

A STUDY OF THE METABOLISM OF 17 α -METHYLANDROSTANE
DERIVATIVES IN RABBITS

by

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ABSTRACT

Because metabolic reduction of the 4-en-3-one system in steroids occurs readily, 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one and 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one can be expected to be primary metabolites from 17 β -hydroxy-17 α -methyl-4-androsten-3-one. The relationship between the metabolites of 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one, 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one and 17 β -hydroxy-17 α -methyl-4-androsten-3-one was studied to determine how the reduction of the 4-en-3-one influences the orientation of hydroxylation around the steroid skeleton. The study also included 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one, which possesses a second conjugated double bond in ring "A". The previously reported unique biotransformation involving C-17 epimerization (17 β -hydroxy/17 α -methyl to 17 α -hydroxy/17 β -methyl) of 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one in man was investigated in the rabbit using the simpler compounds as chemical models. This research consisted of the isolation and identification of the urinary excretion products of these closely related compounds.

The metabolites in urinary excretion from rabbits orally dosed with 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one, 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one, 17 β -hydroxy-17 α -methyl-4-androsten-3-one and 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one were isolated and identified. The diol metabolites were identified by comparison with the authentic synthesized compounds using physical, chromatographic and spectroscopic methods. The hydroxylated triol metabolites were identified by spectroscopic properties (ir, mass, ¹H-nmr) and confirmed by ¹³C-nmr spectra.

The metabolites obtained from 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one were 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one,

17 α -methyl-5 α -androstan-3 α and 3 β ,17 β -diols together with two compounds hydroxylated at the C-6 α and C-15 α positions: 17 α -methyl-5 α -androstan-3 α ,6 α ,17 β -triol and 17 α -methyl-5 α -androstan-3 β ,15 α ,17 β -triol. Formation of the C-6 hydroxylated derivative demonstrated that the 4,6-enolization of the 4-en-3-one is not a necessary requirement for hydroxylation at the C-6 position of the androstan nucleus in the rabbit.

The metabolites of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one were 17 α -methyl-5 β -androstan-3 α ,17 β -diol together with four hydroxylated compounds at C-1 β , C-12 β , C-16 α and 16 β -positions with all C-3 equatorial alcohols (3 α -OH), 17 α -methyl-5 β -androstan-1 β ,3 α ,17 β -triol, 17 α -methyl-5 β -androstan-3 α ,12 β ,17 β -triol, 17 α -methyl-5 β -androstan-3 α ,16 α ,17 β -triol and 17 α -methyl-5 β -androstan-3 α ,16 β ,17 β -triol. The C-1 and C-12 hydroxylations were unique to the 5 β -compound whereas the C-16 hydroxylated metabolites with the 5 β -configuration were also found from 17 β -hydroxy-17 α -methyl-4-androsten-3-one. The 5 α -compound did not give C-16 hydroxylated metabolites.

The common metabolites of 17 β -hydroxy-17 α -methyl-4-androsten-3-one previously identified and also found in this study were 17 α -methyl-5 α -androstan-3 α and 3 β ,17 β -diols and 17 α -methyl-5 β -androstan-3 α ,17 β -diol together with two metabolites hydroxylated at C-16 α and C-16 β positions, 17 α -methyl-5 β -androstan-3 α ,16 α - and 16 β ,17 β -triols. But a new metabolite hydroxylated at the C-6 α position, 17 α -methyl-5 α -androstan-3 β ,6 α ,17 β -triol, was found in this study. As the C-6 hydroxylated metabolite with a C-3 β -hydroxyl group was not obtained from the 5 α -compound, it must be formed as a consequence of the presence of the unsaturated 4-en-3-one system.

The relationship between the metabolites of 17β -hydroxy- 17α -methyl-4-androsten-3-one and 17β -hydroxy- 17α -methyl- 5α - and 5β -androstan-3-one is discussed. Neither C-6 hydroxylated nor hydroxylated 5α -metabolites from 17β -hydroxy- 17α -methyl-4-androsten-3-one have been found previously.

The metabolites from 17β -hydroxy- 17α -methyl-1,4-androstadien-3-one were 17β -hydroxy- 17α -methyl-1,4-androstadien-3-one, the fully reduced derivatives, 17α -methyl- 5α -androstan-3 α -and-3 β ,17 β -diols, and 17α -methyl- 5β -androstan-3 α ,17 β -diol, the partially reduced and hydroxylated metabolites 16 α -and 16 β ,17 β -dihydroxy- 17α -methyl- 5β -androsten-1-en-3-ones, the mono-hydroxylated metabolites, 6 β and 16 β ,17 β -dihydroxy- 17α -methyl-1,4-androstadien-3-ones, and the dihydroxylated metabolite 6 β ,16 β ,17 β -trihydroxy- 17α -methyl-1,4-androstadien-3-one. C-16 Monohydroxylated and C-6, 16-dihydroxylated metabolites, together with compounds having the ring "A" partially reduced and hydroxylated, have not been previously reported from studies of the metabolism of 17β -hydroxy- 17α -methyl-1,4-androstadien-3-one.

The above studies of the metabolism of four compounds showed no evidence for epimerization at the C-17 position.

Three out of four 3-ketones, 17ξ -hydroxy- 17ξ -methyl- 5α - and 5β -androstan-3-ones (except 17β -hydroxy- 17α -methyl- 5α -androstan-3-one), and eight alcohols, 17ξ -methyl- 5α - and 5β -androstan-3 ξ ,17 ξ -diols were synthesized to use as authentic compounds to compare with the possible metabolites. ^1H -nmr spectra of all twelve compounds were recorded in deuteriochloroform and deuteriopyridine. Pyridine induced chemical shifts are discussed. Characteristic ^{13}C -nmr spectra were recorded.

The following seven of the above twelve compounds have not been previously synthesized: 17α -hydroxy- 17β -methyl- 5α - and 5β -androstan-3-

ones, 17 β -methyl-5 α -androstandane-3 α - and 3 β ,17 α -diols, 17 β -methyl-5 β -androstandane-3 α - and 3 β ,17 α -diols and 17 α -methyl-5 β -androstandane-3 β ,17 β -diol. 17 β -Hydroxy-17 α -methyl-5 β -androstandane-3-one, 17 α -methyl-5 α -androstandane-3 α - and 3 β ,17 β -diols, 17 α -methyl-5 β -androstandane-3 α ,17 β -diol, and 17 α -hydroxy-17 β -methyl-4-androsten-3-one were also synthesized.

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GLOSSARY

<u>Common names</u>	<u>IUPAC</u>
Testosterone (IUPAC also)	17 β -Hydroxy-4-androsten-3-one
17 α -Methyltestosterone	17 β -Hydroxy-17 α -methyl-4-androsten-3-one
Danabol ^R , Dianabol ^R , Methandrostenolone	17 β -Hydroxy-17 α -methyl-1,4-androstadien-3-one
5 α -Dihydrotestosterone	17 β -Hydroxy-5 α -androstan-3-one
5 β -Dihydrotestosterone	17 β -Hydroxy-5 β -androstan-3-one
bz	benzene
CHCl ₃ (CDCl ₃)	chloroform (deuteriochloroform)
C ₅ D ₅ N	deuteriopyridine
EtOH	ethanol
EtOAc	ethyl acetate
glc	gas-liquid chromatography
HPLC	high-pressure liquid chromatography
J	coupling constant
J'	apparent coupling constant
MeOH	methanol
mp	melting point
PE	petroleum ether
ppm	parts per million
TMS	tetramethylsilane
v/v	volume/volume

I. INTRODUCTION

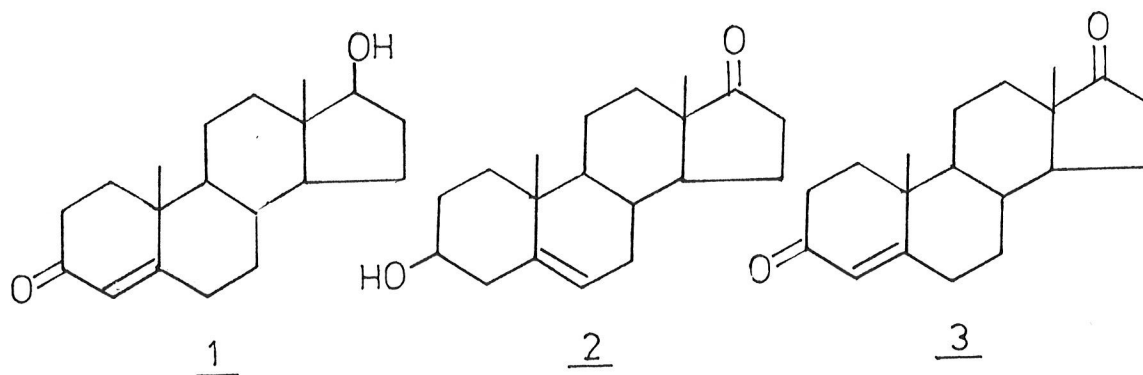
Androgens are defined as a group of biologically active compounds which are responsible for the primary and secondary sex characteristics (anabolic and androgenic properties) of male animals (1,2). The term "anabolic steroids" applies to those steroids that, among other things, promote the synthesis and storage of cytoplasmic protein and stimulate the growth of tissue in general. Androgenic action (virilization) is essentially an anabolic effect confined to specific organs, i.e. those connected with sexual function and characteristics. The distinction of activities between androgenic and anabolic is convenient from a therapeutic standpoint. The search for substances that possess a preponderance of one or the other of these activities has been an ongoing concern of many researchers for the last fifty years. Ideally, for clinical use, anabolic steroids should possess the truly anabolic activity of typical androgens such as testosterone (1) but should lack all of the androgenic properties, such as virilizing effects; however, such compounds have not been reported yet. In general, a compound is considered an anabolic steroid if its anabolic activity prevails and it does not show the virilizing effect of androgens when used at therapeutic dosages.

The anabolic action of the androgens was first documented by Kochakian and Murlin in 1935 (3). In their experiment, extracts of male urine caused a marked retention of nitrogen when injected into dogs fed a normal diet. It was also observed that testosterone propionate produces a similar nitrogen-retaining effect in humans (4). Subsequently, clinical studies demonstrated that testosterone was capable of causing

major acceleration of skeleton growth and a marked increase in muscle mass (5). This action on muscle tissue has been referred to more specifically as the myotropic effect. Anabolic steroids cause positive protein metabolism. In man, treatment with testosterone (1) and related compounds not only brings about nitrogen retention, but causes potassium, phosphorus and calcium retention as well (3,6,7). These elements, together with water, are the main components of the intracellular compartments; hence nitrogen retention is essential in the formation of new protoplasm. Because anabolic steroids promote protein synthesis in the muscle system, these steroids have clinical importance, e.g., in the treatment of underweight children (8) for disturbance of calcium metabolites, and in treating malnutrition, speeding up the healing of wounds, promoting regeneration after surgery, and counteracting the anti-anabolic or catabolic effect of the extensive use of corticoids (9). Some androgenic and anabolic agents are also able to induce remission and prolong life in women with advanced carcinoma of breast (10a-c) and have been widely used and abused by athletes in search of a shortcut to increased muscle mass and strength (10d,11). Such results are not well supported by clinical evidence; and the use of anabolic steroids or androgens for this purpose has been banned in international sports (6,10d, 11). Unfortunately, the clinical use of androgens is limited by side effects in humans, it would be highly desirable to have anabolic compounds that are not androgenic for this would permit their use in women without inducing masculine traits and in children without causing undesirable effects or premature sexual development.

Occurrence and Biosynthesis of Androgens

The androgens are secreted not only by the testes, but also by the ovary and adrenal cortex. Testosterone (1) is the principal circulating androgen and is formed by the Leydig cells of the testes (4). Other tissues, such as liver and human prostate, form testosterone (1) from precursors, but this contribution to the androgen pool is minimal.



3 β -Hydroxy-5-androsten-17-one (2) and 4-androstene-3,17-dione (3) are secreted by the adrenal cortex and ovary, and they contribute indirectly to the testosterone (1) pool because they can be rapidly converted to testosterone (1) by peripheral tissues. It is generally recognized that the synthesis of androgens in the testes is regulated by the trophic hormones, luteinizing hormone (LH) (also called interstitial cell-stimulating hormone; ICSH) and follicle-stimulating hormone (FSH). LH stimulates steroidogenesis in Leydig cells, whereas FSH acts primarily on the germinal epithelium. Testosterone levels are controlled by a feed-back mechanism.

The current hypothesis of steroidogenesis is based largely on investigations with the adrenal cortex and appears to be applicable to the testis and ovary as well (12). Androgen biosynthesis (Figures 1 and 2)

occurs by the following sequence of events known to be involved with steroidogenesis (13): (1) LH activates adenyl cyclase of the Leydig cells; (2) the intracellular concentration of cyclic AMP is increased; (3) cyclic AMP activates certain protein kinases, which are involved in subsequent stages of the steroidogenesis pathway; (4) cholesterol ester (storage form) is converted to free cholesterol (4) which is translocated to the mitochondria; (5) a cytochrome P-450 mixed-function oxidase system converts cholesterol (4) to pregnenolone (5) (14,15,16); and (6) nonmitochondrial enzymatic transformations convert pregnenolone (5) to testosterone (1) (17,18,19,20).

The conversion of cholesterol (4) to pregnenolone (5) has been termed the rate-limiting step in steroid hormone biosynthesis. The reaction requires NADPH and is catalyzed by cytochrome P-450. Among many possible side chain cleavage reactions, the initial events, according to the most widely held hypothesis, involve hydroxylation at C-20 or C-22 followed by the formation of the 20R, 22R-diol (Figure 1). More recent studies, however, cast doubt on this scheme: e.g. Burstein *et al.* (14) obtained kinetic data indicating that 20R, 22R-diol arises directly from cholesterol (4) without the intermediacy of monohydroxylated derivatives. Other studies showed that derivatives of cholesterol (4) having a t-butyl or phenyl in addition to the hydroxyl group at C-20 (i.e. unable to form a C-20,22-dihydroxy derivative) could still give rise to pregnenolone (5) (15,16). Hochberg *et al.* (16) postulated the formation of short-lived radicals or ionic species complexing with the enzyme system. The traditional side chain hydroxylated compounds would then be by-products of the reaction, arising from the transient enzyme-bound radicals or ionic species.

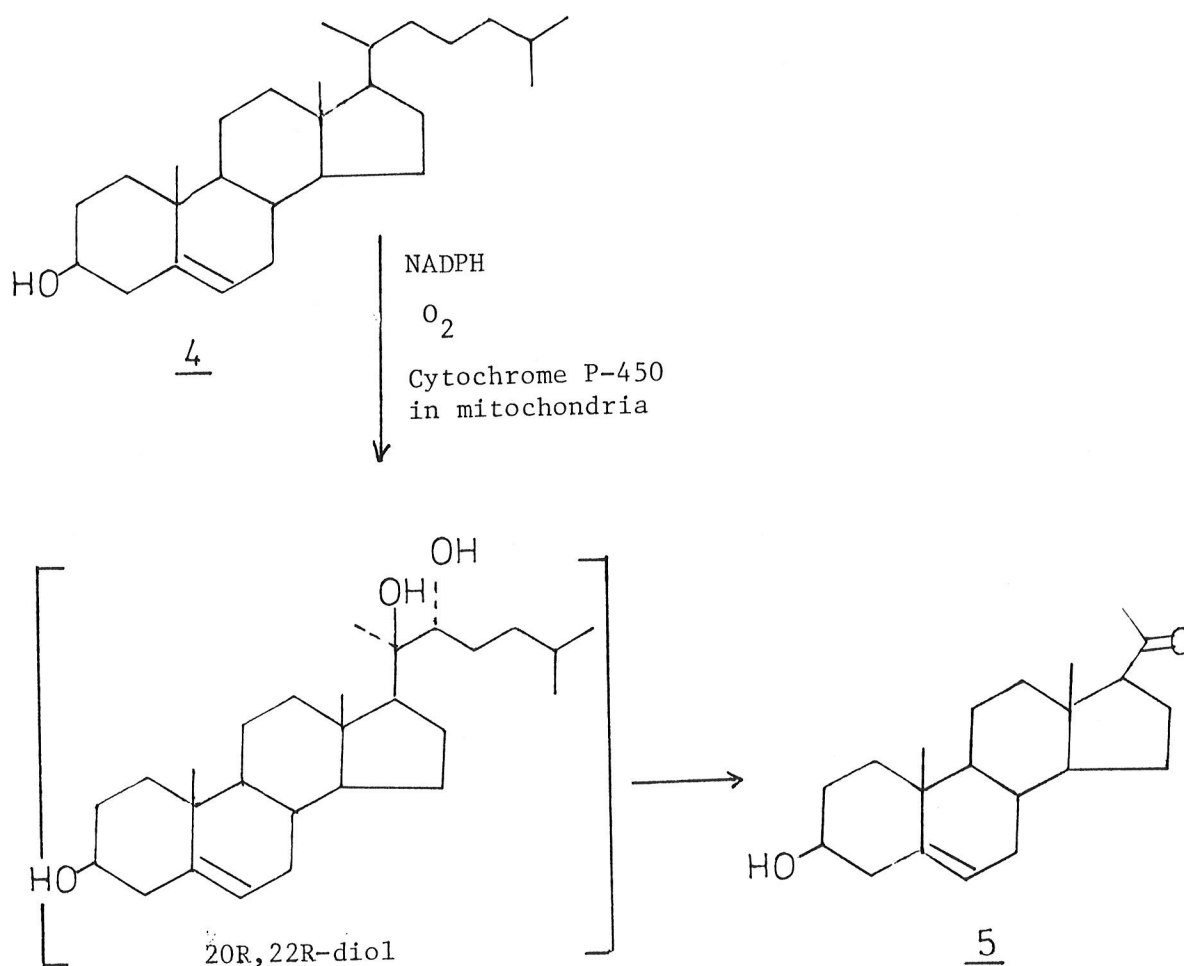


Figure 1. Androgen biosynthesis-side chain cleavage reaction (13).

Tracer studies have shown that two major pathways known as the "4-ene" and "5-ene" pathways are involved in the conversion of pregnenolone (5) to testosterone (1). Both pathways and the required enzymes are shown in Figure 2. Earlier studies favoured the "4-ene" pathway, but more recent work suggests that the "5-ene" pathway is more important in man. The investigations of Vihko and Ruokenen (17) strongly support the "5-ene" pathway. These workers analyzed the spermatic venous plasma for free and conjugated steroids. The unconjugated steroids identified in normal males were all the intermediates of the "5-ene" pathway and no

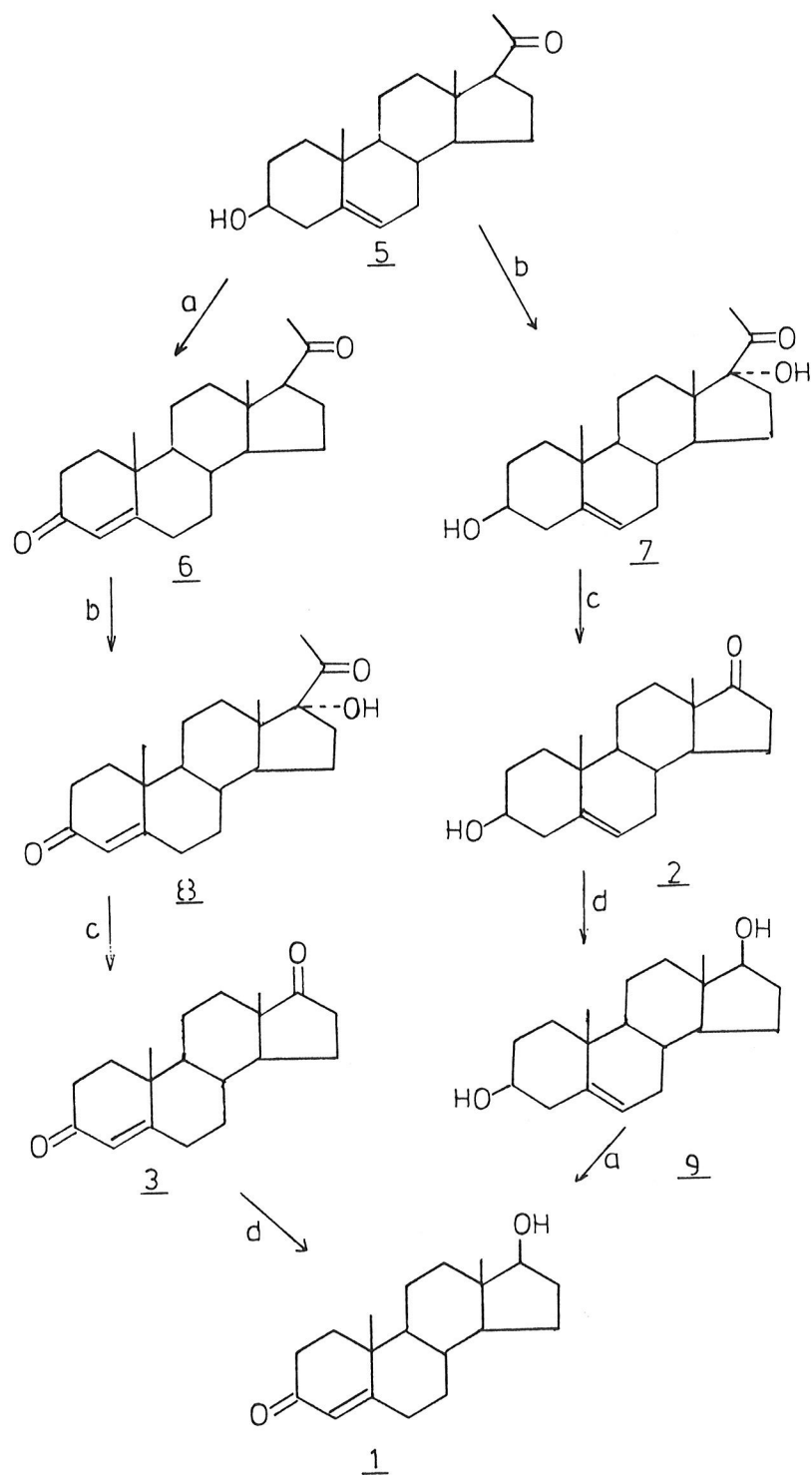


Figure 2. Androgen biosynthesis (13)

- a. 3 β -Hydroxydehydrogenase and isomerase
- b. 17 α -Hydroxylase
- c. C₁₇₋₂₀ Lyase
- d. 17 β -Hydroxydehydrogenase.

intermediate of the "4-ene" pathway was found. In addition, sulfate conjugates were present in significant quantities especially 5-androstene-3 β ,17 β -diol 3-monosulfate. This result strongly suggests that this intermediate and its unconjugated form constitute an important precursor of testosterone (1) in man. This view, however, was not supported by a kinetic analysis of the metabolism of 5-androstene-3 β ,17 β -diol (9) in man (18). Whichever pathway prevails, 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 the mitochondria, those responsible for cleavage of the C₁₇-C₂₀ bond (C₁₇-C₂₀ lyase) reside in the microsomes of the cell. Earlier studies implied 17 α -hydroxypregnenolone (7) or 17 α -hydroxyprogesterone (8) as a necessary intermediate in testosterone biosynthesis (19). Although such a sequence has been accepted dogma for the past quarter-century, the exact details of the lyase reaction are very much in doubt, especially when it can be shown that the C-20 deoxy analog of pregnenolone (5) can serve as a substrate for testosterone biosynthesis (20).

Absorption and Distribution of Androgens

Little is known about the absorption of steroids although considerable research has been carried out on the biochemical mechanism of action of the natural hormones and of the synthesis of modified androgens. It is recognized that a steroid hormone might have high intrinsic activity but exert little or no biological effect because its physicochemical characteristics prevent it from reaching the site of the action (13). This is especially true in man, where slow oral absorption or rapid inactivation may greatly reduce the efficiency of a drug. Even though steroids are commonly given by mouth, little is known of their

intestinal absorption. Results for some steroids indicated that lipid solubility was an important factor for intestinal absorption which may explain the oral activity of certain ethers and esters of testosterone (13).

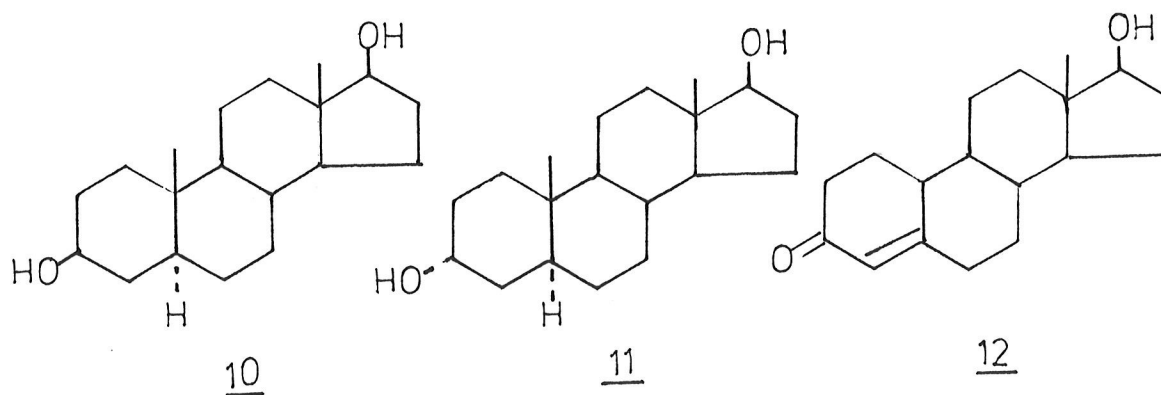
Once testosterone (1) and other androgens enter the circulatory system by either secretion from the testes or absorption of the administered drug, they will reversibly associate with certain plasma proteins, since steroids are biologically active in the unbound form. The extent of this binding is dependent on the structural features of the androgen and the nature of the protein (13).

Albumin was the first protein to be studied and it exhibited a low association constant for testosterone (1) and bound less polar androgens such as 4-androstene-3,17-dione (3) to a greater extent (21). α -Acid glycoprotein (AAG) was shown to bind testosterone with a higher affinity than albumin (22). Another plasma protein that binds testosterone is corticosteroid-binding α -globulin (CBG) (23). However, under normal physiological conditions these plasma proteins are not responsible for binding of androgens in plasma.

A specific protein called sex steroid binding β -globulin (SBG) or testosterone-estradiol binding globulin (TEBG) was found in plasma that bound testosterone with a very high affinity (24). The SBG-sex hormone complex serves several functions, such as acting as a transport or carrier system in the bloodstream, serving as a storage site or reservoir for the hormones, and protecting the hormone from metabolic transformations (25,26). Dissociation constants for binding of testosterone and estradiol to SBG have been reported and are two orders of magnitude less than values reported for the binding of the hormone to the cytosolic

receptor protein (27). The plasma levels of SBG are regulated by the thyroid hormones (28) and remain fairly constant throughout adult life in both the male and female (29). SBG is not present in the plasma of all animals (25,29).

Numerous studies have been carried out on the specificity of the binding of steroids to human SBG (25,26,29,30). The presence of a 17β -hydroxyl group is essential for binding to SBG. In addition to testosterone, 5α -androstane- $3\beta,17\beta$ -diol (10) and 5α -androstane- $3\alpha,17\beta$ -diol (11) bind with high affinity, and these steroids compete for a common binding site. Binding to SBG is decreased by 17α -substituents, e.g. 17α -methyl and 17α -ethynyl groups, and by unsaturation at C-1 or C-6. 19-Nortestosterone (12) derivatives have low affinity.



Another extracellular carrier protein exhibiting high affinity for testosterone, found in seminiferous fluid and epididymis, originates in the testis and is called androgen binding protein (ABP) (31). This protein is produced by the Sertoli cells on stimulation by FSH (32,33) and has very similar characteristics to those of plasma SBG produced in the liver (32). These proteins are distinctly separate from the intracellular cytoplasmic receptor protein (34). It is this cytoplasmic

receptor protein and its high affinity for testosterone and 17β -hydroxy- 5α -androstan-3-one that accounts for the selective accumulation of these hormones in androgen target tissues (35). This selective uptake process is readily observed in rodents following administration of high specific activity tritiated androgens (36).

The absorption of androgens and other steroids from the blood by target cells was assumed to occur by a diffusion of the molecule through the cell membrane. However, studies in the early 1970's using tissue cultures or tissue slices suggested mechanisms of entry for the steroid. Estrogens (37), glucocorticoids (38) and androgens (39-42) exhibit temperature-dependent uptake into target cells, suggesting a protein-mediated process. Among the androgens, 17β -hydroxy- 5α -androstan-3-one exhibited a greater uptake than testosterone in human prostate tissue slices (39), and it was found that estradiol or 4-androstene-3,17-dione interfered with this uptake mechanisms (40,41). Cyproterone, a steroid with antiandrogenic effects, competitively inhibited 4-androstene-3,17-dione (3), testosterone and 17β -hydroxy- 5α -androstan-3-one entry, whereas cyproterone acetate enhanced the uptake of these androgens (43). 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 (44).

Target Cell Androgen Receptor

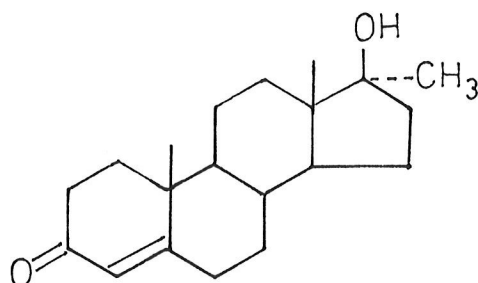
Androgen receptor protein was first found in cellular preparations of rat ventral prostate as a complex of 17β -hydroxy- 5α -androstan-3-one (5α -dihydrotestosterone) and receptor (45,46,47). This finding came from the recognition that, in this target tissue, testosterone is rapidly transformed to 17β -hydroxy- 5α -androstan-3-one, which can be

selectively retained in the prostate cell nuclei (45,48,49). Receptors or receptor-like proteins have been found in a variety of androgen target tissues. Most androgen sensitive tissues appear to have androgen receptor protein (β -protein) that can bind both testosterone and 17 β -hydroxy-5 α -androstan-3-one (43). It was also found that many potent synthetic androgens can bind directly to β -protein and to prostate cell nuclei without a metabolic conversion (50). For this reason, the relative metabolic activities of testosterone and 17 β -hydroxy-5 α -androstan-3-one, and their relative affinities toward the cellular receptors become important factors in providing information about which of the two androgens can play roles in androgenic responses (43).

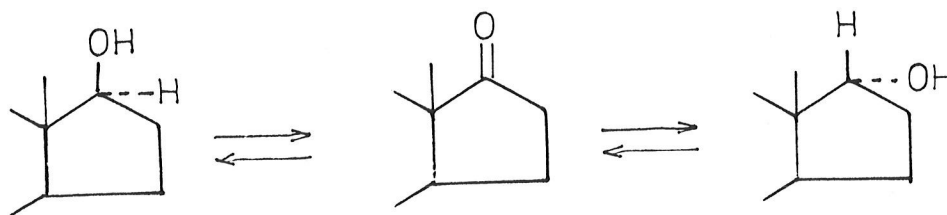
Synthetic Androgens

The main aim of research in synthetic steroids has been structural modification (see structure-activity relationship section) of the basic natural steroid molecule, testosterone, so as to obtain compounds which possess anabolic activity (1) with the least possible androgenic effect (51). However, no such anabolic steroid from which the androgen activity is completely absent has yet been discovered. Other criteria that have guided the search for new anabolic compounds were related to the methods of administration according to the demands of clinical practice. The first steroids used were active only by parenteral administration which presented difficulties in usage. The discovery that 17 α -alkylation led to the products which are active on oral administration opened up the field and 17 α -methyl-5-androstene-3 β ,17 β -diol entered into clinical practice (1). With only a few exceptions, all the anabolic steroids presently in oral use are 17 α -alkylated. Later, in order to achieve slower absorption of steroids so that their action

could be prolonged, esterification of the 17 β -hydroxyl group which is always present in the more active anabolic steroids, was carried out 17 β -Hydroxy-17 α -methyl-4-androsten-3-one (17 α -methyltestosterone, 13) is one of the 17 α -alkyl derivatives retaining androgen potency while at the same time being active orally. The rationale for orally active

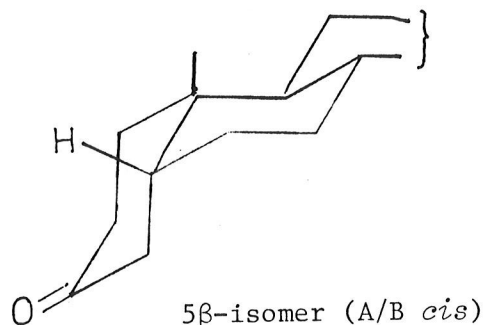
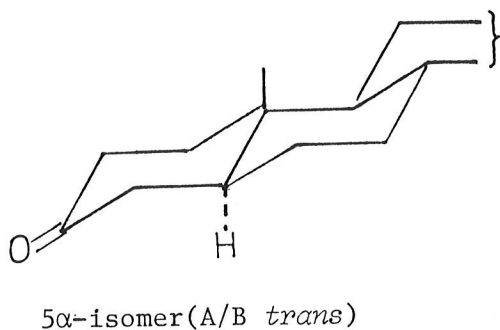
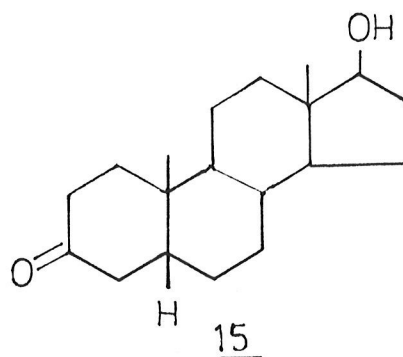
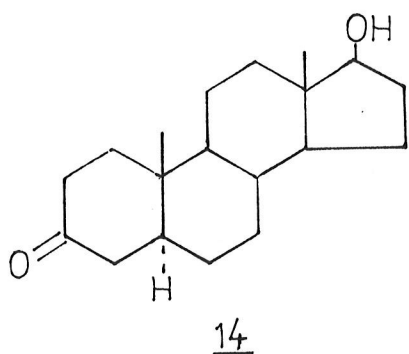
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compounds having a 17 α -methyl group is that the 17 α -methyl group prevents the formation of the 17-keto and epimerization to the inactive 17 α -hydroxyl group as shown below:



The anabolically active steroids can be classified in two large groups: (1) androstane derivatives and (2) 19-norandrostane derivatives (estrans). In this paper only androstane derivatives having 17 β -hydroxy-17 α -methyl groups will be discussed. Efforts to improve upon 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) as an orally active androgen have been actively pursued. 17 α -Methyl-5-androstene-3 β ,17 β -diol was the first synthetic product which showed a differentiation between

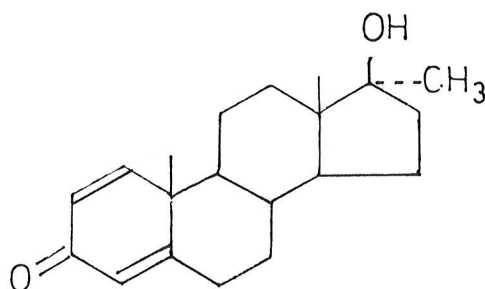
androgenic and anabolic activities (50). One of the earlier modifications of the testosterone molecule was the reduction of the double bond between C-4 and C-5 in which two isomers, 17 β -hydroxy-5 α -(14) and 5 β -androstan-3-one (15) were found (53). The 5 β -isomer, like all other of its derivatives, drastically reduced both the anabolic and androgenic properties (54) whereas the 5 α -isomer was active. These observations established the importance of the A/B *trans* ring junction for activity.



Recently, it has been shown that the C-4 double bond of testosterone is reduced by the target cell to the 5 α -compound and therefore 17 β -hydroxy-5 α -androstan-3-one (14) is considered to be the endogenously active molecule (40,41).

The discovery that C-17 α methylation gave oral activity to testosterone prompted the synthesis of additional C-17 α substituted analogs. Increasing the chain length beyond the methyl group led to a decrease in activity (55).

Marked enhancement in biological activity was shown by the introduction of a double bond at C-1 of cortisone and hydrocortisone. This observation prompted a similar introduction of a double bond at C-1 in the androgens. 17 β -Hydroxy-17 α -methyl-1,4-androstadien-3-one (methandrostenolone, Danabol^R, 16) had one to two times the oral potency of 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) in the rat nitrogen retention (56,57) and levator ani muscle assays (58,59). It is interesting to note that 1,4-androstadiene-3,17-dione, which is similar to compound 16, has been isolated as an endogenous steroid from the urine



16

of a boy with early sexual maturation, although its significance is not known (60). Compound 16 was prepared by the introduction of a double bond between C-1 and C-2 of 17 β -hydroxy-17 α -methyl-4-androsten-3-one (61,62). In clinical studies, compound 16 produced a marked anabolic effect when it was given orally at doses of 1.25-10 mg daily and was several times more potent than 17 β -hydroxy-17 α -methyl-4-androsten-3-one

(66). Desaulles *et al* (64) studied intensively the pharmacological properties of compound 16. The anabolic action of this compound has been demonstrated by the increase in weight obtained in both young and adult rats and by the retention of nitrogen observed in female adult rats. The anabolic action of this compound was also exerted in the presence of catalyzing agents such as the corticoids, the drug antagonizing the true catabolic effect (9a). Compound 16 is the anabolic steroid most widely used clinically. Despite the wide use of this compound, its value as anabolic steroid in sports has not been well established by clinical trials (10d,11). The anabolic activity of compound 16 in man had been shown by an increase in body weight at different doses (65,66,67). The very small androgenic effect shown by this compound has allowed its use in pediatrics.

Androgen activity became evident only when it was used at a 3 mg/kg dose (68) which made the normal dose of 1 mg/kg acceptable (69). Nevertheless, like other 17 α -alkylated steroids, when it was administered orally, it affected liver function; the first case of hepatocellular carcinoma associated with taking an androgenic-anabolic steroid was reported in 1965 (70). Since then, other persons taking C₁₇-alkylated androgenic steroids have developed hepatocellular carcinoma (71). A case of Wilm's tumor (72) and other cases of hepatic angiosarcoma in male adults (73) have been reported. In some cases, patients treated with compound 16 showed a slowing down in the liberation of sulfobromophthalein from liver (74); in others there was also the appearance of jaundice (75). Other researchers reported normal kidney function (76) and some reported a normal histological pattern in biopsies of

the liver (77). Pyörälä *et al.* (78) observed an increased sensitivity to anticoagulants during therapy with the compound 16 which could indicate an impairment of liver function. The use of 17 α -alkylated androgens (e.g. compound 16, ethylestrenol, norethandrolone) has been restricted because this class of compound produces a high incidence of intrahepatic cholestasis (79). These compounds are not converted to estrogens by rat liver slices (80); however, aromatization is brought about by the placental enzyme system (81).

Structure-Activity Relationships of Androgens

Particular chemical structures are known to produce specific biological actions. Many structurally different steroids have been investigated to define the functional relationship between chemical constitution and physiological action.

Structure-activity relationships of androgens have been extensively studied and are well documented (7,46,82,83). The object of these structural studies has been to find chemical specificity or functional estimates of potency and affinity for the construction of theoretical models for an androgen receptor.

Thousands of new steroids have been synthesized during the past three decades. Many of these analogs of natural steroids have enhanced biological activity over the natural molecules, some have been less active or inactive and others have shown activities which are not previously expected on the basis of their structures.

Ringold (82) introduced a theory for biological activity of steroids by systematic examination of effects produced by stereochemical and electronic alterations of the steroid molecules. As the

prototypes of androgens, the natural steroids, testosterone and 17 β -hydroxy-5 α -androstan-3-one, have been used. After examining the androgenic action of α or β -alkyl or halo-substituted androstanes, Ringold has concluded that the interaction of androstanes with a receptor to produce an androgenic response takes place on the α -face of the androgen molecule. Ringold also considered the role of substitution by electronegative groups, such as the α,β -unsaturated ketone group (4-en-3-one system). The α,β -double bond is not required for high biological activity since substituted saturated 5 α -androstan-3-ones are potent as are the 5 α -androstan-3-ols.

After studying the effects of alkyl and electronegative group substitution on the activity of androgenic-anabolic steroids, Zaffaroni's androgenic activity (84) confirmed Ringold's postulate.

Bush (85,86) disagreed with Ringold's theory (82) of α -face attachment to the receptor and suggested that the β surface of the androgenic molecule is responsible for specificity in steroid-receptor association. He also called attention to the fact that substitutions and other modifications in ring A have much more complicated and striking effects on androgenic activity than for other classes of steroids. Furthermore, Bush (85,86) suggested that the 17 β -hydroxyl group plays a specific role in the association of this group with the receptor for androgenic activity, since all active steroids in this group possess a 17 β -hydroxyl group or else a group (17-oxo or 17 α -hydroxy) which can be converted *in vivo* to the 17 β -hydroxyl group. According to Bush, all these data confirm the validity of the broader concept outlined by him that changes in the configuration of the upper half of rings C and D have a far greater

effect on all types of biological activity of steroids than many changes in the configuration of rings A and B.

Bush (85) did not favour a specific interaction of steroids with their receptors and stated that in the nature of drug-receptor interaction, the association between steroid and receptor does not involve chemical reaction. According to Bush, the 17β -hydroxyl group appears to be the crucial polar group in natural androgens, such as testosterone. This group is thought to increase the specificity of the receptor association by increasing the probability that the steroid attaches itself in the correct orientation. This is similar to Sarett's concept (87) of anti-inflammatory steroid action. Bush defined the above mechanism as a blocking mechanism which is based on Paton's rate theory of drug action (88). Paton suggested that a pharmacological effect depends not only upon the number of individual drug-receptor combinations, but also upon the rate of drug-receptor occupation.

Bowers *et al.* (89) studied the structural requirements of androgenic-anabolic activity with the basic concept of varying the electron density pattern in and around ring A because, in conjunction with steric properties, the electron density pattern should be related to the ability to bind to protein-like structures. It was found that C-1, C-2 and C-3 unsaturated 17β -hydroxy- 5α -androsterone all exhibit reasonable anabolic activity, the anabolic-androgenic ratio being more favourable in the latter two compounds, and 17β -hydroxy- 5β -androsterone-3-one was inactive. Bower's study concluded that a high electron density at C-2 and/or C-3 in 17β -hydroxy androsterone is a factor strongly promoting high myotropic activity which may be satisfied by a 3-oxo group. It may be

possible that the 3-oxo group may be active primarily as enols or enolate anions where the 2-ene π -bond is present. Bowers also postulated that "introducing more than one sp^2 -hybridized carbon atom into ring A results in a pronounced flattening of the ring from a cyclohexene chair form to a planar conformation in which the steroid may be better able to rest on a receptor surface with a concomitant increase in the degree of the orbital overlap" (89).

Wolff and Jen (90) presented the hypothesis that androgenic and myotropic responses are initiated by the β -face adsorption of a steroid on the tissue receptor. Later Wolff *et al.* (91) suggested that a requirement for androgenic activity is the presence of high electron density at C-2 and/or C-3 such as is provided by sp^2 -hybridization. This high electron density is responsible for the formation of the π -complex with the receptor site on the basis of biological evaluation of steroids having ethylene oxide (epoxide), cyclopropane or spiro-oxianyl ring fused to C-2 and C-3. This is similar to Bowers *et al.*'s hypothesis discussed above (89). The biological evaluation of a number of these compounds by means of the myotropic-androgenic assay showed that the cyclopropanyl steroids (e.g. $2\alpha,3\alpha$ -cyclopropane- 5α -androstan-17-one) are the most active compounds in the series. This observation resulted in the proposal that anabolic-androgenic androstanes are bound to their receptor by a β -face bond to an sp^2 -system in ring A. To give more information regarding the steric and electronic requirements in ring A of anabolic-androgenic substances, the preparation and androgenic-myotropic testing of analogs of $2\alpha,3\alpha$ -cyclopropane- 5α -androstan-17 β -ol having substituents on the cyclopropyl ring have been studied (91). If the π -bond is important, the strength of the binding would be

different in such analogs having altered electron density at the sp^2 centers. Since β -face binding was assumed to be involved, a substituent group on the cyclopropane ring of analogs of $2\alpha,3\alpha$ -cyclopropane- 5α -androstan- 17β -ol should not interfere sterically with steroid binding. From this work, two main conclusions have been reached: (1) the activity can be related or enhanced; otherwise altered, by substituted compounds and (2) the activity of a given analog is determined by the structure of the substituents on the cyclopropane ring.

