METABOLISM OF 17α-METHYLTESTOSTERONE IN THE RABBIT: C-6 and C-16 HYDROXYLATED METABOLITES

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Abstract - 17α-Methyltestosterone and the reduced metabolites, 17α-methyl-5α-androstane-3α,17β-diol, 17α-methyl-5α-androstane-3β,17β-diol and 17α-methyl-5β-androstane-3α,17β-diol, together with three hydroxylated metabolites, 17α-methyl-5β-androstane-3α,16α,17β-triol, 17α-methyl-5β-androstane-3α,16β,17β-triol and a new metabolite, 17α-methyl-5α-androstane-3β,6α,17β-triol, were isolated and identified in the urine of rabbits dosed with 17α-methyltestosterone. No hydroxylated 5α-metabolite of 17α-methyltestosterone has been identified previously. No evidence for epimerization at the C-17 position was observed.

INTRODUCTION

As part of the investigation of the biotransformations of the two primary metabolites of 17α-methyltestosterone (I), 17α-methyl-5α-dihydrotestosterone (1) and 17α-methyl-5β-dihydrotestosterone (2), an earlier study with 17α-methyltestosterone in the rabbit (3,4) was repeated for direct comparison. Investigation of the metabolism of these closely related substances is of interest in determining the predictability of the relationship between their metabolic products. Possible epimerization of the 17α-methyl/17β-hydroxyl group, which has been reported to occur in humans administered 1-dehydro-17α-methyltestosterone (methandrostenolone) (5,6), was investigated herein with the objective of obtaining both a model compound and animal model to study this unique biotransformation. Hydroxylated metabolites of 17α-methyltestosterone have been reported in man but their structures have not been fully elucidated (7).

EXPERIMENTAL

^{13}C NMR spectra were recorded on a Nicolet modified Brucker WH 90 DS with NTCFT software at 22.63 MHz using polarization transer spectroscopy (8). R_f values are from thin-layer chromatography on precoated silica gel plates (Merck 60 F-254) run in 10% v/v methanol/chloroform. For other instrumentation and methods see reference 1.

Administration of 17α-methyltestosterone (I):

Nine mature male albino rabbits, maintained on a Purina rabbit chow diet and water ad libitum, were housed singly in cages designed for efficient separation of urine and faeces. A controlled illumination environment of 12 hrs light and 12 hrs darkness was maintained. The animals were each dosed four times at two-day intervals with a finely divided slurry of I (0.7 g) in propylene glycol (10 mℓ) by oral administration. A total of 13 ℓ of urine was collected over 10 days under a layer of toluene and stored daily at -5°C. Control urine from rabbits dosed with propylene glycol (10 mℓ) as above was collected similarly prior to dosing. No gross alteration in the appearance, food intake or behaviour of the dosed animals was observed.

Isolation of steroids (enzymatic hydrolysis):

The pooled urine (13 ℓ) was adjusted to pH 4.9 with glacial acetic acid and incubated with bovine liver β-glucuronidase (400 FU/mℓ) at 37°C for 72 hrs followed by ether extraction (3 x 1 ℓ). The combined ether layers were washed successively with saturated aqueous $NaHCO_3$, 1N-NaOH, water and dried over sodium sulfate. Evaporation of the solvent at reduced pressure yielded a crude neutral residue (760 mg/ℓ). A control experiment with urine (1 ℓ) gave a residue (90 mg/ℓ). Acidification of the $NaHCO_3$ and 1N-NaOH extracts followed by ether extraction gave fractions which did not yield any major components on TLC and GLC different from those in the equivalent control experiment. Treatment of the acidic portions with diazomethane did not show any new components.

