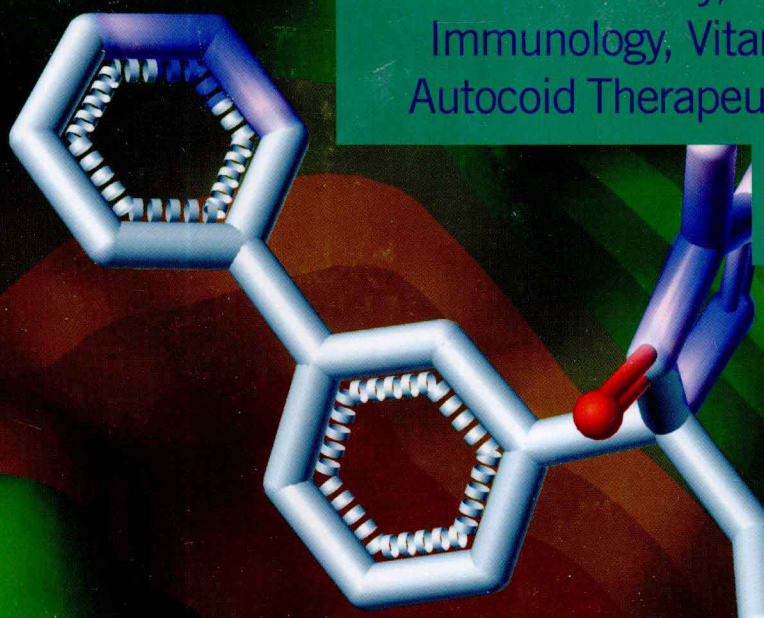
SEVENTH EDITION

BURGER'S MEDICINAL CHEMISTRY, DRUG DISCOVERY, AND DEVELOPMENT

Pulmonary, Bone, Immunology, Vitamins, and Autocoid Therapeutic Agents

Volume 5



Edited by
Donald J. Abraham
David P. Rotella

WILEY

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Volume 5: Pulmonary, Bone, Immunology, Vitamins and Autocoid Therapeutic Agents

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PREFACE

The seventh edition of Burger's Medicinal Chemistry resulted from a collaboration established between John Wiley & Sons, the editorial board, authors, and coeditors over the last 3 years. The editorial board for the seventh edition provided important advice to the editors on topics and contributors. Wiley staff effectively handled the complex tasks of manuscript production and editing and effectively tracked the process from beginning to end. Authors provided well-written, comprehensive summaries of their topics and responded to editorial requests in a timely manner. This edition, with 8 volumes and 116 chapters, like the previous editions, is a reflection of the expanding complexity of medicinal chemistry and associated disciplines. Separate volumes have been added on anti-infectives, cancer, and the process of drug development. In addition, the coeditors elected to expand coverage of cardiovascular and metabolic disorders, aspects of CNS-related medicinal chemistry, and computational drug discovery. This provided the opportunity to delve into many subjects in greater detail and resulted in specific chapters on important subjects such as biologics and protein drug discovery, HIV, new diabetes drug targets, amyloid-based targets for treatment of Alzheimer's disease, high-throughput and other screening methods, and the key role played by metabolism and other pharmacokinetic properties in drug development.

The following individuals merit special thanks for their contributions to this complex endeavor: Surlan Alexander of John Wiley & Sons for her organizational skills and attention to detail, Sanchari Sil of Thomson Digital for processing the galley proofs, Jonathan Mason of Lundbeck, Andrea Mozzarelli of the University of Parma, Alex Tropsha of the University of North Carolina, John Block of Oregon State University, Paul Reider of Princeton University, William (Rick) Ewing of Bristol-Myers Squibb, William Hagmann of Merck, John Primeau and Rob Bradbury of AstraZeneca, Bryan Norman of Eli Lilly, Al Robichaud of Wyeth, and John Lowe for their input on topics and potential authors. The many reviewers for these chapters deserve special thanks for the constructive comments they provided to authors. Finally, we must express gratitude to our lovely, devoted wives, Nancy and Mary Beth, for their tolerance as we spent time with this task, rather than with them.

As coeditors, we sincerely hope that this edition meets the high expectations of the scientific community. We assembled this edition with the guiding vision of its namesake in mind and would like to dedicate it to Professor H.C. Brown and Professor Donald T. Witiak. Don collaborated with Dr. Witiak in the early days of his research in sickle cell drug discovery. Professor Witiak was Dave's doctoral advisor at Ohio State University and provided essential guidance to a young

scientist. Professor Brown, whose love for chemistry infected all organic graduate students at Purdue University, arranged for Don to become a medicinal chemist by securing a postdoctoral position for him with Professor Alfred Burger.

It has been a real pleasure to work with all concerned to assemble an outstanding and up-to-date edition in this series.

DONALD J. ABRAHAM
DAVID P. ROTELLA

March 2010

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CONTENTS

FUNDAMENTALS OF STEROID CHEMISTRY AND BIOCHEMISTRY

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1. INTRODUCTION

Steroids are a unique class of chemical compounds that are found throughout the animal and plant kingdom, and this class includes sterols such as cholesterol and ergosterol, bile acids, and steroid hormones. Modern scientific research on steroid chemistry and biochemistry began in the early twentieth century, and several major treatises on the subject have been published [1–9]. This chapter provides a general summary of the steroid chemistry and biochemistry, and is not a comprehensive review. Subsequent chapters in this volume contain more detailed discussions on the individual classes of steroids.

