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STUDIES ON ANABOLIC STEROIDS—11. 18-HYDROXYLATED METABOLITES OF MESTEROLONE, METHENOLONE AND STENBOLONE: NEW STEROIDS ISOLATED FROM HUMAN URINE

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Summary—New metabolites of mesterolone, methenolone and stenbolone bearing a C_{18} hydroxyl group were isolated from the steroid glucuronide fraction of urine specimens collected after administration of single 50 mg doses of these steroids to human subjects. Mesterolone gave rise to four metabolites which were identified by gas chromatography/mass spectrometry as 18-hydroxy-1α-methyl-5α-androstan-3,17-dione **1**, 3α,18-dihydroxy-1α-methyl-5α-androstan-17-one **2**, 3β,18-dihydroxy-1α-methyl-5α-androstan-17-one **3** and 3α,6ξ,18-trihydroxy-1α-methyl-5α-androstan-17-one **4**. These data suggest that mesterolone itself was not hydroxylated at C_{18}, but rather 1α-methyl-5α-androstan-3,17-dione, an intermediate metabolite which results from oxidation of mesterolone 17-hydroxyl group. In addition to hydroxylation at C_{18}, reduction of the 3-keto group and further hydroxylation at C_6 were other reactions that led to the formation of these metabolites. It is of interest to note that in the case of both methenolone and stenbolone, only one 18-hydroxylated urinary metabolite namely 18-hydroxy-1-methyl-5α-androst-1-ene-3,17-dione **5** and 18-hydroxy-1-methyl-5α-androst-1-ene-3,17-dione **6** were both detected in post-administration urine specimens. These data indicate that the presence of a methyl group at the C_1 or C_2 positions in the steroids studied is a structural feature that seems to favor interaction of hepatic 18-hydroxylases with these steroids. These data provide further evidence that 18-hydroxylation of endogenous steroids can also occur in extra-adrenal sites in man.

INTRODUCTION

It is well known that many endogenous steroids and synthetic anabolic–androgenic steroids are hydroxylated by *P*-450 cytochromes in mammalian systems [1–12]. Hydroxylation can lead to deactivation and elimination or alternatively, may result in production of secondary steroidal metabolites that have unknown physiological functions.

In the biosynthesis of aldosterone, adrenal hydroxylation of corticosterone at the C_{18} position is a typical example illustrating the importance of this general reaction in the *in vivo* production of steroids that have hormonal properties [13]. Several 18-hydroxylated steroids produced from adrenal origin have been described in the literature [14–17]. The first evidence indicating that 18-hydroxylation can also occur in extra-adrenal sites has been reported about three decades ago when 18-hydroxyandrosterone, 18-hydroxyetiocholanolone and 18-hydroxytesterone were isolated and identified from the urine of a patient with a metastatic arrhenoblastoma given large amounts of the parent steroids [18].

Subsequently, the hydroxylation of the angular 18-methyl group of testosterone was demonstrated in human and rat adrenal homogenates [19], and human fetal [20] and rat [21, 22] liver microsomes. Laatikainen and Vihko [23] showed that 18-hydroxyandrosterone disulfate conjugate is the major steroid in human bile. Einarson *et al.* [24] have demonstrated that adult human liver microsomes hydroxylate androsterone, dehydroepiandrosterone (DHEA) and 5α-androstane-3α,17β-diol at the C_{18} position.

In man, hydroxylation of synthetic anabolic–androgenic steroids may occur at positions 2,4,6,12 and 16. Moreover, either α- or β-isomers or both may also arise [4–12]. In preceding papers of this series [4–8], we described the characterization of methenolone and

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stenbolone urinary metabolites, some of which arising from hydroxylation at positions 2,6 and 16. In this study, we report the unexpected isolation and identification of four 18-hydroxylated metabolites of mesterolone (1α-methyl-5α-dihydrotestosterone), a synthetic anabolic compound structurally related to methenolone and stenbolone (Fig. 1). Further investigations of urinary specimens obtained from metabolic studies previously carried out with the two latter steroids showed that both steroids can be hydroxylated at the 18-position with concomitant oxidation of their 17β-hydroxyl function. These 18-hydroxylated steroids were identified in the urine of male volunteers to whom 50 mg of the steroids were administered as a single dose. The identity of the metabolites was ascertained by GC/MS analysis of their TMS ether, TMS enol-TMS ether and corresponding d_9-TMS enol-d_9-TMS ether derivatives and by comparison of the mass spectral data with reference data from the literature and reference steroids. To the best of our knowledge, the hydroxylation of synthetic anabolic–androgenic steroids at the C_{18} position has been reported previously neither in animal nor in human.

EXPERIMENTAL

Reagents

All chemicals and reagents used were described in detail in previous papers from this laboratory [7, 8]. Proviron (mesterolone) was kindly supplied by Schering A. G. (Mexico City, Mexico), Primobolan (methenolone acetate) was supplied by Schering A. G. (Berlin, Germany) and Anatrofin (stenbolone acetate) was obtained from Syntex (Palo Alto, CA, U.S.A.). 5α-androstan-17-one and 19-hydroxy-androst-5-ene-3,17-dione were purchased from Steraloids Inc. (Wilton, MA, U.S.A.). 3β,18-dihydroxy-androst-5-en-17-one was kindly supplied by Dr D. N. Kirk (Steroids Reference Collection, London, England).

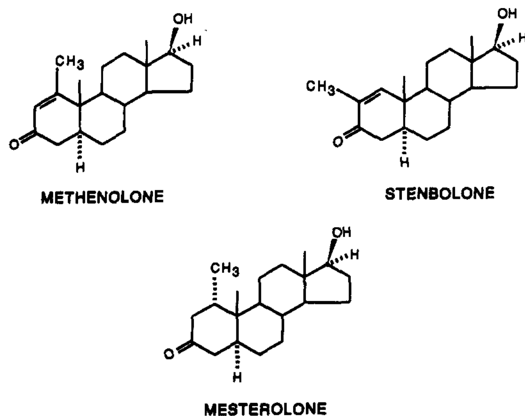

Fig. 1. Chemical structure of the three studied anabolic steroids.

Urine specimens

Blank urine specimens were collected for 24 h before steroid administration. Urine was then systematically collected in sterile plastic containers for seven consecutive days following ingestion of 50 mg of the steroids by healthy male volunteers aged between 25 and 38 years. All urine specimens were kept frozen at −20°C until analyzed.

Analysis of urinary steroids

Methods and techniques used for extraction, fractionation and hydrolysis of steroid conjugates, preparation of derivatives of neutral steroids and experimental conditions for GC/MS analysis were described in detail in previous papers [7, 8]. The α-stereochemistry of the 3-hydroxy group was determined in the case of some mesterolone metabolites by oxidation with the stereoselective 3α-dehydrogenase from *Pseudomonas testosteroni* [8].

RESULTS

Table 1 summarizes some of the chromatographic and mass spectral features of the TMS enol-TMS ether and MO-TMS derivatives of the 18-hydroxylated metabolites of mesterolone, methenolone and stenbolone, all of which were isolated from fractions containing the glucuronic acid conjugates.

