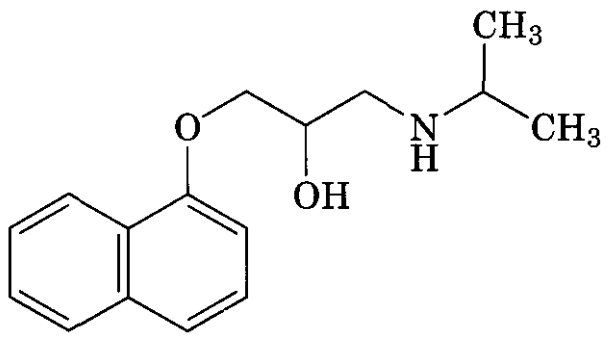
6.3 Adrenaline, Propranolol, and Atenolol

The true clinical potential of β-adrenoceptor blocking agents for treating angina, atrial fibrillation, and tachycardias was first recognized by James Black and colleagues at ICI (174). Black noted a report from Neil Moran of Emory University in 1958, showing that dichloroisoprenaline antagonized the effects of adrenaline on heart rate and muscle tension. The first effective β-adrenoceptor blocker, pronethalol (**133**), was synthesized 2 years later by the ICI group and marketed for limited use in 1963. Toxicity problems soon led pronethalol to be replaced by the 1-naphthyl analog, propranolol (**134**), which became the first β-adrenoceptor antagonist approved for general use, being more potent and yet devoid of the partial agonist or intrinsic sympathomimetic activity shown by many other analogs. Compounds with improved selectivity for the β-adrenoceptor of cardiac muscle (β-1-adreno-

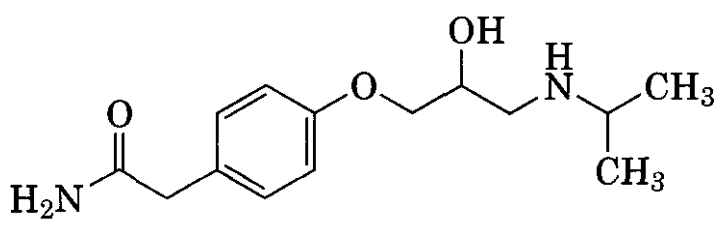


(133) Pronethalol

ceptor blockers) were to follow, including atenolol (**135**), which became the most frequently prescribed β-blocker and one of the best-selling drugs of the time.



(134) Propranolol

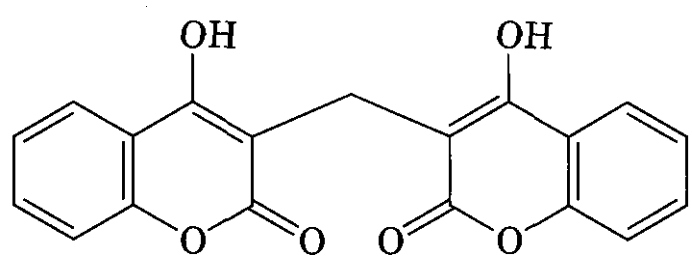


(135) Atenolol

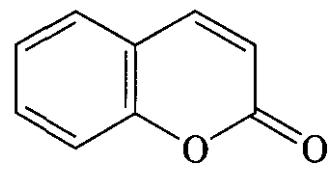
6.4 Dicoumarol and Warfarin

Sweet clover has a long history of medicinal use, often as an antiflammatory or analgesic preparation in the form of ointments and poultices. Melilotus officinalis (yellow sweet clover, or ribbed melilot) was reputed to have been a favorite herbal treatment used by King Henry VIII of England and the plant is still referred to as King's Clover in some publications (175).

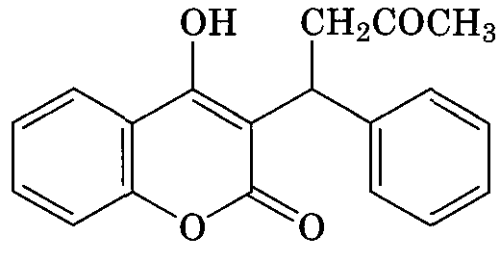
The plant flourishes in poor soil and was cultivated extensively in Europe for cattle fodder and for soil improvement. In the early 1920s M. officinalis was planted on the prairies of North Dakota and Alberta, Canada, but with disastrous consequences. Soon cattle and sheep throughout these regions began literally bleeding to death. The mysterious hemorrhagic disease was traced to clover fodder that had not been stored properly and had become "spoiled," or moldy. However, the insolubility of the anticoagulant component and the difficulty of assaying extracts for biological activity made the task of isolating the active principal component intractable (176). It took almost 20 years before the compound was identified as 3,3′-methylenebis(4-hydroxycoumarin) (**136**), an oxidative degradation metabolite of coumarin (**137**), itself a common component of Melilotus sp. Soon after the compound had been identified, trials were initiated that confirmed the oral anticoagulant activity in humans and in 1942 it was marketed under the name dicoumarol (177). The compound had a slow, erratic onset of action and efforts were initiated to prepare synthetic analogs that acted faster and had longer duration of action. A 4-hydroxycoumarin residue, substituted at the 3-position, proved essential for biological activity and in 1948, after synthesizing over 150 compounds, a 4-hydroxycoumarin derivative that was longer acting and more potent than dicoumarol was selected not for clinical use, but as a rodenticide for development by the Wisconsin Alumni Research Foundation! The compound (**138**),



(136)



(137)



(138)

named warfarin (an acronym derived from the name of the institute coupled with "arin" from coumarin), became a household name for rat poison. Concern over the use of oral anticoagulants and the inherent risk of hemorrhage inhibited the development of warfarin as a therapeutic agent. However, in 1951, a

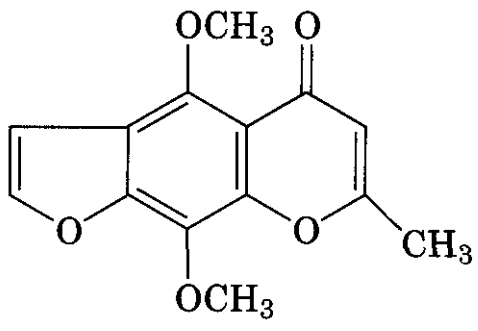
U.S. Army cadet unsuccessfully attempted to commit suicide by taking massive doses of the compound. The incident prompted further clinical trials that resulted in warfarin being used as the anticoagulant of choice for prevention of thromboembolic disease (177).

The mode of action of the coumarin anticoagulants involves blocking the regeneration of reduced vitamin K and induces a state of functional vitamin K deficiency, thus interfering with the blood-clotting mechanism (178).

