Sex Hormones (Male): Analogs and Antagonists

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Keywords

Anabolics
Compounds that demonstrate a marked retention of nitrogen through an increase of protein synthesis and a decrease in protein catabolism in the body.

Androgens
Steroid hormones responsible for the primary and secondary sex characteristics of the male, including the development of the vas deferens, prostate, seminal vesicles, and penis.

Antiandrogens
Agents that compete with endogenous androgens for the hormone binding site on the androgen receptor and blocks androgen action.

Dihydrotestosterone
The C_{19} steroid hormone that is the 5α-reduced metabolite of testosterone produced in certain androgen target tissues and is the most potent endogenous androgen.

5α-Reductase Inhibitors
Compounds that inhibit the conversion of testosterone to its more active metabolite dihydrotestosterone.

Selective Androgen Receptor Modulators
Agents that may act as an androgen antagonist or weak agonist in one tissue but act as strong androgen agonist in another tissue type.

Testosterone
The C_{19} steroid hormone that is the predominant circulating androgen in the bloodstream and is produced mainly by the testis.

The steroid testosterone is the major circulating sex hormone of the male and serves as the prototype for the androgens, the anabolic agents, and androgen antagonists. The endogenous androgens are biosynthesized from cholesterol in various tissues in the body; the majority of the circulating androgens are made in the testes under the stimulation of the gonadotropin LH. The reduction of testosterone to dihydrotestosterone is necessary for the androgenic actions of testosterone in androgen target tissues such as the prostate; the oxidation of testosterone by the enzyme aromatase produces estrogens. The androgenic actions of testosterone are due to the binding of dihydrotestosterone to its nuclear receptor, followed by dimerization of the receptor complex and binding to a specific DNA sequence. This binding of the homodimer to the androgen response element leads to gene expression, stimulation of the synthesis of new mRNA, and subsequent protein biosynthesis. The synthetic androgens and anabolics were prepared to impart oral activity to the androgen molecule, to separate the androgenic effects of testosterone from its anabolic effects and to improve upon its biological activities. Drug preparations are used for the treatment of various androgen-deficient diseases, for the therapy of diseases characterized by muscle wasting and protein catabolism, for postoperative adjuvant therapy, and for the treatment of certain hormone-dependent cancers. The two major categories of androgen antagonists are the antiandrogens, which block interactions of androgens with the androgen receptor, and the inhibitors of androgen biosynthesis and metabolism. Such compounds have therapeutic potential in the treatment of acne, virilization in women, hyperplasia and neoplasia of the prostate, and baldness, and for male contraception.

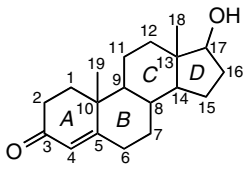
1 Introduction

Androgens are a class of steroids that are responsible for the primary and secondary sex characteristics of the male. In addition, these steroids possess potent anabolic or growth-promoting properties. The general chemical structure of androgens is based upon the androstane C_{19} steroid, consisting of the fused four-ring steroid nucleus (17 carbon atoms, rings A–D) and the two axial methyl groups (carbons 18 and 19) at the A/B and C/D ring junctions. The hormone testosterone (**1**) is the predominant circulating androgen and is produced mainly by the testis. 5α-Dihydrotestosterone (DHT) (**2**) is a 5α-reduced metabolite of testosterone produced in certain androgen target tissues and is the most potent endogenous androgen.

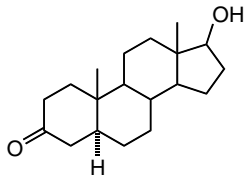
These two steroids and other endogenous androgens influence not only the development and maturation of the male genitalia and sex glands but also affect other tissues such as kidney, liver, and brain. This chapter will discuss the endogenous androgens, synthetic analogs, various anabolic agents, and the androgen antagonists employed in clinical practice or animal husbandry in the United States and elsewhere. Modified androgens that have found use as biochemical or pharmacological tools also are included. More extensive presentations of the topic of androgens, anabolics, and androgen antagonists have appeared in several treatises published over the past three decades.

2 Historical

The role of the testes in the development and maintenance of the male sex characteristics, and the dramatic physiological effects of male castration have been recognized since early time. Berthold was the first to publish in 1849 a report that gonadal transplantation prevented the effects of castration in roosters, suggesting that the testis produced internal secretions exhibiting androgenic effects. However, the elucidation of the molecules of testicular origin responsible for these actions took almost another century. The first report of the isolation of a substance with androgenic activity was made by Butenandt in 1931. The material, isolated in very small quantities from human male urine, was named *androsterone* (**3**). A second weakly androgenic steroid hormone was isolated from male urine in 1934. This substance was named *dehydroepiandrosterone* (**4**) because of its ready chemical transformation and structural similarity to androsterone. A year later, the isolation of the testicular androgenic hormone, testosterone (**1**), was reported, which was 10 times as potent as androsterone in promoting capon comb growth. Shortly after this discovery, the first chemical synthesis of testosterone

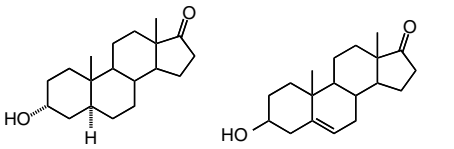


Testosterone (**1**)



5α-Dihydrotestosterone (**2**)

was reported by Butenandt and Hanisch and confirmed by Ruzicka.

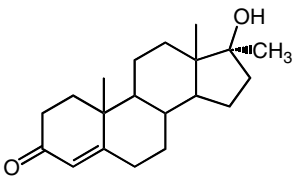


Androsterone (**3**) Dehydroepiandrosterone (**4**)

For many years it was believed that testosterone was the active androgenic hormone in man. In 1968, however, 5α-DHT (**2**), also referred to as *stanolone*, was demonstrated to be the active androgen in target tissues, such as the prostate and seminal vesicles and was formed from testosterone by a reductase present in these tissues. Shortly thereafter a soluble receptor protein was isolated and demonstrated to be specific for DHT and related structures.

The anabolic action of the androgens was first documented by Kochakian and Murlin in 1935. In their experiments, extracts of male urine caused a marked retention of nitrogen when injected into dogs fed a constant diet. Soon afterward testosterone propionate was observed to produce a similar nitrogen-sparing effect in humans. Subsequent clinical studies demonstrated that testosterone was capable of causing a major acceleration of skeletal growth and a marked increase in muscle mass. This action on muscle tissue has been referred to more specifically as the *myotrophic effect*.

The first androgenic-like steroid used for its anabolic properties in humans was testosterone. Unfortunately, its use for this purpose was limited by the inherent androgenicity and the need for parenteral administration. 17α-Methyltestosterone (**5**) was the first androgen discovered to possess oral activity, but it too did not show any apparent separation of androgenic and anabolic activity. The promise of finding a useful, orally effective, anabolic agent free from androgenic side effects prompted numerous clinical and biological studies.



17α-Methyltestosterone (**5**)

3 Endogenous Male Sex Hormones

3.1 Occurrence and Physiological Roles

The hormone testosterone affects many organs in the body. Its most dramatic effects are observed on the primary and secondary sex characteristics of the male. These actions are first manifested in the developing male fetus when the embryonic testis begins to secrete testosterone. Differentiation of the Wolffian ducts into the vas deferens, seminal vesicles, and epididymis occurs under this early androgen influence, as does the development of external genitalia and the prostate. The reductive metabolism of testosterone to 5α-DHT is critical for virilization during this period of fetal development, as dramatically demonstrated in patients with a 5α-reductase deficiency.

At puberty, further development of the sex organs (prostate, penis, seminal vesicles, and vas deferens) is again evident and under the control of androgens. Additionally, the testes now begin to produce mature spermatozoa. Other effects of testosterone, particularly on the secondary sex characteristics, are observed.

Hair growth on the face, arms, legs, and chest is stimulated by this hormone during younger years; in later years, testosterone is responsible for thinning of the hair and recession of the hairline. The larynx develops and a deepening of the voice occurs. The male's skin at puberty thickens, the sebaceous glands proliferate, and the fructose content in human semen increases. Testosterone influences sexual behavior, mood, and aggressiveness of the male at the time of puberty.

In addition to these androgenic properties, testosterone also exhibits anabolic (myotrophic) characteristics. General body growth is initiated, including increased muscle mass and protein synthesis, a loss of subcutaneous fat, and increased skeletal maturation and mineralization. This anabolic action is associated with a marked retention of nitrogen brought about by an increase of protein synthesis and a decrease of protein catabolism. The increase in nitrogen retention is manifested primarily by a decrease in urinary rather than fecal nitrogen excretion and results in a more positive nitrogen balance. For example, intramuscular administration of 25 mg of testosterone propionate twice daily can produce a nitrogen retention to appear within 1 to 3 days, reaching a maximum in about 5 to 8 days. This reduced level of nitrogen excretion may be maintained for at least a month and depends on the patient's nutritional status and diet.

Androgens influence skeletal maturation and mineralization, which is reflected in an increase in skeletal calcium and phosphorus. In various forms of osteoporosis, androgens decrease urinary calcium loss and improve the calcium balance in patients. This effect is not as noticeable in normal patients. Moreover, the various androgen analogs differ markedly in their effects on calcium and phosphorus balance in man. Androgens and their 5β-metabolites (e.g. etiocholanolone) markedly stimulate erythropoiesis, presumably by increasing the production of erythropoietin and by enhancing the responsiveness of erythropoietic tissue to erythropoietin. The effects of androgens on carbohydrate metabolism appear to be minor and secondary to their primary protein anabolic property. The effects on lipid metabolism, on the other hand, seem to be unrelated to this anabolic property. Weakly androgenic metabolites such as androsterone have been found to lower serum cholesterol levels when administered parenterally.

3.2 Biosynthesis

The androgens are secreted not only by the testis but also by the ovary and adrenal cortex. Testosterone is the principal circulating androgen and is formed by the Leydig cells of the testes. Other tissues, such as liver and human prostate, form testosterone from precursors, but this contribution to the androgen pool is minimal. Since dehydroepiandrosterone and androstenedione (see Fig. 2) are secreted by the adrenal cortex and ovary, they indirectly augment the testosterone pool because they can be rapidly converted to testosterone by peripheral tissues.

Plasma testosterone levels for men usually range between 0.61 and 1.1 µg/100 mL and are 5 to 100 times female values. The circulating level of DHT in normal adult men is about one-tenth the testosterone level. Daily testosterone production rates have been estimated to be 4 to 12 mg for young men and 0.5 to 2.9 mg for young women. Although attempts have been made to estimate

the secretion rates for testosterone, these studies are hampered by the number of tissues capable of secreting androgens and the considerable interconversion of the steroids concerned.

The synthesis of androgens in the Leydig cells of the testes is regulated by the gonadotropic hormone, luteinizing hormone (LH), also called *interstitial cell-stimulating hormone* (ICSH). The other pituitary gonadotropin, follicle-stimulating hormone (FSH), acts primarily on the germinal epithelium and is important for sperm development. Both of these pituitary gonadotropins are under the regulation of a decapeptide hormone produced by the hypothalamus. This hypothalamic hormone is luteinizing hormone-releasing hormone (LHRH), also referred to as *gonadotropin-releasing hormone* (GnRH). In adult males, pulsatile secretion of LHRH, and subsequently LH and FSH, occurs at a frequency of 8 to 14 pulses in 24 h. The secretions of these hypothalamic and pituitary hormones are, in turn, regulated by circulating testosterone levels in a negative feedback mechanism. Testosterone will decrease the frequency and amplitude of pulsatile LH secretion, whereas, testosterone and a gonadal peptide, inhibin, are both involved in suppressing the release of FSH.

Our understanding of steroidogenesis in the endocrine organs has advanced considerably during the past three decades, based largely on initial investigations with the adrenal cortex and subsequent studies in the testis and ovary as well. Figure 1 outlines the following sequence of events known to be involved with steroidogenesis in the Leydig cells. LH binds to its receptor located on the surface of the Leydig cell and, via a G-protein-mediated process, activates adenylyl cyclase to result in an increase in intracellular concentrations of cyclic adenosine monophosphate (cAMP). cAMP activates a cAMP-dependent protein kinase,

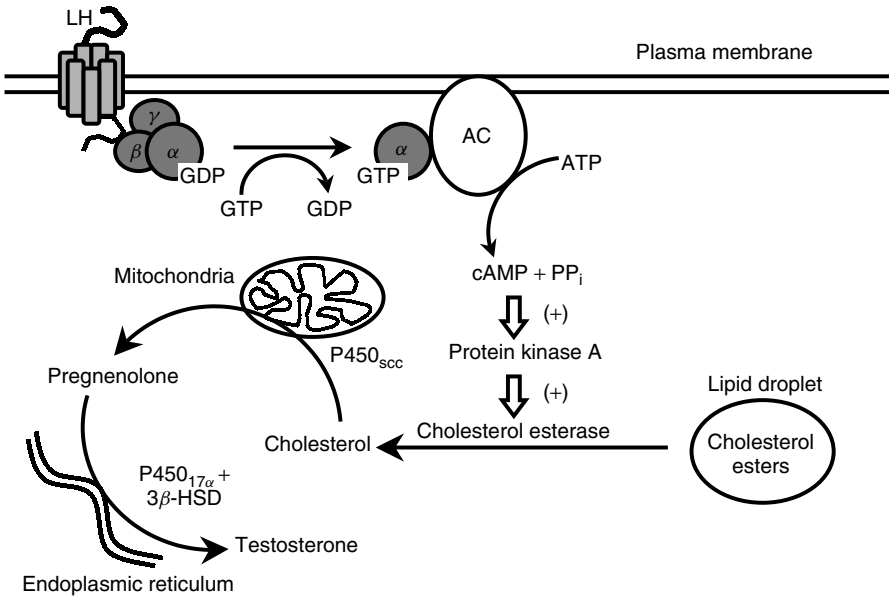


Fig. 1 Cellular events in steroidogenesis in Leydig cell.

which subsequently phosphorylates and activates several enzymes involved in the steroidogenic pathway, including cholesterol esterase and cholesterol side-chain cleavage. Cholesterol esters (present in the cell as a storage form) are converted to free cholesterol by cholesterol esterase, and free cholesterol is translocated to mitochondria. A cytochrome P-450 mixed-function oxidase system, termed *cholesterol side-chain cleavage*, converts cholesterol to pregnenolone. Several nonmitochondrial enzymatic transformations then convert pregnenolone to testosterone, which is secreted.

The conversion of cholesterol (**6**) to pregnenolone (**7**) has been termed the *rate-limiting step* in steroid hormone biosynthesis. The reaction requires NADPH and molecular oxygen and is catalyzed by a cytochrome P-450 enzyme complex termed *cholesterol side-chain cleavage*. This enzyme complex consists of three proteins – cytochrome $P450_{SCC}$ (also called cytochrome P450 11A), adrenodoxin, and adrenodoxin reductase. Three moles of NADPH and oxygen are required to convert one mole of cholesterol into pregnenolone (Fig. 2).

Tracer studies have shown that two major pathways known as the ''4-ene'' and ''5-ene'' pathways are involved in the conversion of pregnenolone to testosterone. Both these pathways and the requisite enzymes are shown in Fig. 2. Earlier studies tended to favor the ''4-ene'' pathway, but more subsequent work has disputed this view and suggests that the ''5-ene'' pathway is quantitatively more important in man. The unconjugated steroids identified in normal males are listed in Table 1 along with the concentration in micrograms per hundred milliliters. All the intermediates of the ''5-ene'' pathway were identified, but progesterone (**8**), an important intermediate of the ''4-ene'' pathway, was not found. In addition, sulfate conjugates were present in significant quantities, especially androst-5-ene-3β,17β-diol 3-monosulfate. The data strongly suggest that this intermediate and its unconjugated form constitute an important precursor of testosterone in man. This view, however, was not supported by a kinetic analysis of the metabolism of androst-5-ene-3β,17β-diol (**12**) in man. Further evidence that the predominant pathway appears to be the ''5-ene'' pathway was provided by *in vitro* studies in human testicular tissues.

Tab. 1 Mean concentration of steroids in spermatic venous plasma from five normal males.

Compound	*Concentration [μg 100 mL^{-1}]*
5-Androstene-3β,17α-diol	4.0
5-Androstene-3β,17β-diol	18.5
Dehydroepiandrosterone	2.2
Androstenedione	2.5
Testosterone	74.0
Pregnenolone	4.8
17α-Hydroxypregnenolone	3.9
17α-Hydroxyprogesterone	6.2

Another important step is the conversion of the C-21 steroids to the C-19 androstene derivatives. Whereas the enzymes for side-chain cleavage are localized in mitochondria, those responsible for cleavage of the $C_{17}-C_{20}$ bond ($C_{17}-C_{20}$ lyase) reside in the endoplasmic reticulum of the cell. Early studies implicated 17α-hydroxypregnenolone (**9**) or 17αhydroxyprogesterone (**10**) as an obligatory intermediate in testosterone biosynthesis, and the $C_{17}-C_{20}$ bond was subsequently cleaved by a second enzymatic process to produce the C-19

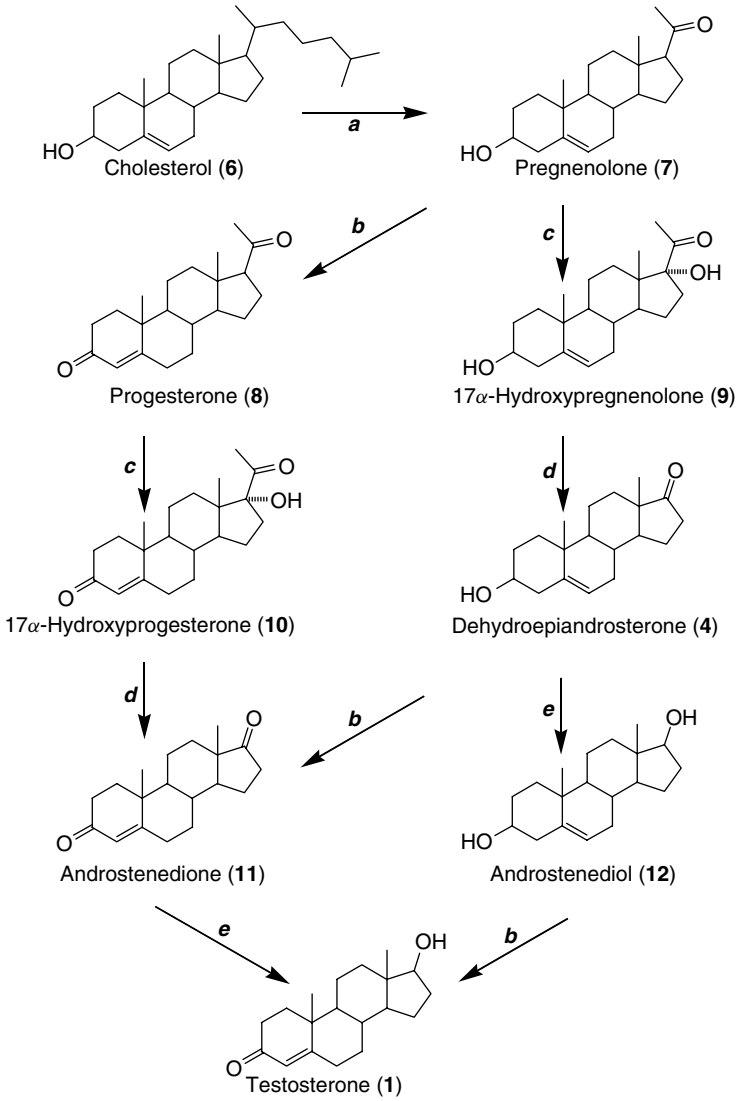


Fig. 2 Enzymatic conversion of cholesterol to testosterone.

androstene molecule. This view of the involvement of two separate enzymes in the conversion of C-21 to C-19 steroids existed until the purification of the proteins in the 1980s. The 17α-hydroxylase/17,20-lyase cytochrome P450 (abbreviated cytochrome P450 17 or cytochrome $P450_{17\alpha}$) was first isolated from neonatal pig testis microsomes, and this cytochrome $P450_{17\alpha}$ possessed both 17α-hydroxylase and 17,20-lyase activity when reconstituted with cytochrome P450 reductase and phospholipid. Identical full-length human cytochrome $P450_{17\alpha}$ cDNA sequences were independently isolated and reported in 1987.