Mesterolone

The potential *in vivo* formation of mesterolone metabolites hydroxylated at positions 1 or 18 was investigated by GC/MS analysis of the free, glucuronic acid and sulfate conjugate fractions. One of the structurally informative mass spectral features of the TMS ether derivatives of 18- and 19-hydroxy steroids [22, 23, 25–27] is the formation of an ion at m/z 103 resulting from the cleavage of the C_9—C_{19} and C_{13}—C_{18} bonds, giving rise to the corresponding CH_2-OTMS ion. Interestingly, the mass spectrum of the TMS enol-TMS ether derivative of 3β,18-dihydroxy-androst-5-en-17-one showed a prominent $[M-103]^+$ ion resulting

Table 1. Partial GC/MS data of the 18-hydroxylated metabolites of mesterolone, methenolone and stenbolone

Steroid[a]	M.U.[b]	Derivative[c]	M^+
(Mesterolone)			
1 18-hydroxy-1α-methyl-5α-androstane-3,17-dione	28.23	TMS E-E	534
	28.28	TMS E-E	534
	28.88	TMS ether	390
	29.23	MO-TMS	448
	29.27	MO-TMS	448
2 3α,18-dihydroxy-1α-methyl-5α-androstan-17-one	27.97	TMS E-E	536
	28.41	TMS ether	464
	28.50	MO-TMS	493
3 3β,18-dihydroxy-1α-methyl-5α-androstan-17-one	28.43	TMS E-E	536
	28.86	TMS ether	464
	28.88	MO-TMS	493
4 3α,6ξ,18-trihydroxy-1α-methyl-5α-androstan-17-one	29.06	TMS E-E	624
	28.94	TMS ether	552
	29.03	MO-TMS	581
(Methenolone)			
5 18-hydroxy-1-methyl-5α-androst-1-ene-3,17-dione	28.84	TMS E-E	532
	30.06	TMS ether	388
(Stenbolone)			
6 18-hydroxy-2-methyl-5α androst-1-ene-3,17-dione	28.24	TMS E-E	532
	28.58	TMS ether	388

[a]Identity of the parent steroid is given in parentheses. In all instances, the metabolites were excreted in urine as glucuronides.
[b]Methylene unit (M.U.) values were calculated by linear interpolation of the retention time of the steroid derivative relatively to that of C_{26}, C_{27}, C_{28}, C_{29}, C_{30} and C_{32} hydrocarbons.
[c]The syn- and anti-forms of some MO-TMS derivatives were resolved chromatographically whereas the TMS enol-TMS ether (TMS E-E) derivatives of some metabolites were a mixture of the isomeric forms.

from the loss of the $^{18}CH_2$-OTMS group. Thus, a series of $[M-103]^+$ ions expected to result from electron-impact ionization of the TMS enol-TMS ether derivatives of hypothetical 18- and/or 19-hydroxylated metabolites were used as probes to detect traces of any of these potential biotransformation products. Solely the monitoring of ions at m/z 431, 433 and 521 provided evidence for the presence of four steroids bearing a hydroxymethyl group (Fig. 2). These steroids were detected only in the glucuronic acid conjugate fraction and were not found in any of the blank urine specimens analyzed concomitantly.

Metabolite 1. This steroid was detected as a pair of two tautomeric TMS enol-TMS ether derivatives (Fig. 2) showing identical mass spectral features [Fig. 3(A)]. The molecular ion at m/z 534 (m/z 543, d_0-TMS enol-d_9-TMS ether mixed derivative) indicated the presence of two keto functions and one hydroxyl group in this compound. The presence of ions characteristic of mesterolone A-ring at m/z 141 and 157 (m/z 141 and 157, d_0-TMS enol-d_9-TMS ether mixed derivative) demonstrated that compound **1** was bearing a 3-keto group and that hydroxylation of the parent steroid did not occur in the A-ring. The prominent ion at m/z 431 (m/z 431, d_9-TMS enol-d_9-TMS ether mixed derivative) results from the loss of a CH_2-OTMS radical from the molecular ion $[M-103]^+$. This fragmentation route is characteristic of steroids bearing a hydroxymethyl group either at the C_{18} [23, 26], C_{19} [27] or C_{21} [22] positions and also of primary alcohol TMS derivatives [25]. Thus, the ion at m/z 431 indicated that the hydroxyl group was located either at the C_{18} or C_{19}

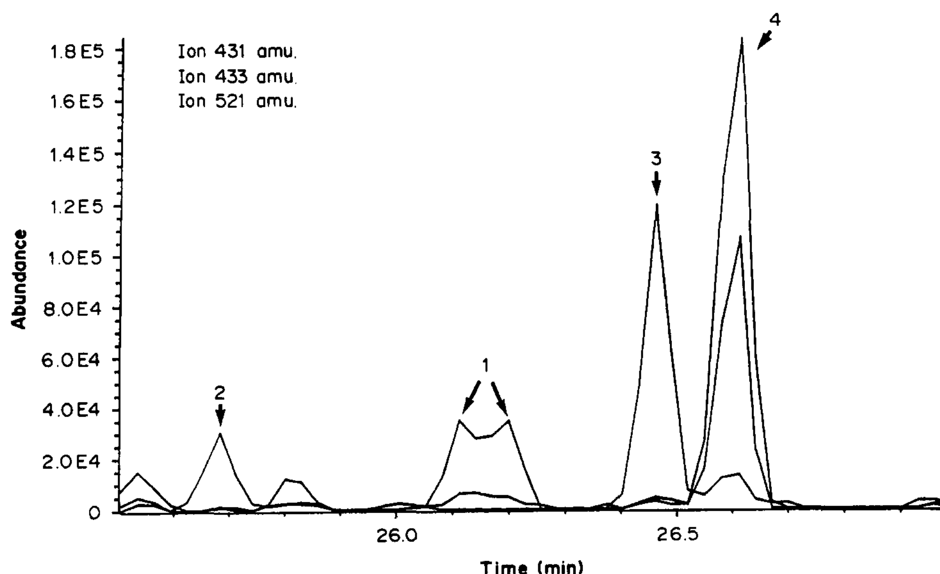

Fig. 2. Reconstructed ion chromatograms from SIM CG/MS analysis of the TMS enol-TMS ether derivatives of mesterolone 18-hydroxylated metabolites isolated from the glucuronide fraction of a urine sample collected 4 h after steroid administration. The characteristic $[M-CH_2OTMS]^+$ ions at m/z 431 (compound **1**), 433 (compounds **2** and **3**) and 521 (compound **4**) were monitored. Compound **1** was detected as two tautomeric forms which are partially resolved on the GC column used. Labeled peak identity is given in Table 1.