7 ANTIASTHMA DRUGS

7.1 Khellin and Sodium Cromoglycate

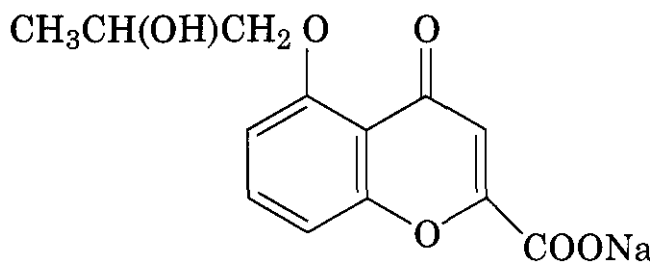
The toothpick plant, *Ammi visnaga*, had been used for centuries in Egypt as an antispasmodic agent to treat renal colic and ureteral spasm. In 1879 one of the plant's main constituents was isolated, crystallized, and named khellin (139) (179). Subsequently, the pure compound was shown to relax smooth muscle and in 1938 the chemical structure was characterized as a chromone derivative (180). In 1945 a medical technician took khellin to treat renal colic and found instead that it acted as a potent coronary vasodilator and relieved his angina (181). This chance discovery, together with earlier observations, led to khellin being used as a coronary artery vasodilator and for treating bronchial asthma (182). However, its clinical use was severely limited by some unpleasant gastrointestinal side effects.



(139)

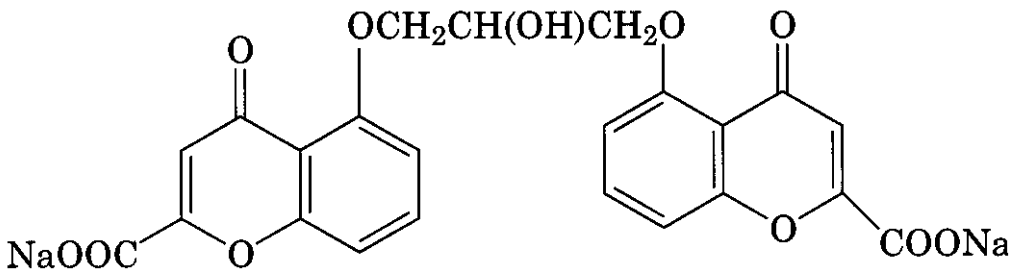
Five years later, a small British pharmaceutical company, called Benger Laboratories, initiated a program to synthesize khellin analogs as potential bronchodilators for treating asthma, and had prepared a series of compounds that relaxed guinea pig bronchial smooth muscle and protected the animals against allergen-induced bronchospasm (183).

A clinical pharmacologist on Benger's staff, who suffered from chronic asthma, questioned the validity of the animal model and decided instead to test the compounds on himself. He then prepared a "soup" of guinea pig fur, inhaled the vapors to induce a reproducible asthma attack, and assessed the effects of the synthesized khellin derivatives. Many of the compounds first prepared were insoluble in water and caused nausea and other unpleasant side effects when taken orally. This led to the test compounds being formulated as aerosol sprays and in 1958, an aerosol preparation of a chromone-2-carboxylic acid derivative (140) was found to exert a protectant effect, albeit short lived, against bronchial allergen challenge without showing the bronchodilator activity seen with other compounds. The compound was completely inactive in the guinea pig asthma model and afforded its protectant effect in humans only when inhaled as an aerosol.

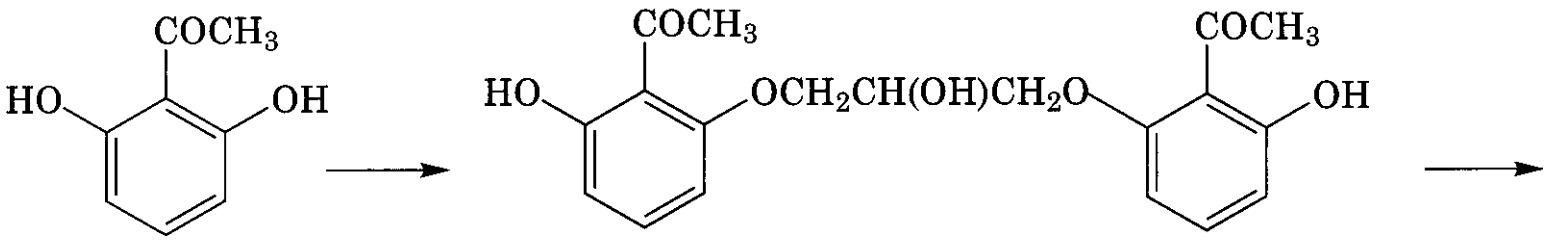


(140)

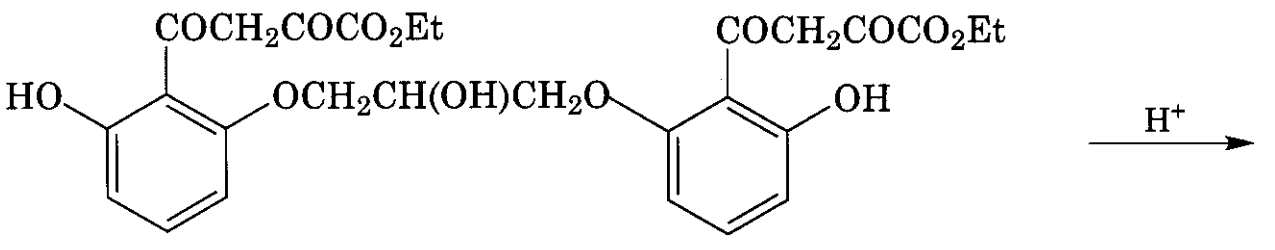
About two new compounds were tested each week and in 1965, after synthesizing some 670 analogs, a bischromone was prepared that gave good protection, even when inhaled up to 6 h before bronchial allergen challenge (184). The compound sodium cromoglycate (141) was obtained by condensing diethyl oxalate with the bis(hydroxy acetophenone) (142) and cyclizing the resultant bis(2,4-dioxobutyric acid) ester (**143**) under acidic conditions (185). The essential chemical features required for activity appeared to be the coplanarity of the chromone nuclei, the flexible dioxyalkyl link, and the carboxyl groups in the 2-positions. It is believed to act by stabilizing tissue mast cells against degranulation, thereby preventing release of inflammatory mediators (186).



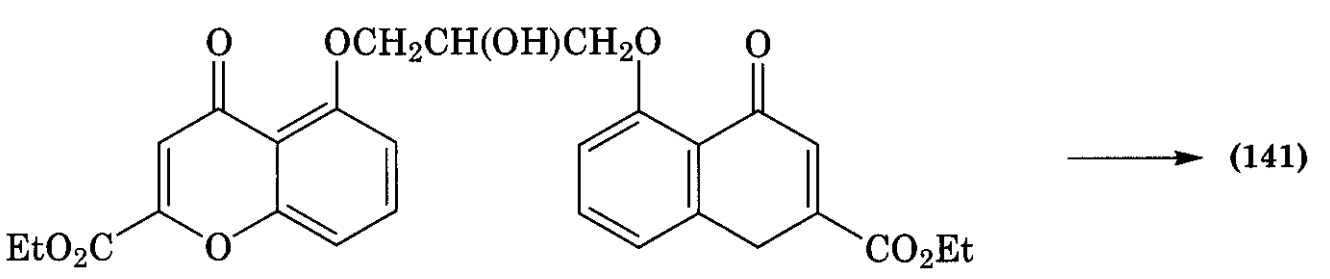
(141)



(142)

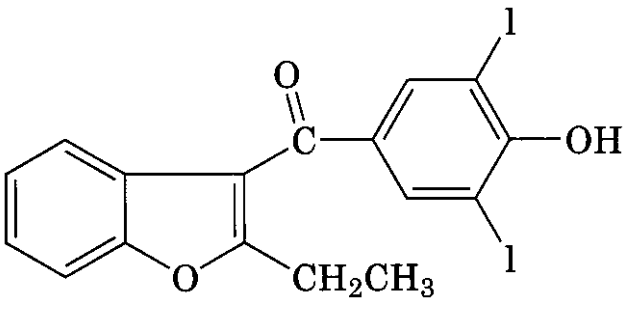


(143)



Sodium cromoglycate entered clinical trials in 1967 and emerged to become a first-line prophylactic treatment for bronchial asthma.

