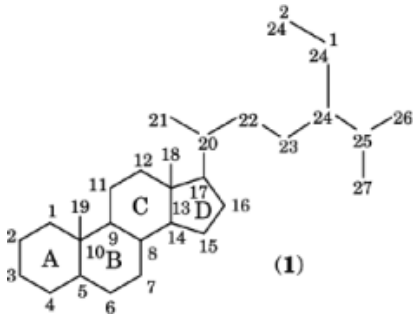
Kirk-Othmer Encyclopedia of Chemical Technology.

STEROIDS

Steroids (1) are members of a large class of lipid compounds called terpenes that are biogenically derived from the same parent compound, isoprene, C_5H_8. Steroids contain or are derived from the perhydro-1,2-cyclopentenophenanthrene ring system **(1)** and are found in a variety of different marine, terrestrial, and synthetic sources. The vast diversity of the natural and synthetic members of this class depends on variations in side-chain substitution (primarily at C17), degree of unsaturation, degree and nature of oxidation, and the stereochemical relationships at the ring junctions.



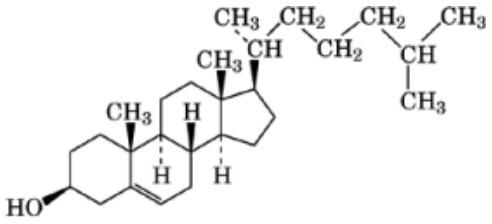
There are many classes of natural and synthetic steroids best known for their wide array of biological activity. The naturally occurring steroids can be subdivided into several categories that include (*1*) nonhormonal, mammalian steroids such as sterols and bile acids; (*2*) vitamin D; (*3*) hormonal steroids such as the human sex hormones (androgen, estrogens, and progestins) and corticosteroids (glucocorticoids and mineralocorticoids); and (*4*) other naturally occurring steroids, such as plant steroids (eg, sapogenins, saponins, withanolides), marine steroids, ecdysteroids, cardiac steroids, steroid alkaloids, and steroid antibiotics (see Hormones; Vitamins, vitamin D; Antibiotics).

The biological activity of the naturally occurring steroids has been exploited in the development of therapeutic agents. Many of these synthetic steroids are the product of pharmaceutical research that has resulted in the development of several steroid-based medicines with a market value over 10 billion U.S. dollars (2). Among the medicinally important synthetic steroids are the antihormones, anesthetics, antiinflammatories, antiasthmatics, contraceptive drugs (qv), antibiotics, anticancer agents, cardiovascular agents (qv), and osteoporosis drugs (see Antiasthmatic agents; Chemotherapeutics, anticancer).

1. History

Initial steroid research involved isolation of sterols and bile acids from natural sources. DeFourcroy is generally credited with the discovery of cholesterol [57-88-5] **(2)** in 1789 (3). In 1848, cholic acid [81-25-4] **(3)** was

isolated from the saponification of ox bile and its elementary composition determined as $C_{24}H_{40}O_5$; 40 years later, Reintzer established the molecular formula of cholesterol as $C_{27}H_{46}O$. Degradative studies revealed the relationship of cholesterol and the bile acids (4).



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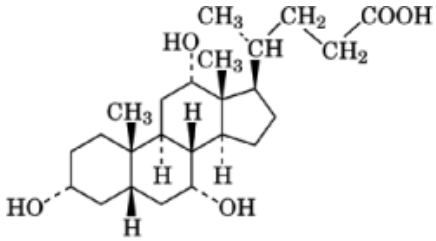
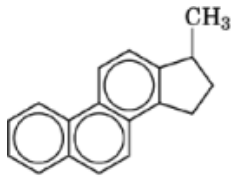


(3)

Although many sterols and bile acids were isolated in the nineteenth century, it was not until the twentieth century that the structure of the steroid nucleus was first elucidated (5). X-ray crystallographic data first suggested that the steroid nucleus was a thin, lath-shaped structure (6). This perhydro-1,2-cyclopentenophenanthrene ring system was eventually confirmed by the identification of the Diels hydrocarbon [549-88-2] **(4)** and by the total synthesis of equilenin [517-09-9] **(5)** (7).



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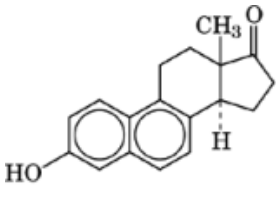


(5)

The period from the 1930s through the 1960s is often referred to as the golden age of steroid research. By the end of the 1930s, the isolation, structural determination, and synthesis of the sex hormones estrogen, progesterone, estradiol, and testosterone were completed (8). Also, investigations of steroid production in the adrenal cortex began in the 1930s. From 1936 to 1942, more than 30 crystalline steroids called corticosteroids were isolated from adrenal gland extracts. Included among these corticosteroids were the potent glucocorticoid cortisol and the mineralocorticoid aldosterone.

Efforts toward producing synthetic steroids, particularly cortisol, expanded during World War II to enable researchers to explore the possibility of medicinal applications of corticosteroids. In 1948, the discovery that cortisone dramatically alleviates the symptoms of arthritis led to intensive research on the antiinflammatory properties of corticosteroids. The development of partial and total syntheses for the commercial preparation of cortisone, alternative methods for producing cortisone, and the search for more potent antiinflammatory analogues greatly stimulated both academic and industrial steroid research.

During this period of intense synthetic research, a search for inexpensive raw materials for the partial synthesis of steroids was initiated. Abundant quantities of the sapogenin diosgenin [512-04-9] were isolated from plant sources and used for the industrial preparation of steroids (9).

This explosion in steroid chemistry both stimulated and was aided by the development of conformational analysis (10). Many basic, physical organic chemistry principles were established as a result of the study of the logically predictable chemistry of the rigid perhydro-1,2-cyclopentenophenanthrene, steroid skeleton.

The 1950s and 1960s saw the development of orally active progestins based on the synthesis of steroids that lack the C19-angular methyl substituent (19-norsteroids). The commercial production of these compounds for the regulation of menstrual disorders began in 1957, and for oral contraception in 1960.

The 1970s were a vibrant period for steroid research. Beginning in the early 1970s, problems with Mexican supplies of the steroid raw material, diosgenin, prompted investigations into alternative sources of starting materials. Chemical methods to degrade the plant sterol stigmasterol [83-48-7] into useful starting materials were developed. In addition, new fermentation methods for the preparation of commercially important steroids from cholesterol and sitosterol were discovered. Also in the 1970s, studies on the control of cholesterol biosynthesis through selective enzymatic inhibition were begun. The active form of vitamin D was determined, and its biochemical effects delineated.

In the 1970s and 1980s, potent antiprogestins were discovered and used as contragestational agents, with possible applications for the treatment of various cancers. The synthesis of the antiprogestin RU-486 demonstrated a versatile way to functionalize the 11-position of a steroid nucleus.

In the 1980s, advances in biotechnology had a considerable impact on steroid research. During this period, the mechanism of steroid hormone-activated gene regulation became more clearly defined. These mechanistic studies still receive considerable attention in the primary literature.

2. Structure and Nomenclature

The long history of steroid nomenclature has been reviewed in "Definitive Rules for Nomenclature of Steroids" in 1972 (11). Most of these rules of steroid nomenclature have been adopted. A general definition of steroids is as follows. "Steroids are compounds possessing the skeleton of cyclopenta[a]phenanthrene or a skeleton derived therefrom by one or more bond scissions or ring expansions or contractions" (12). The position-numbering and ring-lettering conventions for steroids are shown in **(1)**. Positions 18 and 19 are often angular methyl groups; in addition, position 19 can be a hydrogen and is not substituted when the A-ring is aromatic. Position 17 can be substituted, unsubstituted, and/or oxygenated. Compounds are systematically named as derivatives of the parent hydrocarbons shown in Figure 1. Substituents that extend below the plane of the steroid are referred to as α and are designated by a broken line; those attached to the plane of the steroid from above are called β and are shown by a bold or solid line. Substituents of unknown configuration are indicated by a wavy line.

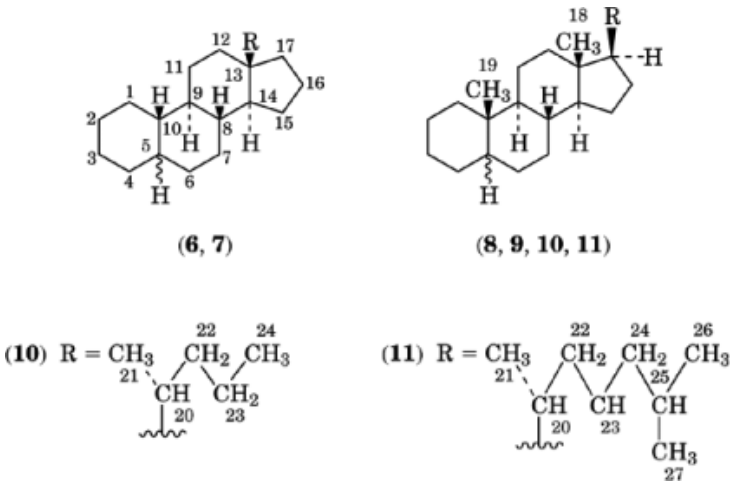


Fig. 1. Nomenclature of the parent hydrocarbon ring skeletons. Gonane [4732-76-7] (6) R=H; estrane [24749-37-9] (7) $R=CH_3$; androstane [24887-75-0] (8) R=H; pregnane [24909-91-9] (9) $R=C_2H_5$; cholane [548-98-1] (10) R as shown; and cholestane [145-53-7] (11) R as shown.

Generally, the ring junctions have an all-trans relationship with the hydrogen attached to C9 on the α-face, unless otherwise indicated. Changes in steroid nomenclature that have been introduced since 1972 include a wider use of the (*R*),(*S*)-system for designating the stereochemistry in the side chain, and four new parent hydrocarbons, ie, campastane, portiferastane, stigmastane, and gorgostane, have been proposed but have yet to receive formal adaptation (13). Although the systematic nomenclature for steroids has been firmly established, the most common and most important steroids are often designated by trivial names.

3. Classification of Biologically Active, Natural Steroids

3.1. Nonhormonal Mammalian Steroids

3.1.1. Sterols and Cholesterol

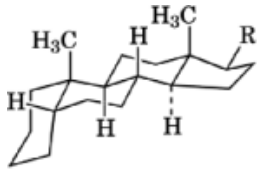
Natural sterols are crystalline C_{26}–C_{30} steroid alcohols containing an aliphatic side chain at C17. Sterols were first isolated as nonsaponifiable fractions of lipids from various plant and animal sources and have been identified in almost all types of living organisms. By far, the most common sterol in vertebrates is cholesterol **(2)**. The total cholesterol content measured in all mammalian species that have been examined is between 1 and 2 g/kg. Cholesterol is found in nearly all tissue types in both an esterified and unesterified form. The bulk of this sterol is in the unesterified form; however, certain tissues, eg, plasma, skin, and hair, contain a considerable portion of cholesterol as an ester (14–18).

Cholesterol serves two principal functions in mammals. First, cholesterol plays a role in the structure and function of biological membranes. The Δ^5 bond, the flat alternating trans–antitrans stereochemistry at the ring junctures, and the C17β-side chain of cholesterol form a rigid nonpolar nucleus. Combined with the hydrophilic C3–OH group, this nucleus has appropriate dimensions to interdigitate between the amphipathic phospholipids in the bilayer of biomembranes. The incorporation of cholesterol into the membrane bilayer changes its physical properties in a way that optimizes the efficiency of the biomembrane functions. Functions such as structural integrity, permeability (eg, optimization of the efficiency of active and passive transport of metabolites), and overall shape are enhanced. Secondly, cholesterol serves as a central intermediate in

the biosynthesis of many biologically active steroids, including bile acids, corticosteroids, and sex hormones (14–19).

3.1.2. Bile Acids and Alcohols

Bile acids have been detected in all vertebrates that have been examined and are a result of cholesterol metabolism. The C_{24} acid, 5β-cholanic acid [546-18-9] **(13)** is the structural derivative of the majority of bile acids in vertebrates. Most mammalian bile acids have a cis-fused A–B ring junction **(12)** resulting in a nonplanar steroid nucleus. Bile acids, like sterols, typically contain a C3α-hydroxyl group (lithocholic acid: 3α-hydroxycholanic acid [434-13-9]). Along with the C3α-hydroxyl group, bile acids may contain a hydroxyl at C7α (chenodeoxycholic acid [474-25-9]), at C12α (7-deoxycholic acid [83-44-3], **(14)**), and at C7α and at C12α (cholic acid, **(3)**) to name but a few. Bile acids with hydroxyl groups in the β-position at C3, C6, or C7 may occur, but in significantly reduced amounts compared to their α-counterparts. In an organism the hydroxyl groups may be progressively oxidized, leading to mixtures of keto acids and keto–hydroxy acids (17, 20–23).



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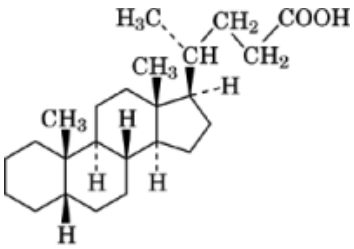

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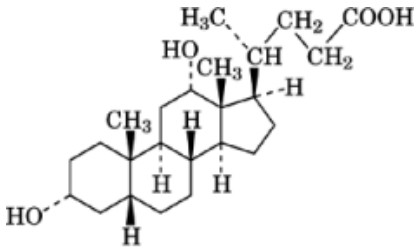


(14)

Bile salts, cholesterol, phospholipids, and other minor components are secreted by the liver. Bile salts serve three significant physiological functions. The hydrophilic carboxylate group, which is attached via an alkyl chain to the hydrophobic steroid skeleton, allows the bile salts to form water-soluble micelles with cholesterol and phospholipids in the bile. These micelles assist in the solvation of cholesterol. By solvating

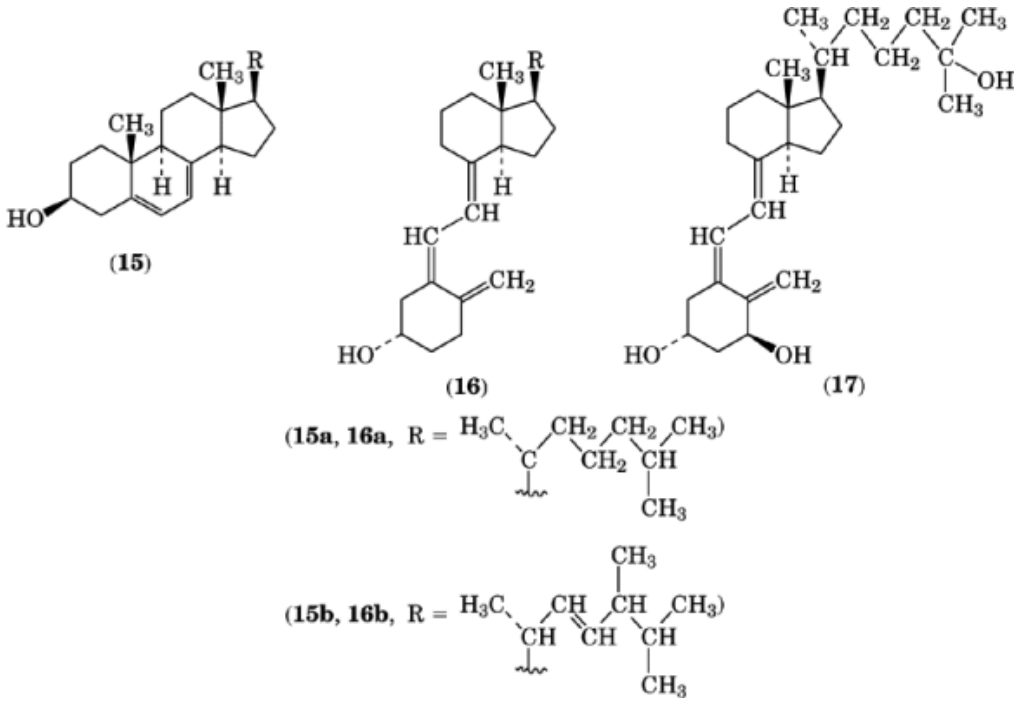


Fig. 2. Vitamin D: precursors (**15**), prohormones (**16**), and active hormone (**17**).

cholesterol, bile salts contribute to the homeostatic regulation of the amount of cholesterol in the whole body. Bile salts are also necessary for the intestinal absorption of dietary fats and fat-soluble vitamins (24–26).

3.2. Vitamin D

Vitamin D refers to a group of seco-steroids that possess a common conjugated triene system of double bonds. Vitamin D_3 [67-97-0] (**16a**) and vitamin D_2 [50-14-6] (**16b**) are the best-known examples (Fig. 2). Vitamin D_3 (**16a**) is found primarily in vertebrates, whereas vitamin D_2 (**16b**) is found primarily in plants. The relationship of vitamin D_2 and vitamin D_3 to the more classical steroid nucleus is demonstrated by their immediate sterol precursors, ergosterol [57-87-4] (provitamin D_2, **15b**) and 7-dehydrocholesterol [434-16-2] (provitamin D_3, **15a**), respectively. Sunlight converts 7-dehydrocholesterol (**15a**) into vitamin D_3 (**16a**) in the skin. The term vitamin is a misnomer. Vitamin D_3 is a prohormone that is converted into physiologically active form, primarily 1,25-dihydroxyvitamin D_3 (**17**), by successive hydroxylations in the liver and kidney. This active form is part of a hormonal system that regulates calcium and phosphate metabolism in the target tissues. In addition, 1,25-dihydroxyvitamin D_3 [32511-63-0] (**17**) has a diverse range of biological actions, including effects on cell differentiation and proliferation and control of other hormonal systems (27, 28). Rickets, a disease of early childhood characterized by faulty ossification of bone, and osteomalacia, a disease characterized by a failure of calcification of bone matrix, are due to a deficiency of vitamin D. Excessive doses of vitamin D can cause hypercalcemia and deposition of bone in the soft tissue (29). Only when skin irradiation is insufficient is there a true dietary requirement for vitamin D (30).

The irradiation pathway of precalciferol [50524-96-4] (**18**) is shown in Figure 3. When irradiated, ergosterol (**15b**) first undergoes a photochemically allowed conrotatory ring opening of the B-ring to form (**18**) as a central intermediate. Triene (precalciferol) (**18**) can undergo a thermally allowed 1,7-sigmatropic shift to form

vitamin D_2. Precalciferol also forms tachysterol [115-61-7] (**19**) by photochemical isomerization of the central triene double bond and lumisterol [474-69-1] (**20**) by conrotatory ring closure (30–33). Although a true steady state of the photochemical reaction is never achieved owing to the formation of over-irradiated products, the experimentally determined quasistationary-state yields are in good agreement with the calculated steady-state values (34, 35).

3.3. Steroid Hormones

Generally, steroid hormones are metabolically short-lived steroids produced in small amounts by various endocrine glands. They serve as chemical messengers that regulate a variety of physiological and metabolic activities in vertebrates. Steroid hormones bind to soluble, intracellular receptor molecules. These steroid hormone receptors, members of the steroid–thyroid–retinoid superfamily, are large proteins that act as ligand-regulated transcription factors controlling specific gene expression. The mechanisms of gene activation of steroid hormones are much too complex for this review and differ slightly for various classes of steroids (36). On a cellular level, however, the mechanism of steroid hormone activation has several common features. The monomeric or untransformed steroid receptor is located in the cytoplasm or the nucleus of almost every living cell. This untransformed receptor is complexed to a variety of heat shock proteins (HSPs), including HSP-90. Upon binding the hormone, the receptors dissociate from the HSPs and dimerize. In the nucleus of the cell, these dimeric steroid receptors bind to DNA and, together with a heteromeric complex of proteins, regulate gene transcription (37). Molecules that interfere with steroid hormone gene regulation are called antagonists or antihormones (38).

Steroid hormones can be subdivided into sex hormones (androgens, estrogens, and progestins) and corticosteroids (glucocorticoids and mineralocorticoids).

3.3.1. Sex Hormones

Androgens, estrogens, and progestins are steroids that are secreted primarily by the genital glands. From a chemical point of view, the division of the sex hormones into these three groups is convenient; however, they may possess common physiological properties. Therefore, the sex hormones are organ-specific rather than sex-specific (39, 40).

3.3.1.1. Androgens. These C_{19} steroids contain the basic perhydro-1,2-cyclopentenophenanthrene ring system with the C18 and C19 angular methyl group. A primary function of androgens is to maintain the male sex organs and secondary sex characteristics. Androgens were first isolated from the urine of males, females, and eunuchs. When androgens from testicular extracts were injected into castrated or immature males, restoration or development, respectively, of the male genital organs and secondary sexual characteristics were observed; hence the term male sex hormone. Examples of androgens are testosterone [58-22-0] **(21)**, dihydrotestosterone (DHT) [521-18-6] **(22)**, androsterone [53-41-8] **(24)**, and dehydroepiandrosterone [53-43-0] (DHEA) **(23)**. DHEA is one of the most abundant steroids in human males; however, it is not a potent androgen. Androsterone **(24)** is not very important in humans, whereas 4-androstene-3,17-dione is important in females. DHT is one of the most potent androgens in humans. Inhibitors of the enzyme 5α-reductase that convert testosterone to DHT are used to treat benign prostate hyperplasia (41–45).

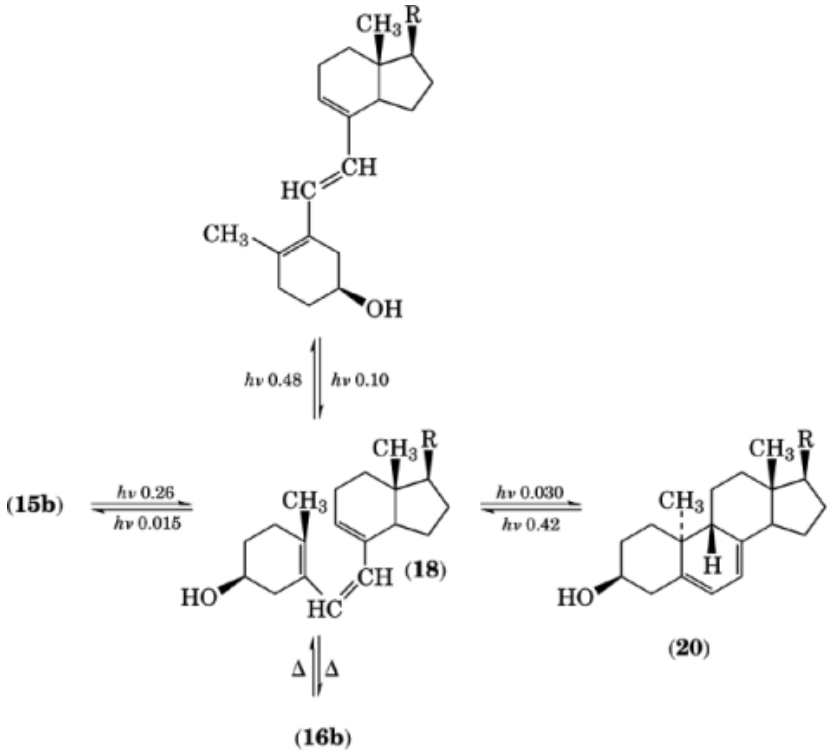
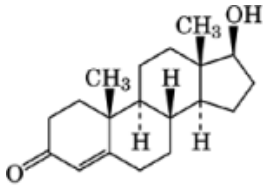


Fig. 3. Photochemical and thermal reactions of previtamin D_2 where the quantum yields for photochemical reactions are given by the arrow. R is as shown in Figure 2 for (**15b**) and (**16b**).



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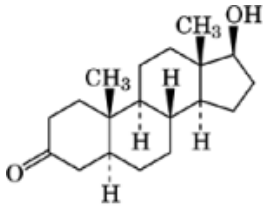

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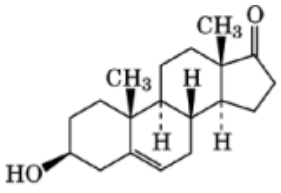
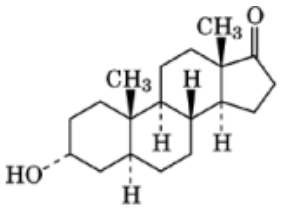


(23)

In addition to their masculinizing effects, androgens are anabolic (tissue-building) agents. Anabolic–androgenic steroids have been abused by athletes with the hope of improving their training, endurance, performance, or physique. Owing to the long-term toxicity of these natural and synthetic anabolic steroids in high doses and the potential for abuse, the U.S. Congress enacted the Anabolic Steroids Control Act in 1990. This Act requires the regulation of anabolic steroids in the same way that various opioid drugs, amphetamines, and barbiturates are regulated (46, 47).



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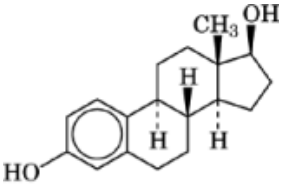

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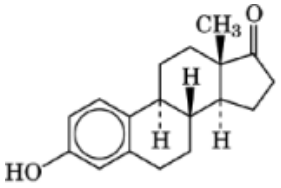
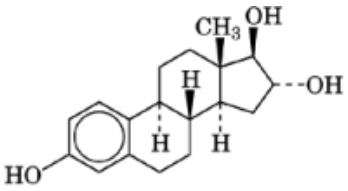


(26)

3.3.1.2. Estrogens. Estrogens were originally isolated between 1929 and 1935 and are characterized by having an aromatic A-ring and thus having a phenolic character. Estrogens stimulate the growth and development of the female reproductive organs and the secondary sex characteristics. Another primary function of estrogens along with progesterone, is to regulate the ovulatory cycle. Estrogens, as with all the steroid

hormones, are important for healthy growth and development in women and men. The main production site of estradiol [50-28-2] **(25)** is the female ovary; however, small amounts of estrogens are produced in testes and the adrenal cortex, and significant amounts of estrogens are produced by peripheral aromatization of local and circulating androgens in skin and fat. Estrone [53-16-7] **(26)** and estriol [50-27-1] **(27)** are estrogenically active metabolites of estradiol. Synthetic and natural estrogens play an important role in the treatment of osteoporosis in post-menopausal women. Antiestrogens are important for the treatment of breast cancer (48–51).



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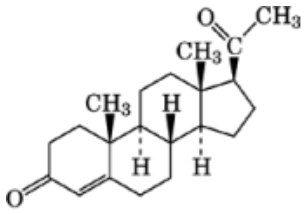


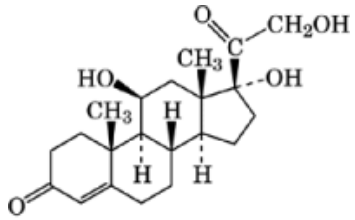
(28)

3.3.1.3. Progestins. Progesterone [57-83-0] **(28)**, the principal progestin in mammals, is secreted primarily by the corpus luteum of the ovary. A main responsibility of progesterone, together with estrogen, is to prepare the endometrium for pregnancy. Synthetic progestins have wide applications in gynecology and contraception (52–57).