The existing hypothesis of the mode of interactions of steroids with receptors and the problem of the structure and mechanism of action of steroids have been restricted to the question of whether the α - or β -face of the steroid is involved in the steroid-receptor interaction and they were based on the changes in the hormonal activity by structural modification. Therefore, another approach was proposed to consider the mechanism of action by considering "the minimal structural requirements necessary to bring about hormonal activity" (7,92). Since the natural androgenic and anabolic agent testosterone does not have the minimum structural requirements of active steroids, it was necessary to start with simpler molecules, such as 5α -androstan- 17β -ol, which displays the minimal structural requirements for androgenic activity. The natural androgen, 5α -androstan- 17β -ol (92) was found to have the androgenic activity, and androstan- 17β -ol (93) containing only the one oxygen at C-17, caused growth of the prostate gland. The ring A saturated compound 17α -methyl- 5α -androstan- 17β -ol (94) was found to have high activity in promoting growth of the kidney, the prostate and seminal vesicles when administered orally (95).

Biological data showed that ring contraction (96) significantly decreased or abolished androgenic-anabolic activity. Ring A of 6-membered or larger carbocyclic rings (e.g. homotestosterone propionate) which flattens the ring A in the vicinity of C-2 and C-3 has increased androgenic activity (97,98). For ring B, seven instead of six carbon atoms also increased the anabolic activity (99). Zanati and Wolff (100) prepared oxa-androstane derivatives in order to investigate the exact area of ring A where the flattening is taking place by systematic substitution of an oxygen atom around the ring A and found that the substitution of oxygen at C-2, 2-oxa-5 α -androstan-17 β -ol, was the most active compound. The C-3 substituted 3-oxa-5 α -androstan-17 β -ol, was less active. These results were similar to those of Bowers *et al.* (89) who introduced a double bond around the ring A in an analogous way. Even though a double bond affects the geometry of two carbon atoms in the ring A and their results cannot be compared directly with Zanati and Wolff (100), considering that the most active olefin was C-1 double bond and the least active one was the C-4 double bond, the two results were in general agreement.

Bowers *et al.* (89) looked into the results of changing the electron density in the ring A, even though they recognized that stereochemistry might be important as well. However, the results obtained by Zanati and Wolff (100) gave strong support for the importance of steric effects in ring A for activity as an androgenic molecule. To support the contention that steric but not electronic effects of ring A are important in androgenic activity, Zanati and Wolff (101) synthesized tricyclic compounds corresponding to steroids lacking a ring A. These compounds were found to be androgenically active also. This finding is important in

connection with the question of whether the intact steroid nucleus is necessary for the production of various kinds of hormonal effects. The C/D ring junction in natural steroids is *trans*. Normal steroids with the C/D ring junction *cis* (13 α -configuration) have been reported to be inactive (102). The C-18 methyl group (103) appears to give high androgenic/anabolic activity. This activity may be due to the fact that the C-18 methyl group locked C/D ring system into *trans* junction and prevents epimerization to the C/D *cis* junction. This C-18 methyl group can be replaced by an ethyl group (104) without decreasing the activity. If ring D were a six membered ring (D-homosteroids), the C/D ring junction would be more stable as the *trans* junction. In this case, the C-18 methyl group can be eliminated without decreasing activity (105). The A/B ring junction in the inactive 5 β -compounds is *cis*. All active anabolic/androgenic steroids have the natural B/C *trans* ring junction (106). Inversion of the configuration at C-8, as in 8 α ,9 α -testosterone (107), causes inactivity because the 8-iso stereoisomer no longer has the natural planar geometry necessary for activity.

One of the main areas of research includes the preparation of aza and oxa steroids produced by replacement of carbon atoms in the steroid skeleton by nitrogen and oxygen. Several attempts have been made to improve hormonal activity by replacing a whole isocyclic ring by a heterocyclic ring like the pyrazole (108), oxazoline (109), pyrimidine (110) or thiopyrimidine (111) ring. All of these modifications lead to compounds which are inactive.

The presence of an oxygen substituent at C-3 of ring A is common to the structure of natural steroids. The C-3 oxo function enhances activity. The importance of the bond angle, distance and direction

related to structure and physiological activity was emphasized by Woodward (112). The introduction of the 3-carbonyl function changes the cyclohexane of ring A to 3-cyclohexanone ring A with very little distortion from cyclohexane geometry as far as the backbone structure of carbons and the attached groups are concerned. The only change brought about is the position of the carbonyl carbon with respect to the rest of the molecule (113). Among the classical endocrine hormones the androgens are unique in that an α,β -unsaturated ketone is not required for high activity and saturated 3-oxo-5 α -androstanes are potent as well as are the 3 α -hydroxy-5 α -androstanes. It is known however that androgenic activity is not dependent on the presence of an oxygen substituent at C-3, although numerous steroids with high activity have a 3-oxo- or 3 α -hydroxy group. Active compounds without a C-3 oxygen function are known, e.g. 17 α -methyl-5 α -androstan-17 β -ol (94, 114), and 5 α -androstan-17 β -ol (93,114,115). However, the possibility of an *in vivo* conversion of these steroids to 3-hydroxy steroids cannot be completely excluded and steroids with an unsaturated ring A may be hydroxylated in animals.

The presence of an oxygen substituent at C-17, specifically a 17 β -hydroxyl group, is more important than the presence of oxygen at C-3 for the activity. All useful anabolic agents and highly active androgens possess the 17 β -hydroxyl group (93). It has been concluded that the 17 β -oxo substituent is responsible for the attachment to the receptor site.

After having examined the effects produced by alterations and substitutions at each carbon atom, Vida (7) postulated a new theory for the steroid hormone-receptor interaction. In considering any kind of

interaction between a steroid and receptor, no matter how attractive it seems to picture the steroid skeleton as having two sides, an α -face and a β -face, and no matter how many attempts there have been in trying to consider steroid-receptor interaction in terms of α - or β -side attachment, one must not forget that the steroid is a three-dimensional entity. Therefore, Vida (7) postulated that the steroid-receptor interaction is a three-dimensional attachment.

The important points of the attachment are ring A on the α -side (through the axially oriented bond attachment at C-1, C-3 and C-5), rings B, C, and D on the β -side [through the axial oriented bonds attached at C-6, C-10, C-13 and secondarily C-8, C-11, and through the bond attached at C-16 and C-17 (16β and 17β)], and on the α -side of ring D (through the bonds attached at C-16 and C-17). In addition to planar, peripheral attachment is made to planes perpendicular to the α - and β -sides of the steroid skeleton. These planes are situated along the equatorially oriented substituents at C-1, C-2, C-11 and C-12 on one side, C-16 and C-17 on the second side, and along the equatorially oriented substituents at C-6 and C-7 on the third side. The important points of attachment are ring A (through the equatorially oriented bonds attached at C-1 and C-2), ring B (through the equatorially oriented bonds attached at C-11 and C-12), ring C (through the equatorially oriented bonds attached at C-6 and C-7) and ring D (through the bond attached at C-16 and C-17).

The interaction of androgens with a receptor necessary to elicit an androgenic and anabolic response is on the α and peripheral sides of ring A; β and peripheral sides of ring B; β and peripheral sides of ring C, and α, β - and peripheral sides of ring D.

The points of attachment at C-8 and C-11 (β -side) are of secondary importance. They are responsible in certain conformational situations (e.g. in the presence of 19-methyl and 18-methyl groups) for inhibiting the steroid-receptor attachments normally present at other positions by steroid interference. Yet, in some other conformational situation (e.g. 19-nor steroids), the absence of other bulky substituents under C-8 and C-11 positions on the β -face become primary points of attachment to the receptor.

Metabolism of Natural Androgens

Present knowledge of the metabolism of natural androgens (e.g. testosterone) is based mainly on a variety of *in vivo* and *in vitro* investigations in man and animals. These results have been reviewed (17, 116, 117). The principal pathways for the urinary metabolism of testosterone in man are shown in Figure 3.

Human liver produces a number of metabolites, including 4-androstene-3,17-dione (3), 4,16-androstadien-3-one, 5 α -androstane-3 β ,17 β -diol (10), 5 α -androstane-3 α ,17 β -diol (11), 5 β -androstane-3 α ,17 β -diol (17), and 3 β -hydroxy-5 α -androstan-17-one (23) (118,119) and also including 6 β - and 16 α -hydroxylated testosterone (120) (Figure 4). As shown in Figure 4, there exists in human liver a reductive as well as an oxidative pathway in the metabolism of testosterone. The reductive pathway leads to the formation of saturated 3,17-diols *via* the ring A saturated 17 β -hydroxy-3-one compound; and the oxidative pathway leads to both unsaturated and saturated ring A 3,17-diones as well as hydroxylated compounds. The fact that ring A saturated 17 β -hydroxy-3-one compounds are formed during the incubation in the liver slices supports the view that the saturated diols arise from ring A saturated 3-keto steroid by

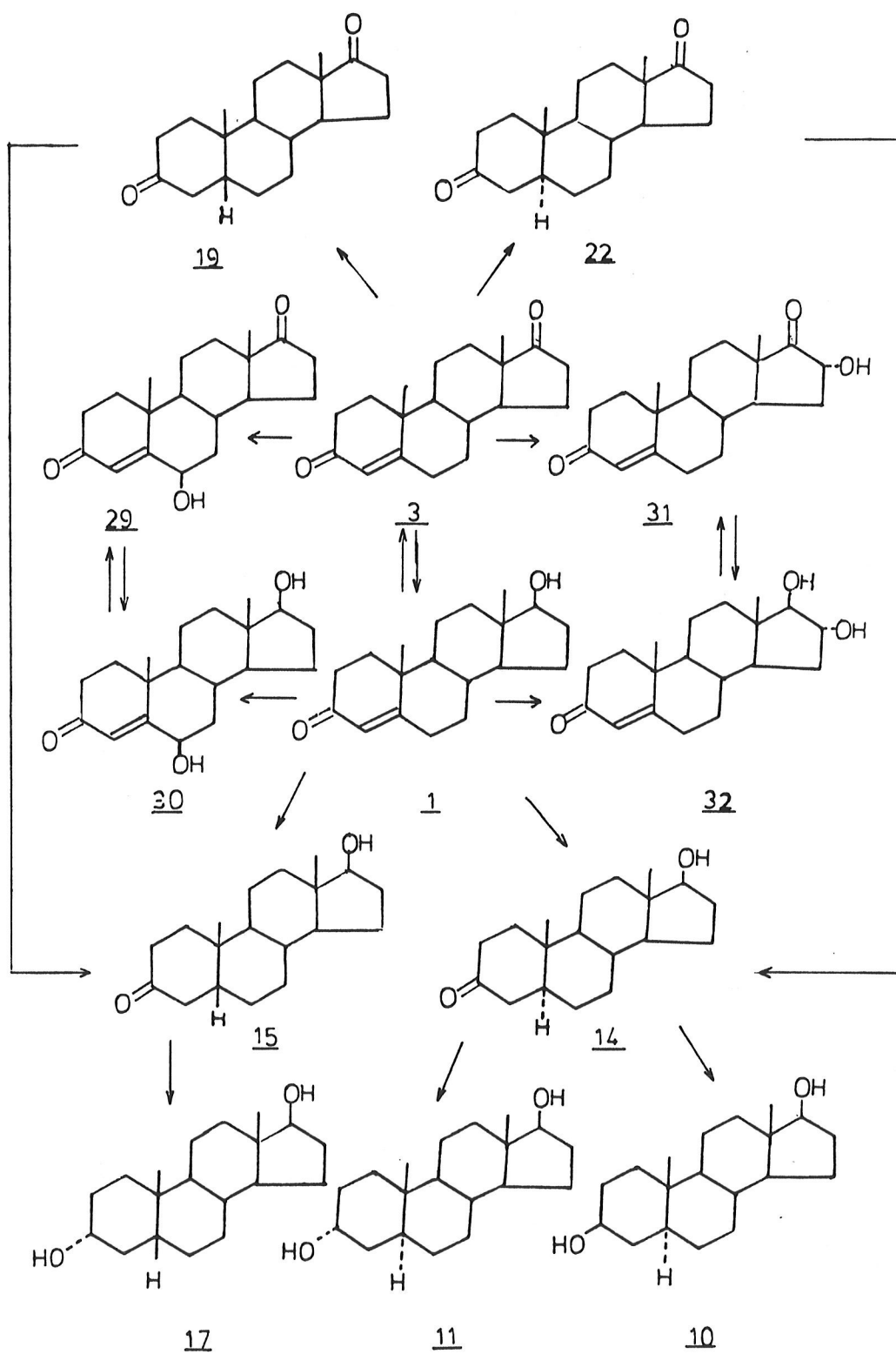


Figure 4. Metabolism of testosterone in slice of human liver (120).

reduction. These facts are in agreement with the finding of Baulieu *et al.* (121) who made the important observation that, under *in vivo* conditions, testosterone is metabolized in man not only to 17-keto steroids but also to the saturated 5 α - and 5 β -androstane diols. The metabolism of testosterone and other related compounds in various liver preparations have been studied extensively and numerous metabolites have been identified (122-127).

It is well established that testosterone can be metabolized in various tissues other than the liver (40,41,128,129). These include human skin where testosterone was found to be actively metabolized under the influence of several enzymes, such as 17 β -dehydrogenase, 5 α - and 5 β -reductase (130,131) and 3 α - and 3 β -oxidoreductase (132,133).

17-Keto steroids and 17 β -hydroxy steroids are interconvertible by the enzyme 17 β -hydroxy steroid dehydrogenase. The 17 β -hydroxy steroids have a greater biological activity than the 17-keto steroids while the 17-keto steroids predominate in urine. The first stage in metabolism is reduction of the double bond in ring A carried out by the 4-ene-5 α - and 4-ene-5 β -reductase, which seems to have some substrate specificity. Reduction of this double bond with production of 5 α - and 5 β -androstane-dione leads to a marked reduction in the biological activity of the steroid. 17 β -Hydroxy-5 α -androstane-3-one (14) is important since this compound has considerable biological activity and seems to be produced from testosterone in target organs for the male sex hormones (133). A number of tissues contain both 3 α - and 3 β -hydroxy steroid dehydrogenases; these enzymes are relatively non-specific regarding their steroid substrate. The amount and/or activity of the former seems to predominate as more 3 α - than 3 β -hydroxy steroids are formed. 6-Hydroxy and

16-hydroxy derivatives (120) of testosterone are known, but are minor, and a small amount of 17 α -hydroxy-4-androsten-3-one is produced, presumably from 4-androstene-3,17-dione (3) by the action of 17 α -hydroxy steroid dehydrogenase. Human adrenal preparations produce 11 β -hydroxy testosterone as a major metabolite (134). Intestinal metabolism of testosterone is similar to liver metabolism (117). The major metabolite in lung is 4-androstene-3,17-dione (135). Studies on testosterone metabolism have been centered on steroid transformation by prostatic tissues. Since the later 1960's, normal prostate, benign prostatic hypertrophy and prostatic carcinoma all involve 3 α ,3 β - and 17 β -hydroxy steroid dehydrogenases and 5 α - and 5 β -reductases, capable of converting testosterone to various metabolites (136).

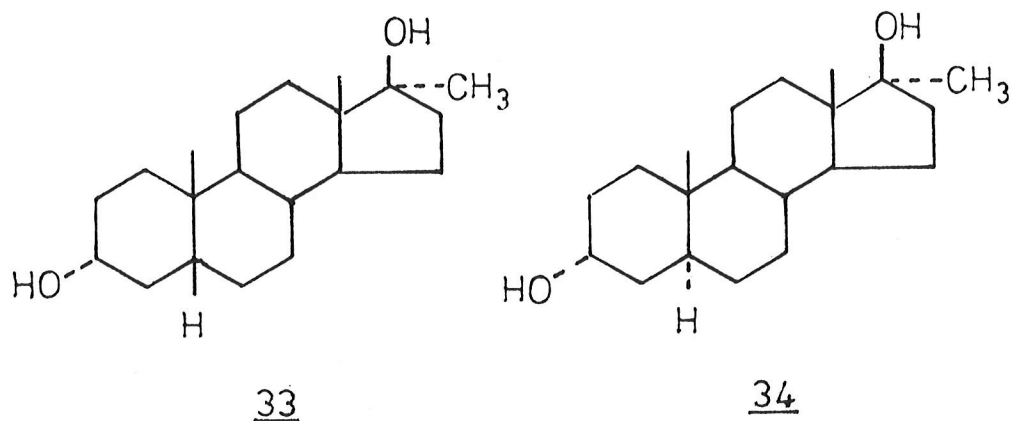
The metabolic products shown in Figure 3 (the urinary metabolites that have been identified following the administration of testosterone in man) are excreted as such or in the form of their glucuronide or sulfate conjugates. The major urinary metabolites (3 α -hydroxy-5 α - and 5 β -androstan-17-one) are excreted predominantly as glucuronides and only about 10% as sulfate (137). These conjugates are capable of undergoing further metabolism, e.g. testosterone glucuronide is metabolized differently from testosterone in man giving rise mainly to 5 β -metabolites (138).

Metabolism of Synthetic 17 α -methylandrosterone Derivatives in Humans

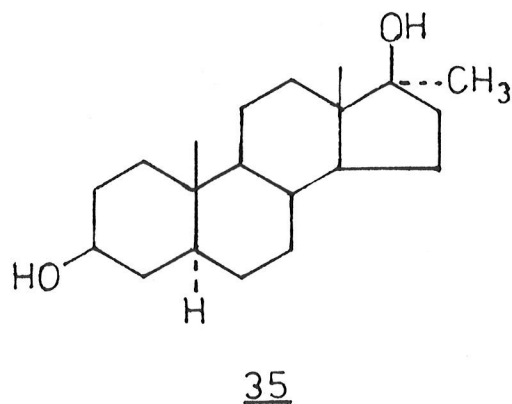
In testosterone, not only is ring A reduced, but the 17 β -hydroxyl group is transformed into the corresponding 17-keto structure providing one source of urinary "17-keto steroids". However, introduction of a 17 α -methyl group into the testosterone molecule slows the rate of metabolism relative to testosterone itself because the oxidation of

17 β -hydroxyl group is prevented (98). In addition to ring A reduction, synthetic androgens may be metabolized by hydroxylation at different sites of the molecule (132).

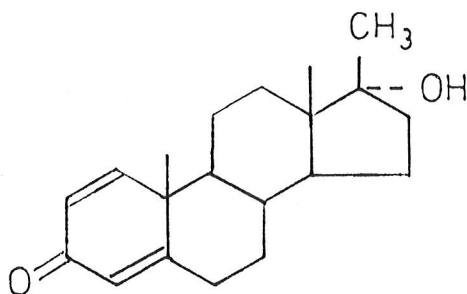
The oral administration of [4-¹⁴C]17 β -hydroxy-17 α -methyl-4-androsten-3-one to a man with carcinoma of the stomach was found to produce complete obstructive jaundice (139) and all of the radioactivity was recovered in urine over 5 days. When [4-¹⁴C]17 β -hydroxy-17 α -methyl-4-androsten-3-one was administered to a woman with breast cancer, the radioactivity was detectable in the faeces after 4 days and it was found that radioactivity in faeces was twice that in the urine. Less conclusive results were obtained by Quincey and Gray (132) who found that on intravenous injection of [1,2-³H]17 β -hydroxy-17 α -methyl-4-androsten-3-one to four patients the radioactivity excreted was 30.6-52.4% in urine and 4.6-7.4% in faeces in 96 hours; 26.7% of radioactivity in urine within 24 hours after the injection. Rongone and Segaloff (140) found that the ratio of metabolic products, 17 α -methyl-5 β -androstane-3 α ,17 β -diol (33) and 17 α -methyl-5 α -androstane-3 α ,17 β -diol (34), which were excreted by a patient who received 1 g per day of 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) orally over four days, was 10:1 in contrast with the ratio of metabolic products from the administration of testosterone. In the case of testosterone, 3 α -hydroxy-5 β -androstan-17-one (21) and 3 β -hydroxy-5 α -androstan-17-one (23) were isolated in equal amounts (128). This suggests that the 17 α -methyl group inhibits the interaction of the steroid with the 5 α -reductase and/or enhances the interaction with the 5 β -reductase. Similar results were obtained from the urinary metabolic products of 1 α -methyl-19-nortestosterone acetate where 3 α ,5 β - and 3 α ,5 α -steroids were excreted in a ratio



of 10:1 (128). 17 α -Methyl-5 β -androstane-3 α ,17 β -diol (33) and 17 α -methyl-5 α -androstane-3 α ,17 β -diol (34) were isolated as metabolic products of 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) from both the unconjugated and enzyme hydrolysed extracts of urine. Quincey and Gray (132) found that 0.3% of unchanged 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) was extracted from human urine and 35% of the urinary metabolic products were present as polar compounds. Segaloff *et al.* (139) isolated unchanged 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) (small amount), 17 α -methyl-5 β -androstane-3 α ,17 β -diol (33) and 17 α -methyl-5 α -androstane-3 β ,17 β -diol (35) in normal adult males. There has not been any evidence of removal of the 17 α -methyl group in these studies.



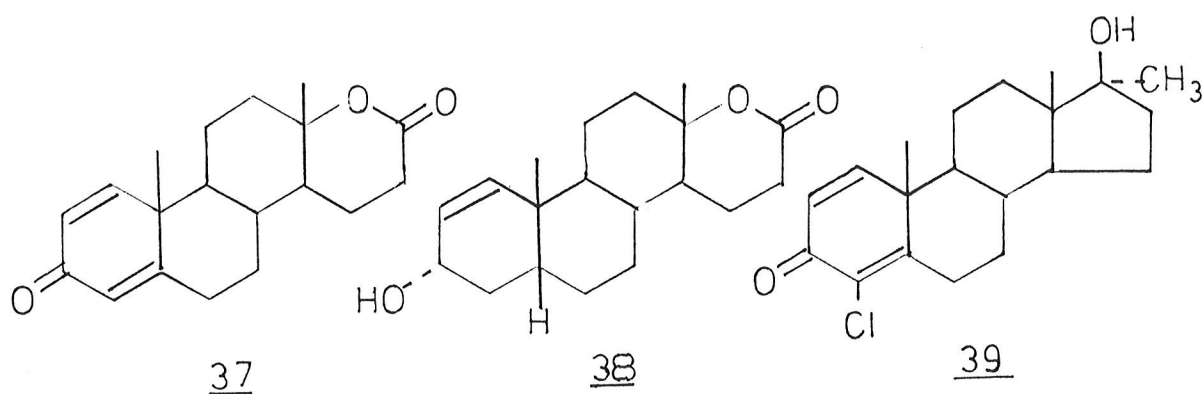
After administration of 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16) (1 g per day orally for 4 days) to a healthy woman, two metabolic products were isolated (141,142). The major one was the 6 β -hydroxylated derivative and the second one was unidentified by them, but it was postulated that it might be a C-17 epimer of the dosed material. This second metabolic product was later identified as the C-17 epimer, 17 α -hydroxy-17 β -methyl-1,4-androstadien-3-one (36) (143). The urinary



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metabolic products after oral administration of 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16) to normal adult men were determined both by chromatography and gas capillary chromatography-mass spectrometry as the C-17 epimer and 6 β -hydroxy-17-epimer of the dosed steroid (144). By the latter method it was also shown that the 6 β -hydroxy derivative of the dosed steroid is obtained (145). A further detailed study of the above metabolic products in normal adult men by computer-aided capillary gas chromatography-mass spectrometry showed them to be the C-17 epimer, 6 β -hydroxy derivative, two unidentified 6-hydroxylated isomers, 17 β -hydroxy-17 α -methyl-1,4,6-androstrien-3-one and 18-nor-17,17-dimethyl-1,4,13(14)-androstatrien-3-one (146). The latter two may have been formed during identification procedures.

From the urine of patients who were treated orally or intramuscularly with 13,17-seco-1,4-androstadien-3-one-17-oic acid lactone (1-dehydrotestolactone) (37), it was possible to isolate 3 α ,13 α -dihydroxy-13,17-seco-5 β -androst-1-en-17-oic acid lactone (38) which showed that the reduction of 4-en-3-one group to the 3 α -hydroxy-5 β -structure had occurred preferentially and that the C-1 double bond remained (128).



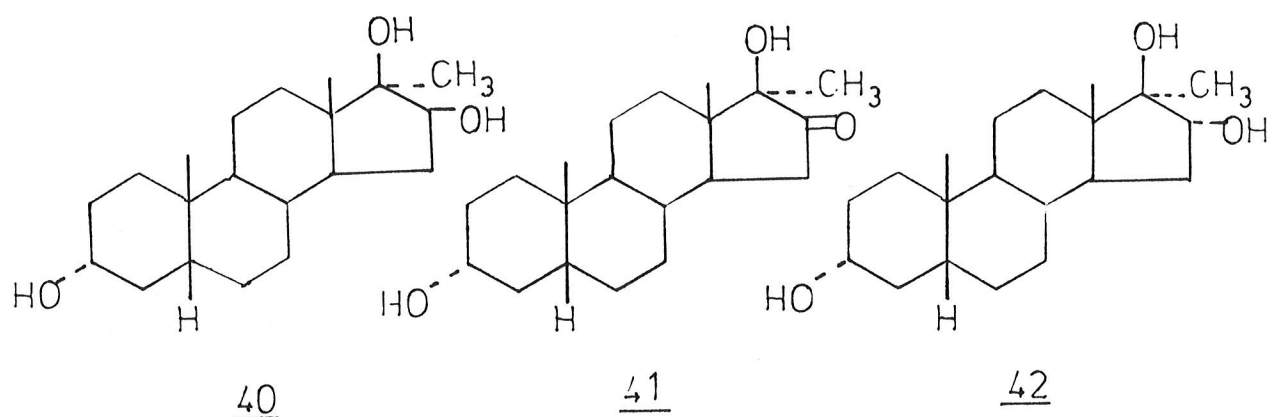
The urinary metabolic products in healthy men after oral administration of 5 β -androst-1-enes (e.g. 17 β -hydroxy-5 β -androst-1-en-3-one) (147), of 5 α -androst-1-enes (e.g. 5 α -androst-1-ene-3,17-dione) (148), and also of 1,4-androstadien-3-ones (e.g. 17 β -hydroxy-1,4-androstadien-3-one) (149) were studied. The main features were that the C-1 double bond proved to be very resistant to metabolic reduction and that 6 β -hydroxylation occurred.

Following oral administration of tritiated 4-chloro-17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (39) to normal females, the radioactivity was mainly in the glucuronide fraction in urine. Compound 39, 4-chloro-6 β ,17 β -dihydroxy-17 α -methyl-1,4-androstadien-3-one, 4-chloro-16 β ,17 β -dihydroxy-17 α -methyl-1,4-androstadien-3-one and 4-chloro-6 β ,16 β ,

17 β -trihydroxy-17 α -methyl-1,4-androstadien-3-one were isolated and identified as urinary metabolites (150,151). Following oral administration of compound 39 to women in their menopause, twenty-one metabolites were observed and 4-chloro-6 β ,17 β -dihydroxy-17 α -methyl-1,4-androstadien-3-one, 4-chloro-16 β ,17 β -dihydroxy-17 α -methyl-1,4-androstadien-3-one and 17 β -hydroxy-17 α -methyl-4,6-androstadien-3-one were identified (152).

Metabolism of Synthetic 17 α -methylandrostane Derivatives in Animals

Administration of 17 β -hydroxy-17 α -[¹⁴C-methyl]-4-androsten-3-one by stomach tube to normal rats led to the excretion of 57 to 90% of the metabolites in faeces and 14 to 41% in urine showing quantitative recovery of the dosed material (153). In animals with a ligated bile duct, 91 to 100% of the dosed material was excreted in the urine and less than 8% in the faeces; when the bile duct was cannulated, one-third of the dose was excreted in the urine and two-thirds in the bile (128). The above results show that absorption of the dosed material from the gastrointestinal tract was complete. It was also observed that the 17 α -methyl group was not lost in either animals or in humans. In the rabbit, as in man, the introduction of a 17 α -methyl group into the testosterone molecule seemed to direct the reduction of the C-4 double bond in ring A preferentially to the 5 β reduced compound and the ratio of the major metabolic products, 17 α -methyl-5 β -androstan-3 α ,17 β -diol (33) to 17 α -methyl-5 α -androstan-3 β ,17 β -diol (35) is 4.6:1 (154). The introduction of the 17 α -methyl group also affected the orientation of hydroxylation; orally dosed 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) yielded mainly 17 α -methyl-5 β -androstan-3 α ,16 β ,17 β -triol (40) as urinary metabolic products as well as the 16-ketone (41) and 16 α -compound (42) (155). In contrast, testosterone was hydroxylated preferentially at the 16 α -



position (155). The metabolic products in faeces after oral administration of 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one to rats were identified as 17 α -methyl-5 β -androstane-3 α ,17 β -diol (33) and 17 α -methyl-5 α -androstane-3 β ,17 β -diol (35) by mass spectral analysis, and only small amounts of metabolic products were excreted in the urine (156).

Metabolism of Synthetic Androgens *In Vitro*

The companion of *in vitro* metabolic studies of 17 β -hydroxy-17 α -methyl-4-androsten-3-one and 17 β -hydroxy-4-androsten-3-one by liver from different species, namely rat, rabbit, guinea pig, dog, monkey, chicken and human, showed that the livers from these species were not able to produce 17-ketosteroids from 17 β -hydroxy-17 α -methyl-4-androsten-3-one whereas all were able to produce 17-ketosteroid from 17 β -hydroxy-4-androsten-3-one (157). The results showed that liver enzymes are not able to split off the 17 α -methyl group.

19-Nortestosterone and 18-nortestosterone were metabolized by the 5 α -reductase present in the microsomal fraction of male and female rat liver at about two-thirds of the rate of testosterone (158). A similar rate was obtained from incubation of 19-nortestosterone with female rat liver. In the latter dose, the metabolic products were 19-nor-5 α -

androstane-3 β ,17 β -diol and 3 β -hydroxy-19-nor-5 α -androstan-17-one. The ratio of 3 α :3 β metabolic products is 5:1 (159).

A number of studies have been carried out concerning the effect of various substituents introduced into the testosterone molecule, on the reduction of the 4-en-3-one group. Rongone (160) studied an enzyme system present in bovine blood albumin which was capable of reducing the double bond at C-4 in the presence of NADPH. In testosterone, 19-nor-testosterone, 17 α -ethyl-19-nortestosterone, testosterone propionate and 2 α -fluorotestosterone, the C-4 double bonds were all reduced up to 90% or more. On the introduction of 17 α -methyl or 6 α -methyl group, the C-4 double bond was reduced 89% and 75% respectively, but on the introduction of 7 α -methyl or 2 α -methyl group, the reduction is 26% and less than 10% respectively (128). With the cytosol fraction of liver homogenate from male rats, the introduction of a halogen atom at the 2 α , 4 α or 6 β -position of testosterone molecule increased not only the rate of reduction of the 4-en-3-one, but also led to reduction of the 3-keto group prior to that of the double bond with the formation of an allyl alcohol (161). These reductions were presumably carried out by the same enzyme as that for the saturated ketone. With 6 α -fluorotestosterone, the reduction of 4-en-3-one to saturated 3-hydroxy steroid occurred more readily than to the 3-hydroxy-4-ene steroid, but with testosterone and androst-4-ene-3,11,17-trione, none of the 3-hydroxy-4-ene compound was produced. The introduction of a methyl group at the 2 α , 4, 6 α or 6 β -position stabilized the 4-en-3-one system and the rate of reduction was decreased. Similar results were obtained with 4-chloro-17 β -hydroxy-4-androsten-3-one using human liver (162), eight metabolic products were

identified and in all the double bond at C-4 was retained. These results indicate that introducing the chlorine atom prevented the reduction of the double bond but not reduction of the 3-keto group or oxidation of the 17 β -hydroxyl group. The introduction of a 17 α -methyl group into testosterone did not influence the 5 α -reductase in the microsomal fraction of female rat liver (163), but this enzyme was inhibited by the introduction of 1 α ,1 β ,16 α or 16 β -methyl groups. However, the 17 α -methyl group did not inhibit the reduction of the 4-en-3-one. The 5 β -reductase in the cytosol fraction of rat liver was affected differently; 1 β - and 16 β -methyl groups had no inhibitory effect, and 16 α - and 17 α -methyl groups had a weak effect, while the 1 α -methyl group had a strong inhibitory effect (163). It was suggested that interaction of testosterone with 5 β -reductase occurs on the α -side which extends to C-16 and C-17 while interaction with 5 α -reductase occurs on β -side (see structure activity relationship section). In contrast to testosterone, 9 β ,10 α -testosterone (retrotestosterone) on incubation with the microsomal and cytosol fraction of rat liver was not attacked by the 5 α - and 5 β -reductases or by the 3 α - and 3 β -hydroxy steroid dehydrogenases, but oxidation of 17 β -hydroxyl group did occur showing that the 17 β -hydroxy steroid dehydrogenase was not inhibited (164).

The major metabolic products of 5 α -androstane-3 β ,17 β -diol on incubation with canine prostate slices were 5 α -androstane-3 β ,7 α ,17 β -triol (80%) and 5 α -androstane-3 β ,6 α ,17 β -triol (20%) (165). This study has two enzymatic hydroxylases responsible for respective triol formation; 6 α -hydroxylase and 7 α -hydroxylase which are both located in the particular fraction of the prostate. Using a variety of C-19 androstane

substrates, 6 α - and 7 α -triols were found to be the major components of the total 3 β -hydroxy-5 α -androstane metabolites produced by canine prostate (165) and by human prostate (166,167). In contrast to the prostates of human and canine, the rat prostate formed predominantly 5 α -androstane-3 β ,6 α ,17 β -triol and a small amount of 5 α -androstane-3 β ,7 α ,17 β -triol (168,169). Because neither 6 α - nor 7 α -triols have any androgenic activities when tested in bioassays in the rat (134) and because the major pathway for triol formation is *via* the 3 β -diol directly, it is possible that the 3 β -triols function as a common final pathway for the termination of androgenic activities of the C-19 androstanes in the canine prostate. This possibility was strengthened by the observation from the *in vitro* study of ³H-testosterone with canine prostate that the major radioactive product was the 5 α -reduced 7-hydroxylated compound (170).

Metabolic Hydroxylation of Androgens *In Vivo* and *In Vitro*

The ability of human foetal liver microsomes to carry out hydroxylations on 4-en-3-one steroids has been demonstrated in both testosterone (171-174) and progesterone (175,176). After incubating testosterone with microsomes of human foetal liver, 6 β ,17 β -dihydroxy-4-androsten-3-one (171), 1 β - and 2 β ,17 β -dihydroxy-4-androsten-3-one (172), 17 β ,18 β -dihydroxy-4-androsten-3-one (173) were isolated and identified. 6 β ,17 β -Dihydroxy-4-androsten-3-one and 6 β -hydroxy-4-androstene-3,17-dione were also found by a study of tissue slices of adult human liver with testosterone (120). The presence of 6 β -hydroxylase activity in the foetal liver microsomes indicates that the foetal liver (171,177), as well as the placenta (178,179), is able to form 6 β -hydroxy C-19 steroids. Hydroxylation of phenolic steroids at the 6 α -position has been

demonstrated to occur in the foetal liver (177) as well as in the placenta (179). Both 6α - and 6β -hydroxy steroid dehydrogenase are present in the human liver (180).

The conversion of testosterone to 1β - and $2\beta,17\beta$ -dihydroxy-4-androsten-3-one by human foetal liver microsomes was demonstrated and they were the first examples of the hydroxylation of a C-19 steroid at 1β -position in an animal tissue and at 2β -position in a primate (172). The microbial hydroxylation of steroids at C-1 is relatively rare (181) and *Xylaria* and *Haplosoyella* species have been found to hydroxylate 4-en-3-one C-19 steroids at 1β -position (182). 1β -Hydroxylation of 5β - C-21 steroids in mammalia has been demonstrated (183). The activity of liver 1β -hydroxylase on testosterone might explain the origin of the C-1 unsaturated steroid isolated (184). Also, the formation of $1\beta,17\beta$ -dihydroxy-4-androsten-3-one by human foetal liver microsome may proceed *via* the introduction of a C-1 double bond during the aromatization of C-19 steroids (172, 185).

2β -Hydroxylation of steroids is much more common than 1β -hydroxylation. 2β -Hydroxy steroids have been isolated from animal tissues (123, 124, 186) as well as from plants after microbial transformation (181). The importance of 2β -hydroxylation is not known, but it may be possible that $2\beta,17\beta$ -dihydroxy-4-androsten-3-one may serve as a precursor of the 2-oxygenated oestrogens eliminated in human urine (171).

The isolation of 1β -hydroxylated C-19 steroids from human urine was first reported by Fukushima *et al.* (187) who suggested that this steroid was of extra-adrenal origin. The finding of $17\beta,18\beta$ -dihydroxy-4-androsten-3-one incubation of testosterone with human foetal liver microsomes demonstrated for the first time that liver enzymes can

hydroxylate a 4-en-3-one C-19 steroid at the C-18 position (173).

The incubation of testosterone with microsomal fraction of human foetal liver yielded 4-androstene-3 α ,17 β -diol. Incubation of testosterone with the supernatant fraction of human foetal liver formed 4-androsten-3 α - and 3 β ,17 β -diols, and incubation with liver slices of human foetal liver yielded 4-androstene-3 α ,17 β -diol and 3 α - and 3 β -hydroxy-4-androsten-17-one (174). In previous similar experiments, testosterone was found to be hydroxylated in several positions (1 β ,2 β ,6 β ,18 β - positions) (171-173). However, it has been demonstrated that 5 α -5 β -oxidoreductases have been absent in some tissues [such as kidney (188)], and as a result, the 4-androstene-3,17-dione was converted to 3-hydroxy-4-ene metabolic products (189). The absence of saturated metabolic products, after incubation of testosterone with fractions of supernatant or of microsomes, or with slices of human foetal liver, indicates that there is a very low activity or absence of 5 α - and 5 β -reductase in the human foetal liver at mid-pregnancy. The absence of ring reductase in the liver preparation probably explains the formation of the allylic alcohols. It was suggested that this metabolic pathway was a detoxification mechanism to inactivate testosterone into a reversible form allowing further metabolism. The conversion of allylic alcohols, 3 α - and 3 β -hydroxy-4-ene steroids, to 4-en-3-one steroids has been demonstrated in liver (190), testis (191) and adrenal preparations (192).

It has been well established that testosterone can be metabolized *in vitro* with rat liver by several enzymes, such as oxido reductase (193), ring reductase (157,188) and hydroxylase (194). However, the nature of the resulting metabolic products of testosterone is not

completely known and some of the possible pathways of testosterone in-activation remain to be investigated. Numerous investigations have been carried out with rat liver microsomes to study the hydroxylation of testosterone and several metabolic hydroxylated testosterone derivatives have been reported; monohydroxylation occurring at $2\beta, 6\beta, 7\alpha, 16\alpha$ -positions (123,124,125,194,195), 6α -position (196), and 18 - position (127); a dihydroxylation occurring at $6\beta, 16\alpha$ -position which was tentatively identified as $6\beta, 16\alpha, 17\beta$ -trihydroxy-4-androsten-3-one (123), while other metabolic products remain unidentified.

After incubation of rat liver microsomes with testosterone and $6\beta, 17\beta$ -dihydroxy-4-androsten-3-one, the metabolite was identified as 5α -androstane- $3\alpha, 6\beta, 17$ -triol (197). From the incubation of testosterone with microsomes of adult male liver, $2\beta, 6\beta, 7\alpha$ - and $16\alpha, 17\beta$ -dihydroxy- 5α -androstane-3-ones were isolated, whereas the incubation of 17β -hydroxy- 5α -androstane-3-one gave $2\beta, 7\alpha$ - and $16\alpha, 17\beta$ -dihydroxy- 5α -andros-
tan-3-one, but no 6β -hydroxy compound was found (124). This result demonstrated that the hydroxylated products of testosterone can be further metabolized by microsomal 5α -reductase to form hydroxylated derivatives of 17β -hydroxy- 5α -androstan-3-one. The presence of 5α -oxido reductase in rat liver microsomes has been demonstrated (198,199); whereas the 5β -oxido reductase activity (199) of rat liver was found in the soluble fraction.

The presence of 16α -hydroxylase in mammalian liver of dog (200) and of rat (201) has been demonstrated by *in vitro* studies with testosterone (123,202).

Studies of the foetal excretion of steroid metabolites in germ



free rats have shown the presence of C-19 5 α -steroids which were hydroxylated at either the 7 α - or 11 β - positions (203). The formation of 7 α ,17 β -dihydroxy-5 α -androstan-3-one in rat liver microsomes indicates one of the possible origins of these foetal metabolites (203).

The formation of androstane-triols after the incubation of testosterone with microsomes of adult male rat liver has been reported and the structures identified as 5 α -androstane-2 β ,3 α ,17 β -triol, 5 α -androstane-3 α ,7 α ,17 β -triol and 5 α -androstane-3 α - and 3 β ,16 α ,17 β -triols (125). Incubation of 2 β - and 7 α ,17 β -dihydroxy-4-androsten-3-one gave the respective androstane-triols with the 3 α -configuration whereas incubation with 6 β - and 16 α ,17 β -dihydroxy-4-androsten-3-one resulted in the formation of both 3 α - and 3 β -isomers of the respective androstane triols (125). After incubation with 17 β -hydroxy-5 α -androstan-3-one, the following compounds were isolated: 5 α -androstane-2 β ,3 α - (and 3 β), 17 β -triol, 5 α -androstane-3 α - (and 3 β), 7 α ,17 β -triol and 5 α -androstane-3 α - (and 3 β), 16 α ,17 β -triol; no 6 β -hydroxy compound was found (125). When 5 α -androstane-3 α ,17 β -diol was incubated, only 5 α -androstane-2 β ,3 α ,17 β -triol was isolated, and the incubation of 5 α -androstane-3 β ,17 β -diol with rat liver microsomes did not produce hydroxylated compounds (125). These findings indicate a substrate specificity for the 2 β ,6 β ,7 α - and 16 α -hydroxylases. The same hydroxylated metabolic products were isolated from testosterone incubated with liver microsomes from male germ free rats as with liver microsomes from conventional rats (203). As shown above, both testosterone and its saturated derivatives are hydroxylated, the latter compounds sometimes to a greater extent than testosterone. It was found that the activity of the 16 α -hydroxylase (204) of lyophilized rat liver microsomes was higher for the 3-hydroxy-5 α - (and 5 β -)-androstan-17-one

epimers than for 4-androsten-3,17-dione and for testosterone. These results are in agreement with the results found earlier that the saturated C-19 steroids are better substrates for hydroxylation than are their 4-en-3-one analogs (205).