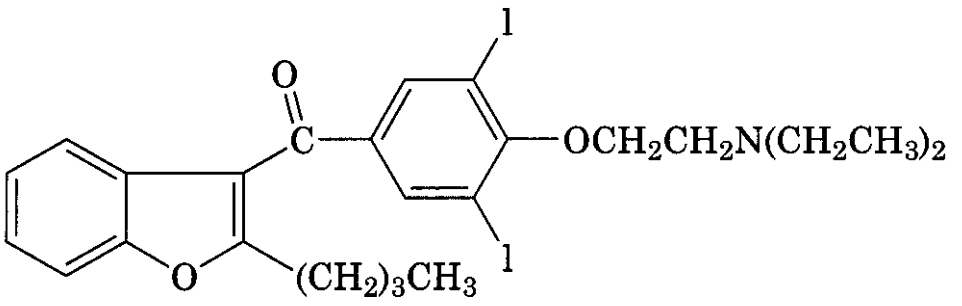
The coronary dilator properties of khellin have not been ignored and at least one successful program was initiated to prepare analogs for testing as potential antiangina drugs (187, 188). Benziodarone (144) was the first useful compound to emerge from the Labaz laboratories in Belgium based on the benzofuran ring system. However, the compound caused hepatotoxicity in man and was soon superseded by amiodarone (145), a more potent coronary dilator for treating angina. In 1970 the first report of antiarrhythmic activity in the clinic was published (189) and amiodarone became established for prophylactic control of supraventricular and ventricular arrhythmias during the 1980s (188).



(144) benziodarone

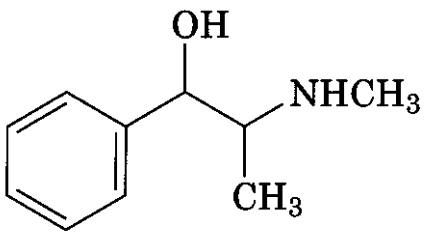
7.2 Ephedrine, Isoprenaline, and Salbutamol

The Chinese have been using a plant extract known as ma huang to treat asthma and hay



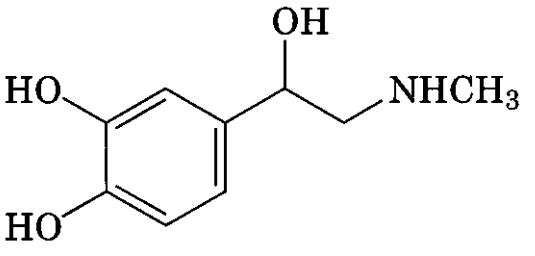
(145) amiodarone

fever for thousands of years. The extract is prepared from several species of ***Ephedra,*** a small leafless shrub found in China. Following experiments at the Peking Union Medical College and then at the University of Pennsylvania and the Mayo Clinic in the United States, the active ingredient, ephedrine (**146**), was introduced into Western medicine in 1926 as an orally active bronchodilator for the treatment of acute asthma (190,191).

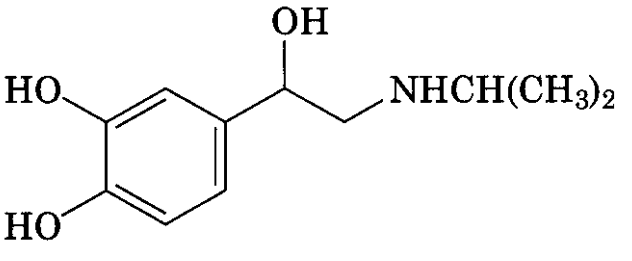


(146)

Ephedrine is related to another natural product that has been used to treat asthma, that is, the adrenal hormone adrenaline (147) (epinephrine). Adrenaline is a potent agonist of both α- and β-adrenoceptors and thus produces arterial hypertension as an undesirable side effect. In 1951 a synthetic alternative, isoprenaline (**148**), was introduced and for almost 20 years it was considered the drug of choice for treating bronchospasm associated with acute asthmatic attack (191). Isoprenaline is a specific β-adrenoceptor agonist and, although it has no vasoconstrictor activity, the compound does have marked cardiac stimulant properties and a short duration of action. Ahlquist's concept (192) of two types of adrenoceptor was developed further by Lands et al. (193), who established the existence of β_1- and β_2-adrenoceptor subtypes. Clear structure-activity relationships emerged with the preparation of compounds related to adrenaline and ephedrine; the basic requirement for β-adrenoceptor agonist activity was an aromatic ring substituted by an ethanolamine side-chain. The branched methyl substituent on the side-chain was associated with prolonged duration of action (i.e., ephedrine), whereas aromatic hydroxylation (in isoprenaline) prevented penetration across the blood–brain barrier and thus prevented stimulation of the CNS (191). However, 1,2-dihydroxy substituents were found to promote enzymic degradation, and replacement of the 3-hydroxy group by a hydroxymethyl substituent was required to extend the duration of action. In 1969 salbutamol (149) was launched by Glaxo as a longer-lasting, selective &-adrenoceptor agonist for the treatment of bronchial asthma (194) and, recently, a lipophilic ether analog, salmeterol (**150**), was introduced with an even longer duration of action that has potential advantage in the prevention of nocturnal asthma.

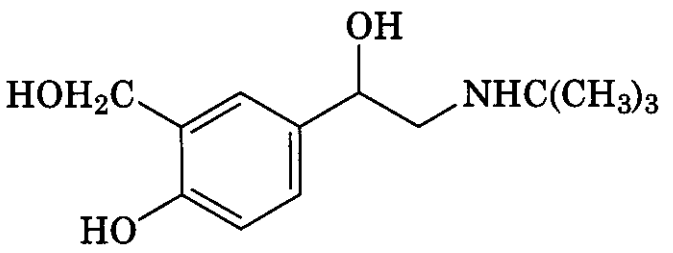


(147)

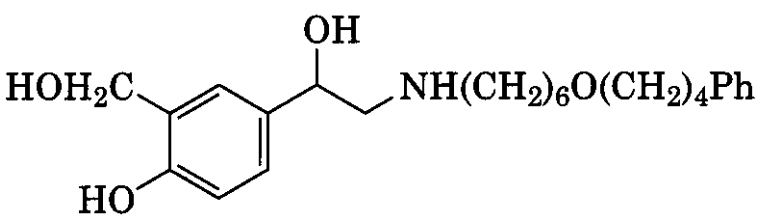


(148)