3.3.2. Corticosteroids

Although the adrenal cortex secretes small amounts of androgens and estrogens, the major secretory steroids from this gland are called corticosteroids. Corticosteroids have several biological activities, including the regulation of electrolyte balance by mineralocorticoids and carbohydrate and protein metabolism by glucocorticoids. Over 30 steroids have been isolated from the adrenal cortex; however, the bulk of the biological activity of the corticosteroids has been attributed to only a few of these compounds. The division between glucocorticoids and mineralocorticoids can be confusing because many corticosteroids have contributing glucocorticoid and mineralocorticoid activities (58–61).

3.3.2.1. Glucocorticoids. Natural, potent glucocorticoids possess a Δ^4-3-one group, an oxygen substituent at C11β (necessary for agonism), and a C17β-2-hydroxyethan-1-one sidechain. Cortisol [50-23-7] **(29)**, corticosterone [50-22-6] **(30)**, and cortisone [53-06-5] **(31)** are typical examples. The principal effects of glucocorticoids are to mobilize fat and protein from tissues, utilize these nutrients to supply energy for the body, and decrease the rate of carbohydrate utilization for energy. Thus, they are diabetogenic and act as functional insulin antagonists. Glucocorticoids are only weakly active in respect to electrolyte metabolism. Glucocorticoids are also potent inhibitors of inflammation and have been the subject of intense synthetic and biological studies. They are used as therapeutics for a variety of different inflammatory diseases (62).



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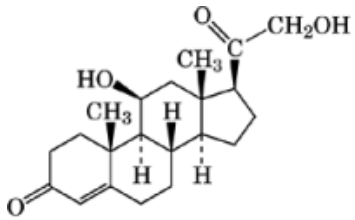

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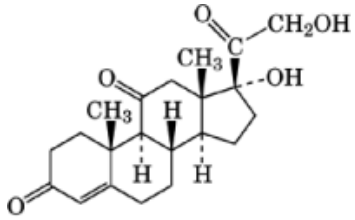
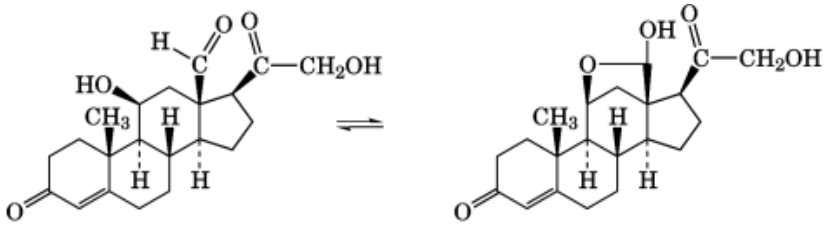


(31)

3.3.2.2. Mineralocorticoids. Aldosterone [6251-69-0] **(32)**, the most potent natural mineralocorticoid, also possesses a Δ^4-3-one group, an oxygen substituent at C11β, and a C17β-2-hydroxyethan-1-one side chain. In addition, the C18 of aldosterone is oxidized to an aldehyde. Mineralocorticoids, particularly aldosterone, act to retain sodium and to prevent the retention of excess potassium. Antimineralocorticoids have been used therapeutically as diuretics and as agents that regulate blood pressure (63–65).



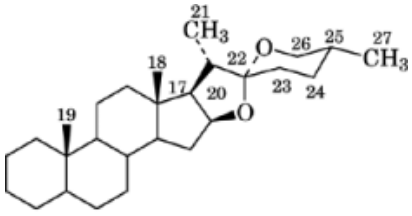
(32)

3.4. Other Natural Steroids

Steroids are nearly ubiquitous to all living organisms and have a variety of structural variations. Herein a brief overview of a few natural steroids from both plant and animal sources that have interesting biological activities or industrial importance is given.

3.4.1. Sapogenins and Saponins

Steroids isolated from a variety of plant sources that contain a spiroketal between hydroxyl moieties at C16 and C26 and a carbonyl at C22 are called sapogenins **(33)**.



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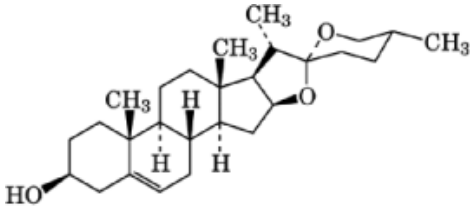
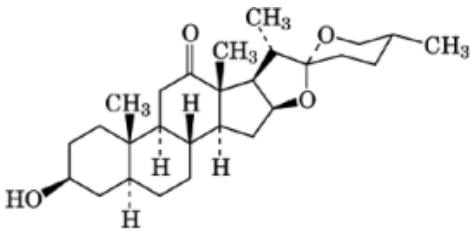
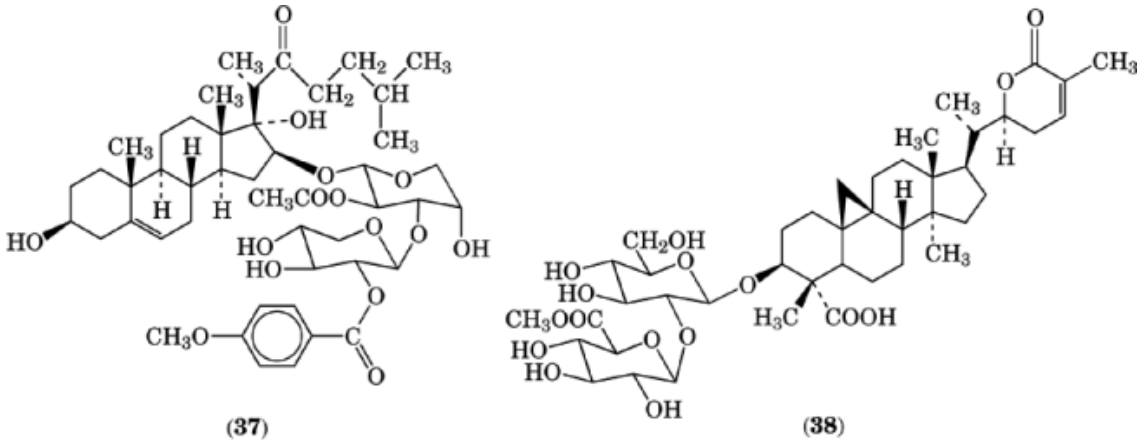


(34)

In addition to this spiroketal moiety, sapogenins generally contain a 3β-hydroxyl group. Since the late nineteenth century, more than 200 sapogenin aglycones have been isolated and characterized. Sapogenin aglycones, particularly diosgenin [512-04-9] **(34)**, hecogenin [467-55-0] **(35)**, and tigogenin [77-60-1] **(36)**, have been an important source of starting materials for the commercial steroid industry owing to their relative abundance in easily cultivated plants and their ease of isolation (66).

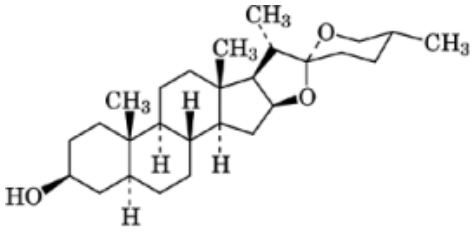


(35)



(37) (38)

Fig. 4. Examples of saponins: OSW-1 [145075-81-6] (**37**) and abrusoside E6″-methyl ester (**38**).



(36)

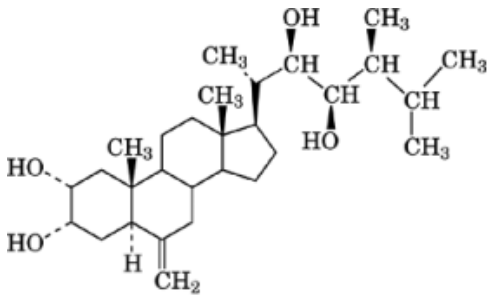
Saponins are widely distributed in plants and marine organisms and consist of a steroid or terpene skeleton attached to a saccharide (Fig. 4). In plants, for example, many sapogenins contain sugar residues attached to the 3β-hydroxyl group. Classical methods of isolation of saponins were inadequate for separating individual components; therefore, the characterization of most of the pure saponins arose only in the late 1970s with the integration of silica gel column chromatography, semipreparative hplc, preparative tlc, and special isolation techniques adapted to particular situations.

Because of diversity in structure, pharmacology, and biological activities, saponins have been studied for a number of different commercial applications. Many of these plant glycosides form a soapy lather when shaken with water and produce hemolysis when water solutions are injected into the blood stream. Thus, saponins have been used as detergents, foaming agents, and fish toxins. Although toxic to fish, saponins are nontoxic when ingested by humans, probably because of nonabsorption by the intestine (67). Another commercial application of saponins is in food flavoring. Depending on the structure, saponins can have either a bitter or sweet taste. For example, (**38**) is a saponin derivative that is 150 times sweeter than sugar. Also, saponins have been studied for their antifungal and anticancer activities. By way of illustration, the saponin OSW-1 (**37**) is extremely toxic to cancer cells but has little toxicity to normal cells when assayed *in vitro* (68) (see Fig. 4).

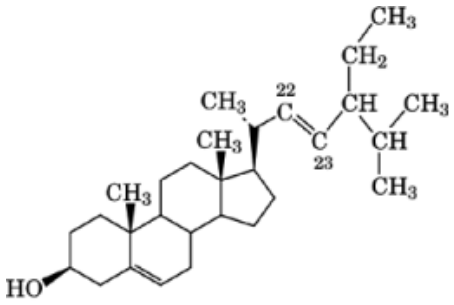
3.4.2. Plant Sterols

Sterols have been identified in almost all types of living organisms and can be isolated, in varying quantities, from many different plants. Similar to cholesterol, plant sterols have a structural and functional role in biological systems and serve as intermediates in the biosynthesis of an assortment of biologically active steroids. The plant sterol brassinolide [72962-43-7] **(39)** is a member of ubiquitous plant steroids termed brassinosteroids.

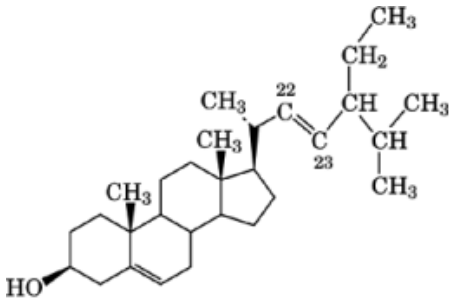
Brassinosteroids may play a role in the regulation of gene expression by light and therefore act as phytohormones (69). Stigmasterol [83-48-7] **(40)** and β-sitosterol [83-46-5] **(41)**, isolated primarily from soybeans, have become an important source of starting materials for the commercial steroid industry (70).



(39)



(40, Δ22,23-)

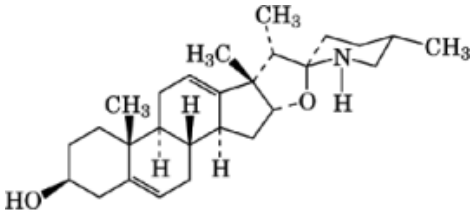


(41, saturated 22,23-)

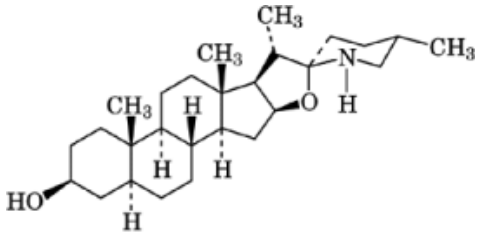
3.4.3. Steroid Alkaloids

Steroid alkaloids are compounds isolated from plants and some higher animals that possess the basic steroidal skeleton with nitrogen(s) incorporated as an integral part of the molecule. The nitrogen can be located within the perhydro-1,2-cyclopentenophenanthrene ring system or in a side chain. Because steroid alkaloids possess several different types of amine nitrogens and may also be conjugated to sugar residues, purification is often difficult. Preparative thin-layer chromatography and column chromatography with a variety of chromatographic materials and eluting solvents are the most common methods employed to isolate the steroidal bases.

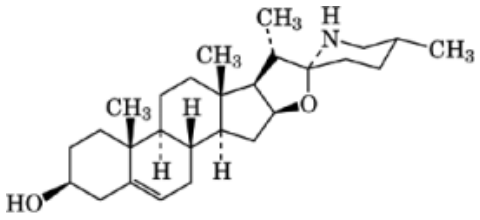
Steroid alkaloids have been isolated from four families of terrestrial plant sources (*Solanaceae*, *Liliaceae*, *Apocynaceae*, and *Buxaceae*), two animal sources (*Salamandra* and *Phyllobates*), and several marine sources. Steroid alkaloids can be classified based on structure and fall into a variety of categories. The spirosolanes contain a C_{27} cholestane skeleton with a C20 spiroaminoketal moiety, as exemplified by the most abundant members of this class, veramine [21059-48-3] **(42)**, tomatidine [77-59-8] **(43)**, and solasodine [126-17-0] **(44)**. Owing to shortages in diosgenin, solasodine and tomatidine have gained importance as alternative raw materials for industrial steroid synthesis. Solanidine-type steroidal alkaloids are a small subclass consisting of only 34 compounds as of 1993. They have three skeletal variations that have a nitrogen-containing 5,6-bicylic ring fused to C16–C17 or C15–C16 of the steroidal skeleton as a common structural feature (Fig. 5). The largest subclass of steroidal alkaloids is the secosoline bases; **(45)** is a general secosolanidine. This class is characterized by a 2-ethyl-piperidine-like base attached to the steroid nucleus at either C16 or C17, exemplified by etioline [29271-49-6] **(46)**. The pregnane-type alkaloids have one or more nitrogens attached to a pregnane skeleton, as illustrated by buxaprogenstine [113762-72-4] **(47)** and irehidiamine-A [3614-57-1] **(48)**. The buxus alkaloids, isolated from evergreen shrubs, contain carbon substitution at C4 and C14 and either a cyclopropane moiety between C9, C10, and C19 or the B-ring expanded diene as demonstrated by cyclobuxine-D [2241-90-9] **(49)** and buxamine-E [14317-17-0] **(50)**, respectively. The carbon substitution at C4 and C14 is indicative of an intermediate in the biogenic scheme between lanosterol and cholesterol-type steroids. Buxus alkaloids have been used as folk remedies for a variety of disorders, including venereal disease, tuberculosis, cancer, and malaria. The samanine, jerveratrum, and ceveratrum-type compounds all have a structurally altered C_{27} steroid skeleton. The samanine alkaloids have an expanded A-ring with the formation of an isoxazoline ring system and a cis-A–B ring junction, as shown by the primary alkaloid of this group, samandarine [467-51-6] **(51)**. The most abundant jerveratrum base, jervine [469-59-0] **(52)**, illustrates the structural variation of this class, including a five-membered C-ring, a six-membered D-ring, and a piperidine ring system fused to C17 of the tetracyclic steroidal system via a spirotetrahydrofuran-linking group. The second-largest subclass of steroid alkaloids, ceveratrum, contains over 100 members. As exemplified by imperialine [61825-98-7] **(53)**, ceveratrum alkaloids consist of a hexacyclic ring system and various degrees of oxidation at C3, C4, C6, C15, C16, and C20 (71–76). Ritterazines and cephalostatins are among steroid alkaloids recently isolated from marine sources that have received considerable attention. These compounds have two steroid nuclei linked at C2 and C3 by a pyrazine ring system, as shown by cephalostatin 1 [11288-65-9] **(54)**. When assayed *in vitro*, cephalostatins are among the most potent cytotoxins ever screened by the National Cancer Institute (77).



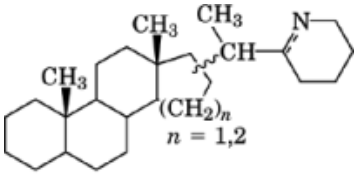
(42)



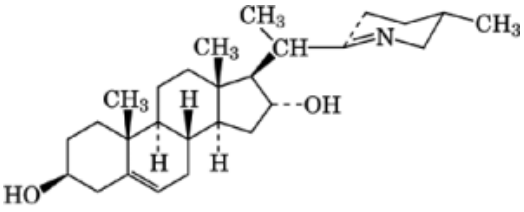
(**43**)



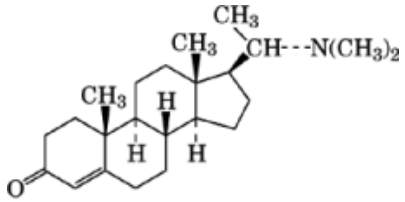
(**44**)



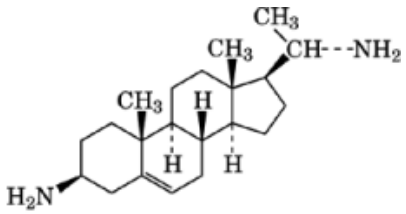
(**45**)



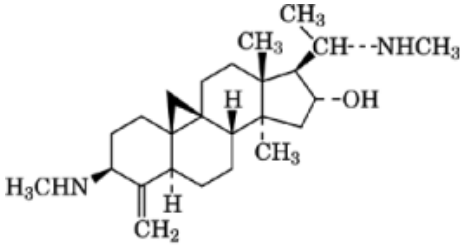
(**46**)



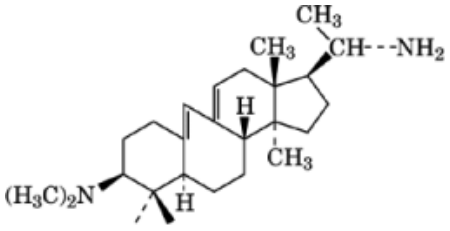
(**47**)



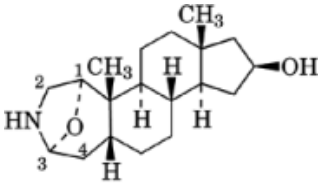
(**48**)



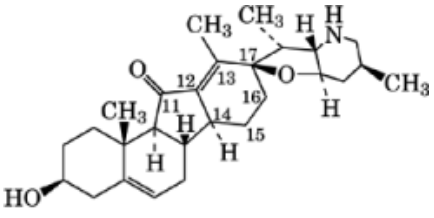
(**49**)



(**50**)



(**51**)



(**52**)

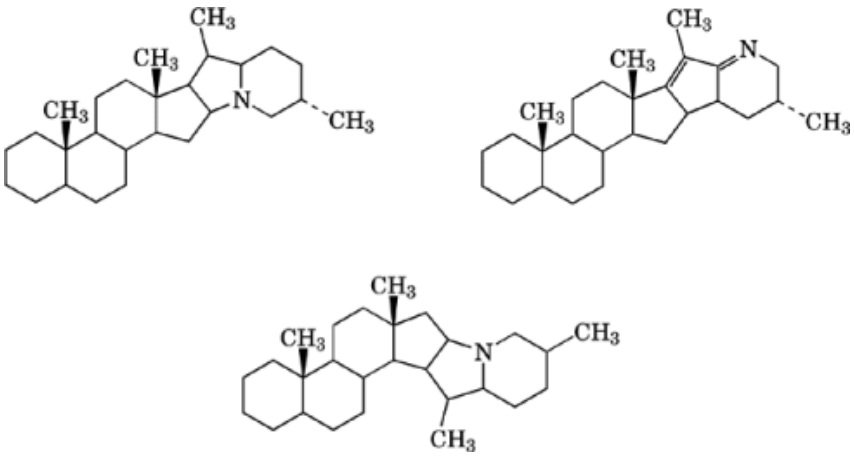
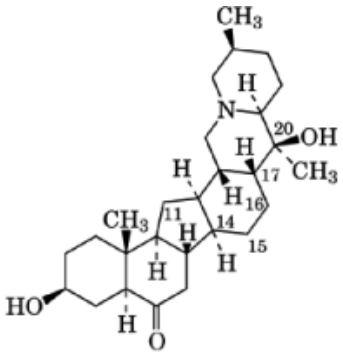
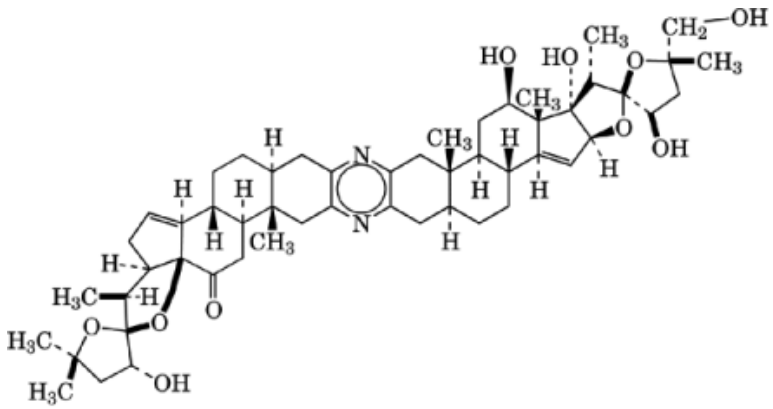


Fig. 5. Solanidine nuclei.



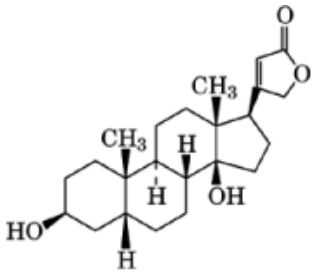
(**53**)



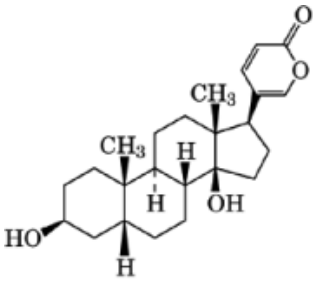
(**54**)

3.4.4. Cardiac Steroids

Cardiac steroids (steroid lactones) and corresponding glycosides are characterized by their ability to exert a powerful inotropic (increasing the force of cardiac contraction) effect, and are used both for their inotropic and antiarrhythmic properties. The two most prevalent cardiac aglycones are the cardenolides and bufadienolides. The cardenolides are C_{23} steroids that have a C17β-substituted five-membered lactone that is generally α,β-unsaturated, an unusual β-faced oxygen on C14, and a bile acid-like cis-A–B ring junction. Cardenolides are exemplified by digitoxigenin [143-62-4] **(55)** which is an active ingredient in digitalis. The bufadienolides differ in that they are C_{24}-steroids that possess a C17β-substituted six-membered lactone ring that generally has two degrees of unsaturation. Bufadienolides can be represented by bufalin [465-21-4] **(56)** which occurs in toad skin secretions.



(55)



(56)

Other structural variations in both series are the stereochemistry at C3 and the degree of oxidation on the nucleus and side chains. Cardiac steroids probably exert their inotropic effects by acting as specific, noncompetitive inhibitors of $Na^+ - K^+ - ATPases$, known as sodium pumps, and thus increasing intracellular Na^+. Elevated levels of Na^+ lead to increases in Ca^{2+} via a sarcolemmal $Na^+ - Ca^{2+}$ exchange; hence, more Ca^{2+} becomes available for the contractile apparatus (78–83).

3.4.5. Withanolides

Withanolides are C_{28}-steroidal lactones that are isolated from the Solanaceae plant family. Withanolides are characterized by an ergostane-type skeleton, the C17-side chain of which is transformed into a six-member lactone ring (Fig. 6). The withanolides and the related ergostanes are the only known natural steroids obtained from the same family that have representatives with both α- and β-orientations of the C17 side chain, such as withanolide D (**58**) and withanolide E (**59**) (84–88). Many biological properties of withanolides have been studied, including antitumor, antibiotic, immunomodulating, and insect antifeeding activities (89).

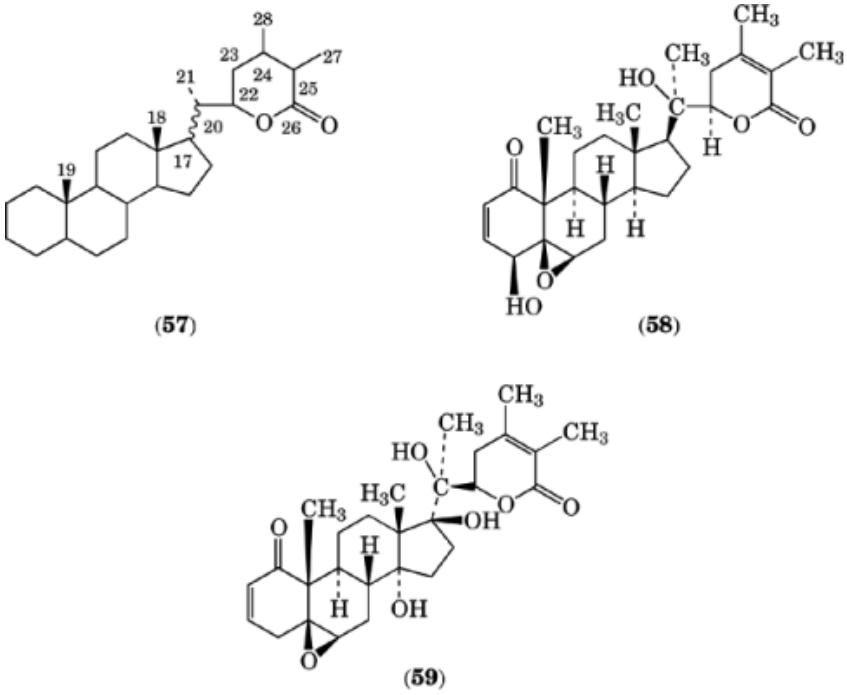
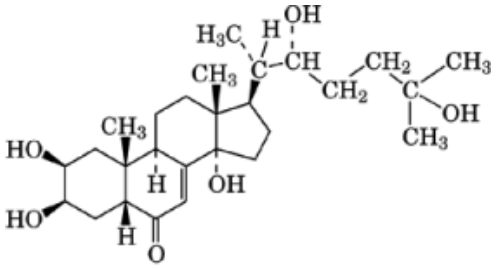


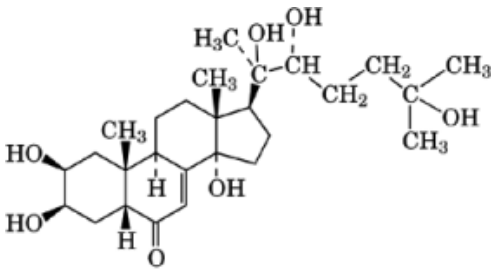
Fig. 6. Examples of withanolides: general structure (**57**); withanolide D [30655-48-2] (**58**); and withanolide E [38254-15-8] (**59**).

3.4.6. Ecdysteroids

Ecdysteroids have been studied since the 1940s. They can be isolated from many species of the animal kingdom that belong to the phyla Protomia, eg, insects, worms, and arthropods, as well as a variety of different plant species. Ecdysteroids include the molting hormones; however, not all the over 60 ecdysteroids that have been isolated are active hormones. Ecdysteroids from animals are referred to as zooecdysteroids and from plants are referred to as phytoecdysteroids. Ecdysteroids all contain a cholestane skeleton with an A–B cis-ring junction and polyhydroxylation, including the 2β-, 3β-diol, 14α-hydroxy, and C17 side-chain hydroxylations, as exemplified by ecdysone [3604-87-3] **(60)** and 20-hydroxyecdysone [5289-74-7] **(61)**. In addition, ecdysteroids are generally oxidized at C6 with a double bond at C7–C8 to form a cyclohexenone in the B-ring. Ecdysone **(60)** was the first insect hormone that was isolated (90) and characterized (91); however, its metabolite, 20-hydroxyecdysone **(61)**, is recognized to be the universal molting hormone of insects and crustaceans. In addition to their molting properties, ecdysteroids have been studied for their antitumor and antimicrobial activities (92–96).