Chromatography of the crude neutral residue:

The crude neutral residue was chromatographed in benzene over ethyl acetate treated alumina (Brockmann Activity II) (9). Fractions were combined on the basis of their TLC, crystallinity and weight. The following components were identified by chromatographic (TLC) and spectroscopic (MS, ^1H NMR, IR) comparison and mixed MP with authentic samples (10): elution with 10% v/v ether/benzene gave 17α-methyltestosterone (I) (10 mg) from methanol, MP 162-3°C and 17α-methyl-5α-androstane-3α,17β-diol (II) which cochromatographed with III; elution with 10-25% v/v ether/benzene gave 17α-methyl-5α-androstane-3β,17β-diol (III) (303 mg) MP 205-8°C from methanol [lit. (11) MP 212-14°C] and 17α-methyl-5β-androstane-3α,17β-diol (IV) (40 mg) MP 161-4°C from ethyl acetate [lit. (12) MP 164-6°C]. Elution with 2.5-5% v/v methanol/ether gave three components: (i) 17α-methyl-5β-androstane-3α,16β,17β-triol (V) (421 mg) MP 255-8°C from methanol [lit. (4) MP 254-7°C and 266-8°C]; R_f=0.23; mixed MP was undepressed and IR and ^1H NMR were identical with the material isolated previously (2); (ii) 17α-methyl-5β-androstane-3α,16α,17β- triol (VI) (192 mg) MP 218-9°C from methanol [lit. (4) MP 221-2°C]; R_f=0.15; mixed MP was undepressed and IR and ^1H NMR were identical with the material isolated previously (2); (iii) 17α-methyl-5α-androstane-3β,6α,17β-triol (VII) (153 mg) MP 212-3°C from dichloromethane/methanol; R_f= 0.05; IR (KBr) ν_{max}: 3230 (OH str.) cm^{-1}; ^1H NMR ($CDCl_3$)δ :0.85 (C-

$10CH_3$ and C-$13CH_3$), 1.21 (C-$17CH_3$), 3.25-3.80, m, (C-3αH and C-6βH), (pyridine-d_5) 0.93 (C-$10CH_3$), 1.10 (C-$13CH_3$), 1.42 (C-$17CH_3$), 3.89, m, (3αH), 3.37, m, (6βH) ppm; MS m/z: 322 (M^+), 307 (M^+-CH_3), 304 (M^+-H_2O), 289 [$M^+-(H_2O + CH_3)$], 286 (M^+-2H_2O).
Anal. Found: C, 74.12; H, 10.64. $C_{20}H_{34}O_3$ requires C, 74.49; H, 10.63. Acetylation gave a non-crystalline diacetate ^1H NMR ($CDCl_3$)δ : 0.84 (C-$13CH_3$), 0.91 (C-$10CH_3$), 1.21 (C-$17CH_3$), 2.02 (C-3β- and C-6α-acetate), 4.67, m, (C-3αH and C-6βH) ppm. Oxidation with Jones reagent (12) gave a non-crystalline dionol, IR (CCl_4) ν_{max}: 3625 (OH str.), 1710 (C-3 and C-6 carbonyl) cm^{-1}; ^1H NMR ($CDCl_3$)δ: 0.89 (C-$13CH_3$), 0.98 (C-$10CH_3$), 1.25 (C-$17CH_3$) ppm; MS m/z: 318 (M^+), 303 (M^+-CH_3).

RESULTS

The acidic and enolic/phenolic fractions obtained from extraction of glucuronidase-treated urine from rabbits orally dosed with 17α-methyltestosterone (I) did not show the presence of metabolites by TLC and GLC examination whereas metabolites were present in the neutral fraction. Column chromatography of the crude neutral fraction gave three diols, 17α-methyl-5α-androstane-3α,17β-diol (II), and 17α-methyl-5α-androstane-3β,17β-diol (III) and 17α-methyl-5β-androstane-3α,17β-diol (IV), identified by comparison with authentic samples. The C-16 hydroxylated compounds, 17α-methyl-5β-androstane-3α,16β- and 16α, 17β-triol (V and VI, respectively) were identified by comparison (IR, ^1H NMR, MS) with samples obtained as metabolites of 17α-methyl-5β-dihydrotestosterone (2) and the structures confirmed by their ^{13}C NMR spectra (see Table I).