However, the results of all saturated metabolic products of testosterone show that they are not hydroxylated to the same extent as testosterone (124,125). These results were summarized in Figure 5. It can be concluded that 17 β -hydroxy-5 α -androstan-3-one cannot serve as a substrate for 6 β -hydroxylases and that 5 α -androstan-3 α ,17 β -diol is only hydroxylated at 2 β position. 5 α -Androstane-3 β ,17 β -diol remains unchanged even after 90 minutes incubation with a NADPH-regenerating system which indicates a substrate specificity of the four hydroxylases and shows that the hydroxylation of C-19 steroid at positions 2 β ,6 β ,7 α , and 16 α occurs at different stages in the reductive metabolism of testosterone. The difference between the hydroxylation of 3 α -hydroxy-5 α (and 5 β)-androstan-17-one (204) and the lack of 16 α -hydroxylation of 5 α -androstan-3 α - (and 3 β), 17 β -diol (125) can only be explained by a different metabolic pathway for 17-keto and 17-hydroxy C-19 steroids ("17-keto and 17 β -hydroxy pathway") (78). To support this different pathway for the 17-keto and 17 β -hydroxy compounds, the metabolism of some 17-keto C-19 steroids, such as 4-androsten-3,17-dione, 5 α -androstan-3,17-dione and 3 α - (and 3 β)-hydroxy-5 α -androstan-17-one has been studied with rat liver microsomes (206) and the following metabolites were isolated: 3 α - and 3 β ,16 α -dihydroxy-5 α -androstan-17-one, 3 α - and 3 β ,7 α -dihydroxy-5 α -androstan-17-one, 3 β ,7 β -dihydroxy-5 α -androstan-17-one and 3 β ,17 β -dihydroxy-5 α -androstan-16-one. For 17 β -hydroxy C-19 steroids, the number of hydroxylation sites decreased with increasing saturation

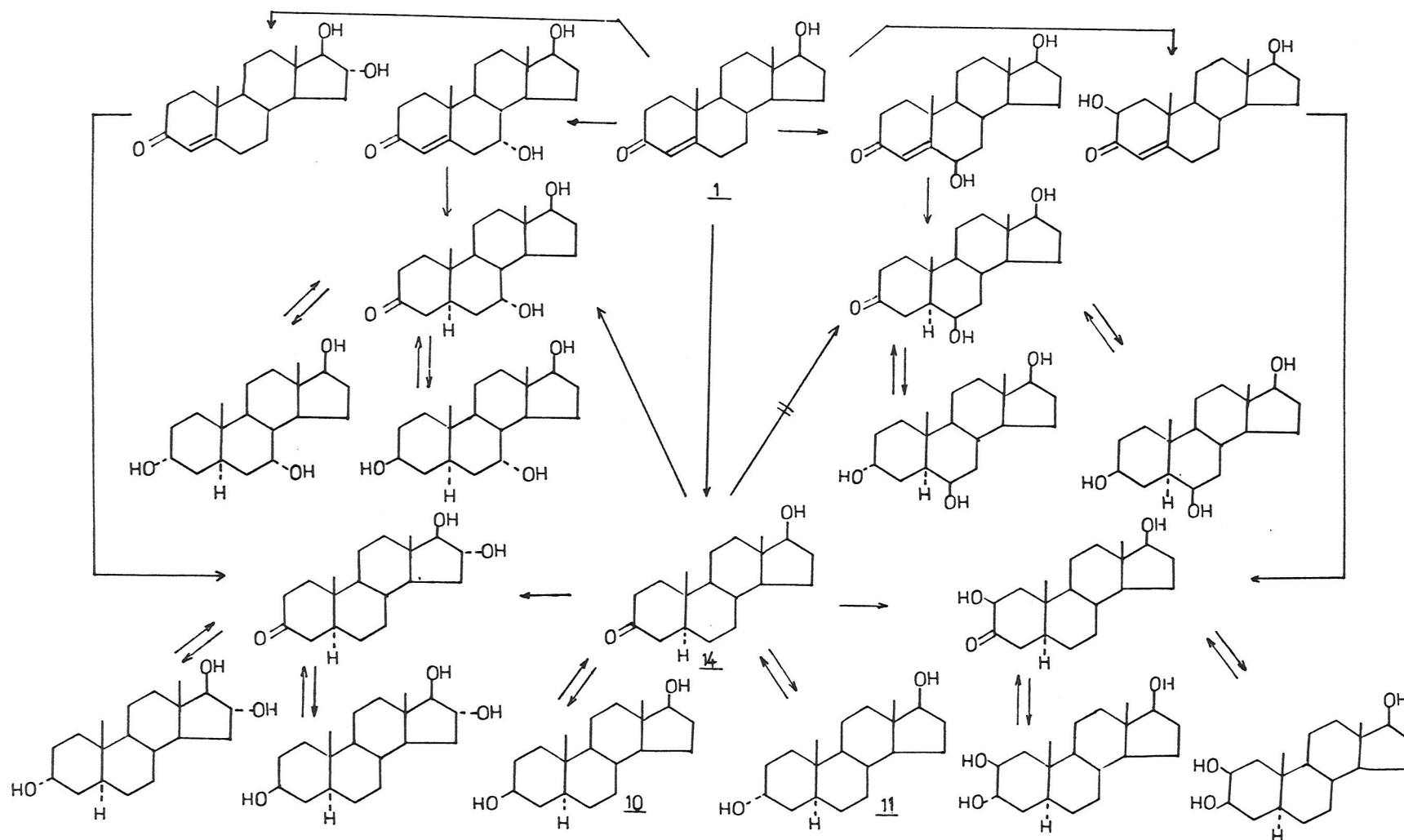


Figure 5. Metabolic interrelationship between testosterone (1), 17β-hydroxy-5α-androstane-3-one (14) and 5α-androstane-3α and 3β,17β-diol (10 and 11), and their hydroxylated derivatives in rat liver microsomes (125).

of ring A (123-125), whereas for 17-keto C-19 steroids the number of hydroxylation sites increased with increasing saturation of ring A.

The 15 α -hydroxylase for C-19 steroids present in rat liver microsomes is not active on 4-androstene-3,17-dione, but is active on ring A saturated steroids (e.g. 5 α -androstane-3,17-dione and 3 β -hydroxy-5 α -androstan-17-one) (207,208). The hydroxylated compound from 5 α -androstane-3,17-dione and 3 β -hydroxy-5 α -androstan-17-one was identified as 3 β , 15 α -dihydroxy-5 α -androstan-17-one and no 3 α -alcohol was observed. However, 5 α -androstane-3 α ,15 α ,17 α -triol and 3 α ,15 α -dihydroxy-5 α -androstan-17-one have been found in faeces from germ free and conventional rats (209). This 3 α ,5 α -configuration could possibly be formed by cytoplasmic isomerization of 15 α -hydroxylated 3 β ,5 α -steroids of microsomal origin. Also 17-keto, but not 17 β -hydroxy steroids, are substrates for rat liver microsomal 15 α -hydroxylases (207). The activity of 15 α - and 15 β -hydroxylases as well as 2 α ,6 α ,6 β ,7 α ,16 α -hydroxylases on progesterone was demonstrated by the incubation of rat liver microsomes (208).

The finding of 6 α ,17 β -dihydroxy-4-androsten-3-one and 6 α -hydroxy-4-androstene-3,17-dione after incubation of testosterone with rat liver microsomes was the first indication of the presence in the rat liver of 6 α -hydroxylase acting on 4-en-3-one C-19 steroids (196). However, the 6 α - and 6 β -hydroxylated steroids and bile acids have been known to be present in the urine and bile of a number of mammalian species; for example, 6 α -hydroxylated C-21 steroids have been found in the urine of humans (210), monkeys (211) and rabbits (212). In addition, 6 α - and 6 β , 17 β -dihydroxy-4-androsten-3-ones have been isolated in human urine after intravenous administration of testosterone (213). Mouse and rat biles contain both 6 α -hydroxylated bile acids and 6 β -hydroxylated bile acids,

whereas hog bile contains only 6 α -hydroxylated bile acids (214,215). 6 α -Hydroxylation of progesterone by rat liver microsomes has been demonstrated (196,216). It has been demonstrated by incubation of 6 β -hydroxy testosterone that 6 α -hydroxylation of 4-en-3-one steroids in rat liver microsomes was not *via* epimerization of 6 β -hydroxylated precursors (124,125). Other mammalian tissues where 6 α -hydroxylation has occurred are human foetal liver microsomes (176), liver homogenate (217) and microsome preparation (218).

6 β -Hydroxylation of testosterone occurred with rat liver microsomes (123-125) but not with 17 β -hydroxy-5 α -androstan-3-one or 5 α -androstane-3 α - and 3 β ,17 β -diols which indicates a substrate specificity of the 6 β -hydroxylating enzyme.

The ability of rat liver microsomes to introduce a 18-hydroxyl group in C-19 steroids has been demonstrated (127). It was also found that the 18-hydroxylase is active on both 4-en-3-one and 5 α -C-19 steroids with a 17 β -hydroxyl function. The presence of 18-hydroxylase activity on both C-19 and C-21 steroids has been shown in mammalian adrenals of several species including adult humans (219), rat liver and in human foetal liver (173).

The presence of testicular 6 α - and 6 β -hydroxylases has been shown by the isolation of 6 α - and 6 β ,17 β -dihydroxy-4-androsten-3-one after incubation of testosterone with testicular preparation of adult rat (220). Their existence has also been demonstrated in rat liver microsomes (123,124,196), and in human foetal liver microsomes (171).

The presence of a 16 α -hydroxylase in testes has also been demonstrated (221). 4-En-3-one C-19 steroids were shown to be metabolized to

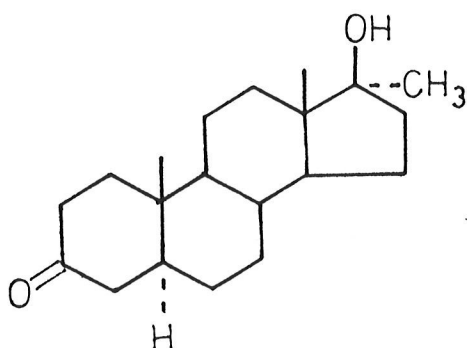
a very small degree by studies with pathological human testis tissue (222). After incubation of minced testicular tumor with $[4-^{14}\text{C}]$ androstene-3,17-dione, the following metabolites were tentatively identified; testosterone, $6\beta,17\beta$ -dihydroxy-4-androsten-3-one and androsterone (223). A 6β -hydroxylated derivative and $[4-^{14}\text{C}]$ -androstene-3,17-dione were isolated after incubation of gonadal homogenates with $[4-^{14}\text{C}]$ -androstene-3,17-dione (224).

Objectives

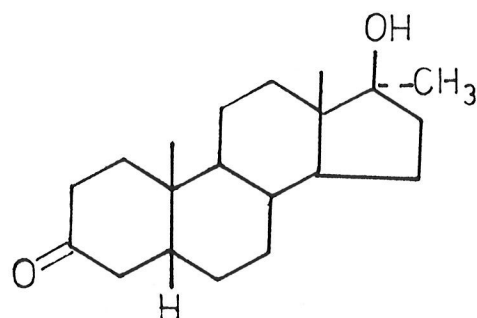
As metabolic reduction of the 4-en-3-one system in steroids occurs readily, 17β -hydroxy- 17α -methyl- 5α -androstan-3-one and 17β -hydroxy- 17α -methyl- 5β -androstan-3-one can be expected to be primary metabolic products of 17β -hydroxy- 17α -methyl-4-androsten-3-one. One of the objectives of this thesis is to study the relationship between the metabolic products of 17β -hydroxy- 17α -methyl- 5α -androstan-3-one (43), 17β -hydroxy- 17α -methyl- 5β -androstan-3-one (44) and 17β -hydroxy- 17α -methyl-4-androsten-3-one (13) to determine how reduction of the 4-en-3-one system influences the orientation of hydroxylation around the steroid rings. The study also includes 17β -hydroxy- 17α -methyl-1,4-androstadien-3-one (16), which incorporates a second conjugated double bond into ring A.

The reported (141-144) C-17 epimerization of the 17β -hydroxy/ 17α -methyl group of 17β -hydroxy- 17α -methyl-1,4-androstadien-3-one (16) in man is an unique biotransformation. Another objective is to study this biotransformation in the rabbit with chemical models (43,44,13) together with 17β -hydroxy- 17α -methyl-1,4-androstadien-3-one (16).

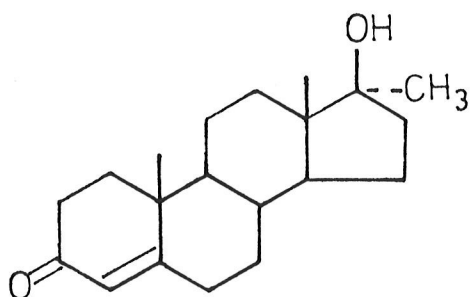
This research consisted of the isolation and identification of the rabbit urinary excretion products from the closely related compounds shown in Figure 6.



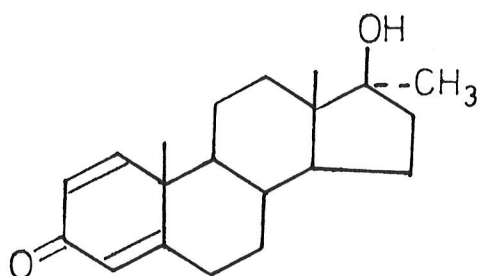
17β-Hydroxy-17α-methyl-
5α-androstan-3-one (43)



17β-Hydroxy-17α-methyl-
5β-androstan-3-one (44)



17β-Hydroxy-17α-methyl-
4-androsten-3-one (13)



17β-Hydroxy-17α-methyl-
1,4-androstadien-3-one (16)

Figure 6. Names and structures of four compounds which were studied in this thesis.

The specific objectives were as follows:

1. To isolate and identify the urinary excretion products of the synthetic androstanes.
2. To synthesize the 17α-methyl-5α- and 5β-androstane-3ξ,17β-diols and the epimeric 17β-methyl-5α- and 5β-androstane-3ξ,17α-diols, the 3-

keto derivatives of 17α -methyl- 5α - and 5β -androstan- 17β -ols and 17β -methyl- 5α - and 5β -androstan- 17α -ols, and also to establish their chromatographic (glc and tlc) and spectroscopic (ir, mass, ^1H -nmr) and ^{13}C -nmr) properties for comparison purposes with the metabolic products.

3. To study whether the metabolic hydroxylation occurs before or after the reduction of the C-4 double bond and also to study the orientation of metabolic hydroxylation of these synthetic steroids.
4. To study whether it is possible to predict the sequence of reduction and hydroxylation in synthetic steroids.
5. To study the metabolic changes caused by introduction of a methyl group at the C-17 position.
6. To compare the metabolism of the rabbit as an animal model to humans and to establish the structure of unidentified human metabolites.
7. To study the potential C-17 epimerization in the rabbit, which is on the basis of the following two assumptions: (1) that the presence of 17β -hydroxyl/ 17α -methyl function is sufficient for this epimerization to occur and is not dependent on the structure of A, B, C or D rings, and (2) that the epimerization can occur in the rabbit as well as in humans.
8. To determine the structure/metabolism relationship of hydroxylation for 17α -methyl androstane derivatives and other alicyclic molecules and to predict the general pattern of metabolic hydroxylation in alicyclic molecules.

Information obtained from metabolic studies and an understanding of the effects of substituents are useful in the designing of new drugs because compounds with altered profiles of activity can be obtained by

altering the biotransformations of a substance. The results of such studies give a better understanding of the structure-activity relationship in anabolic/androgenic molecules and the role which is played by transformation.

II. RESULTS AND DISCUSSION

Metabolism of 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43).

Thin-layer chromatography (tlc) and gas-liquid chromatography (glc) of the total crude neutral, acidic and enolic/phenolic fractions, which were obtained from the ether extraction of β -glucuronidase hydrolyzed urine of rabbits dosed with 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) showed the presence of metabolic products only in the total crude neutral fraction. The neutral fraction contained 25% of the dosed material.

A portion of the total crude neutral fraction (150 mg) was treated with Girard reagent T (225) to separate it into the ketonic (19 mg) and non-ketonic (alcohols, 121 mg) fractions. The glc of the alcohol fraction had peaks corresponding to peaks in the glc of the total crude neutral fraction. The ketonic fraction contained only a negligible quantity of material which was not considered any further.

Column chromatography of the total crude neutral fraction gave five metabolic products 34, 35, 43, 45 and 46 (Figure 7) comprising 75% of this fraction. 17 β -Hydroxy-17 α -methyl-5 α -androstan-3-one (43), 17 α -methyl-5 α -androstan-3 α ,17 β -diol (34) and 17 α -methyl-5 α -androstan-3 β ,17 β -diol (35) were identified by comparison of their melting points, and spectra (ir, mass, ^1H -nmr) with corresponding authentic samples which were synthetic (see syntheses in EXPERIMENTAL section). The two polar compounds were identified as 17 α -methyl-5 α -androstan-3 β ,15 α ,17 β -triol (45) and 17 α -methyl-5 α -androstan-3 α ,6 α ,17 β -triol (46) on the basis of their ir, mass, ^1H -nmr and ^{13}C -nmr spectra and of the ^1H -nmr spectra of their acetylation and oxidation products.

The stereochemistry of the hydroxyl at C-3 was assigned on the basis

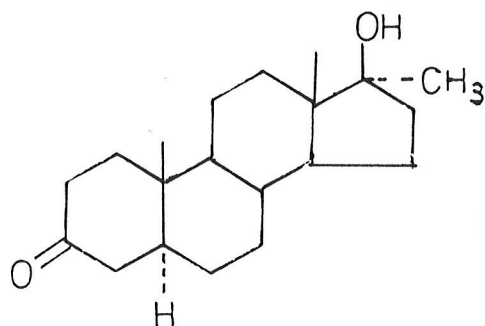
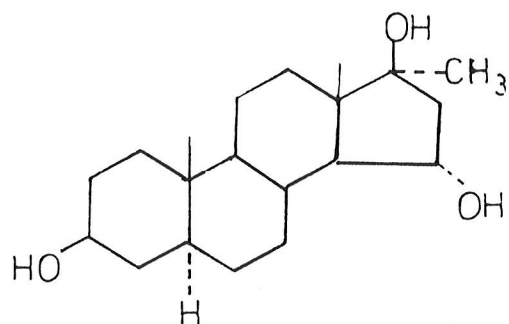
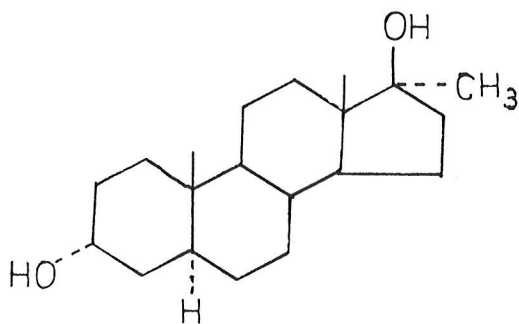
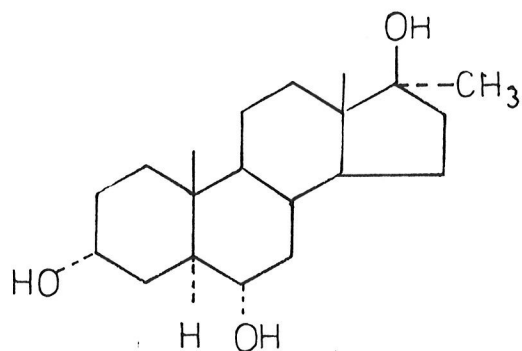
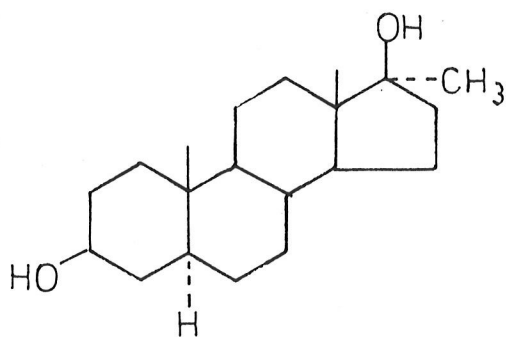
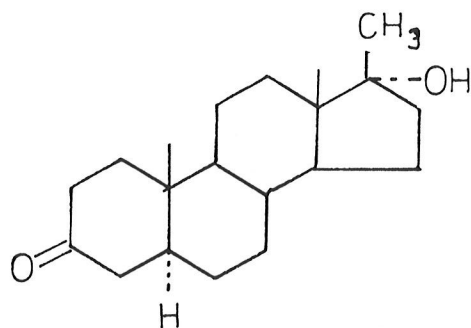
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Figure 7. Five metabolic products of 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) and 17-epimer of compound 43.

of the ^1H -nmr chemical shift and coupling constants of the downfield proton and the C-17 hydroxyl, stereochemistry was assigned on the basis of the chemical shift of the C-17 and C-13 methyl groups. The assignment of the structures were on the basis of the chemical shifts of the C-10 and C-13 methyl protons which are influenced by the new hydroxyl group and by the signals for the proton which is attached to the hydroxyl-group-bearing carbons (226). The assignment of the chemical shifts for the C-10 and C-13 methyl protons were made by comparison with calculations employing Zürcher's additivity tables (227) and the substituent effect on C-10 and C-13 methyl proton signals by Bridgeman *et al.* (226).

Calculations of the expected changes in chemical shifts for the C-10 and C-13 methyl groups of 17α -methyl- 5α -androstande- $3\beta,15\alpha,17\beta$ -triol (45) is as follows using 17α -methyl- 5α -androstande- $3\beta,17\beta$ -diol (35) as a base compound and by introducing the 15α -hydroxyl group as a substituent:

	(ppm)	
	<u>C-10 CH₃</u>	<u>C-13 CH₃</u>
Compound 35	0.82	0.84
15α -OH (226)	0.01	0.03
	0.83	0.87

These calculated values ($\delta = 0.83$ ppm for C-10 CH₃ and 0.87 ppm for C-13 CH₃) agree well with the values of ^1H -nmr obtained for compound 45 ($\delta = 0.83$ ppm for C-10 CH₃ and 0.86 ppm for C-13 CH₃). The pyridine shift of the C-13 CH₃ is 0.32 ($\Delta = \delta_{\text{C}_5\text{D}_5\text{N}} - \delta_{\text{CDCl}_3} = 1.18 - 0.86 = 0.32$ ppm) which is in better agreement with the solvent shift range (0.2 - 0.27 ppm) (228) for a vicinal hydroxyl function as 17β -hydroxy/ 17α -methyl than the 17α -hydroxy/ 17β -methyl (solvent shift range is 0.03 - 0.05 ppm) (228).

The stereochemistry of the hydroxyl groups were assigned from the characteristic chemical shifts, the splitting pattern (226) and coupling constant of the downfield protons (CHOH) and their band width at half-height ($W_{1/2}$) (229). Because the diaxial vicinal coupling constants are much larger than diequatorial or axial-equatorial coupling (230), coupling constants could be used to assign the configuration of hydroxyl groups in steroidal molecules. When an electronegative substituent such as a hydroxyl group, is attached to the steroid molecule, the geminal proton is shifted downfield. Such a proton will usually be adjacent to several others and will give a broad band in which the number of closely spaced lines due to spin-spin couplings are not readily determined. The width of such a band measured at one-half its height ($W_{1/2}$) will reflect the magnitude of the vicinal coupling constants. Therefore, an axial proton split by adjacent axial ($J \approx 9$ Hz) and equatorial protons ($J \approx 2$ Hz) should give a much wider band than an equatorial proton split by adjacent axial ($J \approx 2$ Hz) and equatorial protons ($J \approx 2$ Hz) (229). The band width at half-height due to an axial proton coupling (aa/ae coupling) is broad ($W_{1/2} = 20\text{--}30$ Hz) whereas, that due to an equatorial proton coupling (ae/ee coupling) is narrow ($W_{1/2} = 5\text{--}10$ Hz). These correlations were applied in assigning the stereochemistry of the hydroxyl group of all the metabolites.

17 α - Methyl - 5 α -androsterane-3 β ,15 α ,17 β -triol (45) gave two downfield CHOH absorptions at $\delta = 3.57$ ppm ($W_{1/2} = 27$ Hz) and $\delta = 4.05$ ppm ($J = 4, 10$ and 10 Hz); the absorption at 3.57 ppm corresponds to the 3 α -H which is an axial proton whose coupling constant is large (27 Hz) and the absorption at 4.05 ppm corresponds to the 15 β -H based on its coupling constants and chemical shift (226a). The chemical shift of the C-13 methyl group

effected by introducing the 15 α -hydroxyl substituent on compound 35 is 0.86 ppm and is in agreement with the calculated values of 0.87 ppm. Jones oxidation (231) of compound 45 gave a product whose ir spectrum showed absorptions of both 5- and 6-membered ring ketones; the 6-membered ring ketone corresponds to the 3-position and the 5-membered ring ketone could be either at C-15 or C-16 position in ring D. The chemical shifts of the C-17 and C-13 methyl groups were consistent with a C-15 but not with a C-16 ketone (226, 232). Calculation of the expected change in chemical shift of compound 45 for the C-10 and C-13 methyl groups caused by oxidation of the C-3 hydroxyl to a ketone and introduction of a 15-oxo or 16-oxo groups using 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) as a base compound and 15-oxo and 16-oxo as additional substituents is as follows:

	δ (ppm)	
	C-10 CH ₃	C-13 CH ₃
Compound <u>43</u>	1.03	0.88
15-oxo (226)	0.01	0.08
	1.04	0.96
Compound <u>43</u>	1.03	0.88
16-oxo (226)	0.04	0.17
	1.07	1.05

The chemical shifts obtained from the oxidation product were 0.96 (C-13 CH₃) and 1.03 (C-10 CH₃) ppm which corresponds to the 3-oxo-15-oxo compound, and not the 3-oxo-16-oxo compound. Acetylation of compound 45 gave a diacetate and from its ¹H-nmr spectrum, the chemical shift difference between the hydroxyl and acetate groups, on C-3 and C-15, $\Delta = (\text{>CHOH} - \text{>CHOAc})$ (226) was calculated as - 1.12 ppm for a C-3

hydroxyl group and -0.84 ppm for C-15 hydroxyl group (compare the data with those in Table 1).

Table 1. Chemical shifts of the proton attached to the carbon atom bearing the oxygen atom of alcohols and acetates on the steroid nucleus, and the chemical shift difference (Δ) between the hydroxyl and acetate groups as substrates.

<u>Position of alcohol and acetate</u> <u>Cyclohexane in 5α-series</u>	γ $\text{CHOH}(\delta)$	γ $\text{CHOAc}(\delta)$	Δ
1 α OH, 1 β H	(4.40)	(5.54)	(1.24)
1 β OH, 1 α H	(3.50)	(4.70)	(1.20)
2 α OH, 2 β H	3.79	4.91	1.12
2 β OH, 2 α H	4.17	5.10	0.93
3 α OH, 3 β H	4.05	5.02	0.97
3 β OH, 3 α H	3.55	4.70	1.15
4 α OH, 4 β H	3.43	4.68	1.25
4 β OH, 4 α H	3.82	4.95	1.13
6 α OH, 6 β H	(3.46)	(4.77)	(1.31)
6 β OH, 6 α H	(3.88)	(5.00)	(1.12)
7 α OH, 7 β H ^a	(3.85)	(4.93)	(1.08)
7 β OH, 7 α H	(3.55)	(4.60)	(1.05)
11 α OH, 11 β H	(4.00)	(5.25)	(1.25)
11 β OH, 11 α H	4.34		
12 α OH, 12 β H	3.80	4.99	1.19
12 β OH, 12 α H	(3.46)	(4.72)	(1.10)
<u>Cyclopentane</u>			
15 α OH, 15 β H _b	4.08	4.94	0.86
15 β OH, 15 α H ^b	(2.87)	(3.70)	(0.83)
16 α OH, 16 β H	4.47	5.22	0.75
16 β OH, 16 α H ^c	4.37	5.15	0.78
17 α OH, 17 β H ^c	(3.64)	(5.00)	(1.36)
17 β OH, 17 α H	3.63	4.62	0.95

unless otherwise stated, all data are from Reference 226a.

^aReference 237

^bReference 238

^cReference 232

Data in parenthesis () are from pairs of compounds containing several functional groups.

The calculation of chemical shifts of C-10 and C-13 methyl protons of 17 α -methyl-5 α -androsterane-3 α ,6 α ,17 β -triol (46) using 17 α -methyl-5 α -androsterane-3 α ,17 β -diol (34) as a base molecule and adding a 6 α -hydroxy substituent was as follows:

	δ (ppm)	
	C-10 CH ₃	C-13 CH ₃
Compound <u>34</u>	0.80	0.84
6 α -OH (226)	0.03	0.00
	0.83	0.84

These calculated values agree with those obtained for compound 46 [0.82 (C-10 CH₃) and 0.83 (C-13 CH₃) ppm]. The C-13 methyl pyridine solvent shift ($\Delta = \delta_{\text{CDCl}_3} - \delta_{\text{C}_5\text{D}_5\text{N}} = 0.83 - 1.13 = -0.30$ ppm) establishes the presence of a 17 β -hydroxy/17 α -methyl group.

The two downfield CHOH protons absorbed at 3.74 ppm (unresolved sextet, 3.75 ppm in C₅D₅N, $J = 5, 10.5$ and 10.5Hz) and at 4.13 ppm ($W_{1/2} = 9\text{Hz}$, 4.44 ppm in C₅D₅N, $W_{1/2} = 7\text{Hz}$). The absorption at 4.13 ppm with its narrow half height width corresponds to the equatorial 3 β -H. Therefore, one of the two hydroxylated groups (as indicated by mass spectrum) is 3 α -OH. The second downfield absorption (3.74 ppm) corresponds to the axial 6 β -H; the 6 α -OH is equatorial (226a). The Jones oxidation (231) product from compound 46 showed only a 6-membered ring ketone absorption in the ir spectrum and its ¹H-nmr chemical shifts were at 0.89 (C-13CH₃) and 0.98 (C-10CH₃) ppm.

These correspond to the calculated values shown below using 17 β -hydroxy-17 α -methyl-5 α -androsterane-3-one (43) as a base molecule and addition of a 6-oxo-substituent (226):

	δ (ppm)	
	<u>C-10 CH₃</u>	<u>C-13 CH₃</u>
Compound <u>43</u>	1.03	0.88
6-oxo (226)	-0.05	0.02
	0.98	0.90

These values are identical with the oxidation product of 17 α -methyl-5 α -androstane-3 β ,6 α ,17 β -triol which was isolated as one of the metabolites from 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) (see Metabolism of compound 13). The identification of 17 α -methyl-5 α -androstane-3 α ,6 α ,17 β -triol (46) was confirmed by ¹³C-nmr (APPENDIX). The elemental analysis confirmed the composition of the compound.

A portion of the total crude neutral fraction was treated with excess Jones reagent (231) to give a neutral product mixture. This mixture was separated into its two major components by thick layer plate chromatography. The more polar component gave a molecular ion $m/z = 318$ which corresponded to a diketone from the hydroxylated metabolites. The less polar component corresponded to 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) as demonstrated by glc, tlc, ms and ¹H-nmr.

Thin layer chromatography distinguished 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) from its 17-epimer, 17 α -hydroxy-17 β -methyl-5 α -androstan-3-one (47), whereas the glc system did not separate 43 and 47. The presence of 47 was not detected in the total crude neutral fraction of 43, indicating that no non-hydroxylated epimer was formed.

The major metabolic products found in the urine of rabbits which were dosed with compound 43 contained more of the 3 β -hydroxyl than 3 α -hydroxyl metabolites; the ratio of 3 α :3 β hydroxyl product was 1:2.5 (Table 2).

Table 2: Neutral Urinary Metatolic products from rabbits dosed orally with 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43)

<u>Compound</u>	<u>Relative percentage^a</u>
17 β -Hydroxy-17 α -methyl-5 α -androstan-3-one (43)	2.5
17 α -Methyl-5 α -androstan-3 α ,17 β -diol (34)	5.5
17 α -Methyl-5 α -androstan-3 β ,17 β -diol (35)	57.0
17 α -Methyl-5 α -androstan-3 β ,15 α ,17 β -triol (45)	13.0
17 α -Methyl-5 α -androstan-3 α ,6 α ,17 β -triol (46)	22.6

^a Estimated from column fraction weights. 75% of the neutral fraction was recovered as the above metabolites consisting of 25% of the dosed material.

^b Hydroxylated metabolites (35%)

This result contrasts with that obtained in similar experiments when rabbits were dosed with 17 β -hydroxy-2 α -methyl-5 α -androstan-3-one (232) or 17 β -hydroxy-2 α -hydroxymethylene-5 α -androstan-3-one (233), where the predominantly excreted products were 3 α -hydroxylated metabolites. These results suggested that the presence of the 2 α -substituent sterically favors the formation of the 3 α -hydroxylation which is consistent with earlier work on 2 α -methyl steroids (115).

The two polar metabolites isolated contained hydroxyl groups at C-6 α and C-15 α . The hydroxylation at C-15 rather than at C-16 does not appear to be a consequence of the presence of the 17 α -methyl group alone because an equivalent amount of C-15 α hydroxylation was obtained when 2 α -methyl-17 β -hydroxy-5 α -androstan-3-one was similarly administered to rabbits (115). However, when 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) (155, also see Metabolism of 13) was administered to the rabbit, C-16 hydroxylated metatolites were obtained with the 5 β -configuration.

Therefore, C-15, as opposed to C-16, hydroxylation from compound 43 may be the result of the configuration at C-5. It has been shown earlier that the 15 α -hydroxylase for C₁₉ steroids in rat liver microsomes was not active on 4-androsten-3,17-dione, but active only on the A ring saturated 5 α -androstan-3,17-dione and 3 β -hydroxy-5 α -androstan-17-one. Furthermore, only 3 β ,15 α -dihydroxy-5 α -androstan-17-one was formed, whereas no 3 α ,15 α -dihydroxy-5 α -androstan-17-one could be found in the incubation mixture of 5 α -androstan-3,17-dione and 3 α - and 3 β -hydroxy 5 α -androstan-17-one (207). These results indicate that 15 α -hydroxylase is stereospecific toward the 3 β -hydroxy and 5 α -configuration which was found in our result in compound 45, 17 α -methyl-5 α -androstan-3 β ,15 α , 17 β -triol and also the inactivity of 15 α -hydroxylase toward 4-en-3-one system was shown in metabolism of compound 13.

It is well established that 17 β -hydroxy-4-androsten-3-one is hydroxylated at the 6 β - as well as the 2 β , 7 α , and 16 α -positions on incubation with rat liver microsomes (123, 194, 235, 236). The presence of 6 α -hydroxylase in rat liver microsomes (196), and in testicular preparation of adult rats (220) was established, and its presence has been reported in human foetal liver microsomes (176).

The involvement of the 3,5-dienol system in the metabolic 6 β -hydroxylation of testosterone (235, 239) is generally accepted. Incubation of (6 β -²H) testosterone (48) with rat liver microsomes (239) yielded 6 β -hydroxylated product (30) which did not contain any deuterium (Figure 8). This result is consistent with two possible reaction pathways, either (1) direct replacement of the axial 6 β -deuterium with hydroxyl, or (2) involvement of the 4-en-3-one group *via* an enzymatically induced enolization of the 4-en-3-one system with a stereospecific loss

of the 6β -deuterium to form the enol (49) followed by hydroxylation of the enzyme enol-complex.

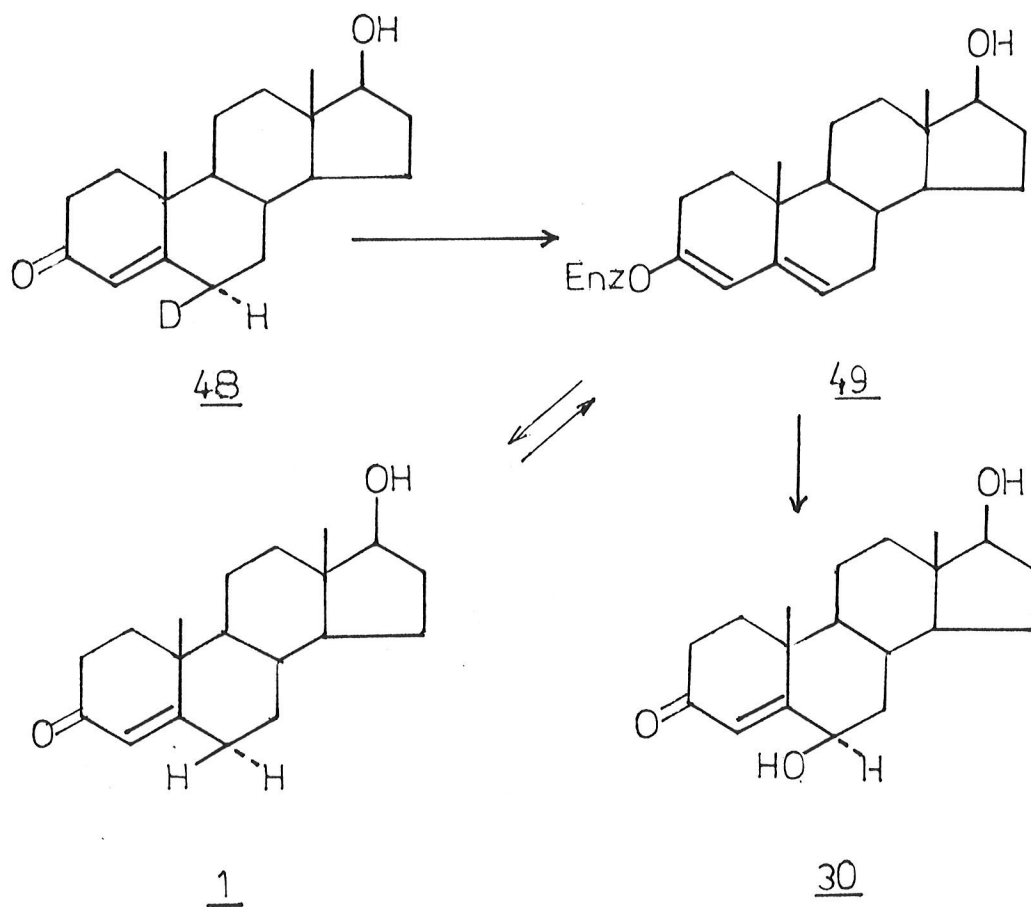


Figure 8. Possible Pathways for 6β -hydroxylation *via* enolization of (6β - ^2H) testosterone in the rat liver microsomes (239).

If the role of an enzyme is merely to induce enolization, it would cause preferential loss of the proton from the 6β -position rather than from the 6α -position, and then the 6β -hydroxy product (30) would contain some deuterium (240,241). Furthermore, Gustafsson (124) have studied the *in vitro* metabolism of testosterone (1) and 17β -hydroxy- 5α -androstan-3-one (14) with rat liver microsomes and hydroxylations occurred at

position 2 β , 7 α and 16 α , but no 6 β -hydroxylation in compound 14 occurred. The fact that testosterone is metabolized by this system to 6 β -hydroxylated products also indicates that the 4-en-3-one system plays a role in 6 β -hydroxylation. It is conceivable that an enzyme-enol complex could serve as an intermediate for other metabolic reactions, such as hydroxylation of the C-4 double bond (239).

The isolation of a 6 α -hydroxylated metabolite, 17 α -methyl-5 α -androsterane-3 α ,6 α ,17 β -triol (46) from 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) in rabbit urine suggests that the C-4 double bond may not be a requirement for the hydroxylation of C-6 in the rabbit and that 6 α -hydroxylation might occur directly on the saturated ring A system followed by the reduction of 3-ketone to 3 α -hydroxy and that it does not occur *via* epimerization of the 6 β -hydroxylated precursor. The latter is supported by the experiment which was carried out by Gustafsson (124, 125) on the incubation of 6 β ,17 β -dihydroxy-4-androsten-3-one (30) with rat liver microsomes which did not result in the formation of any 6 α -hydroxylated metabolite. So far, the mechanism of 6 α -hydroxylation is not known. However, the conversion of testosterone to 6 α ,17 β -dihydroxy-4-androsten-3-one by rat testicular preparation suggests the possibility of a pathway for the formation of 6 α -hydroxy-oestradiol in the rat testis *via* aromatization of hydroxylated 4-en-3-one steroid (220).

Metabolism of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44)

Tlc and glc of the total crude neutral, acidic and enolic/phenolic fractions obtained from ether extraction of β -glucuronidase hydrolyzed urine of rabbits which were dosed with 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44) showed the presence of metabolic products only in the

total crude neutral fraction which was 69% of the dosed steroid. A portion of the total crude neutral fraction (145 mg) was treated with Girard reagent T (225) to separate it into the ketonic (10 mg) and non-ketonic (alcohols, 135 mg) fraction. Glc of the alcohol fractions showed the same components as the glc of the total crude neutral fraction, and the ketonic fraction contained only a negligible quantity of material, which was not considered further. Column chromatography of the total crude neutral fraction gave five metabolic products, 33, 40, 42, 50 and 51 (Figure 9) which consisted of 95% of the total crude neutral fractions (Table 3). The results of the glc of the total crude neutral fraction and isolated metabolic products with the internal standards are in the Table 4.

In glc, the two compounds 50 and 42 co-chromatographed and did not separate even as their TMS derivatives (Table 4) whereas they are separated by column chromatography and by tlc. The relative percentage (Table 3) of the compounds isolated by column chromatography corresponded to the relative percentage of each components calculated by glc results (Table 3). There was no evidence for the presence of the starting material 44.

The major metabolic product 33 (89.5%) was identified by comparison of its melting point (mixture melting point was not depressed) and spectra (ir, mass, ^1H -nmr and ^{13}C -nmr) with the synthesized authentic sample (see syntheses) and with the reported data (140, 155). The four hydroxylated metabolic products 40, 50, 51 and 42 were identified by their spectra (ir, mass, ^1H -nmr and ^{13}C -nmr) and the spectra of their acetylation and oxidation products.

The stereochemistry of the C-3 hydroxyl group and the newly introduced

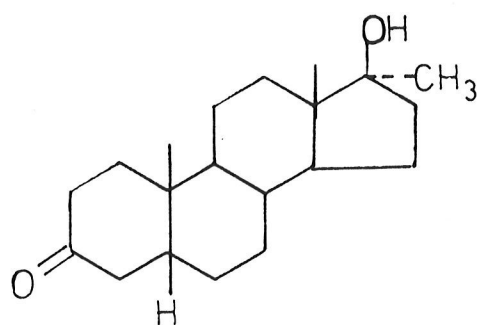
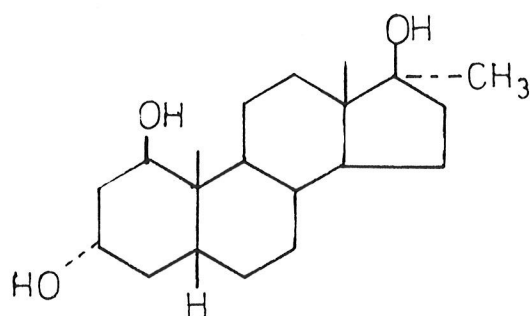
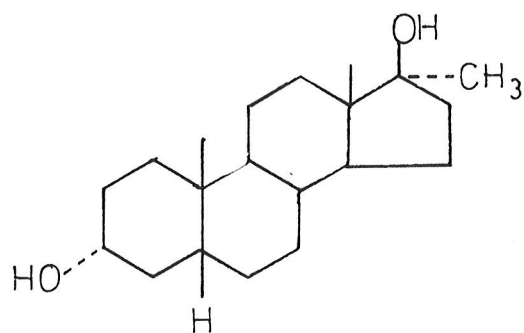
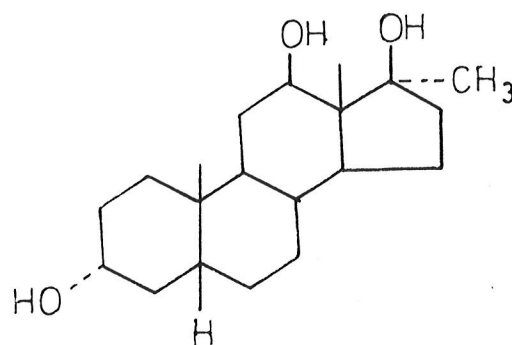
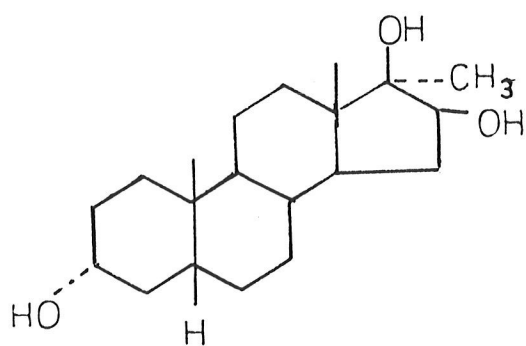
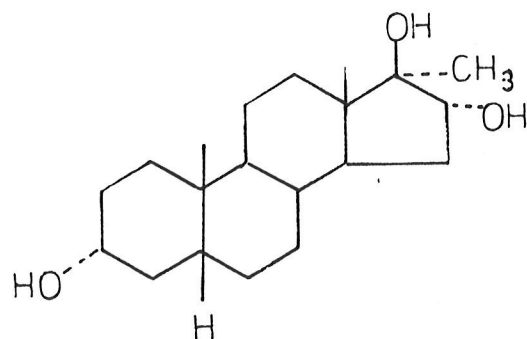
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Figure 9. Five metabolic products of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44).