The biological and medical significance of steroids has been observed since ancient times, even though the exact chemical nature and properties of steroids began to be understood only in the late 1920s and early 1930s. In ancient Greek literature, Hippocrates referred to gallstones, and the term cholesterol is derived from the Greek words for bile (*chole*) and solid (*steros*). Aristotle identified the effects of castration on birds and on humans, and agricultural practices of castration to produce sterility, alter aggressiveness, and affect body size in domestic animals has been known since early times. Egyptians and Romans used extracts of plants such as purple foxglove to treat dropsy. The physical effects of castration were well recognized in eunuchs and in medieval castrato choirs.

Scientific observations in more modern times began to be made about the biological consequences of hormones without the realization of nature of the chemicals involved. Berthold, a Göttingen physiologist, reported the effects of implanted testis in studies with cocks in 1849 [10]. In 1855, Addison discovered the relationship of the adrenal glands with a particular disease characterized by bronze skin color [11], and this disease of chronic adrenal insufficiency is now referred to as Addison's disease. In 1889, Brown-Sequard prepared a testicular extract and tested on himself, reporting enhanced rejuvenation. Although unorthodox, this technique of preparing tissue extracts and evaluating the effects of the extracts was eventually adapted for the isolation of biologically active constituents from the extracts.

The modern era of steroid research began with steroid chemistry in the early 1900s. Dr. Adolf O. Windaus, a Göttingen chemist, worked for over 20 years on the isolation of steroids, development of assays for detecting steroids, and the use of classical chemistry to elucidate steroid structures [12]. Dr. Windaus received the Nobel Prize in 1928 for his research on the "constitution of sterols." During the same period, Dr. Heinrich O. Wieland of Munich was engaged in natural products chemistry, including bile acids [13], and was awarded the Nobel Prize in 1928 for his research on this subject. The original chemical structures of cholesterol and other steroids proposed in 1928 were subsequently found to be incorrect, and correct structures were identified in 1932 [14–16]. Chemical studies on compounds involved in reproduction began in the 1920s, and in 1929 Adolf F. Butenandt and Edward A. Doisy independently reported the isolation of an active steroid sex hormone, estrone, from the urine of pregnant women [17,18]. Throughout the 1930s, many steroid hormones were isolated and structures determined, including progesterone by Butenandt [19] and corticosteroids by Reichstein [20]. The synthesis of steroids followed shortly thereafter by research groups led by W.E. Bachmann, R.B. Woodward, R. Robinson, and J.W. Cornforth [21–23].

Steroid biochemistry began with studies on the biosynthesis and metabolism of steroids, and early studies in the 1930s and 1940s used large amounts of unlabeled compounds [6]. With the production of radiolabeled molecules, studies then used more physiological levels of steroids and steroid precursors, with an early significant demonstration that all the carbon atoms of cholesterol are derived from

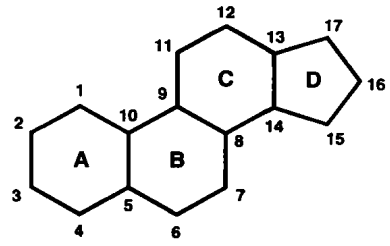
the two carbons present in isotopically labeled acetate [24]. Research on steroid biosynthesis and metabolism began in the 1950s and 1960s and continues to the present time. Studies on the biochemical mechanism of action of steroids began in the late 1950s with the use of tritiated estrogens of high specific activity. Jensen and Jacobson reported the accumulation of physiological levels of estrogen in target organs and postulated the presence of a receptor [25]. Research on the biochemistry and molecular biology of steroid hormone action has exploded in the past few decades and comprises a major effort in the field today.

Two important discoveries in the late 1940s and early 1950s had a dramatic effect not only on steroid research but also on the pharmacological applications of steroids. The first was the clinical report from the Mayo Clinic on the significant improvement in patients with rheumatoid arthritis following cortisone treatment by Hench et al. [26]. The second was the application of estrogen and progestin preparations for contraception, demonstrated by Pincus [27]. These two series of studies showed for the first time that steroids could be considered as drugs. As a result, extensive research on the medicinal chemistry, pharmacology, and clinical studies of steroid agonists and antagonists has evolved and continues to provide new insights and new medicinal agents for therapies in many different diseases and chemoprevention strategies.

2. STEROID CHEMISTRY

2.1. Structure and Physical Properties of Steroids

Steroid molecules possess a common chemical skeleton of four fused rings, consisting three six-membered rings and a five-membered ring (Fig. 1). Chemically, this hydrocarbon scaffold is a cyclopentanoperhydrophenanthrene, describing the three rings of phenanthrene (rings A, B, and C) and the cyclopentane ring (ring D). In steroids, the phenanthrene ring system is completely saturated (hydrogenated) and is thus referred to as a perhydrophenanthrene. This steroid scaffold contains 17 carbon atoms, and the numbering of the carbon atoms begins with the carbons of the



cyclopentanoperhydrophenanthrene

Figure 1. Basic steroid structure.

phenanthrene and then followed by numbering of the remaining carbons of the cyclopentane ring (Fig. 1). Additional carbon atoms on steroids include angular methyl groups attached to C-13 and C-10 and alkyl substituents on C-17 (Fig. 2).