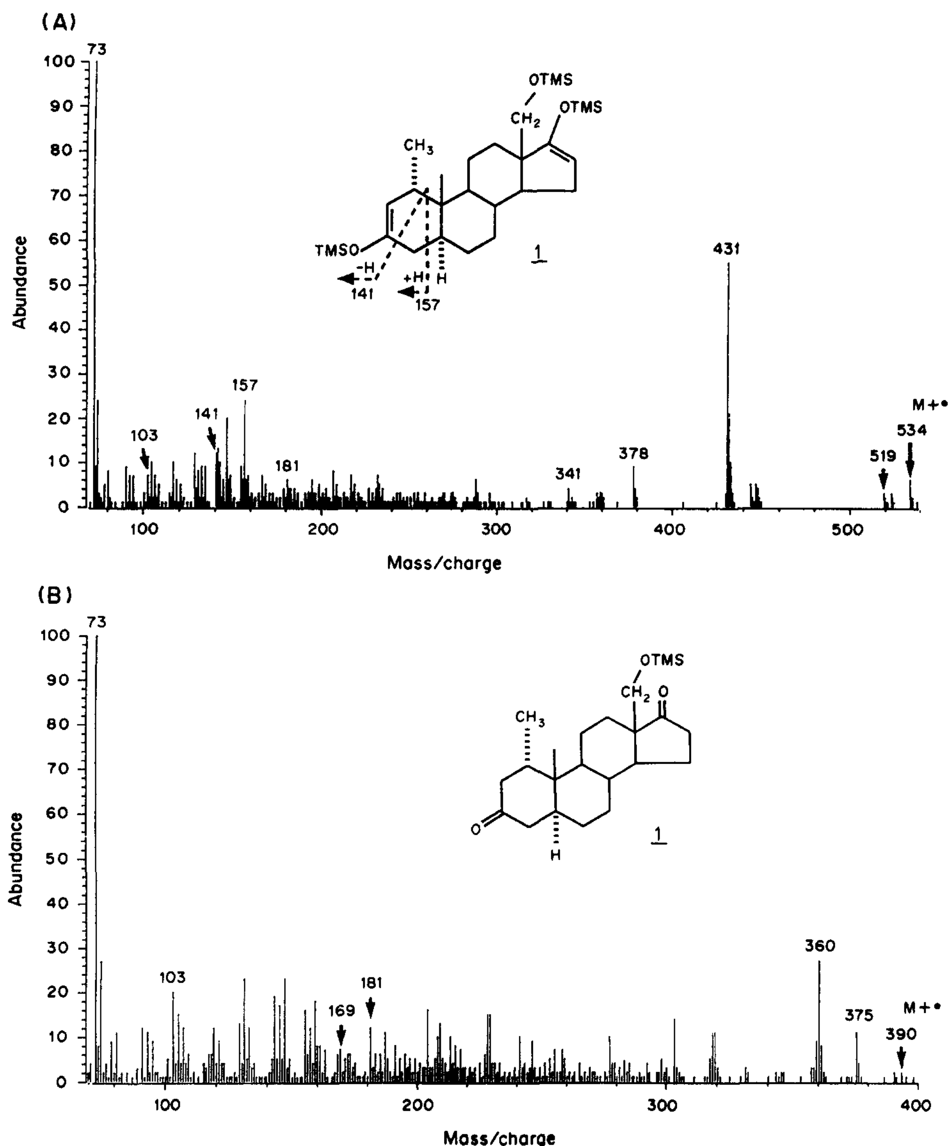

Fig. 3. Mass spectra of 18-hydroxy-1α-methyl-5α-androstane-3,17-dione 1 as (A) TMS enol-TMS ether and (B) TMS ether derivatives.

position. In order to assign the exact location of the hydroxylation site, the TMS ether derivative of this urinary metabolite was prepared and analyzed by GC/MS.

The corresponding mass spectrum [Fig. 3(B)] showed a molecular ion at m/z 390 (m/z 399, d_9-TMS). The presence of an ion of diagnostic importance at m/z 360 (m/z 369, d_9-TMS) indicated that the hydroxyl group is at the C_{18} position. Indeed, this ion arises from a McLafferty-like rearrangement whereby the TMS moiety of the -OTMS group migrates to the oxygen atom of the 17-keto group through a six-membered ring intermediate with concomitant elimination of one molecule of formaldehyde $[M-CH_2O]^+$. This fragmentation is characteristic of steroids bearing 18-hydroxy-17-keto [23, 26–28, 30] and 19-hydroxy-4-en-3-one [26] groups. The presence of a 19-hydroxyl group in **1** is unlikely because the formation of the $[M-30]^+$ ion which involves cleavage of the A-ring would have given rise to a prominent ion at m/z 196 [26] which is not observed in the mass spectrum of **1** TMS derivative [Fig. 3(B)]. Comparison with mass spectra of reference 18-hydroxy-androst-4-ene-3,17-dione and 19-hydroxy-androst-5-ene-3,17-dione TMS ether derivatives and with compound **1** MO-TMS derivative provided further evidence ascertaining the structure of this urinary metabolite of mesterolone which was identified as 18-hydroxy-1α-methyl-5α-androstane-3,17-dione.

Metabolites 2 and 3. The mass spectra of the TMS enol-TMS ether (Fig. 4) and TMS ether (Fig. 5) derivatives of both **2** and **3** were virtually identical, indicating that these steroids were isomers. The molecular ion of their TMS enol-TMS ether derivative at m/z 536 (m/z 554, d_0-TMS enol-d_9-TMS ether mixed derivative) indicated the presence of one keto and two hydroxyl functions. This was further demonstrated by the prominent ion at m/z 433 (m/z 422, d_0-TMS enol-d_9-TMS ether mixed derivative) resulting from the loss of a CH_2-OTMS radical and by the ion at m/z 343 (m/z 343,d_9-TMS; M-CH_2-OTMS − TMSOH). These data suggested that both metabolites were structurally related to compound **1**. Indeed, the mass spectra of their TMS ether derivatives [Fig. 5(A)] showed some similarities with that of compound **1** [Fig. 3(B)], namely the presence of ions at m/z 434 $[M-30]^+$ and m/z 103 ($CH_2 = OTMS^+$) which indicate the presence of 18-hydroxy and 17-keto groups in both isomers.

The presence of a 3-hydroxyl group is demonstrated by the formation of a structurally informative ion at m/z 145 (m/z 154, d_9-TMS) arising from cleavage of the C_1—C_{10} and C_3—C_4 bonds [Fig. 5(A)]. This ion is also prominent in the mass spectrum of the TMS ether derivative of 3α-hydroxy-1α-methyl-5α-androstan-17-one, a urinary metabolite of methenolone, the A-ring of which is structurally similar to that of compounds **2** and **3** [7]. The 3α-configuration of