Table 3. Urinary Metabolic products from rabbits dosed orally with 17 β -hydroxy-17 α -methyl-5 α -androstane-3-one (44)

Compound		Eluted with ^a	R _f ^b	Mp(^o C)	Relative percentage ^c
17 α -Methyl-5 β -androstane-3 α ,17 β -diol	(33)	10% ether/Bz	0.54 (blue-grey)	164-5 ^o	89.5
17 α -Methyl-5 β -androstane-3 α ,16 β ,17 β -triol	(40)	2.5-5% MeOH/ether	0.32 (light-grey)	254-5 ^o	2.4
17 α -Methyl-5 β -androstane-3 α ,1 β ,17 β -triol	(50)	5% MeOH/ether	0.12 (purple)	206-8 ^o	0.8
17 α -Methyl-5 β -androstane-3 α ,12 β ,17 β -triol	(51)	5% MeOH/ether	0.20 (purple or green)	179-180 ^o	3.8
17 α -Methyl-5 β -androstane-3 α ,16 α ,17 β -triol	(42)	5% MeOH/ether	0.11 (dark green)	214-5 ^o	3.5

d

^a Ethyl-acetate washed alumina colum (Brockmann Activity II)

^b 10% MeOH/CHCl₃ on silica plates

^c Estimated from column fraction weight

^d Hydroxylated metabolites (10.5%)

Table 4. Glc results from the total crude neutral urinary excretion products from rabbits dosed orally with 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44), isolated metabolic products and some of the standard compounds.

Standard Compound	Relative Retention Time (RRT)		RRT of TMS derivaties				
	<u>C₂₄</u>	<u>C₃₆</u>	<u>C₂₄</u>	<u>C₃₆</u>			
17β-Hydroxy-17α-methyl-5β-androstan-3-one (44)	2.00	0.68	1.82	0.63			
17α-Methyl-5β-androstane-3α,17β-triol (33)	1.82	0.62	1.53	0.53			
17α-Methyl-5β-androstane-3β,17β-triol (62)	1.81	0.62	1.47	0.51			
<u>Isolated Metabolic products</u>							
Compound <u>33</u>	1.82	0.62	1.53	0.53			
Compound <u>40</u>	2.31	0.79	1.82	0.63			
Compound <u>50</u>	2.38	0.82	1.70	0.58			
Compound <u>51</u>	2.27	0.78	1.64	0.56			
Compound <u>42</u>	2.37	0.81	1.71	0.59			
<u>Total crude neutral fraction</u>							
	<u>RRT</u>			<u>RRT(TMS derivatives)</u>			
<u>Components</u>	<u>C₂₄</u>	<u>C₃₆</u>	<u>Relative %</u>	<u>Components</u>	<u>C₂₄</u>	<u>C₃₆</u>	<u>Relative %</u>
<u>33</u>	1.82	0.62	92	<u>33</u>	1.53	0.53	91
<u>51</u>	2.25	0.77	1	<u>51</u>	1.64	0.57	2
<u>40</u>	2.29	0.79	2	<u>50,42</u>	1.72	0.59	5
<u>50,43</u>	2,38	0.82	5	<u>40</u>	1.83	0.63	2

*C₂₄ and C₃₆ alkanes are used as internal standards.

third hydroxyl group were initially assigned by the chemical shifts and coupling constants of their downfield protons (CHOH); the chemical shifts of the C-10 and C-13 methyl protons were affected by the hydroxyl substituent and the assignments were confirmed by ^{13}C -nmr analysis. ^{13}C -nmr assignments were based on the polarization transfer technique (242), internal consistency with the related spectra, and on chemical shift theory (243, 244). The chemical shift difference of ^{13}C -nmr spectra (APPENDIX) between CDCl_3 and $\text{C}_5\text{D}_5\text{N}$ were within 1 ppm except for carbonyl carbons.

The two C-16 hydroxy epimers (40 and 42) were reported previously as metabolic products of 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) (155). Comparison with published data [mp, ir, chemical analysis, ms, and ^1H -nmr (155)] was made for those compounds (40) and (42). The spectra of their acetate and oxidation products together with ^{13}C -nmr spectral assignments established their structures at 17 α -methyl-5 β -androstan-3 α ,16 β , 17 β -triol (40) and 17 α -methyl-5 β -androstan-3 α , 16 α ,17 β -triol (42).

The remaining two hydroxylated metabolic products (50 and 51) isolated both showed in their ^1H -nmr spectra a broad septet ($W_{1/2} = 22\text{--}24$ Hz) which corresponds to a 3-axial proton (3 α -OH) (226a).

Compound 50 showed an unresolved downfield absorption [$\delta = 3.99$ ppm, $W_{1/2} = 9\text{Hz}$; δ ($\text{C}_5\text{D}_5\text{N}$) = 4.30 ppm, $W_{1/2} = 8\text{Hz}$] which corresponds to an equatorial proton undergoing ee and/or ea coupling. This absorption is assigned to a 1 α -H in the 1 β -hydroxylated compound (50). The chemical shifts of the C-10 methyl protons [$\delta = 1.09$ ppm; ($\text{C}_5\text{D}_5\text{N}$) = 1.41 ppm] and the C-13 methyl protons [$\delta = 0.86$ ppm; δ ($\text{C}_5\text{D}_5\text{N}$) = 1.14 ppm] are in good agreement with the calculated values ($\delta = 1.07$ ppm for C-10 CH_3 ; 0.84 ppm for C-13 CH_3) using the substituent additive rule (226a).

The calculation is as follows using 17 α -methyl-5 β -androstande-3 α ,17 β -diol (33) as the base compound:

	δ (ppm)	
	C-10 CH ₃	C-13 CH ₃
Compound <u>33</u>	0.95	0.84
1 β -OH (226a)	0.12	0.00
	1.07	0.84

Further evidence for the 1 β -OH compound (50) was found in the pyridine-induced shifts in the ¹H-nmr spectrum; both C-10 methyl and C-13 methyl groups underwent a large solvent shift (deshielding) owing to the vicinally situated hydroxyl functions, C-10 methyl by 1 β -OH ($\Delta = \delta \text{CDCl}_3 - \delta \text{C}_5\text{D}_5\text{N} = 1.09 - 1.41 = -0.32$ ppm) and C-13 methyl by 17 β -OH ($\Delta = 0.86 - 1.14 = -0.28$ ppm) which are consistent with the values for a vicinally situated 1 β -hydroxyl group (228), and the chemical shift of 1 α -proton is affected by geminal hydroxyl group ($\Delta = 3.99 - 4.30 = -0.31$ ppm) (228). A large difference in chemical shift (0.52 ppm) was observed between the 3 β -proton ($\delta = 4.16$ ppm) in the 1 β -compound (50) and the 3 β -proton ($\delta = 3.64$ ppm) in the 17 α -methyl-5 β -androstande-3 α ,17 β -diol (33). In the 1 β -hydroxy compound (50), the 3 β -proton is located in 1,3-diaxial position related to the 1 β -hydroxyl group. The observed difference in chemical shift (0.52 ppm) is in good agreement with the 1,3-diaxial effect of a 1 β -hydroxyl group on the 3 β -proton as in the following analogous example in Figure 10. The difference in chemical shift of the 1,3-diaxial interaction is 0.60 ppm in the androstande-3 β ,5 α -diol relation to androstan-3 β -ol (226a). Furthermore, the pyridine-induced solvent shift (228) of the 3 β -proton in compound 50 ($\Delta = \delta \text{CDCl}_3 - \delta \text{C}_5\text{D}_5\text{N} = 4.16 - 4.88 = -0.72$ ppm) was pronounced owing to the 3 β -proton occupying a 1,3-

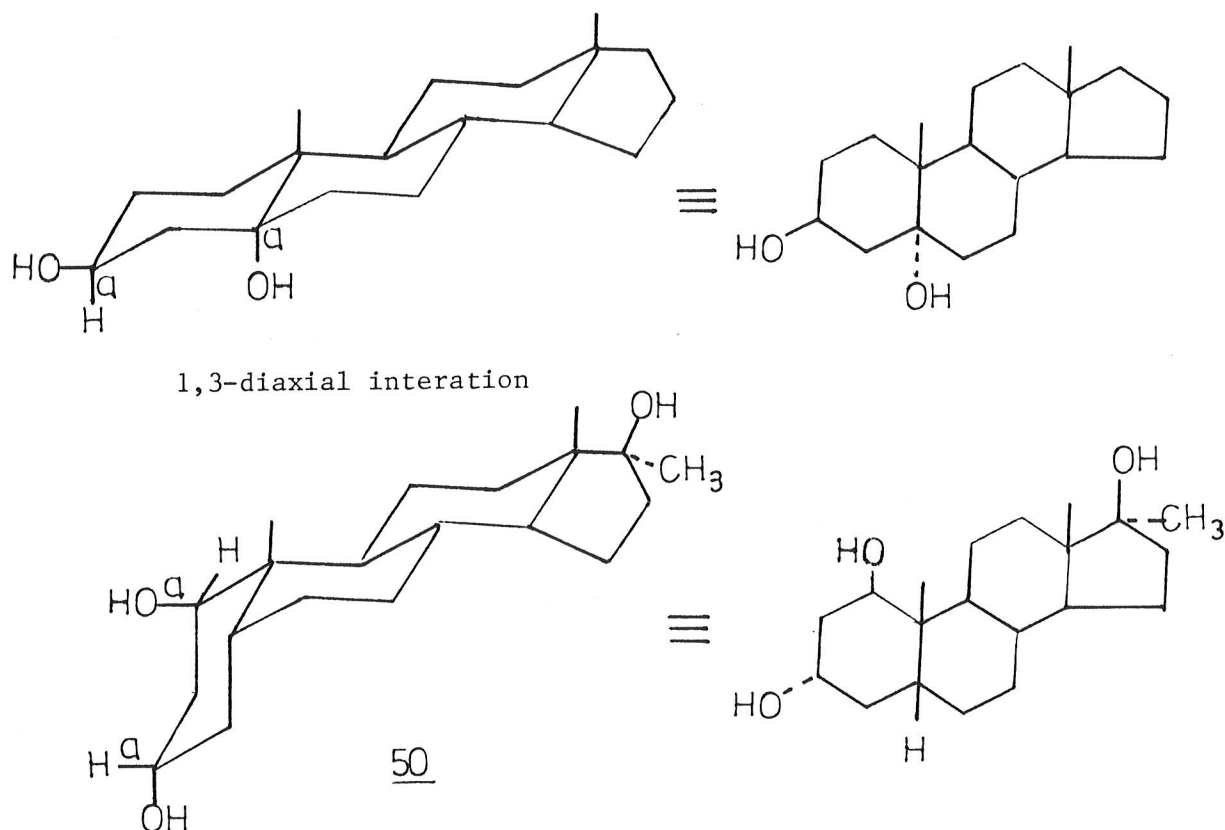


Figure 10: The 1,3 -diaxial interaction of 1 β -hydroxyl group on 3 β -proton

diaxial position relative to the 1 β -hydroxyl function which causes a deshielding effect, whereas the 1 α -proton showed a normal downfield absorption ($\Delta = -0.32$ ppm) (228). Assignment of the ^{13}C -nmr spectrum (APPENDIX) showed that the chemical shift change at C-1 and in the neighbouring carbons are in agreement with the values predicted on introduction of a 1 β -hydroxyl group (244). On the basis of the above evidence, compound 50 has been identified as 17 α -methyl-5 β -androstane-1 β ,3 α ,17 β -triol (50).

The other compound, 51, showed a downfield absorption which appeared as a doublet of doublets ($J = 5$ and 11 Hz) at 3.76 ppm (3.95 ppm in C_5D_5N), which is in agreement only with an axial 12α -proton of the 12β -hydroxylated derivative (226a). The chemical shifts of the C-10 methyl (0.96 ppm) and C-13 methyl (0.92 ppm) groups on introduction of the 12β -hydroxyl group agree with the calculated values (226) as follows:

	δ (ppm)	
	<u>C-10 CH_3</u>	<u>C-13 CH_3</u>
17 α - Methyl-5 β -androstande-3 α ,17 β -diol (<u>33</u>)	0.95	0.84
12 β -OH (226)	0.01	0.05
	0.96	0.89

Acetylation of compound 51 yielded a diacetate which was determined by mass and 1H -nmr spectra. The chemical shifts of the diacetate in $CDCl_3$ for the C-10 methyl (1.00 ppm) and C-13 methyl (0.98 ppm) groups are in agreement with the values for the presence of a 12β -acetyl group (226). The chemical shift (1.38 ppm) of the C-17 methyl group in compound 51 was moved downfield by 0.15 ppm compared with the chemical shift (1.23 ppm) in 17 α -methyl-5 β -androstande-3 α ,17 β -diol (33) and this difference is consistent with 1,3-interaction between the 12β -hydroxyl and the 17 α -methyl group (226), e.g., the 1,3-equatorial/axial interaction between the 11 α -hydroxyl and the C-10 methyl groups (0.12 ppm). The observed downfield pyridine-induced solvent shift for C-13 methyl protons is 0.35 ppm ($\Delta = \delta_{CDCl_3} - \delta_{C_5D_5N} = 0.97 - 1.27 = -0.35$ ppm) which is greater than expected for the 17 β -hydroxyl group alone [the pyridine-induced solvent shift on the C-13 methyl group by a vicinal 17 β -hydroxyl group in 17 α -methyl-5 β -androstande-3 α ,17 β -diol (33) is $\Delta = \delta (0.84 - 1.11) = -0.27$ ppm]. This greater solvent shift for the C-13 methyl protons is

accounted for not only by a vicinal 17 β -hydroxyl group but also by a second vicinal 12 β -hydroxyl group (226a, 228). The normal pyridine-induced solvent shift range (0.15 to 0.25 ppm) (228) was observed for the 3 β -proton ($\Delta = \delta \text{CDCl}_3 - \delta \text{C}_5\text{D}_5\text{N} = 3.67-3.86 = -0.19$ ppm) and in the 12 α -proton ($\Delta = 3.76-3.95 = -0.19$ ppm) as the geminal deshielding of protons situated on a carbon atom bearing the hydroxyl function.

^{13}C -nmr spectrum (APPENDIX) clearly showed that the expected downfield shift for C-12 and the changes of chemical shift in the neighbouring carbons are in agreement with those predicted values on the introduction of a 12 β -hydroxyl group (244). Specifically the γ -gauche effect of the 12 β -hydroxyl group is seen in the chemical shift of the C-18 carbon atom which undergoes a downfield of +5.3 ppm. Based on the above evidence, compound 51 has been identified as 17 α -methyl-5 β -androstan-3 α ,12 β ,17 β -triol (51).

The major urinary excretion product 33 found in rabbits which were dosed with 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44) was the corresponding 3-equatorial alcohol (3 α -OH). The stereochemistry of 3-equatorial alcohol was also present in all four hydroxylated metabolic products 40, 42, 50, and 51 which were isolated. In a similar experiment with 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) (245) (see above Metabolism of compound 43), the 3-equatorial alcohol (3 β ,OH) was the major urinary metabolic product. The 3-axial alcohol (3 α ,OH) was also observed in one hydroxylated metabolite, 17 α -methyl-5 α -androstan-3 α ,6 α ,17 β -triol (46). The 3 α - and 3 β -alcohols were present in the overall ratio of axial: equatorial as 1 : 2.5 (245). A smaller relative percentage of hydroxylated metabolic products (10.5%, in Figure 4) was obtained from 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44) than from 17 β -hydroxy-17 α -methyl-5 α -

androstan-3-one (43) (35% in Table 2) which may be a result of a more effective excretion of the 5β -compound 44 (68%) than 5α -compound 43 (25%) (Table 2 and 3).

Hydroxylation of the 5β -compound 44 occurred mainly at the more hindered methylene positions C-1, C-12 and C-16 which are adjacent to a tertiary methyl group whereas hydroxylation in the 5α -compound 43 occurred at the less hindered positions C-6 and C-15.

No evidence of epimerization of 17β -hydroxy/ 17α -methyl to 17α -hydroxy/ 17β -methyl was observed in the metabolic products from compound 44. The Jones oxidation (231) product of the total crude neutral fraction from compound 44 was compared with a synthesized authentic 17-epimer, 17α -hydroxy- 17β -hydroxy- 5β -androstan-3-one (52), by means of tlc, the two 17-epimers are clearly distinguished by ^1H -nmr by the chemical shift of the C-13 methyl group 17β -hydroxy/ 17α -methyl epimer for C-13 CH_3 : δ (CDCl_3) = 0.89 ppm; ($\text{C}_5\text{D}_5\text{N}$) = 1.12 ppm; 17α -hydroxy/ 17β -methyl epimer for C-13 CH_3 : δ (CDCl_3) = 0.71 ppm; ($\text{C}_5\text{D}_5\text{N}$) = 0.70 ppm (Table 9). ^{13}C -nmr (APPENDIX) distinguishes the two 17-epimer particularly by the chemical shifts of the C-13, C-16, C-18 and C-20 positions.

Metabolism of 17β -hydroxy- 17α -methyl-4-androsten-3-one (13)

Tlc and glc of the total crude neutral, acidic and enolic/phenolic fractions obtained from ether extraction of β -glucuronidase hydrolyzed urine of rabbits which were dosed with 17β -hydroxy- 17α -methyl-4-androsten-3-one (13) showed the presence of the metabolic products only in the total crude neutral fraction which consists of 43% of the dosed steroid.

Column chromatography of the total crude neutral fraction (Table 5) gave seven metabolic compounds 13, 33, 34, 35, 40, 42 and 53 (Figure 11). The glc result of the total crude neutral fraction of 13 is in Table 6.

Table 5. Neutral Urinary Metabolic products from rabbits dosed orally with 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13)^a

Compound	Eluted solvent ^b	R _f ^c	Mp (°C)	Relative Percentage ^d
17 α -Hydroxy-17 α -methyl-4-androsten-3-one (13)	10% ether/Bz	0.61	162-3 ⁰	1
17 α -Methyl-5 α -androstande-3 α ,17 β -diol (34)	25% ether/Bz	0.56	-	> 1
17 α -Methyl-5 α -androstande-3 β ,17 β -diol (35)	25% ether/Bz	0.56	206-8 ⁰	27.5
17 α -Methyl-5 β -androstande-3 α ,17 β -diol (33)	25-75% ether/Bz	0.56	161-4 ⁰	3.0
17 α -Methyl-5 β -androstande-3 α ,16 β ,17 β -triol (40)	2.5% MeOH/ether	0.23	255-8 ⁰	40
17 α -Methyl-5 β -androstande-3 α ,16 α ,17 β -triol (42)	5% MeOH/ether	0.15	218-220 ⁰	12
17 α -Methyl-5 β -androstande-3 β ,6 α ,17 β -triol (53)	5-25% MeOH/ether	0.05	121-213 ⁰	16

^aThe neutral urinary fraction contained 43% of the dosed material; 89% of which consisted of the above metabolites.

^bEthyl acetate washed alumina column (Brockmann Activity II) (239).

^cTlc solvent system: 10% MeOH/CHCl₃

^dEstimated from column fraction weight

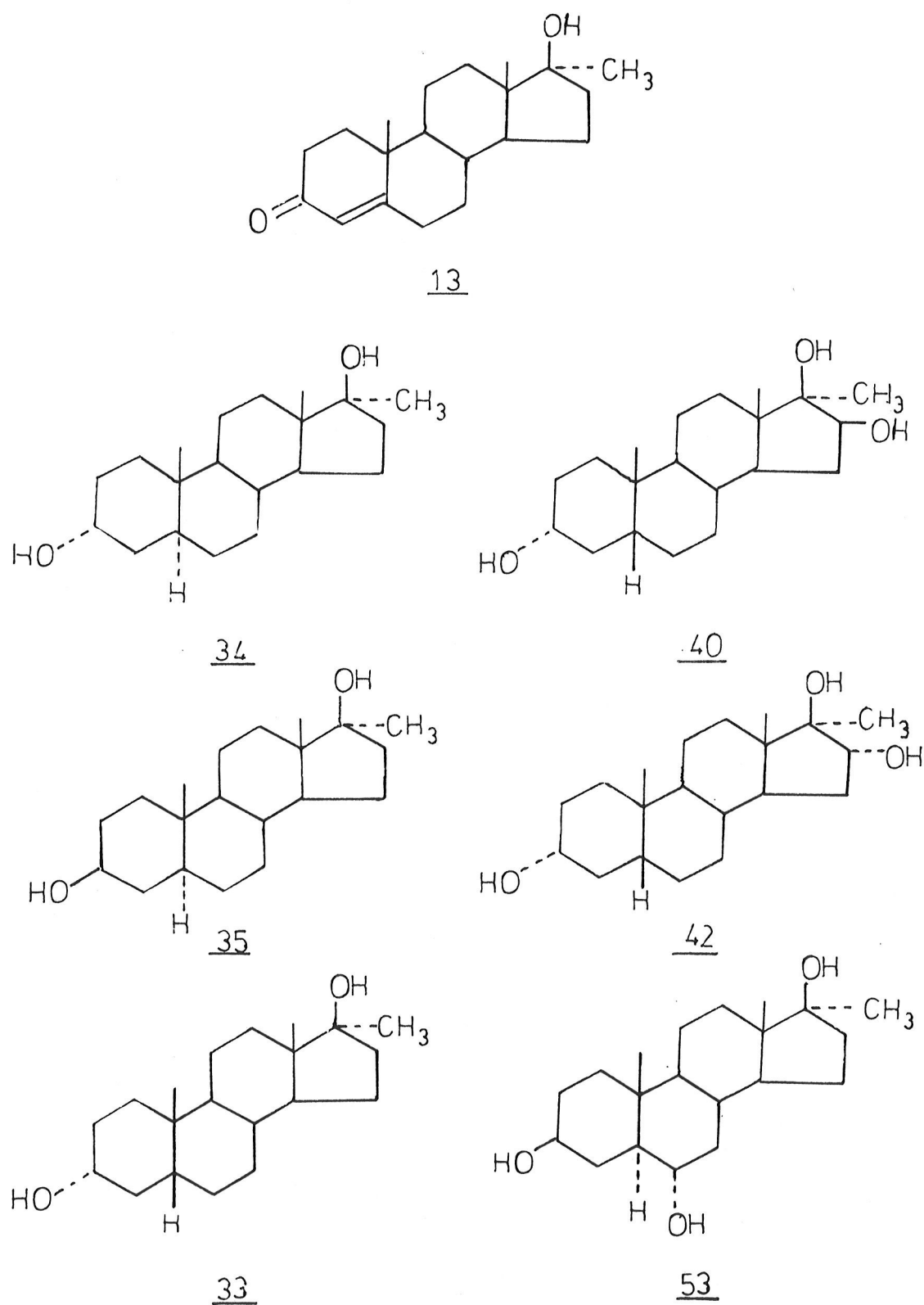


Figure 11. Seven metabolic products of 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13).

Table 6. Glc result of 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13)

Metabolic products isolated	Relative retention Time (RRT)	
	<u>C₂₄</u> *	<u>C₃₆</u> *
17 α -Methyl-5 β -androstan-3 α ,17 β -diol (<u>33</u>)	1.31	0.78
17 α -Methyl-5 α -androstan-3 β ,17 β -diol (<u>35</u>)	1.34	0.80
17 α -Methyl-5 α -androstan-3 α ,17 β -diol (<u>34</u>)	1.34	0.80
17 β -Hydroxy-17 α -methyl-4-androsten-3-one (<u>13</u>)	1.44	0.86
17 α -Methyl-5 β -androstan-3 α ,16 β ,17 β -triol (<u>40</u>)	1.49	0.88
17 α -Methyl-5 β -androstan-3 α ,16 α ,17 β -triol (<u>42</u>)	1.51	0.90
17 α -Methyl-5 β -androstan-3 α ,6 α ,17 β -triol (<u>53</u>)	1.54	0.93

*C₂₄ and C₃₆ hydrocarbons are used as internal standards.

The first four compounds 13, 33, 34 and 35 were identified by the comparison of their melting points (mixture melting points were not depressed and their spectra (ir, mass and ^1H -nmr) with those of the synthesized authentic compounds (see Syntheses) and the reported data (132, 139, 140, 154, 156). The compound 34 was separated from a mixture of 34 and 35 by HPLC to give fractions with retention times (RT) and ^1H -nmr spectra corresponding to compounds 34 and 35. The three hydroxylated compounds 40, 42 and 53 were initially identified by their spectra (ir, mass and ^1H -nmr) and by the spectra of their acetylation and oxidation products, and confirmed by ^{13}C -nmr (APPENDIX). The two C-16 hydroxylated compounds 40 and 42 were isolated as metabolic products of 13 (see also Metabolism of compound 44) and were also previously reported as metabolic products of 13 (155). The comparison of spectra (ir, mass and ^1H -nmr) and melting points (mixture melting points were not depressed) of two C-16 hydroxylated compounds 40, and 42, which were isolated from two different sources, compounds 13 and 44 independently are identical and also the comparison with the reported data (155), proved their identity. ^{13}C -nmr spectra confirmed their identities as 17 α -methyl-5 β -androstane-3 α ,16 β ,17 β -triol(40) and 17 α -methyl-5 β -androstane-3 α ,16 α ,17 β -triol (42).

The third hydroxylated metabolic product 53 was identified by ^1H -nmr spectroscopy; the observed chemical shifts of the C-10 methyl and C-13 methyl groups overlap at 0.85 ppm on introduction of the 6 α -hydroxyl group on 17 α -methyl-5 α -androstane-3 β ,17 β -diol (35). They are in agreement with the following calculated values:

	δ (ppm)	
	<u>C-10 CH₃</u>	<u>C-13 CH₃</u>
Compound <u>35</u>	0.82	0.84
6 α -OH (226)	0.03	0.00
	0.85	0.84

The C-10 and C-13 methyl chemical shifts of the Jones oxidation (231) product of 53, 17 β -hydroxy-17 α -methyl-5 α -androsterane-3,6-dione (δ = 0.89 ppm for C-13 CH₃, 0.98 ppm for C-10 CH₃), are identical with the C-10 and C-13 methyl chemical shifts observed for the Jones oxidation (231) product of 17 α -methyl-5 α -androsterane-3 α ,6 α ,17 β -triol (46), which was isolated from the metabolism of compound 43 (245) (see Metabolism of compound 43). The chemical shifts are in agreement with the calculated values below:

	δ (ppm)	
	<u>C-10 CH₃</u>	<u>C-13 CH₃</u>
17 β -Hydroxy-17 α -methyl-5 α -androsterane-3-one (<u>43</u>)	1.03	0.88
6-oxo (226)	-0.05	0.02
	0.98	0.90

In addition to the ¹H-nmr spectra of 53 and of its oxidation product, the ¹³C-nmr (APPENDIX) confirmed the structure of compound 53 as 17 α -methyl-5 α -androsterane-3 β ,6 α ,17 β -triol (53).

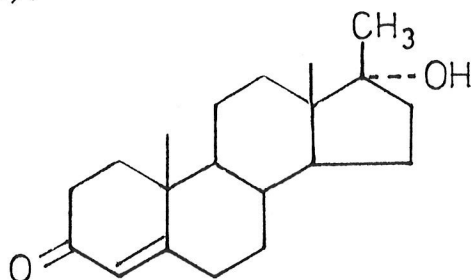
The metabolism of compound 13 in rabbits of the same strain has been studied previously by oral dosing and its urinary metabolic products were identified by Watabe *et al.* (154, 155). The reported metabolic products were two non-hydroxylated diols, 17 α -methyl-5 α -androsterane-3 β , 17 β -diol (35), and 17 α -methyl-5 β -androsterane-3 α ,17 β -diol (33) in the ratio

of 1:1.4 (154) as estimated by glc peak areas, and, from a separate experiment, three hydroxylated metabolic products, 17 α -methyl-5 β -androsterane-3 α ,16 β ,17 β -triol (40), 3 α ,17 β -dihydroxy-5 β -androstan-16-one (41) and a small amount of the 17 α -methyl-5 β -androsterane-3 α ,16 α ,17 β -triol (42) (155). Rongone and Segaloff (140) obtained only two non-hydroxylated diols, 33 and 35 in the ratio of 10 : 1 as the urinary metabolic products of compound 13 when it was administered to a woman with ovarian carcinoma. In our findings, the ratio of the two non-hydroxylated metabolic products 33 and 35 was 1 : 9.6, which is different from the reported result (154). However, the small amount of 5 β -compound diol (35) could be accounted for by the larger percentage of hydroxylated metabolic products (68%) isolated with the 5 β -H and 3 α -OH configuration in compounds 40 (40%), 42 (12%), and 53 (16%), which were isolated and identified. It seems likely that most of the 17 α -methyl-5 β -androsterane-3 α ,17 β -diol (33) is metabolized to hydroxylated compounds such as, 40, 42 and 53 instead of being excreted as the diol itself (33).

The presence of 34 as one of the metabolites from compound 13 was detected by ^1H -nmr spectrum in a very small amount (>1%). Compound 34 was also isolated in a human study (140-142) as one of metabolites of compound 13, but it was not reported in the rabbit by Watabe *et al.* (154). The dosed material, compound 13, was isolated in approximately 1% whereas in previous work it was not isolated (154).

Among the hydroxylated metabolic products 17 α -methyl-5 α -androsterane-3 β ,6 α ,17 β -triol (53) (12%) was isolated and identified in our work, but it has not been reported in a previous similar study (154, 155). However, the previously reported 3 α ,17 β -dihydroxy-5 β -androstan-16-one (155) was not detected.

There was no evidence for the presence of the 17-epimer of compound 13, 17 α -hydroxy-17 β -methyl-4-androstan-3-one (54), in the metabolic products from compound 13. Compounds 13 and 54 (which were synthesized: see below) are not separated by tlc or glc, but their ^1H -nmr spectra are distinct (see Table 9).



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Because the two C-6 α -hydroxy compounds, 46 and 53, which were obtained from 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) and 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) respectively have the same 5 α -configurations, but have different C-3 hydroxy configurations (3 α -OH for 46 and 3 β -OH for 53), it is not likely that they are formed through a common intermediate. A single intermediate would have yielded only one type of hydroxy-6 α -hydroxylated compound. The possibility exists that the formation of 46 by direct hydroxylation at C-6 occurs on the saturated ring A system followed by the reduction of the C-3 ketone to give the 3 α -hydroxy, as discussed earlier with regard to the metabolism of 43. Figure 12 shows the suggested possible pathways for the formation of compound 46 from compound 43, in which the most likely pathway is speculated to be route "a". Therefore, for the formation of 53 from 13, the initial reduction of the C-4 double bond of compound 13 to give compound 43 is unlikely. If this happened, it would give the same 6 α -

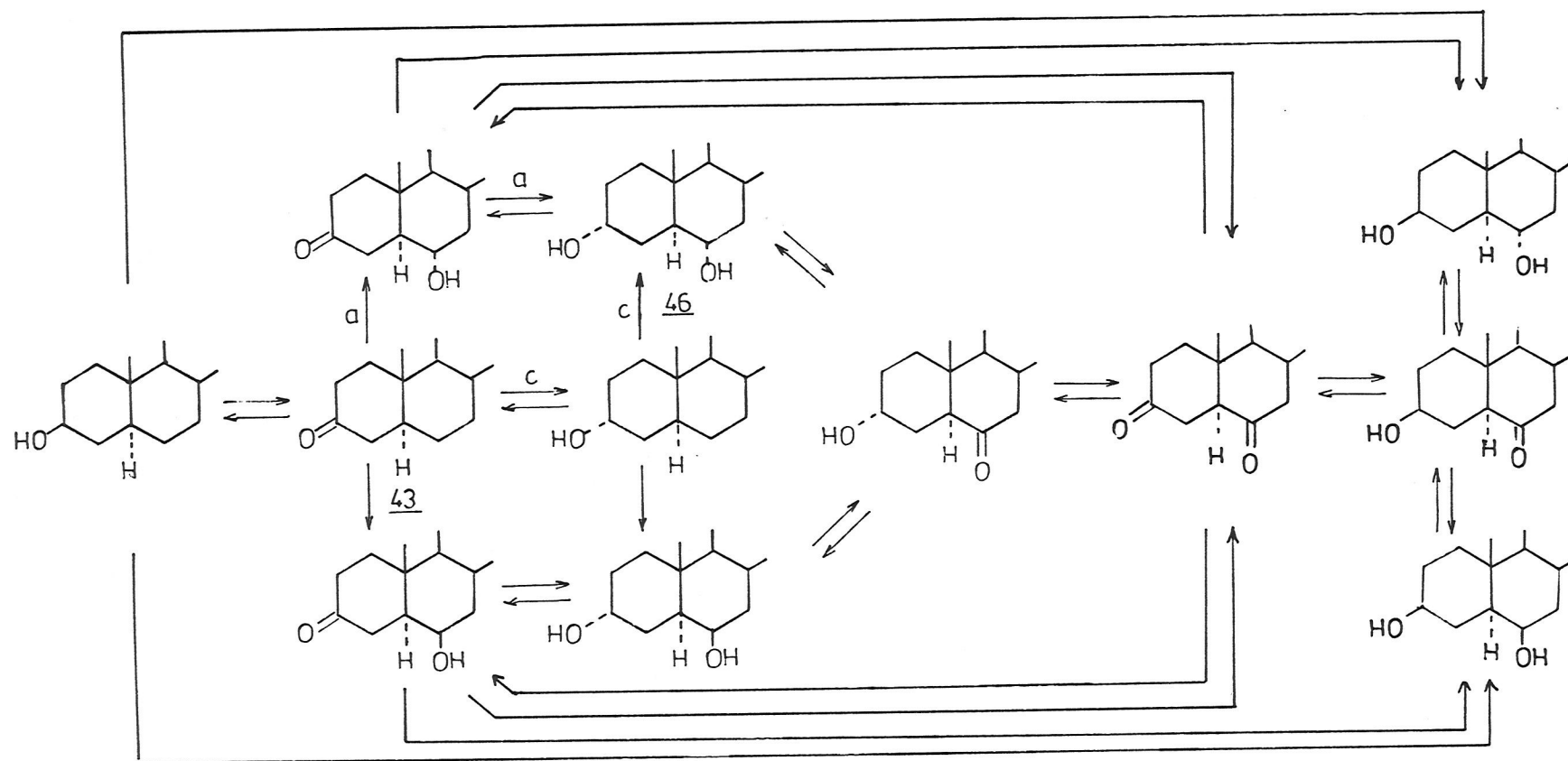


Figure 12. Suggested possible pathways for the formation of 17α-methyl-5α-androstane-3α,6α,17β-triol (46) from 17β-hydroxy-17α-methyl-5α-androstan-3-one (43) in rabbits.

hydroxylated compound. It is probable that C-6 hydroxylation of compound 13 occurs first, followed by reduction of the C-4 double bond and 3-ketone to give the 3 β -hydroxy through route "b" in Figure 13. The stereochemistry of the initial C-6 hydroxylation or its presence during reduction of the 4-en-3-one system cannot be deduced as the C-6 β -alcohol. If it formed, it can be epimerized either through enolization or *via* a 6-ketone intermediate. The C-6 α -hydroxy from compound 13 is not likely formed from the 3 β -alcohol *via* compound 43. From the observation that the 3 β -alcohol is the major metabolite from compound 43, but the C-6 α -hydroxylated compound (46) was 3 α -hydroxyl. However, the results do not rule out C-6 hydroxylation of the 3 α -hydroxy compound 46 from compound 43 through route "c" in Figure 12.

The absence of C-15 α -hydroxylation of 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) suggest that C-15 α -hydroxylation occurs by direct hydroxylation of ring D in the saturated 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) and not through the ring "A" unsaturated molecule.

17 β -Hydroxy-17 α -methyl-4-androsten-3-one (13) undergoes substantial hydroxylation before reduction to the ring "A" saturated derivatives. As 17 β -hydroxy-5 α -androstan-3-one (14) is one of the principle endogenous hormones activating the androgenic receptor, the high activity of many synthetic 5 α -derivatives as androgenic/anabolic agents may in part be a result of decreased metabolic hydroxylation to inactive derivatives, as well as loss by reduction to the inactive 5 β -compounds.

Metabolism of 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16)

Tlc and glc of the total crude neutral, acidic and enolic/phenolic fractions obtained from ether extraction of glucuronidase hydrolyzed urine of rabbits which were dosed with 17 β -hydroxy-17 α -methyl-1,4-

androstadien-3-one (16) showed the presence of the metabolic products only in the total crude neutral fraction, which was 21.4% of the dosed steroid (16).

Column chromatography of the total crude neutral fraction (Table 7) gave 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16), 17 α -methyl-5 α -androstane-3 β ,17 β -diol (35), 17 α -methyl-5 β -androstane-3 α ,17 β -diol (33), 16 β ,17 β -dihydroxy-17 α -methyl-5 β -androst-1-en-3-one (55), 16 β ,17 β -dihydroxy-17 α -methyl-1,4-androstadien-3-one (56), 6 β ,17 β -dihydroxy-17 α -methyl-1,4-androstadien-3-one (57), 16 α ,17 β -dihydroxy-17 α -methyl-5 β -androst-1-en-3-one (58) and 6 β ,16 β ,17 β -trihydroxy-17 α -methyl-1,4-androstadien-3-one (59) (Figure 14). The first three compounds (16, 33, and 35) were identified by comparison with the melting points (mixture melting points) and spectral data (ir, mass and ^1H -nmr) of authentic compounds. HPLC separation gave fractions with retention times and ^1H -nmr spectra corresponding to 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16) and 17 α -methyl-5 α -androstane-3 α ,17 β -diol (34) (trace amount). The three 1,4-dien-3-one hydroxylated compounds (56, 57 and 59) were identified by their ^1H -nmr (Table 8) and mass spectra. All compounds have the expected downfield absorptions in the ^1H -nmr spectra; for C-1H, $\delta = 7.05\text{--}7.08$ ppm (d, $J = 10$ Hz), for C-2H, $\delta = 6.22\text{--}6.25$ ppm [δ ($\text{C}_5\text{D}_5\text{N}$) = $6.42\text{--}6.44$ ppm] and for C-4H, $\delta = 6.07\text{--}6.15$ ppm [δ ($\text{C}_5\text{D}_5\text{N}$) = $6.42\text{--}6.44$ ppm] which overlaps a higher field peak of one of the doublet peaks of the C-2H signals. The above chemical shifts correspond to the protons on the two double bonds at C-1 and C-4 in the ring A as in 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16).

Compound 56 has an absorption at $\delta = 3.61$ ppm (dd, $J = 5$ and 8 Hz) which corresponds to the 16 α -proton; therefore it is the 16 β -hydroxylated

Table 7. Neutral^a urinary metabolic products from rabbits dosed orally with 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16)^b

Compound		R _f ^c	M ⁺	Eluted Solvent ^d	Mp (°C)	wt (mg)	Relative percentage ^e
17 β -Hydroxy-17 α -methyl-1,4-androstadien-3-one	(16)	0.41	300	2.5% EtOAc/CHCl ₃	-	37	3
17 α -Methyl-5 α -androstane-3 β ,17 β -diol	(35)	0.48	306	2.5% Et)Ac/CHCl ₃	209-210.5 ⁰	40	4
17 α -Methyl-5 β -androstane-3 α ,17 β -diol	(33)	0.48	306	5% EtOAc/CHCl ₃	163-164 ⁰	95	10
16 β ,17 β -Dihydroxy-17 α -methyl-5 β -androst -1-en-3-one	(55)	0.48	318	20% EtOAc/CHCl ₃	-	29	3
16 β ,17 β -Dihydroxy-17 α -methyl-1,4-androstadien-3-one	(56)	0.44	316	20% EtOAc/CHCl ₃	201-202 ⁰	49	5
6 β ,17 β -Dihydroxy-17 α -methyl-1,4-androstadien-3-one	(57)	0.40	316	30-40% EtOAc/CHCl ₃	227-228 ⁰	259	27
16 α ,17 β -Dihydroxy-17 α -methyl-5 β -androst -1-en-3-one	(58)	0.36	318	50% EtOAc/CHCl ₃	211-212 ⁰	103	11
6 β ,16 β ,17 β -Trihydroxy-17 α -methyl-1,4-androstadien-3-one	(59)	0.26	332	50-75% EtOAc/CHCl ₃	256-258 ⁰	353	37

^a Glc and tlc indicates neither enolic nor acidic metabolites

^b The neutral urinary fraction contained 21.4% of the dosed material; 71% of which contained the above eight metabolites

^c The solvent system: 10% MeOH/CHCl₃

^d Merck Silica Gel 60H column

^e Estimated from column weight

^f 5 α : 5 β (1:2.5)

^g Hydroxylated metabolites (83%)

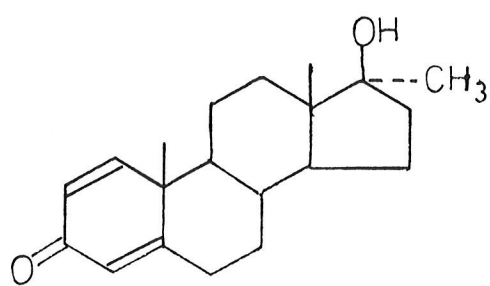
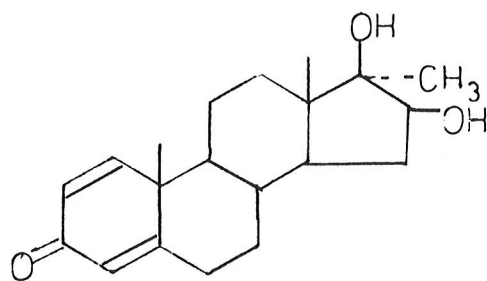
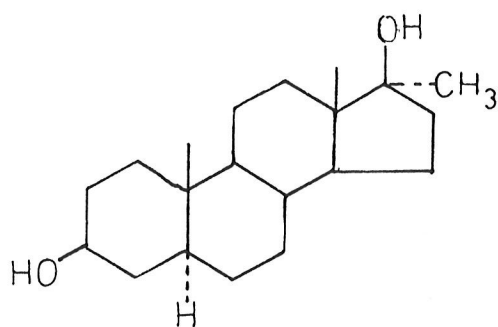
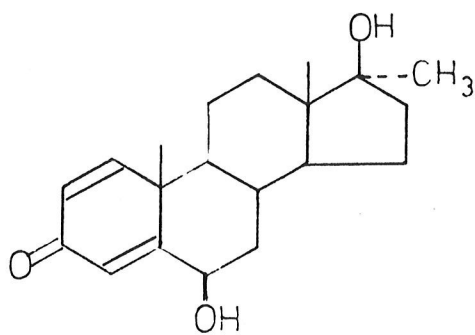
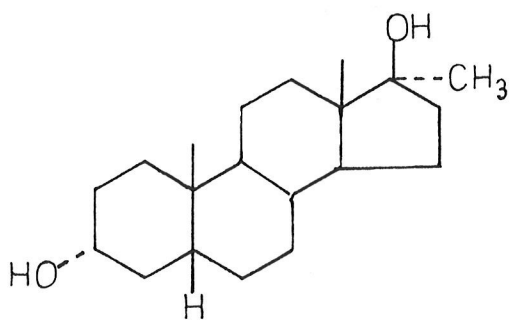
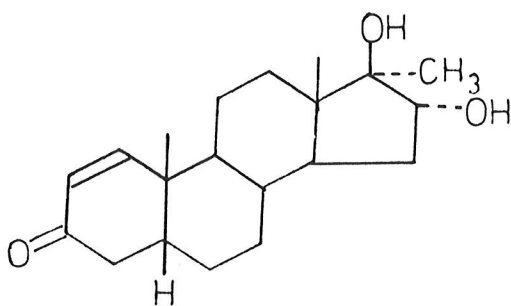
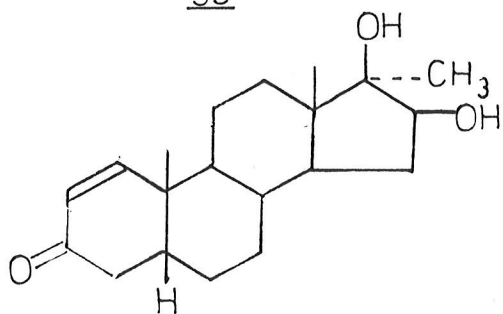
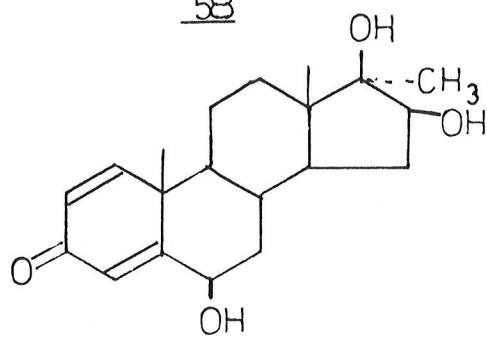
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Figure 14. Eight metabolic products of 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16) in rabbit urine.