Despite the many chemical alterations that have been carried out on the phenylethanolamine "template," the key chemical features associated with modern β-agonists can be seen



(149)

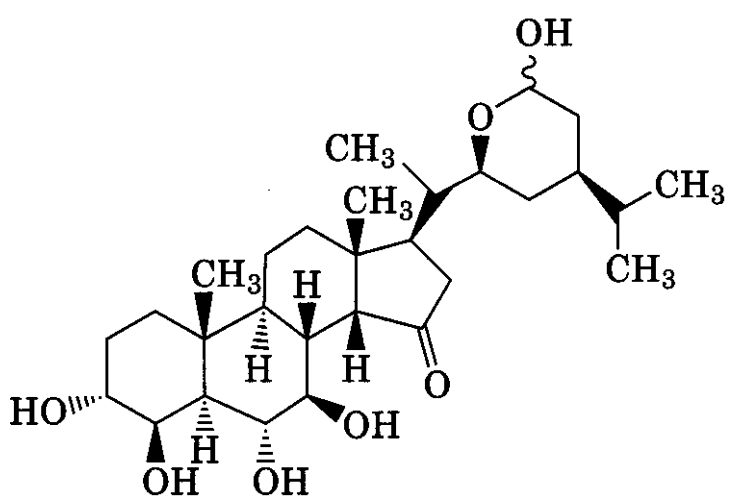


(150)

to have originated from the naturally occurring compounds, adrenaline and ephedrine.

7.3 Contignasterol

The use of inhaled corticosteroids such as fluticasone propionate to treat asthma and rhinitis has been well documented and will not be repeated here. Less well known is an unusual, highly oxygenated marine-derived steroid isolated from the sponge ***Petrosia contignata*** that possesses *a* unique cyclic hemiacyl side-chain (151). The compound was isolated by Andersen and coworkers (195) at the University of British Columbia and found to possess anti-inflammatory properties ***in vivo***. Contignasterol is being developed by Inflazyme, in collaboration with Aventis, for the treatment of asthma and other inflammatory diseases and has progressed to phase II clinical trials.



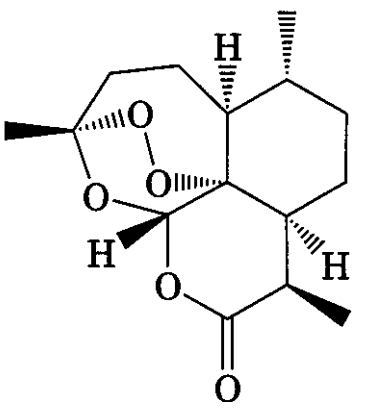
(151) Contignasterol

8 ANTIPARASITIC DRUGS

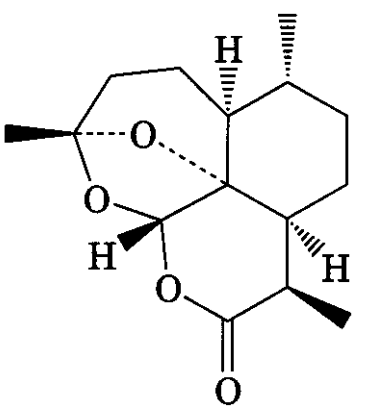
8.1 Artemisinin, Artemether, and Arteether

Artemisia annua (sweet wormwood, qing hao) has been used in Chinese medicine for well over 1000 years. The earliest recommendation is for the treatment of hemorrhoids, but there is a written record of use in fevers dated 340 A.D. Modern development dates from the isolation of a highly active antimalarial, artemisinin (qinghaosu), in 1972, and has been carried out almost entirely in China. Much of the original literature is therefore in Chinese, but there is an excellent review on qinghaosu by Trigg (196) and an account of the uses of *A. annua* (197). This section is largely a summary of these two articles.

Artemisinin (152) is a sesquiterpene lactone with an unusual peroxide bridge. One of the earliest modifications involved catalytic reduction of the peroxide, resulting in loss of one oxygen and total loss of antimalarial activity (196) in the adduct (153). The role of the peroxide bridge in producing antimalarial effects was not fully understood, but it appeared essential for activity, so much of the early work on analogs conserved this structural feature as an empirical finding. The mechanism



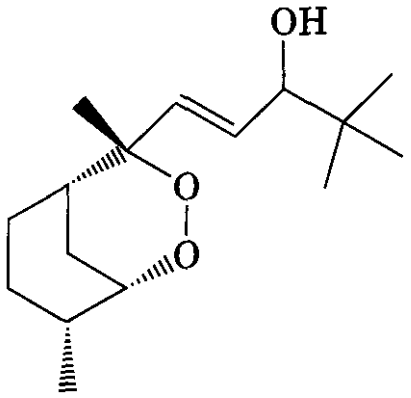
(152) artemisinin



(153)

of action of artemisinin has since been elucidated (198, 199), although it is not without controversy (200, 201). The drug has a high affinity for hemozoin, a storage form of hemin that is retained by the parasite after digestion of hemoglobin, leading to a highly selective accumulation of the drug by the parasite. Artemisinin then decomposes in the presence of iron, probably from the hemozoin, and releases free radicals, which kill the parasite. The peroxide bridge is therefore a crucial part of the drug molecule, as was suspected from structure-activity studies. Elucidation of the mechanism of action has led to the synthesis of a range of simple analogs capable of iron-catalyzed decomposition, some of which have good antimalarial activity (202).

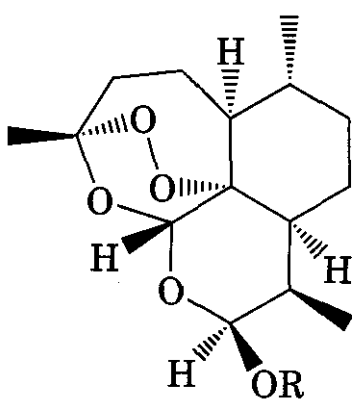
In retrospect, it is not surprising that the peroxide-bridged compound (**154**), isolated from *Artabotrys* uncinatus, also has antimalarial activity (197). Because peroxides of this kind are likely to be formed from a variety of precursors in dried plant material (see below), there may well be many more antimalarials of this kind to be found.



(154)

Artemisinin is an excellent antimalarial, approximately equal in potency to chloroquine, with a good therapeutic index except on the fetus. The preparation of semisynthetic derivatives has been stimulated primarily by a requirement for improved solubility because artemisinin is relatively insoluble in both water and oil.

Reduction of (**152**) with sodium borohydride occurs at the lactone carbonyl, leaving the peroxide intact (196, 197). The resulting cyclic hemiacetal, dihydroartemisinin (**155**), which is a more potent antimalarial than the parent compound, shows typical acetal reactivity. In the presence of acid, a highly reactive carbocation intermediate allows S_N1-type substitution with a variety of nucleophiles. For example, boron trifluoride catalyzes reactions with methanol and ethanol to give artemether (**156**) and arteether (**157**), respectively, two of the most important derivatives (196). Both are more potent than the parent compound and have improved solubility in oil. Artemether has been chosen for development in the West under the name Paluther.