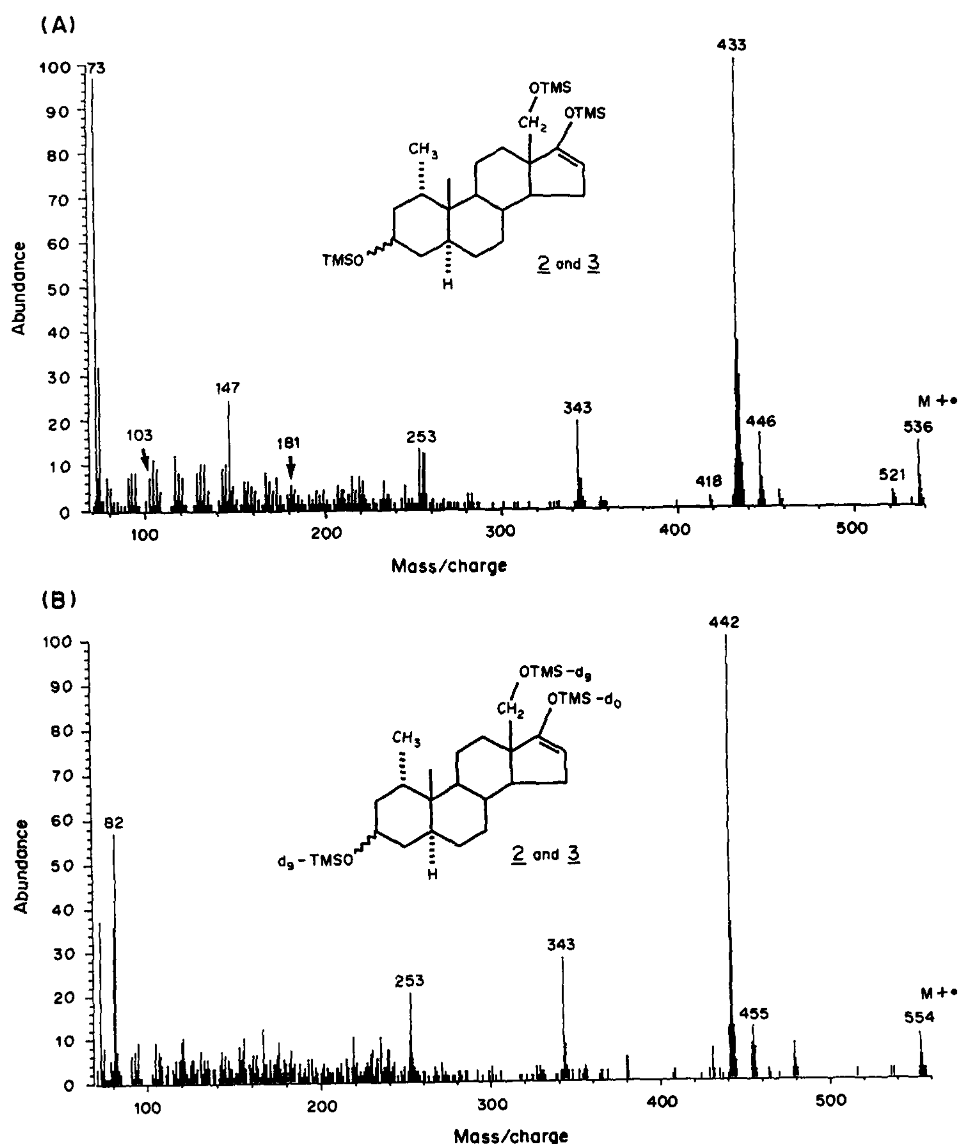

Fig. 4. Mass spectra of 3ξ,18-dihydroxy-1α-methyl-5α-androstan-17-one **2** and **3** (mesterolone) as (A) TMS enol-TMS ether and (B) d_0-TMS enol-d_9-TMS ether derivatives.

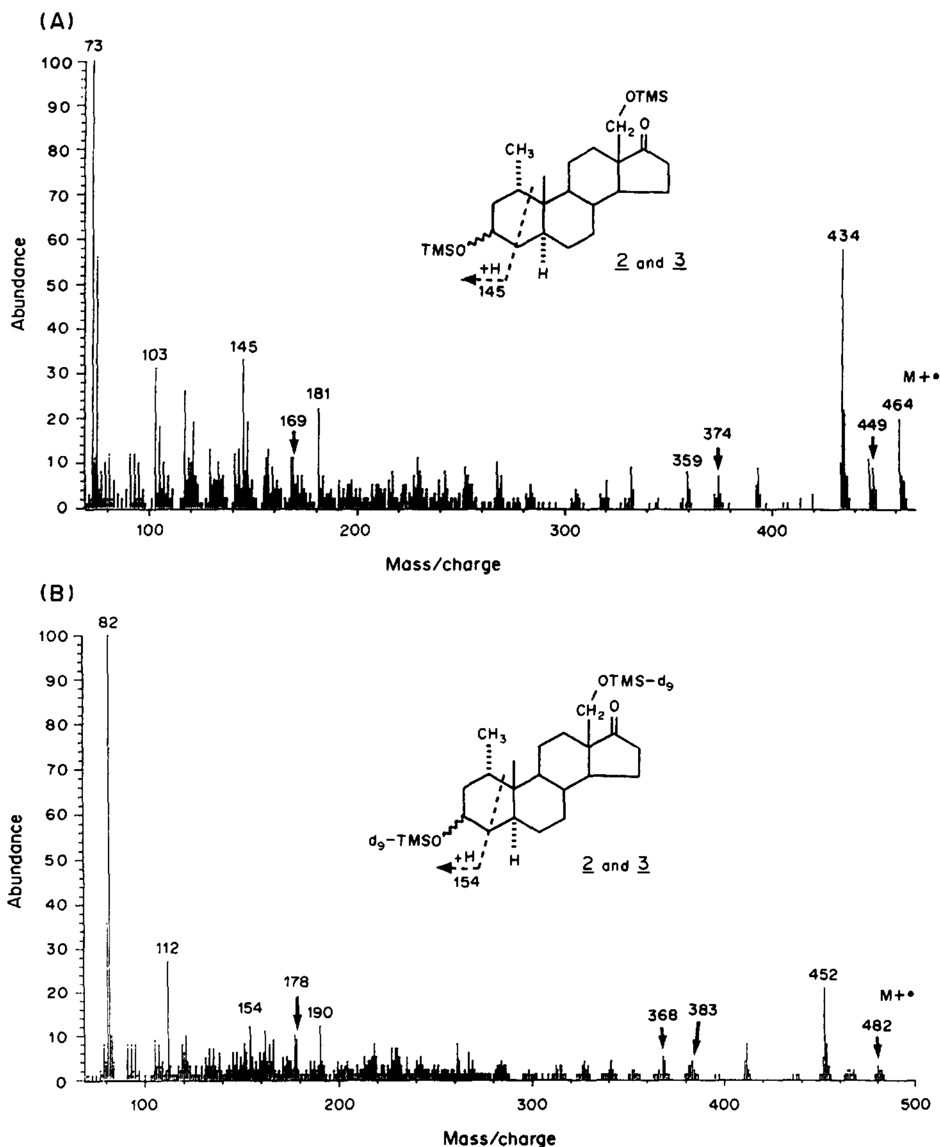

Fig. 5. Mass spectra of 3ζ,18-dihydroxy-1α-methyl-5α-androstan-17-one **2** and **3** (mesterolone) as (A) TMS ether and (B) d_9-TMS ether derivatives.

the 3-hydroxy group in compound **2** was demonstrated by enzymatic oxidation of urinary extracts of mesterolone metabolites with 3α-dehydrogenase. Compound **2** was not detected in the reaction mixture whereas compound **3** was recovered unchanged. The transformation of **2** into 18-hydroxy-1α-methyl-5α-androstan-3,17-dione **1** was noticed by an increase of compound **1** with respect to that from a urinary extract which has not been treated with 3α-dehydrogenase. On the basis of these mass spectral data and other reference data from the literature compounds **2** and **3** were identified as 3α,18-dihydroxy-1α-methyl-5α-androstan-17-one and 3β,18-dihydroxy-1α-methyl-5α-androstan-17-one, respectively.

Metabolite 4. This metabolite was more abundant than compounds **1**, **2** and **3** (Fig. 2). The molecular ions of its TMS enol-TMS ether derivative [Fig. 6(A)] at m/z 624 (m/z 651, d_0-TMS enol-d_9-TMS ether mixed derivative), TMS ether derivative [Fig. 6(B)] at m/z 552 (m/z 579, d_9-TMS) and MO-TMS derivative at m/z 581 (m/z 609, d_9-TMS) indicated the presence of one keto and three hydroxyl functions in this compound. It is of interest to note the presence of ions of diagnostic importance in the mass spectra of the TMS enol-TMS ether and TMS ether derivatives, namely ions at m/z 521 (M-CH_2OTMS^+; m/z 539, d_0-TMS ether mixed derivative) [Fig. 6(A)] and m/z 522 (M-CH_2O^+; m/z 549, d_9-TMS) [Fig. 6(B)] which both