Two additional enzymes are necessary for the formation of testosterone from dehydroepiandrosterone. The first is the 3β-hydroxysteroid dehydrogenase/$\Delta^{4,5}$-isomerase complex, which catalyzes the oxidation of the 3β-hydroxyl group to the 3-ketone and the isomerization of the double bond from C_5-C_6 to C_4-C_5. Again, these processes were originally thought to involve two different enzymes, but purification of the enzymatic activity demonstrated that a single enzyme catalyzes both reactions. The final enzyme in the pathway is the 17β-hydroxysteroid dehydrogenase, which catalyzes the reduction of the 17-ketone to the 17β-alcohol.

3.3 Absorption and Distribution

Although considerable research has been devoted to the biochemical mechanism of action of the natural hormones and the synthesis of modified androgens, little is known about the absorption of these substances. It is well recognized that a steroid hormone might have high intrinsic activity but exerts little or no biological effects because its physicochemical characteristics prevent it from reaching the site of action. This is particularly true in humans, where slow oral absorption or rapid inactivation may greatly reduce the efficacy of a drug. Even though steroids are commonly given by mouth, little is known of their intestinal absorption. One study in rats showed that androstenedione (**11**) was absorbed better than testosterone or 17α-methyltestosterone, and conversion of testosterone to its acetate enhanced absorption. Results with other steroids indicated that lipid solubility was an important factor for intestinal absorption. This may explain the oral activity of certain ethers and esters of testosterone.

Once in the circulatory system by either secretion from the testis or absorption of the administered drug, testosterone and other androgens will reversibly associate with certain plasma proteins, the unbound steroid being the biologically active form. The extent of this binding is dependent on the nature of the proteins and the structural features of the androgen.

The first protein to be studied was albumin, which exhibited a low association constant for testosterone and bound less polar androgens such as androstenedione to a greater extent.

α-Acid glycoprotein (AAG) was shown to bind testosterone with a higher affinity than albumin. A third plasma protein that binds testosterone is corticosteroid-binding α-globulin (CBG). However under normal physiological conditions these plasma proteins are not responsible for extensive binding of androgens in plasma.

A specific protein termed *sex steroid binding β-globulin* (SBG) or testosterone estradiol binding globulin (TEBG) was found in plasma that bound testosterone with a very high affinity. The SBG–sex hormone complex serves several functions, such as transport or carrier system in the bloodstream, storage site or reservoir for the hormones, and protection of the hormone from metabolic transformations. SBG has been purified and contains high affinity, low capacity binding sites for the sex hormones. Dissociation constants of approximately 1×10^{-9} M have been reported for the binding of testosterone and estradiol to SBG and are 2 orders of magnitude less than values reported for the binding of the hormone to the cytosolic receptor protein. The plasma levels of SBG are regulated by the thyroid hormones and remain fairly constant throughout adult

life in both the male and female. SBG is not present in the plasma of all animals. For example, SBG-like activity is notably absent in the rat, and testosterone may be bound in the rat plasma to CBG.

The specificity of the binding of steroids to human SBG has been extensively studied. The presence of a 17β-hydroxyl group is essential for binding to SBG. In addition to testosterone, DHT, 5α-androstane-3β,17β-diol (**19**), and 5α-androstane-3α,17β-diol (**20**) bind with high affinity, and these steroids compete for a common binding site. Binding to SBG is decreased by 17α-substituents such as 17α-methyl and 17α-ethinyl moieties and by unsaturation at C-1 or C-6. Also, 19-nortestosterone derivatives have lower affinity. SBG has been purified to homogeneity by affinity chromatography using a DHT-agarose adsorbent.

Another extracellular carrier protein exhibiting high affinity for testosterone, found in seminiferous fluid and the epididymis, originates in the testis and is called *androgen binding protein* (ABP). This protein is produced by the Sertoli cells on stimulation by FSH and has very similar characteristics to those of plasma SBG produced in the liver.

The absorption of androgens and other steroids from the blood by target cells was usually assumed to occur by a passive diffusion of the molecule through the cell membrane. However, studies in the early 1970s using tissue cultures or tissue slices suggested entry mechanisms for the steroids. Estrogens, glucocorticoids, and androgens exhibit a temperature-dependent uptake into intact target cells, suggesting a protein-mediated process. Among the androgens, DHT exhibited a greater uptake than testosterone in human prostate tissue slices, and it was found that estradiol or androstenedione interfered with this uptake mechanism. In addition, cyproterone competitively inhibited androstenedione, testosterone, and DHT entry, whereas cyproterone acetate enhanced the uptake of these androgens. Little is known about the exit of steroids from target cells; the only reported research has dealt with an active transport of glucocorticoids out of cells.

3.4 Metabolism

For decades, the primary function of metabolism was thought to be the inactivation of testosterone, the increase in hydrophilicity, and the mechanism for the excretion of the steroid into the urine. However, the identification of metabolites of testosterone formed in peripheral tissues and the potent and sometimes different biological activities of these products emphasized the importance of the metabolic transformations of androgens in endocrinology. Two active metabolites of testosterone have received considerable attention – the reductive metabolite 5α-DHT and the oxidative metabolite estradiol (Fig. 3).

3.4.1 Reductive Metabolism

The metabolism of testosterone in a variety of *in vitro* and *in vivo* systems has been reviewed. The principal pathways for reductive metabolism of testosterone in man appear in Fig. 4. Human liver produces a number of metabolites, including androstenedione (**11**), 3β-hydroxy-5α-androstan-17-one (**16**), 5α-androstane-3β,17β-diol (**19**), and 5α-androstane-3α,17β-diol (**20**). Human adrenal preparations, on the other hand, gave 11β-hydroxytestosterone as the major metabolite. (Intestinal metabolism of testosterone is similar to transformations in the

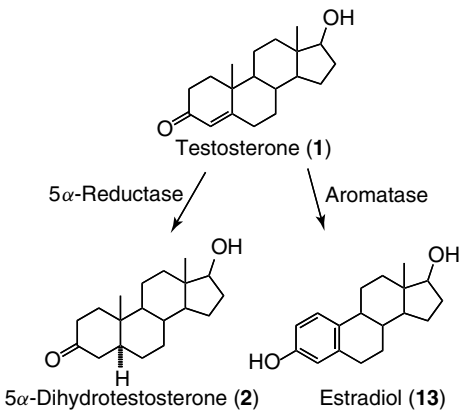


Fig. 3 Enzymatic conversion of testosterone to biologically active metabolites, 5α-dihydrotestosterone and estradiol.

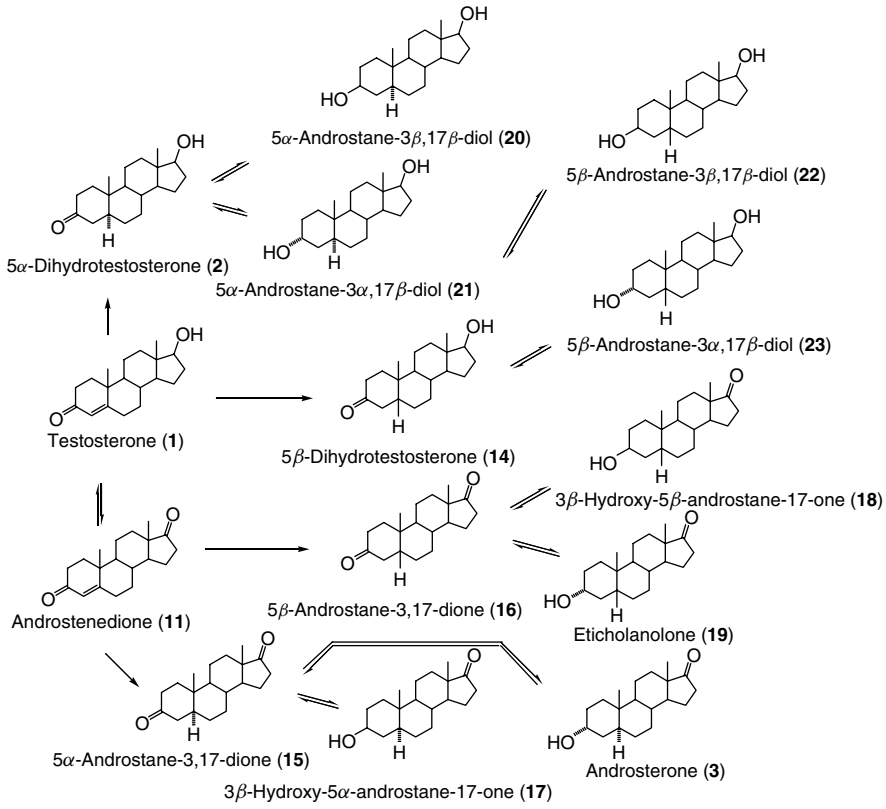


Fig. 4 Reductive metabolites of testosterone.

liver; the major metabolite in lung is androstenedione).

Studies on testosterone metabolism since the late 1960s have centered on steroid transformations by prostatic tissues. Normal prostate, benign prostatic hypertrophy (BPH), and prostatic carcinoma all contain 3α-, 3β-, and 17β-hydroxysteroid dehydrogenases, and 5α- and 5β-reductases, capable of converting testosterone to various metabolites. Prostatic carcinoma metabolizes testosterone more slowly than does BPH or normal prostate. In addition, increased levels of androgens are found in BPH. K. D. Voigt et al. have extensively studied *in vivo* metabolic patterns of androgens in patients with BPH. Tritiated androgens were injected intravenously into these patients 30 min before prostatectomy, and prostatic tissue, tissue from surrounding skeletal muscle, and blood plasma were analyzed for metabolites. The major metabolite of testosterone found in BPH tissues was DHT, with minor amounts of diols isolated. Skeletal muscle and plasma contained primarily unchanged testosterone.

Table 2 lists the urinary metabolites that have been identified following the administration of testosterone to humans (see Fig. 4). These products are excreted as such or in the form of their glucuronide or sulfate conjugates. Androsterone (**3**) and etiocholanolone (**18**), the major urinary metabolites, are excreted predominantly as glucuronides, and only about 10% as sulfates. These conjugates are capable of undergoing further metabolism. Testosterone glucuronide, for example, is metabolized differently from testosterone in man, giving rise mainly to 5β-metabolites. Only a relatively small amount of the urinary 17-ketosteroids is derived from testosterone metabolism. In man at least 67% and in women about 80% or more of the urinary 17-ketosteroids are metabolites of adrenocortical steroids. This explains why a significant increase in testosterone secretion associated with various androgenic syndromes does not usually lead to elevated levels of 17-ketosteroid excretion.

Tab. 2 Urinary metabolites of testosterone.

Metabolite	***Approximate Conversion [%]***
Androsterone	25–50
Etiocholanolone	
5β-Androstane-3α,17β-diol	2
5α-Androstane-3α,17β-diol	1
5α-Androstan-3β-ol-17-one	1
Androst-16-en-3α-ol	0.4
3α,18-Dihydroxy-5β-androstan-17-one	0.3
3α,7β-Dihydroxy-5β-androstan-17-one	Trace
11β-Hydroxytestosterone	Trace
6α-, 6β-Hydroxytestosterone	Trace

Although androsterone and etiocholanolone are the major excretory products, the exact sequence whereby these 17-ketosteroids arise is still not clear. Studies with radiolabeled androst-4-ene-3β,17β-diol and the epimeric 3α-diol in humans showed that oxidation to testosterone was necessary before reduction of the A-ring. Moreover, in rats 5β-androstane-3α,17β-diol (**22**) was the major initial liver metabolite, but this decreased with time with the simultaneous increase of etiocholanolone. This formation of saturated diols agrees with studies using human liver and provides evidence that the initial step in testosterone metabolism is reduction of the α,β-unsaturated ketone to a mixture of diols followed by oxidation to the 17-ketosteroids.

Until 1968, it was generally thought that the excretory metabolites of testosterone were physiologically inert. Subsequent work has shown, however, that etiocholanolone has thermogenic effects when administered to man. Moreover, the hypocholesterolemic effects of parenterally administered androsterone have been described.

The conversion of testosterone to DHT by 5α-reductase has major importance in the mechanism of action of the hormone. This enzymatic activity has been found in the endoplasmic reticulum and in the nuclear membrane of androgen-sensitive cells. In addition, the levels of 5α-reductase are under the control of testosterone and DHT; 5α-reductase activity decreases after castration and can be restored to normal levels of activity with testosterone or DHT administration.

Early biochemical studies of 5α-reductase were performed using a microsomal fraction from rat ventral prostate. The irreversible enzymatic reaction catalyzed by 5α-reductase requires NADPH as a cofactor, which provides the hydrogen for carbon-5. The 5α-reductase from rat ventral prostate tissues exhibited a broad range of substrate specificity for various C_{19} and C_{21} steroids; this broad specificity was also observed in inhibition studies. However, more detailed studies of the enzyme were limited because of the extreme hydrophobic nature of the protein, its instability upon isolation, and its low concentrations in androgen-dependent tissues.

Investigations on the molecular biology of 5α-reductase resulted in the demonstration of two different genes and two different isozymes of the enzyme. The first cDNA isolated and cloned that encoded 5α-reductase was designated type 1, and the second was designated type 2. The gene encoding type 1 is located on chromosome 5, while the gene encoding type 2 is located on chromosome 2. The two human 5α-reductases have approximately 60% sequence homology. These two isozymes differ in their biochemical properties, tissue location, and function. Type 1 5α-reductase exhibits an alkaline pH optimum (6–8.5) and has micromolar affinities for steroid substrates. On the other hand, type 2 5α-reductase has a sharp pH optimum at 4.7–5.5, has higher affinity (lower apparent K_m) for testosterone, and is more sensitive to inhibitors than the type 2 isozyme. The type 2 isozyme is expressed primarily in androgen target tissues, the liver expresses both types, and the type 1 form is expressed in various peripheral tissues. Type 2 5α-reductase appears to be essential for masculine development of the fetal urogenital tract and the external male phenotype, whereas the type 1 isozyme is primarily a catabolic enzyme. In certain cases of human male pseudohermaphroditism, mutations in the type 2 5α-reductase gene are observed and results in significant decreases in DHT levels needed for virilization.

3.4.2 Oxidative Metabolism

Another metabolic transformation of androgens leading to hormonally active compounds is their conversion to estrogens (see Table 3). Estrogens are biosynthesized in the ovaries and placenta and, to a lesser extent, in the testes, adrenals, and certain regions of the brain. The enzyme complex that catalyzes this biosynthesis is referred to as *aromatase*, and the enzymatic activity was first identified by Ryan in the microsomal fraction from human placental tissue. The elucidation of the mechanism of the aromatization reaction began in the early 1960s and continues to receive extensive study. It is a

cytochrome P-450 enzyme complex and requires 3 mol of NADPH and 3 mol of oxygen per mole of substrate. Aromatization proceeds via three successive steps, with the first two steps being hydroxylations. 19-Hydroxyandrostenedione (**23**) was a more active precursor of estrone (**25**) than the substrate androstenedione led to its postulated role in estrogen biosynthesis. This report and numerous studies that followed led to the currently accepted pathway for aromatization, as shown in Fig. 5.

The first two oxidations occur at the C_{19} position, producing the 19-alcohol (**23**) and then the 19-*gem*-diol (**24**), originally isolated as the 19-aldehyde (**25**). The exact mechanism of the last oxidation remains to be fully determined. The final oxidation results in the stereospecific elimination of the 1β and 2β-hydrogen atoms and the concerted elimination of the oxidized C_{19} moiety as formic acid. Investigations using $^{18}O_2$ and isotopically labeled steroidal intermediates failed to show incorporation of the 2β-hydroxyl group into formic acid

Tab. 3 Relative substrate activity in aromatization.

Substrate	***Activity [%]***
C_{19} –Steroids	
Androst-4-ene-3,17-dione	100
19-Hydroxyandrost-4-ene-3,17-dione	184, 133
17β-Hydroxyandrost-4-en-3-one	100
3β-Hydroxyandrost-5-en-17-one	66
5α-Androst-1-ene-3,17-dione	0
5α-Androstane-3,17-dione	0
1α-Hydroxyandrost-4-ene-3,17-dione	0
17β-Hydroxy-1α-methylandrost-4-ene-3-one	0
2β-Hydroxyandrost-4-ene-3,17-dione	15
2α-Hydroxyandrost-4-ene-3,17-dione	0
17β-Hydroxy-2β-methylandrost-4-en-3-one	0
11β-Hydroxyandrost-4-ene-3,17-dione	0
11α-Hydroxyandrost-4-ene-3,17-dione	100
17β-Hydroxy-17α-methylandrost-4-en-3-one	44
6β-Hydroxyandrost-4-ene-3,17-dione	21
6α-Fluoro-17β-hydroxyandrost-4-ene-3,17-dione	0
6β-Fluoro-17β-hydroxyandrost-4-ene-3,17-dione	0
9α-Fluoroandrosta-1,4-diene-3,17-dione	55
Androsta-1,4-diene-3,17-dione	22, 35
Androsta-4,6-diene-3,17-dione	0
Androsta-1,4,6-triene-3,17-dione	0
Androst-4-ene-3,11,17-trione	0
C_{18} Steroids	
Estr-4-ene-3,17-dione	21
17β-Hydroxyestr-4-en-3-one	20
17β-Hydroxy-5α,10β-estr-3-one	0
C_{12} Steroids	
Pregn-4-ene-3,20-dione	0
17α,19,21-Trihydroxypregn-4-ene-3,20-dione	0

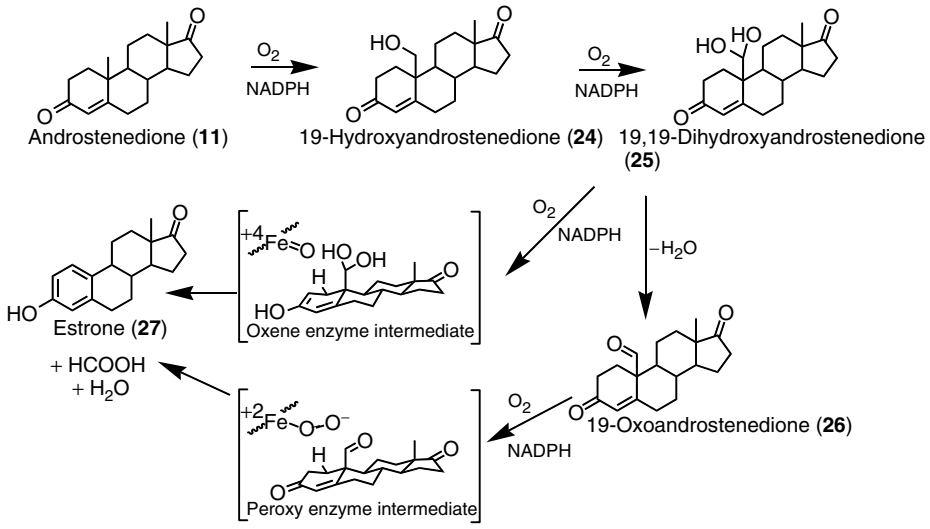


Fig. 5 Aromatization of androgens.