(60)



(61)

3.4.7. Marine Sterols

Throughout the 1980s and 1990s, several hundred unique sterol structures have been elucidated from a variety of marine invertebrates. A single nucleus can be used to describe most terrestrial sterols, but no single template suffices for marine sterols. Basic substructures for marine sterol nuclei have been proposed, eg, (**62**), conventional; (**63**), 19-*nor*; (**64**), A-*nor* (Fig. 7). In addition, seco-sterols, such as 9,11-*seco*-sterols (**65**) and 8,9-*seco*-sterols (**66**), and highly degraded sterol nuclei have been observed. Much of the diversity of this group of sterols is incorporated into the C17 side chain. These variations include cyclopropane, cyclopropene, acetylene, allene, polyalkylated, 26-*nor*, and polyoxygenated side chains. Similar to cholesterol, marine sterols play a critical role in both the physiology and biochemistry of biological systems. In addition, marine sterols may have an ecological role in the marine environment (98–102).

3.4.8. Steroid Antibiotics

The steroid antibiotics are a structurally diverse class of steroids that have a common biological function, ie, antibacterial, antifungal, antiviral, or antitumor activities. This group of compounds can overlap with other steroid classes listed above. Fusidic acid [6990-06-3] **(67)**, helvolic acid [29400-42-8] **(68)**, and cephalosporin P_1 [13258-72-5] **(69)** exemplify a set of antibacterial steroids that contain a prolanostane skeleton with an unique trans–syn–trans–antitrans stereochemistry. This stereochemical relationship forces the B-ring into an unusual "boat" configuration (103). These compounds inhibit the growth of gram-positive bacteria by inhibiting protein synthesis, but have little activity against gram-negative bacteria (104, 105).

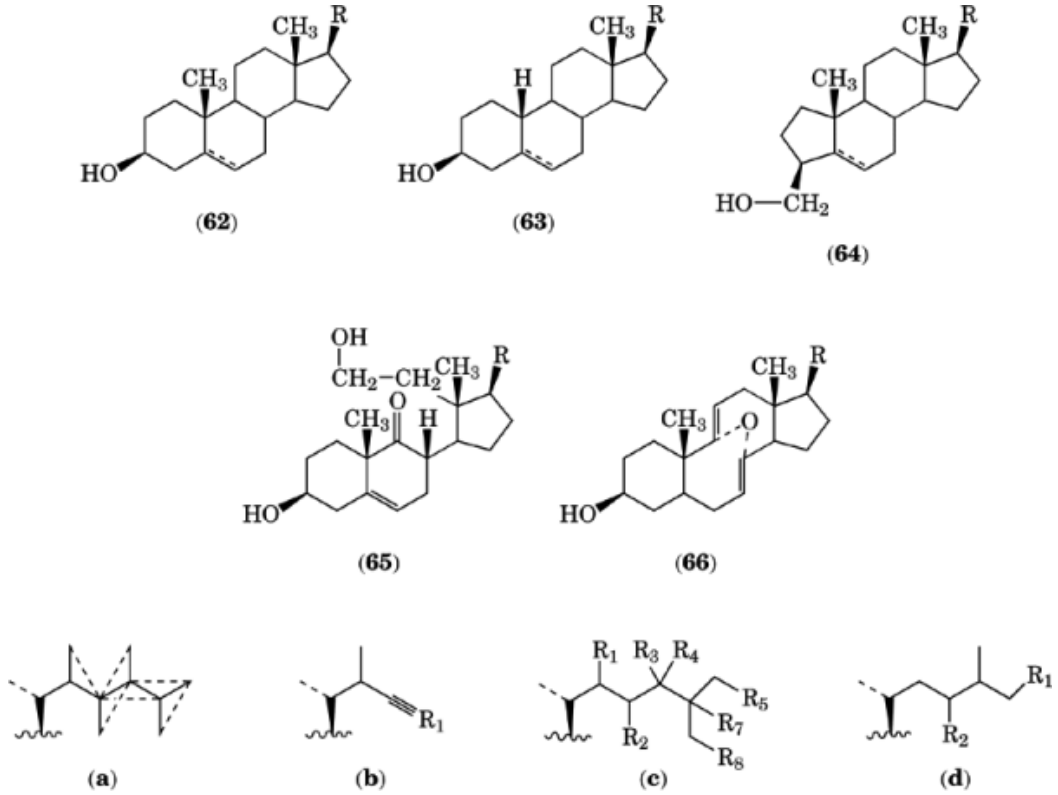
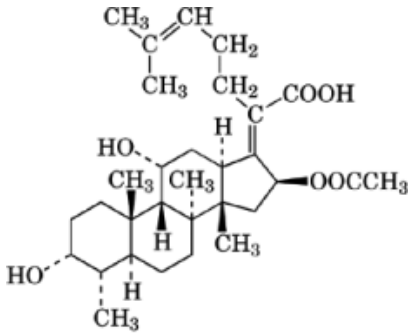
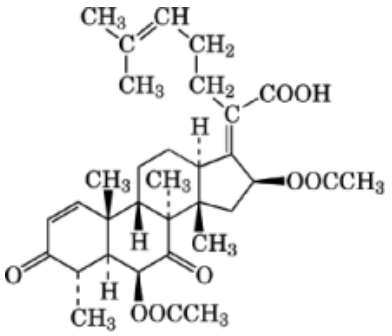


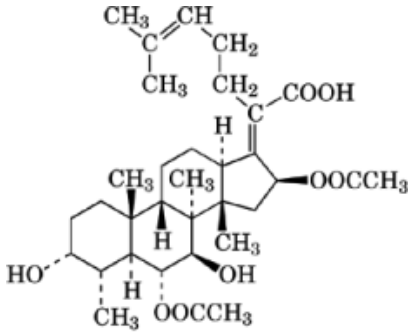
Fig. 7. Structural diversity of marine sterols where R = a variety of unique side chains, including (**a**) cyclopropa(e)ne, (**b**) acetylene, (**c**) polyalkylated, and (**d**) the 26-*nor* side chain.



(67)

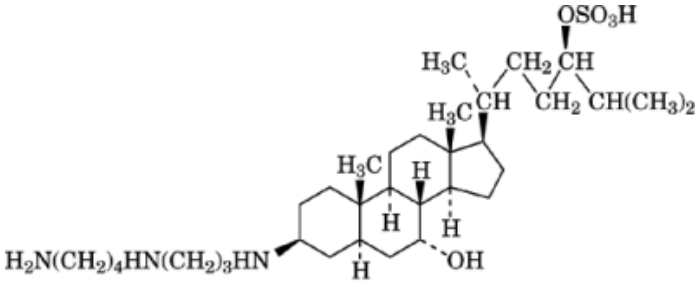


(**68**)

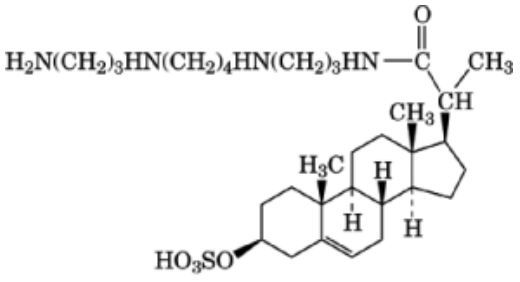


(**69**)

An antibiotic isolated from the tissues of the dogfish shark is the steroid alkaloid squalamine [148717-90-2] **(70)**. Squalamine is a rare adduct of spermidine with an anionic bile acid intermediate. Squalamine is a broad-spectrum antibiotic that exhibits potent antimicrobial activity against fungi, protozoa, viruses, and both gram-negative and gram-positive bacteria (106). The precise mechanism of the antimicrobial activity of squalamine has not been identified. However, squalamine **(70)** could inhibit microbe growth by generating ion channels or pores in microbial membranes or by binding to microbial DNA. The total synthesis of squalamine from commercially available bile acids has been realized and the biological activity of squalamine has been mimicked by analogues that are more easily synthesized **(71)** (107, 108).



(**70**)



(71)

4. Biosynthesis

Steroids are members of a large class of lipid compounds called terpenes. Using acetate as a starting material, a variety of organisms produce terpenes by essentially the same biosynthetic scheme (Fig. 8). The self-condensation of two molecules of acetyl coenzyme A (CoA) forms acetoacetyl CoA. Condensation of acetoacetyl CoA with a third molecule of acetyl CoA, then followed by an NADPH-mediated reduction of the thioester moiety produces mevalonic acid [150-97-0] (**72**). Phosphorylation of (**72**) followed by concomitant decarboxylation and dehydration processes produce isopentenyl pyrophosphate. Isopentenyl pyrophosphate isomerase establishes an equilibrium between isopentenyl pyrophosphate and 3,3-dimethylallyl pyrophosphate (**73**). The head-to-tail addition of these isoprene units forms geranyl pyrophosphate. The addition of another isopentenyl pyrophosphate unit results in the sesquiterpene (C_{15}) farnesyl pyrophosphate (**74**). Both of these head-to-tail additions are catalyzed by prenyl transferase. Squalene synthetase catalyzes the head-to-head addition of two achiral molecules of farnesyl pyrophosphate, through a chiral cyclopropane intermediate, to form the achiral triterpene, squalene (**75**).

Stereospecific 2,3-epoxidation of squalene, followed by a nonconcerted carbocationic cyclization and a series of carbocationic rearrangements, forms lanosterol [79-63-0] (**77**) in the first steps dedicated solely toward steroid synthesis (109, 110). Several biomimetic, cationic cyclizations to form steroids or steroidlike nuclei have been observed in the laboratory (111), and the total synthesis of lanosterol has been accomplished by a carbocation–olefin cyclization route (112). Through a complex series of enzyme-catalyzed reactions, lanosterol is converted to cholesterol **(2)**. Cholesterol is the principal starting material for steroid hormone biosynthesis in animals. The cholesterol biosynthetic pathway is composed of at least 30 enzymatic reactions. Lanosterol and squalene appear to be normal constituents, in trace amounts, in tissues that are actively synthesizing cholesterol.

The conversion of cholesterol **(2)** to pregnenolone [145-13-1] (**78**) is accomplished primarily through enzymatic systems in the adrenocortical and gonadal mitochondria. This conversion appears to be rate-limiting and therefore is regarded as the control point for the entire steroid hormone biosynthetic process. An abbreviated metabolic pathway for the corticosteroids and sex hormones is shown in Figure 9; however, many other metabolites play various roles in biosynthesis and excretion of steroids. The oxidation–reduction steps on the metabolic pathways are generally reversible, whereas the steps that include an oxidative C–C cleavage of the side chain are irreversible (40, 113–119).

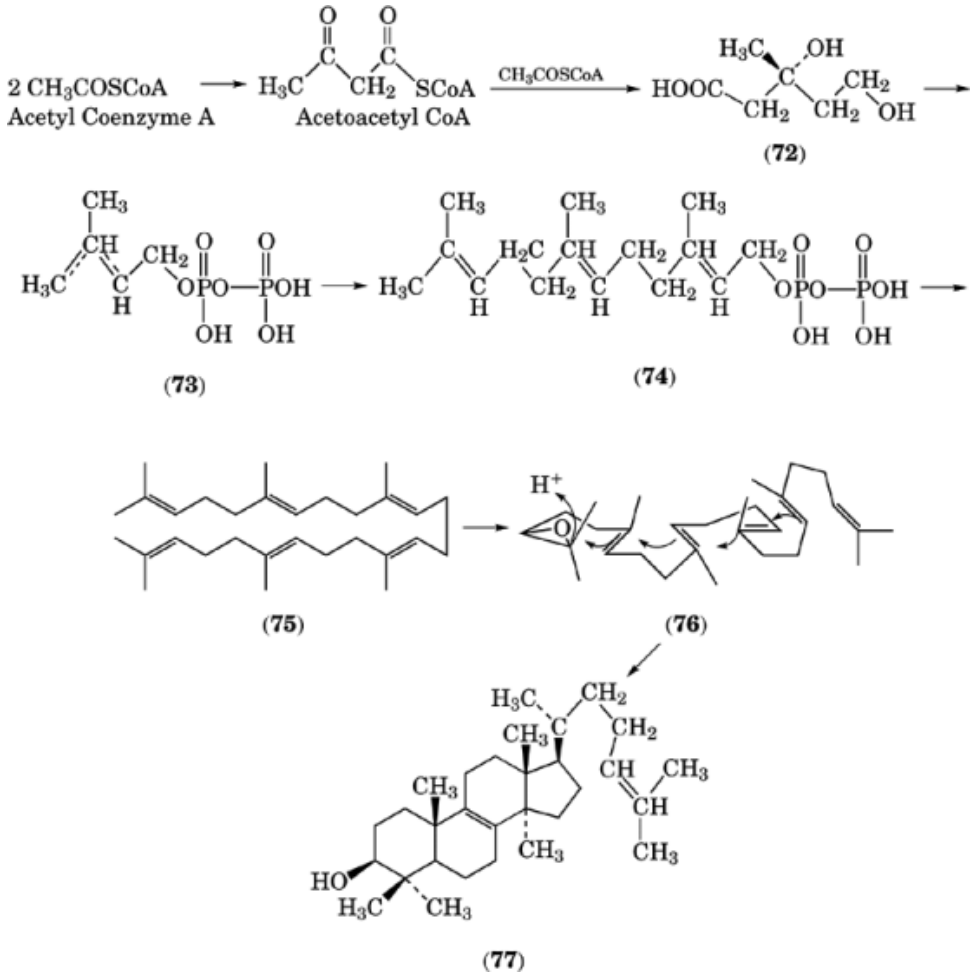


Fig. 8. Abbreviated terpene biosynthesis.

5. Manufacture and Synthesis

There are three general processes that are used, as of ca 1996, worldwide for steroid production: (*1*) direct isolation from natural sources, (*2*) partial synthesis from steroid raw materials that have been isolated from plants and animals, and (*3*) total synthesis from nonsteroidal starting materials (120).

5.1. Direct Isolation

The two most important classes of steroid pharmaceuticals that are isolated directly from natural products are some estrogens and most cardiac steroids. Compounds with estrogenic activity have been isolated from different sources. Urine from pregnant women and from pregnant mares has been used for the commercial production of estrogens. The overall amount of estrogens from pregnant mare urine is generally 10 times greater than that of pregnant human urine; however, the hormone from pregnant mares is more highly conjugated than in

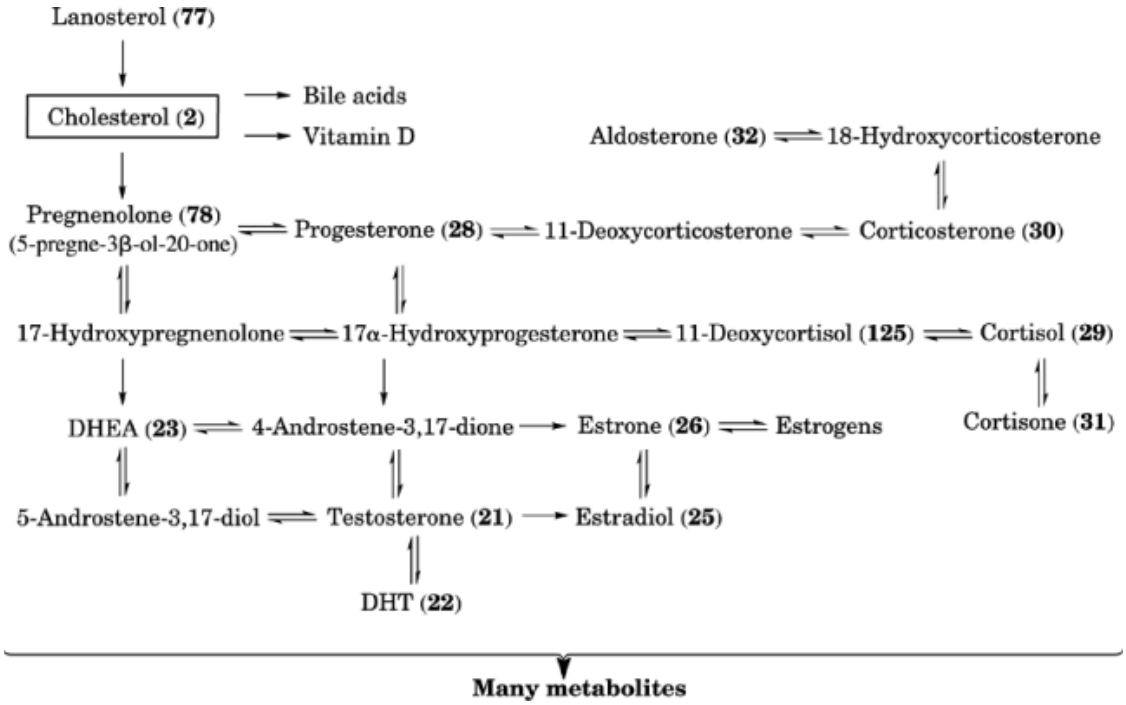


Fig. 9. Abbreviated steroid biosynthesis where DHEA=dehydroepiandrosterone and DHT=dihydrotestosterone.

humans and only 10–25% of the total is directly extractable. The isolation of estrogens from natural sources has been essentially the same since ca 1960 and has been reviewed (121).

Cardiac steroids occur in small amounts in various plants with a wide geographical distribution. The purple foxglove *Digitalis purpuras* has been used for centuries as both a drug and a poison. Isolation and characterization of the various cardiac steroids have been reviewed (122, 123).

5.2. Partial Synthesis

5.2.1. Raw Materials and Extraction

The variety of natural sources of steroid raw materials is vast, and the exact details of manufacturing processes are ambiguous closely held industrial secrets. However, the most widely utilized raw materials for the partial synthesis of steroids appear to be the following: (*1*) the sapogenins, including diosgenin **(34)**, hecogenin **(35)**, and tigogenin **(36)**; (*2*) the structurally related steroid alkaloids, including tomatidine **(43)** and solasodine **(44)**; (*3*) sterols, such as cholesterol **(2)**, stigmasterol **(40)**, and β-sitosterol **(41)**; and (*4*) bile acids, such as deoxycholic acid **(14)** (124).

Plants of the genus *Dioscorea*, which include *D. deltoidea*, *D. prazeri*, and *D. tubers*, are the most common source of diosgenin. This genus occurs abundantly in tropical and subtropical regions throughout the world. A variety of methods are used for the isolation of diosgenin. In a generalized process plant tubers are dried and powdered. This powdered material is first hydrolyzed with aqueous acid or enzymes, then extracted with an organic solvent such as petroleum ether. Diosgenin is isolated after recrystallization (125).

A pilot plant in India has been established to extract fiber, pulp, and juice from the leaves of sisal plants. The fiber is sold directly or used to manufacture rope, the crushed pulp is used in paper processing, and the

juice is an excellent source of hecogenin. During a three- to five-day fermentation of the juice, partial enzymatic hydrolysis causes hecogenin to precipitate as the hemisaponin in the form of a fine sludge. This sediment is hydrolyzed with aqueous hydrochloric acid, neutralized, and filtered. This filter cake is washed with water and extracted with alcohol. The yield of hecogenin varies between 0.05 and 0.1% by the weight of the leaf (126).

Owing to periodic fluctuations in the price of diosgenin, alternative raw materials such as solasodine have been used for the synthesis of steroid drugs. Solasodine can be isolated from a medley of genera and species of plants found worldwide. Generally, solasodine appears in plants as a glycoside at the 3-position of the steroid. Solasodine is isolated by extraction of the fresh or dried glycoalkaloid-containing tissue with either alcohol or aqueous acids. Alcohol extraction yields more total solasodine but with significantly higher levels of contaminants. Therefore, overall yields of the two extraction processes are comparable owing to losses during the purification of the alcohol extracts (127).

In the United States, the plant sterols stigmasterol and β-sitosterol are a significant raw material for the synthesis of antiinflammatory glucocorticoids and other steroid hormones. Extracts from soybean oil by-products contain 12–25% stigmasterol along with large amounts of sitosterols. The industrial separation of these nearly identical sterols was accomplished in high yields and purity using a multistage countercurrent crystallization from selected solvents (70).

In addition to the isolation of steroid raw materials from whole plants, plant tissue cultures have been investigated as an alternative source of these steroids. Despite many advances (128), there are no industrial applications of plant cell cultures for the production of steroids (129, 130).

5.2.2. Methods of Partial Synthesis

Partial syntheses are done typically by chemical degradation or fermentation/biotransformation.

5.2.2.1. Chemical Degradation. Initial efforts toward the synthesis of corticosteroids in Europe and in the United States used 7-deoxycholic acid as a starting material. These syntheses included multistep oxygen transposition and C17 degradation to form various 11-oxy-steroids (131). The first significant breakthrough in the commercial synthesis of steroids was the chemical degradation of diosgenin. The Marker degradation became the principal method for commercial steroid synthesis in the 1940s and 1950s, and modifications of this process are still in use in the 1990s (Fig. 10). When diosgenin is heated to approximately 200°C in acetic anhydride, elimination and acetylation of the oxygen in the F-ring produce the bis-acetylated enol ether (**79**). Oxidative cleavage of the enol ether of (**79**) with chromium trioxide followed by elimination of the C16-acyl-oxygen results in steroid (**80**) [1162-53-4]. Selective hydrogenation of the α,β-unsaturated ketone in the D-ring from the sterically less hindered α-face forms pregnenolone (**78**). Pregnenolone is readily converted into progesterone **(28)** under oxidative conditions (132).

This process was improved and expanded (133) to provide starting materials for the C19-sex hormones that include estrogens and androgens (see Fig. 10). Oxidative cleavage of enol ether (**79**) with chromium trioxide followed by elimination of the C16-acyl-oxygen in hot acetic acid affords pregnenolone acetate (**81**) in over 80% yield. Pregnenolone acetate (**81**) can be converted to progesterone by methods similar to the Marker process. The cleavage of the C17 side chain begins with the treatment of (**81**) with hydroxylamine to afford the C20 oxime (**82**). Beckman rearrangement of (**82**) affords the ene-amide (**83**). Mild acid hydrolysis of (**83**) results in dehydroepiandrosterone acetate [853-23-6] (**84**) (133). The same processes have been applied to the structurally similar steroid alkaloids solasodine **(44)** and tomatidine **(43)**.

Unlike diosgenin, hecogenin possesses a C12-keto group and is saturated at C5–C6. The keto group at C12 in the C-ring allows for a series of chemical steps to transpose this oxygen to the biologically important C11-position (Fig. 11). Bromination of hecogenin acetate (**85**) in either benzene, carbon tetrachloride, or dioxane produces the 11,23-dibromide (**86**) in over 50% recrystallized yield with 32% recovered, usable starting materials. Heating a biphasic mixture of (**86**) and sodium hydroxide in *t*-butyl alcohol and water, followed by acetylation and zinc-mediated debromination forms the bromine-free acetate (**87**) in approximately 80% yield.

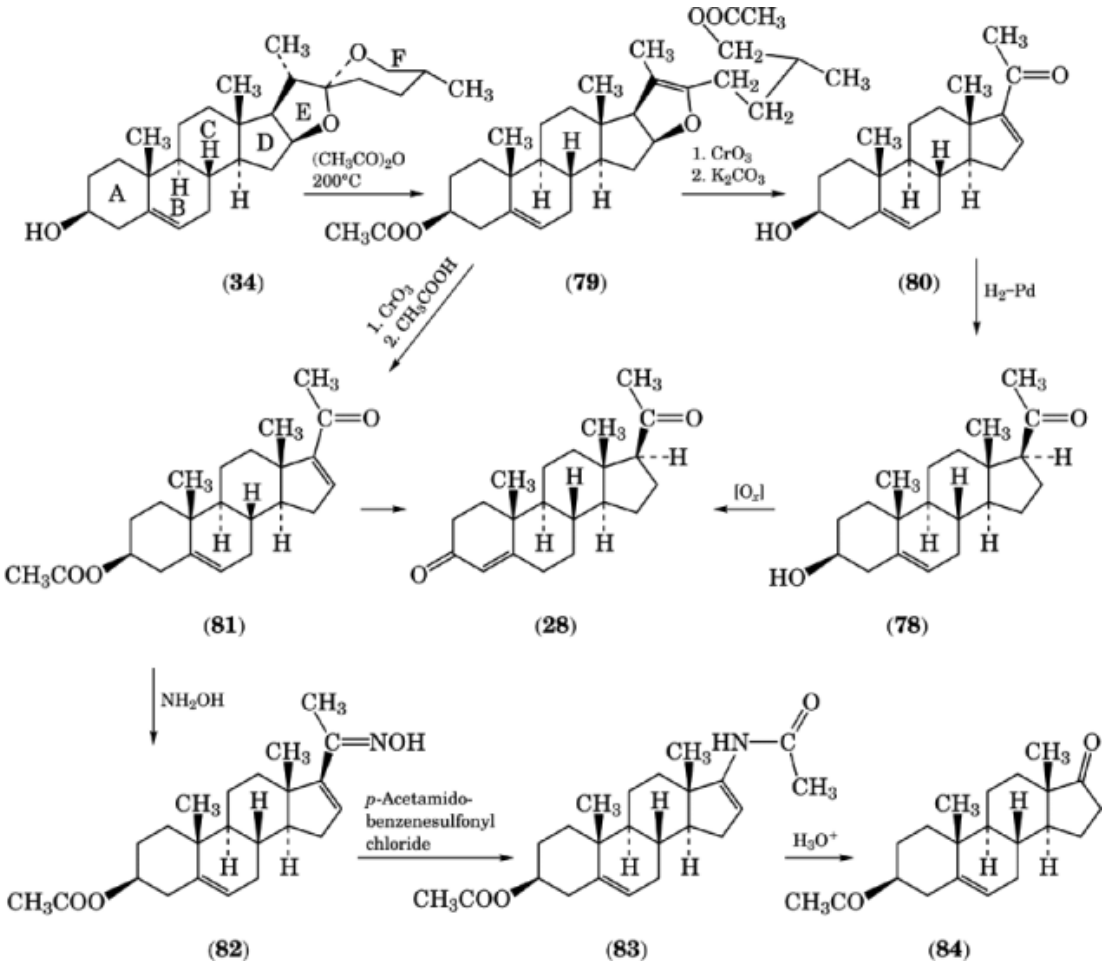


Fig. 10. Chemical degradation of diosgenin.

Finally, a dissolving metal reduction of (**87**) results in 11-ketotigogenin [4802-74-8] (**88**). The spiroketal ring can be degraded by methods similar to the degradations discussed above. The efficient synthesis of 11-ketotigogenin established a commercial production of cortisol from hecogenin (134).

Another commercial method that has been used for the production of progesterone is the chemical degradation of the side chain of stigmasterol (Fig. 12). Oxidation of the C3-hydroxyl of stigmasterol **(40)** with concomitant double-bond migration results in (**89**). The resultant C4–C5 double bond in the A-ring is electron-deficient and less reactive under the subsequent reaction conditions. Selective ozonation and oxidation cleavage of the side chain double bond of (**89**) result in aldehyde (**90**). Enol acetate formation of the C22-aldehyde (**91**), followed by a second ozonation and cleavage of the resultant side-chain double bond, yields progesterone **(28)** (135).