(155) R = H
(156) R = CH_3 artemether
(157) R = CH_2CH_3 arteether
(158) R = $COCH_2CH_2COONa$ sodium artesunate

Water solubility can be greatly improved by the standard ploy of esterification with succinic acid and conversion to the sodium salt. Applied to compound (**155**), this technique gives sodium artesunate (**158**), a water-soluble prodrug that may be given intravenously (196). It may be assumed that hydrolysis occurs in *vivo* to give back (155) as the active antimalarial because (**156**) has been shown to be unstable in aqueous solution and because analogous carboxylic acids with a nonhydrolyzable ether link are relatively inactive.

There are two reasons for the great interest being shown in artemisinin and its derivatives. First, there is little cross resistance with Plasmodium falciparum between the members of this series and the quinoline-based antimalarials like chloroquine (203). On the contrary, significant potentiation of effect is observed in combination with chloroquine analogs such as mefloquine (204). Second, the high lipid solubility of, for example, artemether ensures rapid penetration into the CNS, so these sesquiterpene lactones are first-

line drugs for the treatment of cerebral malaria caused by P. falciparum (197), which is otherwise fatal.

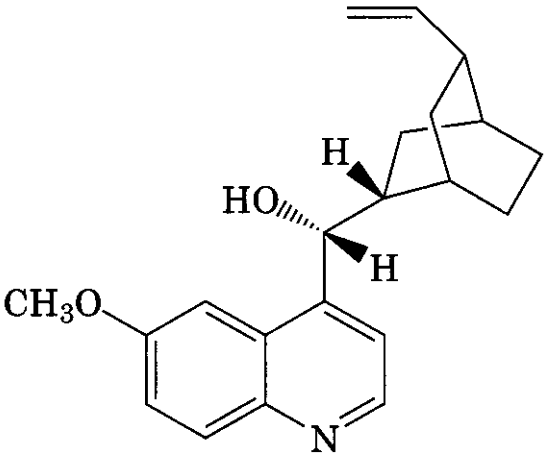
It seems highly likely (205) that most of the artemisinin found in dried plant material is formed by autoxidation after the death of the plant. From the medicinal chemist's point of view this is unimportant, but some plant biochemists might have doubts about the description of artemisinin as a "natural product." In our view, air drying in sunlight is a natural, although not a botanical, process. It is probable that many other plant-derived peroxides are formed in a similar way.

Whole plant extracts often show promising activity that may not be traceable to single components. This is obviously not true of *Artemisia annua* extracts, but it is interesting to note that other constituents, notably methoxylated flavones, have potentiating effects on the antimalarial activity of artemisinin (206).

The reported effect of artemisinin on systemic lupus erythematosus (196) is intriguing, given the history of use of quinine-type antimalarials in this disease.

8.2 Quinine, Chloroquine, and Mefloquine

The use of Cinchona bark (e.g., Cinchona *succirubra*) by South American indians to treat fevers and the subsequent importation of the bark into Europe by Jesuit priests in the 17th century is well known (207). At that time malaria was widespread, even as far north as eastern Scotland, and there was no effective treatment for "the ague." Although quinine (**159**) is not very potent or long acting, a good sample of Cinchona bark contains about 5% of the alkaloid (208). This high concentration permitted genuinely therapeutic doses of bark to be given and allowed the pure alkaloid to be isolated (209) as early as 1820. During the next 100 years quinine was the only effective treatment for malaria known to Europeans. Without quinine, life in the tropics was impossible for those without natural immunity to malaria. "One thing that was compulsory was the taking of five grains of quinine a day. . . . And if you didn't take it and got ill your salary was liable to be stopped" (210). Supplies of quinine to Europe were threatened during World War I, stimulating a major program of research into synthetic analogs.



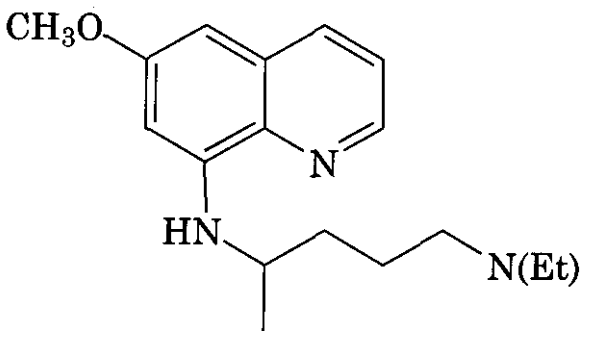
(159) quinine

The chemical techniques available to chemists in the period 1820–1920, although improving rapidly, did not allow a structure to be proposed for quinine with any confidence: the first completely correct proposal (211) came in 1922 and was finally confirmed by total synthesis (212) as late as 1945. However, part structures were known, such as the 6-methoxyquinoline moiety, from long before, and were sufficient to allow the synthesis of mimics. The first clinically successful mimics were the 8-aminoquinolines.

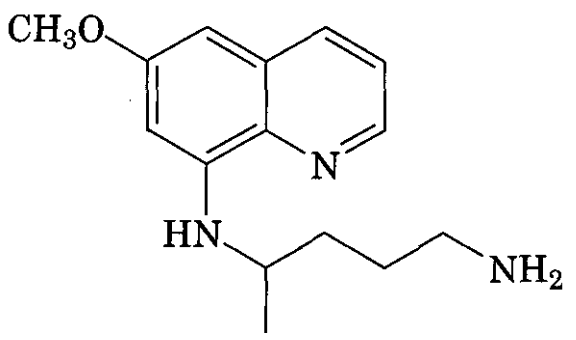
In the early years of the 20th century, synthetic organic chemistry was a young discipline, largely governed by empirical rules. Progress toward synthetic analogs of complex natural structures was governed as much by synthetic feasibility as by a desire for close mimicry. The first quinine analogs were, therefore, a combination of the accessible 6-methoxyquinoline part of the quinine structure, with elements of the first successful antimicrobial agents, such as 9-aminoacridine. Nitration followed by reduction could be used to generate a number of new molecules from a variety of parent heterocycles. It is recorded (213) that 4-, 6-, and 8-aminoquinolines have antimalarial properties and, quite extraordinarily, two of these chemical classes are still used today, have quite different uses as antimalarials, and quite possibly have different modes of action.