Tab. 4 Comparison of the androgenic and myotrophic activities of testosterone derivatives in the chick comb and castrated male rat assays following subcutaneous administration.

Testosterone modification	*Increase in weight [%]*		
	Chick comb	*Rat ventral prostate*	*Rat levator ani*
None	100	100	100
19-Nor	81	42	90
7α-Methyl	11	97	135
7α-Methyl-19-nor	75	218	226
14-Dehydro	128	54	8
14-Dehydro-19-nor	320	69	133
14-Dehydro-7α-methyl-19-nor	435	352	330

under enzymatic or nonenzymatic condition and did demonstrate that the oxygen atoms from the first and third oxidation steps are incorporated into formic acid. These results led to the proposal that the last oxidation step is a peroxidative attack at the C_{19} position. Recent computational chemistry approaches of this third step of aromatase catalysis using density functional theory (DFT) and molecular mechanical methods support the final catalytic step involving the 19-*gem*-diol substrate, a cytochrome P450 oxene intermediate, 1β-hydrogen atom abstraction, and release of formic acid.

Incubation of a large number of testosterone analogs with human placental tissue has provided some insight into the structural requirements for aromatization (see Table 4). Whereas androstenedione was converted rapidly to estrone, the 1-dehydro and 19-nor analogs were

metabolized slowly, and the 6-dehydro isomer and saturated 5α-androstane-3,17-dione remained unchanged. Hydroxyl and other substituents at lα, 2β, and 11β interfered with aromatization, whereas similar substituents at 9α and 11α seemingly had no effect. Of the stereoisomers of testosterone, only the 8β,9β,10β-isomer is aromatized, in addition to compounds having the normal configuration (8β, 9α, 10β). Thus, the substrate specificity of aromatase appears to be limited to C_{19} steroids with the 4-en-3-one system. Inhibition studies with various steroids have provided additional insights into the structural requirements for the enzyme; steroidal aromatase inhibitors are described later in Sect. 6.3.3.

Recent research in aromatase has focused on the biochemistry, molecular biology, and regulation of the aromatase protein. Aromatase is a membrane-bound cytochrome P-450 monooxygenase consisting of two proteins – aromatase cytochrome P-450 (P-450_{arom}) and NADPH-cytochrome P-450 reductase. Cytochrome P-450_{arom} is a heme protein that binds the steroid substrate and molecular oxygen and catalyzes the oxidations. The reductase is a flavoprotein, is found ubiquitously in endoplasmic reticulum, and is responsible for transferring reducing equivalents from NADPH to cytochrome P-450_{arom}. Purification of cytochrome P-450_{arom} proved to be very difficult because of its membrane-bound nature, instability, and low tissue concentration. The combination of hydrophobic chromatography using phenyl Sepharose, nonionic detergent, and the presence of micromolar amounts of substrate androstenedione yielded a highly purified and active cytochrome P-450_{arom}, with the highest specific content of 11.5 nmol of cytochrome P-450 per milligram of protein reported. Reconstitution of this cytochrome P-450_{arom} with NADPH-cytochrome P-450 reductase and phospholipid resulted in complete conversion of androstenedione to estrone, thus, demonstrating that one cytochrome P-450 protein catalyzes all three oxidation steps. Knowledge of the molecular biology of aromatase has advanced greatly in the past five years. A full-length cDNA complementary to mRNA encoding cytochrome P-450_{arom} was sequenced and the open reading frame encodes a protein of 503 amino acids. This cDNA sequence was inserted into COS1 monkey kidney cells, and aromatase mRNA and aromatase enzymatic activity were detected in transfected cells. The entire human cytochrome $P450_{arom}$ gene is greater than 70 kb in size and is located on chromosome 15. Clones have been utilized to examine the regulation of aromatase in ovarian, adipose, and breast tissues.

The metabolism of androgens by the mammalian brain has also been investigated under *in vitro* conditions. In rat brain, testosterone is converted to DHT, androstenedione, 5α-androstane-3,17-dione, and 5α-androstane3β,17β-diol. The aromatization of androgens to estrogens was also found to occur in the hypothalamus and the pituitary gland. The full significance of these metabolites on various neuroendocrine functions, such as regulation of gonadotropin secretion and sexual behavior, is not yet fully understood.

3.5 Mechanism of Action

It would indeed be impossible to explain all the varied biological actions of testosterone by one biochemical mechanism. Androgens, as well as the other steroid hormones adrenocorticoids, estrogens, and progestins, exert potent physiological effects on sensitive tissues and yet are

present in the body in only extremely low concentrations (e.g. 0.1–1.0 nM). The majority of investigations concerning the elucidation of the mechanisms of action of androgens have dealt with actions in androgen-dependent tissues and, in particular, the rat ventral prostate. The results of these studies indicate that androgens primarily act in regulating gene expression and protein biosynthesis by formation of a hormone–receptor complex, analogous to the mechanisms of action of estrogens and progestins. Extensive research activities directed at elucidation of the general mechanism of steroid hormone action have been performed for over three decades, and several reviews have appeared on this subject.

Investigations of a selective uptake of androgens by target cells performed in the early 1960s were complicated by low specific activity of the radiolabeled hormones and the rapid metabolic transformations. Nonetheless, it was noted that target cells retained primarily unconjugated metabolites, whereas conjugated metabolites were present in nontarget cells such as blood and liver. With the availability of steroids with high specific activity, later studies demonstrated the selective uptake and retention of androgens by target tissues. In addition, DHT was found to be the steroidal form selectively retained in the nucleus of the rat ventral prostate. This discovery led to the current concept that testosterone is converted by 5α-reductase to DHT, which is the active form of cellular androgen in androgen-dependent tissues.

The rat prostate has been the most widely examined tissue, and current hypotheses on the mode of action of androgens are based largely on these studies (see Fig. 6). The lipophilic steroid hormones are carried in the bloodstream, with the majority of the hormones reversibly bound to serum carrier proteins and a small amount of free steroids. The androgens circulating in the bloodstream are the sources of steroid hormone for androgen action in target tissues. Testosterone, synthesized

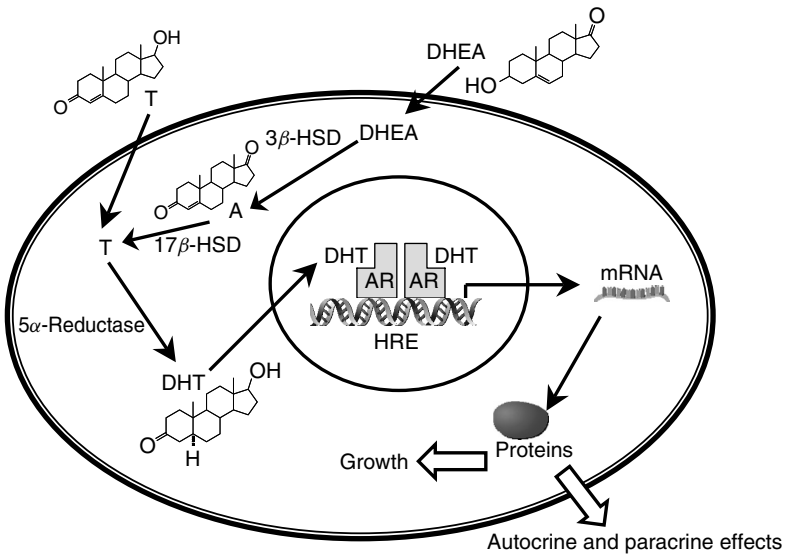


Fig. 6 Mechanism of action of 5α-dihydrotestosterone.

and secreted by the testis, is the major androgen in the blood stream and the primary source of androgen for target tissues in men. Dehydroepiandrosterone (DHEA) and androstenedione also circulate in the bloodstream and are secreted by the adrenal gland under the regulation of adrenocorticotropic hormone (ACTH). DHEA and androstenedione supplement the androgen sources in normal adult men, but these steroids are the important circulating androgens in women. The free circulating androgens steroids passively diffuse through the cell membrane and are converted to the active androgen 5α-DHT within the target cells.

The androgens act on target cells to regulate gene expression and protein biosynthesis via the formation of steroid–receptor complexes. Those cells sensitive to the particular steroid hormone (referred to as *target cells*) contain high-affinity steroid receptor proteins capable of interacting with the steroid. The binding of DHT with the receptor protein is a necessary step in the mechanism of action of the steroid in the prostate cell. Early studies suggested that the steroid receptor proteins were located in the cytosol of target cells and, following formation of the steroid–receptor complex, the steroid–receptor complex translocated into the nucleus of the cell. More recent investigations on androgen action indicate that active, unoccupied receptor proteins are present only in the nucleus of the cell. This nuclear localization of receptor is also observed for estrogens and progestins, while the majority of the glucocorticoid receptor is located in the cytoplasm. In the current model, DHT is formed in the cytoplasm, then enters the nucleus of the cell, and binds to the nuclear steroid receptor protein.

The binding of DHT to the nuclear androgen receptor (AR) initiates a conformational change or activation of the steroid nuclear–receptor complex and results in the formation of a homodimer. The homodimer interacts with particular regions of the cellular DNA, referred to as *androgen-responsive elements* (AREs), and with various nuclear transcriptional factors. Binding of the nuclear steroid–receptor complex to DNA initiates transcription of the DNA sequence to produce messenger RNA (mRNA). Finally, the elevated levels of mRNA lead to an increase in protein synthesis in the endoplasmic reticulum; these proteins include enzymes, receptors, and/or secreted factors that subsequently result in the steroid hormonal response regulating cell function, growth, and differentiation.

Extensive structure–function studies on the androgen receptor or AR have identified regions critical for hormone action. The androgen receptor is encoded by the AR gene located on the X chromosome, and the AR gene is comprised of 8 exons. The human AR contains approximately 900 to 920 amino acids, and the exact length varies because of polymorphisms in the NH_2 terminal of the protein. The primary amino acid sequences of AR, as well as of the various steroid hormone receptors, were deduced from cloned complementary DNAs (cDNAs). The calculated molecular weight of AR is approximately 98 000 kDa based upon amino acid composition; however, the AR is a phosphoprotein and migrates higher at approximately 110 kDa in sodium dodecyl sulfate (SDS) gel electrophoresis. The steroid receptor proteins are part of a larger family of nuclear receptor proteins that also include receptors for vitamin D, thyroid hormones, and retinoids. The overall structure of the androgen receptor (shown in Fig. 7) has

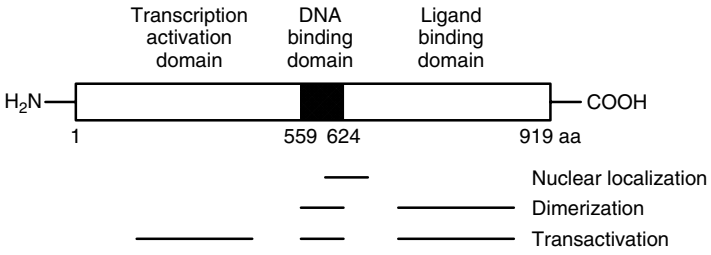


Fig. 7 Schematic diagram of androgen receptor.

strong similarities to the other steroid hormone receptors, with the proteins containing regions that bind to the DNA and bind to the steroid hormone ligand. A high degree of homology (sequence similarities) in the steroid receptors is found in the DNA binding region that interacts with the hormone response elements (HREs). The DNA binding region is rich in cysteine amino acids and chelate zinc ions, forming fingerlike projections called *zinc fingers* that bind to the DNA. The hormone binding domain (or ligand binding domain, LBD) is located on the COOH terminal of the protein. Structure–function studies of cloned receptor proteins also identify regions of the molecules that are important for nuclear localization of the receptor, receptor dimerization, interactions with nuclear transcriptional factors, and activation of gene transcription. Importantly, two regions of the androgen receptor protein are identified as transcriptional activation domains. The domain on the NH_2-terminal region may interact with both coactivators and corepressors, and the COOH-terminal domain initiates transcriptional activation only upon binding of an agonist such as 5α-DHT. The interactions necessary for formation of the steroid–receptor complexes and subsequent activation of gene transcription are complicated, involve multiple protein partners referred to as *coactivators*, and leave many unanswered questions.

Although the tertiary structure of the entire androgen receptor has not been determined, the crystallographic structure of the LBD has recently been reported. The AR LBD consists of an α-helical sandwich, similar to the LBDs reported for other nuclear receptors, and AR LBD contains only 11 helices (no helix 2) and four short β-strands (Fig. 8). Minor differences in the two reported crystallographic structures are likely due to limits of experimental resolution, differences in data interpretation, and the use of different ligands for crystallization. The endogenous ligand DHT (**2**) interacts with helices 3, 5, and 11, and the DHT-bound AR LBD has a single, continuous helix 12 (207). Similar interactions are observed with metribolone (methyltrienolone, **53**); however, helix 12 is split into two shorter helical segments.

Additional information on receptor structure–function has been obtained by analyzing androgen receptor mutations in patients with various forms of androgen resistance and abnormal male sexual development. Two polymorphic regions have been identified in the NH_2-terminal region, encoding a polyglycine repeat and a polyglutamate tract. Currently, these polymorphic regions have not been shown to significantly alter AR levels, stability, or transactivation. These repeats are useful in pedigree analysis of patients. Mutations in the androgen receptor have been identified

Fig. 8 Ribbon diagram of androgen receptor ligand binding domain (LBD) bound with 5α-dihydrotestosterone. Atomic coordinates were obtained from the Protein Data Bank (PDB ID code 1I37; www.rcsb.org) and displayed using ViewerLite 4.2, Accerlys Inc. 5α-Dihydrotestosterone is illustrated as a stick structure behind Helix 12.

in patients with either partial or full androgen insensitivity syndrome (AIS), with the majority of the mutations identified in exons 4 through 8 encoding the DNA binding domain and the hormone binding domain. Finally, studies with the human LNCaP prostate cancer cell line have provided interesting results regarding receptor protein structure and ligand specificity. The LNCaP cells exhibited enhanced proliferation in the presence of androgens, but these cells also unexpectedly proliferated in the presence of estrogens, progestins, cortisol, or the antiandrogen flutamide. Analysis of the cDNA for the LNCaP androgen receptor revealed that a single base mutation in the LBD was present and resulted in the increased affinity for progesterone and estradiol. The crystallographic structures of the LBD with the T877A mutation confirm that the mutated AR LBD can accommodate larger structures at the C-17 position.

The ultimate action of androgens on target tissues is the stimulation of cellular growth and differentiation through regulation of protein synthesis, and numerous androgen-inducible proteins have been identified. One of the prominent androgen-inducible proteins is prostate-specific antigen (PSA), a serine protease expressed by secretory prostate epithelial cells and utilized as blood test in screening for possible prostate diseases such as prostate cancer. Three AREs have been identified in the promoter regions of the PSA gene. Another androgen-regulated gene examined extensively in rats is the gene encoding the protein probasin, a 20-kDa secretory protein from the rat dorsolateral prostate structurally similar to serum globulins. Other proteins induced by androgens include spermine-binding protein, keratinocyte growth factor or fibroblast growth factor (KGF or FGF-7), androgen-induced growth factor (AIGF or FGF-8), nerve-growth factor, epidermal growth factor, c-myc, protease D, β-glucuronidase, and α_{2u}-globulin. Studies of these proteins suggest that the androgens act by enhancing transcription and/or translation of specific RNAs for the proteins. Also, the androgen receptor represses gene expression of certain proteins

such as glutathione S-transferase, TRMP-2 involved in apoptosis, and cytokines such as interleukin-4, interleukin-5, and γ-interferon.

While most biochemical studies focused on the rat ventral prostate, some researchers began to investigate the presence of cellular receptor proteins in other androgen-sensitive tissues. Androgen receptors have been reported in seminal vesicles, sebaceous gland, testis, epididymis, kidney, submandibular gland, pituitary and hypothalamus, bone marrow, liver, and androgen-sensitive tumors. Although DHT is the active androgen in rat ventral prostate, it is not the only functioning form in other androgen-sensitive cells. In ventral prostate and seminal vesicles, DHT is readily formed. It is metabolized only slowly, however, and therefore can accumulate and bind to receptors. Also, comparison of binding kinetics for testosterone and DHT demonstrated that testosterone dissociates faster, implying extended retention of DHT by the androgen receptor. In other tissues, such as brain or kidney, DHT is not readily formed and is metabolized quickly compared to testosterone. Species variations have also been demonstrated. The most striking example is the finding that 5α-androstane-3α, 17α-diol interacts specifically with cytosolic receptor protein from dog prostate and may be the active androgen in this species. Apparently, the need for a 17β-hydroxyl is not essential in all species.

Thus, current findings indicate that androgen receptor proteins vary in steroid specificity among different tissues from the same species as well as among different species. Nevertheless, the basic molecular mechanism of action of the androgens in androgen-sensitive tissues is consistent with the results of the studies on rat ventral prostate.

The manner whereby the androgens exert their anabolic effects has not been as extensively studied. The conversion of testosterone to DHT has been shown to be insignificant in skeletal and levator ani muscles, suggesting that the androgen-mediated growth of muscle is due to testosterone itself. Classical steroid receptors for testosterone are found in the cytoplasm of the levator ani and quadriceps muscles of the rat. Unlike prostate receptor protein, DHT had a lower affinity than testosterone for this protein. Androgen receptors have also been identified in other muscle tissues as well, including cardiac muscle.

4
Synthetic Androgens

4.1
Current Drugs on the Market

See Table 5.

4.2
Therapeutic Uses and Bioassays

The primary uses of synthetic androgens are the treatment of disorders of testicular function and of cases with decreased testosterone production. Several types of clinical conditions result from testicular dysfunction. Information on the biochemistry and mechanism of action of testosterone that has accumulated over the past 20 years has greatly aided in the elucidation of the underlying pathophysiology of these diseases.