Interest in the synthesis of 19-norsteroids as orally active progestins prompted efforts to remove the C19 angular methyl substituent of readily available steroid precursors. Industrial applications include the direct conversion of androsta-1,4-diene-3,17-dione [897-06-3] **(92)** to estrone [53-16-7] **(26)** by thermolysis in mineral

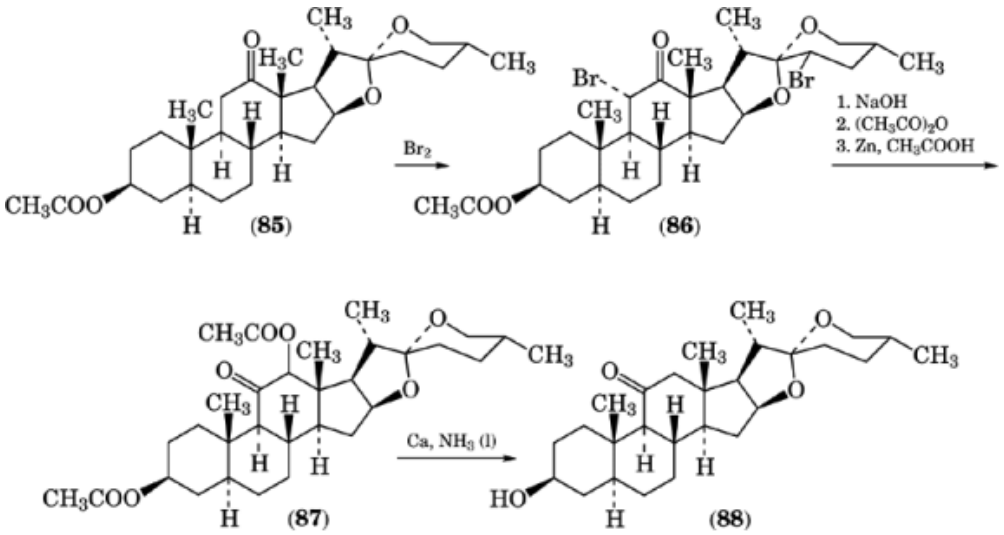


Fig. 11. Carbonyl transposition of hecogenin.

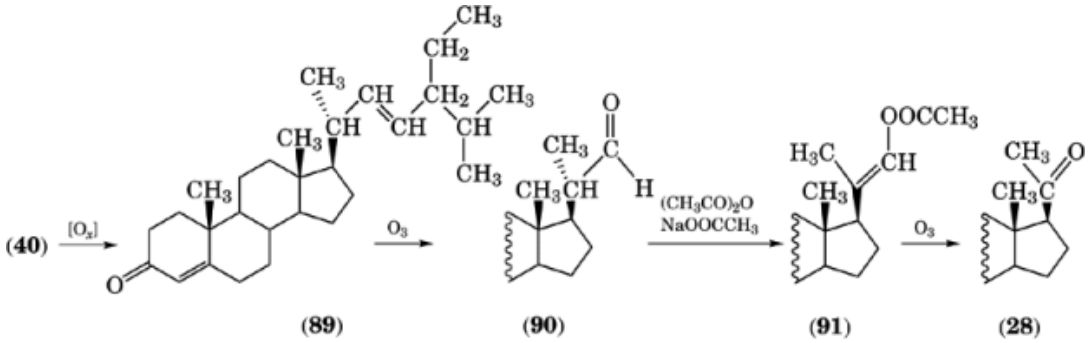
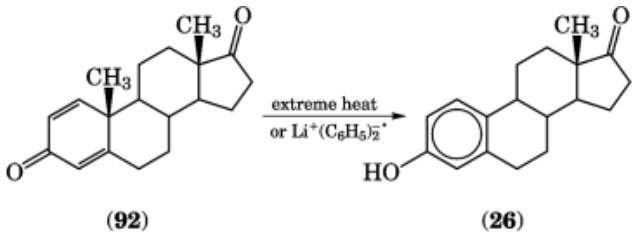


Fig. 12. Chemical degradation of stigmasterol.

oil at about 500°C (136), and reductive elimination of the angular methyl group of the 17-ketal of the dione [2398-63-2] (**93**) with lithium biphenyl radical anion to form the 17-ketal of estrone [900-83-4] (**94**) (137).



Another method to prepare 19-norsteroids is first to oxidize the C19 angular methyl substituent, followed by reductive decarboxylation or decarbonylation of the resultant C19 lactone, carboxylic acid, or aldehyde. All methods of oxidation of angular methyl groups proceed through high energy intermediates capable of oxidizing

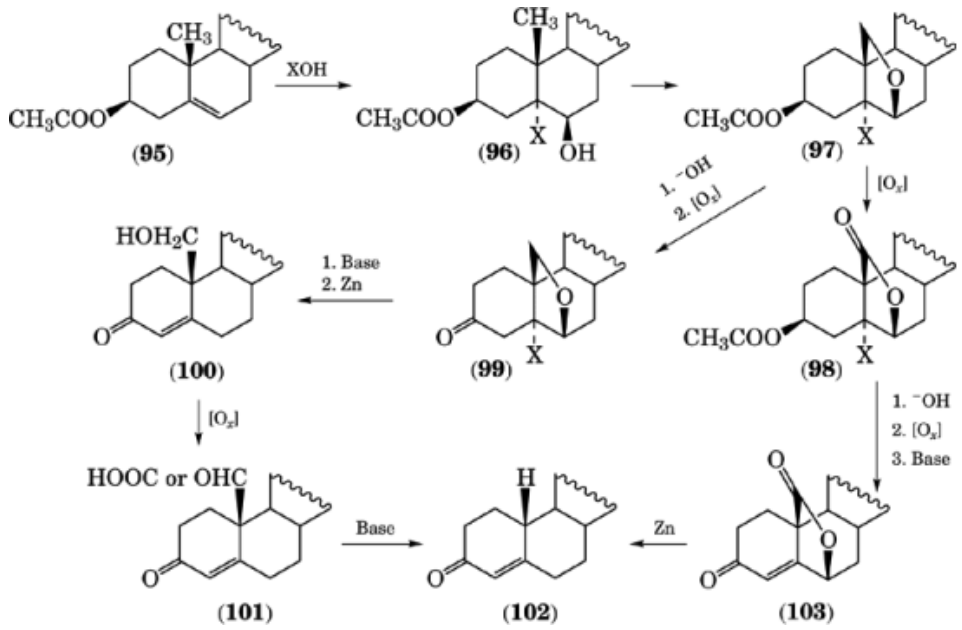


Fig. 13. Chemical degradation to 19-norsteroids where $_{X=Cl}$, Br.

unactivated CH bonds. These high energy intermediates are generated from an intramolecular heteroatom in close proximity to the angular methyl group. Practical routes to 19-norsteroids are shown in Figure 13. The addition of hypohalous acid to Δ^5-steroids (**95**) gives 5α-halo-6β-carbinols (**96**). Cyclization of this 6β-alcohol onto the C19 angular methyl substituent can occur under a variety of different conditions. For example, ether (**97**) is produced by the treatment of (**96**) with lead tetraacetate under thermal or photolytic conditions. In addition, treatment of (**96**) with lead tetraacetate and iodine proceeds though an intermediate hypoiodite (138) that, after homolytic decomposition, results in ether (**97**) (139). Ether (**97**) can be either oxidized to lactone (**98**) or the C3-acetate hydrolyzed and oxidized to the C3-ketone (**99**). Elimination of the halogen of (**99**) followed by C6-oxygen reduction yields the C19 alcohol (**100**). Oxidation of (**100**) to the aldehyde or carboxylic acid (**101**), followed by decarbonylation or decarboxylation, respectively, results in the 19-norsteroid (**102**). Alternatively, acetate hydrolysis, C3-oxidation, and elimination of (**98**) forms lactone (**103**). Concomitant C6-reduction and decarboxylation of (**103**) yield the 19-norsteroid (**102**) (140). In a similar process, the C19 and C18 angular methyl groups can be oxidized by photolytic activation of a nearly nitrite ester (141). A free-radical activation of the C18-angular methyl moiety has been exploited in a number of synthetic approaches to aldosterone (142, 143).

5.2.3. Fermentation/Biotransformation

In a search to decrease the cost and increase the efficiency of steroid synthesis, commercial biotechnology operations have focused on microbial agents for specific transformations of individual steroid substrates. The regio- and stereoselective hydroxylation of every site on virtually every steroid nucleus is possible (144). Many of these hydroxylation steps are of commercial importance. For example, the 9α-, 11α-, 11β-, and 16α-hydroxylations are key steps in the industrial synthesis of synthetic corticosteroid antiinflammatory drugs. These steps are accomplished almost exclusively by microbial transformations. In addition to hydroxylations, other useful microbial oxidations of steroids include alcohol oxidations, epoxidations, oxidative cleavage of carbon–carbon bonds (eg, C17 side-chain cleavage), introduction of double bonds, peroxidations, and heteroatom oxidations.

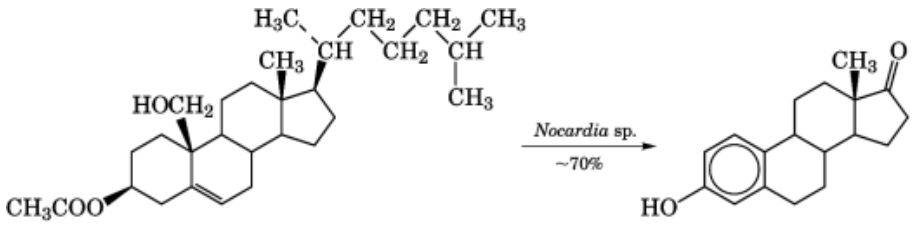
Other invaluable microbial steroid transformations include reductions, degradations, A-ring aromatization, resolutions, isomerizations, conjugations, hydrolyses, heteroatom introduction, and sequential reactions (120, 145, 146).

There are two principal biotechnological applications dealing with steroids. Microbial agents are used for processing raw materials into useful intermediates for general steroid production and for specific transformations of steroids to advanced intermediates or finished products (120, 145).

5.2.3.1. Processing Raw Materials. Along with the aforementioned chemical methods of processing steroid raw materials, microbial transformations have been and are used in a number of commercial degradation processes. The microbial degradation of the C17 side chain of the two most common sterols, cholesterol **(2)** and β-sitosterol **(41)**, is a principal commercial method for the preparation of starting materials in Japan and the United States.

Many microorganisms have been found that partially or completely degrade cholesterol **(2)** (147). Enzyme inhibitors or modified microbial agents have been used to control these degradations in order to form commercially useful steroidal intermediates (Fig. 14). When mixed with metal ions (Ni, Co, Pb, or Se) (148), chelating agents (149), or 8-hydroxyquinoline (150), various mycobacteria have been demonstrated to produce significant quantities of androsta-1,4-diene-3,17-dione [63-05-8] (**104**) from cholesterol (**2**). In a commercialized process for the microbial conversion of cholesterol to androsta-1,4-diene-3,17-dione, the 9α-hydroxylation step was inhibited by α,α-dipyridyl (151). In another commercial process, uv-generated mutations of *Mycobacterium* sp. have been used to produce androsta-4-ene-3,17-dione (**104**) and androsta-1,4-diene-3,17-dione [897-06-3] **(92)** from β-sitosterol (see Fig. 14) (152). In a similar industrial process, a mutant of *Mycobacterium fortuitum* degraded β-sitosterol to 9α-hydroxyandrosta-4-ene-3,17-dione [560-62-3] (**105**) (153). Dehydration of (**105**) to $\Delta^{9(11)}$-derivative (androsta-4,9-diene-3,17-dione [1035-69-4]) (**106**) provided starting material for corticosteroid synthesis (154). The rate of side-chain cleavage of sterols is limited by the low solubility of substrates and products and their low transport rates to and from cells. Cyclodextrins have been used to increase the solubilities of these compounds and to assist in their cellular transport. Cyclodextrins increase the rate and selectivity of side-chain cleavage of both cholesterol and β-sitosterol with no effect on cell growth. Optimal conditions have resulted in enhancement of molar yields of androsta-1,4-diene-3,17-dione **(92)** from 35–40% to $_{>}80\%$ in the presence of cyclodextrins (120, 145, 146, 155).

Besides the aforementioned chemical methods, microbial degradations have been used to remove the C19 angular methyl substituent of readily available steroid precursors. For example, 19-hydroxysterols, such as 3β-acetoxy-19-hydroxy-5-cholestene [750-59-4] **(107)**, can be converted to estrone by *Nocardia* sp. in yields up to 70% (120, 145, 146).



(107) **(26)**

5.2.3.2. Transformations of Steroids to Advanced Intermediates or Finished Products. The most difficult chemical step of the early chemical syntheses of cortisol or corticosteroid analogues was the introduction of an 11-hydroxy moiety. In 1949 the first successful biotransformation of 11-deoxycorticosterone to corticosterone using a perfusion of bovine adrenal glands was reported (156). Similar adrenal perfusions were used by G. D. Searle & Company for the preparation of adequate supplies of hydrocortisone for clinical evaluations (157).

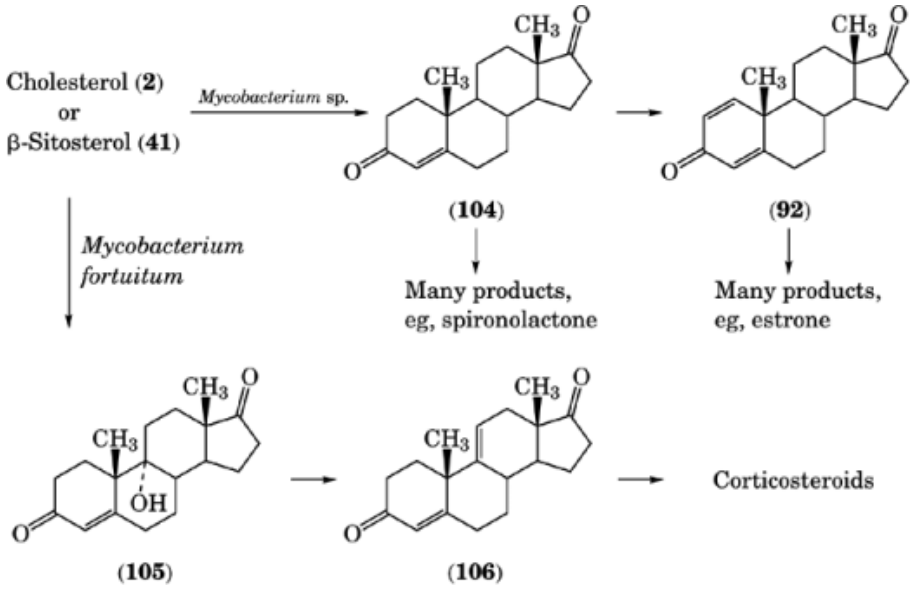


Fig. 14. Commercialized processes for the microbial transformation of readily available sterols to useful synthetic intermediates.

Using microorganisms, workers at Upjohn (1952) found that the specific *Mucorales* fungi grown in aerated cultures were capable of direct 11α-hydroxylation of progesterone in yields as high as 90% (158). Concurrently, workers at the Squibb Institute used *Aspergillus niger* to perform the same reaction on progesterone, 11-deoxycortisol, 11-deoxy-17α-hydroxycorticosterone, and 17α-hydroxyprogesterone (159). The 11α-hydroxy steroids were readily converted to the 11β-hydroxy steroids by first oxidation to the ketone with chromic acid, then sodium borohydride reduction from the sterically less hindered face. However, direct microbial transformation of 11-deoxy steroids to the 11β-hydroxy steroids was still desired. Shortly thereafter, Pfizer investigators found that *Curvularia lunata* converted progesterone, deoxycortisone, 11-deoxy-17α-hydroxycorticosterone, and 17α-hydroxyprogesterone directly to the 11β-hydroxy steroids (160, 161).

Schering investigators uncovered a second significant breakthrough in microbial biotechnology of steroid production. They discovered that *Corynebacterium simplex* converted hydrocortisone (cortisol) **(29)** to prednisolone via a 1,2-dehydrogenation reaction. This $\Delta^{1,4}$-3-ketosteroid is a highly active antiinflammatory commercial product (162).

A third advancement in microbial biotechnology of steroid production was the ability to introduce a 16α-hydroxyl group microbiologically (163). Modifications of the 11β-hydroxylation, 16α-hydroxylation 1,2-dehydrogenation microbial processes are used for the synthesis of hydrocortisone, prednisolone, triamcinolone, and other steroid pharmaceuticals. A few microbial transformations that have been used to manufacture steroids are listed in Table 1 (164).

5.2.4. Representative Partial Syntheses

5.2.4.1. Estranes. The synthesis of 19-norsteroids was stimulated by the development of orally active progestins as birth control agents. The industrial synthesis of pure estrone (120, 165) made conversion to 19-*nor*-A-ring-eneones by the Birch reduction economically feasible (166). An early industrial synthesis of the contraceptive agents norethynodrel (**111**) and norethindrone (**112**) is shown in Figure 15. The aromatic ring of estadiol-3methyl ether is reduced to the 1,4-dihydroestrogen (**108**) with a dissolving metal reduction (167).

Table 1. Commercial Microbial Transformations Used To Produce Advanced Intermediates or Finished Products[a]

Substrate[b]	Transformation	Product	Organism
progesterone	11α-hydroxylation		*Rhizopus nigricans*
	oxidation/lactonization		*Cylindrocarpon radicicola*
11-deoxycortisol (17α-derivatives)	11β-hydroxylation	cortisol/derivatives	*Curvularia lunata*
6α-fluoro-16α-methyl-21-hydroxy-pregn-4-ene-3,20-dione	11β-hydroxylation	Paramethasone	*Curvularia lunata*
11-deoxy-16-methylene-cortisol	11β-hydroxylation	Prednylidene	*Curvularia lunata*
9α-fluorohydro-cortisone	1-dehydrogenation	Triamcinolone	*Arthrobacter simplex*
	16α-hydroxylation		
hydrocortisone	1-dehydrogenation	Prednisolone	*Arthrobacter simplex*
			or
6α-fluoro-16α-methyl cortico-sterone	1-dehydrogenation	Fluocortolone	*Bacillus lentus*
11β,21-dihydroxy-pregna-4,17(20)-dien-3-one	1-dehydrogenation		*Septomyxa affinis*
rac-3-methoxy-8,14-secoestra-1,3,5-(10),9(11)-tetra-ene-14,17-dione(Secosteroid)[c]	17-ketone reduction		*Saccharomyces uvarum*
androst-4-ene-3,17-dione[d]	17-ketone reduction		*Saccharomyces* sp.
21-acetoxy-17α-hydroxy-pregnenolone	Δ^5-3β-alcohol dehydrogenase		*Flavobacterium dehydrogenans*
6α-fluoro-21-hydroxy-16α-methyl-pregn-4-ene-3,20-one	9α-hydroxylation		*Curvularia lunata*

[a]Refs. 120 and 165.
[b]Class is corticosteroid unless otherwise noted.
[c]Class is estrogen–progestin.
[d]Class is androgen.

Careful C17-oxidation of (**108**), followed by addition of acetylene to the resultant C17-ketone (**109**) under basic conditions, yields (**110**). The enol ether of (**110**) is cleaved with mild aqueous acid or strong aqueous acid to give norethynodrel [68-23-5] (**111**) and norethindrone [68-22-4] (**112**), respectively (168–170).

Most 19-norsteroids are produced through total synthesis from nonsteroidal starting materials. One notable exception is the production of the androgen agonist oxendolone (**113**) (Fig. 16). An aldol condensation of acetaldehyde with dehydroepiandrosterone acetate [853-23-6] (**114**) results in ene-one (**115**). Selective hydrogenation of the ene-one from α-face produces (**116**). Reduction of the C17-ketone from the less hindered α-face, followed by acetylation of the C3β- and resultant C17β-alcohols yields a diacetate. Addition of hypochlorous acid to this diacetate first forms an intermediate 5,6-α-chloronium ion, followed by diaxial ring opening produces (**117**). Degradation of the C19-methyl group proceeds as described previously (see Fig. 13), that is, (*1*) a lead tetraacetate-induced free-radical addition of the C6β-hydroxyl moiety to the C19-methyl substituent to form a cyclic ether, (*2*) selective saponification of the C3-acetate, (*3*) oxidation of the C3-alcohol to a ketone, (*4*) base-catalyzed elimination of the C5-chloride to form an A-ring enone, and (*5*) zinc-mediated reduction of the cyclic ether formed the C19 alcohol (**118**). Oxidation of the primary alcohol to an aldehyde, followed by base-catalyzed decarbonylation of this aldehyde and saponification of the C17-acetate, affords oxendolone (**113**) (171).

5.2.4.2. Pregnanes. In 1944, Sarrett completed the first partial synthesis of cortisone (172). Like many of the early syntheses of corticosteroids, Sarrett began with the a bile acid, deoxycholic acid **(14)**. Because bile acids are isolated from animal sources, their supply is by necessity limited (173). Following these early

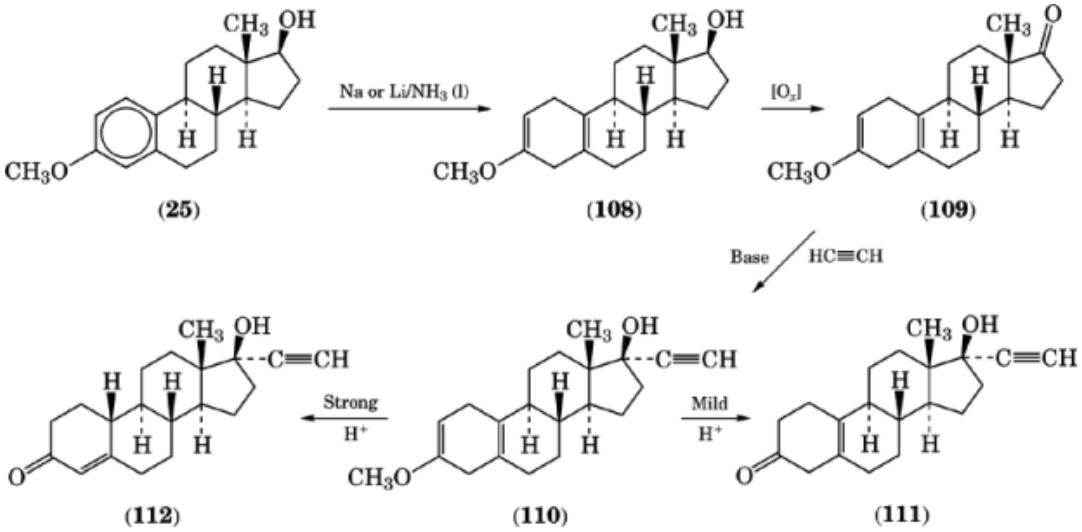


Fig. 15. Partial synthesis of norethynodrel (**111**) and norethindrone (**112**).

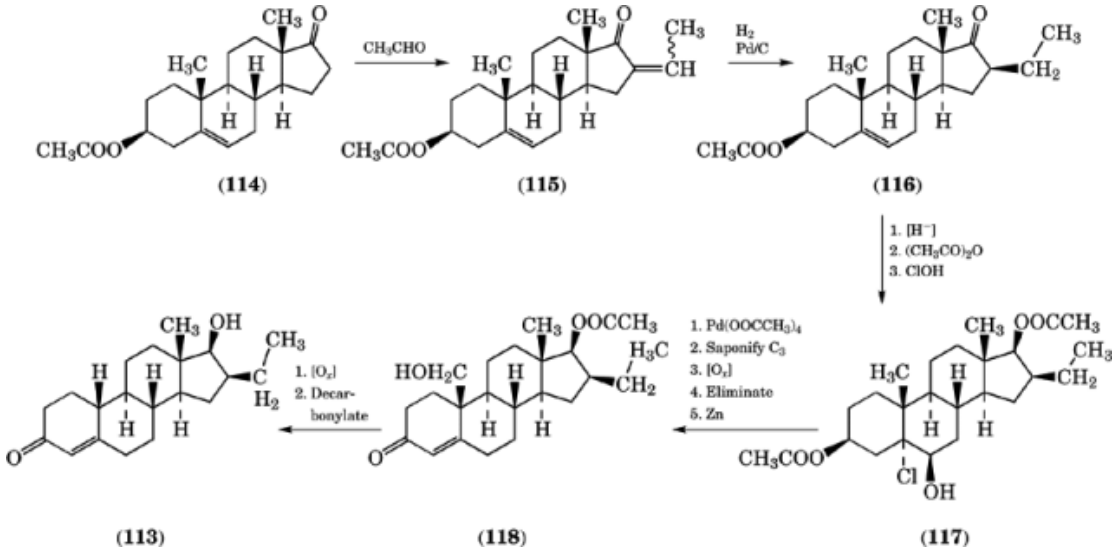


Fig. 16. Partial synthesis of oxendolone [33765-68-3] (**113**).

syntheses, several improvements and innovations have resulted in a number of industrial syntheses of cortisol and other corticosteroids.