The first of the 8-aminoquinolines to be introduced into medicine was pamaquine (**160**), not long after World War I (214). Despite greater toxicity than that of quinine, this class

of drugs was found to have radical curative ability against the relapsing malarias. Several hundred analogs were tested during World War II and of these, primaquine **(161)** survives to the present day for short-term use as a radical curative **(215).**

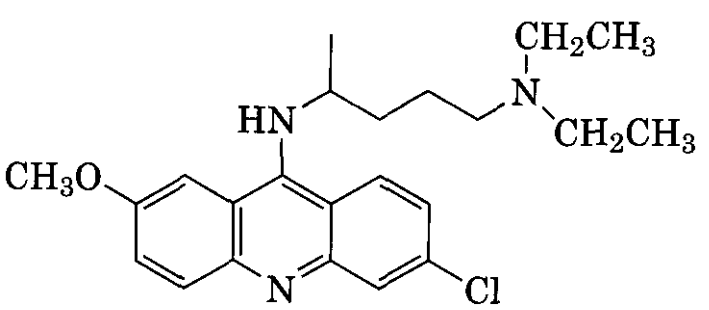


(160) pamaquine



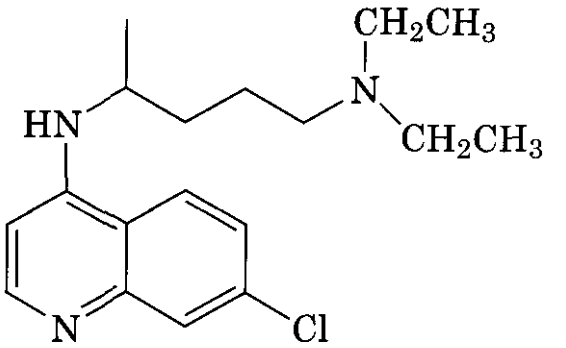
(161) primaquine

Quinacrine **(162)** is an obvious embodiment of the principle outlined above; as a derivative of both quinine and 9-aminoacridine it combined a known antimalarial with a known antimicrobial. The result was a useful, relatively nontoxic antimalarial, although it stained the skin and eyeballs yellow **(216).** Despite this side effect and a high incidence of gastrointestinal disturbance, quinacrine was widely used during World War II by European troops in East Asia. The availability of the results of medicinal chemistry research to both sides in wartime is a curious feature of antimalarial development, highlighted below.



(162) quinacrine (mepacrine)

As has been explained, the major stimulus for research into synthetic antimalarials was not so much the therapeutic inadequacy of quinine as the potential lack of availability in times of social upheaval. During World War II, the United States encouraged the planting of Cinchona in Costa Rica, Peru, and Ecuador (216). The total synthesis of quinine was too difficult in the 1940s and is unlikely to become economically viable even in the new millennium. This problem was partly overcome with quinacrine, which was used widely in World War II, although quinacrine has the defects described above. The conceptual derivation of chloroquine **(163)** from quinacrine is obvious and apparently happened twice, in Germany and the United States, the latter about **10** years after the Germans had discarded the drug as being too toxic! The story of the rediscovery of chloroquine is fascinating, as an account of human muddle and misjudgment, finally leading to an extraordinarily valuable drug **(216).**

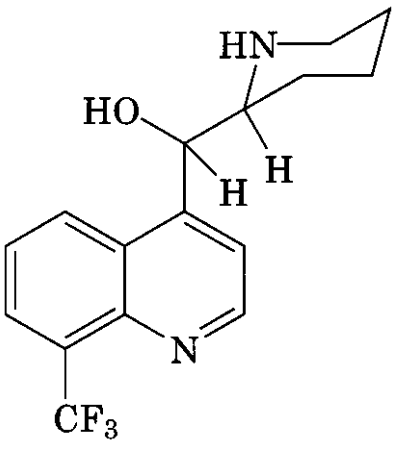


(163) chloroquine

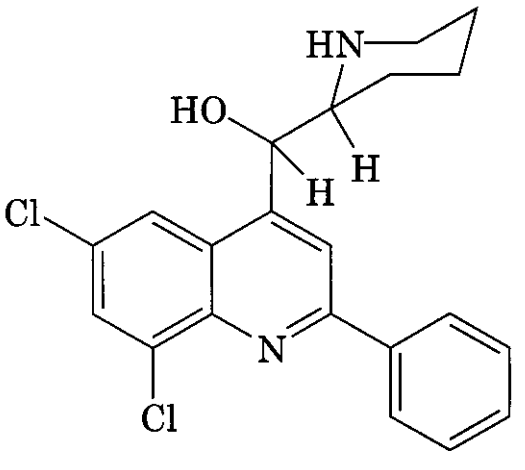
Over decades of sublethal exposure the resistance of **all** types of malaria has increased to a point where chloroquine no longer offers certain protection (217). With the partial exception of quinine and dihydroquinine (218), resistance to antimalarials had reached the stage at the time of the Vietnam war where more research was required. The development of mefloquine (**164**) was a continuation of the World War II effort, with a gap of about **20** years. Resistance to chloroquine had developed widely during that period, but surprisingly less so to quinine, given the obvious similarities in structure. This observation stimulated a reappraisal of quinolines, known as quinoline methanols, which bear a hydroxy group on the α-carbon of a substituent at-

tached to the 4-position (219). Up to 1944, a total of 177 quinoline methanols had been synthesized and tested, resulting in one compound (165) with activity superior to that of quinine. In human volunteers there was a high incidence of phototoxicity associated with (**165**), so research on quinoline methanols in 1944 had ceased in favor of the 4-amino series, which included chloroquine. Reappraisal of about 100 of the World War II compounds confirmed the high activity and phototoxicity of (165) and also showed the high potency of an analog (**166**), which had reduced phototoxicity (219). These data, together with results from about 200 newer compounds, fostered the belief that phototoxicity was separable from antimalarial activity. Extensive evaluation of (**166**) in humans with chloroquine-resistant *Plasmodium falciparum* infections showed promise, but with a significant incidence of toxic reactions; the dose required was also inconveniently large.

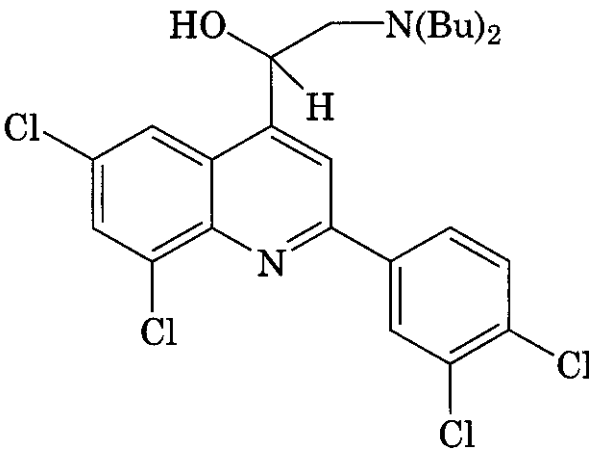
Two hypotheses concerning the effect of the 2-phenyl substituent were proposed. One was that metabolic oxidation was blocked at



(164) mefloquine



(165)



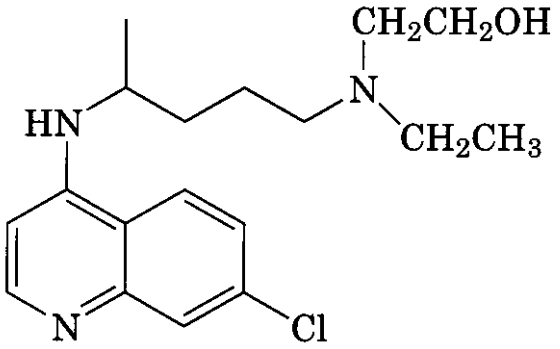
(166)

this position, so that duration of action was prolonged, which was considered desirable. Second, the UV chromophore was enlarged, which would increase the likelihood of drug-induced photosensitivity. The phenyl substituent was thus replaced by trifluoromethyl in the 2-position (220). Before the first such derivatives were tested, further analogs were prepared with an additional trifluoromethyl group on the benzene ring. This was serendipitous because the first series of 2-trifluoromethyl analogs had low potency and were also photosensitizing. The series with two trifluoromethyl groups, one at position 2 and another in the 6-, 7-, or 8-position were all potent and free from phototoxicity (221). The most potent was mefloquine (**164**), a very successful drug but one that produces unacceptable CNS effects in a small proportion of users (222); parasite resistance has also been observed in parts of Southeast Asia (217). There is now a serious attempt by the World Health Organization to find new antimalarials.