Table 8. ^1H -nmr metabolic products of 17β -hydroxy- 17α -methyl- $1,4$ -androstadien- 3 -one (16)
(δ value in ppm, solvent is CDCl_3 , unless otherwise mentioned)

Metabolic products		C-10 CH_3	C-13 CH_3	C-17 CH_3	3 CHOH	6 CHOH	16 CHOH	C-1H	C-2H	C-4H
17β -Hydroxy- 17α -methyl- $1,4$ -androstadien- 3 -one	(16)	1.254	0.959	1.194				7.057 (d, J=10 Hz)	6.222 (dd, J=2&10Hz)	6.070 (m)
17α -Methyl- 5α -androstane- 3β , 17β -diol	(35)	0.846	0.828	1.204	3.565 (m)					
17α -Methyl- 5β -androstane- 3α , 17β -diol	(33)	0.943	0.838	1.222	3.589 (m)					
16β , 17β -Dihydroxy- 17α -methyl- 5β -androst- 1 -en- 3 -one	(55)	1.218	0.894	1.130			3.667 (16 α -H) (dd, J=5&8Hz)	6.929 (d, J=10Hz)	5.894 (dd, J=1&10Hz)	2.676 (4 α -H) (dd, J'=15&17Hz)
16β , 17β -Dihydroxy- 17α -methyl- $1,4$ -androstadien- 3 -one	(56)	1.252	0.941	1.116			3.611 (16 α -H) (dd, J=5&8Hz)	7.045 (d, J=1-Hz)	6.250 (dd, J=2&10Hz)	6.066 (s)
6β , 17β -Dihydroxy- 17α -methyl- $1,4$ -androstadien- 3 -one	(57)	1.460	0.972	1.203	4.542 (6 α -H) (m)			7.056 (d, J=10Hz)	6.222 (d, J=2&10Hz)	6.150 (s)
6β , 17β -Dihydroxy- 17α -methyl- $1,4$ -androstadien- 3 -one	(57)*	1.665	1.165	1.432	4.734 (6 α -H)			7.009 (d, J=10Hz)	6.438 (dd, 2&10Hz)	6.438 (d, J=2.5Hz)
16α , 17β -Dihydroxy- 17α -methyl- 5β -androst- 1 -en- 3 -one	(58)	1.209	0.914	1.179			4.285 (16 β -H) (dd, J=3&10Hz)	6.813 (d, J=10Hz)	5.889 (dd, J=10Hz)	2.792 (4 α -H) (dd, J'=15&17Hz)
6β , 16β , 17β -Trihydroxy- 17α -methyl- $1,4$ -androstadien- 3 -one	(59)	1.650	1.202	1.316	4.722 (6 α -H) (t, J=2.5Hz)		3.910 (16 α -H) (dd, J=5&9Hz)	7.076 (d, J=10Hz)	6.417 (dd, J=2&10Hz)	6.417 (d, J=2Hz)
<u>Derivative of metabolic product</u>										
6β , 16β -Diacetoxy- 17β -hydroxy- 17α -methyl- $1,4$ -androstadien- 3 -one		1.350	1.223	1.256	2.059 (6 β -OAc) 5.499 (6 α -H) (t, J=2.5Hz)	2.105 (16 β -OAc) 4.691 (16 α -H) (dd, J=5&7.5Hz)	7.000 (d, J=1-Hz)	6.290 (dd, J=2&10Hz)	6.222 (d, J=2Hz)	6.065 (m)
<u>Standards</u>										
17β -Hydroxy- 17α -methyl- $1,4$ -androstadien- 3 -one		1.251	0.940	1.194				7.056 (d, J=10Hz)	6.221 (dd, J=2&10Hz)	6.065 (m)
17β -Hydroxy- 17α -methyl- 5β -androst- 1 -en- 3 -one		1.217	0.890	1.217				6.837 (d, J=10Hz)	5.890 (dd, J=1&10Hz)	2.784 (4 α -H) dd, J=15&17Hz)
17β -Acetoxy- 5α -androst- 1 -en- 3 -one		1.019	0.828	2.043 (17 β -OAc) 4.623 (17 α -H) (t, J=7.5&10Hz)				7.131 (d, J=10Hz)	5.852 (d, J=10Hz)	

*solvent is $\text{C}_5\text{D}_5\text{N}$.

derivative of compound 16. Compound 56 is therefore identified as 16 β , 17 α -dihydroxy-1,4-androstadien-3-one.

Compound 57 has an absorption at $\delta = 4.54$ ppm (4.75 ppm in C_5D_5N) which corresponds to the 6 α -proton. The observed chemical shifts of compound 57 are 1.46 ppm for C-10 CH_3 and 0.97 ppm for C-13 CH_3 . The calculated values are as follows:

	δ (ppm)	
	<u>C-10 CH_3</u>	<u>C-13 CH_3</u>
17 β -Hydroxy-17 α -methyl-1,4-androstadien-3-one (<u>16</u>)	1.25	0.94
6 β -OH (226)	0.23	0.04
	1.48	0.98

The above calculated values are in agreement with the observed values as well as the previously reported data (143, 246, 247). The mass spectrum for compound 57 has m/z 316 corresponding to M^+ . On the basis of the above evidence, compound 57 is identified as 6 β ,17 β -dihydroxy-1,4-androstadien-3-one (57).

Compound 59 has two downfield absorptions at $\delta = 3.91$ ppm in C_5D_5N (dd, $J = 5$ and 9 Hz) which corresponds to the 16 α -proton. A m/z of 332 (M^+) corresponds to a dihydroxylated derivative of compound 16. The structure of compound 59 is confirmed by ^{13}C -nmr (APPENDIX) as 6 β ,16 β ,17 β -tri-hydroxy-17 α -methyl-1,4-androstadien-3-one.

The remaining two hydroxylated compounds, 55 and 58, each have two absorptions at $\delta = 6.81$ ppm (d, $J = 10$ Hz) for C-1H and at $\delta = 5.89$ ppm (dd, $J = 1$ and 10 Hz) for C-2H, which corresponds to the C-1 double bond in ring "A" as observed in an authentic sample of 17 β -hydroxy-17 α -methyl-5 β -

androst-1-en-3-one* [δ = 0.89 ppm for C-13 CH₃, 1.22 ppm for C-10 CH₃ and C-17 CH₃, 5.89 ppm for C-2H (dd, J = 1 and 10 Hz) and 6.84 ppm for C-1H (d, J = 10 Hz) (Table 8) together with 2.68-2.79 ppm (dd, J' = 15 and 17 Hz for the 4 α -H)]. In compound 55, the absorption at δ = 3.67 ppm (dd, J = 5 and 8 Hz) corresponds to the 16 α -H which is coupled with the 15 α -H dihedral angle θ , Ca.0°(248)] and with the 15 β -H [θ , Ca.120°(248)]. Therefore, compound 55 is identified as 16 β ,17 β -dihydroxy-5 β -androst-1-en-3-one. Compound 58 has an absorption at δ = 4.29 ppm (dd, J = 3 and 10 Hz) corresponding to the 16 β -H which is coupled with the 15 β -H [θ , Ca.0°(248)] and with 15 α -H [θ , Ca.120°(248)]. Compound 58 is, on the above evidence, identified as 16 α ,17 β -dihydroxy-5 β -androst-1-en-3-one.

The metabolism of the anabolic steroid 16 has been studied previously by Rongone and Segaloff (141) who isolated two metabolites (7.25% of the dosed material) from a woman with advanced adenocarcinoma of the lung. One of the metabolites was identified as the 6 β -hydroxylated derivative of compound 16, and it was suggested that the other was 17-epimer of compound 16. Metabolic studies of 16 by Adhikary and Harkness (249) in normal man gave 5% of the dosed material as two major metabolites from the extractable neutral fraction of urine. These authors found the two compounds in a ratio of 2:1; the minor one was identified as the 6 β -hydroxylated 16 and the major one as the 17-epimer of 16 which was identified by comparison with the synthetic substance.

The determination of the metabolic products from 16 in human urine by gas chromatography and capillary column gas chromatography-mass

*The compound was obtained from rat metabolism of compound 16 which was donated by Dr. J.W. Steele.

spectrometry (144-146) has been studied. After oral administration to man, two major metabolites were detected and were identified as the 17-epimer of 16 and the 6 β -hydroxylated derivative of 16, which were recovered as 5% of the dosed amount.

In comparison with the above studies, this study in the rabbit gave a 21.4% recovery of the metabolites after β -glucuronidase hydrolysis. Of these metabolites, 5.8% was the 6 β -hydroxylated compound 57. The formation of 6 β -hydroxylated derivatives has been seen in other studies of related conjugated ketones in addition to the above studies (141, 144-146, 249); 6 β -hydroxylation of triamcinolone (9 α -fluoro-11 β ,16 α ,17 α , 21-tetrahydroxy-pregn-1,4-dien-3,20-dione) in the dog (250); 6 β -hydroxy-cortisol (6 β ,11 β ,17 α ,21-tetrahydroxy-pregn-4-en-3,20-dione) from the urine of a patient after administration of cortisol (251); presence of 6 β -hydroxy-cortisol in urine of pregnant women (252); presence of a 6 β -hydroxylating system in the placenta (253); demonstration of the capacity for 6-hydroxylation of cortisone (17,21-dihydroxy-pregn-4-ene-3,11,20-trione) by human adrenal slices (254); neoplastic testicular tissue's capacity for 6 β -hydroxylation of several steroid (255); suggestion of liver as one of major organs involved in 6 β -hydroxylation of cortisol (256).

In this study, 6 β -hydroxylation of the 1,4-dien-3-one system in compound 57 is consistent with previous work (141, 144-146, 249), considering the difference in the species used. The total metabolites which were isolated and identified in the present experiment were eight compounds totalling 21.4% of the dosed material. Of the isolated metabolic products, the large amounts of C-4 and/or C-1 unsaturated ring A compounds (83%) (Table 7) are a result of the limited

susceptibility of the 1,4-dien-3-one system to enzymatic reduction. This is in agreement with the results obtained after administration of other 1,4-dien-3-one steroids such as prednisone (149c) prednisolone (149c) and 1-dehydrotestololactone (37), 17 β -hydroxy-1,4-androstadien-3-one (149a) and 4-chloro-17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (150, 151) as well as previous work with compound 16 (128, 141-146, 246, 249).

The olefinic bond in the C-1 position largely survives the metabolic process. On the basis of the isolation of substantial amounts of 5 β -1-en-3-one compounds 55 and 58 (Table 7) together with 1,4-dien-3-one compounds 56, 57 and 59 after administration of 1,4-dien-3-one compound 16, the C-1 double bond proved to be resistant to reduction by body enzymes, unlike the reduction of the 4-en-3-one system. Similar results were observed in the metabolism of other 1,4-dien-3-one steroids (148, 150-152). The selective stereospecific reduction of the C-4 double bond to 5 β -compounds is also in accordance with the chemical behavior of the 1,4-dien-3-one system.

Compounds 55 and 58 with C-1 double bond intact were 3% and 11% of dosed material respectively (Table 7). The ratio of 16 α to 16 β -hydroxylated compounds was 1 : 3.6. Compounds 55 and 58 with C-1 double bond intact, were not previously isolated in metabolic study in man (142a, 144-146, 249) nor in rat (156).

The smaller amount of ring "A" saturated diol compounds 35 and 33, 4% and 10% respectively, and the ratio of 5 α -H, 3 β -OH (35) to 5 β -H, 3 α -OH (33) of 1 : 2.5 are in agreement with the findings of metabolic studies with compound 16 in the rat (156) in which no ring "A" unsaturated compounds were detected as metabolites. However, in the study of metabolism of

compound 16 in man (141, 144-146, 249), no ring 'A' saturated compounds were found.

The 6β -and 16β -dihydroxylated derivative of 16, $6\beta, 16\beta, 17\beta$ -trihydroxy-1,4 - androstadien-3-one (59), is unique because such dihydroxylation of compound 16 has not been reported previously. However, a $6\beta, 16\alpha$ -dihydroxylated structure has been tentively assigned to a metabolite from testosterone after incubation with the rat liver (123).

Syntheses

As part of this study to identify and compare the metabolic products derived from 17β -hydroxy- 17α -methyl- 5α -androstan-3-one (43), 17β -hydroxy- 17α -methyl- 5β -androstan-3-one (44), 17β -hydroxy- 17α -methyl-4-androsten-3-one (13) and 17β -hydroxy- 17α -methyl-1,4 - androstadien-3-one (16), it was considered necessary to prepare the four C-3 ketones and eight isomers of the C-3 epimeric alcohols with both 17β -hydroxy- 17β -methyl- 5α -and 5β -configurations. In consideration of the reported epimerization of the 17β -hydroxy/ 17α -methyl to the 17α -hydroxy/ 17β -methyl group from the administration of compound 16 in human (141, 144-146, 249), it was also desirable to prepare the corresponding C-3 ketones and epimeric C-3 alcohols in the 17α -hydroxy/ 17β -methyl- 5α -and 5β -configurations. The twelve compounds (Figure 15) and 17α -hydroxy- 17β -methyl-4-androsten-3-one (54) synthesized are standards for chromatographic and spectroscopic comparison purposes.

Of the four ketones, 17β -hydroxy- 17α -methyl- 5α -androstan-3-one (43) was available commercially whereas the remaining three (44, 47 and 52) (Figure 15) had to be synthesized. Compound 44 was prepared by the method of Surorov and Yaraslavitseva (257); hydrogenation of 17β -hydroxy- 17α -methyl-4-androsten-3-one over 10% Pd/CaCO₃ in dry pyridine. This

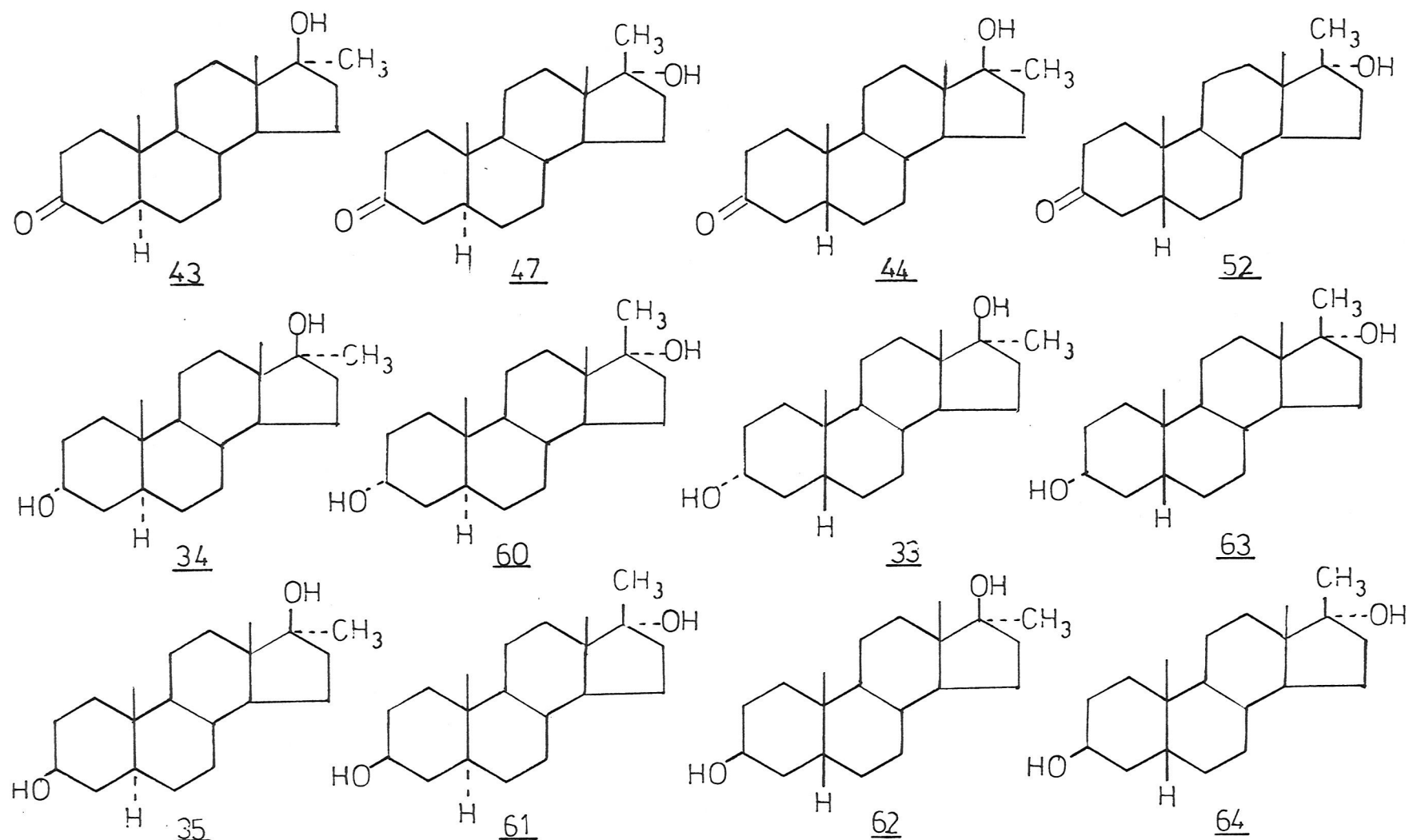


Figure 15. Prepared four C-3 ketones and eight C-3 epimeric alcohols with 17ξ-hydroxy-17ξ-methyl-5α and 5β-configurations.

hydrogenation occurs through a kinetically controlled 2,4-dien-3-ol intermediate in which the reduction takes place on the top side of the molecule to give the 5 β -compound (87% yield) as the predominant product (Figure 16).

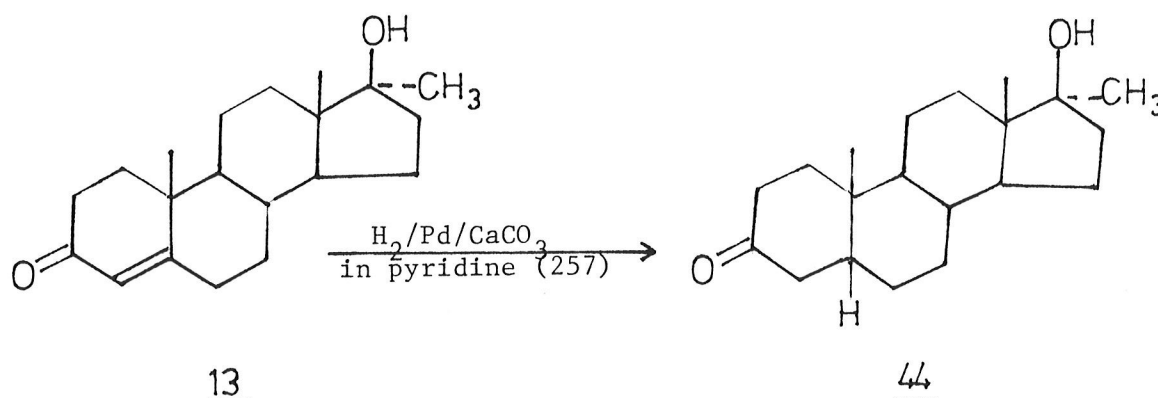


Figure 16. Preparation of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44)

The product, 44, was identified by comparison of its melting point and ir spectrum with previously reported data (154, 258). The assigned structures were confirmed by ^{13}C -nmr (APPENDIX), ^1H -nmr (Table 9) and mass (Table 10) spectra.

17 α -Hydroxyl-17 β -methyl-5 α -androstan-3-one (47) was prepared by the scheme outlined in Figure 17. Compound 65 was prepared by the Wittig reaction (143, 259, 260) with 3 β -hydroxy-5 α -androstan-17-one (23) in good yield (84%). Its melting point corresponded to the reported value (260) and its structure was confirmed by ^1H -nmr, mass and ir spectra (EXPERIMENTAL). Oxidation of 65 with *meta*-chloroperbenzoic acid (143, 259) produced 17 α ,20-oxido-17 β -methyl-5 α -androstan-3 β -ol (66) in quantitative yield. Lithium aluminum hydride reduction (143, 259) of 66 yielded 17 α -hydroxy-17 β -methyl-5 α -androstan-3-ol (61) (93%). Jones oxidation (231) of 61 yielded 17 α -hydroxy-17 β -methyl-5 α -androstan-3-one

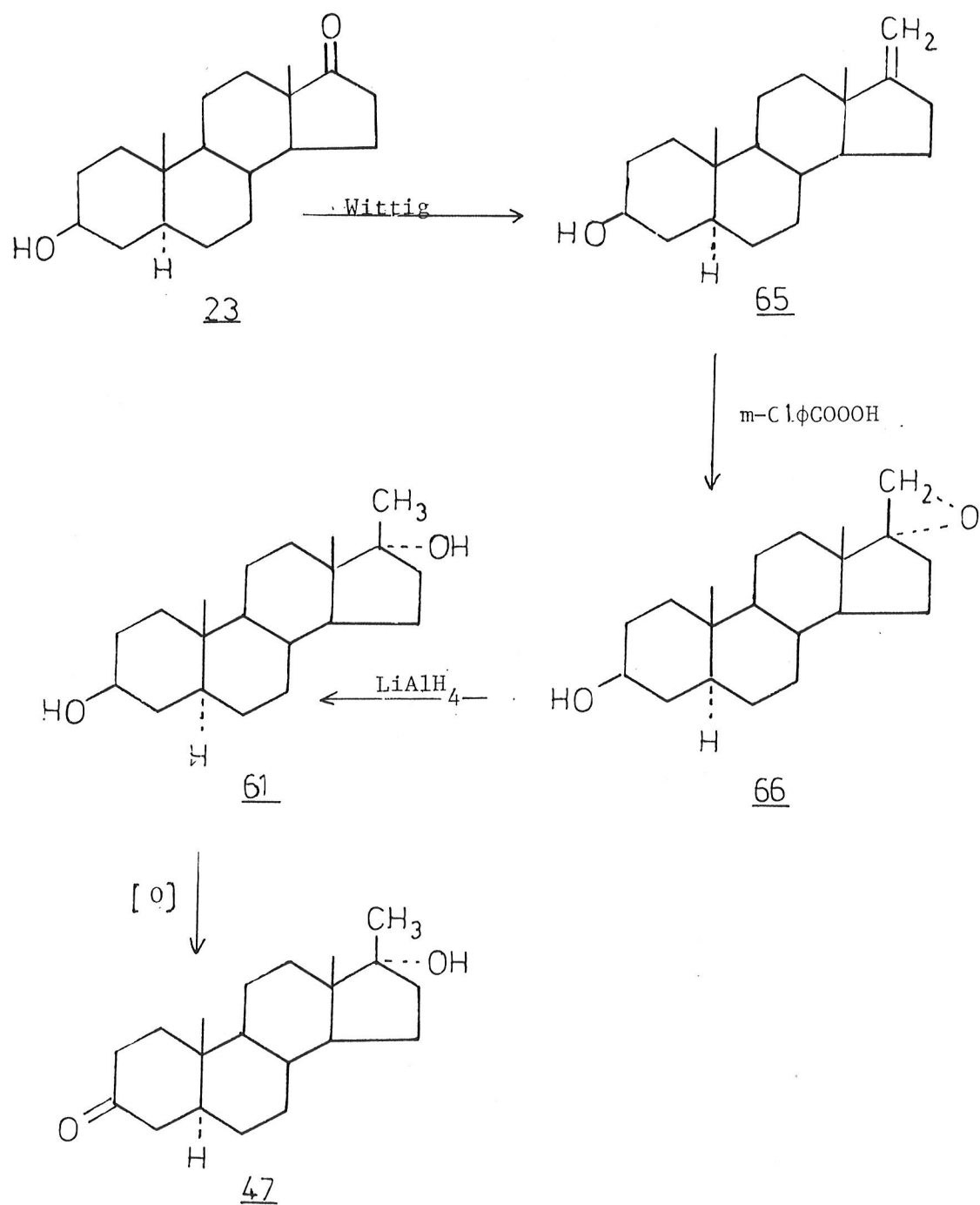


Figure 17. Scheme for the preparation of 17 α -hydroxy-17 β -methyl-5 α -androstan-3-one (**47**).

(47) in 53% yield. The above three compounds (47, 61, and 66) were new compounds whose structures were identified by spectroscopic data [ir, ^1H -nmr (Table 9) and mass (Table 10) spectra] and their compositions were confirmed by elemental analyses.

The preparation of 17 α -hydroxy-17 β -methyl-5 β -androstan-3-one (52) is outlined in Figure 18. 17-Methylene-5-androsten-3-ol (67) was prepared by the Wittig reaction (143, 259, 260) with 3 β -hydroxy-5-androsten-17-one (2) in 91% yield. It was identified by the comparison of its melting point, and ir and ^1H -nmr spectra with previously reported data (143, 259). The Oppenauer oxidation of 67 yielded 17-methylene-4-androsten-3-one (68) (77% yield). Compound 68 was identified by comparison of its melting point and ir spectrum with the previously reported data (143, 259) and the assignment was confirmed by ^1H -nmr (Table 9) and mass (Table 10) spectra. Hydrogenation of 68 over Pd/CaCO₃ in dry pyridine (257) gave 17-Methylene-5 β -androstan-3-one (69) in quantitative yield. *Meta*-chloroperbenzoic acid oxidation (143, 259) of 69 yielded 17 α ,20-oxido-17 β -methyl-5 β -androstan-3-one (70) (43% yield), and lithium aluminum hydride reduction (143, 259) of 70 yielded a mixture of 17 β -methyl-5 β -androstan-3 α - and 3 β ,17 β -diols (63 and 64) in quantitative yield. This mixture gave a 31% yield of 17 α -hydroxy-17 β -methyl-5 β -androstan-3-one (52) on Jones oxidation (231). Compounds 52, 63, 64, 69 and 70 were new compounds and were identified by spectroscopic data [ir, ^1H -nmr (Table 9) and mass (Table 10) spectra]. The structure of 52 was confirmed by ^{13}C -nmr (APPENDIX). Their compositions were confirmed by their elemental analyses.

The preparation of the C-3 axial and C-3 equatorial alcohols are outlined in Figure 19. The C-3 axial alcohols, which are the thermody-

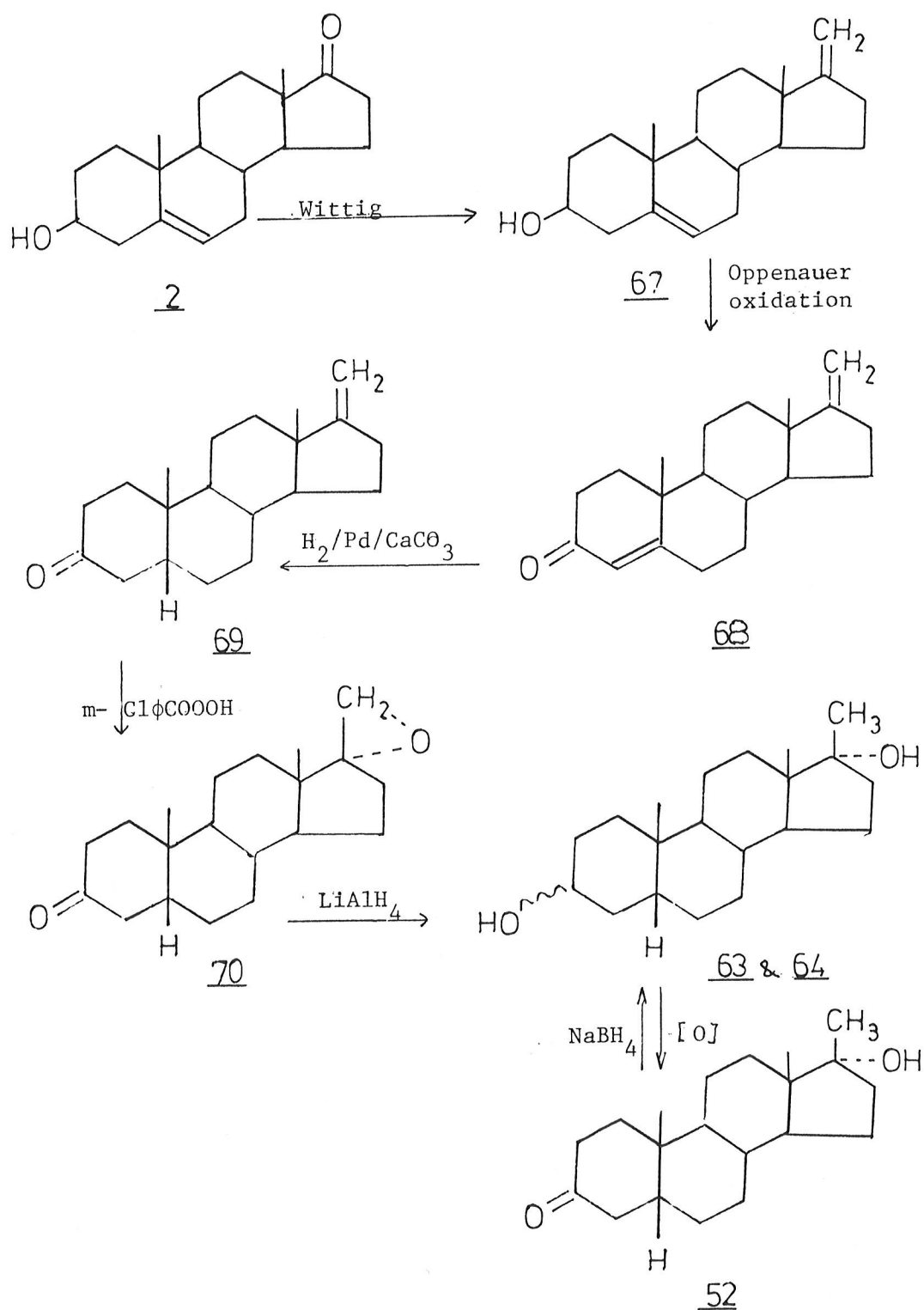


Figure 18. Scheme for the preparation of 17 α -hydroxy-17 β -methyl-5 β -androstan-3-one (**52**).

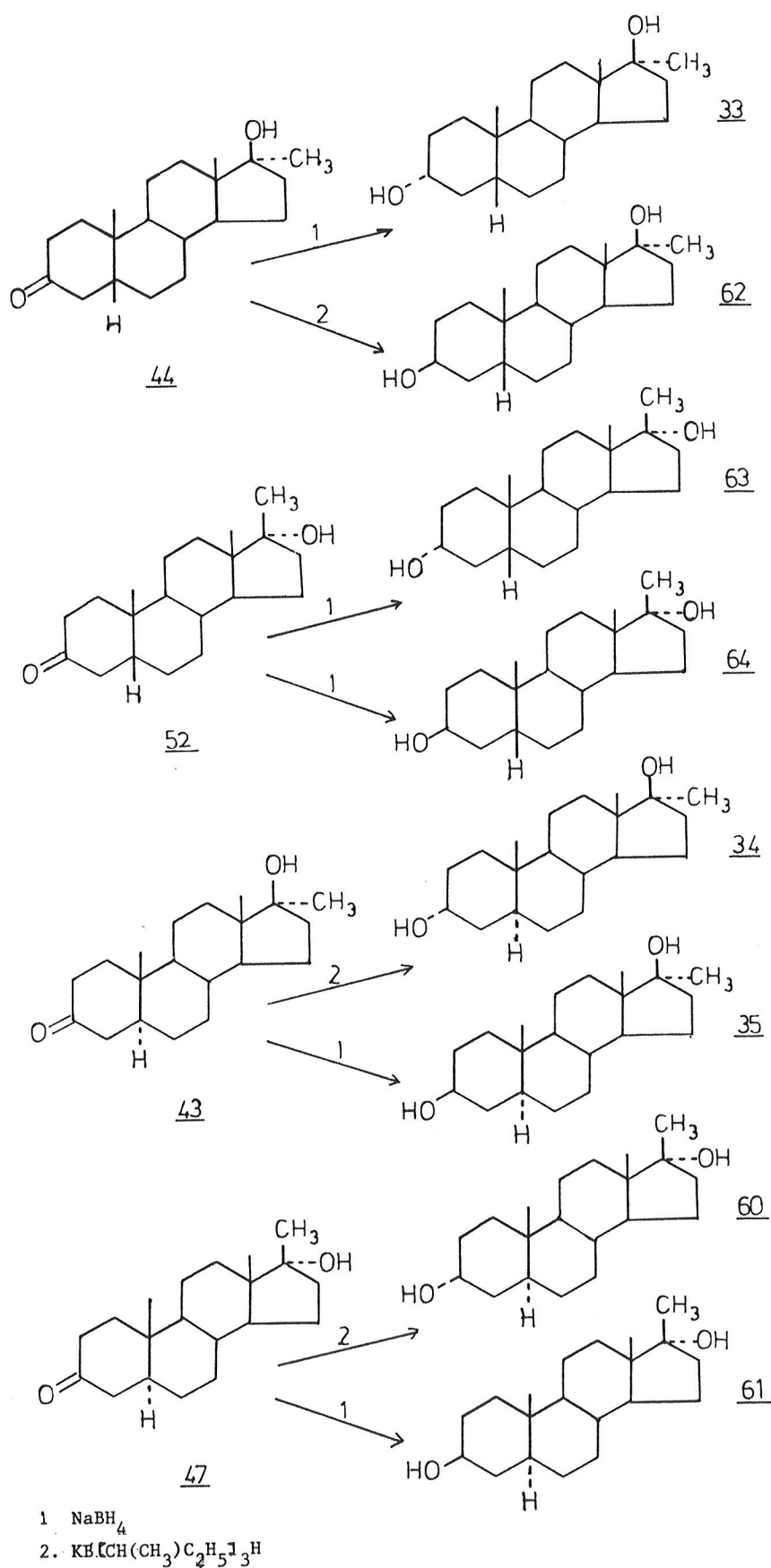
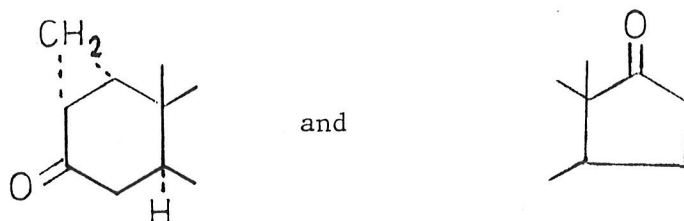


Figure 19. The preparation of C-3 axial and C-3 equatorial alcohols, 17 ξ -methyl-5 α and 5 β -androstane 3 ξ ,17 ξ -diols.

namically less stable epimers, are not readily available synthetically. Application of the recently available reagent, potassium tri-(*sec*-butyl)-borohydride [K-selectride, KB $[\text{CH}(\text{CH}_3)\cdot\text{C}_2\text{H}_5]_3\text{H}$] (261-263), which had been shown to yield principally the axial epimer alcohols with the cyclohexane derivatives, reacted with 3-keto steroids to give essentially quantitative yields of the C-3 axial alcohols in both the 5α - and 5β -series provided a means of obtaining the desired compounds. However, the above reagent did not react with ketones possessing the following structures:



The C-3 equatorial alcohols, which are more stable thermodynamically, were prepared by sodium borohydride (NaBH_4) reduction (264, 265) which gave a ratio of 9:1 mixture of C-3 equatorial : C-3 axial diols in good yield. The four C-3 ketones and eight C-3 epimeric alcohols with both 17β -hydroxy/ 17α -methyl and 17α -hydroxy/ 17β -methyl configurations are shown in Figures 15 and 19.

17α -Hydroxy- 17β -methyl-4-androsten-3-one (54)

17α -Hydroxy- 17β -methyl-4-androsten-3-one (54) was prepared by the method described previously (143, 259) (Figure 20).

The *meta*-chloroperbenzoic acid oxidation of compound 68 yielded 71, which was reacted with lithium aluminum hydride to give a mixture of 17β -methyl-4-androstene- 3α - and $3\beta,17\beta$ -diols (72 and 73). Jones oxidation (231) of the above mixture yielded compound 54 which was identified by the comparison of its melting point and ir spectrum with

previously reported data (259). The structure of 54 was confirmed by ^1H -nmr (see EXPERIMENTAL) and ^{13}C -nmr (APPENDIX) spectra.

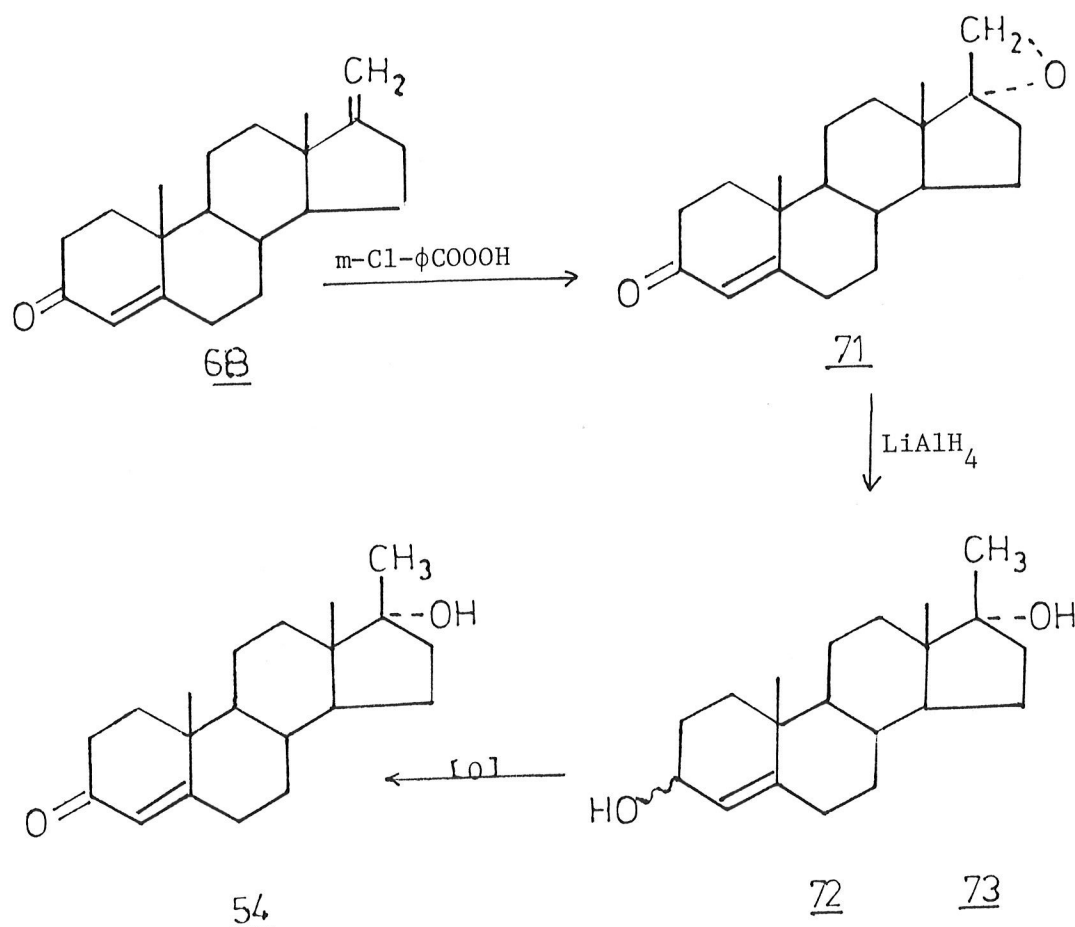


Figure 20. The preparation of 17 α -hydroxy-17 β -methyl-4-androsten-3-one (54)

The ^1H -nmr data of the twelve isomers are recorded in both deuteriochloroform and deuteriopyridine (Table 9).

17 α -Hydroxy-17 β -methyl-5 α -androstan-3-one (47), 17 α -hydroxy-17 β -methyl-5 β -androstan-3-one (52) and their corresponding C-3 axial and equatorial alcohols (60, 61, 63, 64) are new compounds whose syntheses have not been reported previously. All synthesized compounds have been characterized by their spectroscopic data ^1H -nmr (Table 9), mass (Table 10) and ir and elemental analyses. The structures of some of their

Table 9. ^1H -nmr spectra of 17 ξ -hydroxy-17 ξ -methyl-5 α -and 5 β -androstan-3-one and 17 ξ -methyl-5 α -and 5 β -androstan-3 ξ ,17 ξ -diol epimers in deuteriochloroform (CDCl_3) and deuteriopyridine ($\text{C}_5\text{D}_5\text{N}$) with solvent differences^{a,b}

Compounds	C-3 H^c			C-10 CH_3			C-13 CH_3			C-17 CH_3		
	CDCl_3	$\text{C}_5\text{D}_5\text{N}$	Δ	CDCl_3	$\text{C}_5\text{D}_5\text{N}$	Δ	CDCl_3	$\text{C}_5\text{D}_5\text{N}$	Δ	CDCl_3	$\text{C}_5\text{D}_5\text{N}$	Δ
17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43)				1.033	0.950	+0.083	0.882	0.119	-0.237	1.218	1.436	-0.218
17 α -hydroxy-17 β -methyl-5 α -androstan-3-one (47)				1.032	0.932	+0.100	0.705	0.736	-0.031	1.208	1.390	-0.182
17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44)				1.057	0.950	+0.107	0.886	1.117	-0.231	1.250	1.477	-0.227
17 α -hydroxy-17 β -methyl-5 β -androstan-3-one (52)				1.040	0.910	+0.130	0.710	0.700	-0.010	1.212	1.38	-0.168
17 α -methyl-5 α -androstan-3 α ,17 β -diol (34)	4.023	4.295	-0.272	0.795	0.870	-0.075	0.841	1.125	-0.284	1.205	1.428	-0.223
17 α -methyl-5 α -androstan-3 β ,17 β -diol (35)	3.600	3.897	-0.297	0.818	0.870	-0.052	0.841	1.110	-0.269	1.193	1.441	-0.248
17 β -methyl-5 α -androstan-3 α ,17 α -diol (60)	4.040	4.276	-0.236	0.800	0.866	-0.066	0.675	0.750	-0.075	1.188	1.375	-0.187
17 β -methyl-5 α -androstan-3 β ,17 α -diol (61)	3.618	3.868	-0.250	0.839	0.848	-0.009	0.682	0.720	-0.038	1.192	1.390	-0.198
17 α -methyl-5 β -androstan-3 α ,17 β -diol (33)	3.641	3.917	-0.276	0.947	0.957	-0.010	0.840	1.105	-0.265	1.229	1.450	-0.221
17 α -methyl-5 β -androstan-3 β ,17 β -diol (62)	4.125	4.382	-0.257	0.980	1.089	-0.109	0.841	1.130	-0.289	1.218	1.485	-0.267
17 β -methyl-5 β -androstan-3 α ,17 α -diol (63)	3.620	3.870	-0.250	0.950	0.950	0	0.640	0.700	-0.060	1.190	1.370	-0.180
17 β -methyl-5 β -androstan-3 β ,17 α -diol (64)	4.150	4.316	-0.301	0.980	1.050	-0.070	0.690	0.720	-0.030	1.190	1.380	-0.190

^aTMS as internal standard

^bSpectra were recorded on either a Varian 220 MHz, Bruker 90 MHz, NIC 220 MHz or NTC 360 MHz instrument.