Although there are many variations on industrial partial synthesis of corticosteroids, two basic processes are shown in Figure 17. Stigmasterol **(40)** is converted to 11-oxoprogesterone [516-15-4] (**119**) by methods that have been discussed. Under controlled conditions, base-catalyzed acylation of (**119**) forms a 21-monoglyoxylated compound as the primary product and a 2,21-bisglyoxylated compound (**120**) as a by-product. The preferred production process treats (**119**) with two or more moles of diethyl glyoxylate to form (**120**), exclusively. Treatment of (**120**) with bromine followed by alkaline cleavage of the glyoxylates produces intermediate (**121**). Without

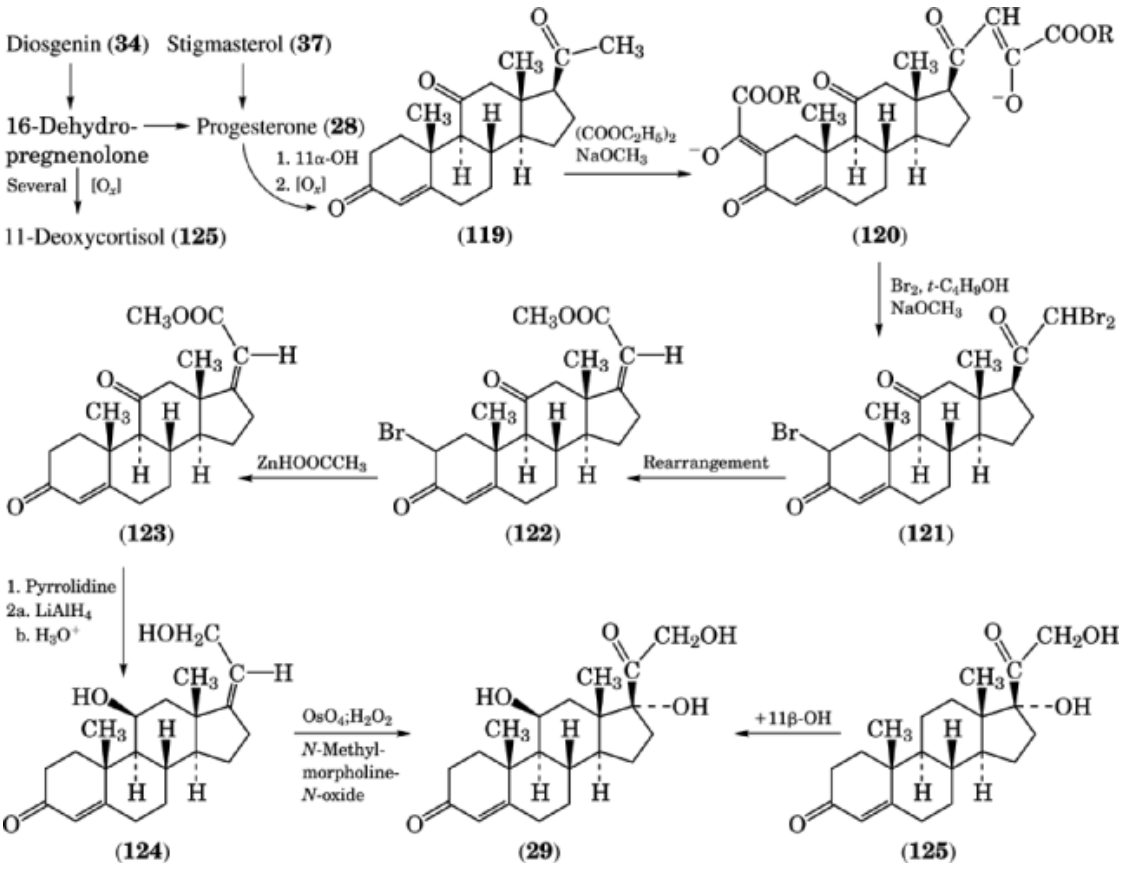


Fig. 17. Early industrial syntheses of cortisol (**29**).

isolation of (**121**), Favorskii rearrangement forms (**122**). Direct debromination of (**122**) with Zn yields (**123**). In addition, (**122**) can be taken directly to the potent corticosteroid prednisolone in three chemical steps. Protection of the C3-ketone of (**123**) by formation of the ene-amine with pyrrolidine, followed by C11 and C21 reduction with lithium aluminum hydride and hydrolysis of the ene-amine results in (**124**). Osmium tetroxide-catalyzed oxidation of (**124**) yields cortisol (hydrocortisone, **29**) (174).

In another process, diosgenin is degraded to 16-dehydropregnenolone by chemical methods. Conversion of 16-dehydropregnenolone to 11-deoxycortisol (**125**) can be accomplished in 11 chemical steps. These steps result in hydroxylations at C21 and C17, oxidation at C3, and Δ^5 to Δ^4 double-bond isomerization (175). Microbial oxidation of (**125**) also produces cortisol **(29)**.

Several cortisol analogues have become important therapeutically. Among the useful cortisol derivatives are the following: (*1*) dehydrogenation at C1–C2, (*2*) addition of fluorine to C6α and C9α, (*3*) addition of a methyl substituent to C6α, C16α, and C16β, and/or (*4*) hydroxylation at C16α (174). Commercial syntheses of a few of the C9α-fluoro analogues are shown in Figure 18. Tosylation of the C11α-hydroxyl substituent of (**126**), followed by elimination of this moiety in acidic acid/sodium acetate produces the $\Delta^{(9,11)}$-steroid (**127**). Bromohydrin formation on the $\Delta^{(9.11)}$-steroid (**127**) forms 9α-bromocortisol acetate. Treatment of this 9α-bromo-11β-carbinol with potassium acetate in boiling alcohol yields a 9β,11β-epoxy steroid. Treatment of the 9β,11β-epoxy steroid with hydrogen fluoride results in the corresponding 9α-fluorohydrin (9α-fluorohydro cortisone [127-31-1]) (**128**)

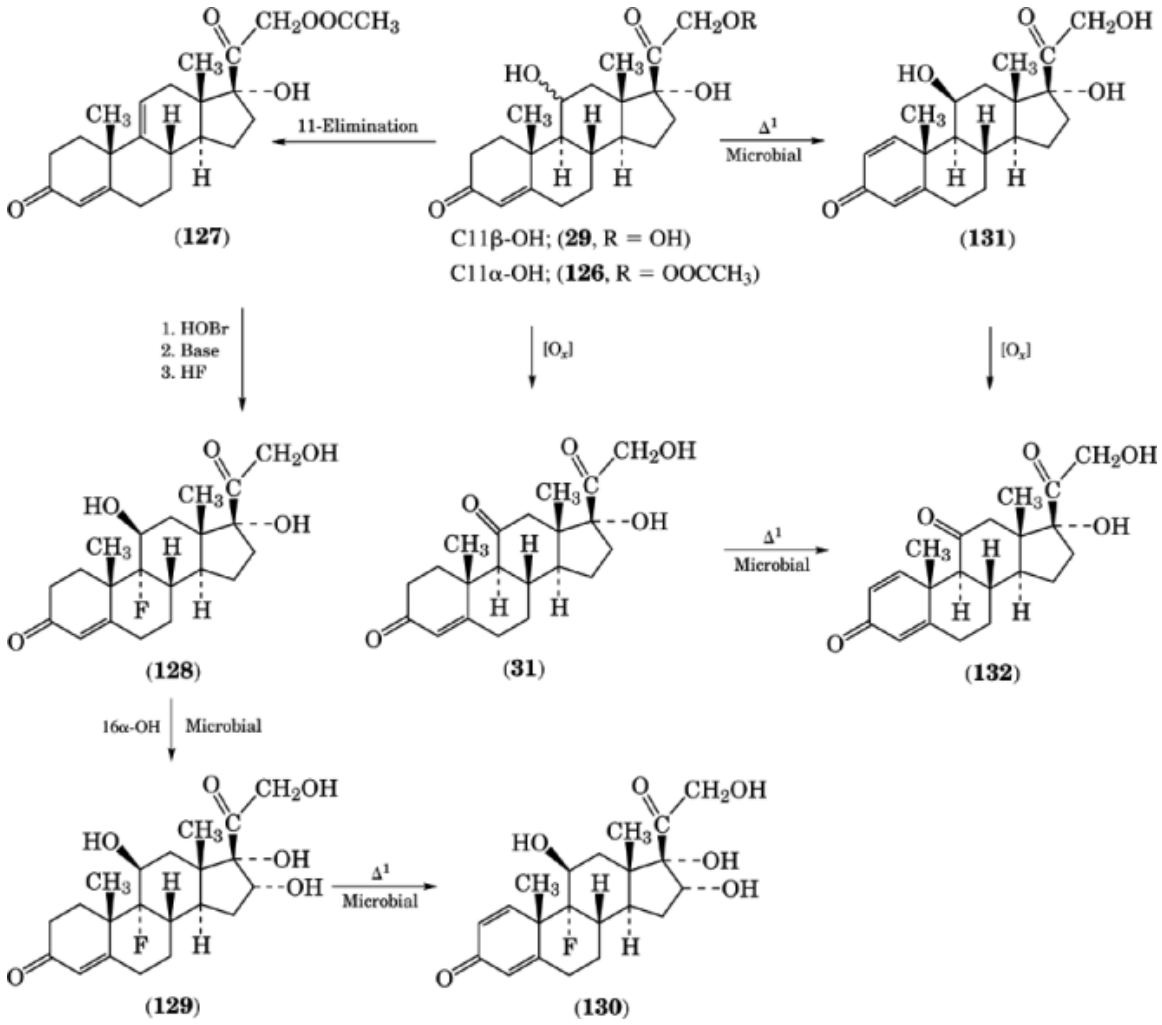


Fig. 18. Partial synthesis of corticosteroid drugs: 9α-fluoro-16α-hydroxyhydrocortisone [337-02-0] (**129**), triamcinolone [124-94-7] (**130**), prednisolone [50-24-8] (**131**), and prednisone [53-03-2] (**132**).

(176, 177). The other therapeutically important steroid syntheses shown in Figure 18 have been discussed in the biotransformations section.

Following the development of the efficient synthesis of the microbial degradation of cholesterol and sitosterol (177), the transformation of the C17-ketone of the resultant androstanes into the corticoid side chain were studied extensively (178). What appear to be among the most efficient of these pathways are shown in Figure 19. Treatment of androst-4-ene-3,17-dione (**106**) or androsta-1,4-diene-3,17-dione (**131**) with potassium cyanide in acetic acid forms the desired β-cyano-α-hydrin (**133**) through an equilibration of the two cyanohydrin epimers and selective crystallization of (**133**). The C17-hydroxyl moiety is protected and activated as the (chloromethyl)dimethylsilyl ether (**135**). Treatment of (**135**) with a strong base, eg, lithium diisopropylamide (LDA), results in deprotonation of the chloromethyl group and cyclization of this anion onto the adjacent nitrile to form a spirocyclic intermediate with a C20-imine. When the reaction mixture is quenched with aqueous acid, this C20-imine is hydrolyzed, the silyl ether is cleaved, and protodesilylation occurs resulting in (**140**) without

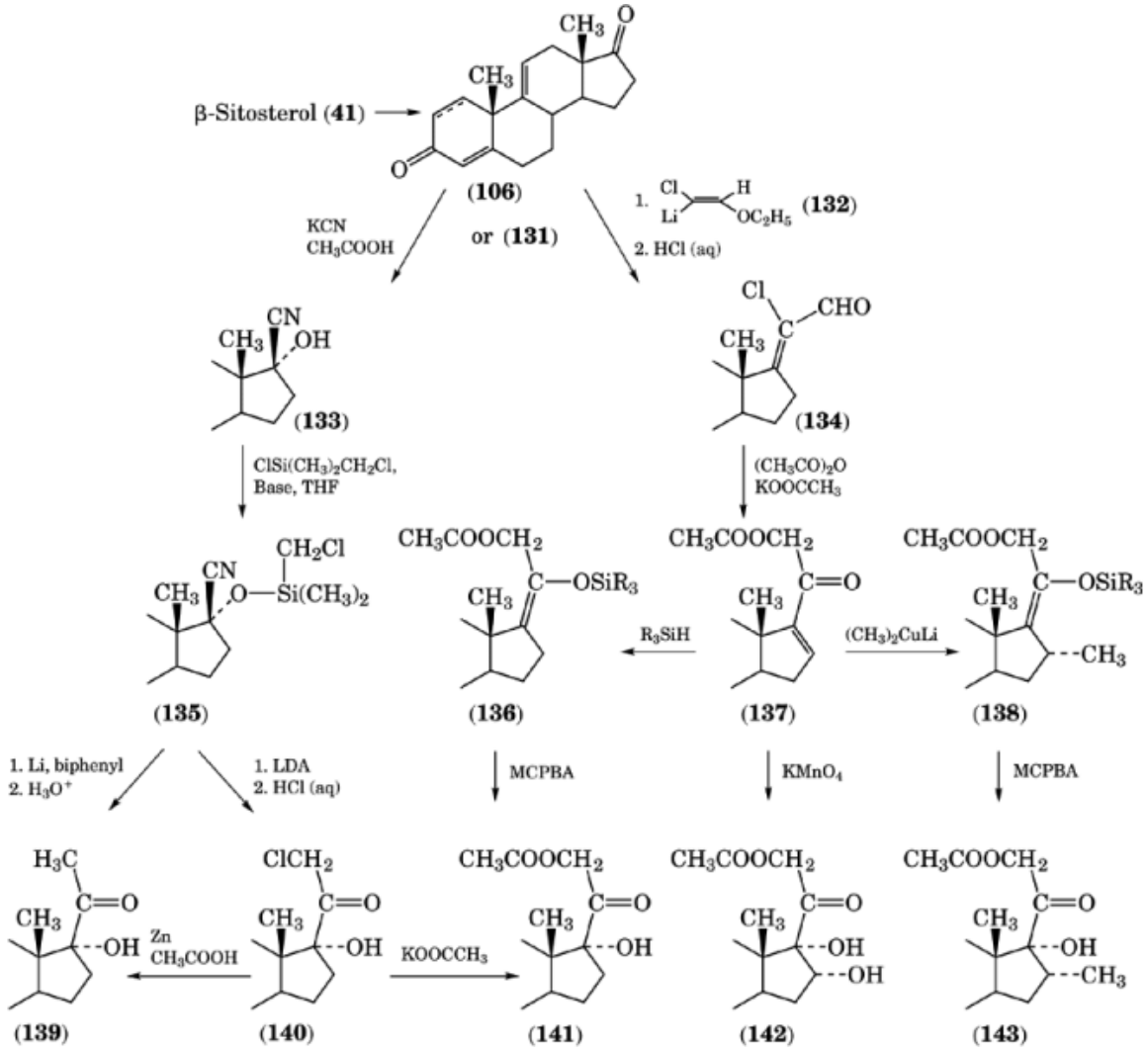


Fig. 19. C_{17} chemistry.

the need for a protecting group on the C3-ketone. The resultant C21-chloride of (**140**) can be displaced with acetate anion or reduced with zinc in acetic acid to produce (**141**) and (**139**), respectively. Alternatively if the C3-ketone is first protected, (**139**) is formed directly from (**135**) by reductive cyclization followed by hydrolysis (174, 179).

A second industrial process for the conversion of androst-4-ene-3,17-dione (**106**) or androsta-1,4-diene-3,17-dione (**131**) into corticosteroid drugs is also shown in Figure 19. After selective protection of the C3-ketone, addition of the lithium compound (**132**) followed by an aqueous acid quench results in the chloro aldehyde (**134**). Treatment of (**134**) with potassium acetate and acetic anhydride forms the Δ^{16}-corticoid (**137**). Steroid (**137**) is a versatile intermediate for the synthesis of a variety of the aforementioned, potent glucocorticoids. Conjugate reduction of the C16–C17 double bond of (**137**) with trialkylsilane yields enol ether (**136**). *meta*-Chloroperbenzoic acid (MCPBA) mediated epoxidation of (**136**) produces a C17α,C20α-epoxide. Desilylation

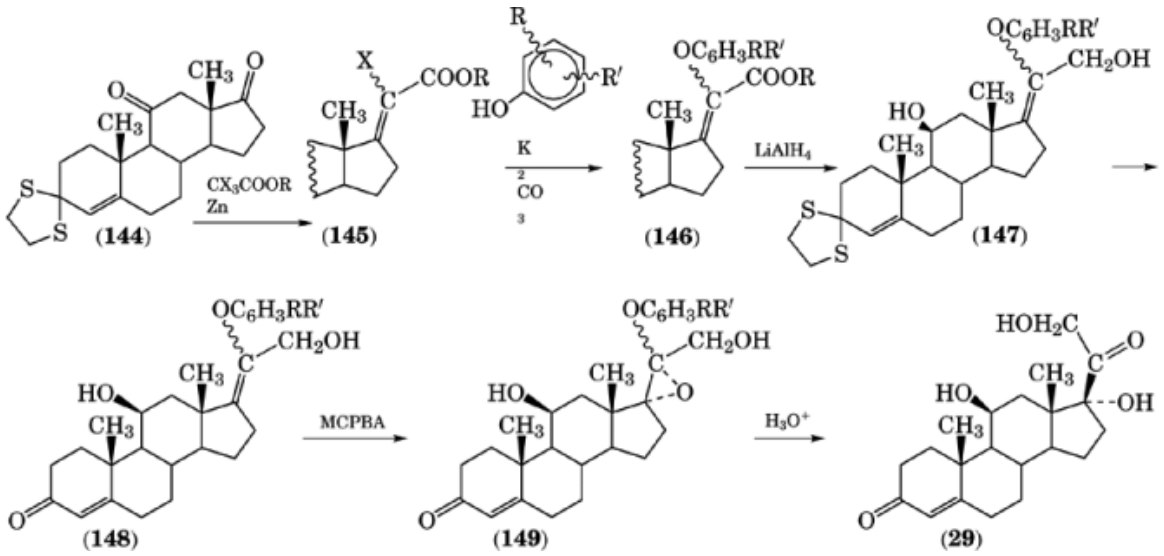


Fig. 20. Further C_{17} chemistry. The disubstituted phenol that reacts with (**145**) may be represented as $C_6H_3RR'OH$.

of the C20 silyl-enol-ether yields the C20-acylated glucocorticoid side chain (**141**). Treatment of (**137**) with potassium permanganate forms the α16,α17-diol (**142**). Conjugate addition of a methyl cuprate to the α-face of C16 of (**137**) followed by *O*-silylation of the resultant enolate results in the substituted enol ether (**138**). Epoxidation and desilylation, as before, yield (**143**) (174, 180).

Another synthesis of the cortisol side chain from a C17-keto-steroid is shown in Figure 20. Treatment of a C3-protected steroid 3,3-ethanedyldimercapto-androst-4-ene-11,17-dione [112743-82-5] (**144**) with a trihaloacetate, zinc, and a Lewis acid produces (**145**). Addition of a phenol and potassium carbonate to (**145**) in refluxing butanone yields the aryl vinyl ether (**146**). Concomitant reduction of the C20-ester and the C11-ketone of (**146**) with lithium aluminum hydride forms (**147**). Deprotection of the C3-thioketal, followed by treatment of (**148**) with *meta*-chloroperbenzoic acid, produces epoxide (**149**). Hydrolysis of (**149**) under acidic conditions yields cortisol **(29)** (181).

Mineralocorticoid antagonists are used as diuretics and antihypertensive agents. The introduction of a spirolactone function at C17 provides for the potent antimineralocorticoid activity. The commercial synthesis of the potassium-sparing diuretic spironolactone is outlined in Figure 21. The C17-ethynylated compound 17β*H*-pregn-5-en-20-yne-3β-dio [3604-60-2] (**151**) is carboxylated with carbon dioxide and base to provide (**152**). Catalytic reduction of (**152**) followed by acid-catalyzed cyclization forms (**153**). A second catalytic hydrogenation produces the spirolactone (**154**). Direct oxidation of (**154**) yields diene (**155**). Conjugate addition of thiolacetic acid to (**155**) affords the orally active spironolactone (**150**) (182).

In addition to its antimineralocorticoid activity, spironolactone (**150**) also possesses to some degree such undesirable effects as progestinal and antiandrogenic activity. Newer mineralocorticoid antagonists have been prepared in efforts to design agents free of these usually unwanted side effects (183). The synthesis of one such agent, spirorenone (**156**), is shown in Figure 22. This synthesis demonstrates an efficient method for C17-spirolactone construction. Microbiological oxidation of the dehydroepiandrosterone derivative (**157**) [67572-65-0] provides the 7β-hydroxylated compound (*158*). Selective acylation of the C3-hydroxyl group (**159**), followed by vanadium-catalyzed epoxidation affords (**160**) as the β-epoxide. Chlorination of the 7-hydroxyl moiety with triphenyl phosphine and carbon tetrachloride forms (**161**). Sequential reductive elimination (**162**) followed by saponification yields (**163**). Cyclopropanation of (**163**) with the Simmons-Smith reagent gave exclusively the

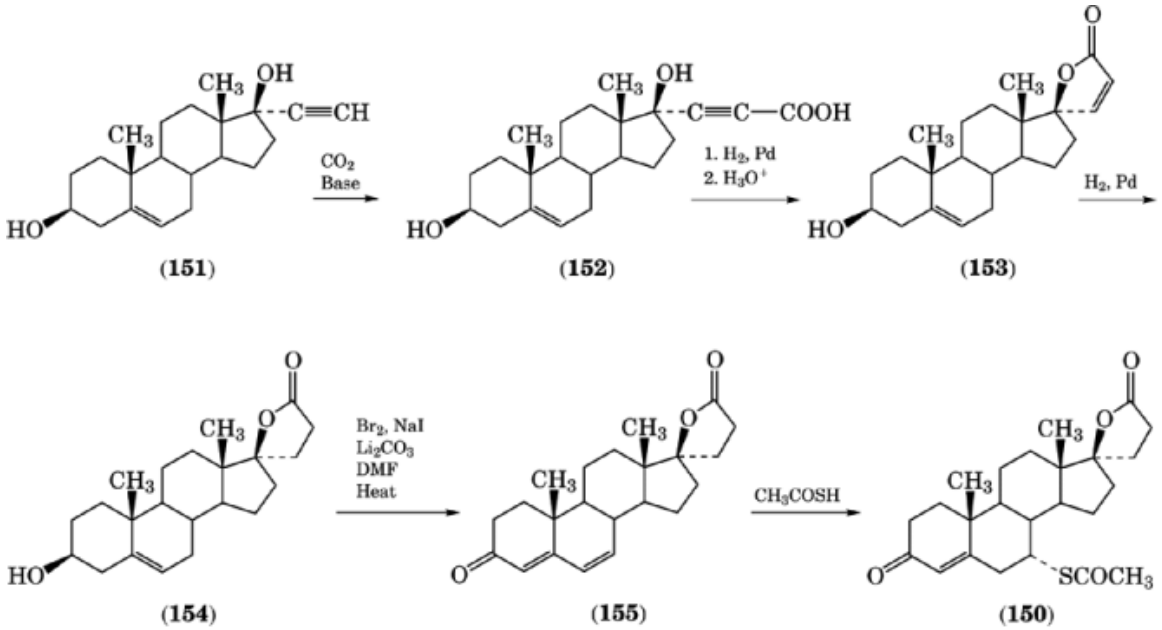


Fig. 21. Industrial synthesis of spironolactone [52-01-7] (**150**).

β-cyclopropane (**164**). The C17-spirolactone construction begins with the addition of the dianion of propargyl alcohol to the C17-ketone of (**164**) to form (**165**). Palladium-catalyzed reduction of (**165**) forms (**166**). Treatment of (**166**) with pyridinium chlorochromate simultaneously oxidizes the C3-alcohol to a ketone, oxidizes the primary alcohol to an acid, eliminates the 5-hydroxy-3-ketone to an ene-one, and cyclizes the D-ring hydroxy acid to a lactone to form (**167**). Dehydrogenation of the C1–C2 bond with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) yields spirorenone (**156**) (184).

The stereocontrolled syntheses of steroid side chains for ecdysone, crustecdysone, brassinolide, withanolide, and vitamin D_3 have been reviewed (185). Also, other manuscripts, including reviews on the partial synthesis of steroids (186), steroid drugs (187–189), biologically active steroids (190), heterocyclic steroids (191), vitamin D (192), novel oxidations of steroids (193), and template-directed functionalization of steroids (194), have been published.

5.3. Total Synthesis

5.3.1. Estranes

Investigations into the total synthesis of steroids began in the 1930s shortly after the precise formula for cholesterol was established. The earliest studies focused on equilenin **(5)** because of its relative stereochemical simplicity when compared to other steroid nuclei. Initially, equilenin was synthesized in 20 chemical steps with an overall yield of 2.7%. This synthesis helped to confirm the perhydro-1,2-cyclopentenophenanthrene ring system of the steroid nucleus (195). Estrone was the second natural steroid to be synthesized from nonsteroidal starting materials in 0.1% overall yield in 18 steps (196). Many of the latter steps in this synthesis of estrone were essentially the same as those for the earlier synthesis of equilenin.

Since these original processes, a vast number of total syntheses of aromatic A-ring steroids have appeared (197, 198). A highly efficient synthesis of (±)-9,11-dehydroestrone methyl ether **(177)**, involving a tandem Cope-Claisen rearrangement, has been reported (199). Alkylation of the anion of methyl

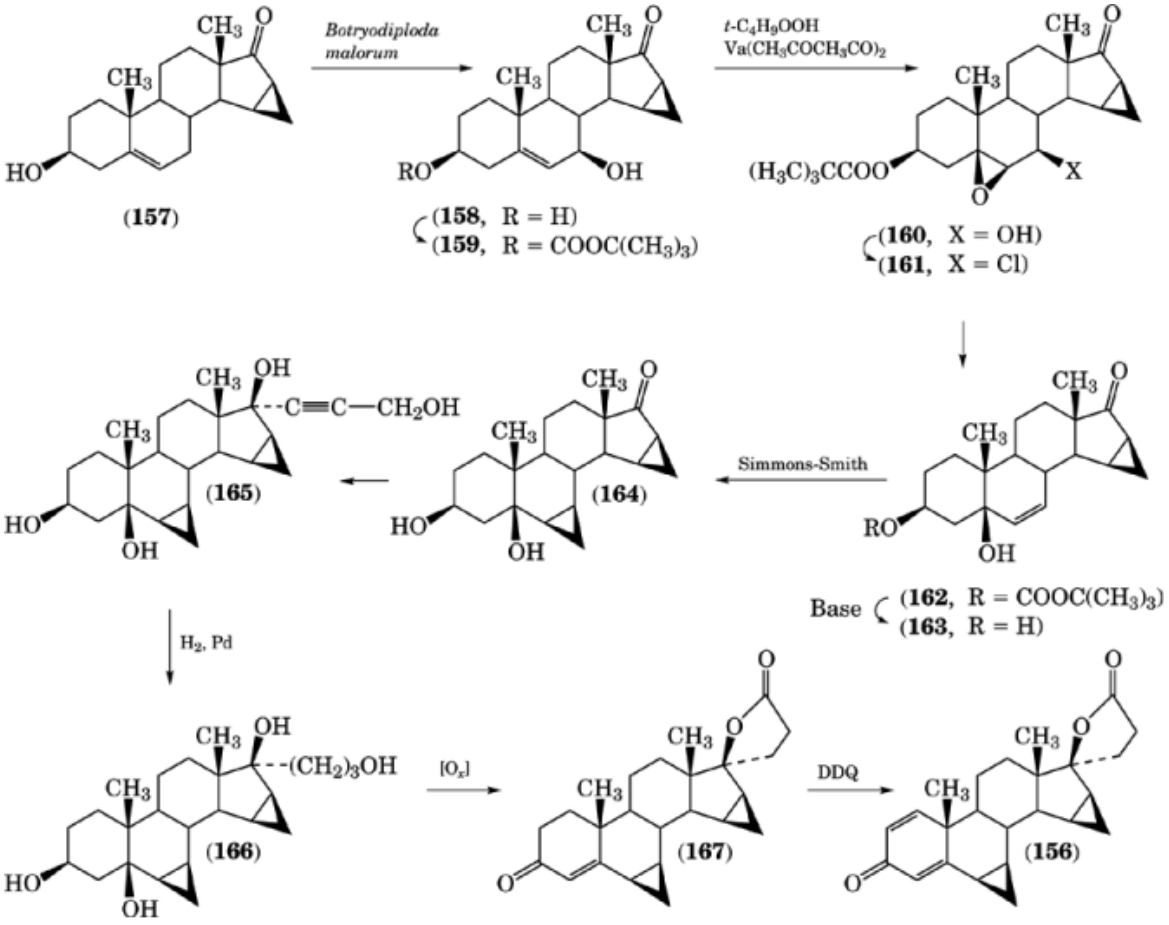
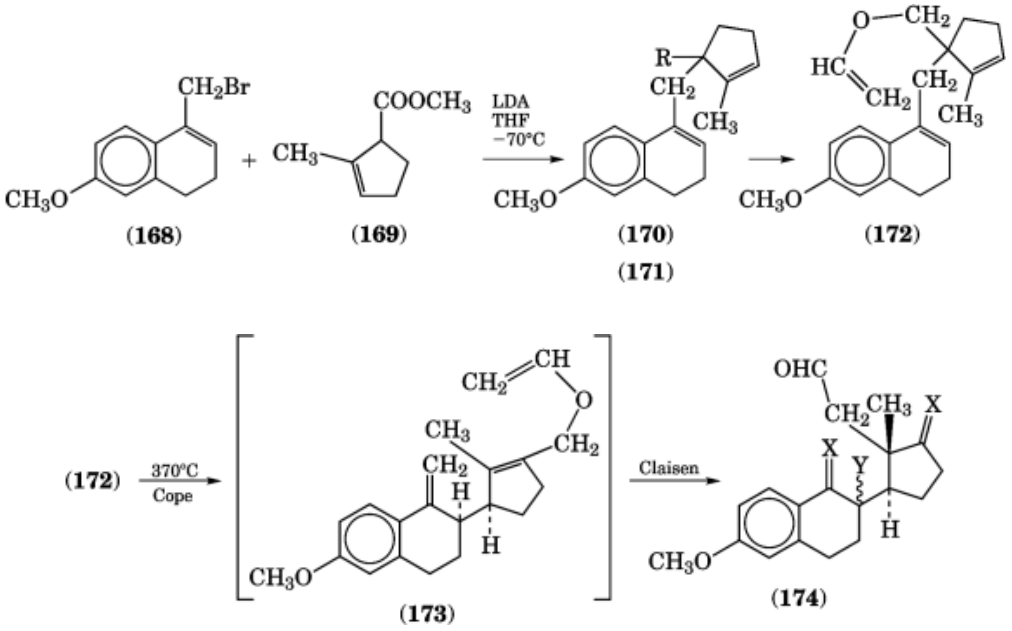
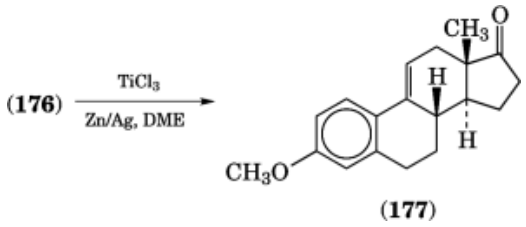


Fig. 22. Synthesis of spirorenone [74220-07-8] (**156**) where DDQ= 2,3-dichloro-5,6dicyano-1,4-benzoquinone.