Hypogonadism arises from the inability of the testis to secrete androgens and can be caused by various conditions. These hypogonadal diseases can, in many cases, result in disturbances in

Tab. 5

Generic name (structure)	*Trade name*	*US manufacturer*	*Chemical class*	*Dose*
Testosterone enanthate (**31**)	Delatestryl	BTG Pharm	Androstane	Injection: 200 mg mL^{-1}
Testosterone cypionate (**33**)	Depo-Testosterone	Pharmacia	Androstane	Injection: 100 mg mL^{-1}; 200 mg mL^{-1}
Testosterone pellets (**1**)	Testopel	Bartor Pharmacal	Androstane	Pellets: 75 mg
Testosterone transdermal system (**1**)	Testoderm	Alza	Androstane	Transdermal: 10 mg; 15 mg
	Testoderm TTS	Alza	Androstane	Transdermal: 328 mg
	Testoderm with Adhesive	Alza	Androstane	Transdermal: 15 mg
	Androderm	Watson Pharma	Androstane	Transdermal: 12.2 mg; 24.3 mg
Testosterone gel (**1**)	AndroGel 1%	Unimed Pharm	Androstane	Gel: 1% testosterone
Methyltestosterone (**5**)	Methyltestosterone	Various suppliers	Androstane	Tablets: 10 mg; 25 mg; Tablets (buccal): 10 mg
	Testred	ICN Pharm	Androstane	Capsules: 10 mg
	Android	ICN Pharm	Androstane	Capsules: 10 mg
	Virilon	Star	Androstane	Capsules: 10 mg
	Virilon IM	Star	Androstane	Injection: 200 mg mL^{-1}
Fluoxymesterone (**36**)	Halotestin	Pharmacia	Androstane	Tablets: 2 mg; 5 mg; 10 mg
	Fluoxymesterone	Various suppliers	Androstane	Tablets: 10 mg
Danazol (**77**)	Danazol	Various suppliers	Androstane	Capsules: 50 mg; 100 mg; 200 mg
	Danocrine	Sanofi-Synthelabo	Androstane	Capsules: 50 mg; 100 mg; 200 mg
Testolactone (**38**)	Teslac	Bristol-Myers Squibb	Androstane	Tablets: 50 mg

sexual differentiation and function and/or sterility. Primary hypogonadism is the result of a basic disorder in the testes, while secondary hypogonadism results from the failure of pituitary and/or hypothalamic release of gonadotropins and thus diminished stimulation of the testis. Usually primary hypogonadism is not recognized in early childhood (with the exception of cryptorchidism) until the expected time of puberty. This testosterone deficiency is corrected by androgen treatment for several months, at which time the testes are evaluated for possible development. Long-term therapy is necessary if complete testicular failure is present. Patients with Klinefelter's syndrome, a disease in which a genetic male has an extra X chromosome, have low testosterone levels and can also be treated by androgen replacement.

Male pseudohermaphroditism are disorders in which genetically normal men do not undergo normal male development. One type, testicular feminization, is observed in patients who have normal male XY chromosomes, but the male genitalia and accessory sex glands do not develop. Rather, the patients have female external genitalia. These patients are unresponsive to androgens and have defective androgen receptors. Another type of male pseudohermaphroditism results from a deficiency of the enzyme 5α-reductase. Since DHT is necessary for early differentiation and development, the patients again develop female genitalia; later, some masculinization can occur at the time of puberty due to elevated testosterone levels in the blood. A third disorder is Reifenstein syndrome, an incomplete pseudohermaphroditism. In these patients, the androgen levels are normal, 5α-reductase is present, and elevated LH levels are found. Partially deficient androgen receptors are present in these patients. In most cases of male pseudohermaphroditism, androgen replacement has little or no effect and thus steroid treatment is not recommended.

Deficiencies of circulating gonadotropins lead to secondary hypogonadism. This condition can be caused by disorders of the pituitary and/or hypothalamus resulting in diminished secretions of neurohormones. The lack of stimulation of the seminiferous tubules and the Leydig cells due to the low levels of these neurohormones decreases androgen production. Drugs such as the neuroleptic phenothiazines and the stimulant marijuana can also interfere with release of gonadotropins. The use of androgens in secondary hypogonadism is symptomatic.

Synthetic androgens have also been used in women for the treatment of endometriosis, abnormal uterine bleeding, and menopausal symptoms. However, their utility is severely limited by the virilizing side effects of those agents. Two weak androgens, calusterone, and 1-dehydrotestolactone, are used clinically in the treatment of mammary carcinoma in women. The mode of action of these drugs in the treatment of breast cancer is unknown, and is not simply related to their androgenicity. More recent evidence on the ability of these compounds to inhibit estrogen biosynthesis catalyzed by aromatase suggests that they effectively lower estrogen levels *in vivo*.

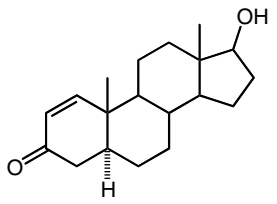
Traditionally, androgens have been assayed by the capon comb growth method and by the use of the seminal vesicles and prostate organs of the rodent. An increase in weight and/or growth of the capon comb have been used to denote androgenic activity following injection or topical application of a solution of the test compound in oil. A number of minor modifications of this test have been

described. The increase in weight of the seminal vesicles and the ventral prostate of the immature castrated male rat has provided another measure of androgenic potency. The test compound is administered either intramuscularly or orally, and the weight of the target organs is compared with those of control animals. *In vitro* evaluation of the relative affinity of potential androgens for the androgen receptor has also become an important tool in assessing biological activity of androgens.

4.3 Structure–Activity Relationships for Androgens

4.3.1 Early Modifications

Most of the early structure–activity relationship studies concerned minor modifications of testosterone and other naturally occurring androgens. Studies in animals and humans showed the 17β-hydroxyl function to be essential for androgenic and anabolic activity. In certain cases, esterification of the 17β-hydroxyl group not only enhanced but also prolonged the anabolic and androgenic properties. The 1-dehydro isomer of testosterone (**28**) and related compounds are potent androgenic and anabolic steroids.

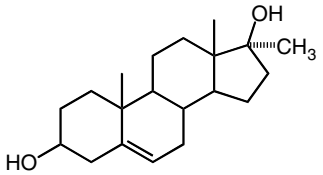


(**28**)

Reduction of the A-ring functional groups has variable effects on activity. For example, conversion of testosterone to DHT has little effect or may increase potency in a variety of bioassay systems. On the other hand, changing the A/B trans stereochemistry of known androgens such as androsterone (**3**) and DHT to the A/B *cis*-etiocholanolone (**18**) and 17β-hydroxy-5β-androstan-3-one (**13**), respectively, drastically reduces both the anabolic and androgenic properties. These observations established the importance of the A/B trans ring juncture for activity.

4.3.2 Methylated Derivatives

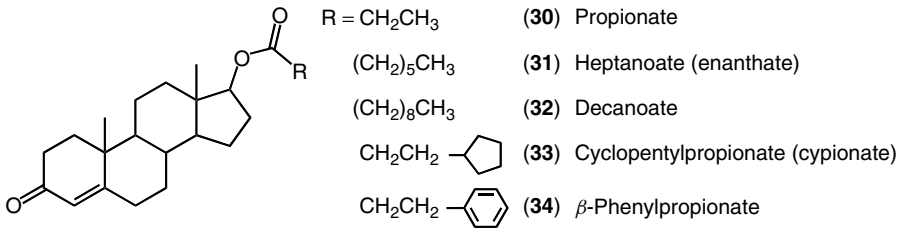
The discovery that C-17α-methylation conferred oral activity on testosterone prompted the synthesis of additional C-17α-substituted analogs. Increasing the chain length beyond methyl invariably led to a decrease in activity. As a result of these studies, however, 17α-methylandrost-5-ene-3β,17β-diol (methandriol, **29**) was widely evaluated in humans as an anabolic agent. Clinical studies showed no advantage of methandriol over 17α-methyltestosterone (**5**).



(**29**)

4.3.3 Ester Derivatives

As early as 1936 it was known that esterification of testosterone markedly prolonged the activity of this androgen when it was administered parenterally. This modification enhances the lipid solubility of the steroid and, after injection, permits a local depot effect. The acyl moiety is usually derived from a long-chain aliphatic or arylaliphatic acid such as propionic, heptanoic



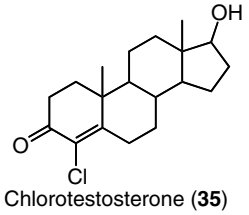
(enanthoic), decanoic, cyclopentylpropionic (cypionic), or β-phenylpropionic acid (**30**–**34**).

4.3.4 Halo Derivatives

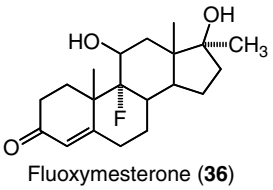
In general, the preparation of halogenated testosterone derivatives has been therapeutically unrewarding. 4-Chloro-17β-hydroxyandrost-4-en-3-one (chlorotestosterone, **35**) and its derivatives are the only chlorinated androgens that have been used clinically, albeit sparingly. The introduction of a 9α-fluoro and an 11β-hydroxy substitutent (analogous to synthetic glucocorticoids) gives 9α-fluoro-11β,17β-dihydroxy-17α-methylandrost-4-en-3-one (fluoxymesterone, Halotestin, **36**), which is an orally active androgen exhibiting approximately fourfold greater oral activity than 17α-methyltestosterone. Early clinical studies with fluoxymesterone indicated an anabolic potency that was 11 times that of the unhalogenated derivative. Nitrogen balance studies, however, revealed an activity only 3 times that of 17α-methyltestosterone. Because of the lack of any substantial separation of anabolic and androgenic activity, halotestin is used primarily as an orally effective androgen, particularly in the treatment of mammary carcinoma.

4.3.5 Other Androgen Derivatives

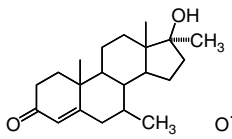
Several synthetic steroids having weak androgenic activity are being utilized in patients. 7β,17α-Dimethyltestosterone (calusterone, **37**) and 1-dehydrotestolactone (Testlac, **38**) are very weak androgenic agents that have been used in the treatment of advanced metastatic breast cancer.



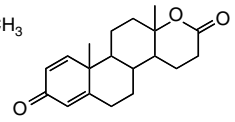
Chlorotestosterone (**35**)



Fluoxymesterone (**36**)



Calusterone (**37**)



1-Dehydrotestolactone (**38**)

4.3.6 Summary of Structure–Activity Relationships

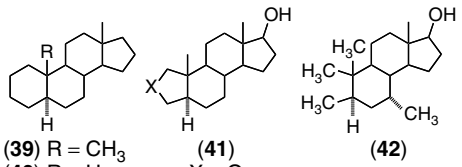
As with other areas of medicinal chemistry, the desire to relate chemical structure to androgenic activity has attracted the attention of numerous investigators. Although it is often difficult to interrelate biological results from different laboratories, androgenicity data from the same laboratory afford useful information. In evaluating the data, one must be careful to note not only the animal model employed, but also the mode of administration. For example, marked differences in androgenic activity can be found when compounds are evaluated in the chick comb assay (local application) as opposed to the rat ventral prostate assay (subcutaneous or oral). The chick comb assay measures ''local androgenicity'' and is believed to minimize such factors as absorption, tissue distribution, and metabolism, which complicate the interpretation of *in vivo* data in terms of hormone–receptor interactions.

Furthermore, although the rat assays correlate well with what one eventually finds in humans, few studies of comparative pharmacology have been performed. Indeed, DHT may not be the principal mediator of androgenicity in all species. For example, a cytosol receptor protein has been found in normal and hyperplastic canine prostate that is specific for 5α-androstane-3α,17α-diol.

Since the presence of the 17β-hydroxyl group was demonstrated very early to be an important feature for androgenic activity in rodents, most investigators interested in structure–activity relationships maintained this function and modified other parts of the testosterone molecule. Three observations can be made on the basis of these studies: (1) The 1-dehydro isomer of testosterone is at least as active as testosterone; (2) the 1- and 4-keto isomers of testosterone and DHT have variable activity; (3) the 2-keto isomers of testosterone and DHT consistently lack appreciable activity.

The oxygen function at position 3 could be removed from testosterone with little reduction in androgenic activity, while removal of the hydroxyl group from position 17 sharply reduced the androgenicity. As a continuation of these studies, the hydrocarbon nucleus, 5α-androstane (**39**), was synthesized. It too was found to possess androgenicity when applied topically or given intramuscularly in the chick comb assay, albeit at high doses. On the other hand, it was learned later that the 19-nor analog, 5α-estrane (**40**), had less than 1% of the androgenic activity of testosterone propionate in castrated male rats.

The relative androgenicity of the isomeric A-ring olefins of 3-deoxy testosterones was the order $\Delta^1 > \Delta^2 > \Delta^3 > \Delta^4$. The Δ^2-isomer displayed the greatest anabolic activity and the best anabolic-to-androgenic ratio. On the basis that sulfur is bioisosteric with CH=CH, the thia, seleno, and tellurio androstanes were synthesized, which displayed androgenic activity. When the heteroatom was oxygen, however, the compound **(41)** was essentially devoid of



(**39**) R = CH_3
(**40**) R = H

(**41**)
X = O

(**42**)

androgenicity. The oxygen analog was said to be inactive because oxygen is isosteric with CH_2 rather than $CH_2{=}CH_2$. Thus, a minimum ring size was found to be required for activity. When the oxygen atom was introduced as part of a six-membered A-ring, an active androgen resulted.

As with the case of the double-bond isomers, the position of the oxygen atom was found to be important. The substitution of oxygen at C-2 gives rise to the most active compound, and the order of activity was $2 > 3 \gg 4$. The full steroid nucleus is not essential for androgen activity, since 7α-methyl 1,4-seco-2,3-bisnor-5α-androstan-17β-ol (**42**) has 50% of the anabolic activity of testosterone.

Both 14-dehydrotestosterone and the corresponding 19-nor analog were found to be potent androgens when applied topically. An extension of this series ascertained the effect of introducing a 7α-methyl. The results of this study are listed in Table 4, in terms of percentage increases in the weights of chick combs, rat ventral prostates, and rat levator ani induced by the test compounds as related to a similar dose of testosterone, the responses to the latter being described as 100%.

The effects of either 7α-methyl or 14- dehydro modification are more pronounced for 19-nortestosterone than for testosterone. The 14-dehydro modification had a greater effect on local androgenicity, whereas 7α-methylation had a more positive effect on systemic androgenicity. A marked synergism resulted when both the 14-dehydro and 7α-methyl modifications were present. The resultant compound, 7α-methyl-14-dehydro-19-nortestosterone, represents one of the most potent androgens reported to date.

The characterization of a specific receptor protein in androgen target tissues has made it possible to directly analyze the receptor affinity of various testosterone analogs. Table 6 shows the receptor affinity and androgenic activity of a variety of androgens relative to DHT. The ability of the various steroids to be retained by prostate nuclei is also indicated. As would be expected, the receptor affinity data did not necessarily correlate with the systemic androgenicity. In some cases, such as with 7α-methyl-19-nortestosterone, there was good agreement. Such was not the case, however, for 19-nortestosterone. Table 7 summarizes their findings. Whereas the importance of the A/B trans ring fusion

Tab. 6 Relative androgenicity and receptor binding capacity of various androgens.

Steroid	*Androgenicity in rat (s.c.)*	*Cytosol receptor*	*Nuclear retention*
DHT	1.0	1.0	1.0
Testosterone (T)	0.4	<0.1	0.7
5α-Androstanedione	0.2	0.0	0.2
19-NorDHT	0.1	0.5	0.6
19-NorT	0.2	0.9	0.7
7α-CH_3-DHT	1.2	0.4	0.4
7α-CH_3-T	0.4	0.2	0.2
7β-CH_3-T	0.1	<0.1	<0.1
7α-CH_3—19-NorDHT	0.3	0.6	0.4
7α-CH_3—19-NorT	2.6	2.6	1.8

Tab. 7 Binding affinity of various androgens for rat ventral prostate receptor protein.

Steroid	K_B $[M^{-1}]$
5α-DHT	6.9×10^8
5β-DHT	6.4×10^7
17β-Testosterone	4.2×10^8
17α-Testosterone	2.1×10^7
Androstenedione	1.3×10^7
5α-Androstanedione	3.5×10^7
19-Nortestosterone	8.6×10^8
14-Dehydrotestosterone	4.4×10^8
14-Dehydro-19-nortestosterone	5.9×10^8
7α-CH_3-14-Dehydro-19-nortestosterone	5.0×10^8

and 17β-hydroxyl prevailed, the data failed to demonstrate the potency previously noted for 7α-methyl-14-dehydro-19-nortestosterone. Moreover, 19-nortestosterone displayed a receptor affinity greater than that of DHT, yet its androgenicity is much less than DHTs.

These differences in correlations between receptor assays and *in vivo* data should not cloud the importance of the receptor studies. The receptor assays measure affinity for the receptor protein, and this property is shared by androgens as well as antiandrogens. Moreover, such assays cannot predict the disposition and metabolic fate of an androgen following administration. Figure 9 contains a summary of the structure–activity relationships for androgens.

4.4 Absorption, Distribution, and Metabolism

Numerous factors are involved in the absorption, distribution, and metabolism of the synthetic androgens and the physicochemical properties of these steroids greatly influence the pharmacokinetic parameters. The lipid solubility of a synthetic steroid is an important factor in its intestinal absorption. The acetate ester of testosterone demonstrated enhanced absorption from the gastrointestinal tract over both testosterone and 17α-methyltestosterone. Injected solutions of testosterone in oil result in the rapid absorption of the hormone from the injection site; however, rapid metabolism greatly decreases the biological effects of the injected testosterone. The esters of testosterone are much more nonpolar and, when injected intramuscularly, are absorbed more slowly. As a result, the commercial preparations of testosterone propionate are administered

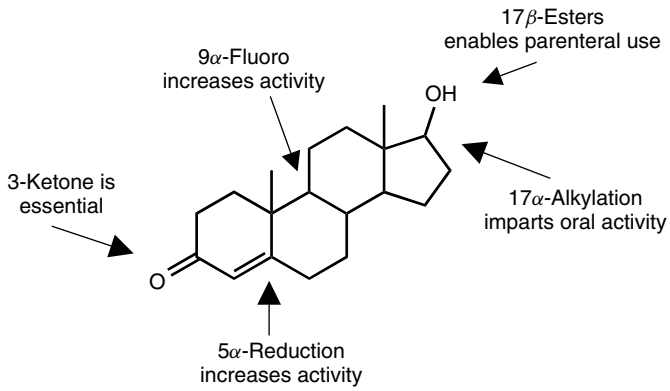


Fig. 9 Summary of structure–activity relationships for androgens.

every 2 or 3 days. Increasing the size of the ester functionality enables the testosterone esters such as the ethanate or cypionate to be given in a depot injection lasting 2 to 4 weeks.

Once absorbed, the steroids are transported in the circulation primarily in a protein-bound complex. Testosterone and other androgens are reversibly associated with certain plasma proteins and the unbound fraction can be absorbed into target cells to exert its action. The structure-binding relationships of the natural and synthetic androgens to sex SBG have been extensively investigated. A 17α-hydroxyl group is essential for binding and the presence of a 17α-substituent such as the 17α-methyl moiety decreases its affinity. The 5α-reduced androgens bind with the highest affinity. A much smaller quantity of the androgen is bound to other plasma proteins, principally albumin and CBG or transcortin.

The metabolism of the synthetic androgens is similar to that of testosterone. The introduction of the 17α-methyl group greatly retards the metabolism, thus providing oral activity. Reduction of the 4-en-3-one system in synthetic androgens to give the various α- and β-isomers occurs *in vivo*. Finally, aromatization of the A-ring can also occur. One analog that demonstrates an alternate metabolic pattern is 4-chlorotestosterone, which in humans gave rise to an allylic alcohol, 4-chloro-3α-hydroxyandrost-4-en-17-one. A number of other halogenated testosterone derivatives subsequently were found to take this abnormal reduction path *in vitro*. It was proposed that fluorine or chlorine substituents at the 2-, 4-, or 6- position in testosterone interfere with the usual α,β-unsaturated ketone resonance so that the C-3 carbonyl electronically resembles a saturated ketone.