^cAll axial (35,61,33,63) C-3 protons show broad ($W_1 \approx 21\text{Hz}$) aa/ae coupling and equatorial (34,60,62,64) C-3 protons narrow ($W_1 = 7\text{Hz}$) ae/ee coupling.

$\Delta = \delta (\text{CDCl}_3) - \delta (\text{C}_5\text{D}_5\text{N})$; minus signs indicate a downfield shift.

Table 10. Mass spectra of 17 ξ -hydroxy-17 ξ -methyl-5 α -and 5 β -androstan-3-ones^a and 17 ξ -methyl-5 α and 5 β -androstan-3 ξ ,17 ξ -diol epimers

Compounds	Percentage abundance (m/z 43 = 100%)				Abundance ratio	
	M ⁺	M ⁺ -15	M ⁺ -18	M ⁺ -15-18	M ⁺ -18/M ⁺	M ⁺ -15-18/M ⁺
17 β -hydroxy-17 α -methyl-5 α -androstan-3-ones (43)	10.8	14.8	6.6	8.4	0.61	1.28
17 α -hydroxy-17 β -methyl-5 α -androstan-3-one (47)	18.6	25.0	10.6	11.0	0.57	1.04
17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44)	1.7	1.5	1.4	2.6	0.83	1.85
17 α -hydroxy-17 β -methyl-5 β -androstan-3-one (52)	3.9	1.8	2.4	4.8	0.63	1.95
17 α -methyl-5 α -androstan-3 α ,17 β -diol (34)	11.8	15.5	8.1	9.8	0.68	1.21
17 α -methyl-5 α -androstan-3 β ,17 β -diol (35)	5.8	8.4	2.8	4.3	0.48	1.53
17 β -methyl-5 α -androstan-3 α ,17 α -diol (60)	13.6	20.1	8.0	12.4	0.59	1.54
17 β -methyl-5 α -androstan-3 β ,17 α -diol (61)	10.5	16.1	3.7	7.5	0.35	2.03
17 α -methyl-5 β -androstan-3 α ,17 β -diol (33)	3.0	5.2	11.3	4.4	3.7	0.86
17 α -methyl-5 β -androstan-3 β ,17 β -diol (62)	5.3	12.6	17.2	7.9	3.3	0.46
17 β -methyl-5 β -androstan-3 α ,17 α -diol (63)	1.4	2.9	7.6	5.7	5.3	0.75
17 β -methyl-5 β -androstan-3 β ,17 α -diol (64)	2.8	8.0	10.0	6.9	3.6	0.69

^aSpectra were recorded on a Finnigan Quadrapole Model 1015 instrument at 70 eV using a direct probe method.

Table 11. R_f value of 17 ξ -Hydroxy-17 ξ -methyl-5 ξ -androstan-3-ones and 17 ξ -Methyl-5 ξ -androstan-3 ξ ,17 ξ -diol epimers in silica gel tlc plates, pre-coated* and laboratory made plates(LMP#) in two different solvent systems. (Plates were sprayed with 4% EtOH in cone sulfuric acid and heated 100-110°C for 10 min.

Compounds	R_f (50% EtOAc/PE)		R_f (10% MeOH/CHCl ₃)	
	LMP	Pre-coated	LMP	Pre-coated
17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43)	0.49(yellow)	0.42(yellow)	0.76	0.51(yellow)
17 α -hydroxy-17 β -methyl-5 α -androstan-3-one (47)	0.52(yellow)	0.54(yellow)	0.78	0.58(yellow)
17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44)	0.47(brown)	0.40(brown)	0.75	0.49(yellow beige)
17 α -hydroxy-17 β -methyl-5 β -androstan-3-one (52)	0.49(brown)	0.45(brown)	0.77	0.53(yellow beige)
17 α -methyl-5 α -androstan-3 α ,17 β -diol (34)	0.37(beige)	0.36(beige)	(0.64)	0.34(beige)
17 α -methyl-5 α -androstan-3 β ,17 β -diol (35)	0.32(beige)	0.30(beige)	0.56	0.26(beige)
17 β -methyl-5 α -androstan-3 α ,17 α -diol (60)	0.31(beige)	0.28(beige)	0.48	0.18(beige)
17 β -methyl-5 α -androstan-3 β ,17 α -diol (61)	0.31(beige)	0.33(beige)	0.57	0.37(beige)
17 α -methyl-5 β -androstan-3 α ,17 β -diol (33)	0.27(beige)	0.26(beige)	0.57	0.24(beige)
17 α -methyl-5 β -androstan-3 β ,17 β -diol (62)	0.36(beige)	0.35(beige)	(0.76)	0.32(beige)
17 β -methyl-5 β -androstan-3 α ,17 α -diol (63)	0.17(beige)	0.18(beige)	0.50	0.12(beige)
17 β -methyl-5 β -androstan-3 β ,17 α -diol (64)	0.28(beige)	0.36(beige)	0.58	0.33(beige)

*Merck Type-40

#Merck Type-40

compounds were confirmed by ^{13}C -nmr (APPENDIX). Their tlc results are given in Table 11.

Examination of the ^1H -nmr spectra (Table 9) of the twelve isomers of 17 ξ -hydroxy/17 ξ -methyl-5 ξ -androstan-3-ones and 17 ξ -hydroxy/17 ξ -methyl-5 ξ -androstane-3 ξ ,17 ξ -diols have been recorded both in deuteriochloroform (CDCl_3) and deuteriopyridine ($\text{C}_5\text{D}_5\text{N}$). The solvent shifts ($\Delta = \delta \text{CDCl}_3 - \delta \text{C}_5\text{D}_5\text{N}$) are interpreted in terms of specific solute-solvent complexes between pyridine molecules and the polar hydroxyl function in the solute molecule. Pyridine has for some time been recognized as a useful solvent for nmr analysis of molecules containing polar functional groups for solubility and for increased spectral simplification relative to chloroform or carbon tetrachloride (CCl_4) (266-268). Because of the ability of the aromatic system of pyridine to coordinate at electron-deficient sites within a solute molecule, protons situated in the vicinity of a polar functional group invariably experience large screening effects as a result of the large anisotropy in the magnetic susceptibility of the aromatic system (269). The correlation of chemical shifts induced in benzene and pyridine relative to chloroform with specific structural features in a variety of carbonyl containing compounds is well established, and the potential of this technique for use in structural analysis has been demonstrated (270). When pyridine is used as a solvent for the hydroxylated compounds, the hydrogen-bonding of the pyridine to C-3 and C-17 hydroxyl groups would be expected to alter the magnetic environment selectively around the molecule, thereby inducing structural differences.

^1H -nmr of 17 ξ -methyl-5 α -and 5 β -androstane-3 ξ ,17 ξ -diols:

C-17 Methyl group - The stereochemistry of the C-17 methyl (α or β)

cannot be distinguished by the ^1H -nmr spectra recorded in CDCl_3 (1.19-1.26 ppm) (Table 9), whereas the difference is small but significant in $\text{C}_5\text{D}_5\text{N}$, in which the 17α -methyl proton resonance is in the range of 1.43-1.49 ppm and the 17β -methyl proton resonance is in the range of 1.37-1.39 ppm (Table 9). The distinction is reflected in the solvent shift ($\Delta = \delta \text{CDCl}_3 - \delta \text{C}_5\text{D}_5\text{N}$); for the 17α -methyl proton in 17β -hydroxy/ 17α -methyl the solvent shift is $-(0.22-0.27)$ ppm and for 17β -methyl proton in 17α -hydroxy/ 17β -methyl the solvent shift is $-(0.18-0.19)$ ppm. The solvent shift 0.18-0.27 ppm is consistent with geminal deshielding (228); protons and methyl groups situated on a carbon atom bearing a hydroxyl function are deshielded by 0.15-0.26 ppm in $\text{C}_5\text{D}_5\text{N}$ related to CDCl_3 .

C-13 Methyl group - The effect on the chemical shift of the C-13 methyl proton resonance caused by the 17ξ -hydroxy/ 17ξ -methyl epimers was measured in CDCl_3 as well as $\text{C}_5\text{D}_5\text{N}$ and is given by the solvent shift ($\Delta = \delta \text{CDCl}_3 - \delta \text{C}_5\text{D}_5\text{N}$). For 17β -hydroxy/ 17α -methyl group, the chemical shift for C-13 methyl is at 0.84 ppm in CDCl_3 and 1.11-1.13 ppm in $\text{C}_5\text{D}_5\text{N}$ (Table 9). The solvent shift is in the range 0.27-0.29 ppm, which is in good agreement with results expected for a methyl group deshielded by a vicinal hydroxyl group (0.20-0.27 ppm) with a dihedral angle of 60° between the methyl and hydroxyl group (228), whereas for 17α -hydroxy/ 17β -methyl, the chemical shift of the C-13 methyl group is in the range of 0.64-0.69 ppm in CDCl_3 and 0.70-0.75 ppm in $\text{C}_5\text{D}_5\text{N}$ (Table 9). The solvent shift in this latter case is in the range of 0.03-0.08 ppm, which is in strongly suggests vicinal deshielding of a proton and methyl group caused by a hydroxyl function (228). The extent of the deshielding seems to depend on the magnitude of the dihedral angle θ , subtended

between the methyl group (or proton) and the hydroxyl function [if θ is ca. $180-160^\circ$; the solvent shift is $-(0.03-0.05)$ ppm]. The solvent shifts of larger dihedral angles between methyl and hydroxyl groups are much smaller than those of smaller dihedral angles. These differences in chemical shifts for the C-13 methyl proton resonance in both CDCl_3 and $\text{C}_5\text{D}_5\text{N}$ as well as the solvent shifts which are directly influenced by the stereochemistry of 17ξ -hydroxy/ 17ξ -methyl groups clearly distinguish the 17β -hydroxy/ 17α -methyl from the 17α -hydroxy/ 17β -methyl group. The chemical shifts and the solvent shifts of the C-13 group in the 5α - and 5β -configurations are so similar that 5α - or 5β -configurations cannot be differentiated by the chemical shifts of the C-13 methyl proton resonance.

C-10 Methyl group - The chemical shifts of the C-10 methyl proton resonance for the 5β -compounds are at significantly higher fields both in CDCl_3 (0.95-0.98 ppm) and in $\text{C}_5\text{D}_5\text{N}$ (0.95-1.09 ppm) than those of 5α -compounds, which are 0.80-0.84 ppm in CDCl_3 and 0.85-0.87 ppm in $\text{C}_5\text{D}_5\text{N}$ (Table 9). These differences in chemical shifts for the C-10 methyl proton resonance can be used to distinguish between the 5α - and 5β -configuration.

C-3 Proton (C-3 hydroxyl group) - The 3α - and 3β -alcohols in both the 5α - and 5β -compounds are clearly distinguished by the chemical shifts of C-3 proton and their coupling constants (reflected in band width at half height, $W_{1/2}$) of the equatorial protons (4.02-4.15 ppm in CDCl_3 ; 4.28-4.38 ppm in $\text{C}_5\text{D}_5\text{N}$; $W_{1/2} = 7$ Hz; narrow ea/ee coupling) and the axial protons (3.60-3.64 ppm in CDCl_3 ; 3.87-3.92 ppm in $\text{C}_5\text{D}_5\text{N}$; $W_{1/2} = 23 \sim 27$ Hz; broad aa/ae coupling). The solvent shifts are all similar for the eight alcohols ($-0.24 \sim -0.30$ ppm).

^1H -nmr of 17 ξ -hydroxy-17 ξ -methyl-5 α -and 5 β -androstan-3-one:

C-17 Methyl group - The chemical shifts of the C-17 methyl proton resonance in CDCl_3 are similar in all four ketones. However, in $\text{C}_5\text{D}_5\text{N}$, for the 17 β -hydroxy/17 α -methyl-5 α -and 5 β -configurations of 3-ketones (43 and 44) the C-17 methyl proton resonance are higher (1.44 and 1.48 ppm respectively) than for the 17 α -hydroxy/17 β -methyl-5 α -and 5 β -configuration of 3-ketones (47 and 52) (1.39 and 1.38 ppm respectively) (Table 9). Accordingly, the solvent shifts for 43 and 44 are higher (-0.22 and -0.23 ppm respectively) than for 47 and 52 (-0.18 and -0.17 ppm respectively). These differences are in agreement with the results from the eight alcohols.

C-13 Methyl group - For 17 β -hydroxy/17 α -methyl-5 α -and 5 β -configurations of 3-ketones (43 and 44), the chemical shifts of the C-13 methyl proton resonance are 0.88 and 0.89 ppm in CDCl_3 , and 1.12 and 1.12 ppm in $\text{C}_5\text{D}_5\text{N}$ respectively. For 17 α -hydroxy/17 β -methyl-5 α -and 5 β -configurations 3-ketones (47 and 52), the chemical shifts of C-13 methyl proton resonance are 0.71 and 0.71 ppm in CDCl_3 , and 0.74 and 0.70 ppm in $\text{C}_5\text{D}_5\text{N}$ respectively. The solvent shifts for 43 and 44 are 0.23-0.24 ppm whereas for 47 and 52 are 0.01 - 0.03 ppm (Table 9).

C-10 Methyl group - The chemical shifts are all similar in both CDCl_3 and $\text{C}_5\text{D}_5\text{N}$.

The above results allow a clear distinction to be made between the 17 β -hydroxy/17 α -methyl and 17 α -hydroxy/17 β -methyl 3-ketones; between the 3 ξ , 17 ξ -diols and between the 5 α and 5 β -configurations. Also they provide a firm basis for identification of metabolic products.

^{13}C -nmr spectra (APPENDIX)

In the ^{13}C -nmr spectra, the 5 α -and 5 β -configurations were differ-

enciated by the chemical shift difference of 15 ppm at C-9 positions (40.4-41.0 ppm for the 5 β -compounds compared with 54.8-54.9 ppm for 5 α -compounds) and of 11 ppm at the C-19 position (22.7-24.4 ppm for the 5 β -compounds compared with 11.7-12.6 ppm for 5 α -compounds) which reflects the γ -gauche effect of C-4 in these epimers. Also the C-3 axial proton is differentiated from the C-3 equatorial proton in both 5 α - and 5 β -configurations by a chemical shift difference of 5-6 ppm at C-3 (70.7-71.1 ppm for the C-3 axial protons and 65.6-66.2 ppm for the C-3 equatorial protons) and at C-5 (42.7-45.2 ppm for the C-3 axial protons and 37.3-39.6 ppm for the C-3 equatorial protons). The chemical shift of C-3 and C-5 for the C-3 equatorial alcohol are both higher by about 5-6 ppm than for the C-3 axial alcohol.

Comparison of the effects caused by the introduction of the 17 α -methyl group on the ^{13}C -nmr spectrum of 5 β -androsterane-3 α ,17 β -diol (244) shows no significant change in the chemical shift at C-17 (<0.5 ppm). The β -carbon atoms, C-13 and C-16, undergo downfield shifts of 2.4 and 8.6 ppm, respectively. The γ -gauche effect at C-12 causes a 5.3 ppm downfield shift whereas the γ -carbons at C-14 and C-15 show no change; an upfield shift of 3.0 ppm is seen for the C-18 methyl group. No pair of compounds are available for comparison of the effect of introduction of a 17 β -methyl group on the ^{13}C -nmr spectrum of 5 β -androsterane-3 α ,17 α -diol. Nevertheless comparison of the ^{13}C -nmr spectra of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44) and 17 α -hydroxy-17 β -methyl-5 β -androstan-3-one (52) reveals that the α , β and γ -effects of both C-17 epimers are in all cases <2 ppm. This difference is not large enough to be a reliable indicator of the stereochemistry for structural assignment of substituted derivatives.

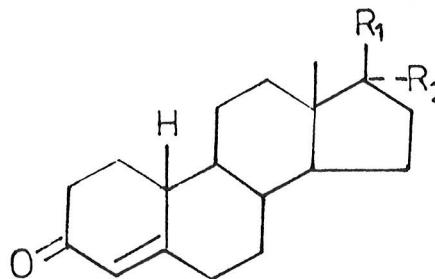
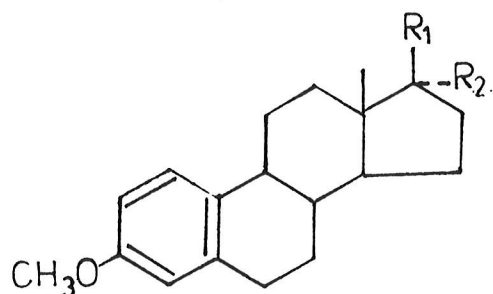
The application of ^{13}C -nmr of the standard compounds with the 17 β -hydroxy/17 α -methyl and 17 α -hydroxy/17 β -methyl groups and all the hydro-

xylated metabolic products aided in resolving difficulties in assigning their structures and confirming the structure of others. In particular it was not possible to distinguish clearly between 17 β -hydroxy-17 α -methyl-5 α -androsterane-3 α ,6 α ,17 β -triol 46 and its isomer 17 β -hydroxy-17 α -methyl-5 α -androsterane-3 β ,6 α ,17 β -triol 53, except through the ^{13}C -nmr spectra. It has been noted that the identification of a steroid metabolite had not been established solely by ^{13}C -nmr spectra and this is the first use of ^{13}C -nmr for this purpose. By using the polarization transfer technique (242), CH_3 and CH are distinguished from CH_2 carbons, and quaternary carbons are also differentiated. For purposes of comparison, ^{13}C -nmr shielding data for steroids (243, 244) were used as references and substituent effects (243, 244) at saturated carbons in 5 α - and 5 β -androsteranes were used to confirm the assignments of the structures.

Mass spectra (Table 10):

Zaretskii *et al* (271), on the basis of mass spectra results of the following 17 ξ -hydroxy-17 ξ -alkyl steroid derivatives, 3-methoxy-17-alkyl-1,3,5-estratrien-17-ol and 17-alkyl-19-nortestosterone, concluded that the ratio $\text{M}^+ - \text{H}_2\text{O}/\text{M}^+$ is higher for the 17 α -alcohol than for the 17 β -alcohol.

Other workers (246) used the ratio of $\text{M}^+ - \text{H}_2\text{O}/\text{M}^+$ as an empirical basis to distinguish between 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16) and its 17 α -hydroxy/17 β -methyl epimer. This ratio was further used to suggest the absence of the C-17 epimer in the metabolic products isolated from rat faeces (156).



	R_1	R_2	$\text{M}^+ - \text{H}_2\text{O} / \text{M}^+ (\text{in}\%)$		R_1	R_2	$\text{M}^+ - \text{H}_2\text{O} / \text{M}^+ (\text{in}\%)$
1.	CH_3	OH	44.6		CH_3	OH	35.5
2.	OH	CH_3	14.5		OH	CH_3	17.3
3.	C_2H_5	OH	41		C_2H_5	OH	21
4.	OH	C_2H_5	21		OH	C_2H_5	15
5.	$\text{CH}_2 - \underset{\text{CH}_2}{\underset{\parallel}{\text{C}}} - \text{CH}_3$	OH	62.5				
6.	OH	$\text{CH}_2 - \underset{\text{CH}_2}{\underset{\parallel}{\text{C}}} - \text{CH}_3$	21				

Although in theory it is reasonable to expect that the loss of water from the hydroxyl group at the C-17 position might be a useful indicator of stereochemistry at that position, our results obtained from the complete set of twelve isomers (Table 10) show that this generalization is misleading. As a basis for a generalization with respect to the stereochemistry at C-17, the loss of a methyl group from the steroid molecule is complicated by the presence of the C-10 and C-13 methyl groups which will fragment to a greater or lesser degree, depending on the presence of other functional groups in the molecule regardless of any influence on the C-17 methyl group. A similar difficulty affects the loss of water involving the C-17 hydroxyl group as many metabolites may be expected to contain new hydroxyl groups.

The only useful correlation obtained from the mass spectra data is

the relationship between the M^+-H_2O/M^+ ratio, for the 5α -and 5β -configuration. The M^+-H_2O/M^+ ratio for the 5β -series is consistently less (0.35-0.68) than unity, whereas for the 5α -series, the range is approximately an order of magnitude greater (3.3-5.3) than for 5α -series (Table 10). The M^+-H_2O/M^+ ratio, therefore, is a useful indicator for the stereochemistry of the A/B ring junction in the diol compounds, but it is of no value in assigning the configuration of the C-17 position. Among the four ketones, the M^+-H_2O/M^+ ratio showed no correlation with any structural features.

As the differences between the spectra of the eight $3\xi,17\xi$ -diols are largely a result of different intensities rather than different fragmentation pathways, the similarity between the spectra is marked. Therefore, care is required in making the comparison, especially with spectra obtained from biological samples which may contain mixtures of isomers which are difficult to separate.

III. EXPERIMENTAL

Instruments:

Melting points (mp) were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (ir) spectra were recorded with a Perkin-Elmer Model 237 Infrared spectrophotometer either in a KBr disc or in CCl_4 solution as indicated below. Proton magnetic resonance (^1H -nmr) spectra were recorded using deuteriochloroform (CDCl_3) and/or deuteriopyridine ($\text{C}_5\text{D}_5\text{N}$) as the solvent and tetramethylsilane (TMS) as the internal standard using the following instruments: (1) Varian HR 220 for all the metabolic products and the derivatives of 17β -hydroxy- 17α -methyl- 5α - and 5β -androstan-3-one and all the synthetic compounds of 17β -hydroxy- 17α -methyl- 5α - and 5β -androstane derivatives; (2) NIC (220 MHz) or NTC (360 MHz) for the synthetic compounds of 17α -hydroxy- 17β -methyl- 5α - and 5β -androstane derivatives at the Canadian 220 MHz NMR Centre, Ontario Research Foundation, Medical Science Building, University of Toronto, Toronto, Ontario; (3) Nicolet modified Bruker WH 90 DS using NTCFT software at 90 MHz for all the metabolic products and derivatives of 17β -hydroxy- 17α -methyl-4-androsten-3-one and 17β -hydroxy- 17α -methyl-1,4-androstadien-3-one at the Department of Chemistry, University of Manitoba, Winnipeg, Manitoba. The following designations are used in characterizing ^1H -nmr signals: singlet (s), doublet (d), triplet (t), doublet of doublet (dd), multiplet (m), broadened (br) and width-half height ($W_{1/2}$). Carbon-13 magnetic resonance (^{13}C -nmr) spectra were recorded on a Nicolet modified Bruker WH 90 DS with NTCFT software at 22.63 MHz using the polarization transfer spectroscopy technique (242) in either CDCl_3 (77.1 ppm peak as the internal standard) or $\text{C}_5\text{D}_5\text{N}$ (149.9 ppm peak as the internal

standard). Mass spectra (ms) were recorded on a Finnigan Quadrupole Model 1015 mass spectrophotometer at 70 eV using the direct probe method at the Department of Chemistry, University of Manitoba, Winnipeg, Manitoba. Elemental analyses were performed by Mr. G. Crouch or Mr. B. Baldeo at the Microanalytical Laboratory, School of Pharmacy, University of London, England. Gas-liquid chromatography (glc) was carried out on a Varian Aerograph Series 1800 chromatograph equipped with hydrogen flame ionization detector and a pyrex glass column of internal diameter 2 mm and length 180 cm packed with 3% OV-17 on chromosorb GHP 100/120 mesh. The detector temperature was 308–340°C, column temperature 180°C increasing 10°/min to 310° and the flow rates of carrier gases N₂, H₂, and air were 45 mL/min, 45 mL/min, and 300 mL/min. In some cases mass spectrometry was performed on the effluents of the column using a capillary collector attached to a glc stream splitter. As required, steroids were treated with trimethylsilyl chloride in pyridine before injection so that 1 µL contained 25 µg of steroid. Relative retention times were related to known amounts of internal standards (C₂₄ and C₂₆ hydrocarbons in 0.5% w/v chloroform) to avoid error due to injection volume (272). The steroids were injected as 0.5% w/v pyridine solutions. In all cases, straight lines passing through the origin were obtained, indicating that glc response was proportional to the amount of metabolic product present. High pressure liquid chromatography (HPLC) was carried out on a Waters Scientific Ltd. - M 45 G solvent delivery system with U6K Universal liquid chromatogram injector using RCM-100 Radial Compression Separation System (RCSS), and with 10 µ Radial-PAKTM Si column (8 mm ID x 10 cm) and RCSS silica guard pak. The detector used was R 401 refractive index detector-differential refractometer. The sample solution injected had a

concentration of about 50 $\mu\text{g}/\mu\text{L}$ in 5% acetone/dichloromethane (filtered and degassed) and the flow rate was 1.0-1.5 mL/min. The standards and their retention times (RT) were as follows: 17 α -methyl-5 α -androstande-3 β , 17 β -diol (14.3 min), 17 α -methyl-5 α -androstande-3 α , 17 β -diol (11.6 min), 17 α -methyl-5 β -androstande-3 β , 17 β -diol (11.1 min), 17 α -methyl-5 β -androstande-3 α , 17 β -diol (15.9 min) and 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (17.3 min).

Materials:

Thin-layer chromatography (tlc) was carried out on Merck 60 F-254 precoated silica gel plates 0.25 mm thick using one of the following liquid phases: (1) 10% methanol/chloroform; (2) 25% ethyl acetate/petroleum ether; or (3) 50% ethyl acetate/petroleum ether (the petroleum ether being in the boiling range of 60° to 90°C). The tlc plates were made visible by irradiating with an ultraviolet (uv) source and/or spraying with a 4% v/v solution of concentrated sulfuric acid in 95% ethanol followed by heating at approximately 100°C in an oven for 5 to 10 minutes. Thick-layer chromatography was carried out on silica gel coated (Merck silica gel 60H) preparatory plate 1 mm thick. After developing the plate in a specified liquid phase, bands were removed from the plate and dissolved in warm methanol and chloroform (1:1), filtered through a sintered glass funnel containing a celite layer, and taken to dryness at reduced pressure. The residue was dissolved in ether and filtered and the solvent was evaporated. For olefins, the plates were sprayed with silver nitrate (AgNO_3) solution, dried at 100°C for 30 minutes and stored in the dark. Column chromatography was carried out with alumina (Brockmann activity II aluminum oxide for chromatographic adsorption analysis, BDH Chemicals Ltd.), silica gel (Merck Silica Gel 60H for tlc

from BDH Chemicals Ltd.), or ethyl acetate treated alumina prepared by immersing the alumina in ethyl acetate (273), stirring and letting the mixture stand for at least 2 days with occasional shaking, filtering and drying at 80°C.

Albino male rabbits used in the experiments were purchased from Canadian Breeding Laboratories, St. Constant, P.Q., Canada. β -Glucuronidase (β -D-glucuronide glucuronohydrolase, Type B-I, bovine liver) with an activity of 1×10^6 Fishman Units (FU)/g was supplied by Sigma Chemical Co., St. Louis, Missouri. Of the steroids used in the metabolism studies, 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one was synthesized (257), 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one was obtained from Sigma, 17 β -hydroxy-17 α -methyl-4-androsten-3-one was from Searle Labs., and 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one was from Ciba. The solvents - benzene, chloroform, dichloromethane, dioxane, pyridine, t-butyl alcohol, and tetrahydrofuran [epoxide free (274)] - were distilled before use.

Methods:

Administration of the steroids and collection of urine:

Albino male rabbits (2.3-3.0 kg) were maintained on a Purina rabbit chow diet and water *ad libitum* and were housed singly in cages which were designed for efficient separation of urine and faeces. A controlled illumination environment of 12 hours of light and 12 hours of darkness was maintained. The animals were each dosed four times at two-day intervals with a well-mixed slurry of steroid (0.7 g) in propylene glycol (11 mL) by oral administration (the steroid left in the tube or syringe was collected in order to estimate the actual weight administered). The night before each dosing, solid food was removed and only water was available

to the animals. Urine was collected from the first day until 72 hours after the last dose under a layer of toluene. Daily samples were pooled and stored at -5°C . (The crude residue obtained from the freshly collected urine dosed with 17β -hydroxy- 17α -methyl- 5α -androstan-3-one showed an identical glc pattern to the residue obtained from urine which was left at room temperature for 7 days under a layer of toluene). Two control urine collections were carried out with the same rabbits prior to the dosing with steroids; the first control urine was collected under the layer of toluene for 7 days after keeping the rabbits in metabolism cages for 3-4 days to become adapted to the environment, and the second control urine was collected after administering propylene glycol (11 mL) four times at two-day intervals for 8 days. In repeated experiments with different animals, no significant differences in weight of total crude neutral fraction, glc, and tlc between the two control experiments were observed. No gross alteration in the appearance, food intake, or behaviour of the dosed animals was observed.

Enzymatic hydrolysis of urine and isolation of steroids:

The urine was adjusted to pH 4.9 with glacial acetic acid and incubated with bovine liver β -D-glucuronidase (400 FU/mL of urine) in a water bath (37°C) for 72 hours. The enzyme-hydrolyzed urine (275) was extracted with ether three times (the urine and ether formed an emulsion on shaking, requiring centrifugation for efficient separation). The combined ether layer, which constituted the total crude ether extract, was separated into the total crude neutral (TCN), acidic (TCA), and enolic/phenolic (TCE/P) fractions by successive extraction with 0.5N HCl, 1N NaOH and water (see Figure 21) followed by the general work-up

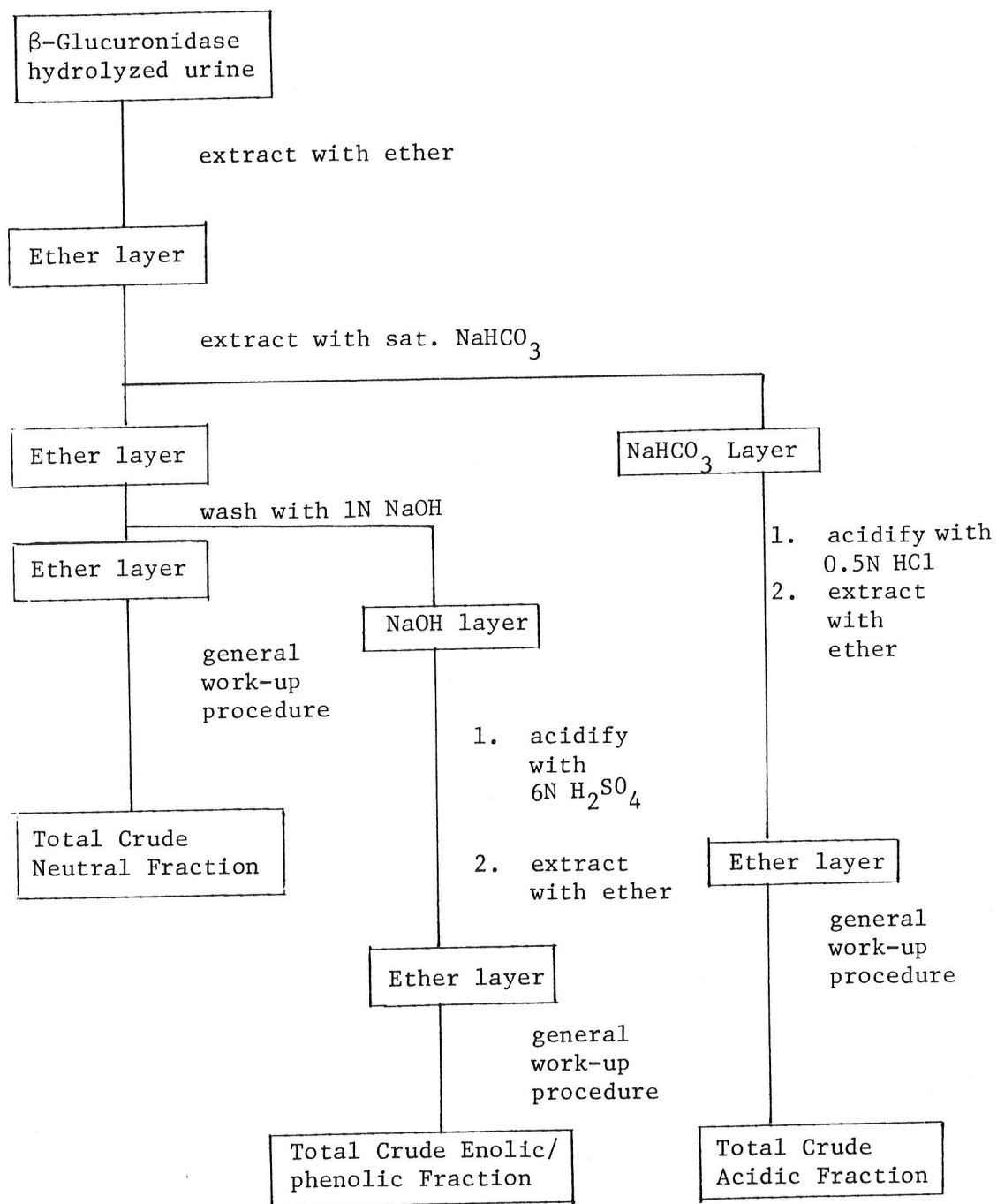


Figure 21. Scheme to Separate TCN, TCA, and TCE/P from the Total Crude Urine Extract

procedure* which is as follows: the ether layer was washed with water, saturated aqueous bicarbonate (to remove acidic material), or with dilute hydrochloric acid (to remove basic material), again with water and finally with saturated sodium chloride solution and the ether layer was dried over anhydrous sodium sulfate, filtered through cotton wool, and evaporated to dryness using a rotary evaporator at reduced pressure. Tlc and glc of TCN, TCA and TCE/P fractions of controls and steroid dosed urine showed the presence of metabolic products only in the TCN fraction.

Separation of steroids into ketonic and non-ketonic fractions:

TCN (0.5 mmoles, 145.2 mg) from dosed urine and Girard reagent-T (225) [N-hydrazinocarbonyl methyl-trimethylammonium chloride, $\text{NH}_2\text{NHCO-CH}_2\text{N}(\text{CH}_3)_3\text{Cl}$, 92 mg, 0.55 mmoles] in 1M- $\text{CH}_3\text{COOH}/95\% \text{C}_2\text{H}_5\text{OH}$ (1.5 mL) was heated to reflux for one hour. The reaction mixture was poured into excess cold saturated aqueous sodium bicarbonate solution and the product extracted with ether. The ether layer was washed with saturated sodium bicarbonate solution and followed by the general work-up procedure to give a crude non-ketonic fraction (alcohols). The combined sodium bicarbonate extractions and washings were acidified with concentrated hydrochloric acid, heated on the steam bath for 30 minutes, cooled, extracted with ether and followed by the general work-up procedure to give a crude ketonic fraction. Equivalent control experiment was carried out with TCN from control urine and the results were compared

* This procedure was applied to other extractions of steroids as products from reaction mixtures or from other enzyme hydrolysis, as noted below.

with steroid dosed urine by tlc and glc.

Jones oxidation (chromic acid/acetone oxidation):

A 0.5-5% solution of the steroid in acetone was treated dropwise with Jones reagent (231) until a permanent orange color was obtained. After 5 to 10 minutes, excess methanol was added until the color turned green, and was washed with water, and excess saturated NaHCO_3 solution was added. Acetone was removed at reduced pressure. Ether extraction followed by the general work-up procedure yielded the product (ketone).

Acetylation:

To a 10% solution of the steroid in dry pyridine, acetic anhydride (one-half the volume of pyridine) (276) was added. After standing overnight, the reaction mixture was poured into an ice-water mixture and allowed to stand for 30 minutes. The reaction mixture was acidified with dilute hydrochloric acid (2N), and extracted with ether, followed by the general work-up procedure.

Metabolism of 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43):

Administration of 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43):

Three rabbits were each dosed three times at two-day intervals with a well-mixed slurry of compound 43 (0.7 g) in propylene glycol (11 mL) by oral administration. A total of 5 L of urine was collected over seven days. Blank urine was collected from the rabbits which were dosed with propylene glycol (11 mL) as described above prior to dosing with the steroid (43).

Enzymatic hydrolysis of urine and isolation of steroids:

A portion of the pooled urine (4.3 L) was adjusted to pH 5.0 with glacial acetic acid and incubated with β -glucuronidase (400 FU/mL) at 37°C for 72 hours, followed by ether extraction (3 x 500 mL) (275). The combined ether layers were washed successively with saturated aqueous NaHCO_3 , 1N NaOH, water and dried over sodium sulfate (anhydrous) as outlined in Figure 21 and separated into TCN, TCA and TEC/P fractions. From extraction of compound 43, TCN (0.46 g/L), TCA (1.47 g/L) and TCE/P (0.27 g/L) were obtained and from the propylene glycol dosed control urine, TCN (0.14 g/L), TCN (0.4 g/L) and TCE/P (0.26 g/L) were obtained. TCA and TCE/P fractions did not yield any major components which are different from those in the equivalent control experiment on tlc and glc. Treatment of the acidic portions with diazomethane did not show any new components on tlc and glc.

Separation of steroids into ketonic and non-ketonic fractions:

A portion of the TCN (150 mg) was treated with Girard reagent-T (225) to give an alcohol fraction (121 mg, 80.7%) and a ketonic fraction (19 mg, 12.7%). Glc of the alcohol fraction showed the same component as the TCN; the ketonic fraction contained only a negligible quantity of material which was not considered any further.

Chromatography of the total crude neutral fraction:

The total crude neutral fraction (1.80 g) was dissolved in benzene (100 mL) and chromatographed over ethyl acetate treated alumina (273). The fractions were combined on the basis of tlc and crystallinity. The following three components were isolated by eluting with 10% v/v ether/benzene and they were identified by tlc and spectroscopic [ir, ^1H -nmr

(Table 9), ms (Table 10)] comparison with authentic samples.

17 β -Hydroxy-17 α -methyl-5 α -androstan-3-one (43); mp 183-185°C, [lit. 188-191°C (279)].

17 α -Methyl-5 α -androstan-3 α ,17 β -diol (34); mp 176-180°C [lit. 189-190°C (metabolite) and 189-190°C (synthetic compound) (140); 188-190°C (154); 180-181°C (authentic sample) (277); 181-182°C (synthetic sample, see SYNTHESSES)].

17 α -Methyl-5 α -androstan-3 β ,17 β -diol (35); mp 209-211°C [lit. 211-212°C (278), 212-214°C (279); 212-214°C (154); 209-210°C (synthetic sample, see SYNTHESSES)], mixture mp with an authentic sample was not depressed; R_f : 0.48 (10% MeOH/CHCl₃); ms m/z : 306 (M^+), 219 (M^+-CH_3), 288 (M^+-H_2O), 273 ($M^+-CH_3-H_2O$), 255 ($M^+-CH_3-2H_2O$); 1H -nmr (CDCl₃) δ : 0.83 (3H, C-10CH₃,s), 0.85 (3H, C-13CH₃,s), 1.20 (3H, C-17CH₃,s), 3.56 (1H, 3 α -H, br m, $W_{1/2}$ =21 Hz) ppm (Table 9; p 100).

17 α -Methyl-5 α -androstan-3 β ,15 α ,17 β -triol (45) (31 mg) was eluted with 2.5% v/v methanol/ether and was recrystallized from methanol/ethyl acetate, mp 220-223°C; R_f : 0.20 (5% MeOH/CHCl₃); ir (KBr) ν_{max} : 3380 (OH) cm⁻¹; ms m/z : 332 (M^+); 307 (M^+-CH_3), 304 (M^+-H_2O), 289 ($M^+-CH_3-H_2O$), 286 (M^+-2H_2O), 271 ($M^+-CH_3-2H_2O$), 268 (M^+-3H_2O); 1H -nmr (CDCl₃) δ : 0.83 (3H, C-10CH₃,s) 0.86 (3H, C-13CH₃,s), 1.33 (3H, C-17CH₃,s), 2.38 (1H, 16 β -H, dd, J =10 and 15 Hz), 3.57 (1H, 3 α -H, m, $W_{1/2}$ =27 Hz), 4.05 (1H, 15 β -H, sextet, J =4, 10 and 10 Hz); (C_5D_5N) δ 0.92 (3H, C-13CH₃,s), 1.18 (3H, C-10CH₃,s), 1.67 (3H, C-17CH₃,s), 2.91 (1H, 16 β -H, dd, J =10 and 15 Hz), 3.89 (1H, 3 α -H, m, $W_{1/2}$ =27 Hz), 4.36 (1H, 15 β -H, sextet, J =4, 10 and 10 Hz) ppm. Acetylation (276) gave a non-crystalline diacetate, 1H -nmr (CDCl₃) δ : 0.84 (3H, C-13CH₃,s), 0.89 (3H, C-10CH₃,s), 1.32 (3H, C-17CH₃,s), 2.00 and 2.02 (each 3H, 3 β - and

15 α -OCOCH₃,s,s) 2.50 (1H,16 β -H,dd,J=10 and 15 Hz), 4.69 (1H,3 α -H,m,W_{1/2}=27 Hz), 4.89 (1H,15 β -H, sextet,J=4,10 and 10 Hz) ppm: oxidation with Jones reagent (231) gave a non-crystalline dionol, RRT 4.20; ir (KBr) $\nu_{\max}$: 3610 (OH), 1738 (5-membered ring C=O), 1710 (6-membered ring C=O) cm⁻¹; ms m/z: 318 (M⁺), 303 (M⁺-CH₃), 300 (M⁺-H₂O), 285 (M⁺-CH₃-H₂O); ¹H-nmr (CDCl₃) δ : 0.96 (3H,C-13CH₃,s), 1.03 (3H,C-10CH₃,s), 1.25 (3H,C-17CH₃,s) ppm.