When the steroid nucleus is drawn in a two-dimensional representation, the steroid scaffold appears planar and substituents on carbons of the steroid scaffold may be located either above or below the "plane" of the steroid. Substituents located above the plane are drawn with solid lines or with solid wedges, and these moieties are referred to as being in the β-configuration. Substituents located below the plane are drawn with dashed lines and are referred to as having the α-configuration. The angular methyl groups numbered 18 and 19 are attached in the β-configuration (above the steroid plane) to C-13 and C-10, respectively. Side chains at position 17 are always β unless indicated by dotted lines or in the nomenclature of the steroid. The stereochemistry of the rings and the substituents on the steroid scaffold markedly affects the biological activity of a given class of steroids.

The three-dimensional shapes of the rings in steroid scaffold are actually not planar. The cyclohexane rings of steroids exist in the preferred chair conformation. As a result, substituents on the cyclohexane rings can be located in axial or equatorial positions. The cyclopentane ring exists in a half-chair or open envelope conformation. Although the cyclohexane ring may undergo a flip in conformation, steroids are rigid structures because they generally have at least one *trans* fused ring system, and these rings must be diequatorial to each other. Endogenous steroids contain two *trans*

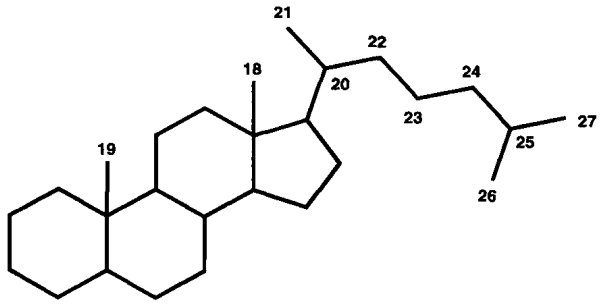


Figure 2. Side chains on steroid scaffold.

fused rings, one diequatorial *trans* fusion between rings B and C (carbons 8 and 9) and the other diequatorial *trans* fusion between rings C and D (carbons 13 and 14). Two possible ring fusions are observed in endogenous steroids between rings A and B. The diequatorial *trans* fusion between rings A and B results in the hydrogen atom at position 5 being on the opposite side of the rings from the angular methyl group at position 19; and the 5α notation is used for this hydrogen. The overall three-dimensional shape of the 5α-steroid is nearly flat and pleated. The axial–equatorial *cis* fusion between rings A and B results in the hydrogen atom at position 5 being on the same side of the rings with the angular methyl group at position 19; and the 5β-notation is used for this hydrogen. The two-dimensional and three-dimensional representations for the 5α-steroid and the 5β-steroid are illustrated in Fig. 3. These ring junctions (A/B and B/C) and the chair conformation of the six-membered rings result in an overall topography of the steroid scaffold that is rather rigid. Some minor flexibility is observed in the conformation of the D ring.

Since the angular methyl groups at positions 18 and 19 are β and have an axial orientation (i.e., perpendicular to the plane of the rings), the conformational orientation of the remaining bonds of a steroid can be easily assigned. The orientation of the remaining bonds on a steroid may be determined if one recalls that groups on a cyclohexane ring that are positioned on adjacent carbon atoms (vicinal, C_1HC_2H) of the ring (i.e., 1, 2 to each other) are *trans* if their relationship is 1,2-diaxial or 1,2-diequatorial and are *cis* if their relationship is 1,2-equatorial–axial.

The backbone of the steroid molecule can be referred to by a series of carbon–carbon bonds and the *cis* or *trans* relationship of the four rings (Fig. 4). The 5α-steroid molecule has a *trans–anti–trans–anti–trans* backbone. In this structure, all the fused rings have *trans* (diequatorial) stereochemistry, that is, the A/B

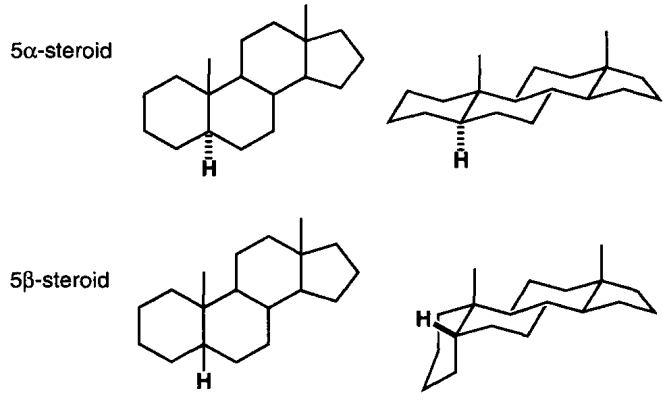
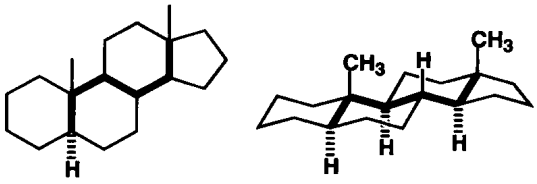


Figure 3. Representations of 5α-steroid and 5β-steroid.



trans-anti-*trans*-anti-*trans*

Figure 4. Steroid backbone.