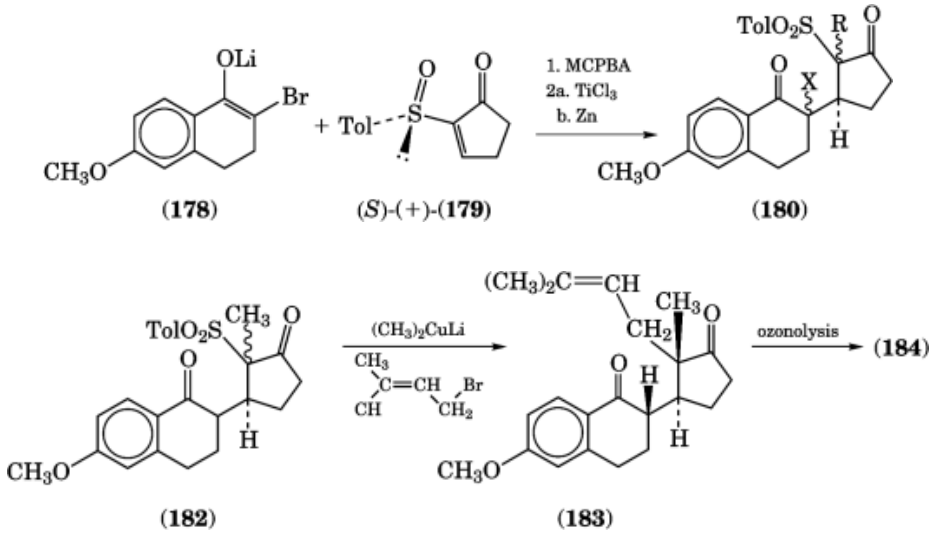
2-methyl-2-cyclopentene-1-carboxylate [25662-31-1] **(169)** with bromide 4-bromomethyl-7-methoxy-1,2-dihydronaphthalene [83747-47-1] **(168)** produces ester (**170**, $R = COOCH_3$) in 94% yield. Reduction of **(170)** with lithium aluminum hydride yields (**171**, $R = CH_2OH$). Treatment of alcohol **(171)** with vinyl ethyl ether and mercury(II) acetate provides **(172)** in an overall yield of 87% from **(170)**. Thermolysis of **(172)** at approximately 370°C undergoes a Cope rearrangement to form **(173)**, followed by a Claisen rearrangement producing a 2/1 mixture of diastereomeric aldehydes at C13. Separation of the major product from this rearrangement affords the desired diastereomer (**174**, $X = CH_2$; $Y = \alpha H$) in 35% yield. Ozonolysis of **(174)** results in tricarbonyl (**175**, $X = O$; $Y = \alpha H$) in 70% yield. Epimerization of the hydrogen on the α-face of C8 of (**175**) with a methanol–sodium methoxide solution yields (**176**, $X = O$; $Y = \beta H$) in 69% yield. This compound contains the desired C8, C13, and C14 relative stereochemistry.



A McMurry coupling of (**176**, X = O; Y = βH) provides (±)-9,11-dehydroesterone methyl ether [1670-49-1] **(177)** in 56% yield. 9,11-Dehydroestrone methyl ether **(177)** can be converted to estrone methyl ether by stereoselective reduction of the C_9–C_{11} double bond with triethyl silane in trifluoroacetic acid. In turn, estrone methyl ether can be converted to estradiol methyl ether by sodium borohydride reduction of the C17 ketone (199, 200).



An asymmetric synthesis of estrone begins with an asymmetric Michael addition of lithium enolate **(178)** to the scalemic sulfoxide **(179)**. Direct treatment of the crude Michael adduct with *meta*-chloroperbenzoic acid to oxidize the sulfoxide to a sulfone, followed by reductive removal of the bromine affords (**180**, X = α and βH; R = H) in over 90% yield. Similarly to the conversion of **(175)** to **(176)**, base-catalyzed epimerization of **(180)** produces an 85% isolated yield of (**181**, X = βH; R = H). C8 and C14 of **(181)** have the same relative and absolute stereochemistry as that of the naturally occurring steroids. Methylation of **(181)** provides **(182)**. A $(CH_3)_2CuLi$-induced reductive cleavage of sulfone **(182)** followed by stereoselective alkylation of the resultant enolate with an allyl bromide yields **(183)**. Ozonolysis of **(183)** produces (**184**) (wherein the aldehydric oxygen is by isopropylidene) in 68% yield. Compound (**184**) is the optically active form of Ziegler's intermediate **(176)**, and is converted to (+)-estrone in 6.3% overall yield and >95% enantiomeric excess (200).



The most recent, and probably most elegant, process for the asymmetric synthesis of (+)-estrone applies a tandem Claisen rearrangement and intramolecular ene-reaction (Fig. 23). Stereochemically pure (**185**) is synthesized from (2*R*)-1,2-*O*-isopropylidene-3-butanone in an overall yield of 86% in four chemical steps. Heating a toluene solution of (**185**), enol ether (**187**), and 2,6-dimethylphenol to 180°C in a sealed tube for 60 h produces (**190**) in 76% yield after purification. Ozonolysis of (**190**) followed by base-catalyzed epimerization of the C8α-hydrogen to a C8β-hydrogen (again similar to conversion of **(175)** to **(176)**) produces (**184**) in 46% yield from (**190**). Aldehyde (**184**) was converted to 9,11-dehydroestrone methyl ether **(177)** as discussed above. The overall yield of 9,11-dehydroestrone methyl ether **(177)** was 17% in five steps from 6-methoxy-1-tetralone (**186**) and (**185**) (201).

Most 19-norsteroid contraceptive agents are produced by total synthesis from nonsteroidal starting materials. A large number of syntheses of 19-norsteroids have been reported (202). An industrial synthesis of 19-norsteroids that is based on the classical Torgov (203) synthesis of aromatic steroids is shown in Figure 24. The addition of vinyl magnesium chloride (**192**) to 6-methoxy-1-tetralone (**186**) produces (**191**). The acidity of the 1,3-dione in 2-alkyl cyclopentadione (**200**, R = CH_3 or C_2H_5) catalyzes the addition of (**200**) to the vinyl carbinol (**191**), forming the tricyclic compound (**193**). The alkyl substituent or R-group of the cyclopentadione is either methyl or ethyl for the synthesis of estradiol and *d*-norgestrel **(209)**, respectively. Microbial reduction of the prochiral secosteroid (**193**) produces the 13(*R*),17(*S*)-hydroxyketone (**194**). Acid-mediated cyclization of (**194**) forms steroids (**195**). Catalytic hydrogenation of the 14–15 double bond from the face opposite to the C18 substituent yields (**196**). Compound (**196**) contains the natural steroid stereochemistry around the D-ring. A metal-ammonia reduction of (**196**) forms the most stable product (**197**) thermodynamically. When R is equal to methyl, this process comprises an efficient total synthesis of estradiol methyl ester. Birch reduction of the A-ring of (**197**) followed by acid hydrolysis of the resultant enol ether allows access into the 19-norsteroids (**198**) (204).

Another efficient synthesis of *d*-norgestrel **(209)** begins with the condensation of 2-ethyl-1,3-cyclopentanedione (**200**, R = C_2H_5) with methyl vinyl ketone **(199)**, producing **(201)**. An asymmetric, intramolecular aldol condensation of **(201)** that is catalyzed by (*S*)-(−)-proline followed by an acid-catalyzed dehydration yields hydrindandione **(202)** in 97% optical purity (205). Condensation of **(202)** with formaldehyde and benzenesulfinic acid generates **(203)** in 85% yield.

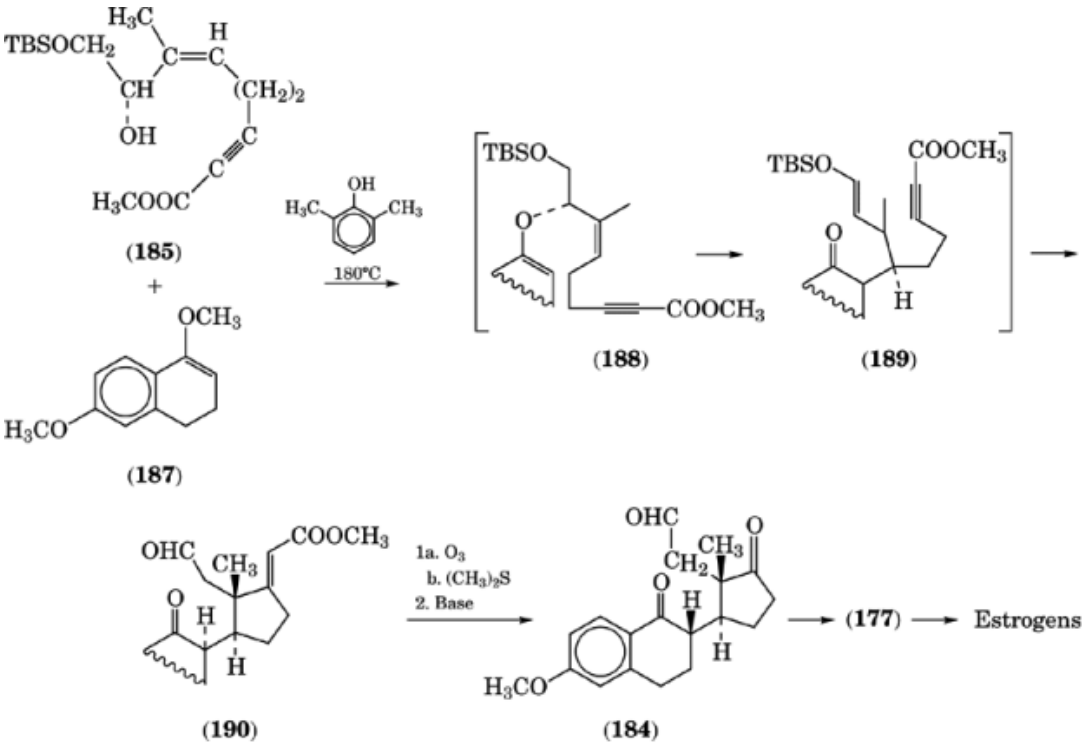
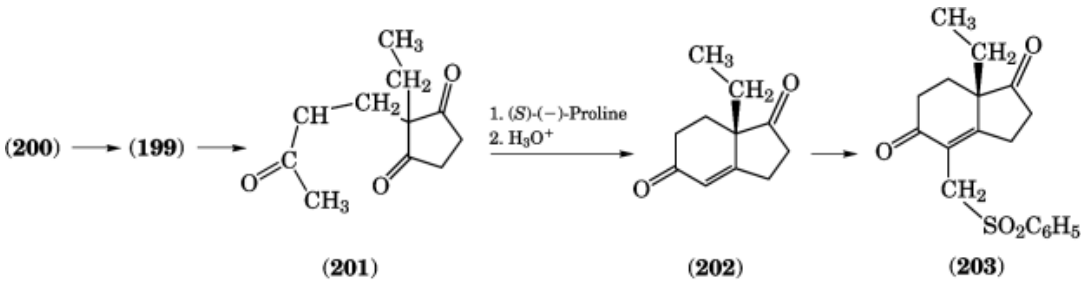


Fig. 23. Asymmetric total synthesis of estrogens, where TBS=*tert*−butyldimethylsilane.



In acid solution, the double bond of **(203)** is hydrogenated to the trans-fused sulfone **(204)**. Presumably, this hydrogenation goes through a cis-fused intermediate that is rapidly epimerized to **(204)** under the acidic conditions of the reaction. Condensation of the sodium salt of 7,7-ethylenedioxy-3-oxooctanoate **(205)** with **(204)** produces **(206)**. Crude **(206)** is cyclized, hydrolyzed, and decarboxylated, producing the tricyclic compound **(207)**. Hydrogenation of **(207)** followed by ketal hydrolysis and cyclization affords **(208)** in an overall yield of 35% from hydrindandione **(203)**. *d*-Norgestrel [797-63-7] **(209)** is obtained by ethynylation of **(208)** (206).

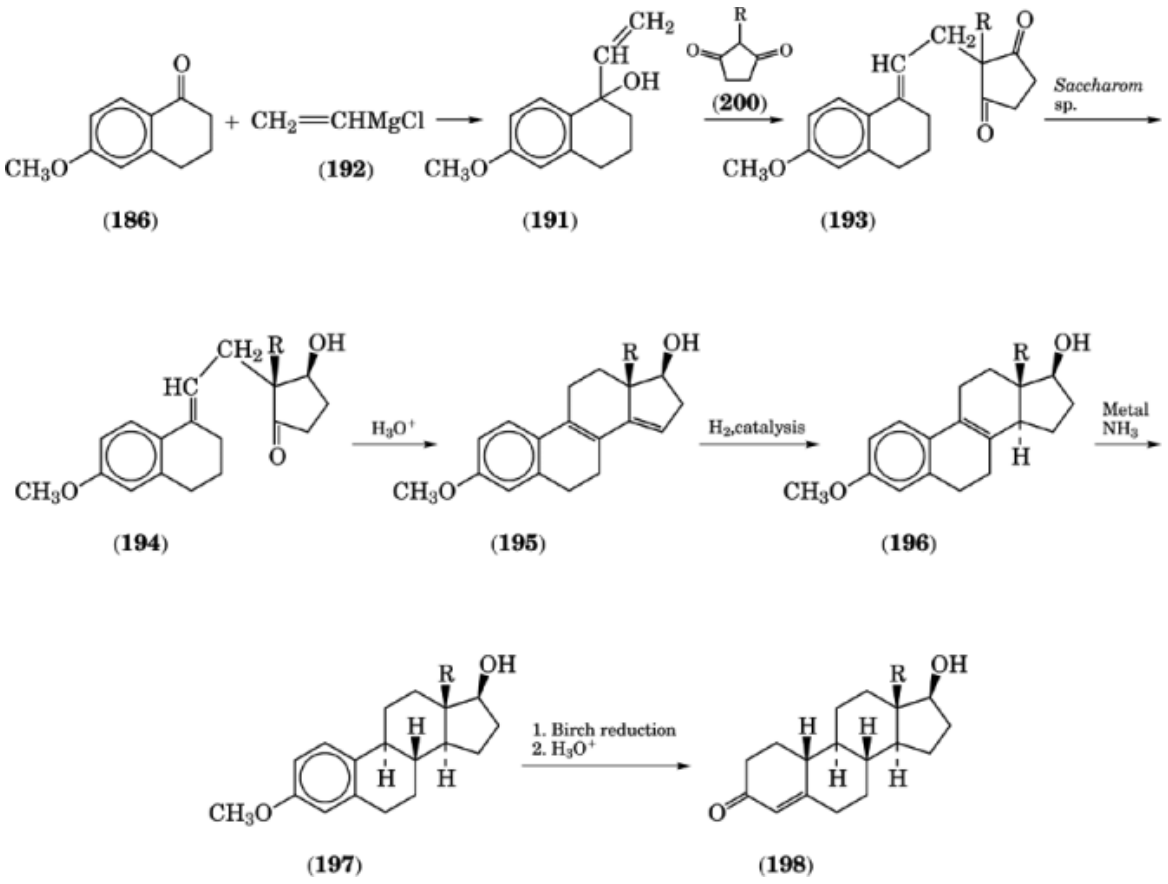
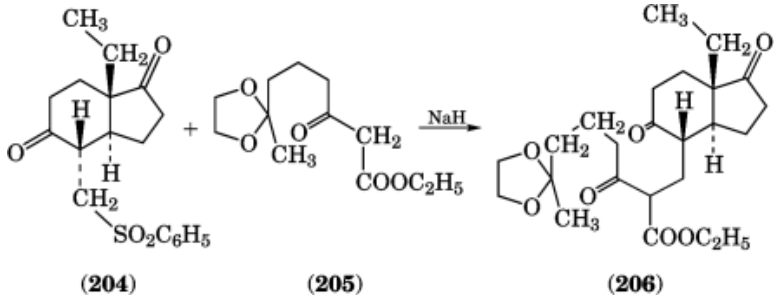
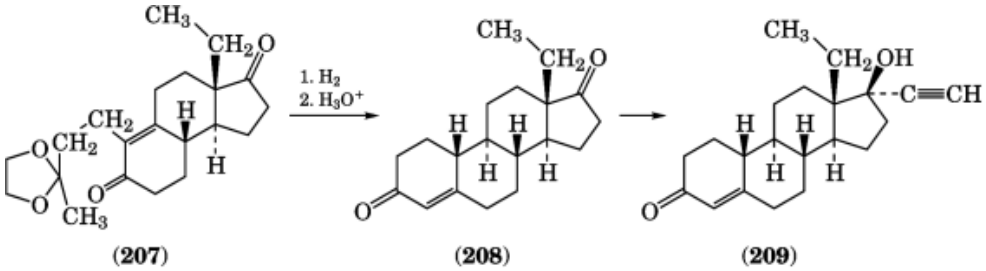


Fig. 24. Industrial total synthesis of gestrogens. For (**195**, $R=CH_3$) [4858-90-6]; (**195**, $R=C_2H_5$) [14507-45-0]. For (**196**, $R=CH_3$) [6733-79-5]; (**196**, $R=C_2H_5$) [7443-72-3].





(207) (208) (209)

5.3.2. Androstanes and Pregnanes

The first total syntheses of nonaromatic steroids that contain the C19-angular methyl substituent were accomplished in the early 1950s. These syntheses all began with starting materials containing a two-ring system. The remaining two rings were appended through annulation sequences. For example, the Robinson (207) total synthesis began with the B/C-ring system followed by annulation of the A-ring and finally the D-ring. Although this synthesis consisted of approximately 40 chemical steps in an overall yield below $10^{-4}\%$, by demonstrating the possibility of the total synthesis of nonaromatic steroids it nevertheless has great historical value. Likewise, the basic plan of Sarrett's (208) first total synthesis of cortisol was based on the B–C to A–D-ring strategy in an overall yield of about 0.14% in approximately 30 chemical steps. The Woodward (209) total synthesis began with the C–D-ring system followed sequentially by the formation of the B- and A-ring systems (210).

A more recent ring annulation strategy for the total synthesis of steroids from the Stork group is shown in the following equation (**210** ⟶ **211** ⟶ **212**) and Figure 25. This synthesis begins with the formation of the C–D-ring system as a suitably functionalized indane. Condensation of the pyrrolidine enamine of cyclopentanone with ene-one **(210)** results in the bicyclic keto-ester **(211)** in 60–70% yield. Treatment of **(211)** with isopropenyl acetate and sulfuric acid produces a dienol acetate. Treatment of this dienol acetate with acetic anhydride and boron trifluoride etherate forms **(212)** as the major product. Reduction of ene-one **(212)** to an allylic alcohol with sodium borohydride, followed by enol acetate cleavage and conjugation of the double bond produces (**213**). Conjugate addition of diethyl aluminum cyanide to (**213**), followed by protection of the ketone as the 1,3-dioxolane, affords (**214**) as a single diastereomer. Concomitant reduction of the cyano and ester moieties of (**214**) with lithium aluminum hydride forms a diol with an angular imine moiety. Wolff-Kishner reduction of this resultant imine converts the angular imine to a methyl group. Oxidation with pyridinium dichromate (PDC) yields the keto acid (**215**). Treatment of (**215**) with isopropenyl lithium affords the isomeric diene (**216**) after dehydration. This mixture of dienes (**216**) is isomerized to diene (**217**) with catalytic ruthenium trichloride. Diene (**217**) is arranged for an intramolecular Diels-Alder reaction. More conveniently, treatment of dienes (**216**) with a catalytic amount of ruthenium trichloride in refluxing ethanol forms the endo Diels-Alder adduct (**218**) as the major isomer in 93% yield. Ozonolysis of (**218**) affords the trione (**219**) that contains the unnatural stereocenter at C9. Treatment of (**219**) with sodium methoxide in methanol epimerizes C9 to the thermodynamically most stable stereoisomer and cyclizes the A-ring to form 11-keto-progesterone (**220**) in 60% overall yield from indanonepropionic acid (**215**) (211).

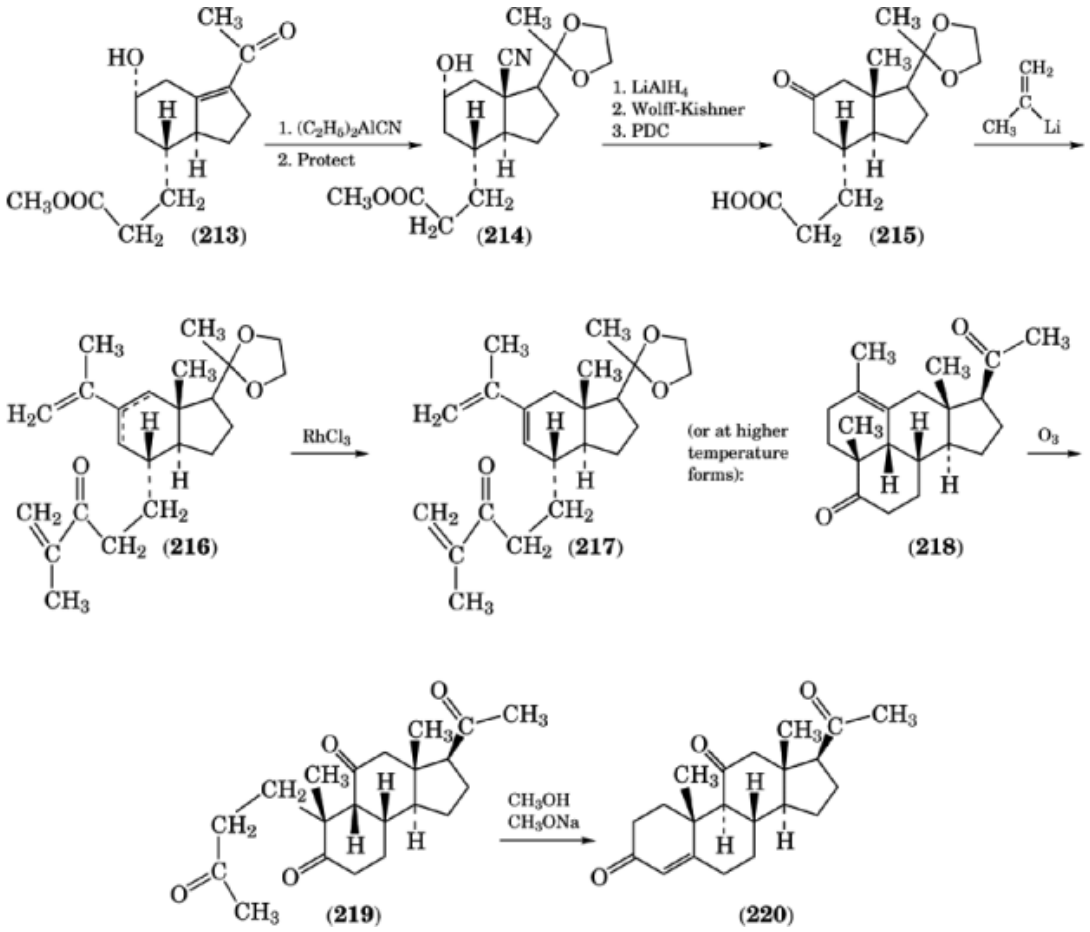
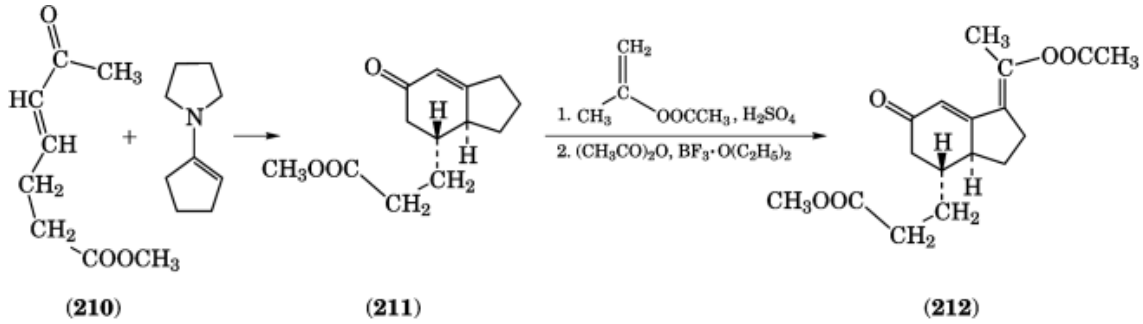


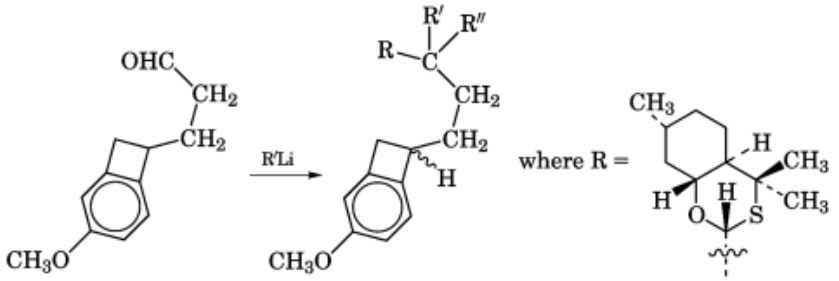
Fig. 25. Total synthesis of 11-keto-progesterone [516-15-4] (**220**).