17 α -Methyl-5 α -androstand-3 α ,6 α ,17 β -triol (46) (160 mg) was eluted with 5% v/v methanol/ether and was recrystallized from methanol, mp 252-254°C; R_f: 0.30 (0.5% MeOH/CHCl₃); ir (KBr) $\nu_{\max}$: 3360, 3285, 3150 (OH) cm⁻¹; ms m/z: 322 (M⁺ absent), 307 (M⁺-CH₃), 304 (M⁺-H₂O), 289 (M⁺-CH₃-H₂O), 286 (M⁺-2H₂O), 271 (M⁺-CH₃-2H₂O), 268 (M⁺-3H₂O), 258 (M⁺-CH₃-3H₂O); ¹H-nmr (CDCl₃) δ : 0.82 (3H,C-10CH₃,s), 0.83 (3H,C-13CH₃,s), 1.22 (3H,C-17CH₃,s), 3.74 (1H,6 β -H, unresolved sextet), 4.13 (1H,3 β -H,m,W_{1/2}=9 Hz); (C₅D₅N) δ : 0.94 (3H,C-10CH₃,s), 1.13 (3H,C-13CH₃,s), 1.42 (3H,C-17CH₃,s), 2.86 (1H,4 α -H,ddd,J=2.5,5 and 14 Hz), 3.75 (1H, 6 β -H,sextet,J=4.5,10.5 and 10.5 Hz), 4.44 (1H,3 β -H,m,W_{1/2}=7 Hz) ppm; ¹³C-nmr (C₅D₅N) (APPENDIX); Anal. Found: C, 74.49; H, 10.66. C₂₀H₃₄O₃ requires C, 74.49; H, 10.63; acetylation (276) yielded a diacetate which was crystallized from dichloromethane/ethyl acetate, mp 202-203°C; ir (KBr) $\nu_{\max}$: 3568 (free OH), 3450 (H-bonded OH), 1740, 1725 (ester C=O), 1258, 1235 (ester C=O) cm⁻¹; ¹H-nmr (CDCl₃) δ : 0.84 (3H,C-13CH₃,s), 0.88 (3H,C-10CH₃,s), 1.21 (3H,C-17CH₃,s), 2.02 and 2.06 (each 3H, 3 α - and 6 α -OCOCH₃,s and s), 4.66 (1H,6 β -H,sextet,J=5,11 and 11 Hz), 5.06 (1H,3 β -H, unresolved m) ppm; Anal. Found: C, 70.85; H, 9.44; C₂₄H₃₈O₅ requires C, 70.90; H, 9.42. Oxidation with Jones reagent (231) gave a non-crystalline

dionol, R_f : 0.75 (5% MeOH/CHCl₃); ir (KBr) $\nu_{\max}$: 3420 (OH), 1706 (6-membered ring C=O) (cm⁻¹; ms m/z: 318 (M⁺), 303 (M⁺-CH₃), 300 (M⁺-H₂O), 285 (M⁺-CH₃-H₂O); ¹H-nmr (CDCl₃) δ : 0.89 (3H, C-13CH₃, s), 0.98 (3H, C-10CH₃, s), 1.23 (3H, C-17CH₃, s); (C₅D₅N) δ : 0.95 (3H, C-13CH₃, s); 1.08 (3H, C-10CH₃, s), 1.45 (3H, C-17CH₃, s) ppm.

Metabolism of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44):

Preparation of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44):

To a solution of compound 13 (25 g, 82 mmol) in dry pyridine (114 mL), 10% Pd/CaCO₃ (2.5 g) (257) was added. The mixture was stirred in a hydrogen atmosphere at room temperature and atmospheric pressure. When uptake of hydrogen ceased after the equivalent number of moles of hydrogen (2L) was absorbed (several hours), the reaction mixture was filtered through a sintered funnel containing a celite layer under suction and washed with chloroform. The collected solvents were evaporated under pressure. The residue was redissolved in chloroform (200 mL) and the solution was washed with 2N H₂SO₄. The chloroform layer was washed with water and poured through a filter paper to remove water. The chloroform was evaporated and the residue was crystallized* to give compound 44 (21.8 g, 87%) mp 78–80°C and 117–117.5°C on recrystallization from ether/hexane [lit. 74–76°C and 119–121°C (dil. EtOH) and anal. sample 120–121°C (258)]; R_f : 0.42 (50% EtOAc/PE); ir (KBr) $\nu_{\max}$: 3625 (OH),

* Three out of five preparations of compound 44 did not crystallize at this stage and the non-crystalline residue was chromatographed over alumina using the following solvents: 50% bz/PE, 75% bz/PE, 95% bz/ether and ether which yielded a crystalline product (50–54%), mp 78–80°C and 117–117.5°C.

1720 (6-membered ring C=O) cm^{-1} ; ms m/z: 304 (M^+), 289 ($\text{M}^+ - \text{CH}_3$), 286 ($\text{M}^+ - \text{H}_2\text{O}$), 271 ($\text{M}^+ - \text{CH}_3 - \text{H}_2\text{O}$), 253 ($\text{M}^+ - \text{CH}_3 - 2\text{H}_2\text{O}$); ^1H -nmr (CDCl_3) δ : 0.87 (3H, C-13 CH_3 , s), 1.06 (3H, C-10 CH_3 , s), 1.25 (3H, C-17 CH_3 , s); ($\text{C}_5\text{D}_5\text{N}$) δ : 0.95 (3H, C-10 CH_3 , s), 1.12 (3H, C-13 CH_3 , s), 1.48 (3H, C-17 CH_3 , s) ppm; ^{13}C -nmr (APPENDIX).

Administration of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44):

Six rabbits (2.3–2.6 kg) were each dosed four times at two day intervals with a well-mixed slurry of compound 44 (0.7 g)*, in propylene glycol (11 mL) by oral administration. The total urine collected over 10 days was 8750 mL. Blank urine (4162 mL) was collected from four animals over 8 days and the propylene glycol administered blank urine (2816 mL) was collected for three animals over eight days prior to dosing of the steroid.

Enzymatic hydrolysis of urine and isolation of steroids:

Blank, propylene glycol administered blank, and steroid dosed urines were individually adjusted to pH 4.9 with glacial acetic acid and incubated with β -glucuronidase as described above (275). These enzyme hydrolyzed urines were separated into the TCN, TCA and TCE/P fractions (Table 12) as outlined in Figure 21.

TCA and TCE/P fractions did not show any major components on tlc and glc and they were not significantly different from those in the equivalent blank urines. Treatment of the acidic portions with diazomethane did not show any new components on tlc.

*The amount weighed out for six animals was 16.8 g, but the actual weight dosed was 15.833g because 0.967 g was left on the syringe and tube which was washed out and collected.

Table 12. Weights of TCN, TCA and TCE/P from enzyme hydrolyzed urines of blank, propylene glycol (PG) blank and 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44).

Wt (g)/L of urine	TCN	TCA	TCE/P
Compound <u>44</u>	1.25 g/L	0.24 g/L	0.37 g/L
Blank urine	0.07 g/L	0.36 g/L	0.37 g/L
PG blank	0.09 g/L	0.40 g/L	0.26 g/L

Separation of steroids into ketonic and non-ketonic fractions:

A portion of TCN (142.2 mg) was treated with Girard reagent-T (92 mg) (225) to give a crude non-ketonic fraction (alcohols) (135 mg, 94.7%) and a ketonic fraction (10 mg, 0.7%).

Jones oxidation:

A 20% solution of TCN of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44) (97 mg) in acetone (231) gave 82.2 mg of neutral fraction and 7.1 mg of acidic and enolic/phenolic fractions. With 100 mg of TCN from propylene glycol dosed urine gave 62.4 mg of neutral and 19.9 mg of acidic and enolic/phenolic fractions. Glc and tlc data for the oxidation products of TCN of compound 44 and of PG dosed urine were compared with data for oxidation products of the isolated metabolites of compound 44. The latter corresponded to the neutral fraction of TCN from the compound 44, but not to the acidic and enolic/phenolic fractions, nor to the oxidation products of PG dosed urine.

Chromatography of the total crude neutral fraction:

The total crude neutral fraction (10.31 g) from rabbits dosed with compound 44 was dissolved in benzene (250 mL) and chromatographed over

ethyl acetate washed alumina (272). Eluted fractions (45 mL) of benzene, ether and methanol mixtures of gradually increasing polarity were collected. Fractions were combined on the basis of tlc, crystallinity and weight. Each component was identified by chromatographic (tlc and glc) and spectroscopic (ir, mass, ^1H -nmr and ^{13}C -nmr) methods; diols were directly compared with authentic compounds. The following compounds were isolated and identified:

17 α -Methyl-5 β -androstand-3 α ,17 β -diol (33)

Crystalline material (8.77 g) was obtained on elution with 10-25% v/v ether/benzene and was recrystallized from dichloromethane/methanol to give compound 33 (5.5 g) which was identified as 17 α -methyl-5 β -androstand-3 α ,17 β -diol (33), mp 164-165°C [lit. 165-168°C (metabolite) and 164-166°C (synthetic) (140); 164-165°C (154)]; mixture mp with an authentic sample (see SYNTHESSES) 164-165°C; R_f : 0.54 (10% MeOH/ CHCl_3); ir (KBr) ν_{max} : 3350 (OH), 1090, 1080, 1045 ($\text{C}=\text{O}$) cm^{-1} ; ms m/z: 306 (M^+), 271 (M^+-CH_3), 291 (M^+-CH_3), 288 ($\text{M}^+-\text{H}_2\text{O}$), 273 ($\text{M}^+-\text{CH}_3-\text{H}_2\text{O}$), 270 ($\text{M}^+-2\text{H}_2\text{O}$), 255 ($\text{M}^+-\text{CH}_3-2\text{H}_2\text{O}$); ^1H -nmr (CDCl_3) δ : 0.84 (3H, C-13 CH_3 , s), 0.95 (3H, C-10 CH_3 , s), 1.23 (3H, C-17 CH_3 , s), 3.64 (1H, 3 β -H, br m, $W_{1/2}$ =21 Hz); ($\text{C}_5\text{D}_5\text{N}$) δ : 0.96 (3H, C-10 CH_3 , s), 1.11 (3H, C-13 CH_3 , s), 1.45 (3H, C-17 CH_3 , s), 3.92 (1H, 3 β -H, br m, $W_{1/2}$ =21 Hz) ppm. ^{13}C -nmr ($\text{C}_5\text{D}_5\text{N}$) (APPENDIX).

17 α -Methyl-5 β -androstand-3 α ,16 β ,17 β -triol (40)

A more polar compound 40 (231 mg) was eluted with 2.5-5% v/v methanol/ether and was recrystallized from dichloromethane/acetone to give compound 40 (25 mg), which was identified as 17 α -methyl-5 β -androstand-3 α ,16 β ,17 β -triol (40), mp 254-255°C [lit. 254-257°C turning to needle 266-268°C (155)]; R_f : 0.32 (10% MeOH/ CHCl_3); ir (KBr) ν_{max} : 3406, 3306 (OH),

1060, 1036, 1020 (C=O) cm^{-1} ; ms m/z: 322 (M^+), 304 ($\text{M}^+ - \text{H}_2\text{O}$), 286 ($\text{M}^+ - 2\text{H}_2\text{O}$), 271 ($\text{M}^+ - \text{CH}_3 - 2\text{H}_2\text{O}$); ^1H -nmr (CDCl_3) δ : 0.84 (3H, C-13 CH_3 , s), 0.96 (3H, C-10 CH_3 , s), 1.16 (3H, C-17 CH_3 , s), 3.61 (1H, 3 β -H, br septet, $W_{1/2} = 23$ Hz), 3.65 (1H, 16 α -H, dd, $J = 5.5$ and 8 Hz); ($\text{C}_5\text{D}_5\text{N}$) δ : 0.95 (3H, C-10 CH_3 , s), 1.14 (3H, C-13 CH_3 , s), 1.34 (3H, C-17 CH_3 , s), 3.91 (1H, 3 β -H, br septet, $W_{1/2} = 23$ Hz), 3.92 (1H, 16 α -H, dd, $J = 5.5$ and 8.5 Hz) ppm; ^{13}C -nmr ($\text{C}_5\text{D}_5\text{N}$) (APPENDIX); Anal. Found: C, 74.65; H, 10.70 $\text{C}_{20}\text{H}_{34}\text{O}_3$ requires C, 74.49; H, 10.63. Acetylation (276) yielded non-crystalline diacetate, ^1H -nmr (CDCl_3) δ : 0.88 (3H, C-13 CH_3 , s), 0.98 (3H, C-10 CH_3 , s), 1.25 (3H, C-17 CH_3 , s), 2.07 and 2.14 (each 3H, 3 α - and 16 β -OCO CH_3 , s and s), 4.66-4.84 (2H, 3 β -H and 16 β -H, m) ppm. Oxidation with Jones reagent (231) gave a non-crystalline product ir (KBr) ν_{max} : 1720 (br. 6-membered ring C=O) cm^{-1} .

17 α -Methyl-5 β -androsterane-3 α ,1 β ,17 β -triol (50)

One of the more polar compounds (80.5 mg) was eluted with 5% methanol/ether and was recrystallized from dichloromethane/acetone to give compound 50 (17 mg), which was identified as 17 α -methyl-5 β -androsterane-3 α ,1 β ,17 β -triol (50), mp 206-208°C; R_f : 0.12 (10% MeOH/ CHCl_3); ir (KBr) ν_{max} : 3120 (OH), 1049, 1038, 1024 (C=O) cm^{-1} ; ms m/z: 322 (M^+), 304 ($\text{M}^+ - \text{H}_2\text{O}$), 286 ($\text{M}^+ - 2\text{H}_2\text{O}$), 271 ($\text{M}^+ - \text{CH}_3 - 2\text{H}_2\text{O}$); ^1H -nmr (CDCl_3) δ : 0.86 (3H, C-13 CH_3 , s), 1.09 (3H, C-10 CH_3 , s), 1.23 (3H, C-17 CH_3 , s), 3.99 (1H, 1 α -H, m, $W_{1/2} = 9$ Hz), 4.16 (1H, 3 β -H, m, $W_{1/2} = 23$ Hz); ($\text{C}_5\text{D}_5\text{N}$) δ : 1.14 (3H, C-13 CH_3 , s), 1.41 (3H, C-10 CH_3 , s), 1.48 (3H, C-17 CH_3 , s), 4.30 (1H, 1 α -H, m, $W_{1/2} = 9$ Hz), 4.88 (1H, 3 β -H, m, $W_{1/2} = 23$ Hz) ppm; ^{13}C -nmr ($\text{C}_5\text{D}_5\text{N}$) (APPENDIX). Anal. Found: C, 72.12; H, 10.69, $\text{C}_{20}\text{H}_{34}\text{O}_3 \cdot \frac{1}{2}\text{H}_2\text{O}$ requires C, 72.46; H, 10.64. Acetylation (272) yielded non-crystalline diacetate, ^1H -nmr (CDCl_3) δ : 0.84 (3H, C-13 CH_3 , s), 0.94 (3H, C-10 CH_3 , s), 1.26 (3H, C-17 CH_3 , s), 2.07 (6H, 1 β - and

$3\alpha\text{-OCOCH}_3$, s and s), 5.06 (1H, $3\beta\text{-H}$, m, $W_{\frac{1}{2}}=23$ Hz), 5.25 (1H, $1\alpha\text{-H}$, m, $W_{\frac{1}{2}}=9$ Hz) ppm. Oxidation with Jones reagent (231) yielded non-crystalline product, ir (CCl_4) ν_{max} : 1720 (6-membered ring C=O) cm^{-1} .

17 α -Methyl-5 β -androstand-3 α ,12 β ,17 β -triol (51)

A more polar compound was eluted with 5% v/v methanol/ether and was recrystallized from dichloromethane/acetone to give compound 51 (67 mg), which was identified as 17 α -methyl-5 β -androstand-3 α ,12 β ,17 β -triol (51), mp 179-179.5°C; R_f : 0.20 (10% MeOH/ CHCl_3); ir (KBr) ν_{max} : 3392 (OH), 1073, 1045, 1014 (C-O) cm^{-1} ; ms m/z : 322 (M^+), 304 ($M^+-\text{H}_2\text{O}$), 286 ($M^+-2\text{H}_2\text{O}$), 271 ($M^+-\text{CH}_3-2\text{H}_2\text{O}$); ^1H -nmr (CDCl_3) δ : 0.92 (3H, C-13 CH_3 , s), 0.96 (3H, C-10 CH_3 , s), 1.39 (3H, C-17 CH_3 , s), 3.68 (1H, $3\beta\text{-H}$, septet, $W_{\frac{1}{2}}=24$ Hz), 3.77 (1H, $12\alpha\text{-H}$, dd, $J=5$ and 11 Hz), ($\text{C}_5\text{D}_5\text{N}$) δ : 0.98 (3H, C-10 CH_3 , s), 1.27 (3H, C-13 CH_3 , s), 1.64 (3H, C-17 CH_3 , s), 3.86 (1H, $3\beta\text{-H}$, m, $W_{\frac{1}{2}}=24$ Hz), 3.95 (1H, $12\alpha\text{-H}$, dd, $J=5$ and 11 Hz) ppm. ^{13}C -nmr ($\text{C}_5\text{D}_5\text{N}$) (APPENDIX). Anal. Found: C, 73.34; H, 10.69. $\text{C}_{20}\text{H}_{34}\text{O}_3 \cdot \frac{1}{2}\text{H}_2\text{O}$ requires C, 73.46; H, 10.64. Acetylation (272) yielded non-crystalline diacetate, ^1H -nmr (CDCl_3) δ : 0.98 (3H, C-13 CH_3 , s), 1.00 (3H, C-10 CH_3 , s), 1.28 (3H, C-17 CH_3 , s), 2.03 and 2.06 (each 3H, 3α - and $12\beta\text{-OCOCH}_3$, s and s), 4.73 (1H, $3\beta\text{-H}$, septet), 4.89 (1H, $12\alpha\text{-H}$, dd, $J=5$ and 11 Hz) ppm. Oxidation with Jones reagent (231) yielded a non-crystalline dionol, ir (KBr) ν_{max} = 3600, 3440 (OH), 1715 (6-membered ring C=O) cm^{-1} ; ^1H -nmr (CDCl_3) δ : 1.19 (3H, C-10 CH_3 , s), 1.27 (3H, C-13 CH_3 , s), 1.48 (3H, C-17 CH_3 , s) ppm.

17 α -Methyl-5 β -androstand-3 α ,16 α ,17 β -triol (42)

A more polar compound (338 mg) was eluted with 5% v/v methanol/ether and was recrystallized from methanol to give compound 42 (42 mg) which was identified as 17 α -methyl-5 β -androstand-3 α ,16 α ,17 β -triol (42),

mp 214-215°C [lit. 221-222°C (155); 220-221°C (280)], R_f : 0.11 (10% MeOH/ CHCl_3); ir (KBr) ν_{max} : 3400 (OH), 1095, 1060, 1043 (C-O) cm^{-1} ; ms m/z: 322 (M^+), 304 ($\text{M}^+ - \text{H}_2\text{O}$), 286 ($\text{M}^+ - 2\text{H}_2\text{O}$), 271 ($\text{M}^+ - \text{CH}_3 - 2\text{H}_2\text{O}$); ^1H -nmr (CDCl_3) δ : 0.88 (3H, C-13 CH_3 , s), 0.96 (3H, C-10 CH_3 , s), 1.22 (3H, C-17 CH_3 , s), 3.68 (1H, 3 β -H, br septet, $W_{1/2}$ =22 Hz), 4.33 (1H, 16 β -H, dd, J=3 and 10 Hz); ($\text{C}_5\text{D}_5\text{N}$) δ : 0.98 (3H, C-10 CH_3 , s), 1.19 (3H, C-13 CH_3 , s), 1.71 (3H, C-17 CH_3 , s), 3.96 (1H, 3 β -H, septet, $W_{1/2}$ =22 Hz), 4.94 (1H, 16 β -H, dd, J=3 and 10.5 Hz) ppm; ^{13}C -nmr (APPENDIX). Anal. Found: C, 74.61; H, 10.72. $\text{C}_{20}\text{H}_{34}\text{O}_3$ requires C, 74.49; H, 10.63. Acetylation (272) yielded a non-crystalline diacetate, ^1H -nmr (CDCl_3) δ : 0.93 (3H, C-13 CH_3 , s), 0.97 (3H, C-10 CH_3 , s), 1.09 (3H, C-17 CH_3 , s), 2.07 and 2.15 (each 3H, 3 α - and 16 α - OCOCH_3 ; s and s), 4.76 (1H, 3 β -H, br septet, $W_{1/2}$ =23 Hz), 5.10 (1H, 16 β -H, dd, J=3 and 10 Hz) ppm; ir (CCl_4) ν_{max} : 3540 (OH), 1760 (acetate C=O), 1380, 1365 (ter. OH), 1238 (acetate C-O) cm^{-1} . Oxidation with Jones reagent (231) yielded a non-crystalline dionol, ir (CCl_4) ν_{max} : 3360 (OH), 1750 (5-membered ring C=O), 1717 (6-membered ring C=O) cm^{-1} ; ^1H -nmr (CDCl_3) δ : 0.84 (3H, C-13 CH_3 , s), 1.09 (3H, C-10 CH_3 , s), 1.23 (3H, C-17 CH_3 , s) ppm.

Metabolism of 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13):

Administration of 17 β -hydroxy-17 α -methyl-4-androstan-3-one (13):

Nine rabbits (2.2-3.0 kg) were each dosed four times at two-day intervals with a well-mixed slurry of compound 13 (0.7 g)* in propylene glycol (11 mL) by oral administration and the total collected urine over 10 days was 13 L.

*The amount weighed out for nine animals was 25.2 g, but the actual weight dosed was 22.845 g because 2.355 g was left on the syringe and tube which was washed out and collected.

Enzymatic hydrolysis of urine and isolation of steroids:

Owing to the large volume of urine (13 L), it was worked up in four portions. The urine was adjusted to pH 4.9 with glacial acetic acid and incubated with β -glucuronidase (400 FU/mL) in 37°C water bath (275). The TCN, TCA and TCE/P fractions (Table 13) were separated as outlined in Figure 15.

Table 13. Weights of TCN, TCA, TCE/P from enzyme hydrolyzed urines of PG blank and 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) dosed.

	TCN	TCA	TCE/P
Compound <u>13</u>	9.90 g/13 L	5.974 g/13 L	4.780 g/13 L
Compound <u>13</u>	0.76 g/L	0.46 g/L	0.37 g/L
PG Blank	0.09 g/L	0.40 g/L	0.26 g/L

TCA and TCE/P fractions did not show any major components on tlc and glc and they were not significantly different from those in the equivalent blank urines. Treatment of the acidic portions with diazomethane did not show any new compounds on tlc and glc.

Chromatography of the total crude neutral fraction:

The total crude neutral fraction (9.503 g) was dissolved in benzene (230 mL) and chromatographed over ethyl acetate washed alumina (272).

Eluted fractions (45 mL) with benzene, ether and methanol mixtures of gradually increasing polarity was collected. The fractions were combined on the basis of tlc, crystallinity and weight. Each component was identified by chromatographic (tlc and glc) and spectroscopic (ir, mass, ^1H -nmr and ^{13}C -nmr) methods; diols were identified by direct comparison with the authentic samples. The following compounds

were identified:

17 β -Hydroxy-17 α -methyl-4-androsten-3-one (13)

A crystalline material (114 mg) was eluted from 10% v/v ether/benzene and was recrystallized from methanol to give compound 13 (10 mg), which was identified as 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13), mp 162-163°C [lit. 162-164°C (66b)]; mixture mp with an authentic sample was not depressed; R_f : 0.61 (10% MeOH/CHCl₃); ir (KBr) $\nu_{\max}$: 3430 (OH), 1660, 1610 (conj C=O) cm⁻¹; ms m/z: 302 (M⁺); ¹H-nmr (CDCl₃) δ : 0.91 (3H, C-13CH₃, s), 1.21 (6H, C-10 and C-17CH₃, s), 5.75 (1H, C-4H, s) ppm.

17 α -Methyl-5 α -androstan-3 α ,17 β -diol (34)

A crystalline material 34 (40 mg) was eluted with 10% ether/benzene together with compound 35. HPLC separation gave fractions with retention times (RT) and ¹H-nmr spectra correspond to compounds 34 and 35. Compound 34 was identified as 17 α -methyl-5 α -androstan-3 α ,17 β -diol 34; RT (HPLC): 11.6 min; ms m/z: 306 (M⁺), 291 (M⁺-CH₃), 288 (M⁺-H₂O), 273 (M⁺-CH₃-H₂O), 255 (M⁺-CH₃-2H₂O); ¹H-nmr (CDCl₃) δ : 0.80 (3H, C-10CH₃, s), 0.85 (3H, C-13CH₃, s), 1.21 (3H, C-17CH₃, s), 4.04 (1H, 3 β -H, m, $W_{1/2}$ =7 Hz) ppm.

17 α -Methyl-5 α -androstan-3 β ,17 β -diol (35)

A crystalline compound (2.315 g) was eluted with 25% v/v ether/benzene and was recrystallized from methanol to give compound 35 (303 mg) which was identified as 17 α -methyl-5 α -androstan-3 β ,17 β -diol (35), mp 205-208°C [lit. 212-214°C (154), 211-212°C (278), 212-214°C (279), 206-209°C (282), 208-210°C (283)], mixture mp with an authentic sample (see SYNTHESSES) was not depressed; R_f : 0.56 (10% MeOH/CHCl₃); ir (KBr) $\nu_{\max}$: 3460, 3380, 3190 (OH), 1074, 1035 (C-O), 855 [characteristic peak

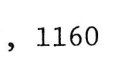
for 5 α -compound and absent for 5 β -compound (258)]; RT (HPLC): 14.3 min; ms m/z: 306 (M^+), 291 (M^+-CH_3), 288 (M^+-H_2O), 273 ($M^+-CH_3-H_2O$), 255 ($M^+-CH_3-2H_2O$); 1H -nmr ($CDCl_3$) δ : 0.83 (3H, C-10 CH_3 , s), 0.84 (3H, C-13 CH_3 , s), 1.20 (3H, C-17 CH_3 , s), 3.57 (1H, 3 α -H, septet, $W_{1/2}$ =24 Hz) ppm; ^{13}C -nmr (APPENDIX 1).

17 α -Methyl-5 β -androsterane-3 α ,17 β -diol (33)

A crystalline material (240 mg) was eluted with 25% ether/benzene and was recrystallized from dichloromethane to give compound 33 (40 mg) which was identified as 17 α -methyl-5 β -androsterane-3 α ,17 β -diol (33); mp 161-164°C [synthetic authentic sample 164-165°C (see SYNTHESSES): lit. 165-168°C (metabolite) and 164-166°C (synthetic sample) (140); 164-165°C (154)]; R_f : 0.56 (10% MeOH/ $CHCl_3$); ms m/z: 306 (M^+), 291 (M^+-CH_3), 288 (M^+-H_2O), 273 ($M^+-H_2O-CH_3$), 270 (M^+-2H_2O), 255 ($M^+-2H_2O-CH_3$); 1H -nmr ($CDCl_3$) δ : 0.84 (3H, C-13 CH_3 , s), 0.94 (3H, C-10 CH_3 , s), 1.22 (3H, C-17 CH_3 , s), 3.63 (1H, 3 β -H, septet, $W_{1/2}$ =26 Hz) ppm; ^{13}C -nmr (APPENDIX).

17 α -Methyl-5 β -androsterane-3 α ,16 β ,17 β -triol (40)

One of the more polar materials (3.324 g) was eluted with 2.5-5% v/v methanol/ether and was recrystallized from methanol to give compound 40, which was identified as 17 α -methyl-5 β -androsterane-3 α ,16 β ,17 β -triol (40), mp 255-257°C; mixture mp with this compound 40 from metabolism of compound 44 was not depressed, [lit. 254-257°C (turning into needles) and 266-268°C (155)]; R_f : 0.23 (10% MeOH/ $CHCl_3$); ir (KBr) ν_{max} : 3405, 3305 (OH), 1060, 1038, 1020 (C-O) cm^{-1} ; ms m/z: 322 (M^+), 307 (M^+-CH_3), 304 (M^+-H_2O), 286 (M^+-2H_2O), 271 ($M^+-2H_2O-CH_3$); 1H -nmr ($CDCl_3$) δ : 0.85 (3H, C-13 CH_3 , s), 0.95 (3H, C-10 CH_3 , s), 1.14 (3H, C-17 CH_3 , s), 3.5-3.8 (2H, 3 β and 16 α -H, unresolved m); (C_5D_5N) δ : 0.93 (3H, C-10 CH_3 ,

s), 1.12 (3H, C-13CH₃, s), 1.31 (3H, C-17CH₃, s), 3.86 (1H, 3β-H, m, $W_{1/2}$ = 23 Hz), 3.94 (1H, 16α-H, dd, J = 5.5 and 8.5 Hz) ppm, which is identical to the compound 40 isolated from compound 44 and previously reported one (155); ¹³C-nmr (APPENDIX). Acetylation (276) yielded a non-crystalline diacetate, ¹H-nmr (CDCl₃) δ: 0.86 (3H, C-13CH₃, s), 0.95 (3H, C-10CH₃, s), 1.24 (3H, C-17CH₃, s), 2.03 and 2.09 (each 3H, 3α and 16β-OCOCH₃, both s), 4.67 (1H, 16α-H, unresolved m), 4.71 (3β-H, m, $W_{1/2}$ = 21 Hz) ppm. Oxidation with Jones reagent (231) yielded a non-crystalline 3-one-16,17-acetonide; ms m/z: 360 (M⁺); ir (CCl₄) $\nu_{\max}$: 1720 (6-membered ring C=O), 1465, 1428, 1389, 1360 [(CH₃)₂C<], 1160 ( cyclic ether) cm⁻¹, ¹H-nmr (CDCl₃) δ: 1.01 (3H, C-13CH₃, s), 1.11 (3H, C-10CH₃, s), 1.20 (3H, C-17CH₃, s), 1.43 [6H, (CH₃)₂C<] ppm.

17α-Methyl-5β-androstane-3α,16α,17β-triol (42)

One of the more polar compounds (1.345 g) was eluted with 2.5-5% v/v methanol/ether and was recrystallized from methanol to give compound 42 (192 mg) which was identified as 17α-methyl-5β-androstane-3α,16α,17β-triol (42), mp 218-219°C [lit. 221-222°C (155), 220-221°C (280)], mixture mp with this compound 42 isolated from metabolism of compound 44 was not depressed; R_f: 0.15 (10% MeOH/CHCl₃); ir (KBr) $\nu_{\max}$: 3440, 3360 (OH), 1058, 1038, 1008 (C-O) cm⁻¹; ms m/z: 322 (M⁺), 307 (M⁺-CH₃), 304 (M⁺-H₂O), 286 (M⁺-2H₂O), 271 (M⁺-CH₃-2H₂O); ¹H-nmr (CDCl₃) δ: 0.86 (3H, C-13CH₃, s), 0.93 (3H, C-10CH₃, s), 1.20 (3H, C-17CH₃, s), 3.64 (1H, 3β-H, m, $W_{1/2}$ = 23 Hz), 4.33 (1H, 16β-H, dd, J = 3 and 10 Hz), which is identical with compound 42 isolated from metabolism of compound 44 and a previously reported one (155); ¹³C-nmr (APPENDIX). Anal. Found: C, 74.44; H, 10.76; C₂₀H₃₄O₃ requires C, 74.49; H, 10.63. Acetylation (276) yielded a non-

crystalline diacetate, $^1\text{H-nmr}$ (CDCl_3) δ : 0.92 (3H, C-13 CH_3 , s), 0.95 (3H, C-10 CH_3 , s), 1.07 (3H, C-17 CH_3 , s), 2.03 and 2.11 (each 3H, 3 α - and 16 α - OCOCH_3 , s and s), 4.67 (1H, 3 β , septet, $W_{\frac{1}{2}}=22$ Hz), 5.06 (1H, 16 β -H, dd, $J=3$ and 10 Hz) ppm. Oxidation with Jones reagent (231) yielded a non-crystalline dionol, ir (CCl_4) ν_{max} : 3350 (OH), 1742 (5-membered ring C=O), 1712 (6-membered ring C=O), cm^{-1} ; $^1\text{H-nmr}$ (CDCl_3) δ : 0.83 (3H, C-13 CH_3 , s), 1.07 (3H, C-10 CH_3 , s), 1.20 (3H, C-17 CH_3 , s) ppm.

17 α -Methyl-5 α -androsterane-3 β ,6 α ,17 β -triol (53)

A more polar compound (1.035 g) was eluted with 2.5-5% v/v methanol/ether and was recrystallized from methanol to give compound 53 (153 mg) which was identified as 17 α -methyl-5 α -androsterane-3 β ,6 α ,17 β -triol (53), mp 212-213°C; R_f : 0.05 (10% MeOH/ CHCl_3); ir (KBr) ν_{max} : 3228 (OH), 1078, 1030 (C-O), 859 [characteristic peak for 5 α -compound (258)]; ms m/z: 322 (M^+), 307 (M^+-CH_3), 304 ($\text{M}^+-\text{H}_2\text{O}$), 289 ($\text{M}^+-\text{CH}_3-\text{H}_2\text{O}$), 286 ($\text{M}^+-2\text{H}_2\text{O}$); $^1\text{H-nmr}$ (CDCl_3) δ : 0.85 (6H, C-10 and C-13 CH_3 , s), 1.21 (3H, C-17 CH_3 , s), 3.25-3.80 (2H, 3 α -H and 6 β -H br unsymm. m), ($\text{C}_5\text{D}_5\text{N}$) δ : 0.92 (3H, C-10 CH_3 , s), 1.10 (3H, C-13 CH_3 , s), 1.42 (3H, C-17 CH_3 , s), 3.0-4.0 (2H, 3 α -H and 6 β -H, unresolved m) ppm. Anal. Found: C, 74.12; H, 10.64; $\text{C}_{20}\text{H}_{34}\text{O}_3$ requires C, 74.49; H, 10.63. $^{13}\text{C-nmr}$ ($\text{C}_5\text{D}_5\text{N}$) (APPENDIX). Acetylation (276) yielded a non-crystalline diacetate, $^1\text{H-nmr}$ (CDCl_3) δ : 0.84 (3H, C-13 CH_3 , s), 0.91 (3H, C-10 CH_3 , s), 1.21 (3H, C-17 CH_3 , s), 2.02 (6H, 3 β - and 6 β - OCOCH_3 , s), 4.67 (2H, 3 α -H and 6 β -H, m) ppm. Oxidation with Jones reagent (231) yielded a non-crystalline dionol; ir (CCl_4) ν_{max} : 3625 (OH), 1710 (6-membered ring C=O, both C-3 and C-6 carbonyl groups) cm^{-1} ; ms m/z: 318 (M^+), 303 (M^+-CH_3); $^1\text{H-nmr}$ (CDCl_3) δ : 0.89 (3H, C-13 CH_3 , s), 0.98 (3H, C-10 CH_3 , s), 1.25 (3H, C-17 CH_3 , s) ppm.

Metabolism of 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16):

Administration of 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16):

Three rabbits (2.2-2.3 kg) were dosed four times at two day intervals with a well-mixed slurry of compound 16 (0.66 g) in propylene glycol (11 mL) by oral administration. The total urine collected for 11 days was 5100 mL and the total amount of steroid dosed was 7.6963 g*.

Enzymatic hydrolysis of urine and isolation of steroids:

After adjustment of its pH to 4.9, the urine was incubated with β -glucuronidase (400 FU/mL) at 37°C (275). The enzyme hydrolyzed urines were separated into total crude neutral (1.647 g; 0.32 mg/L), acidic (1.657 g; 0.32 mg/L), and enolic/phenolic (1.04 g; 0.20 mg/L) fractions. TCA and TCE/P fractions did not show any major components on tlc and glc, and they were not significantly different from those in the equivalent blank urines. Treatment of the acidic portions with diazomethane did not show any new compounds on tlc and glc.

Chromatography of the total crude neutral fraction:

The total crude neutral fraction (1.346 g) was dissolved in chloroform (10 mL) and chromatographed over Silica gel (thin-layer chromatography silica gel 60 H) column by eluting with chloroform, ethyl acetate and methanol with gradual increase in polarity. The rate of flow was adjusted by the fish tank pump, and 25 mL fractions were collected. The following compounds were isolated and identified by chromatographic (tlc and glc) and spectral (ir, ms, ^1H -nmr, ^{13}C -nmr) methods and diols

* The amount weighed out for three animals was 7.962 g, but the actual weight dosed was 7.6963 g because 0.2657 g was left on the syringe and tube which was washed out and collected.

were identified by the direct comparison with authentic samples.

17 β -Hydroxy-17 α -methyl-1,4-androstadien-3-one (16)

A crystalline compound (37 mg) was eluted with 2.5% v/v ethyl acetate/chloroform and was identified as 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16), R_f : 0.41 (10% MeOH/CHCl₃); ir (KBr) $\nu_{\max}$: 3440 (OH str), 1655, 1620 and 1600 (1,4-dien-3-one) cm⁻¹; ¹H-nmr (CDCl₃) δ : 0.96 (3H, C-13CH₃,s), 1.20 (3H, C-17CH₃,s), 1.25 (3H, C-10CH₃,s), 6.07 (1H, C-6H,m), 6.22 (1H, C-2H,dd,J=2 and 10 Hz), 7.06 (1H, C-1H,d,J=10 Hz)ppm (Table 8).

17 α -Methyl-5 α -androstande-3 α ,17 β -diol (34)

HPLC separation of the crystalline material (20 mg) using 2.5% v/v ethyl acetate/chloroform as the eluant gave fractions with retention times and ¹H-nmr spectra corresponding to 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16) (RT: 17.3 min) and 17 α -methyl-5 α -androstande-3 α ,17 β -diol (34) (RT: 11.6 min) in trace amount.

17 α -Methyl-5 α -androstande-3 β ,17 β -diol (35)

A crystalline compound (40 mg) was eluted with 2.5% v/v ethyl acetate/chloroform and was recrystallized from dichloromethane to give compound 35 (9 mg), which was identified as 17 α -methyl-5 α -androstande-3 β ,17 β -diol (35), mp 209-210°C [lit. 212-214°C (154,279), 211-212°C (278), 209-210°C, (synthetic sample, see SYNTHESSES)], mixture mp with an authentic sample was not depressed; R_f : 0.48 (10% MeOH/CHCl₃); ms m/z: 306 (M⁺), 219 (M⁺-CH₃), 288 (M⁺-H₂O), 273 (M⁺-CH₃-H₂O), 255 (M⁺-CH₃-2H₂O); ¹H-nmr (CDCl₃) δ : 0.83 (3H, C-13CH₃,s), 0.85 (3H, C-10CH₃,s), 1.20 (3H, C-17CH₃,s), 3.56 (1H, 3 α -H, br m, W_{1/2}=21 Hz) ppm (Table 9, p. 100).

17 α -Methyl-5 β -androsterane-3 α ,17 β -diol (33)

A crystalline compound (95 mg) was eluted with 5% ethyl acetate/chloroform and was recrystallized from dichloromethane to give compound 33 (30 mg), which was identified as 17 α -methyl-5 β -androsterane-3 α ,17 β -diol (33), mp 163-164°C [lit. 165-168°C (metabolite) and 164-166°C (synthetic) (140), 164-165°C (154)], mixture mp with an authentic sample was not depressed; R_f : 0.48 (10% MeOH/CHCl₃); ms m/z: 306 (M⁺), 291 (M⁺-CH₃), 288 (M⁺-H₂O), 273 (M⁺-H₂O-CH₃), 255 (M⁺-2H₂O-CH₃); ¹H-nmr (CDCl₃) δ : 0.84 (3H, C-13CH₃, s), 0.94 (3H, C-10CH₃, s), 1.22 (3H, C-17CH₃, s), 3.59 (1H, 3 β -H, m, $W_{1/2}$ =21 Hz) ppm.

16 β ,17 β -Dihydroxy-17 α -methyl-5 β -androster-1-en-3-one (55)

A non-crystalline material (29 mg) was eluted with 20% ethyl acetate/chloroform and was identified as 16 β ,17 β -dihydroxy-17 α -methyl-5 β -androster-1-en-3-one (55); R_f : 0.48 (10% MeOH/CHCl₃); ms m/z: 318 (M⁺); ¹H-nmr (CDCl₃) δ : 0.89 (3H, C-13CH₃, s), 1.13 (3H, C-17CH₃, s), 1.22 (3H, C-10CH₃, s), 2.68 (1H, 4 α -H, dd, J =15 and 17 Hz), 3.67 (1H, 16 α -H, dd, J =5 and 8 Hz), 5.90 (1H, C-2H, dd, J =1 and 10 Hz) and 6.93 (1H, C-1H, d, J =10 Hz) ppm (Table 8).

16 β ,17 β -Dihydroxy-17 α -methyl-1,4-androstadien-3-one (56)

A crystalline compound (42 mg) was eluted with 20% ethyl acetate/chloroform and was recrystallized from ethyl acetate to give compound 56 (16 mg), which was identified as 16 β ,17 β -dihydroxy-17 α -methyl-1,4-androstadien-3-one (56), mp 201-202°C; R_f : 0.44 (10% MeOH/CHCl₃); ms m/z: 316 (M⁺), 298 (M⁺-H₂O), 283 (M⁺-CH₃-H₂O), 265 (M⁺-CH₃-2H₂O); ¹H-nmr (CDCl₃) δ : 0.94 (3H, C-13CH₃, s), 1.12 (3H, C-17CH₃, s), 1.25 (3H, C-10CH₃, s),

3.61 (1H, 16 α -H, dd, J=5 and 8 Hz), 6.07 (1H, C-4H), 6.25 (1H, C-2H, dd, J=2 and 10 Hz) and 7.05 (1H, C-1H, d, J=10 Hz) ppm (Table 8); Anal. Found: C, 75.32; H, 8.72; C₂₀H₂₈O₃ requires C, 75.91; H, 8.92.

6 β ,17 β -Dihydroxy-17 α -methyl-1,4-androstadien-3-one (57)

A crystalline compound (259 mg) was eluted with 30-40% v/v ethyl acetate/chloroform and was recrystallized from ethyl acetate to give compound 57 (46 mg), which was identified as 6 β ,17 β -dihydroxy-17 α -methyl-1,4-androstadien-3-one (57), mp 227-228°C [lit. 228-229°C (aq EtOH) (141)] R_f: 0.40 (10% MeOH/CHCl₃); ir (KBr) $\nu_{\max}$: 3490, 3380 (OH str), 1655 and 1620 (1,4-dien-3-one) cm⁻¹; ms m/z: 316 (M⁺), 298 (M⁺-CH₃), 283 (M⁺-CH₃-H₂O), 265 (M⁺-CH₃-2H₂O); ¹H-nmr (CDCl₃) δ : 0.97 (3H, C-13CH₃, s), 1.20 (3H, C-17CH₃, s), 1.46 (3H, C-10CH₃, s), 4.54 (1H, 6 α -H, t, J=2.5 Hz), 6.15 (1H, C-4H, s, partially overlapping with C-2H peak), 6.22 (1H, C-2H, dd, J=2 and 10 Hz), 7.06 (1H, C-1H, d, J=10 Hz); (C₅D₅N) δ : 1.17 (3H, C-13CH₃, s), 1.43 (3H, C-17CH₃, s), 1.67 (3H, C-10CH₃, s), 4.74 (1H, 6 α -H, s), 6.44 (1H, C-4H, d, J=2.5 Hz), 6.44 (1H, C-2H, dd, J=2 and 10 Hz), 7.01 (1H, C-1H, d, J=10 Hz) ppm (Table 8, p. 86).

16 α ,17 β -Dihydroxy-17 α -methyl-5 β -androst-1-en-3-one (58)

A crystalline compound (103 mg) was eluted with 50% v/v ethyl acetate/chloroform and was recrystallized from ethyl acetate to give compound 58 (34 mg), which was identified as 16 α ,17 β -dihydroxy-17 α -methyl-5 β -androst-1-en-3-one (58), mp 211-212°C; R_f: 0.36 (10% MeOH/CHCl₃); ms m/z: 318 (M⁺), 303 (M⁺-CH₃), 300 (M⁺-H₂O), 285 (M⁺-H₂O-CH₃), 267 (M⁺-2H₂O-CH₃); ¹H-nmr (CDCl₃) δ : 0.91 (3H, C-13CH₃, s), 1.18 (3H, C-17CH₃, s), 1.21 (3H, C-10CH₃, s), 2.79 (1H, 4 α -H, dd, J'=15 and 17 Hz), 4.29 (1H, 16 β -H,

dd, $J=3$ and 9.5 Hz), 5.89 (1H, C-2H, dd, $J=1$ and 10 Hz) and 6.81 (1H, C-1H, d, $J=10$ Hz ppm (Table 8); Anal. Found: C, 75.41 ; H, 9.48 , $C_{20}H_{30}O_3$ requires C, 75.43 ; H, 9.50 .