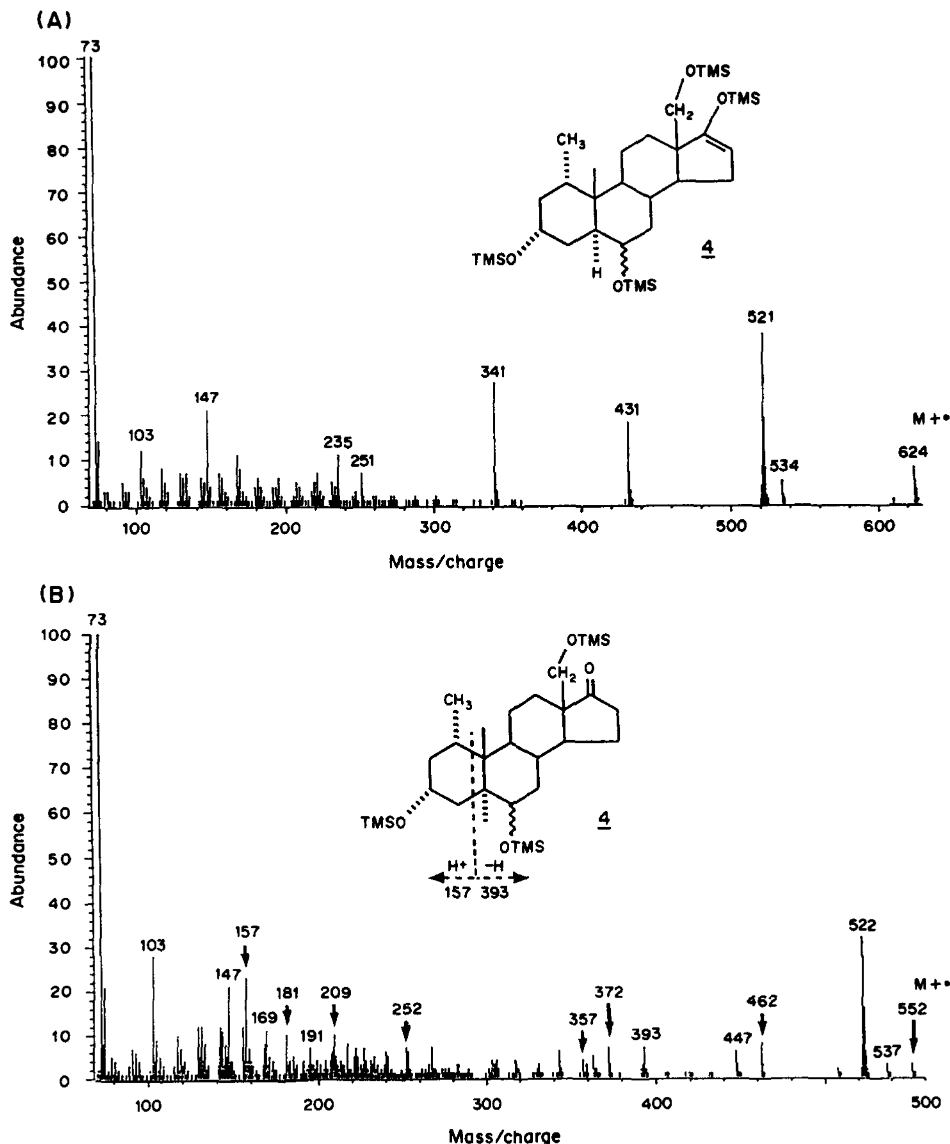

Fig. 6. Mass spectra of 3α6ξ,18-trihydroxy-1α-methyl-5α-androstan-17-one 4 (mesterolone) as (A) TMS enol-TMS ether and (B) TMS ether derivatives.

indicate the presence of 18-hydroxyl and 17-keto functions as in the metabolites discussed above.

The mass spectrum of the TMS ether derivative provided structural evidence suggesting that the two other hydroxyl groups were at the C_3 and C_6 positions. As illustrated in Fig. 6(B), ions at *m/z* 393 (*m/z* 411, d_9-TMS) and *m/z* 157 (*m/z* 166, d_9-TMS) are likely to arise from the cleavage of the C_1—C_{10} and C_4—C_5 bonds according to mechanisms proposed by Harvey and Vouros [29] for the formation of analogous ions in 3,6-dihydroxy-steroids. Other TMS-containing ions which are characteristic of 3,6-dihydroxy-steroids [29] were present at low mass in the TMS ether derivative mass spectrum and the most characteristic are observed at *m/z* 103 (*m/z* 112, d_9-TMS); *m/z* 169 (*m/z* 178, d_9-TMS); *m/z* 181 (*m/z* 190, d_9-TMS) and *m/z* 209 (*m/z* 218, d_9-TMS).

Enzymatic oxidation of 4 with 3α-hydrogenase yielded 6ξ,18-dihydroxy-5α-androstan-3,17-dione, thus demonstrating that compound 4 bears a 3α-hydroxyl group. The mass spectrum of the oxidation product showed a molecular ion at *m/z* 622 (*m/z* 640, d_0-TMS enol-d_9-TMS ether mixed derivative) and characteristic ions at *m/z* 519 (M-CH_2OTMS)$^+$, 429 (M-CH_2OTMS-TMSOH)$^+$ and 339 (M-CH_2OTMS-2TMSOH)$^+$ which were consistent with the proposed structure. Given the fact we had neither undisputed mass spectral evidence nor reference steroid to irrefutably

demonstrate the presence of a 6-hydroxyl group and establish its stereochemistry, compound **4** was tentatively identified as 3α-6ξ-18-trihydroxy-5α-androstan-17-one.

Methenolone

Because of the structural similarity between mesterolone and methenolone (Fig. 1), we expected that the latter steroid could also undergo 18-hydroxylation in human. Urinary specimens collected after administration of a single 50 mg dose of the steroid taken orally were analyzed by GC/MS according to a procedure analogous to that used to mesterolone.

*Metabolite **5**.* Contrary to mesterolone, only one compound with mass spectral features consistent with those of a 18-hydroxy steroid was detected in urine. The mass spectra of the TMS enol-TMS ether [Fig. 7(A)] and TMS ether [Fig. 7(B)] derivatives were consistent with the structure of 18-hydroxy-1-methyl-5α-androst-1-en-3,17-dione **5**. The presence of methenolone A-ring characteristic ions at *m/z* 179, 195 and 208 [7] demonstrated that hydroxylation occurred neither at the C_{19} nor at the C_1 position [Fig. 7(A)]. The prominent ion at *m/z* 429 ($M-CH_2OTMS^+$; *m/z* 429, d_0-TMS enol-d_9-TMS ether mixed derivative) analogous to the corresponding ions observed in the mass spectra of metabolites **1–4** indicates the presence of 18-hydroxyl and 17-keto functions. This is corroborated by mass spectral evidence from the