Physicians are pragmatic when choosing therapy for patients whose suffering is not alleviated by accepted methods. A drug that has been shown to be toxicologically safe may be utilized in a new area for the flimsiest of reasons. Thus Page (223) described his use of quinacrine in two cases of lupus erythematosus as being based on "[a] chance observation . . . ," although he did not describe the observation that led to his decision. He did, however, record that quinine had been tried previously and "prevented extension of the lesions," so this may have been the basis for his rationale. In any event, the beneficial effects of quinacrine were remarkable and ap-

peared to be related to the degree of yellowing of the skin that, as described earlier, is a common side effect of the use of quinacrine in malaria.

Among Page's group of patients with lupus erythematosus were two with rheumatoid arthritis, whose symptoms also responded to treatment with quinacrine. The following year, other physicians (224) conducted a trial of quinacrine on a larger group of patients with rheumatoid arthritis; the results encouraged Haydu (225) to test chloroquine on similar patients, again with positive results. A year later, two more physicians (226) compared quinacrine with chloroquine and found the latter to be better tolerated, the majority of patients gaining some benefit. Both quinacrine and chloroquine caused gastrointestinal disturbances, which led to a trial (227) of hydroxychloroquine (167), an unsuccessful antimalarial but with less effect on the gut, thus allowing larger doses to be given. Hydroxychloroquine has remained part of the standard drug therapy for rheumatoid arthritis ever since.



(167) hydroxychloroquine

So far, the choice of quinine-like drugs to treat rheumatoid arthritis has been based on preliminary selection as antimalarials. Because the two types of action are presumably unconnected, there might be some value in a screening program aimed directly at rheumatoid disease.

8.3 Avermectins and Milbemycins

There is no major distinction between the avermectins and milbemycins, which are based on the same complex polyketide macrocycle (168): the avermectins are oxygenated at C-13 and bear a disaccharide on this oxygen. They have been isolated from cultures of a number of *Streptomyces* species, obtained from all over the world (228).

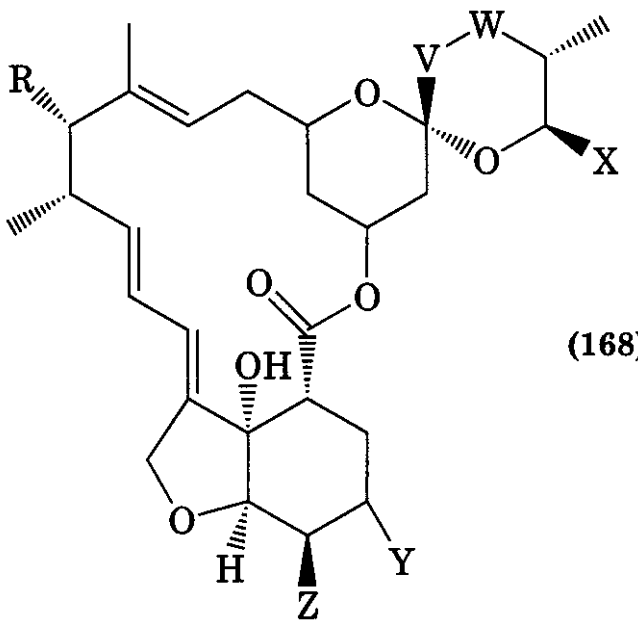
The avermectins, particularly, have been the subject of intense commercial interest because they possess potent activity against both nematode and arthropod parasites of livestock (229). A full discussion of structure-activity relationships would be out of place here, not least because the data are voluminous. so we shall concentrate on the development of ivermectin, which has been a major success.

Structural designation of avermectins is quaintly based on three series: A, B; a, b; and 1, 2. These are illustrated diagrammatically. Greater activity resides in the B series, with a free OH at position 5. There is little difference in potency between the a and b series. In the more potent B series there are important differences between the 1 series and the 2 series; B_1 is the more active orally, whereas B_2 is the more potent by injection. There are also differences in their spectrum of activity (230). The spectrum of activity was kept as broad as possible by hydrogenation of a mixture of avermectins B_1a and B_1b to give ivermectin (169), which contains at least 80% of 22,23-dihydroavermectin B_1a and not more than 20% of 22,23-dihydroavermectin B_1b.

Ivermectin was developed for, and has been highly successful in, the treatment and control of parasites in cattle, horses, sheep, pigs, and dogs. Following studies in humans with river blindness (onchocerciasis) (231–233), the developers of ivermectin (Mectizan) have participated in a major program aimed at eradication of the disease. The sufferers inhabit some of the poorest parts of Africa and cannot pay for their treatment, so the drug has been donated by Merck and Co. Since 1996 more than 20 million treatments have been given (234). The drug does not kill the adult worms that cause onchocerciasis (235), but is useful in interrupting the life cycle (236). Ivermectin is also of value in treatment of scabies (237). A great deal of information on the biological aspects of the use of ivermectin has recently been summarized (238).

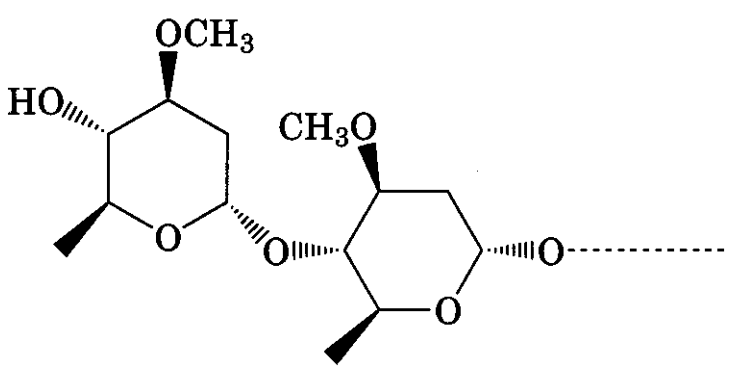
9 CONCLUSION

Natural product research has been the single most successful strategy for discovering new