4.5 Toxicities

The use of androgens in women and children can often result in virilizing or masculinizing side effects. In boys, an acceleration of the sexual maturation is seen, while in girls and women growth of facial hair and deepening of the voice can be observed. These effects are reversible when medication is stopped; however, prolonged treatment can produce effects that are irreversible. Inhibition of gonadotropin secretion by the pituitary can also occur in patients receiving androgens.

Both males and females experience salt and water retention resulting in edema. This edema can be treated by either maintaining a low-salt diet or by using diuretic agents. Liver problems are also encountered with some of the synthetic androgens. Clinical jaundice and cholestasis can develop after the use of the 17α-alkylated products. Various clinical laboratory tests for hepatic function such as bilirubin concentrations, sulfobromophthalein retention, and glutamate transaminase and alkaline phosphatase activities are affected by these androgen analogs.

5 Anabolic Agents

5.1 Current Drugs on the Market

See Table 8.

5.2 Therapeutic Uses and Bioassays

Many synthetic analogs of testosterone were prepared in order to separate the anabolic activity of the C_{19} steroids from

Tab. 8

Generic name (structure)	*Trade name*	*US manufacturer*	*Chemical class*	*Dose*
Nandrolone decanonate (**43**)	Deca-Durabolin	Organon	Estrane	Injection (in oil): 100 mg mL^{-1} 200 mg mL^{-1}
Oxandrolone (**65**)	Oxandrin	Gynex	Androstane	Tablets: 2.5 mg
Oxymetholone (**63**)	Anadrol-50	Syntex	Androstane	Tablets: 50 mg
Stanozolol (**75**)	Winstrol	Winthrop Pharm	Androstane	Tablets: 2 mg

their androgenic activity. Although the goal of a pure synthetic anabolic that retains no androgenic activity has not been accomplished, numerous preparations are now available on the market that have high anabolic/androgenic ratios.

The primary criterion for assessing anabolic activity of a compound is the demonstration of a marked retention of nitrogen. This nitrogen-retaining effect is the result of an increase in protein synthesis and a decrease in protein catabolism in the body. Thus, the urinary nitrogen excretion, particularly urea excretion, is greatly diminished. The castrated male rat serves as the most sensitive animal model for nitrogen retention, although other animals have been utilized. Another bioassay for anabolic activity involves examination of the increase in mass of the levator ani muscle of the rat upon administration of an anabolic agent. This measure of myotrophic effect correlates well with the nitrogen retention bioassay and the two are usually performed in the determination of anabolic activity.

Anabolic steroids exert other effects on the body as well. Skeletal mineralization and bone maturation are enhanced by androgens and anabolics. These agents will decrease calcium excretion by the kidney and result in increased deposition of both calcium and phosphorus in bone. Androgenic and anabolic agents also can influence red blood cell formation. Two mechanisms of action of this erythropoiesis-involving increased erythropoietin production and enhanced responsiveness of the tissue have been described.

These various biological activities of the anabolics have prompted the use of these agents in treatment protocols, with varying success. Clinical trials have demonstrated the effectiveness of the anabolic steroids in inducing muscle growth and development in some diseases. Anabolic steroids are effective in the symptomatic treatment of various malnourished states due to their ability to increase protein synthesis and decrease protein catabolism. Treatment of diseases such as malabsorption, anorexia nervosa, emaciation, and malnutrition as a result of psychoses includes dietary supplements, appetite stimulants, and anabolics. Improved postoperative recovery with adjunctive use of anabolic agents has been demonstrated in numerous clinical studies. However, their usefulness in other diseases such as muscular dystrophies and atrophies and in geriatrics has not been observed.

Anabolic steroids also have the ability to lower serum lipid levels *in vivo*. The most

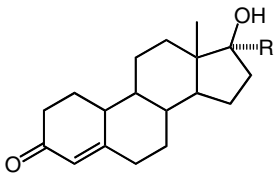
widely studied agent is oxandrolone, which dramatically lowers serum triglycerides and, to a lesser extent, cholesterol levels at pharmacological doses. The proposed mechanism of this hypolipidemic effect includes both an inhibition of triglyceride synthesis and an increased clearance of the triglycerides. The androgenic side effects of the anabolics and the lack of superiority over conventional hypolipidemic agents have curtailed its use for treatment of these conditions.

The stimulation of erythropoiesis by anabolics has resulted in the use of these agents for the treatment of various anemias. Anemias arising from deficiencies of the bone marrow are particularly responsive to pharmacological doses of anabolic agents. Treatment of aplastic anemia with anabolics and corticosteroids has been proven effective. Secondary anemias resulting from inflammation, renal disease, or neoplasia are also responsive to anabolic steroid administration. Synthetic anabolics have been prescribed for women with osteoporosis and for children with delayed growth. These applications have produced limited success; however, the virilizing side effects severely limit their usefulness, particularly in children.

The methods employed to determine the anabolic or myotrophic properties of steroids are based on an increase in nitrogen retention and/or muscle mass in various laboratory animals. The castrated male rat is presently the most widely used and most sensitive laboratory animal for nitrogen balance studies. Dogs and ovariectomized monkeys have also been employed. Although it is generally agreed that variations in urinary nitrogen excretion relate to an increase or decrease in protein synthesis, nitrogen balance assays are not without their limitations. This is partly because such studies fail to describe the shifts in organ protein and measure only the overall status of nitrogen retention in the animal.

The easily accessible levator ani muscle of the rat has provided a valuable index for measuring the myotrophic activity of steroidal hormones. By comparing the weight of levator ani muscle, seminal vesicles, and ventral prostate with controls, one can obtain a ratio of anabolic-to-androgenic activity. There also appears to be some correlation between the levator ani response and urinary nitrogen retention. A modification of this muscle assay utilizes the parabiotic rat and allows for the simultaneous measurement of pituitary gonadotropic inhibition and myotrophic activity. The suitability of the levator ani assay has been questioned for the possibility that its growth is more a result of androgenic sensitivity than of any steroid-induced myotrophic effect. Thus, this assay is usually performed in conjunction with nitrogen balance studies or acceleration of body growth.

Finally, the myotrophic effects have also led to the use and the abuse of these agents by athletes. Conflicting reports on the effectiveness of anabolics to increase strength and power in healthy males have resulted from clinical trials. Several groups reported no significant differences between groups of male college-age students receiving anabolics and weight training and those groups receiving placebo plus the weight training in double-blind studies. Other reports cited some improvement in strength and power, but they utilized small numbers of subjects or were only single blind studies. ''Supraphysiologic'' doses of anabolics have demonstrated enhancement of muscle size and strength. Overall, the consensus of these various studies are that anabolic steroids provide only limited improvement in strength and



R = H (**43**) Nandrolone
R = CH_3 (**44**) Normethandrone
R = CH_2CH_3 (**45**) Norethandrone

power in healthy males. Anabolic steroids also exhibit an anticatabolic effect, that is, reversing the catabolic effects of glucocorticoids released in response to stress. Such effects would enable individuals to recover more quickly following strenuous workouts. An alarming percentage of amateur and professional athletes utilize anabolic steroids, which are readily available ''on the street.'' The use of these steroids for increasing strength and power is banned in intercollegiate and international sports, and very sensitive assays (radioimmunoassay (RIA), gas chromatography-mass spectrometry (GS-MS)) have been developed for measuring anabolic levels in urine and blood. Nonetheless, the recent discovery of ''designer steroids'' such as tetrahydrogestrinone (THG), a new chemical entity synthesized specifically for illicit anabolic use to avoid standard antidoping tests, is an alarming trend and highlights the importance of aggressive detection approaches for monitoring drug doping in sports.

5.3 Structure–Activity Relationships for Anabolics

5.3.1 19-Nor Derivatives

An important step toward developing an anabolic agent with minimal androgenicity occurred when 19-nortestosterone (17β-hydroxyestr-4-en-3-one, nandrolone, **43**) was found to be as myotrophic but only about 0.1 as androgenic as testosterone. This observation prompted the synthesis and evaluation of a variety of 19-norsteroids, including the 17α-methyl (normethandrone, **44**) and the 17α-ethyl (norethandrolone, **45**) homologs of 19-nortestosterone.

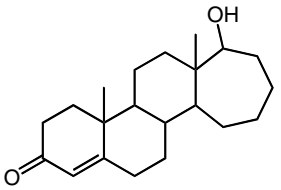
Nandrolone in the form of a variety of esters (such as decanoate and β-phenylpropionate) and norethandrolone have been widely used clinically. The latter, under the name Nilevar®, was the first agent to be marketed in the United States as an anabolic steroid. Androgenic and progestational side effects, however, led to its eventual replacement by other agents.

Nonetheless, these studies did stimulate the synthesis of other norsteroids. Interestingly, both 18-nortestosterone and 18,19-bisnortestosterone were essentially devoid of both androgenic and anabolic properties. Contraction of the B-ring led to B-norsteroids, which were also lacking in androgenicity, but unlike the foregoing, this modification led to compounds with antiandrogenic activity.

Of the number of homoandrostane derivatives (those having one or more additional methylene groups included in normal tetracyclic ring system) that have been synthesized, only B-homo and D-homodihydrotestosterone have shown appreciable androgenic activity. A D-bishomo analog (**46**) was reported to be weakly androgenic.

5.3.2 Dehydro Derivatives

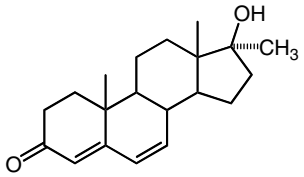
The marked enhancement in biological activity afforded by introduction of a double bond at C_1 of cortisone and hydrocortisone



(**46**)

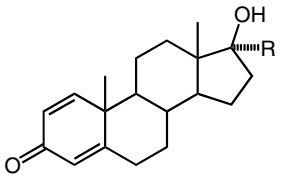
prompted similar transformations in the androgens. The acetate of 17β-hydroxyandrosta-1,4-dien-3-one (**47**) was as myotrophic as testosterone propionate but was much less androgenic. Furthermore, 17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one (methandrostenolone, **48**) had 1 to 2 times the oral potency of 17α-methyltestosterone in the rat nitrogen retention and levator ani muscle assays. In clinical studies, methandrostenolone produced a marked anabolic effect when given orally at doses of 1.25 to 10 mg daily and was several times more potent than 17α-methyltestosterone.

In contrast with the 1-dehydro analogs, introduction of an additional double bond at the 6-position (**49**) markedly decreased both androgenic and myotrophic activity in the rat. Moreover, removal of the C_{19}-methyl, inversion of the configuration at C_9 and C_{10} and at C_8 and C_{10}, and reduction of the C_3-ketone failed to improve the biological properties.



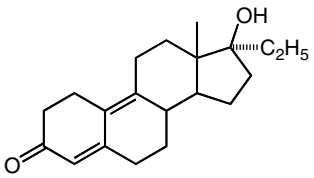
(**49**)

On the other hand, introduction of unsaturation into the B-, C-, and D-rings has given rise to compounds with significant androgenic or anabolic activity. Ethyldienolone (**50**), for example, displayed an anabolic-to-androgenic ratio of 5 and was slightly more active than methyltestosterone when both were given orally. Introduction of a 14–15 double bond (**51**) increased androgenicity when compared with testosterone by local application in the chick comb assay. On the other hand, there was a 25% decrease in androgenicity when measured by the rat ventral prostate following subcutaneous administration. Conversion to the 19-nor analog (**52**) increased

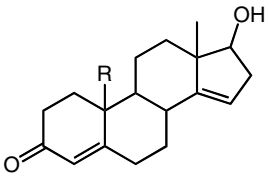


R = H (**47**)
R = CH_3 (**48**)

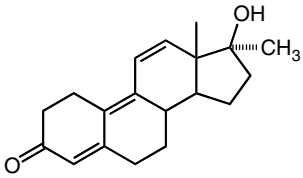
Methandrostenolone



(**50**)



(**51**) R = CH_3
(**52**) R = H



(**53**)

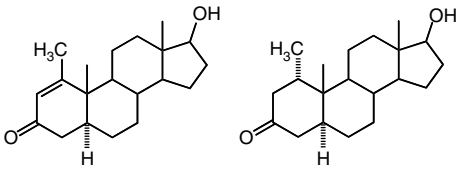
androgenicity, but the anabolic activity was significantly enhanced.

Of a variety of triene analogs of testosterone that have been tested, only 17α-methyl-17β-hydroxyestra-4,9,11-trien-3-one (methyltrienolone, **53**) showed significant activity in rats. Surprisingly, this compound had 300 times the anabolic and 60 times the androgenic potency of 17α-methyltestosterone when administered orally to castrated male rats. In this instance, however, the potent hormonal properties on rats did not correlate with later studies in humans. One study in patients with advanced breast cancer found methyltrienolone to have weak androgenicity and to produce severe hepatic dysfunction at very low doses.

5.3.3 Alkylated Analogs

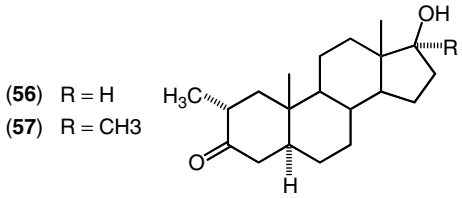
An extensive effort has been directed toward assessing the physiological effect of replacing hydrogen with alkyl groups at most positions of the steroid molecule. Although methyl substitution at C_3, C_4, C_5, C_6, C_{1l}, and C_{16} has generally led to compounds with low anabolic and androgenic activity, similar substitutions at C_1, C_2, C_7, and C_{18} have afforded derivatives of clinical significance.

1-Methyl-17β-hydroxy-5α-androst-l-en-3-one (**54**) as the acetate (methenolone acetate) was about 5 times as myotrophic, but only 0.1 as androgenic, as testosterone propionate in animals. In addition, this compound or the free alcohol represented one of the few instances of a C_{17} nonalkylated steroid that possessed significant oral anabolic activity in animals and in man. This effect may be related to the slow *in vivo* oxidation of the 17β-hydroxyl group when compared with testosterone. At a daily dose of 300 mg, methenolone acetate caused little virilization. By contrast, the dihydro analog, 1α-methyl-17β-hydroxy-5α-androstan-3-one (mesterolone, **55**), was found to possess significant oral androgenic activity in the cockscomb test and in clinical assays. A comparison of the anabolic and androgenic activity of (**54**) with its A-ring congeners revealed that the double bond was necessary at C_1 for anabolic activity. For example, 1α-methyl-17β-hydroxyandrost-4-en-3-one had a much lower activity. Furthermore, either reduction of the C_3 carbonyl group of (**55**) or removal of the C_{19} methyl group greatly reduced both anabolic and androgenic activity in this series.



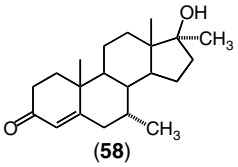
Methenolone (**54**) Mesterolone (**55**)

Among the C_2-alkylated testosterone analogs, 2α-methyl-5α-androstan-17β-ol-3-one (drostanolone, **56**) and its 17α-methylated homolog (**57**) have displayed anabolic activity both in animals and in man. In contrast, 2,2-dimethyl and 2-methylenetestosterone or their derivatives showed only low anabolic or androgenic activity in animals. The most interest in drostanolone has been in relation to its potential as an antitumor agent with decreased masculinizing propensity.

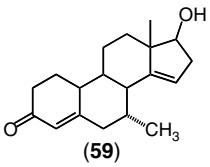


7α,17α-Dimethyltestosterone (bolasterone, **58**) had 6.6 times the oral anabolic

potency of 17α-methyltestosterone in rats. Similar activity was observed in man at 1 to 2 mg day^{-1} without many of the usual side effects. Moreover, the corresponding 19-nor derivative was 41 times as active as 17α-methyltestosterone as an oral myotrophic agent in the rat. 7α-Methyl-14-dehydro-19-nortestosterone (**59**) was found to be approximately 1000 times as active as testosterone in the chick comb assay and about 100 times as active as testosterone in the ventral prostate assay.

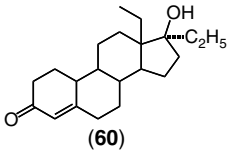


(**58**)

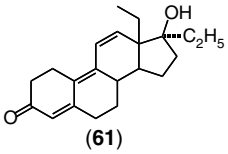


(**59**)

Certain totally synthetic 18-ethylgonane derivatives possessed pronounced anabolic activity. Similar to other 19-norsteroids, 13β,17α-diethyl-17β-hydroxygon-4-en-3-one (norbolethone, **60**) was found to be a potent anabolic agent in animals and in man. Since it is prepared by total synthesis, the product is isolated and marketed as the racemic DL-mixture. The hormonal activity resides in the D-enantiomer.



(**60**)

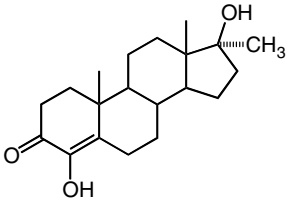


(**61**)

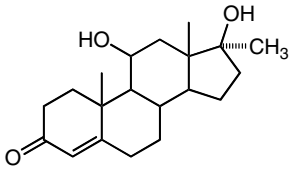
Tetrahydrogestrinone or THG (**61**), a "designer steroid" for illicit anabolic use, is synthesized from the progestin, gestrinone, by reduction of the 17α-ethinyl group. THG demonstrates potent androgenic and progestational activity in *in vitro* receptor bioassay systems. No animal or human studies with THG are known, since this agent was never prepared as a pharmaceutical for medical or veterinary use.

5.3.4 Hydroxy and Mercapto Derivatives

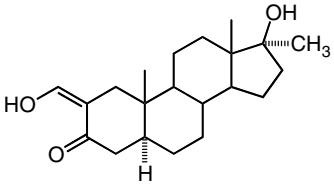
Testosterone has been hydroxylated at virtually every position on the steroid nucleus. For the most part, nearly all these substances possess no more than weak myotrophic and androgenic properties. Two striking exceptions to this, however, are 4-hydroxy- and 11β-hydroxytestosterones. 4-Hydroxy-17α-methyltestosterone (oxymesterone, **62**), for instance, had 3 to 5 times the myotrophic and 0.5 times the androgenic activity of 17α-methyltestosterone in the rat. In clinical studies, oxymesterone produced nitrogen retention in adults at a daily dose of 20 to 40 mg, and no adverse liver function was observed. Introduction of an 11β-hydroxyl group in many instances resulted in a favorable effect on biological activity. 11β-Hydroxy-17α-methyltestosterone (**63**) was more anabolic in the rat than 17α-methyltestosterone and 1.5 times as myotrophic in humans.



(**62**)



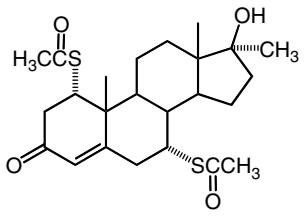
(**63**)



(**64**)

One of the most widely studied anabolic steroids has been 2-hydroxymethylene-17α-methyl-5α-androstan-17β-ol-3-one (oxymetholone, **64**). In animals, it was found to be 3 times as anabolic and 0.5 times as androgenic as 17α-methyltestosterone. Clinical studies confirmed these results.

The substitution of a mercapto for a hydroxyl group has generally resulted in decreased activity. However the introduction of a thioacetyl group at C_1 and C_7 of 17α-methyltestosterone afforded lα,7α-bis(acetylthio)-17α-methyl-17β-hydroxyandrost-4-en-3-one (thiomesterone, **65**), a compound with significant activity. Thiomesterone was 4.5 times as myotrophic and 0.6 times as androgenic as 17α-methyltestosterone in the rat and has been used clinically as an anabolic agent.