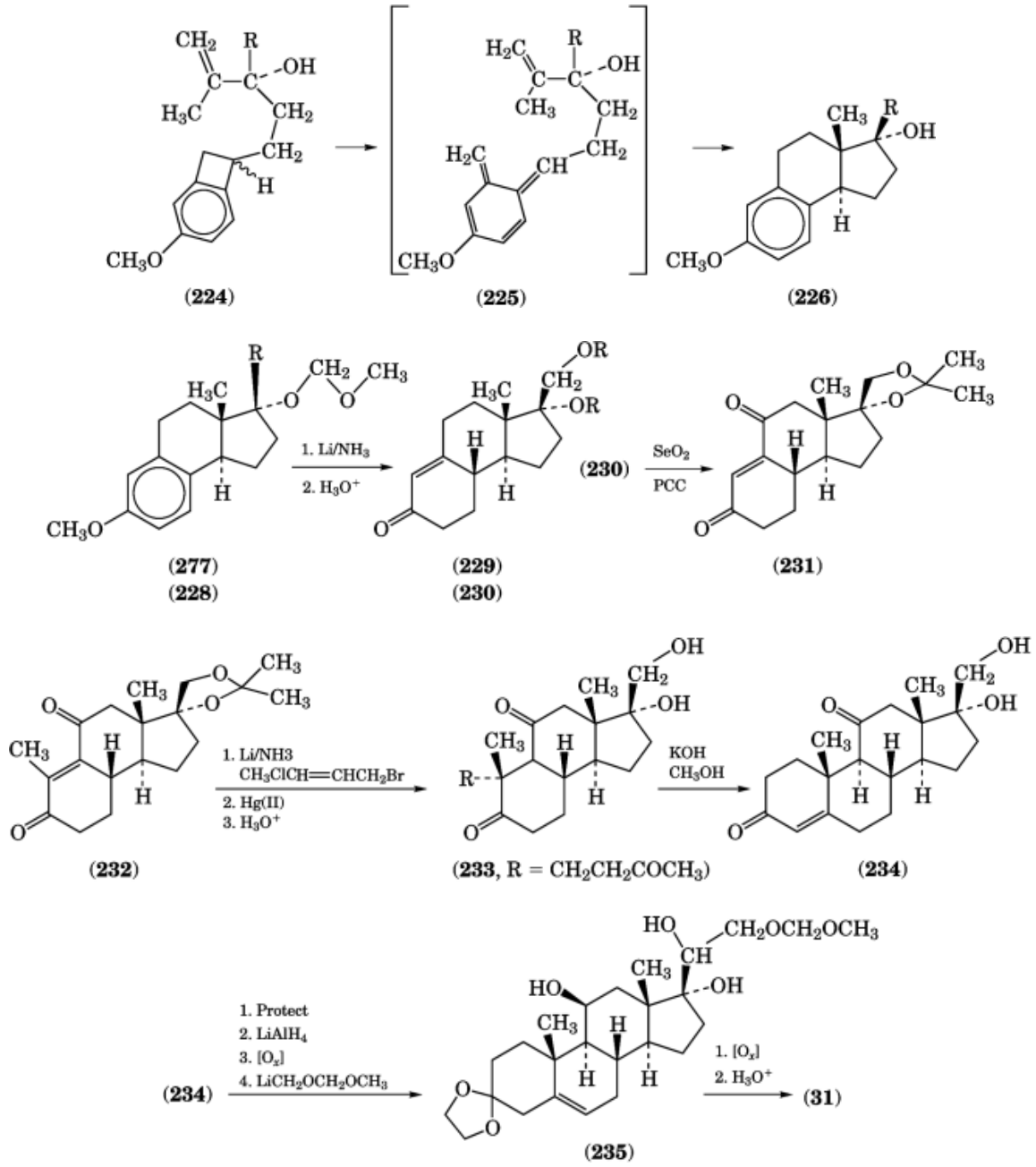
A similar intramolecular Diels-Alder strategy was employed in an efficient synthesis to an appropriately functionalized hydrindanone nucleus (212). After functionalization, Diels-Alder cyclization, and appropriate functional group manipulation, this hydrindanone was converted into (±)-cortisone. The overall process afforded (±)-cortisone in a total of 18 chemical steps in approximately 3% yield.

A third variation of this strategy has been applied to an enantioselective total synthesis of cortisone. From an appropriately functionalized, scalemic hydrindan that possessed an 11-oxo-group and a masked corticoid side-chain, (+)-cortisol was produced in an 11-step total synthesis (213).

Several additional Diels-Alder cycloaddition strategies have been applied to the total synthesis of the steroid skeleton (214). For example, the first enantioselective synthesis of (+)-cortisone **(31)** was accomplished by the intramolecular [4 + 2] cycloaddition of an olefinic *o*-quinodimethane that contained an optically active stereodirecting group as the key chemical step. Condensation of aldehyde **(221)** with a lithiated oxathiane forms a 96% yield of alcohol (**222**, $R' = OH$; $R'' = H$); Swern oxidation of **(222)** produces ketone (**223**, $R' = R'' = O$). Addition of 2-propene magnesium bromide to ketone **(223)** yields **(224)** as a single stereoisomer at the isopropenyl alcohol and as a mixture of two diastereomers at the benzocyclobutenylic position; the absolute configuration at the benzocyclobutenylic position is inconsequential because this stereocenter is lost during thermolysis of **(224)**. The thermal reaction of **(224)** proceeds through an intermediate *o*-quinodimethane ***(225)***, followed by a Diels-Alder [2 + 4] cycloaddition, producing the tricyclic compound **(226)** as the only detectable isomeric product in quantitative yield. Protection of the C17 tertiary alcohol with methoxymethyl chloride (MOMCl) followed by oxidative hydrolysis of the C18 chiral auxiliary produces aldehyde (**227**, R = CHO). Reduction of **(227)** with sodium borohydride affords alcohol (**228**, $R = CH_2OH$). Birch reduction of **(228)** followed by acid-catalyzed hydrolysis of the resultant enol ether affords, in about 75% yield, the thermodynamically favored stereoisomer (**229**, R = H) as a single product. Diol **(229)** is converted into acetonide (**230**, R = isopropylidene), quantitatively. Allylic oxidation of **(230)** followed by pyridinium chlorochromate (PCC) mediated oxidation of the resultant mixture of allylic alcohols produces ene-dione **(231)**. A 1,3-dipolar cycloaddition of diazomethane to **(231)** followed by thermolysis forms **(232)**. Compound **(232)** contains the C19 angular methyl substituent and an oxygen at C11. Stereoselective A-ring formation, through a known procedure (215), produces **(234)**. The steroid (**234** [128802-55-1]) contains the basic skeleton of (+)-cortisone **(31)**, therefore the total synthesis of **(31)** is completed by C3-ketone protection, side-chain manipulations, and final deprotection **(234)** $\longrightarrow$ **(235)** $\longrightarrow$ **(31)** (216, 217).



(**221**) (**222**) (**223**)



Other approaches to the stereoselective total synthesis of nonaromatic steroids include the carbocationic, biomimetic cyclization reactions. Generally, these cyclizations begin with the synthesis of an appropriately functionalized cyclopentenol. Acid-catalyzed cyclization forms the B–C–D rings of the steroid nucleus with the natural relative stereochemistry in a single step. Methods for the stereoselective synthesis of acyclic and monocyclic substrates and reaction conditions for their cyclization have been established (218). Optimization of these carbocationic cyclizations is accomplished by manipulating the initiating and the terminating

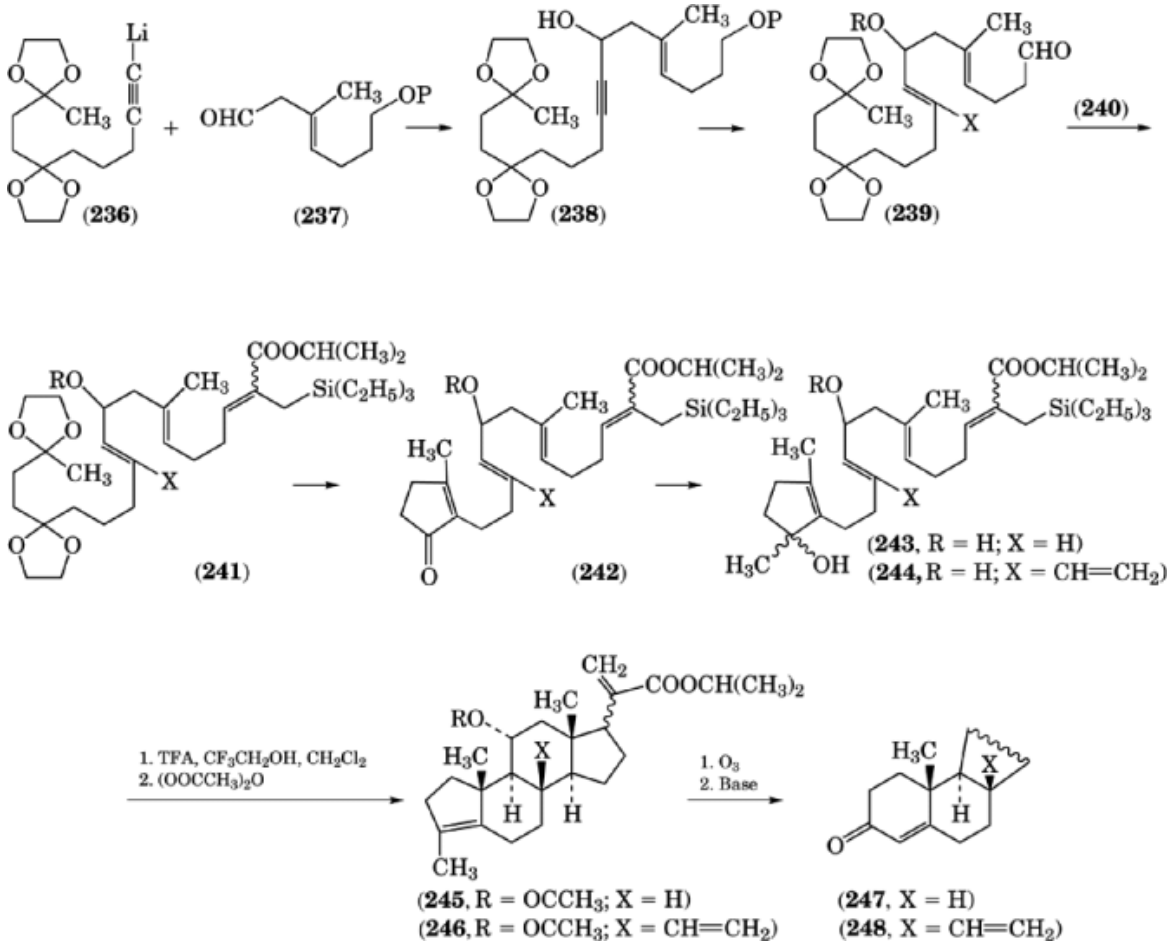


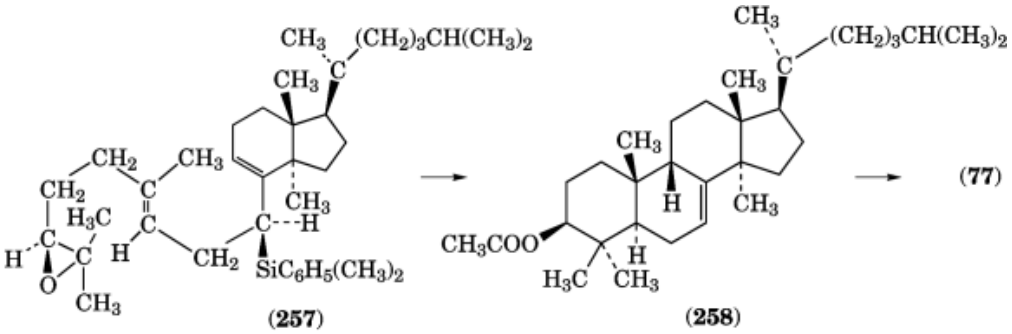
Fig. 26. Representative biomimetic cyclization where P=a protecting group, TFA=trifluoroacetic acid, and **(240)** $(CH_3)_2CHOOC \quad O$ | ‖ $(C_2H_5)_3SiCH_2CH—P(OC_2H_5)_2$

groups. Figure 26 illustrates a carbocationic cyclization approach. The addition of diketal (**236**) to aldehyde (**237**) produces alcohol (**238**). Compound (**238**) is converted into aldehyde (**239**) after several functional group manipulations. Treatment of (**239**) with the anion of phosphonate **(240)** forms (**241**) as a 2:3 mixture of (*E*)- and (*Z*)-isomers. Compound (**241**) is converted to enone (**242**) and then to the desired carbinols (**243**) and (**244**) through established methodology (219). Compounds (**243**) and (**244**) contain an allylic cyclopentenol as a carbocation initiating group and allylic silanes as a terminating group. Treatment of (**243**) and (**244**) with trifluoroacetic acid (TFA) in 1:1 2,2,2-trifluororethanol and dichloromethane, followed by acetylation of the C17-alcohol, produces (**245**) and (**246**) in 20 and 80% yields, respectively (220). Previously, similar steroids that contain this methyl cyclopentene A-ring have been converted to the natural steroid A-ring by oxidative cleavage of the double bond, followed by base-catalyzed cyclization (221).

In the above carbocationic cyclizations (see Fig. 26), a C8β-vinyl substituent enhances the rate and overall yield of the reaction by providing a cation-stabilizing auxiliary. Chemical routes were explored to remove this

C8β-vinyl substituent. These pathways required many chemical steps and were not very efficient. Therefore, fluoro-olefins were studied as removable cation-stabilizing auxiliaries (Fig. 27) (222). These studies culminated in the synthesis of *dl*-β-amyrin (**256**). Starting with mesityl oxide, fluoro-dienol (**249**) was prepared in nine steps in a 20% overall yield. Compound (**249**) was converted to cyclopentenol (**250**) in approximately 18% overall yield in 15 chemical steps. Compound (**250**) contains an allylic cyclopentenol initiating group, a fluoro-olefin cation-stabilizing auxiliary, and a propargyl silane-terminating group. Treatment of (**250**) with trifluoroacetic acid in dichloromethane produced the pentacyclic compound (**251**) in 65–70% yield. Four rings of (**251**) bearing seven chiral centers were formed during this cyclization step. Compound (**251**) was converted to *dl*-β-amyrin (**256**) by a series of chemical steps that included oxidative cleavage of the double bonds, base-catalyzed cyclization to form the six-membered A-ring, elimination of the C13-fluorine group, and dimethylation at C4 (223). The completion of the A-ring transformations, especially dimethylation at C4, was problematic. Therefore, other carbocation-initiating groups were studied. First, an epoxide was used as an initiating group. Treatment of polyene (**252**) with a Lewis acid in dichloromethane at $-78^{\circ}C$ provides pentacycle (**253**) in 10% yield. Steroid (**253**) contains the A-ring functionality of β-amyrin (**256**). In an improved process, an allylic alcohol-initiated polycyclization was investigated. Treatment of polyene (**254**) with a Lewis acid in dichloromethane at $-78^{\circ}C$ forms pentacycle (**255**) in 31% yield. This cyclization product (**255**) is suitably functionalized at C3, C13, and C22 to allow conversion to β-amyrin (**256**) with a minimum of synthetic manipulations (224).

Other, removable cation-stabilizing auxiliaries have been investigated for polyene cyclizations. For example, a silyl-assisted carbocation cyclization has been used in an efficient total synthesis of lanosterol. The key step, treatment of **(257)** with methyl aluminum chloride in methylene chloride at $-78^{\circ}C$, followed by acylation and chromatographic separation, affords **(258)** in 55% yield (two steps). When this cyclization was attempted on similar compounds that did not contain the C7β-silicon substituent, no tetracyclic products were observed. Steroid **(258)** is converted to lanosterol (**77**) in three additional chemical steps (225).



In addition to cationic cyclizations, other conditions for the cyclization of polyenes and of ene-ynes to steroids have been investigated. Oxidative free-radical cyclizations of polyenes produce steroid nuclei with exquisite stereocontrol. For example, treatment of **(259)** and **(260)** with Mn(III) and Cu(II) afford the D-homo-5α-androstane-3-ones **(261)** and **(262)**, respectively, in approximately 30% yield. In this cyclization, seven asymmetric centers are established in one chemical step (226, 227). Another intramolecular cyclization reaction of iodo-ene poly-ynes was reported using a carbopalladation cascade terminated by carbonylation. This carbometalation–carbonylation cascade using CO at 111 kPa (1.1 atm) at 70°C converted an acyclic iodo–tetra-yne **(263)** to a D-homo-steroid nucleus **(264)** [162878-44-6] in approximately 80% yield in one chemical step (228). Intramolecular annulations between two alkynes and a chromium or tungsten carbene complex have been examined for the formation of a variety of different fused-ring systems. A tandem Diels-Alder–two-alkyne annulation of a triynylcarbene complex demonstrated the feasibility of this strategy for the synthesis of steroid nuclei. Complex **(265)** was prepared in two steps from commercially available materials.

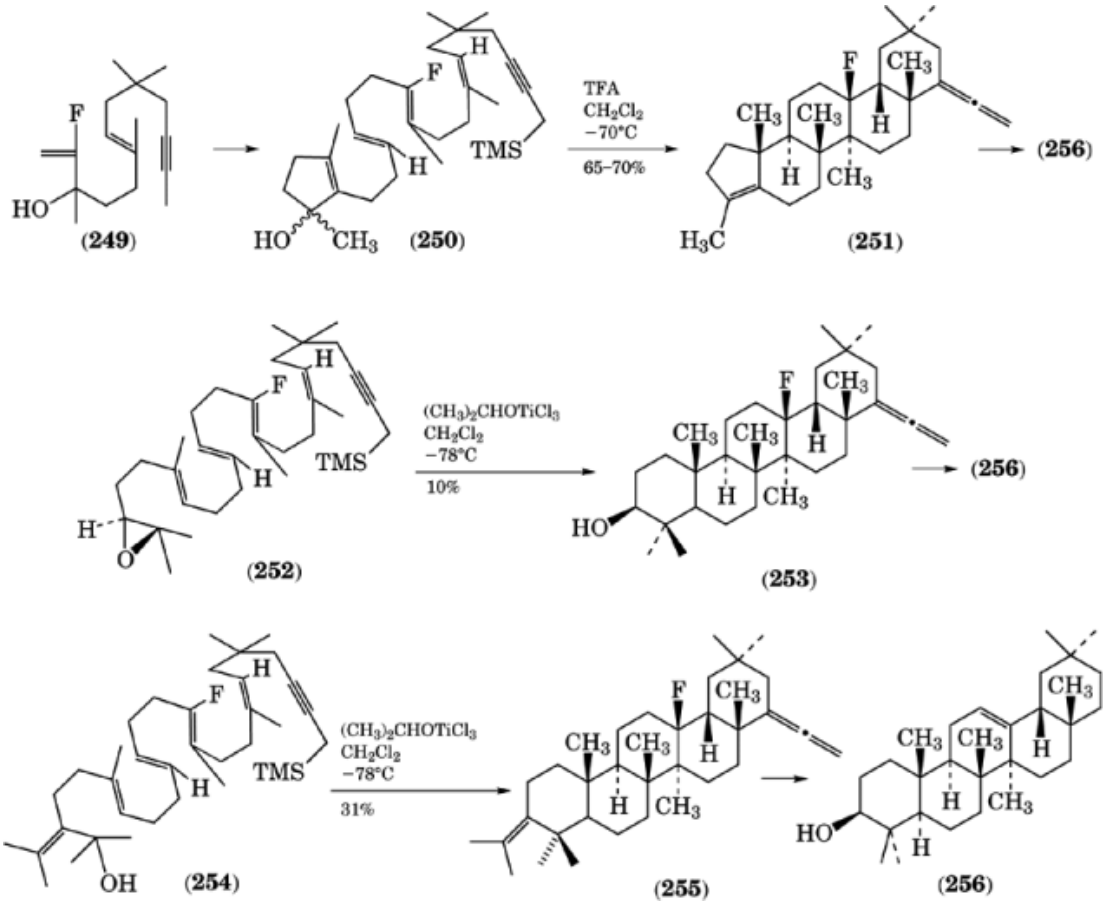
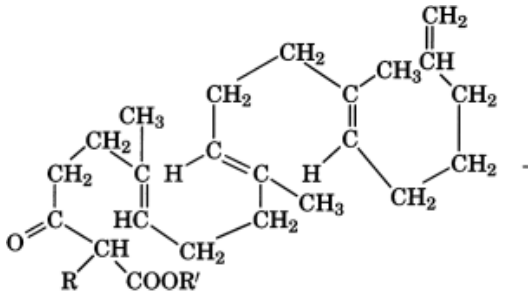
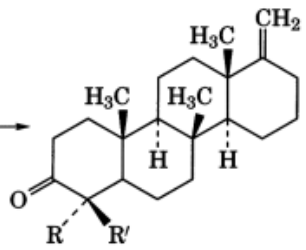


Fig. 27. Total synthesis of β-amyrin [559-70-6] (**256**) via a carbocationic cyclization, where $_{TMS=}$ trimethylsilyl.

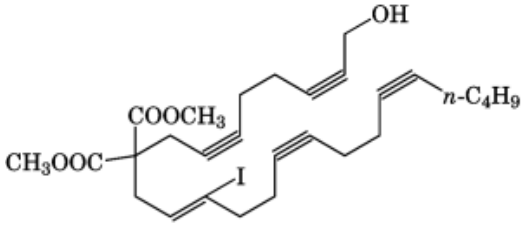
Treatment of **(265)** with Danishefsky's diene in CH_3CN at room temperature under an atmosphere of carbon monoxide (101.3 kPa = 1 atm), followed by heating the reaction mixture to 110°C, provided **(266)** in 62% yield (TBS = *tert* − butyldimethylsilyl). In a second experiment, a sequential Diels-Alder–two-alkyne annulation of triynylcarbene complex **(267)** afforded a nonaromatic steroid nucleus **(269)** in approximately 50% overall yield from the acyclic precursors (229).



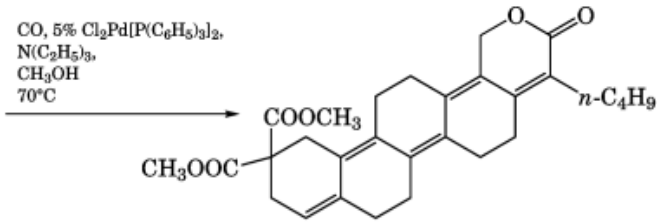
(**259**, R = CH_3; R′ = CH_3)
(**260**, R = H; R′ = C_2H_5)



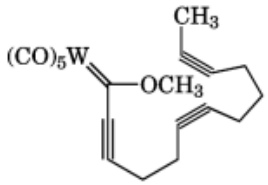
(**261**, R = CH_3; R′ = $COOCH_3$)
(**262**, R = $COOC_2H_5$; R′ = H)



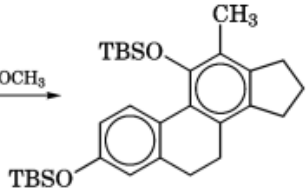
(**263**)



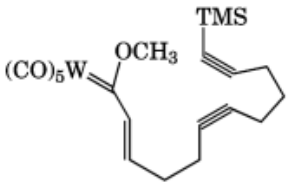
(**264**)



(**265**)



(**266**)



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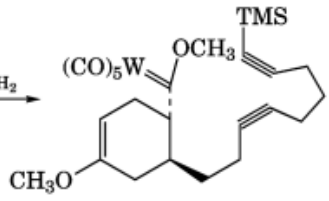
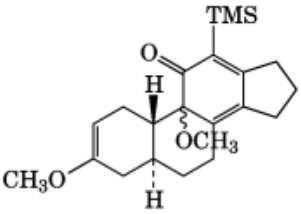
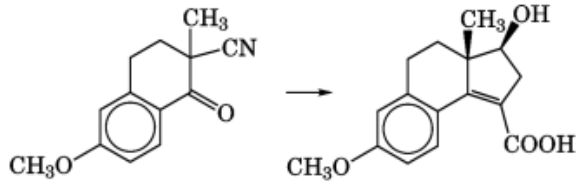


(**268**)

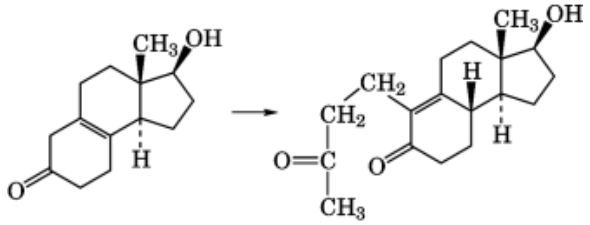


(**269**)

Besides the aforementioned A-ring aromatic steroids and contraceptive agents, partial synthesis from steroid raw materials has also accounted for the vast majority of industrial-scale steroid synthesis. One notable exception, however, was the first industrial-scale synthesis of optically active steroids performed by workers at Roussel-UCLAF. The linear synthesis began with a suitable B–C-ring synthon, 6-methoxy-1-tetralone (**186**). In a series of steps, tetralone (**186**) was converted to 2-methyl-2-cyanotetralone **(270)**. Condensation of **(270)** with dimethyl succinate followed by carbonyl reduction, saponification, and resolution produced the optically active tricyclic acid **(271)**. A series of reductions, a decarboxylation, and a hydrolysis produced **(272)**. Appendage of the A-ring functionality by alkylation produced intermediate **(273)**. Compound **(273)** was used as a common intermediate for the synthesis of 19-norsteroids, estrogens, and corticosteroids (230).



(270) **(271)**



(272) **(273)**

An interesting breakthrough in steroid endocrinology occurred with the discovery of a novel class of steroid antihormones. Several 11β-substituted 19-norsteroids display potent antiprogestinal activity. For example, RU-486 [84371-65-3] (**278**) is marketed in Europe as a contragestive agent. The synthesis of RU-486 demonstrates a unique method for functionalization of the 11β-position of a steroid nucleus. Condensation of the mono-ketal (**274**), available by either partial synthesis or total synthesis methods discussed above, with lithium propyne forms (**275**). Regiospecific epoxidation of (**275**) with hydrogen peroxide and hexafluoroacetone in methylene chloride produces epoxide (**276**) as a 3:1 mixture of 5α,10α-epoxide (**276**) and 5β,10β-epoxide, respectively. Pure epoxide (**276**) is isolated in approximately 60% yield after chromatographic purification. More recently, similar dienes have been epoxidized using a readily accessible pigment, Fe(II)-phthalocyane, as a catalyst to form a 10:1 mixture of a 5α,10α-epoxide and a 5β,10β-epoxide, respectively (231). In the key step, addition of a Grignard reagent to the unsaturated epoxide (**276**), via copper-catalyzed conjugate addition, produces the 11β-substituted steroid (**277**) in high yield. This reaction is extremely versatile for a variety of different copper-catalyzed organometallic reagents regardless of steric hindrance. Concomitant acid-catalyzed ketal hydrolysis and dehydration produces RU-486 (**278**) (232) (Fig. 28).