$6\beta, 16\beta, 17\beta$ -Trihydroxy- 17α -methyl- $1,4$ -androstadien- 3 -one (59)

A crystalline compound (353 mg) was eluted with 50 - 75% v/v ethyl acetate and was recrystallized from methanol to give compound (59) (109 mg), which was identified as $6\beta, 16\beta, 17\beta$ -trihydroxy- 17α -methyl- $1,4$ -androstadien- 3 -one (59), mp 256 - 258°C ; R_f : 0.26 (10% MeOH/ CHCl_3); ir (KBr) ν_{max} : 3410 (OH str), 1660 and 1615 ($1,4$ -dien- 3 -one) cm^{-1} ; ms m/z : 332 (M^+), 314 ($M^+ - H_2O$), 299 ($M^+ - H_2O - CH_3$), 296 ($M^+ - 2H_2O$), 281 ($M^+ - 2H_2O - CH_3$), 278 ($M^+ - 3H_2O$), 263 ($M^+ - 3H_2O - CH_3$); ^1H -nmr (C_5D_5N) δ : 1.20 ($3H, C-13CH_3, s$), 1.32 ($3H, C-17CH_3, s$), 1.65 ($3H, C-10CH_3, s$), 3.91 (1H, 16α -H, dd, $J=5$ and 9 Hz), 4.72 (1H, 6α -H, t, $J=2.5$ Hz), 6.42 (1H, C-4H, d, $J=2$ Hz), 6.42 (1H, C-2H, dd, $J=2$ and 10 Hz) and 7.08 (1H, C-1H, d, $J=10$ Hz) ppm (Table 8); ^{13}C -nmr (C_5D_5N) (APPENDIX). Anal. Found: C, 70.99 ; H, 8.64 . $C_{20}H_{28}O_4 \cdot \frac{1}{2}H_2O$ requires C, 70.35 ; H, 8.56 . Acetylation (276) yielded a non-crystalline diacetate, ^1H -nmr (CDCl_3) δ : 1.22 ($3H, C-13CH_3, s$), 1.26 ($3H, C-17CH_3, s$), 1.35 ($3H, C-10CH_3, s$), 2.06 and 2.11 (each $3H, 6\beta$ - and 16β -OCOCH $_3, s, s$), 4.69 (1H, 16α -H, dd, $J=5$ and 7.5 Hz), 5.50 (1H, 6α -H, t, $J=2.5$ Hz), 6.22 (1H, C-4H, d, $J=2$ Hz), 6.29 (1H, C-2H, dd, $J=2$ and 10 Hz) and 7.00 (1H, C-1H, d, $J=10$ Hz) ppm.

Syntheses:

Preparation of 17α -methyl- 5β -androstan- 3 -one (44):

Compound 44 was prepared by the hydrogenation of 17β -hydroxy- 17α -methyl- 4 -androsten- 3 -one (13) with 10% pd/ CaCO_3 in pyridine (257); mp 78 - 80°C and 117 - 117.5°C (87%); [lit. 74 - 76°C and 119 - 121°C (dil EtOH) and anal. sample 120 - 121°C (258)]. For detailed procedure, see Metabolism

of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44).

Preparation of 17 α -hydroxy-17 β -methyl-5 β -androstan-3-one (52)

(Figure 18, p. 96):

17-Methylene-5-androsten-3 β -ol (67)

To a solution of triphenyl methyl phosphonium bromide ($\text{Ph}_3\text{PCH}_3\text{Br}$, 150 g, 2.8 mmoles), potassium tertiary butoxide (39 g) and tertiary butyl alcohol (39 mL) in dry dimethyl sulfoxide (DMSO, 540 mL) in a N_2 atmosphere was added to the solution of 3 β -hydroxy-5-androsten-17-one (2) (24.24 g, 0.084 mmoles) in DMSO (540 mL). The reaction mixture was heated at 80-85°C in an oil bath with continuous stirring for one hour while still in a N_2 atmosphere. After cooling the reaction mixture, which contained a yellow precipitate, it was poured into a mixture of 10% sodium chloride solution (3 L) and crushed ice. The mixture was extracted with ether and followed by the general work-up procedure. The crystalline product was recrystallized from dichloromethane/methanol to give compound 67 (22.1 g, 91.2%), mp 133-134°C [lit. 134-135°C (143), 133-134°C (259)]; R_f : 0.30 (50% EtOAc/PE); ir (CCl_4) ν_{max} : 3625 (OH), 1057, 890 (17-methylene), 1040, 1018 (C-O) cm^{-1} .

17-Methylene-4-androsten-3-one (68)

The Oppenauer oxidation (143,259,281) was carried out as follows: to a 2 L round bottom flask was equipped with a steam distillation, was added 17-methylene-5-androsten-3 β -ol (67) (22.1 g, 0.077 mmoles), freshly distilled cyclohexanone (138 mL) and distilled toluene (506 mL), and the steam distillation was continued. When 50 mL of toluene was distilled, aluminum isopropoxide [$\text{Al} [\text{OCH}(\text{CH}_3)_2]_3$, 22.1 g, 0.108 moles] in toluene (366 mL) was added slowly through a separatory funnel. Toluene was

continuously distilled by the steam distillation with addition of 60 mL fresh toluene. The reaction was monitored by the Zimmerman test on tlc for the presence of acetone produced. When 850 mL of toluene was distilled, the Zimmerman test was negative, and the presence of the starting material was negligible on tlc. To the reaction mixture, saturated potassium sodium tartrate solution (Rochelle salt solution, 190 mL) was added, and the steam distillation was continued until the distillate contained only aqueous layer and no more organic layer. When the reaction mixture was cooled, the product was extracted with ether and washed with 2N hydrochloric acid followed by the general work-up procedure. The product was recrystallized from dichloromethane/methanol to give compound 68 (17 g, 77%), mp 133-134°C [lit. 129-131°C (acetone) (143); 129-131°C (acetone/pentane) (259); 134-135°C (acetone/hexane or methanol) (259); 135-136°C (281)]; R_f : 0.38 (25% EtOAc/PE); ir (CCl_4) ν_{max} : 1678 (6-membered ring C=O), 1680 and 880 (17-methylene) cm^{-1} .

17-Methylene-5 β -androstan-3-one (69)

17-Methylene-4-androsten-3-one (68) (12 g, 41.89 mmol) was hydrogenated with Pd/10CaCO₃ (1.392 g) in dry pyridine (62 mL) at atmospheric pressure. The product in pyridine was filtered through sintered glass funnel containing a layer of celite under the vacuum and was washed with chloroform to prevent the catalyst catching fire. Pyridine was evaporated, the crude product was dissolved in ether (500 mL) and acidified with 2N H₂SO₄ followed by the general work-up procedure. Recrystallization of the crude product from dichloromethane/methanol yielded compound 69 (6.6 g, 55%); mp 97-98°C; R_f : 0.79 (50% EtOAc/PE; AgNO₃ sprayed tlc plate); ir (CCl_4) ν_{max} : 1715 (6-membered ring C=O), 1650 and 880 (17-

methylene) cm^{-1} ; ms m/z: 286 (M^+); 271 ($\text{M}^+ - \text{CH}_3$); ^1H -nmr (CDCl_3) δ : 0.81 (3H, C-13 CH_3 , s), 1.05 (3H, C-10 CH_3 , s), 4.64 (2H, 17= CH_2 , m, $W_{1/2}$ =5 Hz) ppm;
 Anal. Found: C, 83.54; H, 10.67; $\text{C}_{20}\text{H}_{30}\text{O}$ requires C, 83.86; H, 10.58.

17 α ,20-Oxido-17 β -methyl-5 β -androstan-3-one (70)

A solution of 17-methylene-5 β -androstan-3-one (69) (4.484 g, 15.65 mmoles) in dichloromethane (40 mL) was added to the solution of m-chloroperbenzoic acid (143) (85% purity; 3.496 g, 10% excess) in dichloromethane (37.4 mL) and was stirred at room temperature for 15 minutes. The reaction mixture was poured into 10% Na_2SO_3 solution (100 mL) in a separatory funnel and the organic layer was washed with saturated NaHCO_3 solution followed by the general work-up procedure and yielded compound 70 (4.5 g, ~100%) after recrystallization from ethyl acetate, mp 150-151°C; R_f : 0.81 (50% EtOAc/PE); ir (CCl_4) ν_{max} : 1715 (6-membered ring C=O), 1267 (epoxide) cm^{-1} ; ms m/z: 302 (M^+); ^1H -nmr (CDCl_3) δ : 0.84 (3H, C-13 CH_3 , s), 1.03 (3H, C-10 CH_3 , s), 2.74 (2H, C-17 CH_2 -O, AB quartet, $J_{\text{AB}}=4$ and 10z) ppm; Anal. Found: C, 78.89, H, 9.88, $\text{C}_{20}\text{H}_{30}\text{O}_2$ requires C, 79.42, H, 10.00.

17 β -Methyl-5 β -androstan-3 ξ ,17 α -diol (63 and 64)

To a solution of 17 α ,20-oxido-17 β -methyl-5 β -androstan-3-one (70) (4.5 g, 1.49 mmoles) in dry ether (200 mL), lithium aluminum hydride (4.5 g) in dry ether (100 mL) was added slowly (143) and the mixture was heated to reflux for 1 hour. Excess LiAlH_4 was decomposed by careful addition of ethanol followed by 10% NH_4Cl solution until no more hydrogen gas evolved. The ether layer was separated and extracted by the general work-up procedure to give a mixture of compound 63 and 64; (4g,

89%); R_f : 0.29 (both alcohols run together) (50% EtOAc/PE); ^1H -nmr (Table 9). This crude product was used for the next step.

17 α -Hydroxy-17 β -methyl-5 β -androstan-3-one (52)

The crude product from the above reaction, 17 β -methyl-5 β -androstan-3 ξ ,17 α -diol (63 and 64) (3.5 g, 11.4 mmoles), was dissolved in acetone (250 mL) and the oxidation was carried out with Jones reagent (231). The non-crystalline ketone product (3.31 g) was chromatographed on alumina using 20-40% ether/benzene as the eluant to give compound 52 (1.086 g, 31%) which was recrystallized from acetone, mp 133-134°C; R_f : 0.45 (50% EtOAc/PE); ir (CCl_4) ν_{max} : 3620 (OH), 1715 (6-membered ring C=O) cm^{-1} ; ms m/z: 304 (M^+), 289 ($\text{M}^+ - \text{CH}_3$), 286 ($\text{M}^+ - \text{H}_2\text{O}$), 271 ($\text{M}^+ - \text{H}_2\text{O} - \text{CH}_3$); ^1H -nmr (CDCl_3) δ : 0.71 (3H, C-13 CH_3 , s), 1.04 (3H, C-10 CH_3 , s), 1.21 (3H, C-17 CH_3 , s) ppm; Anal. Found: C, 78.81, H, 10.23; $\text{C}_{20}\text{H}_{32}\text{O}_2$ requires C, 78.90, H, 10.59; ^{13}C -nmr (APPENDIX).

Preparation of 17 α -hydroxy-17 β -methyl-5 α -androstan-3-one (47)

(Figure 17, p. 94):

17-Methylene-5 α -androstan-3 β -ol (65)

A solution of 3 β -hydroxy-5 α -androstan-17-one (23) (5 g, 17.2 mmoles) in DMSO (110 mL) was added dropwise to a mixture of $\text{Ph}_3\text{PCH}_3\text{Br}$ (31 g, 86.6 mmoles), t-BuOK (8.1 g) and t-BuOH (8.1 mL) in DMSO (110 mL) under N_2 (109). The stirred reaction mixture was stirred and heated at 82°C for 1 hour. After cooling to room temperature, 10% NaCl solution and crushed ice (618 mL) was added. The product was extracted with ether followed by the general work-up procedure to give compound 65 (4.2 g, 84%); mp 141-142.5°C [lit. 144-145°C (MeOH) (260)].

17 α ,20-Oxido-17 β -methyl-5 α -androstan-3 β -ol (66)

A solution of 17-methylene-5 α -androstan-3 β -ol (4 g, 13.89 mmoles) in dichloromethane (40 mL) was added to the solution of m-chloroperbenzoic acid (143) (85% purity, 3.02 g, 10% excess) in dichloromethane (37 mL) and was stirred at room temperature for 10-15 minutes. By that time the reaction was completed. The reaction mixture was poured into 10% Na₂SO₃ solution (89 mL) in a separatory funnel, and the organic layer was washed with saturated NaHCO₃ solution followed by the general work-up procedure and yielded compound 66 (2.2 g, 54%) after recrystallization from dichloromethane, mp 140-142°C, R_f: 0.31 (50% EtOAc/PE); ir (KBr) $\nu_{\max}$: 3370 (OH), 930, 755 (epoxide) cm⁻¹; ms m/z: 304 (M⁺); ¹H-nmr (CDCl₃) δ : 0.81 (6H, C-10CH₃, C-13CH₃, s), 2.69 (2H, C-17, CH₂-O, AB quartet, J_{AB} = 4 and 9 Hz), 3.55 (1H, 3 α -H, m, W₂ = 24 Hz) ppm; Anal. Found: C, 77.94; H, 10.71; C₂₀H₃₂O₂ · ½H₂O requires C, 77.20; H, 11.09.

17 β -Methyl-5 α -androstan-3 β ,17 α -diol (61)

To a solution of 17 α ,20-oxido-17 β -methyl-5 α -androstan-3 β -ol (66) (2.15 g, 7.1 mmoles) in dry ether (200 mL), was added slowly LiAlH₄ (2.15 g) in dry ether (100 mL) (109) and the mixture was heated to reflux for 45 minutes. Excess LiAlH₄ was decomposed by careful addition of ethanol followed by 10% NH₄Cl solution. The ether layer was separated and extracted by the general work-up procedure to give the compound 61 (2 g, 93%), which on recrystallization from acetone gave a mp 194.5-195.5°C; ir (KBr) $\nu_{\max}$: 3380 (OH), 1070, 1065, 1030 (C-O) cm⁻¹; ms m/z: 306 (M⁺), 291 (M⁺-CH₃), 288 (M⁺-H₂O), 273 (M⁺-H₂O-CH₃), 255 (M⁺-2H₂O-CH₃); ¹H-nmr (CDCl₃) δ : 0.68 (3H, C-13CH₃, s), 0.84 (3H, C-10CH₃, s), 1.19 (3H, C-17CH₃, s), 3.62 (1H, 3 α -H, m, W₂ = 24 Hz); (C₅D₅N) δ : 0.72 (3H, C-13CH₃, s), 0.85 (3H,

C-10CH₃,s), 1.39 (3H, C-17CH₃,s) 3.87 (1H, 3 α H,m,W_{1/2}=24Hz) ppm;

Anal. Found: C, 77.98; H, 11.03, C₂₀H₃₄O₂ requires C, 78.38; H, 11.18 (see NaBH₄ reduction of 47).

17 α -Hydroxy-17 β -methyl-5 α -androstan-3-one (47)

17 β -Methyl-5 α -androstan-3 β ,17 β -diol (61) (1.5 g, 4.9 mmol) was dissolved in acetone (350 mL) and treated with Jones reagent (231). Ether extraction followed by the general work-up procedure yielded compound 47 (800 mg, 53%), which, on recrystallization from dichloromethane/ethyl acetate, gave mp 224–226°C [lit. 224–226°C (245)]; R_f: 0.54 (50% EtOAc/PE); ir (KBr) $\nu_{\max}$: 3620 (OH), 1715 (6-membered ring C=O) cm⁻¹; ms m/z: 304 (M⁺), 289 (M⁺-CH₃), 286 (M⁺-H₂O), 271 (M⁺-H₂O-CH₃); ¹H-nmr (CDCl₃) δ : 0.71 (3H, C-13CH₃,s), 1.03 (3H, C-10CH₃,s), 1.213 (3H, C-17CH₃,s); (C₅D₅N) δ : 0.74 (3H, C-13CH₃,s), 0.93 (3H, C-10CH₃,s), 1.39 (3H, C-17CH₃,s) ppm; Anal. Found: C, 79.35; H, 10.85. C₂₀H₃₂O₂ requires C, 78.90; H, 10.59.

Sodium borohydride (NaBH₄) reduction of the 3-ketosteroids to the 3-equatorial alcohol steroids (Figure 19, p. 97):

To a solution of the 3-ketosteroid (1 g, 3.3 mmol) in 95% ethanol (15 mL) at room temperature was added powdered NaBH₄ (125 mg, 3.3 mmol) (264, 265), and the mixture was stirred for 1 hour. The reaction mixture was gradually added to water (200 mL) in a separatory funnel. When all the hydrogen gas had evolved, it was acidified with 2N H₂SO₄. The reduced product was extracted with dichloromethane and dried by pouring through filter paper; this treatment was followed by the general work-up procedure to give a mixture of the 3-axial and 3-equatorial alcohols (1:9 ratio) (264). The desired 3-equatorial alcohol was separated by crystallization and the

mixture of two alcohols in the mother liquor was oxidized by Jones reagent (231) to give the 3-ketosteroid which was later used for reduction to the 3-axial alcohol selectively with K-selectride (261-263).

17 α -Methyl-5 β -androstan-3 α ,17 β -diol (33)

The NaBH₄ reduction of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44) (1 g) yielded compound 33 (0.667 g, 67%), which was recrystallized from dichloromethane to give a mp 164-165°C [lit. 165-168°C (metabolite) and 164-166°C (synthetic sample) (PE/ether) (106); 164-165°C (154)]; R_f: 0.26 (50% EtOAc/PE); ir (KBR) $\nu_{\max}$: 3320 (OH), 1090, 1065, 1040 (C-O) cm⁻¹; ms m/z: 306 (M⁺), 291 (M⁺-CH₃), 288 (M⁺-H₂O), 273 (M⁺-H₂O-CH₃), 270 (M⁺-2H₂O), 255 (M⁺-2H₂O-CH₃); ¹H-nmr (CDCl₃) δ : 0.84 (3H, C-13CH₃, s), 0.95 (3H, C-10CH₃, s), 1.23 (3H, C-17CH₃, s), 3.64 (1H, 3 β -H, m, W_{1/2}=23 Hz); (C₅D₅N) δ : 0.96 (3H, C-10CH₃, s), 1.11 (3H, C-13CH₃, s), 1.45 (3H, C-17CH₃, s), 3.92 (1H, 3 β -H, m, W_{1/2}=23 Hz) ppm; ¹³C-nmr (APPENDIX).

17 β -Methyl-5 β -androstan-3 α ,17 α -diol (63)

The NaBH₄ reduction of 17 α -hydroxy-17 β -methyl-5 β -androstan-3-one (52) (0.9 g) yielded compound 63 (0.59 g, 66%), which was recrystallized from dichloromethane to give mp 195-196°C; R_f: 0.18 (50% EtOAc/PE); ir (CCl₄) $\nu_{\max}$: 3620, 3480 (OH), 1085, 1064, 1040 (C-O) cm⁻¹; ms m/z: 306 (M⁺), 291 (M⁺-CH₃), 288 (M⁺-H₂O), 273 (M⁺-H₂O-CH₃), 270 (M⁺-2H₂O), 255 (M⁺-2H₂O-CH₃); ¹H-nmr (CDCl₃) δ : 0.64 (3H, C-13CH₃, s), 0.95 (3H, C-10CH₃, s), 1.19 (3H, C-17CH₃, s), 3.62 (1H, 3 β , m, W_{1/2}=23 Hz), (C₅D₅N) δ : 0.70 (3H, C-13CH₃, s), 0.95 (3H, C-10CH₃, s), 1.37 (3H, C-17CH₃, s), 3.87 (1H, 3 β -H, m, W_{1/2}=23 Hz) ppm; Anal. Found: C, 76.03; H, 10.65; C₂₀H₃₄O₂ requires C, 76.14; H, 11.18.

17 β -Methyl-5 β -androsterane-3 β ,17 α -diol (64)

The mother liquor from the above NaBH₄ reduction of 17 α -hydroxy-17 β -methyl-5 β -androsterane-3-one (52) after isolation of 17 β -methyl-5 β -androsterane-3 α ,17 α -diol (63) contained a mixture of 17 β -methyl-5 β -androsterane-3 α and 3 β -17 α -diols (63 and 64) and was chromatographed over alumina. Elution with 5-10% v/v ether/benzene gave the 17 β -methyl-5 β -androsterane-3 β ,17 α -diol (64) (95 mg, 10.6%), which was recrystallized from dichloromethane/methanol to give mp 194-195°C; R_f: 0.36 (50% EtOAc/PE); ir (KBr) $\nu_{\max}$: 3360 (OH), 1090, 1074, 1030 (C-O) cm⁻¹; ms m/z: 306 (M⁺), 291 (M⁺-CH₃), 288 (M⁺-H₂O), 273 (M⁺-H₂O-CH₃), 270 (M⁺-2H₂O), 255 (M⁺-2H₂O-CH₃); ¹H-nmr (CDCl₃) δ : 0.69 (3H, C-13CH₃, s), 0.98 (3H, C-10CH₃, s), 1.19 (3H, C-17CH₃, s), 4.15 (1H, 3 α -H, m, W_{1/2}=9 Hz), (C₅D₅N) δ : 0.72 (3H, C-13CH₃, s), 1.05 (3H, C-10CH₃, s), 1.38 (3H, C-17CH₃, s), 4.32 (1H, 3 α -H, m, W_{1/2}=9 Hz) ppm; Anal. Found: C, 78.35; H, 11.23; C₂₀H₃₄O₂ requires C, 78.38; H, 11.18.

17 α -Methyl-5 α -androsterane-3 β ,17 β -diol (35)

The NaBH₄ reduction of 17 β -hydroxy-17 α -methyl-5 α -androsterane-3-one (43) (912 mg, 3 mmoles) yielded compound 35 (495 mg, 54%), which was recrystallized from chloroform to give a mp 209-210°C [lit. 212-214°C (154), 211-212°C (278), 212-214°C (279), 206-209°C (282), 208-210°C (283)]; R_f: 0.30 (50% EtOAc/PE); ir (KBr) $\nu_{\max}$: 3480, 3400, 3200 (OH), 1078, 1040 (C-O) cm⁻¹; ms m/z: 306 (M⁺), 291 (M⁺-CH₃), 288 (M⁺-H₂O), 273 (M⁺-H₂O-CH₃), 255 (M⁺-2H₂O-CH₃); ¹H-nmr (CDCl₃) δ : 0.82 (3H, C-10CH₃, s), 0.84 (3H, C-13CH₃, s), 1.19 (3H, C-17CH₃, s), 3.60 (1H, 3 α -H, m, W_{1/2}=24 Hz); (C₅D₅N) δ : 0.87 (3H, C-10CH₃, s), 1.11 (3H, C-13CH₃, s), 1.44 (3H, C-17CH₃, s), 3.90 (1H, 3 α -H, m, W_{1/2}=24 Hz) ppm; ¹³C-nmr (APPENDIX).

17 β -Methyl-5 α -androstan-3 β ,17 α -diol (61)

The NaBH₄ reduction of 17 α -hydroxy-17 β -methyl-5 α -androstan-3-one (47) (800 mg, 2.6 mmoles) yielded compound 61 (600 mg, 75%), which was recrystallized from acetone to give mp 194-195°C; R_f: 0.33 (50% EtOAc/PE); ir (KBr) $\nu_{\max}$: 3380 (OH), 1070, 1065, 1030 (C-O) cm⁻¹; ms m/z: 306 (M⁺), 291 (M⁺-CH₃), 288 (M⁺-H₂O), 273 (M⁺-H₂O-CH₃), 255 (M⁺-2H₂O-CH₃); ¹H-nmr (CDCl₃) δ : 0.72 (3H, C-13CH₃, s), 0.85 (3H, C-10CH₃, s), 1.19 (3H, C-17CH₃, s), 3.62 (1H, 3 α -H, m, W_{1/2}=24 Hz); (C₅D₅N) δ : 0.72 (3H, C-13CH₃, s), 0.85 (3H, C-10CH₃, s), 1.39 (3H, C-17CH₃, s), 3.87 (1H, 3 α -H, m, W_{1/2}=24 Hz) ppm; Anal. Found: C, 77.89; H, 11.06; C₂₀H₃₄O₂· $\frac{1}{2}$ H₂O requires C, 77.24; H, 11.18.

Potassium tri-(sec-butyl)-borohydride [K-selectride, KB[CH(CH₃)C₂H₅]₃H]

reduction of the 3-ketosteroids to the 3-axial alcohol steroids

(Figure 19, p. 97):

To a stirred solution of the 3-ketosteroid (304 mg, 1 mmole) in freshly distilled (peroxide free) tetrahydrofuran (THF) (200 mL) (274) at -78°C (acetone/dry-ice bath) under N₂ was added K-selectride (1.1 mL of 1 M solution in THF $\equiv$ 1.1 mmoles) (261-263). The reaction, monitored by tlc, was completed in 1-2 hours. The reaction mixture was allowed to warm to room temperature, and then 10% NaOH solution (7 mL) and 30% H₂O₂ (5 mL) were added, and the mixture was stirred overnight. The reaction mixture was extracted with ether followed by the general work-up procedure to give the crystalline 3-axial alcohol.

17 α -Methyl-5 β -androstan-3 β ,17 β -diol (62)

The K-selectride reduction (261, 262) of 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44) (304 mg) yielded compound 62 (277 mg, 89%) which

was recrystallized from ethyl acetate to give a mp 178-179°C; R_f : 0.35 (50% EtOAc/PE); ir (KBr) $\nu_{\max}$: 3380 (OH), 1092, 1080, 1031 (C-O) cm^{-1} ; ms m/z: 306 (M^+), 291 ($M^+ - \text{CH}_3$), 288 ($M^+ - \text{H}_2\text{O}$), 273 ($M^+ - \text{H}_2\text{O} - \text{CH}_3$), 270 ($M^+ - 2\text{H}_2\text{O}$), 255 ($M^+ - 2\text{H}_2\text{O} - \text{CH}_3$); ^1H -nmr (CDCl_3) δ : 0.84 (3H, C-13 CH_3 , s), 0.98 (3H, C-13 CH_3 , s), 1.22 (3H, C-17 CH_3 , s), 4.13 (1H, 3 α -H, m, $W_{1/2}$ =8 Hz); ($\text{C}_5\text{D}_5\text{N}$) δ : 1.09 (3H, C-10 CH_3 , s), 1.13 (3H, C-13 CH_3 , s), 1.49 (3H, C-17 CH_3 , s), 4.38 (1H, 3 α -H, m, $W_{1/2}$ =8 Hz) ppm; Anal. Found: C, 75.98; H, 10.69; $\text{C}_{20}\text{H}_{34}\text{O}_2 \cdot \frac{1}{2}\text{H}_2\text{O}$ requires C, 76.14; H, 11.18; ^{13}C -nmr (APPENDIX).

17 α -Methyl-5 α -androstan-3 α ,17 β -diol (34)

The K-selectride reduction (261,262) of 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) (304 mg) yielded compound 34 (267 mg, 88%), which was recrystallized from acetone/ethyl acetate to give a mp 181-182°C [lit. 188-190°C (106), 181-182°C (245), 180-181°C (277); 180-182°C (283)]; R_f : 0.36 (50% EtOAc/PE); ir (KBr) $\nu_{\max}$: 3425, 3360 (OH), 1075, 1025, 1010 (C-O) cm^{-1} ; ms m/z: 306 (M^+), 291 ($M^+ - \text{CH}_3$), 288 ($M^+ - \text{H}_2\text{O}$), 273 ($M^+ - \text{H}_2\text{O} - \text{CH}_3$), 255 ($M^+ - 2\text{H}_2\text{O} - \text{CH}_3$); ^1H -nmr (CDCl_3) δ : 0.80 (3H, C-10 CH_3 , s), 0.84 (3H, C-13 CH_3 , s), 1.21 (3H, C-17 CH_3 , s), 4.02 (1H, 3 β -H, m, $W_{1/2}$ =7 Hz); ($\text{C}_5\text{D}_5\text{N}$) δ : 0.87 (3H, C-10 CH_3 , s), 1.13 (3H, C-13 CH_3 , s), 1.43 (3H, C-17 CH_3 , s), 4.30 (1H, 3 β -H, m, $W_{1/2}$ =7 Hz) ppm; ^{13}C -nmr (APPENDIX).

17 β -Methyl-5 α -androstan-3 α ,17 α -diol (60)

The K-selectride reduction (261,262) of 17 α -hydroxy-17 β -methyl-5 α -androstan-3-one (47) (421 mg, 1.38 mmoles) yielded compound 60 (355 mg, 84%), which was recrystallized from acetone to give mp 188-189°C; R_f : 0.28 (50% EtOAc/PE); ir (KBr) $\nu_{\max}$: 3400 (OH), 1080, 1060, 1003 (C-O) cm^{-1} ; ms m/z: 306 (M^+), 291 ($M^+ - \text{CH}_3$), 288 ($M^+ - \text{H}_2\text{O}$), 273 ($M^+ - \text{H}_2\text{O} - \text{CH}_3$), 270 ($M^+ - 2\text{H}_2\text{O}$), 255 ($M^+ - 2\text{H}_2\text{O} - \text{CH}_3$); ^1H -nmr (CDCl_3) δ : 0.68 (3H, C-13 CH_3 , s),

0.80 (3H, C-10CH₃, s), 1.19 (3H, C-17CH₃, s), 4.24 (1H, 3β-H, m, $W_{\frac{1}{2}} = 7$ Hz);
 (C₅D₅N) δ: 0.75 (3H, C-13CH₃, s), 0.87 (3H, C-10CH₃, s), 1.38 (3H, C-17CH₃, s),
 4.28 (1H, 3β-H, m, $W_{\frac{1}{2}} = 7$ Hz) ppm; Anal. Found: C, 74.17; H, 11.00;
 C₂₀H₃₄O₂·H₂O requires C, 74.02; H, 11.18.

17α-Hydroxy-17β-methyl-4-androsten-3-one (54) (Figure 20, p. 99):

17α,20-Oxido-17β-methyl-4-androsten-3-one (71) was prepared as follows (143,259): Compound 68 (2 g, 7.04 mmoles) and m-chloroperbenzoic acid (1.57 g, 9.04 mmoles) in chloroform (36 ml) was cooled in an ice salt mixture for 1 hour. To the reaction mixture, 10% Na₂CO₃ (50 mL) was added and the organic layer washed with saturated NaHCO₃ solution and water. The organic layer was dried over Na₂SO₄ and the solvent evaporated. The crude product (mp 165-169°C) was used in the next reaction [lit. mp 180-184°C (143); 179-182°C (259)].

17β-Methyl-4-androstene-3α and 3β,17α-diols (72 and 73) were prepared as follows (143,259): The above crude product (71) was dissolved in ether (200 ml) and LiAlH₄ (2 g) added and the reaction mixture refluxed for 1 hour. Excess LiAlH₄ was destroyed by addition of EtOAc, 95% EtOH and water. The product was extracted with ether followed by the general work-up procedure.

17α-Hydroxy-17β-methyl-4-androsten-3-one (54) was prepared by Jones oxidation (231) of the above crude product (72 and 73). Recrystallization from acetone/ether yielded crystalline material (469 mg), mp 178-179°C [lit. mp 175-177°C (acetone/hexane); analytical sample 181-182°C (ethanol) (259)]; R_f: 0.58 (50% EtOAc/PE) [cf: compound 13 (17 epimer of 54), R_f: 0.53]; ¹H-nmr (CDCl₃) δ: 0.73 (3H, C-13CH₃, s), 1.21 (6H, C-10 and C-17CH₃, s) and 5.93 (1H, C-4H, s). ¹³C-nmr (APPENDIX).

IV. CONCLUSION

Metabolites obtained in urinary excretion experiments from rabbits orally dosed with 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43), 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44), 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13), and 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16) were isolated and identified by their spectra (ir, mass, ^1H -nmr, and ^{13}C -nmr).

The metabolites obtained from 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) were the dosed compound (43), 17 α -methyl-5 α -androstan-3 α - and 3 β ,17 β -diols together with compounds hydroxylated at the C-6 α and C-15 α positions: 17 α -methyl-5 α -androstan-3 α ,6 α ,17 β -triol and 17 α -methyl-5 α -androstan-3 β ,15 α ,17 β -triol.

The metabolites obtained from 17 β -hydroxy-17 α -methyl-5 β -androstan-3-one (44) were 17 α -methyl-5 β -androstan-3 α ,17 β -diol together with compounds hydroxylated at C-1 β , C-12 β , C-16 α and C-16 β -positions, all with the C-3 equatorial alcohol (3 α -OH): 17 α -methyl-5 β -androstan-1 β ,3 α ,17 β -triol, 17 α -methyl-5 β -androstan-3 α ,12 β ,17 β -triol, 17 α -methyl-5 β -androstan-3 α ,16 α ,17 β -triol, and 17 α -methyl-5 β -androstan-3 α ,16 β ,17 β -triol.

The metabolites obtained from 17 α -methyl-4-androsten-3-one (13) were 17 α -methyl-5 α -androstan-3 α and 3 β ,17 β -diols and 17 α -methyl-5 β -androstan-3 α ,17 β -diol together with compounds hydroxylated at C-6 α , C-16 α and C-16 β positions: 17 α -methyl-5 α -androstan-3 β ,6 α ,17 β -triol, 17 α -methyl-5 β -androstan-3 α ,16 α ,17 β -triol, and 17 α -methyl-5 β -androstan-3 α ,16 β ,17 β -triol.

Formation of the C-6 hydroxylated derivative (46) obtained from 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43) demonstrated that the 4,6-

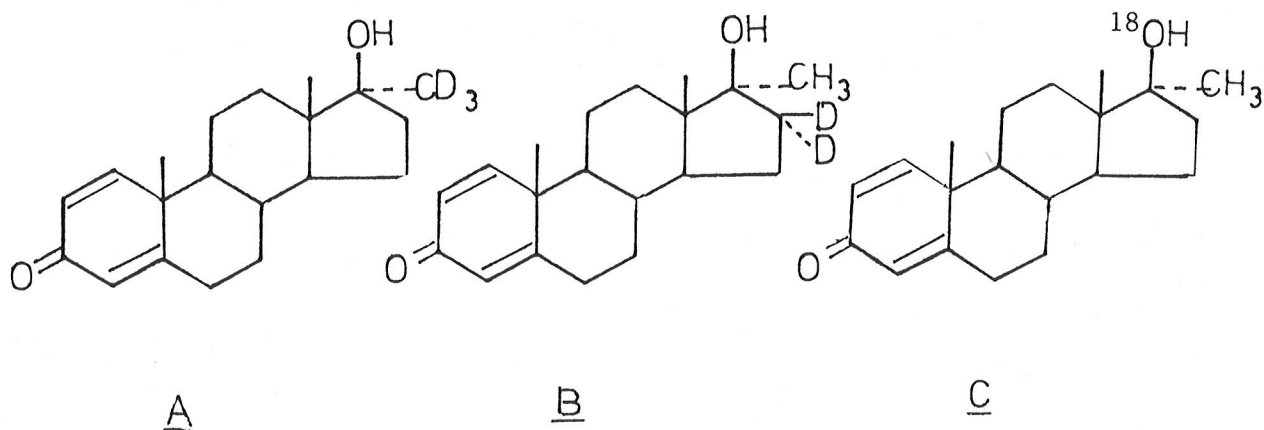
enolization of the 4-en-3-one system is not a necessary requirement for hydroxylation at the C-6 position of the androstane nucleus in the rabbit.

The C-1 (50) and C-12 (51) hydroxylations were unique to the 5 β -compound (44) whereas the C-16 hydroxylated metabolites (40, 42) with 5 β -configuration were also found from 17 β -hydroxy-17 α -methyl-4-androst-3-one (13). The 5 α -compound (43) did not give C-16 hydroxylated derivatives (40, 42), but give a C-15 (45) hydroxylated metabolite. A new metabolite hydroxylated at the C-6 α position, 17 α -methyl-5 α -androst-3 β ,6 α ,17 β -triol (53) was obtained from 17 β -hydroxy-17 α -methyl-4-androst-3-one (13). As the C-6 hydroxylated metabolite (53) with a C-3 β -hydroxyl group was not obtained from the 5 α -compound (43), it must be formed as a consequence of the presence of the unsaturated 4-en-3-one system. Neither C-6 α -hydroxylated nor hydroxylated 5 α -metabolites from 17 β -hydroxy-17 α -methyl-4-androst-3-one (13) has been isolated previously.

The metabolites obtained from 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16) were the dosed compound, the fully reduced derivatives, 17 α -methyl-5 α -androstane-3 α and 3 β ,17 β -diol, the partially reduced and hydroxylated metabolites, 16 α - and 16 β ,17 β -dihydroxy-17 α -methyl-5 β -androst-1-en-3-ones, the monohydroxylated metabolites, 6 β - and 16 β ,17 β -dihydroxy-17 α -methyl-1,4-androstadien-3-one and the dihydroxylated metabolite 6 β ,16 β ,17 β -trihydroxy-17 α -methyl-1,4-androstadien-3-one. C-16 monohydroxylated and 6,16-dihydroxylated metabolites together with the partially reduced and hydroxylated ring 'A' compounds have not been previously reported from studies of the metabolism of 17 β -hydroxy-17 α -methyl-1,4-androstadien-3-one (16).

Three of the four ketones, 17 ξ -hydroxy-17 ξ -methyl-5 α - and 5 β -androstan-3-ones [except 17 β -hydroxy-17 α -methyl-5 α -androstan-3-one (43)] and eight alcohols, 17 ξ -methyl-5 α and 5 β -androstan-3 ξ ,17 ξ -diols were synthesized for comparison purposes with the metabolites. Seven of the twelve compounds had not been synthesized previously. The spectral properties (ir, mass, ^1H -nmr and ^{13}C -nmr) of the twelve compounds aided in the assignment of stereoisomers and the position of hydroxylation of the metabolic products.

No epimerization of the C-17 position (17 β -hydroxy/17 α -methyl to 17 α -hydroxy/17 β -methyl) was observed in the metabolic studies with the above four compounds (43, 44, 13, 16) in the rabbit. As the rabbit has not proved to be a useful model to study this metabolic transformation, a study in man would be a logical necessity for further investigation of this epimerization, which seems to be species dependent. If this study on the four compounds were carried out in man, it would also test the hypothesis that dependent on the structure of the ring "A". The mechanism of this epimerization can be studied in man by means of the deuterium and ^{18}O oxygen derivatives of 17 β -hydroxyl-17 α -methyl-1,4-androstadien-3-one shown below:



Administration of these labelled compounds (A, B and C) would yield the information necessary to formulate a mechanism for the epimerization.

Administration of compound A (the 17 α -trideuteriomethyl derivative) would establish whether the methyl group was hydroxylated or exchanged during the inversion process. Similarly, compound B (the C-16 deuteriated derivative) would demonstrate the involvement of that position in the process. The ¹⁸O derivative (C) would establish whether the oxygen atom was exchanged during the epimerization.

The information obtained may allow a consistent mechanism to be proposed. At present no mechanism suggests itself strongly and the complete lack of information permits only speculation.

The possibility of 17 β -hydroxy/17 α -methyl epimerization of 17 β -hydroxy-17 α -methyl-4-androsten-3-one (13) has not been investigated in man.

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VI. APPENDIX ¹³C-nmr spectra of synthetic and hydroxylated products
(values in ppm in C₅D₅N unless otherwise mentioned)

Name of compounds		C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
17β-Hydroxy-17α-methyl-5β-androstan-3-one	(44)	*37.1 (37.3)	37.1 (37.4)	213.1 211.5	42.3 42.6	44.3 44.5	25.6 25.9	26.6 27.0	36.5 36.8	40.9 41.0	35.0 35.2	20.9 21.3	31.9 32.5	45.7 46.3	50.7 51.0	23.3 23.8	39.0 39.6	81.6 80.7	14.0 14.8	22.7 22.7	25.9 26.7
17α-Hydroxy-17β-methyl-5β-androstan-3-one	(54)	*37.2 37.3	37.2 37.3	213.4 211.6	42.4 42.5	44.5 44.6	26.0 26.4	26.6 27.0	36.0 36.3	40.7 40.8	35.0 35.2	20.8 21.2	30.1 30.6	46.8 47.3	50.0 50.0	23.9 24.5	38.5 39.3	81.2 80.9	15.9 16.2	22.7 23.0	22.7 22.7
17β-Hydroxy-17α-methyl-4-androsten-3-one	(13)	36.1	34.4	198.3	124.1	170.7	32.9	(32.1)	36.6	54.1	38.9	21.1	(32.1)	46.0	50.7	23.7	39.5	80.5	14.7	17.3	26.7
17α-Hydroxy-17β-methyl-4-androsten-3-one	(52)	36.0	34.4	198.3	124.0	170.8	33.0	32.7	36.2	54.1	38.8	21.0	30.3	47.0	49.7	24.4	39.1	80.6	16.0	17.3	23.0
17β-Hydroxy-17α-methyl-1,4-androstadien-3-one	(16)	156.0	127.7	185.5	124.1	169.1	32.9	32.0	36.4	52.8	43.8	22.8	33.6	46.2	50.2	22.8	39.3	80.4	14.7	18.7	26.7
17α-Methyl-5α-androstane-3α,17β-diol	(34)	32.4	29.9	65.6	37.0	39.6	29.2	33.0	36.8	54.9	36.6	21.0	32.4	46.2	51.3	23.8	39.6	80.7	14.9	11.6	26.8
17α-Methyl-5α androstane-3β,17β-diol	(35)	37.7	32.4	70.7	39.4	45.2	29.3	32.5	36.8	54.8	36.0	21.4	32.3	46.2	51.2	23.9	39.5	80.7	14.9	12.6	26.8
17α-Methyl-5β-androstane-3α,17β-diol	(33)	37.4	31.5	71.1	36.1	42.7	27.7	26.8	37.2	41.1	35.1	20.9	32.6	46.3	51.2	23.9	39.6	80.7	14.8	23.8	26.8
17α-Methyl-5β-androstane-3β,17β-diol	(62)	30.8	28.7	66.2	34.5	37.3	27.3	26.7	37.1	40.4	35.7	21.3	32.7	46.4	51.3	23.9	39.7	80.8	14.8	24.4	26.8
17α-Methyl-5β-androstane-1β,3α,17β-triol	(50)	73.0	39.0	66.2	37.4	42.7	27.4	26.5	36.3	37.4	39.8	21.1	32.7	46.1	51.2	23.9	39.5	80.7	14.9	19.1	26.8
17α-Methyl-5β-androstane-3α,12β,17β-triol	(51)	37.4	31.4	71.0	36.2	42.3	27.7	26.4	36.3	39.8	35.1	30.5	73.9	50.5	50.1	23.7	39.1	81.6	9.5	23.7	26.6
17α-Methyl-5β-androstane-3α,16α,17β-triol	(42)	37.4	31.5	71.2	36.0	42.7	27.7	26.8	36.8	41.2	35.2	20.5	32.9	47.0	49.0	35.1	80.2	83.9	15.3	23.8	19.2
17α-Methyl-5β-androstane-3α,16β,17β-triol	(40)	37.4	31.5	71.2	36.1	42.6	27.7	27.0	36.7	41.2	35.1	20.7	33.4	45.7	47.8	36.0	77.6	78.9	14.5	23.7	24.7
17α-Methyl-5α-androstane-3α,6α,17β-triol	(46)	33.4	29.7	65.3	31.4	47.0	69.3	42.8	35.7	54.5	37.3	20.9	32.5	46.2	51.2	23.9	39.5	80.6	14.9	12.9	26.8
17α-Methyl-5α-androstane-3β,6α,17β-triol	(53)	38.2	32.4	71.1	33.8	53.0	68.8	42.7	35.7	54.4	36.7	21.4	32.4	46.3	51.1	23.9	39.5	80.7	14.9	13.8	26.8
6β,16β,17β-Trihydroxy-17α-methyl-1,4-androstadien-3-one	(59)	157.5	127.0	186.5	125.3	168.2	73.4	41.1	31.1	52.7	41.1	22.5	32.8	45.7	46.8	36.0	77.2	78.8	14.4	20.8	24.6

*CDC1₃

Values in parenthesis () are interchangeable.