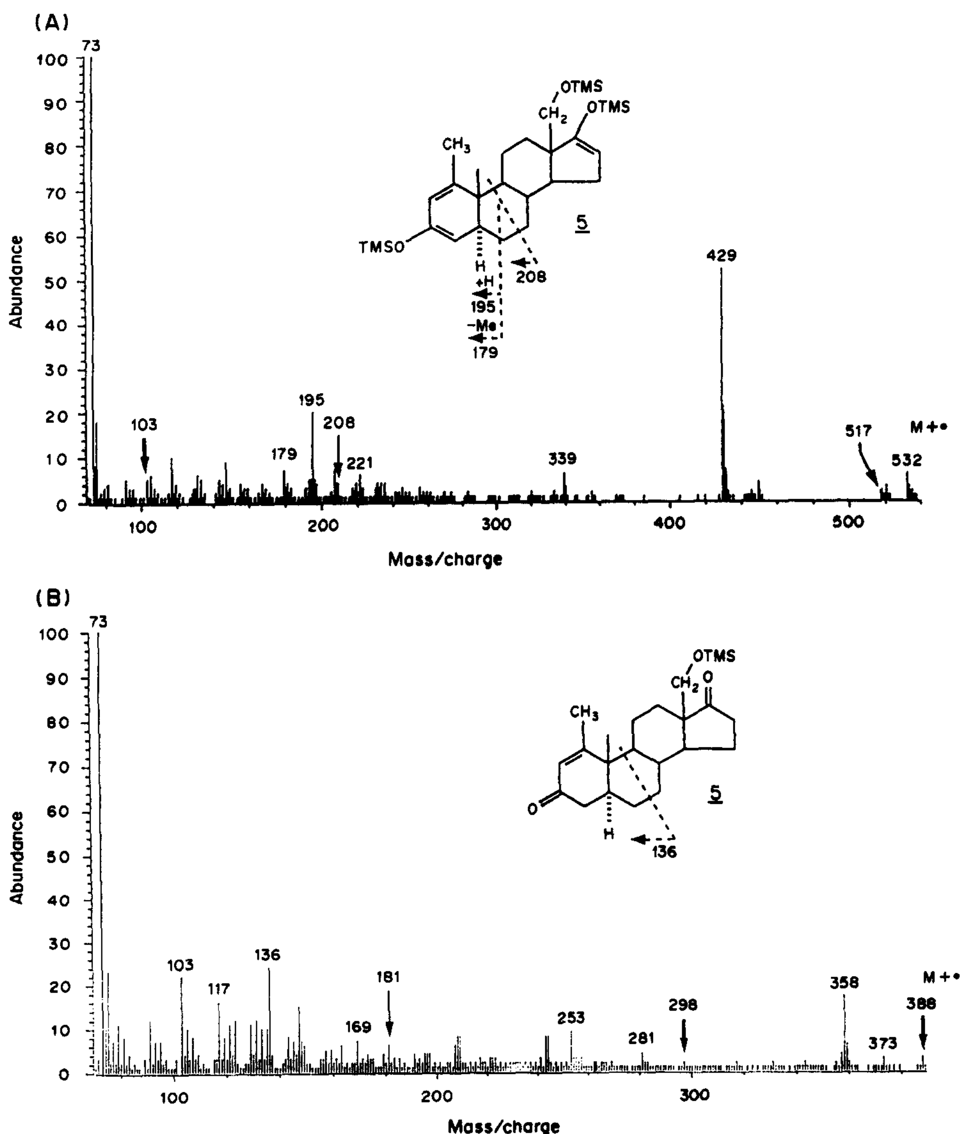

Fig. 7. Mass spectra of 18-hydroxy-1-methyl-5α-androst-1-ene-3,17-dione **5** as (A) TMS enol-TMS ether and (B) TMS ether derivatives.

TMS ether derivative [Fig. 7(B)]. Diagnostic ions at m/z 103 $(CH_2=OTMS)^+$ and m/z 358 ($M\text{-}CH_2O^+$; m/z 367, d_9-TMS) are characteristic of 18-hydroxy-17-keto steroids, whereas an ion at m/z 136 (m/z 136, d_9-TMS) indicates that methenolone A-ring was not affected by any metabolic reaction [7]. Thus, compound **5** was identified as 18-hydroxy-1-methyl-5α-androst-1-en-3,17-dione.

Stenbolone

Contrary to both mesterolone and methenolone, stenbolone bears a C_2 instead of a C_1 methyl group. We are interested in determining whether such a small structural modification would affect the prospensity of stenbolone to undergo hydroxylation at C_{18} as it is the case for its C_1 methyl analogs.

*Metabolite **6**.* Interestingly, one urinary metabolite, the TMS enol-TMS ether of which [Fig. 8(A)] is very similar to that of metabolite **5** was detected in post-administration urine specimens. The molecular ion at m/z 532 (m/z 541, d_0-TMS enol-d_9-TMS ether mixed derivative), the diagnostic ion at m/z 429 ($M\text{-}CH_2OTMS^+$; m/z 429, d_0-TMS enol-d_9-TMS ether mixed derivative) and ions characteristics of stenbolone A-ring at m/z 193, 208 and 221 [8] were consistent with the proposed structure. Further mass spectral data were provided by analysis of the TMS ether derivative [Fig. 8(B)]. The corresponding mass spectrum

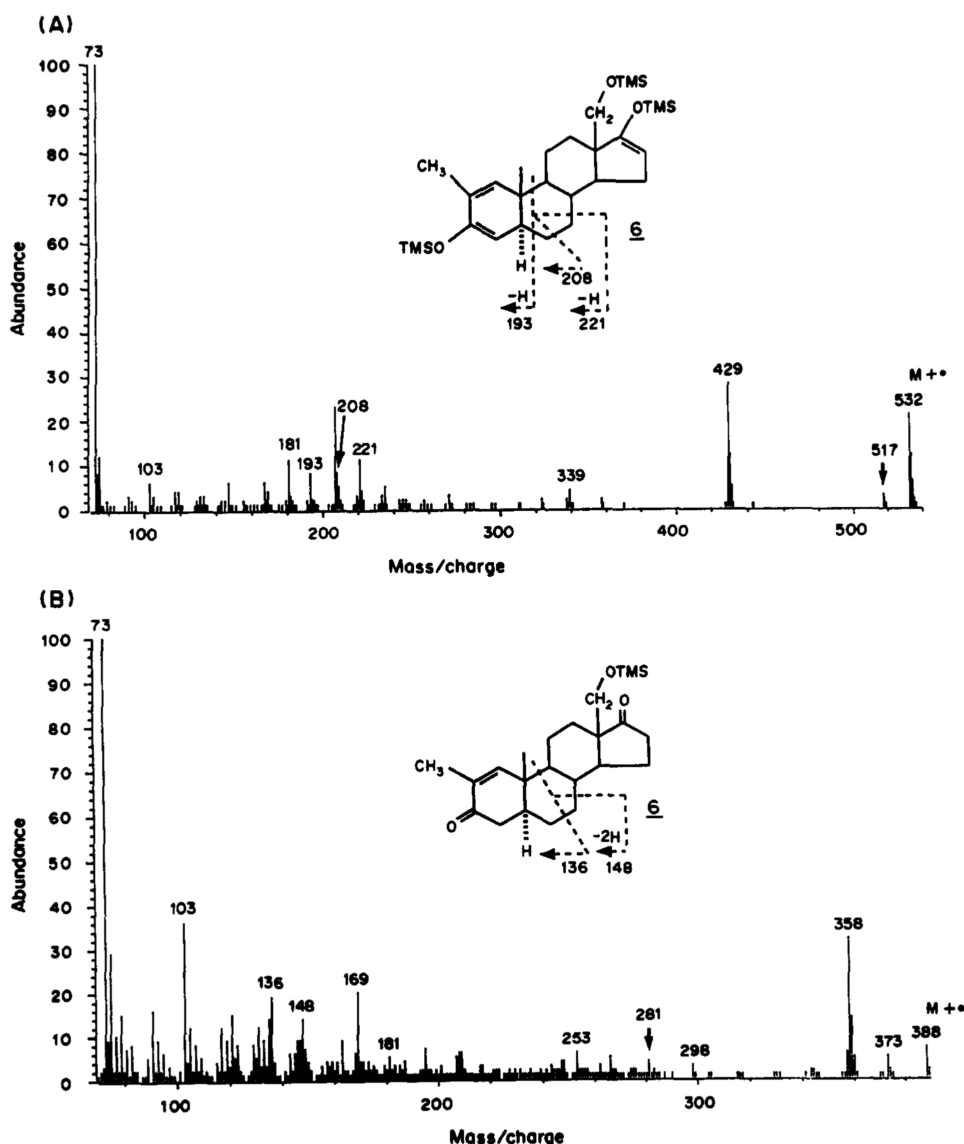

Fig. 8. Mass spectra of 18-hydroxy-2-methyl-5α-androst-1-ene-3,17-dione **6** as (A) TMS enol-TMS ether and (B) ether derivatives.

showed a molecular ion at m/z 388 (m/z 397, d_9-TMS) and structurally informative ions at m/z 358 ($M\text{-}CH_2O^+$; m/z 367, d_9-TMS), m/z 148 (m/z 148, d_9-TMS), 136 (m/z 136, d_9-TMS) and 103 (m/z 112, d_9-TMS). Compound **6** was identified as 18-hydroxy-2-methyl-5α-androst-1-ene-3,17-dione.