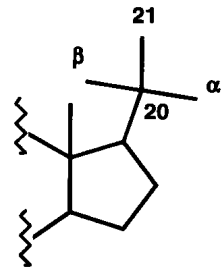


Figure 5. Substituents on carbon 20.

fused ring, the B/C fused ring, and the C/D fused ring are *trans*. The term *anti* is used in backbone notation to define the orientation of rings that are connected to each other and have a *trans*-type relationship. For example, the bond equatorial to ring B, at position 9, which forms part of ring C, is *anti* to the bond equatorial to ring B, at position 10, which forms part of ring A. A 5β-steroid has a *cis–anti–trans–anti–trans* backbone, in which the A/B rings are fused *cis*. The term *syn* is used to define a *cis*-type relationship in a similar fashion to *anti*. No naturally occurring steroids exist with a *syn*-type geometry, although such compounds can be chemically synthesized. Thus, the conventional drawing of the steroid nucleus is the natural configuration and does not show the hydrogens at 8β-, 9α-, or 14α-positions. If the carbon at position 5 is saturated, the hydrogen is always drawn, either as 5α or as 5β. Also, the conventional drawing of a steroid molecule has the C-18 and C-19 methyl groups shown only as solid lines.

Many of the biologically important steroids contain a carbon–carbon double bond between positions 4 and 5 or 5 and 6, and consequently there is no *cis* or *trans* relationship between rings A and B. The symbol Δ is often used to designate a carbon–carbon double bond (C=C) in a steroid. If the carbon–carbon double bond is between positions 4 and 5, the compound is referred to as a Δ^4-steroid. If the carbon–carbon double bond is between positions 5 and 10, the compound is designated as a $\Delta^{5(10)}$-steroid. Addition of a double bond also increases the flexibility of the ring, for example, the A ring of a Δ^4-steroid exists primarily in a half-chair conformation.

Aliphatic side chains at position 17 are always assumed to be β configuration, which is the configuration found in endogenous steroids. In the long-standing convention, the α and β terms have been applied to substituents on carbon number 20 (Fig. 5) on steroids containing a two-carbon side chain (i.e., containing carbons 20 and 21). Because C-20 is not a ring carbon, the preferred designation for the stereochemistry on C-20 is determined according to the Cahn–Ingold–Prelog sequence rules (the *R,S* system). The *R,S* system is also used to designate the stereochemistry of other positions on the steroid side chains.

Important physical properties of steroids include the physical state of the molecules and the solubility of steroids. The overall class of steroids is found almost entirely as solids. The melting points for steroids range from approximately 100–250°C. Molecules of one particular steroid compound may crystallize in either an anhydrous form or in a hydrated or solvated form, resulting in different melting points observed for the two forms. Also, individual molecules of one steroid compound may pack in different arrangements in crystals from different solvents, resulting in polymorphic forms [5]. Regarding solubility, steroids are generally insoluble in water while reasonably soluble in organic solvents such as ethanol, acetone, chloroform, and dioxane. Steroids with a phenolic hydroxyl group on the aromatic A ring (estrogens) are soluble in dilute sodium hydroxide.

2.2. Steroid Nomenclature

Many naturally occurring steroids are referred to by their common or trivial names, such as cholesterol, cortisol, progesterone, testosterone, and estradiol. As more steroid molecules were being discovered and/or synthesized, it became clear that a more systematic method for naming steroids was needed.

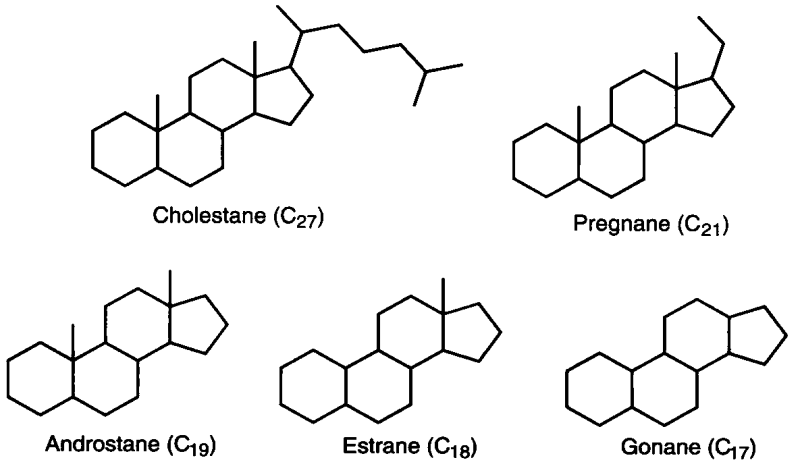


Figure 6. Structures of steroid stem names.

Starting in the 1950s, nomenclature rules for steroids began to be developed, and the most recent IUPAC-IUB rules for systematic steroid nomenclature were published in 1989 [28,29]. The systematic names for steroids are based upon the steroid hydrocarbon system, and the particular systematic name begins by selection of the stem name based upon the hydrocarbon system (Fig. 6). Cholestane is the term used for steroids with 27 carbon atoms (i.e., the C_{27} steroid structure). Pregnanes are steroids with 21 carbon atoms, androstanes have 19 carbon atoms, estranes have 18 carbon atoms, and gonanes have 17 carbon atoms.

If there are any double bonds present in the steroid scaffold, the "ane" ending of the stem name is replaced with "ene" if one double bond is present, "diene" if two double bonds are present, "triene" if three double bonds are present, and so on. The position(s) of the double bond is indicated by placing the lowest number of the carbon atom of the double bond in front of the "ene" ending. If the number of the first carbon atom indicates ambiguous positions, then this first number is followed by the number of the other carbon atom, placed in parentheses. When saturation is present at position 5, a designation of either 5α- or 5β- is required and is placed before the stem name. A suffix for the stem name is selected based upon the following priorities:

carboxylic acid (or derivative) > carbonyl > alcohol > amine > ether

In adding the suffix to the stem name, the final "e" in the stem name is always dropped when the suffix begins with a vowel. The carbon number of the substituent (and stereochemistry, if present) is placed in front of the suffix. Remaining substituents are denoted as prefixes, are preceded by the position number and stereochemistry, and are placed in alphabetical order.