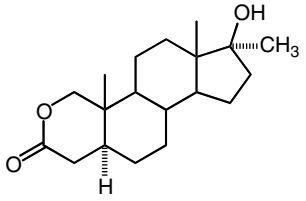
(**65**)

Moreover, numerous 7α-alkylthio androgens have exhibited anabolic-androgenic activity similar to that of testosterone propionate when administered subcutaneously. Even though no clinically useful androgen resulted, similar 7α-substitutions were advantageous in the development of radioimmunoassays now employed in clinical laboratories. In addition, certain 7α-arylthioandrost-4-ene-3,17-diones are effective inhibitors of estrogen biosynthesis (see Sect. 6.3.3).

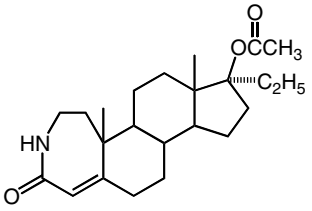
5.3.5 Oxa, Thia, and Aza Derivatives

A number of androgen analogs in which an oxygen atom replaces one of the methylene groups in the steroid nucleus have been synthesized and biologically evaluated. Of these derivatives, 17β-hydroxy-17αmethyl-2-oxa-5α-androstan-3-one (oxandrolone, **66**) was 3 times as anabolic and only 0.24 times as androgenic as 17α-methyltestosterone in the oral levator ani assay. By contrast, only minimal responses were obtained following intramuscular administration. The 2-thia and 2-aza analogs were essentially devoid of activity by both routes. The 3-aza-A-homoandrostene derivative (**67**) displayed only 5% the anabolic-to-androgenic activity of methyltestosterone.

The clinical anabolic potency of oxandrolone was considerably more active than 17α-methyltestosterone and provided perceptible nitrogen sparing at a dose as low as 0.6 mg day^{-1}. Moreover, at dosages of 0.25 to 0.5 mg kg^{-1}, oxandrolone was effective as a growth-promoting agent without producing the androgenically induced bone maturation. Because of this



(**66**)

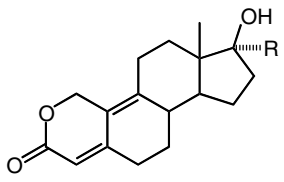


(**67**)

favorable separation of anabolic from androgenic effects, oxandrolone has been one of the most widely studied anabolic steroids. Its potential utility in various clinical hyperlipidemias was discussed in Sect. 5.2.

The significant hormonal activity noted for estra-4,9-dien-3-ones such as (**50**) (see Sect. 5.3.2) prompted the synthesis of the 2-oxa bioisosteres in this series. Despite the lack of a 17α-methyl group, (**68**) had 93 times the oral anabolic activity of 17α-methyltestosterone. It was also 2.7 times as androgenic. As might be expected, the corresponding 17α-methyl derivative, (**69**), was the most active substance in this series. It had 550 times the myotrophic and 47 times the androgenic effect of 17α-methyltestosterone. These two compounds differed dramatically in progestational activity, however. The activity of (**68**) was only 0.1 times, whereas the activity of (**69**) was 100 times that of progesterone in the Clauberg assay. The pronounced oral activity of (**68**) suggests that it is not a substrate for the 17β-alcohol dehydrogenase and represents an interesting finding.

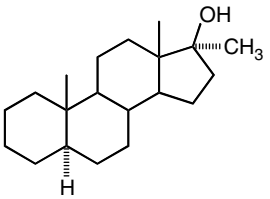
(**68**) R = H
(**69**) R = CH_3



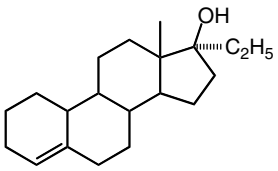
5.3.6 Deoxy and Heterocyclic-fused Analogs

Early studies indicated that the 17β-hydroxyl group and the 3-keto group were essential for maximum androgenic activity. On the basis of this observation, the C_3 oxygen function was removed in the hope of decreasing the androgenic potency while maintaining anabolic activity. Unfortunately, the results failed to substantiate the rationale, and 17α-methyl-5α-androstan-17β-ol (**70**) was found to be a potent androgen in animals and humans. However, this substance is extensively metabolized to the 3-keto derivative by rabbit liver homogenate. Other deoxy analogs of testosterone have been synthesized and tested. A 19-nor derivative, 17α-ethylestr-4-en-17β-ol (estrenol, **71**) had at least 4 times the anabolic and 0.25 times the androgenic activity of 17α-methyltestosterone in animals and was effective in humans at a daily dose of 3 to 5 mg. In addition, 17α-methyl-5α-androst-2-en-17β-ol (**72**) offered a good separation of anabolic from androgenic activity.

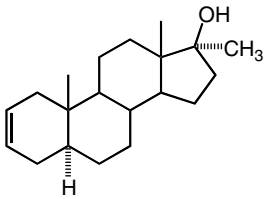
Since sulfur is considered to be isosteric with $-CH{=}CH-$, the 2-thia-A-nor-5α-androstane derivatives such as (**73**) possessed high androgenic and anabolic activity and served to verify that steric rather than electronic factors are important in connection with the structural requirements at C-2 and/or C-3 in androgens. Interestingly, the selenium and tellurium isosteres in the same series were found to



(**70**)

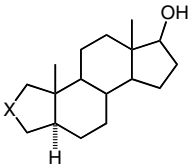


(**71**)

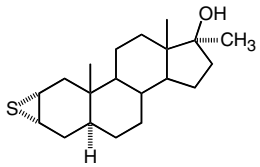


(**72**)

have good androgenic activity. Moreover, experiments with a ^{75} Se-labeled analog have shown (**74**) to selectively bind with the specific cytosol receptor for DHT in rat prostate.



(**73**) X = S
(**74**) X = Se

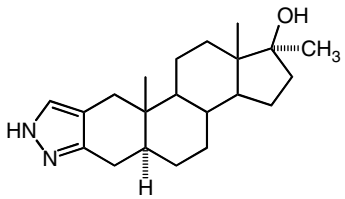


(**75**)

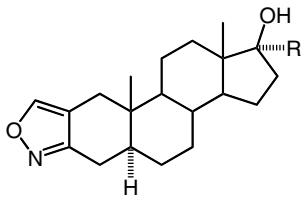
The high biological activity noted for the 3-deoxy androstanes prompted numerous investigators to fuse various systems to the A-ring. The simplest such changes were 2,3-epoxy, 2,3-cyclopropano, and 2,3-epithioandrostanes. The 2α,3α-cyclopropano-5α-androstan-17β-ol was as active as testosterone propionate as an anabolic agent. While the epoxides had little or no biological activity, certain of the episulfides possessed pronounced anabolic/androgenic activity. For example, 2,3α-epithio-17α-methyl-5α-androstan17β-ol (**75**) was found to have approximately equal androgenic and 11 times the anabolic activity of methyltestosterone after oral administration to rats. The 2,3-β-episulfide, on the other hand, was much less active. 2,3α-Epithio-5α-androstan17β-ol has been shown to have long-acting antiestrogenic activity, as well as some beneficial effects in the treatment of mammary carcinoma.

Other heterocyclic androstane derivatives have included the pyrazoles. Thus, 17β-hydroxy-17α-methylandrostano[3,2-*c*] pyrazole (stanozolol, **76**) was 10 times as active as 17α-methyltestosterone in improving nitrogen retention in rats. The myotrophic activity, however, was only twice that of 17α-methyltestosterone. Stanozolol at a dose of 6 mg day^{-1} produced an adequate anabolic response with no lasting adverse side effects.

The high activity of the pyrazoles instigated the synthesis of other heterocyclic fused androstane derivatives including isoxazoles, thiazoles, pyridines, pyrimidines, pteridines, oxadiazoles, pyrroles, indoles, and triazoles. One of the most potent was 17α-methylandrostan-17β-ol[2,3-*d*]isoxazole (androisoxazol, **77**), which exhibited an oral anabolic-to-androgenic ratio of 40. The corresponding 17α-ethynyl analog (danazol, **78**) has been of most interest clinically. This compound has impeded androgenic activity and inhibits pituitary gonadotropin secretion. Since it depresses blood levels of androgens and gonadotropins, it has been studied as an antifertility agent in males. At doses of 200 or 600 mg daily, danazol lowered plasma testosterone and androstenedione levels, and this effect was dose related. In addition to an inhibition in gonadotropin release, a direct inhibition of Leydig cell



(**76**)



(**77**) R = H
(**78**) R = C≡CH

androgen synthesis was observed. Other studies have shown danazol to be effective for the treatment of endometriosis, benign fibrocystic mastitis, and precocious puberty. Several reports have appeared relating to its disposition and metabolic fate.

5.3.7 Esters and Ethers

Since esterification of testosterone markedly prolonged its activity, it was only natural that this approach to drug latentiation would be extended to the anabolic steroids. The acyl moiety is usually derived from a long-chain aliphatic or arylaliphatic acid such as heptanoic (enanthoic), decanoic, cyclopentylpropionic, and β-phenylpropionic. For example, no less than 12 esters of 19-nortestosterone (nandrolone) have been used clinically as long-acting anabolic agents.

In the case of nandrolone, the duration of action and the anabolic-to-androgenic ratio increased with the chain length of the ester group. The decanoate and laurate esters, for instance, were active 6 weeks after injection. Clinically, nandrolone decanoate appeared to be the most practical, since a dose of 25 to 100 mg/week produced marked nitrogen retention.

Since the 17α-alkyl group has been implicated as the cause of the hepatotoxic side effects of oral preparations, the effect of esterification on oral efficacy has attracted attention. For example, esterification of DHT with short chain fatty acids resulted in oral anabolic and androgenic activity in rats. Moreover, esters of methenolone possessed appreciable oral anabolic activity. Unfortunately, follow-up studies in humans have not been reported.

The manner in which the steroid esters evoke their enhanced activity and increased duration of action has puzzled investigators for many years. The classical concept has been that esterification delays the absorption rate of the steroid from the site of injection, thus preventing its rapid destruction. Other factors must be involved, however, since the potency and prolongation of action vary markedly with the nature of the esterifying acid.

The effect of various aliphatic esters of testosterone on rat prostate and seminal vesicles were studied and correlated androgenicity with lipophilicity and rate of ester hydrolysis by liver esterase. The peak androgenic response was observed with the butyrate ester, which was also the most readily hydrolyzed. The more lipophilic valerate ester was slightly less androgenic in a quantitative sense, but its action was longer lasting. It was concluded that the ease of hydrolysis controls the weight of the target organs, whereas lipophilicity was responsible for the duration of androgenic effect. These results explain the low androgenic activity previously noted for hindered trimethylacetate (pivalate) esters, which would be expected to be resistant to *in vivo* hydrolysis.

The effect of etherification on anabolic or androgenic activity has been studied less rigorously. Replacement of the 17β-OH with 17β-OCH_3 markedly reduced androgenic activity but did not greatly affect the ability to counteract cortisone-induced adrenal atrophy in male rats. A series of 17β-acetals, alkyl ethers, and 3-enol ethers, however, showed significant activity when given orally. The cyclohexyl enol ether of 17α-methyltestosterone, for example, was orally 5 times as myotrophic as 17α-methyltestosterone.

Other ethers such as the tetrahydropyranol and trimethylsilyl have oral anabolic and androgenic activity in animals. The

trimethylsilyl ether of testosterone (silandrone) had protracted activity following injection and orally had twice the anabolic and androgenic activities of 17α-methyltestosterone.

5.3.8 Summary of Structure–Activity Relationships

Synthetic modifications of C_{19} steroids have resulted in the enhancement of anabolic activity, even though a pure synthetic anabolic agent that retains no androgenic activity has not been accomplished. Structural changes in two regions of the testosterone molecule have resulted in the greatest enhancement of the anabolic/androgenic ratio. The first region is the C-17 position of the testosterone molecule. Introduction of the 17α-alkyl functionality, such as a 17α-methyl or a 17α-ethyl group, greatly increases the metabolic stability of the anabolic and decreases *in vivo* conversion of the 17β-alcohol to the 17-ketone by 17β-hydroxysteroid dehydrogenases. In addition, esterification of the 17β-alcohol enhances the lipid solubility of the steroids and provides injectable preparations for depot therapy.

The A-ring of testosterone is the second region in which structural modifications can be made to increase anabolic activity. Removal of the C-19 methyl group results in the 19-nortestosterone analogs, which have slightly higher anabolic activity. A major impact on the structure–activity relationships of anabolics can be observed with modifications at the C-2 position. Bioisosteric replacement of the carbon atom at position 2 with an oxygen provides a threefold increase in anabolic activity, as is seen with oxandrolone. Finally, the greatest effects were observed with the addition of heterocyclic rings fused at positions 2 and 3 of the A-ring. The two heterocycles that have lead to the greatest changes are the pyrazole and the isoxazole rings, as seen in stanozolol and androisoxazole, respectively. In these anabolics, the 3-ketone of testosterone is replaced by the bioisosteric 3-imine. Stanozolol, which contains the pyrazole ring at C-2 and C-3, shows the greatest increases when compared to testosterone. Table 9 compares the anabolic activities of nitrogen retention and myotrophic activity for several common anabolics. Figure 10 contains a summary of the structure–activity relationships for anabolic agents.

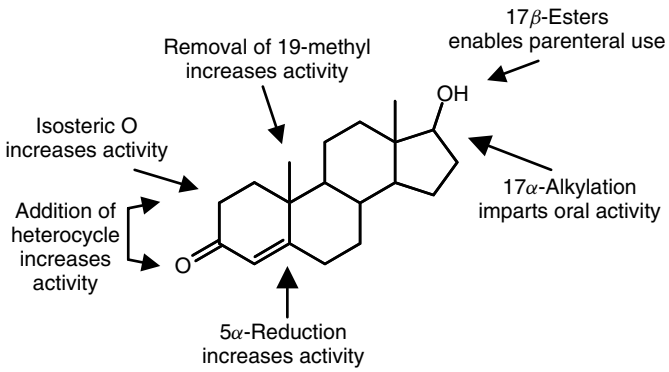


Fig. 10 Summary of structure–activity relationships for anabolic agents

Tab. 9 Comparison of anabolic activities.

Compound	*Number*	*Trade names*	*Anabolic activity*	
			Nitrogen retention	*Myotrophic activity*
Testosterone	(**1**)	Android-T Malestrone Oreton Primotest Virosterone	1.0	1.0
19-Nortestosterone Nandrolone	(**43**)	Nerobolil Nortestonate	0.8	1.0
Normethandrone	(**44**)	Methalutin Orgasteron	4.0	4.5
Norethandrolone	(**45**)	Nilevar Solevar	3.9	4.0
Methandrostenolone Methandienone	(**48**)	Danabol Dianabol Nabolin Nerobil	0.6	1.4
Drostanolone	(**56**)	Drolban Masterone	–	1.3
Oxymetholone	(**63**)	Adroyd Anadrol Anadroyd Anapolon Anasterone Nastenon Protanabol Synasteron	2.75	2.8
Oxandrolone	(**65**)	Anavar Provita	3.0	3.0
Estrenol	(**70**)	Duraboral-O Maxibolin Orabolin Orgaboral Orgabolin	1.7	2.0
Stanozolol	(**75**)	Stanozol Winstrol Tevabolin	10.0	7.5
Androisoxazole	(**76**)	Androxan Neo-ponden	1.5	1.7

5.4 Absorption, Distribution, and Metabolism

The absorption, distribution, and metabolism of the various anabolic steroids is quite similar to those pharmacokinetic properties of the endogenous and synthetic androgens discussed earlier in the chapter. Again, lipid solubility is critical for the absorption of these agents following oral or parenteral administration. The 17α-methyl group retards the metabolism of the compounds and provides orally active agents. Other anabolics such as methenolone are orally active without a 17α-substituent, indicating that these steroids are poor substrates for 17α-hydroxysteroid dehydrogenase. Reduction of the 4-en-3-one system in synthetic anabolics to give the various α- and β-isomers occurs *in vivo*. The 3-deoxy agent 17α-methyl-5α-androstan-17α-ol was shown to be extensively converted to the 3-keto derivative by liver homogenate preparations. The metabolic fates of stanozolol and danazol have been reported, with the major metabolites being heterocycle-ring opened derivatives and their deaminated products. Finally, both the unchanged anabolics and their metabolites are primarily excreted in the urine as the glucuronide or sulfate conjugates.

5.5 Toxicities

The major side effect of the anabolic steroids is the residual androgenic activity of the molecules. The virilizing actions are undesirable in adult males as well as in females and children. In addition, many anabolic steroids can suppress the release of gonadotropins from the anterior pituitary and lead to lower levels of circulating hormones and potential reproductive problems. Headaches, acne, and elevated blood pressure are common in individuals taking anabolics. The salt and water retention induced by these agents can produce edema.

The most serious toxicities resulting from the use of anabolic steroids are subsequent liver damages. Liver damage including jaundice and cholestasis can occur after use of the 17α-alkylated C_{19} steroids. Also, individuals who have received anabolic agents over an extended period have developed hepatic adenocarcinomas. Such clinical reports serve to underscore the inherent risks associated with anabolic steroid use in amateur athletes for no demonstrable benefits.

6 Androgen Antagonists

A majority of the recent research efforts in the area of androgens has concentrated on the preparation and biological activities of androgen antagonists. An androgen antagonist is defined as a substance that antagonizes the actions of testosterone in various androgen-sensitive target organs and, when administered with an androgen, blocks or diminishes the effectiveness of the androgen at various androgen-sensitive tissues. Androgen antagonists may act to block the action of testosterone at several possible sites. First, such compounds could interfere with the entrance of the androgen into the target cell. A second site of action of androgen antagonists may be to block the conversion of testosterone to its more active metabolite dihydrotestosterone or DHT. Third, competition for the high-affinity binding sites on the androgen receptor molecule may account for antiandrogenic effects. Finally, certain agents such as LHRH agonists can act in the

pituitary to lower gonadotropin secretion via receptor downregulation and thus diminish the production of testosterone by the testis. The substances described in this section act through at least one of these mechanisms.

6.1 Current Drugs on the Market

See Table 10.

6.2 Antiandrogens

6.2.1 Therapeutic Uses

Antiandrogens are agents that compete with endogenous androgens for the hormone binding site on the androgen receptor. These agents have therapeutic potential in the treatment of acne, virilization in women, hyperplasia and neoplasia of the prostate, baldness and male contraception, and clinical studies have demonstrated the potential therapeutic benefits of the antiandrogens. The applications of antiandrogens for the treatment of prostatic carcinoma and for the treatment of BPH have also been investigated. Antiandrogens are effective for the treatment of prostate cancer when combined with androgen ablation, such as surgical (orchiectomy) or medical (LHRH agonist) castration.

6.2.2 Structure–Activity Relationships for Antiandrogens

6.2.2.1 **Steroidal agents.** Several steroidal and nonsteroidal compounds with demonstrated antiandrogenic activity have been utilized clinically. The first compounds used as antiandrogens were the estrogens and progestins. Steroidal estrogens and diethylstilbesterol are used in the treatment of prostatic carcinoma and exert their action via suppression of the release of pituitary gonadotropins. Progestational compounds have also been utilized for antiandrogenic actions with limited success. The inherent hormonal activities of these compounds and the development of more selective antiandrogens have limited the clinical applications of estrogens and progestins as antiandrogens.