(168)

avermectins R =



milbemycins R = H

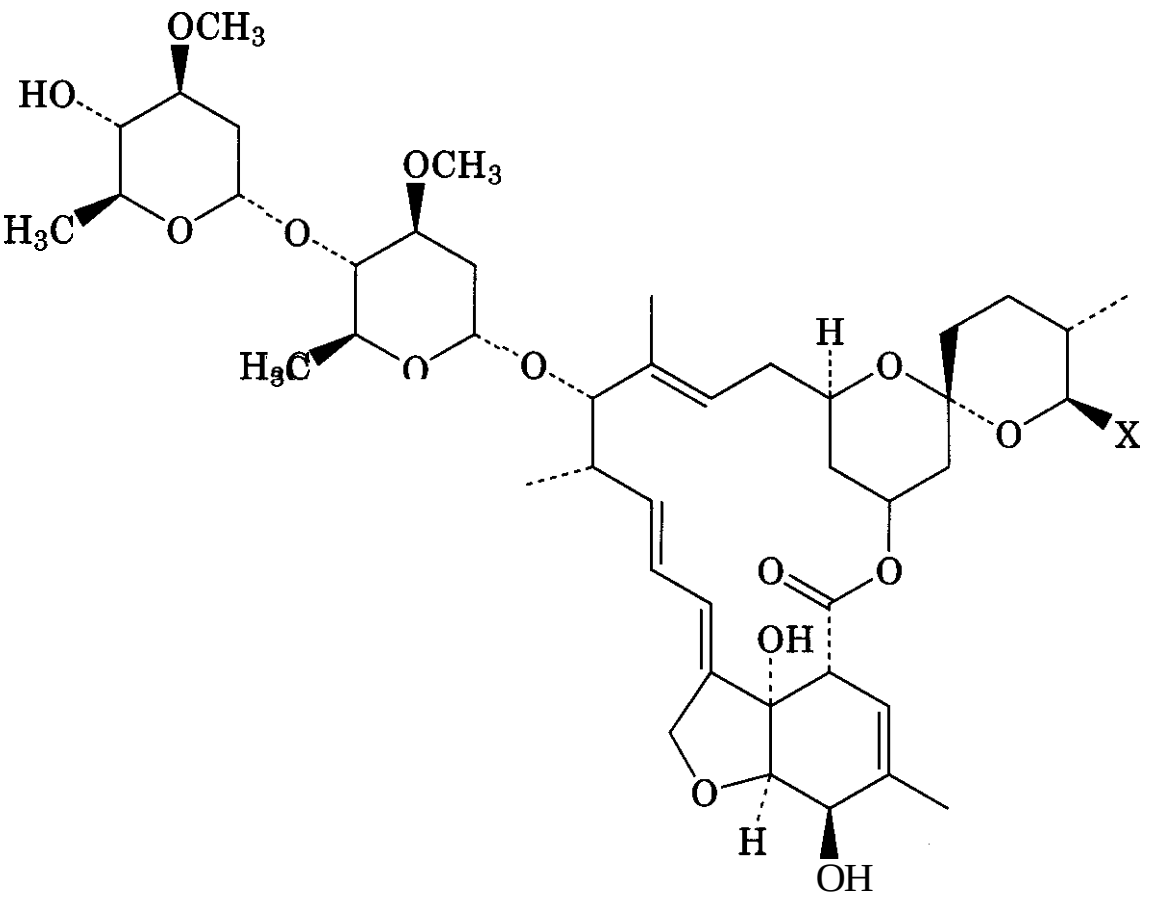
In the avermectins the series are designated as follows (Y = CH_3):

A, Z = CH_3
B, Z = H
a, X = $CH(CH_3)CH_2CH_3$
b, X = $CH(CH_3)_2$
1, V-W = CH=CH
2, V-W = $CH_2CH(OH)$

For further details of these descriptors, in the milbemycins, see Ref. 228.

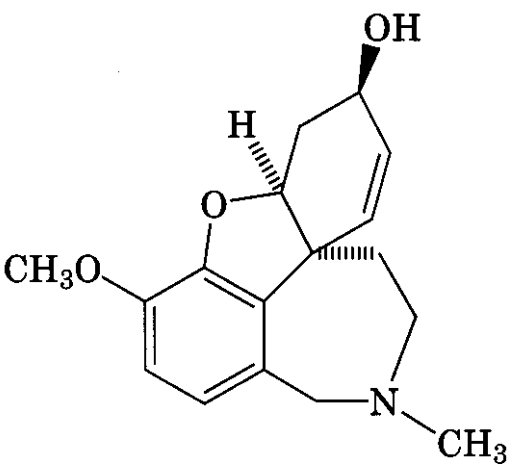
In ivermectin **(169)**, V-W = CH_2CH_2, X = $CH(CH_3)CH_2CH_3$ (major) or $CH(CH_3)_2$ (minor), Y = CH_3 and Z = H

pharmaceuticals and has contributed dramatically to extending human life and improving clinical practice. As long as Nature continues to yield novel, diverse chemical entities possessing selective biological activities, natural products will play an important role as leads for new pharmaceuticals. An interesting recent example is the alkaloid galantamine (**Nivalin, Reminyl**) (**170**), originally isolated from the bulbs of the *Amaryllidaceae* family (snowdrops, daffodils, etc.), which has found use in the symptomatic treatment of Alzheimer's Disease (239). It is a reversible and competitive inhibitor of acetylcholinesterase that also interacts allosterically with nicotinic acetylcholine receptors to potentiate the action of



(169) X = $CH(CH_3)CH_2CH_3$ (major)or $CH(CH_3)_2$ (minor)

agonists. By acting to enhance the reduced central cholinergic function associated with this disease, significant improvements in cognition and behavioral symptoms have been observed in patients. In this case it is the alkaloid itself that is used as the active compound and it will be interesting to see whether development leads to better drugs. There are as yet relatively few publications in this area, although Sanochemia is interested (240,241).



(170)

Over 90% of bacterial, fungal, and plant species are still waiting to be investigated (242). High throughput screening methods will allow even greater numbers of samples to be tested against more biological targets (243, 244), although this approach sometimes produces more data than can be conveniently integrated into a research program. An alternative view is that the elucidation of the biological effects of chosen compounds, in some detail, will yield insight into biological processes that may open avenues for medicinal chemistry research that is not based on pure chance. This view is based on the recognition that secondary metabolites have been produced and ruthlessly selected, by evolution, over a long period of time. Either way, the medicinal chemist has a wonderful opportunity to continue utilizing the rich chemical diversity offered by nature, as is shown in two recent reviews that explore this topic in some detail (245,246).

The best approach for the identification of natural product leads is a matter of debate. Some very inventive techniques have been used in the bioassay-guided method; for example, by spraying TLC plates with reactive media that respond by producing a color change in the presence of an active compound. An alternative is to use an ethnobotanical or ethnopharmacological technique, whereby the accumulated wisdom of many generations of native plant users may be harnessed in the

search for better medicines for all. These two techniques may be combined, so that the native people describe the uses to which they put the plant and the researchers devise a bioassay that is used to find the active components. The problem with any bioassay-guided technique, however, is that the inactive constituents are not identified. This represents a considerable waste, given that the plant has had to be collected, preserved, and identified. An alternative view is that it is best to extract all the constituents, with a view to screening in whichever way is appropriate, at that time or in the future. With modern high-performance liquid chromatography facilities it is possible to reduce a plant to its secondary metabolites, as single compounds, in a few days: the products are then able to be screened in a high throughput manner in an equally short time and the compounds can be reevaluated when new screens become available. One thing is certain: the variety of natural product structures, after perhaps 300 million years of natural selection, far exceeds the bounds of human imagination, unlike the typical output from combinatorial chemistry!

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