Examples of the trivial names, systematic names, and chemical structures for common steroids are illustrated in Fig. 7. Cholesterol is the central steroid of the animal kingdom and functions as an essential component of cell membranes and as a biosynthetic precursor to other steroids in the body. Cholesterol has 27 carbon atoms, a hydroxyl group in the α-configuration at carbon 3, and contains a carbon–carbon double bond between carbons 5 and 6. Cholesterol is referred to as a Δ^5-steroid or, more specifically, a Δ^5-sterol because it is an unsaturated alcohol. The systematic name for cholesterol is cholest-5-en-3β-ol. The adrenocorticoids (adrenal cortex hormones) are pregnanes and are exemplified by cortisol, which is a 11β,17α,21-trihydroxypregn-4-ene-3,20-dione. Progesterone (pregn-4-ene-3,20-dione), a female sex hormone synthesized by the corpus luteum, is also a pregnane analog. The male sex hormones (androgens) are based on the structure of 5α-androstane.

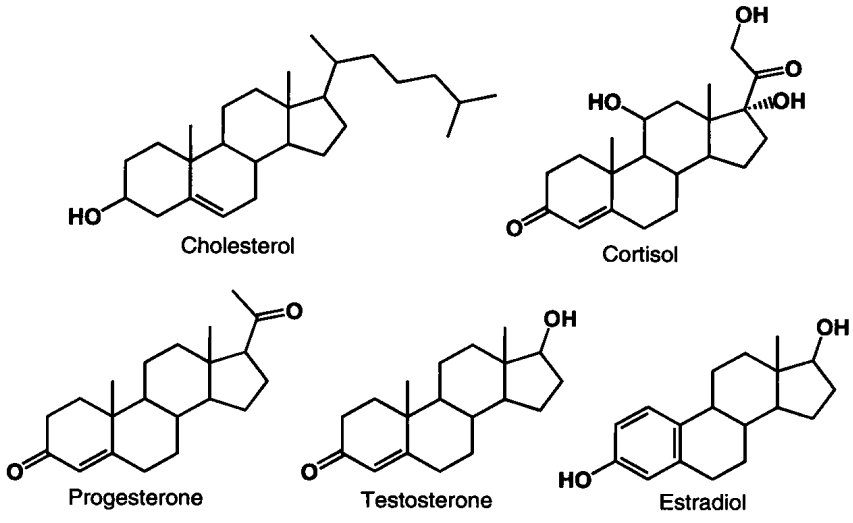


Figure 7. Structures of common steroids.

Testosterone, an important naturally occurring androgen, is named 17β-hydroxyandrost-4-en-3-one. Finally, the estrogens are female sex hormones synthesized by the follicular cells of the ovaries and are estrane analogs containing an aromatic A ring. Although the A ring does not contain isolated carbon–carbon double bonds, these analogs are named as if the bonds were in the positions shown in estradiol. Estradiol, a typical member of this class of drugs, is named estra-1,3,5(10)-triene-3,17β-diol.

2.3. Chemical Synthesis and Microbial Transformations of Steroids

A total of six Nobel Prizes were awarded to scientists working in the area of steroids, with four out of the six prizes in steroid chemistry. The pioneer work of Adolf Windaus and Heinrich Wieland in the 1920s in structural elucidation of a number of important steroids set the stage for the many significant discoveries in the steroid area from 1930s to 1950s. The total synthesis of equilenin was first reported by Bachmann et al. [21] and later by Johnson et al. [30]. The total synthesis of estrone was reported by Anner and Miescher [31], followed by the work of other well-known chemists on the synthesis of cholesterol and sex hormones [32–35]. In addition to the total synthesis of steroids, Marker reported the use of sapogenins as the starting material for the synthesis of corticosteroids and sex hormones [36]. In a series of studies reported in numerous brief communications in the early 1940s, Marker reported a chemical degradation process that converted diosgenin, a sapogenin from the Mexican yam *Cabeza de negro*, to pregnonolone acetate in essentially two steps [37,38]. His work had significant impact on the industrial production of steroids. One unique feature of research on steroid chemistry is the equal contribution to the field from both academia and pharmaceutical industry. Well-known scientists such as Ralph Hirschmann, Carl Djerassi, Hershel Herzog, Josef Fried, Arthur Birch, Seymour Bernstein, Vladimir Petrow, and George Rosenkranz and pharmaceutical companies such as Syntex, Searle, Schering, Upjohn, Merck, Lederle, and Squibb made significant contributions to the steroid field. The historical perspective on steroid chemistry has been described in detail by a series of papers [7–9].

The development of corticosteroids and oral contraceptives in the late 1940s and early 1950s reflected the significance of chemical synthesis in steroid research. In this chapter, we will use the chemical synthesis of cortisone as an example to illustrate some of the synthetic transformations in steroid chemistry. In addition, the high costs of the chemical synthesis of corticosteroids eventually led to

the research on microbial biotransformation of steroids described in the next section.