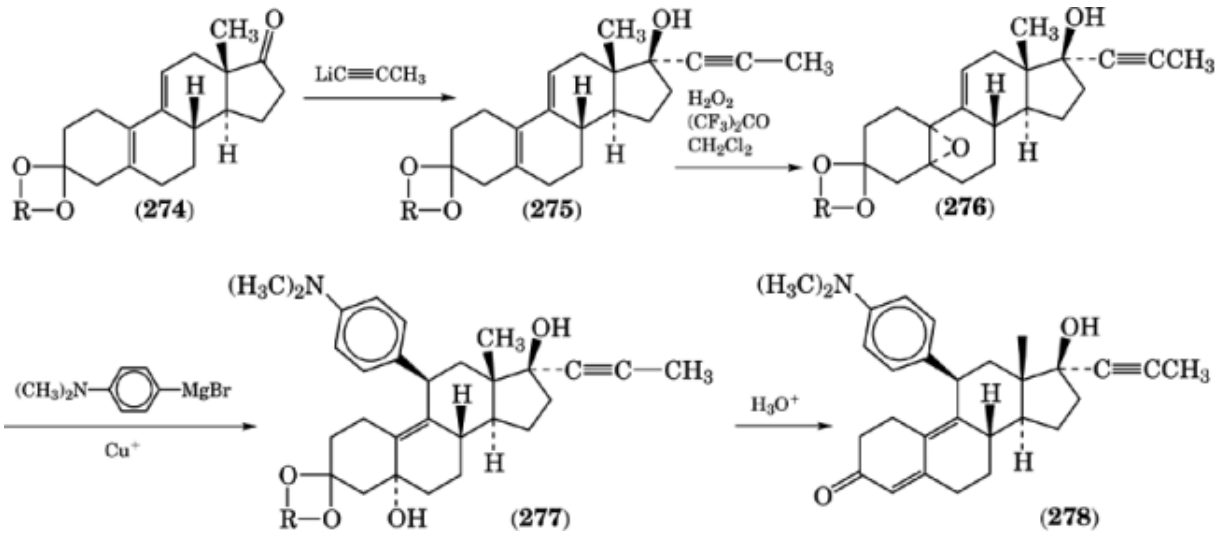


Fig. 28. Industrial synthesis of RU-486.

6. Uses: Therapeutics and Toxicology

6.1. Steroid Hormones

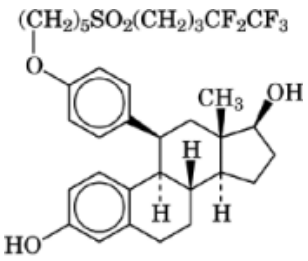
6.1.1. Sex Hormones

The largest economic impact of synthetic estrogen and progestin production has been for use as contraceptive agents and for treatment and prevention of osteoporosis. Mixtures of estrogens and progestins have been used as contraceptive agents since the early 1960s. The principal mode of steroid contraceptive action is exerted at the hypothalamic–pituitary–ovarian and uterine sites. Thus, contraceptive steroid mixtures have been used to treat a variety of related abnormal states including endometriosis, dysmenorrhea, hirsutism, polycystic ovarian disease, dysfunctional uterine bleeding, benign breast disease, and ovarian cyst suppression (233). One of two estrogens, ethinylestradiol [57-63-6] ((17α)-19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol) or mestranol [72-33-3] ((17α)-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yne-17-ol) is contained in most combination oral contraceptives. The progestin component in oral contraceptive pills is more variable. The progestin component can be progesterone derivatives that contain a C6-methyl group such as medroxyprogesterone [520-85-4] ((6α)-17-hydroxy-6-methyl-4-ene-3,20-dione) and megestrol acetate [595-33-5] ((17-hydroxy-6-methylpregna-4,6-diene-3,20-dione acetate). Also, 19-norsteroids, such as norethindrone [68-22-4] ((17α)-17-hydroxy-19-norpregn-4-en-20-yn-3-one) (**112**), norgestrel [6533-00-2] (13-ethyl-17-hydroxy-18,19-dinorpregn-4-en-20-yn-3-one) **(208)**, norgestimate [35189-28-7] ((17α)-17-(acetyloxy)-13-ethyl-18,19-dinorpregn-4-20-yn-3-one) and gestodene [60282-87-3] ((17α)-13-ethyl-17-hydroxy-18,19-dinorpregnane-4,15-diene-20-yne-3-one) are used as orally active progestins. The C17-ethinyl moiety protects these steroids from metabolism and assists in their excellent pharmacokinetic profile (234–236).

Estrogens are routinely prescribed to post-menopausal women to prevent the development and exacerbation of osteoporosis because it can increase bone density and reduce fractures. Any increased risk of uterine cancer with the use of estrogen alone is practically eliminated by cyclic therapy with a progestinal agent. Estradiol **(25)** or conjugated estrogens, isolated from the urine of pregnant mares, are typical agents used for the prevention and treatment of osteoporosis (237).

Antiprogestins, such as RU-486 [84371-65-3] (17β-hydroxy-11β-(4-dimethylaminophenyl-1)-17α-(prop-1-ynyl)-estra-4,9-diene-3-one) (**278**) and ZK98299 [096346-61-1], (11β-(4-dimethylaminophenyl)-17α-hydroxy-17β-(3-hydroxypropyl-13α-methyl-4,9-gonadien-3-one) (**237**) represent a new class of drugs for fertility regulation. Also, these drugs have potential applications in the treatment of uterine cancer. An RU-486 and prostaglandin combination is accepted in Western Europe as a low resource method for early pregnancy termination (239).

During the 1960s and 1970s a wide range of estrogens and antiestrogens were synthesized primarily to study reproductive endocrinology. The focus of clinical applications of many of these antiestrogens has shifted to breast cancer therapy. These antiestrogens possess both steroidal, such as RU-58668 [151555-47-4] (11β-4-[5-(4,4,5,5,5-pentafluoropentylsulfonyl)pentyloxy]penyl]estra-1,3,5(10)-triene-3,17β-diol **(279)** (240) and nonsteroidal, such as tamoxifen [10540-29-1] (**280**, R = H), droloxifene [82413-20-5] (**281**, R = OH), and raloxifene [84449-90-1] **(282)**, structures. Although structurally different, all of these



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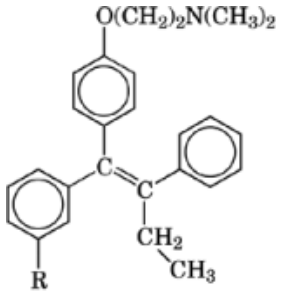

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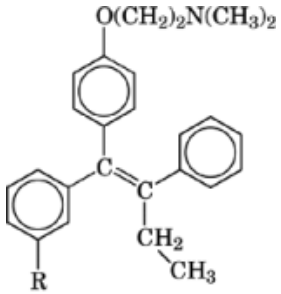
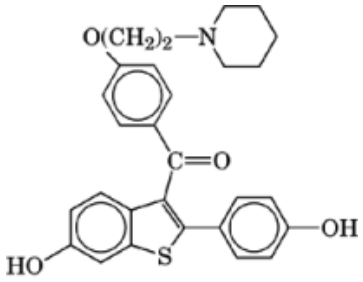


(**281**)



(282)

antiestrogens bind to the estrogen receptor in the breast cancer cell and exert a profound influence on cell replication. However, many of these compounds, eg, tamoxifene, may have other indirect mechanisms to control cell replication. Along with acting as estrogen antagonists in the breast, droloxifene and raloxifene are estrogen agonists in bone and are undergoing clinical trials for the treatment of osteoporosis (241, 242).

Testosterone, alkylated testosterone, or testosterone esters are the primary anabolic–androgenic steroid drugs. Most of these synthetic testosterone derivatives were developed in the 1950s in failed attempts to separate the hormones' masculinizing (androgenic) and skeletal muscle-building (anabolic) effects. The difficulty in separating the androgenic and anabolic effects was probably experienced because both actions of these steroid derivatives are mediated by the same intracellular receptor. The medicinal uses for these drugs include treatment of certain types of anemias, hereditary angioedema, certain gynecological conditions, protein anabolism, certain allergic reactions, and use in replacement therapy in gonadal failure states. However, anabolic–androgenic steroids are best known for their nonmedical, and illegal, use to aid in body-building or to increase skeletal muscle size, strength, and endurance. The doses used by strength athletes often exceed testosterone-equivalent doses for replacement therapy by 10- to 100-fold. Consequently, a vast number of toxic and deleterious effects from these drugs have been reported. Among these adverse effects are impaired liver function including malignant hepatic tumors, impaired growth of prepubescent boys, altered sexual characteristics in both men and women, and prostatic enlargement. Other possible adverse effects include an increased risk for cardiovascular disease, colonic cancer, adenocarcinoma of the prostate, fatal rupture of a hepatic tumor, severe cystic acne, and psychosis (243).

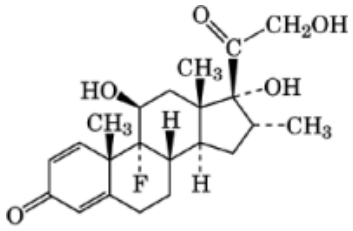
6.1.2. Corticosteroids

The greatest portion of steroid drug production is aimed at the synthesis of glucocorticoids (244), which are highly effective agents for the treatment of chronic inflammation. Glucocorticoids exert their effects by binding to the cytoplasmic glucocorticoid receptor within the target cell and thus either increase or decrease transcription of a number of genes involved in the inflammatory process. Specifically, glucocorticoids down-regulate potential mediators of inflammation such as cytokines, certain cytokine receptors, inducible nitric oxide synthase (NOS), cyclooxygenase-2, endothelin-1, and phospholipase A_2 (PLA_2) and up-regulate agents that are potential inhibitors of inflammation such as lipocortin-1, β_2-adrenoreceptor, endonucleases, neutral endopeptidase (NEP), and angiotensin converting enzyme (ACE) (245).

The discovery that cortisone dramatically alleviates the symptons of arthritis led to intensive research on the antiinflammatory properties of corticosteroids. Glucocorticoids are used (ca 1996) to treat a variety of different diseases that are exacerbated by inflammation, such as arthritis, asthma, rhinitis, and skin irritations. Typical oral glucocorticoids used to treat rheumatoid arthritis are prednisone (**132**), 6α-methylprednisolone [83-43-2], and dexamethasone [50-02-2] **(283)**. Systemic side effects of exogenous glucocorticoids include suppression of the pituitary–adrenal axis, resulting in a decreased response to stress which eventually normalizes after discontinuation, although the recovery can be delayed. An excess of exogenous glucocorticoids can result

in symptoms of Cushing's disease, including weight gain, weakness, hypertension, and diabetes. In addition, excess exogenous glucocorticoid ingestion can cause increased bone reabsorption because of a negative calcium balance, delayed union fractures, secondary hyperparathyroidism, reduced bone formation, and sex hormone disturbances, all contributing to a significant increase in the risk of osteoporosis (246).

Corticosteroids are the most efficacious treatment available for the long-term treatment of asthma, and inhaled corticosteroids are considered to be a first-line therapy for asthma (247). In the early 1950s, cortisone **(31)** and cortisol **(29)** were used to treat asthma. However, drugs with fewer side effects and with



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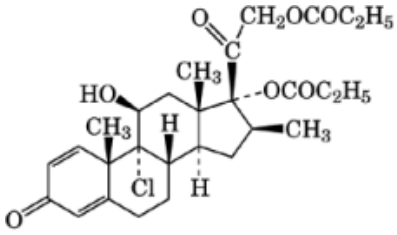
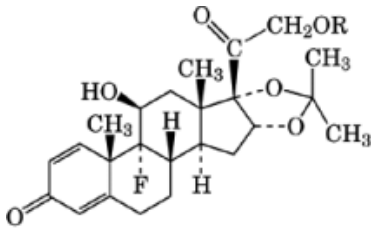


(284)

a higher therapeutic index were sought. Prednisolone (**131**) and dexamethasone **(283)** were developed as orally active corticosteroids with increased glucocorticoid activity but decreased mineralocorticoid activity. Still, these drugs possessed unwanted systemic side effects. In 1972, betamethasone valerate and beclomethasone dipropionate [55340-19-8] **(284)** were introduced. These topically active corticosteroids had few side effects when administered by inhalation. Since 1972



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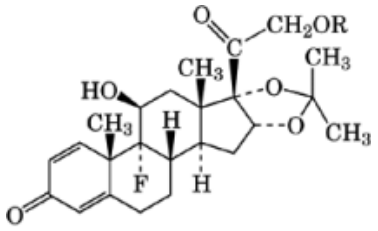

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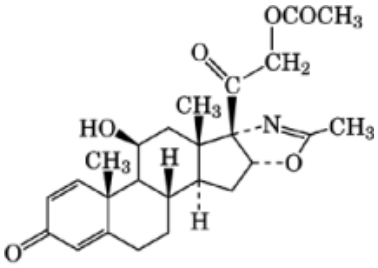


(287)

several corticosteroids have been designed to break down rapidly after reaching the systemic circulation. Therefore, these novel corticosteroids are less likely to suppress the hypothalamic–pituitary–adrenal axis. Some of these drugs that are in clinical development include fluticasone (**288**), mometasone (**289**), SQ-27239 (**290**), and JO-1222 (**291**) (Fig. 29) (248, 249). Other corticosteroids include [76-25-5] (**285**, R = H triamcinolone acetonide), [5611-51-8] (**286**, R = $OCCH_2C(CH_3)_3$ triamcinolone hexacetonide), and dexflazacort [14484-47-0] **(287)**.

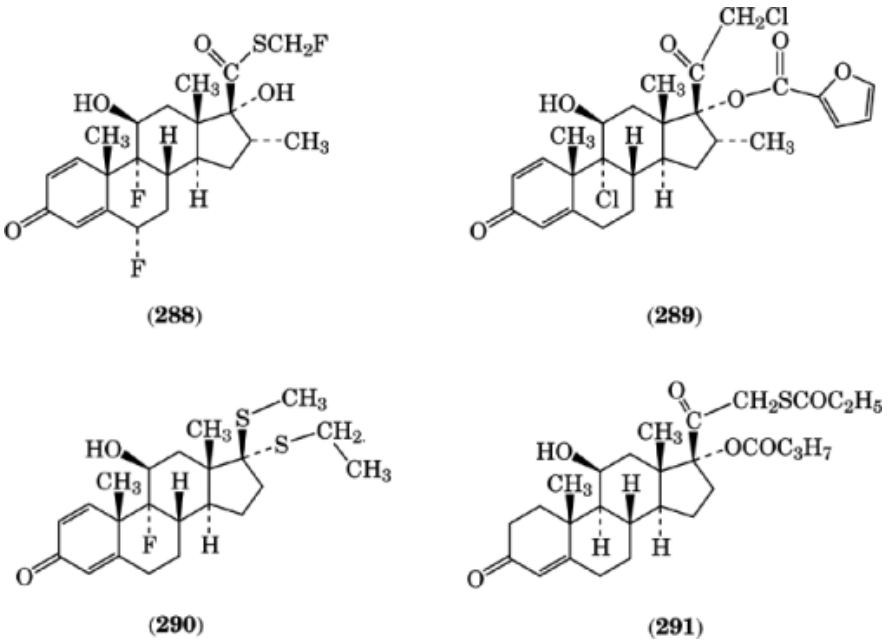
Rhinitis is characterized by nasal stuffiness with partial or full obstruction, and itching of the nose, eyes, palate, or pharynx, sneezing, and rhinorrhoea. If left untreated it can lead to more serious respiratory diseases such as sinusitis or asthma. Although several types of drugs are available for treatment, nasal spray topical corticosteroids are widely regarded as the reference standard in rhinitis therapy (250).

There are hundreds of topical steroid preparations that are available for the treatment of skin diseases. In addition to their aforementioned antiinflammatory effects, topical steroids also exert their effects by vasoconstriction of the capillaries in the superficial dermis and by reduction of cellular mitosis and cell proliferation especially in the basal cell layer of the skin. In addition to the aforementioned systemic side effects, topical steroids can have adverse local effects. Chronic treatment with topical corticosteroids may increase the risk of bacterial and fungal infections. A combination steroid and antibacterial agent can be used to combat this problem. Additional local side effects that can be caused by extended use of topical steroids are epidermal atrophy, acne, glaucoma and cataracts (thus the weakest concentrations should be used in and around the eyes), pigmentation problems, hypertrichosis, allergic contact dermatitis, perioral dermatitis, and granuloma gluteale infantum (251).

6.2. Other Therapeutic Steroids

6.2.1. Saponins

Although the hypocholesterolemic activity of saponins has been known since the 1950s, their low potency and difficult purification sparked little interest in natural saponins as hypolipidemic agents. Synthetic steroids (**292**, **293**) that are structurally related to saponins have been shown to lower plasma cholesterol in a variety

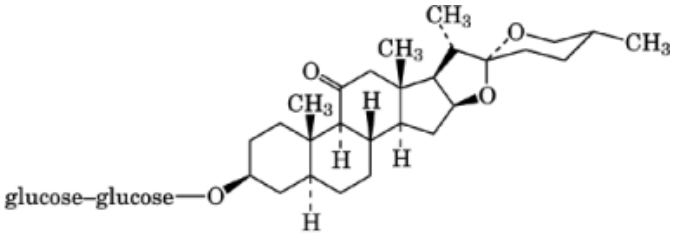


(288) (289)

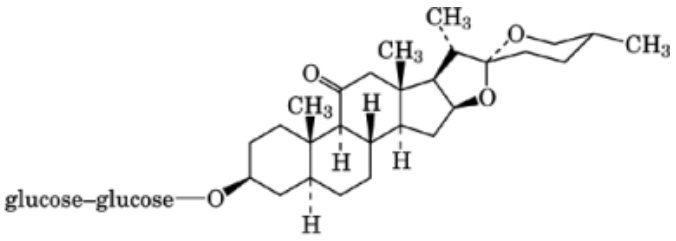
(290) (291)

Fig. 29. Examples of topical antiasthma corticosteroids that are rapidly broken down in the circulation: fluticasone [90566-53-3] (**288**), monetasone [83919-23-7] (**289**), SQ-27239 [85197-77-9] (**290**), and JO-1222 [98449-05-9] (**291**).

of different species (252). Steroid **(292)** is designated CP-88,818 [99759-19-0]. The hypocholesterolemic agent CP-148,623 [150332-35-7] **(293)** is not absorbed into the systemic circulation and does not inhibit enzymes involved in cholesterol synthesis, release, or uptake. Rather, **(293)** specifically inhibits cholesterol absorption into the intestinal mucosa (253). As of late 1996, CP-148,623 is in clinical trials as an agent that lowers blood concentrations of cholesterol (254).



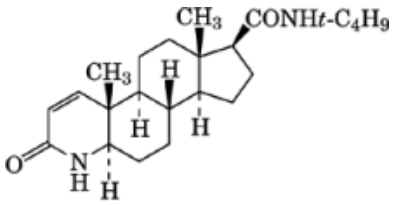
(**292**, no carbonyl at C-11)



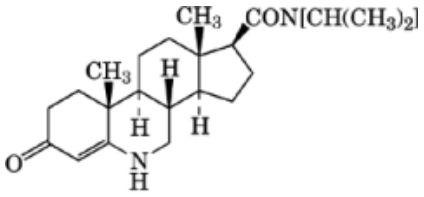
(293)

6.2.2. Heterocyclic Steroids

Steroid 5α-reductase (types 1 and 2) converts testosterone **(21)** to the physiologically more potent androgen dihydrotestosterone (DHT) **(22)**. The type 1 isoform occurs in nongenital skin, whereas the type 2 isoform is the predominant form in the prostate (the type 1 isoform is present in a lesser extent) and genital skin fibroblasts. There has been much interest in developing inhibitors of steroid 5α-reductase as a therapy for a variety of disorders associated with elevated levels of DHT including benign prostatic hyperplasia (BPH), some prostatic cancers, certain skin disorders, and male pattern baldness. Among these inhibitors are 4-aza and 6-aza steroids. The 4-aza



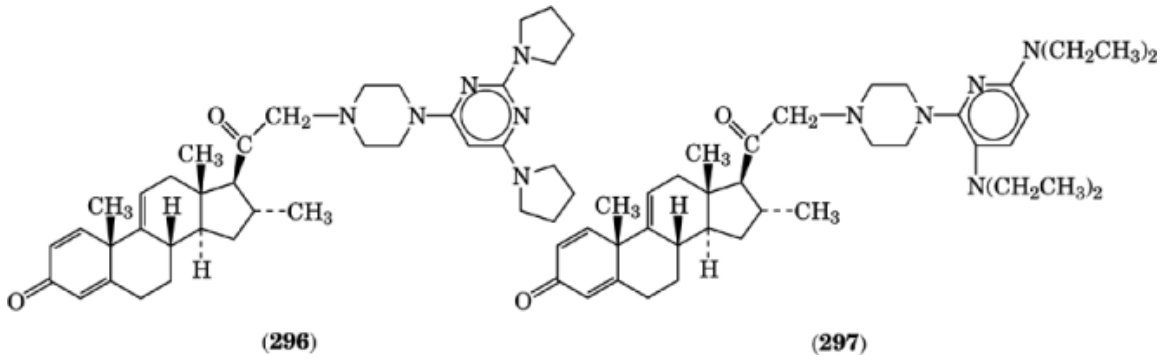
(294)



(295)

steroid, finasteride [98319-26-7] **(294)**, shows selectivity for the human type 2 isoform and has been approved for the treatment of benign prostate hyperplasia (255).

Several steroids that contain C21 heterocycles (lazaroids) have been reported to be potent inhibitors of iron-dependent lipid peroxidation (Fig. 30). These compounds were evaluated as antioxidants, CNS neuroprotective agents, and antiasthmatic agents. This class of compounds does not appear to have any glucocorticoid activity. Thus, the precise mode of action for the antiasthmatic activity of lazaroids is under investigation. Tirilazad mesylate [110101-67-2] (U-74006F) (**296**) is undergoing clinical trials for head injury, subarachniod hemorrhage, and spinal cord trauma (256, 257).



(296) (297)

Fig. 30. C_{21}-Heterocyclic steroids that are inhibitors of iron-dependent lipid peroxidation: U-74006F [110101-67-2] (**296**) and U-74500A [110101-65-0] (**297**).

7. Economic Aspects

7.1. Raw Materials

In 1984, diosgenin accounted for at least 50% of the total steroid drug output worldwide (258); 1994 estimates suggest that approximately 60% of all steroids used as drugs are synthesized using diosgenin as the starting material (259). Until 1970, Mexico was the main source of diosgenin production. In the early 1970s the Mexican government's nationalization of the collection of *Dioscorea* plants together with a decrease in diosgenin content (6–4%) by overharvesting and increases in transportation costs, caused the price of a kilogram of diosgenin to rise from \$10 to >\$100 by the mid-1970s. Because of competition with other steroid raw materials, such as β-sitosterol, stigmasterol, and the solasidine alkaloids, and competition from other diosgenin-producing countries, the price of a kilogram of diosgenin fell to \$25 by the early 1980s (260). As of 1991, the leading producers of diosgenin were China, Mexico, India, Guatemala, and Costa Rica. China, Brazil, and India are the leading exporters of hecogenin (261).

7.2. Therapeutics

The sale of steroid drugs is a multibillion dollar industry. The two largest selling groups of steroid drugs are the systemic sex hormones, including agents such as hormonal contraceptives, estrogens, progestins, and androgens, and the corticosteroids (Table 2). The 12-month period ending December 1994 saw systemic sex hormones account for approximately \$5.4 billion whereas total corticosteroids accounted for about \$5.7 billion in sales. Disregarding inflation, the sales of steroid drugs have climbed since the 1970s. For example, total worldwide sales of the systemic sex hormones and corticosteroids was around \$7.4 billion in 1990 and approximately \$11.2 billion in 1994.

In addition to the sex hormones and corticosteroids, other steroid drugs have substantial worldwide markets. For example, cytostatic hormones had worldwide sales of approximately \$1.8 billion in 1994. Included in these \$1.8 billion are several steroids or steroid-mimetics such as megestrol acetate and tamoxifen **(282)**, respectively (262).

Table 2. Total Worldwide Sales of Systemic Sex Hormones and Corticosteroids

	Sales, $\$ \times 10^6$	
Steroid class	1990	1994
sex hormones, systemic	3,582	5,436
corticosteroids		
topical	1,558	1,891
systemic	903	1,181
respiratory	988	2,170
nasal	382	665
inhalants, systemic	606	1,505
steroids for sensory organs	396	507
Total	*7,427*	*11,185*

8. Analytical Methods

The field of steroid analysis includes identification of steroids in biological samples, analysis of pharmaceutical formulations, and elucidation of steroid structures. Many different analytical methods, such as ultraviolet (uv) spectroscopy, infrared (ir) spectroscopy, nuclear magnetic resonance (nmr) spectroscopy, x-ray crystallography, and mass spectroscopy, are used for steroid analysis. The constant development of these analytical techniques has stimulated the advancement of steroid analysis.

Data collected on the uv spectra of steroids are available in several books, spectrum atlases, and review articles (263). The most characteristic absorptions in steroid hormones include α,β-unsaturated ketones, conjugated dienes, and phenolic A-rings (264).

The most powerful method for structure elucidation of steroid compounds during the classical period of steroid chemistry ($\sim 1940 - 1950\text{s}$) was ir-spectroscopy. As with the ultraviolet spectra, data collected on the infrared spectra of steroids are available in several books, spectrum atlases, and review articles (265, 266). Unlike ultraviolet spectroscopy, even the least substituted steroid derivatives are relatively rich in characteristic absorption bands in infrared spectroscopy (264).

Generally, the most powerful method for structural elucidation of steroids is nuclear magnetic resonance (nmr) spectroscopy. There are several classical reviews on the one-dimensional (1-D) proton ^{1}H-nmr spectroscopy of steroids (267). ^{13}C-nmr, a technique used to observe individual carbons, is used for structure elucidation of steroids. In addition, ^{13}C-nmr is used for biosynthesis experiments with ^{13}C-enriched precursors (268). The availability of higher magnetic field instruments coupled with the arrival of 1-D and two-dimensional (2-D) techniques such as DEPT, COSY, NOESY, 2-D J-resolved, HOHAHA, etc, have provided powerful new tools for the structural elucidation of complex natural products including steroids (269).

A definitive method for structural determination is x-ray crystallography. Extensive x-ray crystal structure determinations have been done on a wide variety of steroids and these have been collected and listed (270). In addition, other analytical methods for steroid quantification or structure determination include, mass spectrometry (271), polarography, fluorimetry, radioimmunoassay (264), and various chromatographic techniques (272).

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