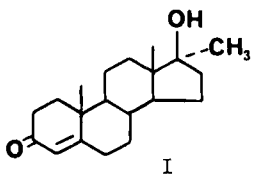
A third hydroxylated metabolite, 17α-methyl-5α-androstane-3β,6α,-17β-triol (VII), was identified by ^{13}C NMR spectroscopy (see Table I) and confirmed by ^1H NMR. ^{13}C NMR spectral assignments for this compound are consistent with those for authentic 17α-methyl-5α-androstane-3α,17β-diol (II) and the C-6 epimer, 17α-methyl-5α-androstane-3α,-6α,17β-triol (VIII) previously identified as a metabolite of 17α-methyl-

TABLE I

^{13}C NMR SPECTRA OF 17α-METHYL-5ξ-ANDROSTANE DERIVATIVES

C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
II	[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
III	37.7	(32.3)	70.7	[39.4]	45.4	29.2	(32.6)	36.8	54.8	36.0	21.4	(32.4)	46.2	51.2	23.9	[39.6]	80.7	14.9	12.6	26.8
VII	33.4	29.7	65.3	31.4	47.0	69.0	42.8	35.7	54.5	37.3	20.9	32.4	46.3	51.2	23.9	39.5	80.6	14.9	12.9	26.8
VIII	38.2	32.4	71.1	33.8	53.0	68.8	42.7	35.7	54.5	36.7	21.4	32.4	46.3	51.1	23.9	39.5	80.7	14.9	13.8	26.8

Spectra are recorded in pyridine-d_5.
Enclosed numbers in a row indicate interchangeable or overlapping pairs of values.



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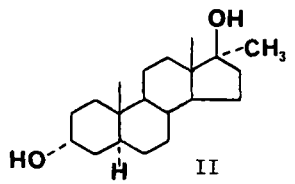

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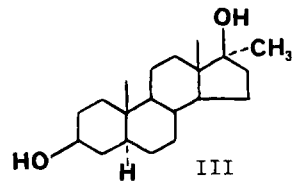

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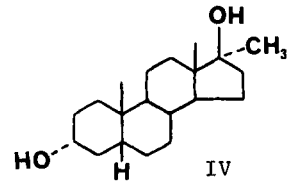

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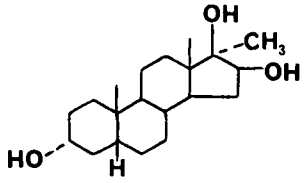

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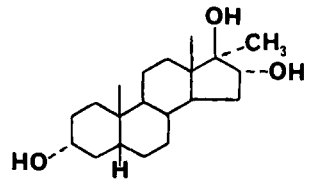

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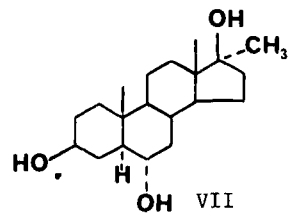

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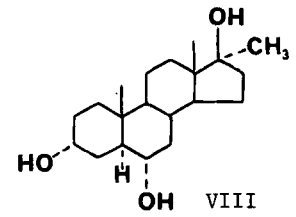

VIII

5α-dihydrotestosterone (1). Both triols were converted to the same diketone on oxidation with Jones reagent (13) as shown by ^{1}H NMR.

DISCUSSION

The metabolism of 17α-methyltestosterone (I) in the rabbit has been reported by Watabe et al. (3,4) in which urinary metabolites from the same strain of rabbits used in the present work and similarly dosed were identified. The three non-hydroxylated metabolites, 17α-methyl-5α-androstane-3α,17β-diol (II), 17α-methyl-5α-androstane-3β,17β-diol (III) and 17α-methyl-5β-androstane-3α,17β-diol (IV) found in this work were the same as those previously reported except that an increased ratio of 5α:5βisomers (9:1) compared with (1:4.6) was obtained (see Table II). Among the hydroxylated metabolites, the results were different in that a new metabolite, 17α-methyl-5α-androstane-3β,6α,17β-triol (VII), was identified. The major hydroxylated metabolites were the 16β- and 16α-alcohols (V) and (VI) but the C-16 ketone previously obtained (4) was not found in this study.