2.3.1. Synthesis of Cortisone The synthesis of cortisone was first reported by Sarett and is shown in Fig. 8a [39,40]. Methyl bisnordesoxycholate (I) was used as the starting material for the synthesis. The synthetic scheme constitutes three basic operations: transposition of 12α-hydroxy group to the C-11 position (from compound I to V), cleavage of the bile acid side chain and incorporation of the dihydroxyacetone moiety (from V to XIII), and generation of the 4-ene-3-one system (from XIII to cortisone). The yield for the conversion of V to IX was low. Eventually, an improved synthesis of cortisone was reported by Sarett [41,42]. The improved synthesis began with deoxycholic acid I. Protection of the 3α-hydroxy group in I followed by oxidation yielded ketone III. The next step involved the formation of the unsaturated ketone IV with selenium dioxide [43]. The next few steps required the transposition of the ketone at C-12 to C-11. The unusual step is the conversion of VII to VIII to form the 3α,9α-epoxide [44]. The epoxide ring was opened with HBr and subsequent transformations yielded the important intermediate XIII. The remaining steps are the improved procedure for the introduction of the dihydroxyacetone side chain as compared to the previous procedure (Fig. 8b). Reacting XIII with HCN formed the cyanohydrin XIV. Dehydration followed by acetylation obtained XVI. Reacting XVI with osmium tetroxide yielded the osmate ester XVII. The osmate ester served as a protecting group for the C_{17}–C_{20} double bond in addition to the introduction of C-17α-alcohol group. Oxidation of the 3α-hydroxy group followed by the introduction of 3,4-double bond afforded cortisone acetate. The total synthesis of cortisone was reported in 1951 by Woodward et al. [45]. For a complete account of the era of cortisone syntheses, please refer to the review article by Hirschmann [46].

2.3.2. Microbial Steroid Biotransformations The research on microbial transformation of steroid was stimulated after World War II when the anti-inflammatory properties of cortisone was reported [26]. Efficient synthesis of corticosteroids was then required both for scale-up synthesis and for structure–activity relationship studies. As shown in Fig. 8, the synthesis of cortisone required a total of 31 steps [39,40]. One of the particular challenging conversions was the transposition of 12α-hydroxy group in the bile acid to the C-11 that required 12 steps. In 1952, Peterson and Murray of Upjohn reported the first patented process of direct 11α-hydroxylation of progesterone using *Rhizopus arrhizus* and *R. nigricans* [47,48] (Fig. 9). In the same year, Fried et al. of Squibb Institute reported the similar microbial transformation with *Aspergillus niger* [49]. In addition to progesterone, deoxycorticosterone, 11-deoxy-17α-hydroxycorticosterone, and 17α-hydroxy-progesterone could also be substrates for the transformation. Three years later, Schull and Kita of Pfizer reported the stereoselective 11β-hydroxylation with *Curvularia lunata* using the same progesterone substrates as reported by Fried [5] (Fig. 9). As a result of the incorporation of the microbial transformation in the synthesis, the cost of production of hydrocortisone was lowered from $200 per gram in 1948 to $3.50 per gram in 1955.

In this chapter, only selected microbial transformations of steroids will be described. Please refer to more extensive reviews on this topic [50–56]. The microbial transformations are described in regard to the type of reaction and the carbon of the steroid skeleton bearing the reaction. Figure 10 is a diagrammatic illustration of the selected site of microbial transformations of steroids using a cholestane steroid skeleton.

16α-Hydroxylation Every carbon in a steroid molecule is accessible for microbial transformation [57]. Hydroxylations at the 11α-, 11β-, and 16α-positions of steroids are routinely used industrially through microbial transformation. Hydroxylations at the 11α- and 11β-positions of progesterone have been described above. The first example of microbial 16α-hydroxylation was the conversion of progesterone to 16α-hydroxyprogesterone reported in *Streptomyces argenteolus* by Perlman et al. [58] (Fig. 11). In addition to progesterone, 16α-hydroxylation on synthetic corticosteroid was also accomplished with *S. roseochromogenus* [59] and subsequently led to the