DISCUSSION

Although adrenal and hepatic 18-hydroxylation of endogenous androgenic steroids such as androsterone and testosterone has been demonstrated in several animal species and in human [18–24, 31], the presence of an 18-hydroxyl group in urinary metabolites of synthetic anabolic steroids has not previously been described in animal or in human. *In vivo* and *in vitro* studies have revealed that 18-hydroxylase activity is not confined to the adrenal cortex but is also displayed by extra-adrenal tissues [18, 21, 31].

The isolation of the 18-hydroxylation steroids **1–6** as minor urinary metabolites of mesterolone, methenolone and stenbolone indicates the presence in human hepatic tissue of an 18-hydroxylase active for 3,17-diketo and/or 17-hydroxy-3-keto C_{19} steroids bearing a methyl group at the C_1 or C_2 positions. The structural similarity of the studied anabolic–androgenic steroids with testosterone, particularly that of their B-, C- and D-rings, suggests that the synthetic steroids studied are probably hydroxylated at C_{18} by the same hepatic androgen-specific 18-hydroxylases that give rise to 18-hydroxytestosterone in human [18]. Given the multiplicity of mammalian microsomal cytochromes *P*-450 [32], it is also possible that mesterolone, methenolone and stenbolone are 18-hydroxylated by distinct forms of *P*-450. It is interesting to note that metabolites **1–4** were detected in urine only for discrete periods of time after administration of mesterolone. Compound **1** which appears to be the first metabolite produced in this metabolic cascade was only detected during the first 12 h after administration, whereas both compounds **2** and **3** and **4** were found until 24 h and 48 h, respectively after administration. This suggests enterohepatic circulation of the lipophilic **1** which favors further biotransformation into **2**, **3** and **4**, thus preventing its excretion over a prolonged period of time.

On the other hand, compound **5**, as well as other minor metabolites of methenolone [7] was detected until 70 h after administration. Finally, **6** was still detected in urine as long as 120 h after administration of stenbolone. The excretion data can be rationalized by the fact that lipophilic steroids such as methenolone and

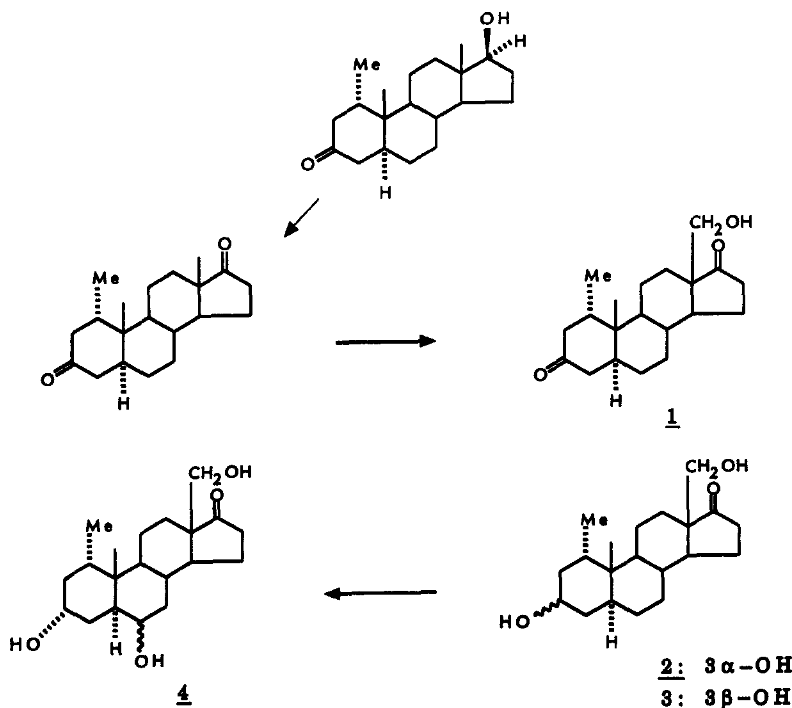

Fig. 9. Proposed pathways for the formation of 18-hydroxylated metabolites of mesterolone.

stenbolone with 3-keto and 1-ene groups are apparently metabolized at slower rates than steroids such as mesterolone (Fig. 1) bearing only a 3-keto function and which is rapidly metabolized to yield a 3α-hydroxy-1α-methyl-5α-androstan-17-one as the major urinary metabolite [33].

The 18-hydroxylated metabolites of mesterolone originate from a biotransformation route that encompasses a series of hydroxylation and reduction reactions. Mesterolone is partly oxidized at C_{17} to give the corresponding 3,17-diketo steroid, which is converted by hydroxylation at C_{18} to compound **1** and subsequent reduction of the 3-keto group afforded the isomeric diols **2** and **3** and further hydroxylation of **2** yielded the triol **4**. The fact that these metabolites as well as compounds **5** and **6** are produced from relatively hydrophobic precursors (Figs 1 and 9) led us to speculate that they may be formed as detoxification products.

Since these biosynthetic routes are minor pathways in the biotransformation of these steroids in human, it is difficult to comment on their biological significance. However, there may be some specific biological role associated to the 18-hydroxylation of these anabolic steroids as certain hydroxylated steroids such as 18-hydroxyandrostenedione can be aromatized by human placenta [34]. Like testosterone which can be aromatized into estrogens in human [35, 36], anabolic–androgenic steroids that possess the proper structural requirements can also undergo aromatization into estrogens [37, 38]. Aside from C_{19}-hydroxylation, which is an essential reaction for aromatization of androgens [35], 18-hydroxylation of mesterolone, methenolone and stenbolone yields 18-hydroxysteroids which could be substrates for aromatizing systems [34].