TABLE II
NEUTRAL URINARY METABOLITES FROM THE RABBIT DOSED ORALLY WITH 17α-METHYLTESTOSTERONE

Compound	Relative Percentage[a]
17α-methyltestosterone (I)	1
17α-methyl-5α-androstane-3α,17β-diol (II)	1
17α-methyl-5α-androstane-3β,17β-diol (III)	28
17α-methyl-5β-androstane-3α,17β-diol (IV)	3
17α-methyl-5β-androstane-3α,16β,17β-triol (V)	40
17α-methyl-5β-androstane-3α,16α,17β-triol (VI)	12
17α-methyl-5α-androstane-3β,6α,17β-triol (VII)	16

[a]Estimated from column fraction weights. 89% of the neutral fraction was recovered as the above metabolites consisting of 43% of the dosed substance.

17α-Methyl-5α-androstane-3β,6α,17β-triol (VII) is formed from 17α-methyltestosterone by C-6-hydroxylation either before or after

reduction of the unsaturated ketone function. As the C-6α-alcohols (VII and VIII) obtained from 17α-methyltestosterone (I) and 17α-methyl-5α-dihydrotestosterone (1) have different configurations of the C-3 alcohols (3β and 3α, respectively) it is unlikely that they are formed through a common metabolic intermediate. Therefore, initial reduction of the double bond of 17α-methyltestosterone to give 17α-methyl-5α-dihydrotestosterone followed by hydroxylation is an unlikely route to the C-6α-alcohol and it is more probable that C-6 hydroxylation occurs before reduction of the double bond.

A small preponderance of 5β-metabolites (55%) over 5α-metabolites (44%) was obtained from 17α-methyltestosterone (I) (see Table II). More hydroxylated metabolites with the 5β-configuration were obtained than with the 5α-configuration. As the same C-16-alcohols were formed from 17α-methyl-5β-dihydrotestosterone (2) they may be formed after reduction of 17α-methyltestosterone to 17α-methyl-5β-dihydrotestosterone. The absence of C-16-alcohols and the presence of the C-15 α-alcohol from 17α-methyl-5α-dihydrotestosterone (1) shows that the 5α-compound is preferentially hydroxylated at C-15. Hydroxylation of the D-ring therefore appears to be a function of A/B ring stereochemistry. C-15-Hydroxylated androstane derivatives have been isolated from saturated but not unsaturated 3-ketosteroids in rats (14).

17α-Methyltestosterone (I) apparently undergoes substantial hydroxylation at C-6 before reduction to the saturated derivative. As 5α-dihydrotestosterone is one of the principal endogenous hormones activating the androgenic receptor, the high activity of many synthetic 5α-derivatives as androgenic/anabolic agents (15) may be in part a result of decreased metabolic hydroxylation to inactive derivatives.

Activity of 17α-methyltestosterone derivatives may also be decreased by reduction to the inactive 5β-isomer.

No evidence for the presence of a C-17 epimer was observed in the ^{1}H NMR spectra which clearly distinguishes the epimers (10).

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GLOSSARY

17α-Methyl-5α-dihydrotestosterone = 17β-hydroxy-17α-methyl-5α-androstan-3-one
17α-Methyl-5β-dihydrotestosterone = 17β-hydroxy-17α-methyl-5β-androstan-3-one
17α-Methyltestosterone = 17β-hydroxy-17α-methyl-4-androsten-3-one
1-Dehydro-17α-methyltestosterone = 17β-hydroxy-17α-methyl-1,4-androstadien-3-one.