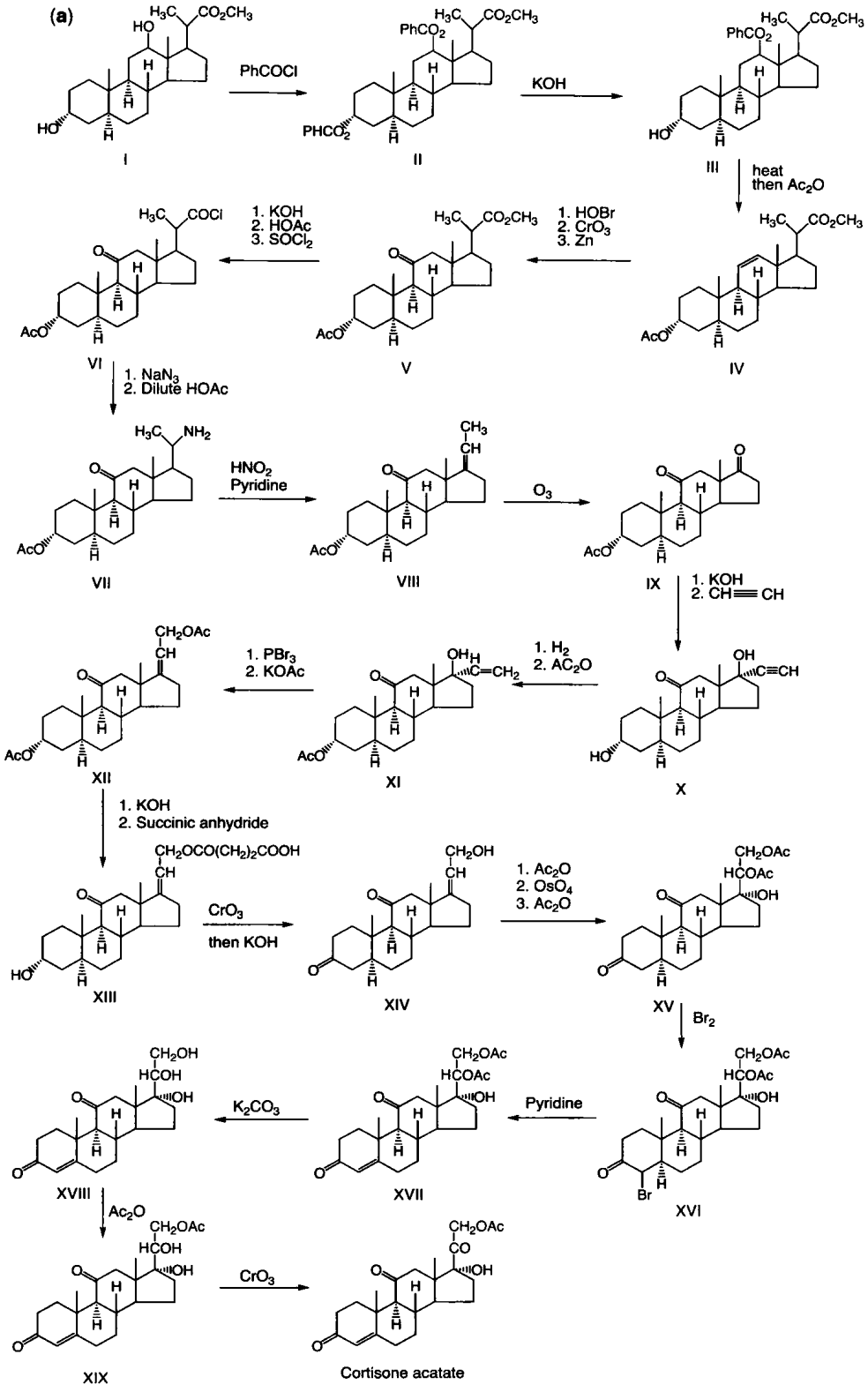


Figure 8. (a) Synthesis of cortisone acetate. (b) Improved synthesis of cortisone acetate.