REFERENCES

1. Hobkirk R.: Hydroxylated C_{18} and C_{19} steroids; their significance and factors related to their biosynthesis. In *Steroid Biochemistry* (Edited by R. Hobkirk). CRC Press, Boca Raton, Vol 2 (1979) pp. 134–179.
2. Waxman D. J.: Interactions of hepatic cytochromes *P*-450 with steroid hormones: regioselectivity and stereospecificity of steroid metabolism and hormonal regulation of rat *P*-450 enzyme expression. *Biochem. Pharmac.* **37** (1988) 71–84.
3. Gower D. B.: The role of cytochrome *P*-450 in steroidogenesis and properties of some of the steroid-transforming enzymes. In *Biochemistry of Steroid Hormones* (Edited by H. L. J. Makin). Blackwell Scientific Publications, Oxford, 2nd Edn (1984) pp. 230–280.
4. Massé R., Ayotte C. and Dugal R.: Studies on anabolic steroids. 1. Integrated methodological approach to the gas chromatographic-mass spectrometric analysis of anabolic steroid metabolites in urine. *J. Chromat.* **489** (1989) 23–50.
5. Massé R., Bi H., Ayotte C. and Dugal R.: Studies of anabolic steroids. 2.: Gas chromatographic-mass spectrometric characterization of oxandrolone urinary metabolites in man. *Biomed. Environ. Mass Spectrom.* **18** (1989) 429–438.
6. Massé R., Ayotte C., Bi H. and Dugal R.: Studies of anabolic steroids. 3. Detection and characterization of stanozolol urinary metabolites in humans by gas chromatography-mass spectrometry. *J. Chromat.* **497** (1989) 17–37.
7. Gouldreault D. and Massé R.: Studies on anabolic steroids—4. Identification of new urinary metabolites of methenolone acetate (Primobolan®) in human by gas chromatography/mass spectrometry. *J. Steroid. Biochem. Molec. Biol.* **37** (1990) 137–154.
8. Goudreault D. and Massé R.: Studies on anabolic steroids—6. Identification of urinary metabolites of stenbolone acetate (17β-acetoxy-2-methyl-5α-androst-1-en-3-one) in human by gas chromatography/mass spectrometry. *J. Steroid. Biochem. Molec. Biol.* **38** (1991) 639–655.
9. Dürbeck H. W., Büker I., Scheulen B. and Telin B.: GC and capillary column CG/MS determination of synthetic anabolic steroids II. 4-chloro-methandienone (oral turinabol) and its metabolites. *J. Chromat. Sci.* **21** (1983) 405–410.
10. Kammerer R. C., Merdink J. L., Jagels M. and Catlin D. H.: Testing for fluoxymesterone (Halotestin®) administration to man: Identification of urinary metabolites by gas chromatography-mass spectrometry. *J. Steroid. Biochem.* **36** (1990) 659–666.
11. Schänzer W., Opfermann G. and Donike M.: Metabolism of stanozolol: identification and synthesis of urinary metabolites. *J. Steroid. Biochem.* **36** (1990) 153–174.
12. Schänzer W., Geyer H. and Donike M.: Metabolism of methandienone in men: identification and synthesis of conjugated excreted urinary metabolites, determination of excretion rates and gas chromatographic-mass spectrometric identification of bis-hydroxylated metabolites. *J. Steroid. Biochem. Molec. Biol.* **38** (1991) 441–464.
13. Gower D. B.: Biosynthesis of the corticosteroids. In *Biochemistry of Steroid Hormones* (Edited by H. L. J. Makin). Blackwell Scientific Publication, Oxford, 2nd Edn (1984) pp. 117–169 and reference cited therein.
14. Chu M. D. and Ulick S.: Isolation and identification of 18-hydroxycortisol from the urine of patients with primary aldosteronism. *J. Biol. Chem.* **257** (1982) 2218–2224.
15. Ulick S., Chu M. D. and Land M.: Biosynthesis of 18-oxocortisol by aldosterone-producing adrenal tissue. *J. Biol. Chem.* **258** (1983) 5498–5502.
16. Fujii S., Momoi K., Okamoto M., Yamano T., Okada T. and Terasawa T.: 18,19-dihydroxydeoxycorticosterone, a new metabolite produced from 18-hydroxydeoxycorticosterone by cytochrome P-$450_{11\beta}$. Chemical synthesis and structural analysis by H NMR. *Biochemistry* **23** (1984) 2558–2564.
17. Gomez-Sanchez C. E., Foecking M. F., Shackleton C. H. L., Chavarri M. R. and Gomez-Sanchez E. P.: 18-hydroxy-11-deoxycortisol: a new steroid isolated from incubations of the adrenal with 11-deoxycortisol. *J. Steroid. Biochem.* **26** (1987) 105–111.
18. Fukushima D. K., Bradlow H. L. and Hellman L.: Isolation and characterization of 18-hydroxy-17-ketosteroids. *J. Biol. Chem.* **237** (1962) 3359–3363.
19. Lucis R., Carballeria A. and Venning E. H.: Biosynthesis of 19-hydroxy-steroids and 18-hydroxytestosterone by human and rat adrenal tissue. *Excerpta Medica Congr. Series* **157** (1968) 183.

20. Ingelman-Sunberg M., Rane A. and Gustafsson J.-Å.: Properties of hydroxylase systems in the human fetal liver active on free and sulfoconjugated steroids. *Biochemistry* **14** (1975) 429–438.
21. Gustafsson J. Å. and Lisboa B. P.: Studies on the metabolism of C_{19} steroids in rat liver. 5. 18-hydroxylation of 17β-hydroxy-C_{19} steroids in rat liver microsomes. *Steroids* **14** (1969) 659–674.
22. Lisboa B. P., Gustafsson J. A. and Sjövall.: Studies on the metabolism of C_{19} steroids in rat liver. 1. Hydroxylation of testosterone in rat liver microsomes. *Eur. J. Biochem.* **4** (1968) 496–505.
23. Laatikainen T. and Vihko R.: Identification of 18-hydroxyandrosterone in human bile. *Steroids* **13** (1969) 615–621.
24. Ingleman-Sundberg M.: Specific metabolic pathways of steroid sulfates in human liver microsomes. *J. Clin. Endocr.* **43** (1976) 56–63.
25. Sharkey A. G., Friedel R. A. and Langer S. H.: Mass spectra of trimethylsilyl derivatives. *Analyt. Chem.* **29** (1957) 770–776.
26. Brooks C. J. W.: Some aspects of mass spectrometry in research on steroids. *Phil. Trans. Soc. Lond. A* **293** (1979) 53–67.
27. Brooks C. J. W., Harvey D. J., Middleditch B. S. and Vouros P.: Mass spectra of trimethylsilyl ethers of some δ-5-3β-hydroxy C_{19} steroids. *Org. Mass Spectrom.* **7** (1973) 925–948.
28. Weber W. P., Felix R. A. and Willard A. K.: Mass spectra rearrangements. A silyl McLafferty rearrangement. *J. Am. Chem. Soc.* **92**(5) (1970) 1420–1421.
29. Harvey D. J. and Vouros P.: Influence of the 6-trimethylsilyl group on the fragmentation of the trimethysilyl derivatives of some 6-hydroxy and 3,6-dihydroxy-steroids and related compounds. *Biomed. Mass. Spectrom.* **6**(4) (1979) 135–143.
30. Gustafsson J. Å. and Lisboa B. P.: Studies on the metabolism of C_{19} steroids in rat liver. 7. 18-hydroxylation of 17-oxo-C_{19}-steroids in rat liver microsomes. *Steroids* **15** (1970) 723–735.
31. Lisboa B. P. and Gustafsson J. Å.: Biosynthesis of 18-hydroxytestosterone in the human foetal liver. *Eur. J. Biochem.* **9** (1969) 402–405.
32. Lu A. Y. H. and West S. B.: Multiplicity of mammalian microsomal cytochromes *P*-450. *Pharmac. Rev.* **31** (1980) 277–295.
33. Massé R. and Goudreault D.: Studies on anabolic steroids. 13. Investigation of mesterolone (17β-hydroxy-17α-methyl-5α-androstan-3-one) metabolism in man by GC/MS. Submitted.
34. Tan I. K. and Loke K. H.: Aromatization of 18-hydroxyandrostendione to 18-hydroxyestrone by human placental tissue. *Steroids* **8** (1968) 348–354.
35. Fotherby K.: Biosynthesis of the oestrogens. In *Biochemistry of Steroid Hormones* (Edited by H. L. J. Makin). Blackwell Scientific Publications, Oxford, 2nd Edn (1984) pp. 209–229.
36. Martini L.: The 5α-reduction of testosterone in the neuroendocrine structure. Biochemical and physiological implications. *Endocrine Rev.* **3** (1982) 1–25.
37. Kruskemper H. L.: Metabolism of anabolic steroids. In *Anabolic Steroids* (Edited by H. L. Kruskemper). Academic Press, New York (1968) pp. 21–30.
38. Rahwan R. G.: The pharmacology of androgens and anabolic steroids. *Am. J. Pharm. Educ.* **52** (1988) 167–177.