A modified progestin that is a potent antiandrogen and has minimal progestational activity is the agent cyproterone acetate (**79**). This compound was originally prepared in search of orally active

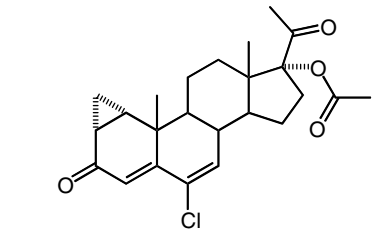
Tab. 10

Generic name (structure)	*Trade name*	*US manufacturer*	*Chemical class*	*Dose*
Antiandrogens				
Flutamide (**91**)	Eulexin	Schering-Plough	Nonsteroidal	Tablets: 125 mg
Bicalutamide (**97**)	Casodex	AstraZeneca	Nonsteroidal	Tablets: 50 mg
Nilutamide (**93**)	Nilandron	Aventis	Nonsteroidal	Tablets: 50 mg 150 mg
Cyproterone acetate (**78**)	Androcur	Schering AG	Pregnane	
5α-Reductase inhibitors				
Finasteride (**104**)	Proscar	Merck	Androstane	Tablets: 5 mg
	Propecia	Merck	Androstane	Tablets: 1 mg

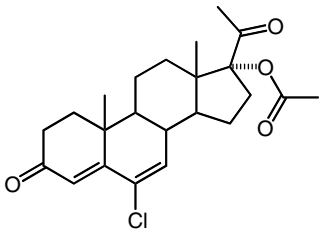
progestins, but was quickly recognized for its ability to suppress gonadotropin release. It was later demonstrated that this compound also bound with high affinity to the androgen receptor and thus competed with DHT for the binding site. Cyproterone acetate has received the most clinical attention in antiandrogen therapy. Cyproterone acetate has produced quite satisfactory results in the treatment of acne, seborrhea, and hirsutism. Therapeutic effectiveness of this agent in the treatment of prostatic carcinoma has been reported. Cyproterone acetate was reported to be a good alternative to estrogens for the treatment of prostate cancer when combined with androgen ablation. However, this combination did not improve disease-free survival or overall survival when compared to castration alone.

Other pregnane compounds that exhibit antiandrogenic actions by binding to the androgen receptor are chlormadinone acetate (**80**), medroxyprogesterone acetate (**81**), medrogesterone (**82**), A-norprogesterone (**83**) and gestonorone capronate (**84**). In addition, medrogesterone exerts antiandrogenic effects by inhibiting 5α-reductase and thus preventing the formation of DHT. Gestonorone capronate interferes with the uptake process in target cells.

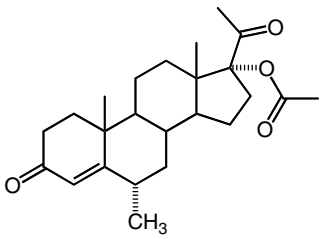
Several androstane derivatives demonstrate antiandrogenic properties. 17α-Methyl-B-nortestosterone (**85**) was prepared and first tested for antihormonal



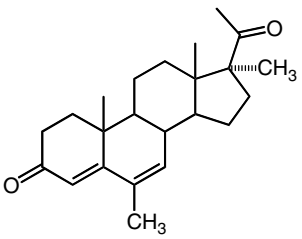
(**79**)



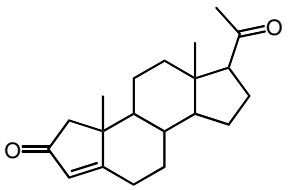
(**80**)



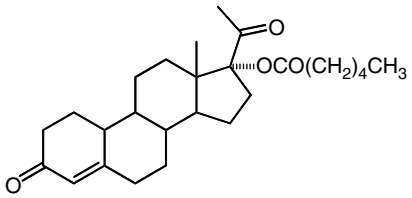
(**81**)



(**82**)



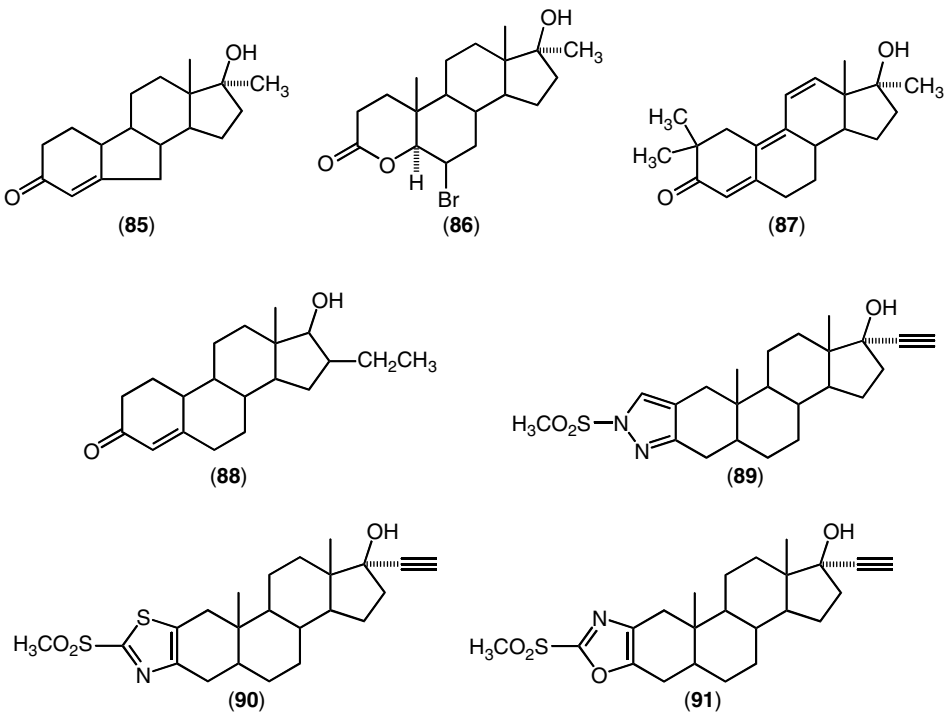
(**83**)

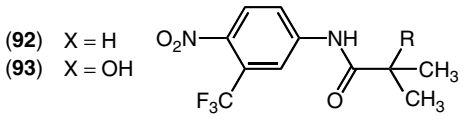


(**84**)

activity. Several other androstane analogs were found to possess antiandrogenic activity, including BOMT (**86**), R2956 (**87**), and oxendolone (**88**). As expected, the mechanism of antiandrogenic action of these synthetic steroids is the competition with androgens for the binding sites on the receptor molecule. Numerous A- and B-ring modified steroids were examined for antiandrogenic activity and the ability to bind to the androgen receptor, demonstrating that the structural requirements of receptor binding site can accommodate some degree of flexibility in the A- and/or B-rings of antiandrogenic molecules. Heterocyclic-substituted A-ring antiandrogens such as zanoterone (WIN 49,596) (**89**) further support these conclusions on the structure-activity relationships of steroidal antiandrogens. Additional A-ring heterocycles identified as novel antiandrogens are the thiazole (**90**) and oxazole (**91**). The optimal substitutions on the A-ring heterocyclic androstanes for *in vivo* antiandrogenic activity are the methylsulfonyl group at the N-1′ position and a 17α-substituent (e.g. 17α-methyl or 17α-ethinyl).

6.2.2.2 **Nonsteroidal agents.** The absolute requirement of a steroidal compound for interaction with the androgen receptor was invalidated when the potent nonsteroidal antiandrogen flutamide (Eulexin, **92**) was introduced. Subsequent receptor studies demonstrated that this compound competed with DHT for the binding sites. The side chain of flutamide allows sufficient flexibility for the molecule to assume a structure similar to an androgen. In addition, a hydroxylated metabolite (**93**) has been identified, which is a more

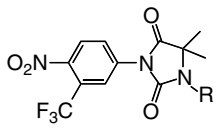




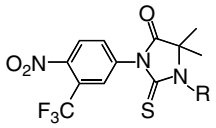
powerful antiandrogen *in vivo*, and has a higher affinity for the receptor than the parent compound. Important factors in the structure–activity relationships of flutamide and analogs are the presence of an electron-deficient aromatic ring and a powerful hydrogen-bond donor group.

Flutamide has been extensively evaluated for the treatment of prostate cancer. Large double-blind studies in prostate cancer patients using a combination of flutamide with an LHRH agonist (as a medical castration) resulted in an increased number of favorable responses and increased overall survival when compared to an LHRH agonist or surgical castration.

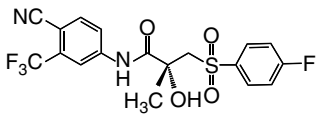
Nilutamide (Anandron, **94**), and related nilutamide analogs (**95**–**97**), and bicalutamide (Casodex, **98**) are other nonsteroidal antiandrogens with a similar electron-deficient aromatic ring and



(**94**) R = H
(**95**) R = $(CH_2)_4OH$



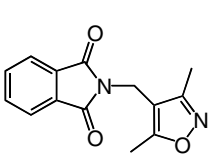
(**96**) R = CH_3
(**97**) R = $(CH_2)_4OH$



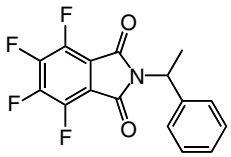
(**98**)

have been shown to interact with the androgen receptor to varying degrees. Nilutamide and bicalutamide are pure antiandrogens and are effective in suppressing testosterone-stimulated cell proliferation. Both nilutamide and bicalutamide have demonstrated effectiveness against prostate cancer.

Other aryl substituted nonsteroidal compounds have also been identified as antiandrogens. DIMP (**99**) is a phthalimide derivative that showed weak affinity for the androgen receptor and poor *in vivo* activity. A series of tetrafluorophthalimides such as (**100**) demonstrated moderate activity as antiandrogens in cell proliferation assays.



DIMP (**99**)



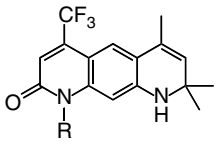
(**100**)

6.2.3 Selective Androgen Receptor Modulators (SARMs)

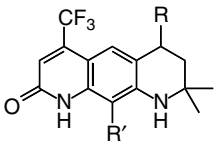
Current research efforts in the medicinal chemistry and pharmacology of androgens and antiandrogens have focused on the development of ligands that produce tissue-selective interactions with the androgen receptor. These ligands, referred to as *selective androgen receptor modulators* (SARMs), may act as antagonists or weak agonists in one tissue but act as strong agonists in another tissue type. Such SARMs would theoretically enable effective treatment of certain disorders affecting bone or muscle with little or no effect on the prostate.

A series of 1,2-dihydropyridono[5,6-*g*]quinolines were identified as novel nonsteroidal antiandrogens on the basis of a cell-based screening approach. Several analogs (**101**–**104**) demonstrated excellent *in vivo* activity, reducing rat ventral prostate weight without affecting serum

gonadotropins and serum testosterone levels.



(**101**) R = H
(**102**) R = CH_3



(**103**) R,R′ = H
(**104**) R,R′ = CH_3

Structural modifications of the antiandrogen bicalutamide have resulted in molecules (**105**–**107**) that produced weak partial agonist activities in the prostate and seminal vesicles (androgen-responsive tissues) and full agonist activity in the levitor ani muscle (anabolic effect) in male rats. Furthermore, these agents did not affect serum gonadotropins and serum testosterone levels, again emphasizing the tissue selectivity for their actions.

6.2.4 Absorption, Distribution, and Metabolism

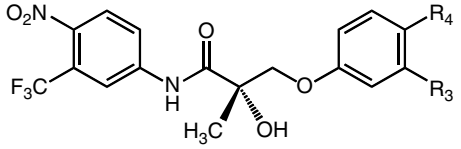
The steroidal antiandrogens exhibit similar pharmacokinetic properties to the androgens and anabolic agents. The lipophilicity of the compounds influences absorption both orally and from injection sites. Reduction of the 3-ketone and 4,5 double bond are common routes of metabolism. An unusual metabolite of cyproterone acetate, 15α-hydroxycyproterone acetate, was isolated and identified in both animals and man. The nonsteroidal antiandrogen flutamide is rapidly absorbed and extensively metabolized *in vivo*. As described earlier, the hydroxy metabolite (**93**) of flutamide is a more potent antiandrogen. The major metabolite of bicalutamide is the sulfone, which has comparable *in vivo* activity. Finally, the antiandrogens are primarily excreted as the glucuronide and sulfate conjugates in the urine.

6.2.5 Toxicities

Side effects of these agents have been identified from various clinical trials. Testicular atrophy and decreased spermatogenesis have been observed during treatment with cyproterone acetate. Certain antiandrogens such as cyproterone and medrogesterone also exhibit inherent progestational activity, suppress corticotropin release, and have some androgenic effects. No hormonal activities were observed for the nonsteroidal antiandrogens, such as flutamide. On the other hand, many nonsteroidal antiandrogens exhibit other endocrine side effects, such as elevated serum gonadotropins and serum testosterone levels. Gynecomastia, nausea, diarrhea, and liver toxicities have been observed in patients on nonsteroidal antiandrogens. Also, resistance to antiandrogen therapy has been observed in prostate cancer patients.

6.3 Enzyme Inhibitors

Enzymes involved in the biosynthesis and metabolism of testosterone are attractive targets for drug design and drug development. Suppression of the synthesis

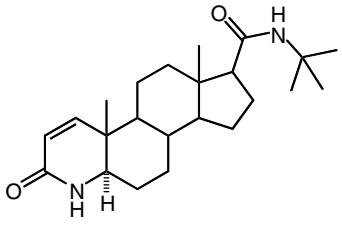


(**105**) R_3 = H, R_4 = F
(**106**) R_3 = H, R_4 = NHC(O)CH_3
(**107**) R_3 = F, R_4 = Cl

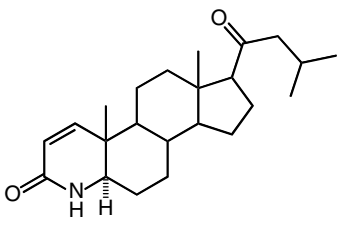
of androgenic hormones and androgen precursors is a viable therapeutic approach for the treatment of various androgen-mediated disease processes and an important endocrine treatment for prostate cancer. Potent inhibition of type 2 5α-reductase in androgen target tissues and the resultant decrease in DHT levels will provide selective interference with androgen action within those target tissues and no alterations of other effects produced by testosterone, other structurally related steroids, and other hormones such as corticoids and progesterone. The cytochrome $P450_{17\alpha}$ enzyme complex displays two enzymatic activities – 17α-hydroxylation to produce 17α-hydroxysteroids and $C_{17}-C_{20}$ bond cleavage (17,20-lyase activity) to produce androgens. In the male, this enzyme is found in both testicular and adrenal tissues, with these organs providing circulating androgens in the blood. Effective inhibition of this microsomal enzyme complex would eliminate both testicular and adrenal androgens and remove the growth stimulus to androgen-dependent prostate carcinoma. Synthetic androgen analogs that inhibit the oxidative metabolism of androgens testosterone and androstenedione to estrogens estradiol and estrone can serve as potential therapeutic agents for controlling estrogen-dependent diseases such as hormone-dependent breast cancer.

6.3.1 5α-Reductase Inhibitors

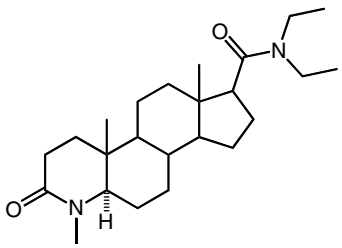
The most extensively studied class of 5α-reductase inhibitors is the 4-azasteroids, which includes the drug finasteride (Proscar, **108**). Finasteride is the first 5α-reductase inhibitor approved in the United States for the treatment of BPH. This drug has approximately a 100-fold greater affinity for type 2 5α-reductase than for the type 1 enzyme, demonstrating an IC_{50} value of 4.2 nM for type 2 5α-reductase. In humans, finasteride decreases prostatic DHT levels by 70 to 90% and reduces prostate size, while testosterone tissue levels increased. Clinical trials demonstrated sustained improvement in BPH disease and reduction in PSA levels. Related analogs (**109–111**)



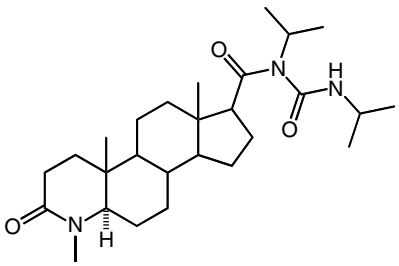
Finasteride (**108**)



(**109**)



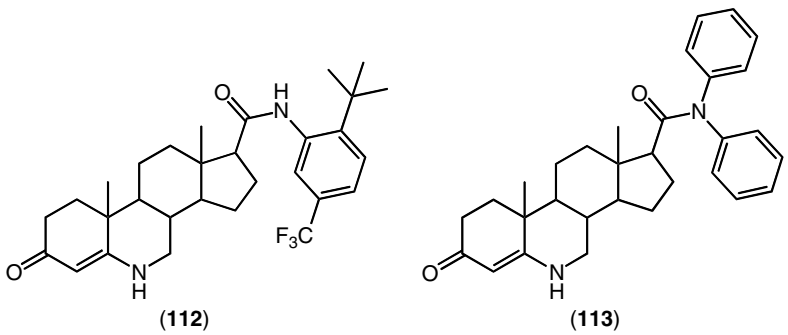
(**110**)



(**111**)

have also demonstrated effectiveness *in vitro* and *in vivo*. These agents were originally designed to mimic the putative 3-enolate intermediate of testosterone and serve as transition-state inhibitors. Subsequently, finasteride was shown to produce time-dependent enzyme inactivation and function as a mechanism-based inactivator. The structure–activity relationships for the 4-azasteroids illustrate the stringent requirements for inhibition of human type 2 5α-reductase. The 5α-reduced azasteroids are preferred, a 1,2-double bond can be tolerated, and the nitrogen can be substituted with only hydrogen or small lipophilic groups. Lipophilic amides or ketones are preferred as substituents at the C-17β position.