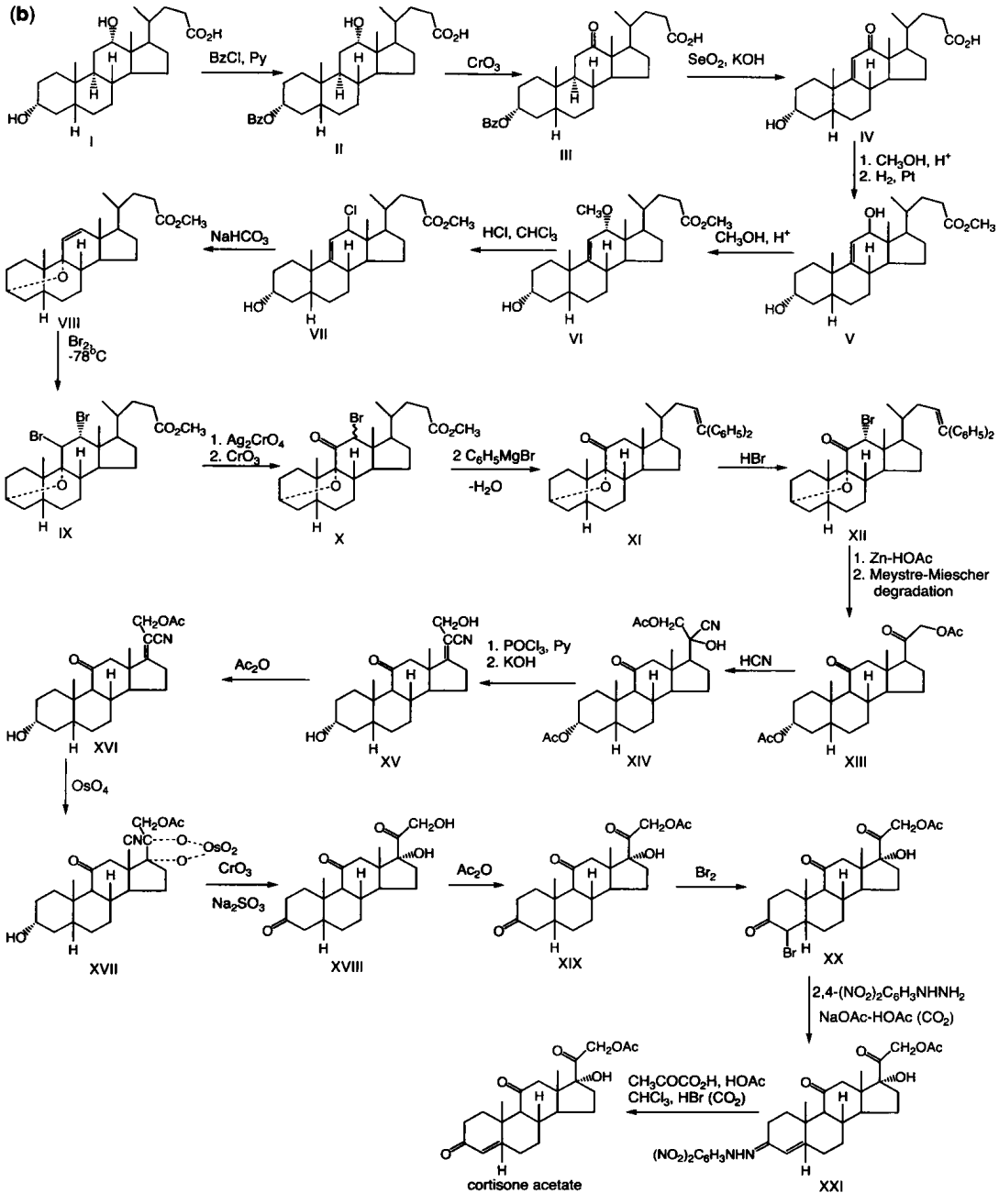


Figure 8. (*Continued*)

discovery of triamcinolone, a widely used anti-inflammatory steroid.

7α- and 9α-Hydroxylations 7α-Hydroxyandrostenedione is an important intermediate in the production of diuretics. Its production with microbial transformation from 3α,7α-dihydroxy-5β-cholanic acid has been reported [60]. Microbial 9α-hydroxylation of steroids was first observed by Peterson and coworkers [61]. This type of transformation

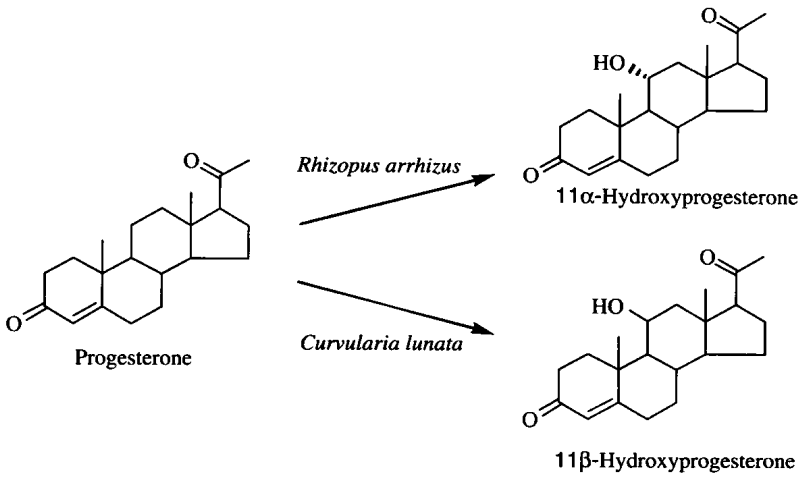


Figure 9. 11-Hydroxylation of progesterone.

has practical significance since the 9α-hydroxy moiety can be easily converted to the 9,11-dehydro functionality. The 9,11-dehydro system is an important intermediate in the production of 9α-fluoro and/or 11 hydroxy steroids.

14α-Hydroxylation 14α-Hydroxy steroids are of practical significance. 14α-Hydroxyandrost-4-ene-3,6,17-trione was recently shown to have aromatase inhibitory activity in human placenta and uterine tumors [62–64]. The derivative was obtained through microbial oxidation of androst-4-ene-3,17-dione with *Acremonium strictum*.

Many fungi, such as *Mucor griseocyanus* and *Actinomucor elegans*, have been shown to introduce a 14α-hydroxy group to progesterone and other steroids [61,65]. The 14α-Hydroxy steroids can serve as important intermediates in the production of steroids with 14β-hydroxy-5β-pregnane nuclei; a common structural framework existed in many cardioactive steroids. Chemical transformation of steroids from 14α-hydroxy configuration to 14β-hydroxy substituents can be accomplished through 14β,15β-epoxide intermediates.

Side-Chain Cleavage Many of the cheap and readily available natural products such as sitosterol, campesterol, and cholesterol have been considered waste products because of the lack of efficient methods for the cleavage of their saturated side chain. A process for the conversion of the steroids to androst-1,4-diene-3,17-dione by *Mycobacterium* has

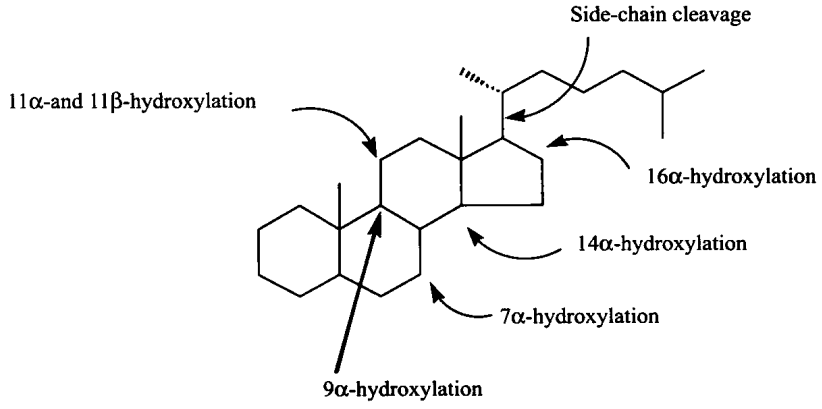


Figure 10. Selected site of microbial transformation of steroids.