Several 6-azasteroids, such as **(112)** and**(113)**, were prepared as extended mimics of the enolate transition state and have also demonstrated potent inhibition of 5α-reductase. The 6-azasteroids are more effective inhibitors of type 2 5α-reductase, but some analogs also exhibit good inhibition of type 2 5α-reductase. Alkylation of the nitrogen can be tolerated; however, a 1,2-double bond decreases inhibitory activity in this series. The best inhibitors contain large lipophilic substituents at the C-17β position. Figure 11 contains a summary



(**112**)

(**113**)

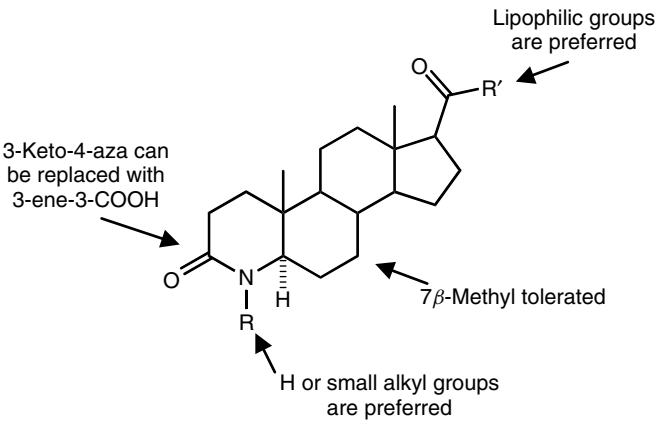
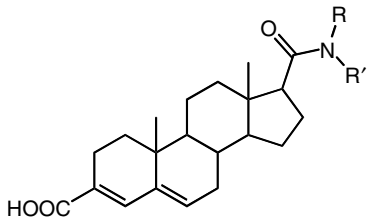
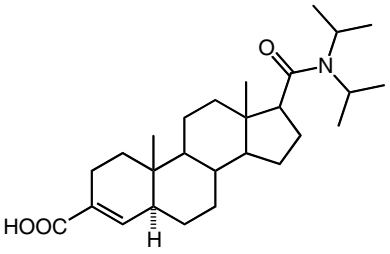


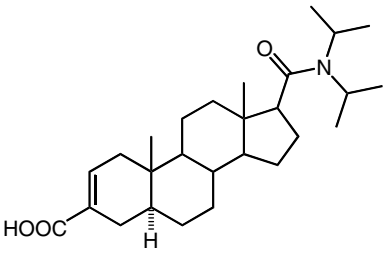
Fig. 11 Summary of structure–activity relationships for 5α-reductase inhibitors.



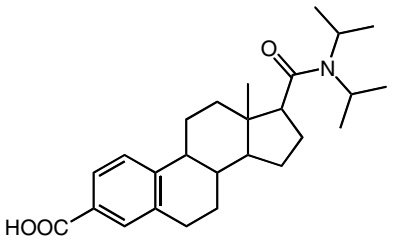
(**114**) R = H; R′ = $C(CH_3)_3$
(**115**) R = R′ = $CH(CH_3)_2$



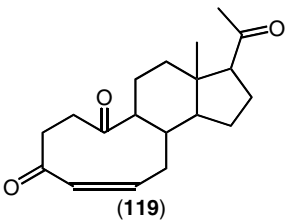
(**116**)



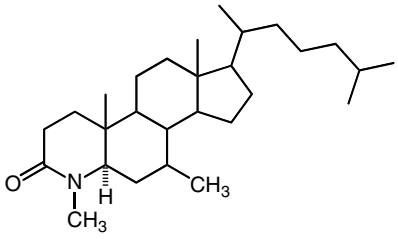
(**117**)



(**118**)



(**119**)

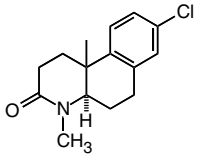


(**120**)

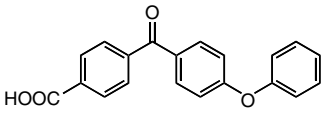
of the structure–activity relationships for steroidal 5α-reductase inhibitors.

Androstadiene 3-carboxylic acids **(114)** and **(115)** were recently designed as transition-state inhibitors and have demonstrated potent uncompetitive inhibition of type 2 5α-reductase. Epristeride (SK&F 105,657, **114**) has demonstrated the ability to lower serum DHT levels by 50% in clinical trials. Other analogs with acidic functionalities at the C-3 position include other androstene carboxylic acids (**116, 117**) and estratriene carboxylic acids (**118**). The allenic secosteroid (**119**) has demonstrated potent irreversible inhibitor of 5α-reductase, even though it was originally developed as an irreversible inhibitor of 3β-hydroxysteroid dehydrogenase/$\Delta^{4,5}$-isomerase. Finally, selective and potent inhibitors of type 1 5α-reductase were developed on the basis of the 4-azacholestane MK-386 (**120**).

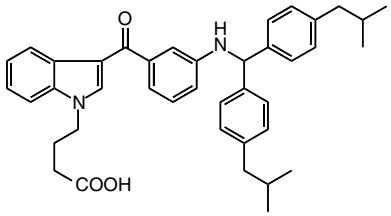
Several nonsteroidal 5α-reductase inhibitors have been developed on the basis of the azasteroid molecule or from high-throughput screening methods. Examples of these nonsteroidal inhibitors include the benzoquinolinone (**121**), an aryl carboxylic acid (**122**), and FK143 (**123**).



(**121**)



(**122**)

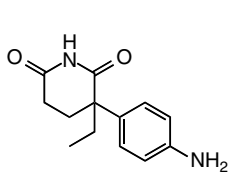


FK143 (**123**)

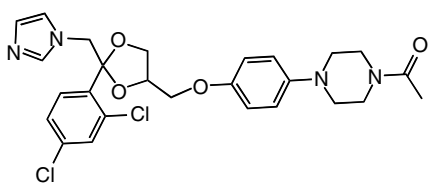
6.3.2 17,20-Lyase Inhibitors

Both nonsteroidal and steroidal agents have been examined as inhibitors of 17α-hydroxylase/17,20-lyase. The nonsteroidal agents studied most extensively are aminoglutethimide (**124**) and ketoconazole (**125**), both *in vitro* and in clinical trials. Objective response rates for treatment of prostate cancer in relapsed patients were observed with high doses of aminoglutethimide and high doses of ketoconazole, but both agents produce frequent side effects. A third nonsteroidal agent that has received extensive preclinical evaluation is the benzimidazole analog, liarozole (**126**), which produced reduction in plasma testosterone and androstenedione levels *in vivo*. Other nonsteroidal agents reported to exhibit 17α-hydroxylase/17,20-lyase inhibitory activity *in vitro* include other imidazole analogs, nicotine, bifluranol analogs, and pyridylacetic acid esters. In general, high doses of nonsteroidal agents are needed to produce significant *in vitro or in vivo* activity. Another potential problem with these agents is nonspecific inhibition of other cytochrome P450 enzymes involved in either steroidogenesis or liver metabolism.

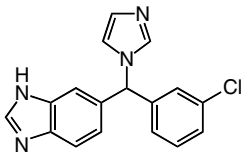
A few studies of steroidal inhibitors of 17α-hydroxylase/17,20-lyase have been reported. An extensive analysis of the specificity of steroid binding to testicular microsomal cytochrome P450 identified several steroids exhibiting binding affinity. One of these, promegestrone (**127**), has been utilized in kinetic analysis of purified cytochrome $P450_{17\alpha}$. An affinity label inhibitor, 17-bromoacetoxyprogesterone (**128**), alkylates a unique cysteine residue on purified cytochrome $P450_{17\alpha}$. Potential mechanism-based inhibitors include 17β-(cyclopropylamino)-5-androsten-3β-ol (**129**) and 17β-vinylprogesterone (**130**). To date, all of these inhibitors exhibit apparent K_i's in the micromolar (μM) range, while the apparent K_m for progesterone is 140 nM. The 17β-aziridinyl analog (**131**) and 17β-pyridyl derivative (**132**) also exhibited similar inhibitory activity.



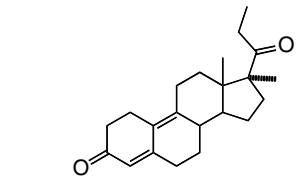
Aminoglutethimide (**124**)



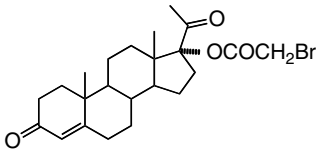
Ketoconazole (**125**)



Liarozole (**126**)



(**127**)



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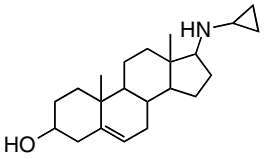

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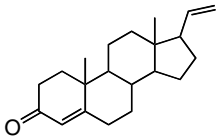

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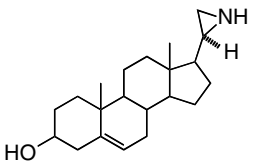

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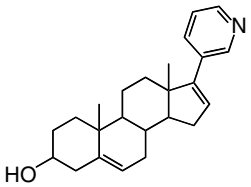


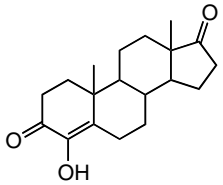
(**132**)

6.3.3 C_{19} Steroids as Aromatase Inhibitors

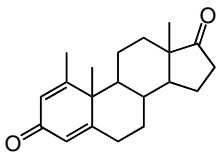
Aromatase is the enzyme complex that catalyzes the conversion of androgens into the estrogens. This enzymatic process is the rate-limiting step in estrogen biosynthesis and converts C_{19} steroids, such as testosterone and androstenedione, into the C_{18} estrogens, estradiol, and estrone, respectively. Inhibition of aromatase has been an attractive approach for examining the roles of estrogen biosynthesis in various physiological or pathological processes. Furthermore, effective aromatase inhibitors can serve as potential therapeutic agents for controlling estrogen-dependent diseases such as hormone-dependent breast cancer. Investigations on the development of aromatase inhibitors began in the 1970s and have expanded greatly in the past two decades.

Steroidal inhibitors that have been developed to date build upon the basic androstenedione nucleus and incorporate chemical substitutents at varying positions on the steroid. These inhibitors bind to the aromatase cytochrome P450 enzyme in the same manner as the substrate androstenedione. Even though the steroidal aromatase inhibitors are C_{19} steroids, these agents exhibit no significant androgenic activity. A limited number of effective inhibitors with substituents on the A-ring have been reported. Several steroidal aromatase inhibitors contain modifications at the C-4 position, with 4-hydroxyandrostenedione (**133**) (4-OHA; formestane) being the prototype agent. Initially, 4-OHA was thought to be a competitive inhibitor, but was later demonstrated to produce enzyme-mediated inactivation. *In vivo*, 4-OHA inhibits reproductive process and causes regression of hormone-dependent mammary rat tumors. 4-OHA is effective in the treatment of advanced breast cancer

in postmenopausal women, and this drug is approved in the United Kingdom for breast cancer therapy. Thus, the spacial requirements of the A-ring for binding of the steroidal inhibitor to aromatase are rather restrictive, permitting only small structural modifications to be made. Incorporation of the polar hydroxyl group at C-4 enhances inhibitory activity. 1-Methyl-1,4-androstadiene-3,17-dione **(134)** is a potent inhibitor of aromatase *in vitro* and *in vivo*; on the other hand, bulky substitutents at the 1α-position are poor inhibitors. At the C-3 position, replacement of the ketone with a methylene provided effective inhibitors.



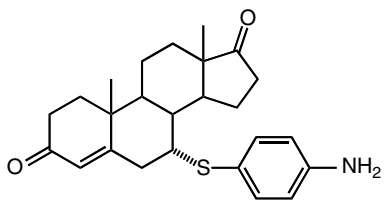
(**133**)



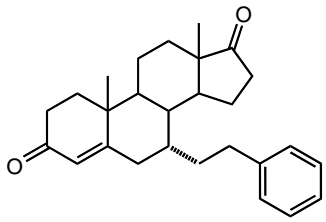
(**134**)

More extensive structural modifications may be made on the B-ring of the steroid nucleus. Bulky substitutions at the C-7 position of the B-ring have provided several very potent aromatase inhibitors. 7α-(4′-Amino)phenylthio-4-androstene-3,17-dione (**135**) (7α-APTA) is a very effective competitive inhibitor, with an apparent K_i of 18 nM. This inhibitor has also demonstrated effectiveness in inhibiting aromatase in cell cultures and in treating hormone-dependent rat mammary tumors. Evaluation of various substituted aromatic analogs of 7α-APTA provided no correlation between the electronic character of the substituents and inhibitory activity. Investigations of various seven-substituted 4,6-androstadiene-3,17-dione derivatives suggest that only those derivatives that can project the 7-aryl substitutent into the 7α pocket are effective inhibitors. More metabolically stable inhibitors were synthesized by replacing the thioether linkage at the 7α-position with a carbon–carbon linkage, such as 7α-phenethylandrost-4-ene-3,17-dione (**136**). Overall, the most effective B-ring modified aromatase inhibitors are those with 7α-aryl derivatives, with several analogs having 2 to 10 times greater affinity for the enzyme than the substrate. These results suggest that additional interactions occur between the phenyl ring at the 7α-position and amino acids at or near the enzymatic site of aromatase, resulting in enhanced affinity.

Numerous modified androstenedione analogs have been developed as effective mechanism-based inhibitors of aromatase. The first compound designed as a mechanism-based inhibitor of aromatase was 10-propargyl-4-estrene-3,17-dione **(137)** (PED; MDL 18,962). MDL 18,962 has an electron-rich alkynyl function on the C-19 carbon atom, the site of aromatase-mediated oxidation of the



(**135**)

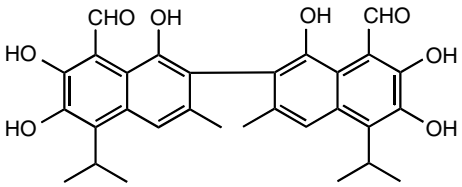


(**136**)

substrate. Although the identity of the reactive intermediate formed is not known, an oxirene and a Michael acceptor have been suggested. This agent is an effective inhibitor *in vitro* and *in vivo*. Other approaches to C-19 substituted mechanism-based inhibitors containing latent chemical groups have provided a limited number of inhibitors. These agents include the difluoromethyl analog **(138)** and a thiol **(139).** Another series of mechanism-based inhibitors have developed from more detailed biochemical investigations of several inhibitors originally thought to be competitive inhibitors. These inhibitors can be grouped into the general categories of 4-substituted androst-4-ene-3,17-diones, such as 4-hydroxyandrostenedione **(133)**, and substituted androsta-1,4-diene-3,17-diones, such as 7α-(4′-amino)-phenylthioandrosta-4,6-diene-3,17-dione (7α-APTADD; **140**), 7α-phen-propylandrosta-1,4-diene-3,17-dione (**141**), and 6-methyleneandrost-4-ene-3,17-dione (exemestane; **142)**. Exemestane (Aromasin) is marketed as second-line therapy for the treatment of breast cancer patients who failed tamoxifen and is effective as first-line therapy in women with advanced breast cancer.

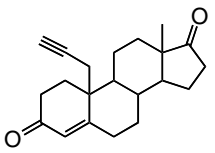
6.3.4 Other Agents

An interesting natural product that lowers circulating androgen levels *in vitro* is the nonsteroidal agent gossypol. Gossypol, (2,2′-binaphthalene)-8,8′-dicarboxaldehyde-1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl **(143)** is a

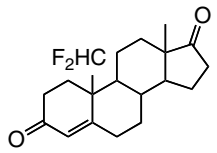


(**143**)

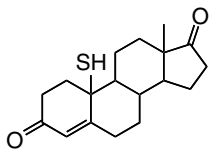
polyphenolic compound contained in the pigment of cottonseed. This natural product has been used in fertile men in China as an effective male contraceptive agent for many years; its antifertility effects on reproductive endocrine tissues are observed at 1000-fold lower doses than its toxic effects in other tissues. Gossypol has been shown to disrupt spermatogenesis by inhibiting lactate dehydrogenase-X (LDH-X), to interfere with steroidogenesis in testicular Leydig cells, and to hinder the function of primary cultures of Leydig and Sertoli



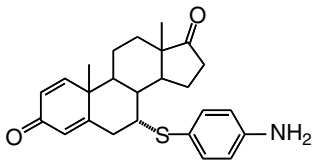
(**137**)



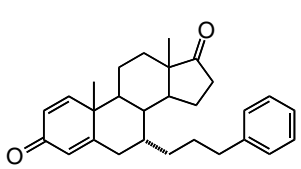
(**138**)



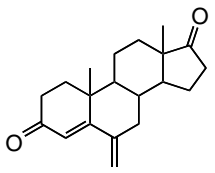
(**139**)



(**140**)



(**141**)



(**142**)

cells. In addition, gossypol is also capable of altering steroid biosynthesis in the female reproductive systems. The antisteroidogenic effect of gossypol in cultured bovine luteal cells involves suppression of the activities of adenylate cyclase and 3β-hydroxysteroid dehydrogenase (3β-HSD). Reproductive endocrine tissues are also sensitive to metabolites of gossypol, such as gossypolone. Gossypolone, a major metabolite formed by gossypol oxidation, inhibits both 3β-HSD and cytochrome P450scc activities in cultured bovine luteal cells and suppresses adrenocorticotropic hormone-induced corticosterone secretion in cultured rat adrenocortical cells at a similar potency as gossypol. Gossypol metabolites have also demonstrated inhibitory action on hCG-induced testosterone production in young male rats. The major side effects of gossypol therapy fatigue, gastrointestinal upset, weakness, and hypokalemia thus limit its therapeutic usefulness.

7 Summary

The steroid testosterone is the major circulating sex hormone of the male and serves as the prototype for the androgens, the anabolic agents, and androgen antagonists. The endogenous androgens are biosynthesized from cholesterol in various tissues in the body; the majority of the circulating androgens are made in the testes under the stimulation of the gonadotropin LH. A critical aspect of testosterone and its biochemistry is that this steroid is converted in various cells to other active steroidal agents. The reduction of testosterone to dihydrotestosterone is necessary for the androgenic actions of testosterone in androgen target tissues such as the prostate. On the other hand, oxidation of testosterone by the enzyme aromatase to yield the estrogens is crucial for certain CNS actions. Investigations of these enzymatic conversions of circulating testosterone continue to be a fruitful area of biochemical research on the roles of the steroid hormones in the body. Additionally, the elucidation of the mechanism of action of the androgens in various target tissues receives ongoing emphasis. The androgenic actions of testosterone are due to the binding of DHT to its nuclear receptor, followed by dimerization of the receptor complex and binding to a specific DNA sequence. This binding of the homodimer to the androgen response element leads to gene expression, stimulation of the synthesis of new mRNA, and subsequent protein biosynthesis. Other actions of testosterone, particularly the anabolic actions, appeared to be mediated through a similar nuclear receptor-mediated mechanism. Many of the intricate biochemical events that occur during the action of the androgens in their target cells remain for further clarification. Nevertheless, receptor studies of new agents are an important biological tool in the evaluation of the compounds for later, in-depth pharmacological testing.

The synthetic androgens and anabolics were prepared to impart oral activity to the androgen molecule, to separate the androgenic effects of testosterone from its anabolic effects and to improve upon its biological activities. These research efforts have provided several effective drug preparations for the treatment of various androgen-deficient diseases, for the therapy of diseases characterized by muscle wasting and protein catabolism, for postoperative adjuvant therapy, and for the treatment of certain hormone-dependent cancers. The synthetic anabolics have also resulted in the abuse of these agents in

athletics. Finally, the most recent area of research emphasis is the development of the androgen antagonists, both steroidal and nonsteroidal agents. The two major categories of these antagonists are the antiandrogens, which block interactions of androgens with the androgen receptor, and the inhibitors of androgen biosynthesis and metabolism. Such compounds have therapeutic potential in the treatment of acne, virilization in women, hyperplasia and neoplasia of the prostate, and baldness and for male contraception. The current focus on the development of SARMs enables tissue-selective activation of the androgen receptor, such that these ligands may act as strong agonists in one tissue while being antagonists or weak agonists in another tissue type. A number of androstane derivatives are also being developed as inhibitors of aromatase for the treatment of hormone-dependent breast cancer. Thus, the numerous biological effects of the male sex hormones testosterone and dihydrotestosterone and the varied chemical modifications of the androstane molecule have resulted in the development of effective medicinal agents for the treatment of androgen-related diseases.

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See also Male Reproductive System: Testis Development and Spermatogenesis; Medicinal Chemistry; Receptor Biochemistry.

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