Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 933–936. Pergamon Press. Printed in Great Britain

METABOLISM OF 1-DEHYDROANDROSTANES IN MAN

III—METABOLISM OF 17β-HYDROXY-5α-ANDROST-1-EN-3-ONE, 17β-(1′-METHOXY-CYCLOHEXYLOXY)-5α-ANDROST-1-EN-3-ONE (MESABOLONE) AND 5α-ANDROST-1-ENE-3, 17-DIONE†

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(*Received* 19 *May* 1972)

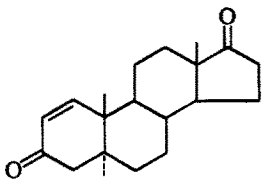
SUMMARY

In a program of studies on the metabolism of 1-dehydroandrostanes, the urinary metabolites of 5α-androst-1-ene-3,17-dione (I), 17β-hydroxy-5α-androst-1-en-3-one (II) and 17β-(1′-methoxy-cyclohexyloxy)-5α-androst-1-en-3-one (Mesabolone, III) have been investigated in humans.

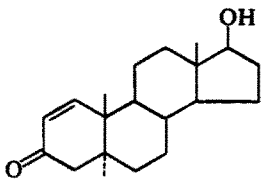
In the case of all compounds significant amounts of C_1-C_2 unsaturated 17-ketones and 17-alcohols were recovered in urines. The excretion of androsterone was also important. The very similar urinary metabolites of II and III suggest the lability *in vivo* of ether III to give II.

IN PREVIOUS papers[1, 2] of this series we reported the results of our investigation on the metabolism of 17β-hydroxy-1,4-androstadien-3-one, 17β-hydroxy-5β-androst-1-en-3-one and related compounds. In both the 1,4-dien-3-one and the 1-en-5β-3-one series it was found that: (a) unsaturation in ring A greatly affects 17β-hydroxy/17-keto ratio in urine metabolites, (b) C_1-C_2 double bond largely survives metabolic reduction.

In the present paper we will report the result of the investigation carried out in order to verify to which extent the metabolic fate of 5α-androst-1-en-3-ones matches that of the previously investigated classes of compounds. In this connection we studied the metabolism in man of 5α-androst-1-ene-3,17-dione,I, and 17β-hydroxy-5α-androst-1-en-3-one, II. In the meantime, in the framework of our research on orally active labile ethers of 17β-hydroxysteroids, we also investigated the metabolism of 17β-(1′-methoxycyclohexyloxy)-5α-androst-1-en-3-one,



I



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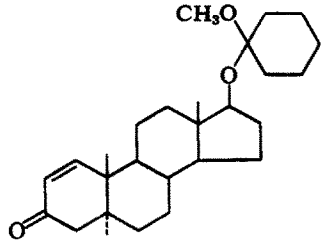


III

Key words: 5α-Androst-1-ene-3,17-dione metabolism of, in man; 17β-Hydroxy-5α-androst-1-en-3-one metabolism of, in man; 17β-(1′-Methoxycyclohexyloxy)-5α-androst-1-en-3-one metabolism of, in man; 3α-Hydroxy-5α-androst-1-en-17-one as metabolite of 5α-androst-1-en-3-ones in man; Mesabolone, see 17β-(1′-methoxycyclohexyloxy)-5α-androst-1-en-3-one.

†The results of this paper were partially presented at the "Third International Congress on Hormonal Steroids", International Congress Series No. 210. Excerpta Medica Found., Amsterdam, 1970. Abstract No. 357, p. 172.

mesabolone,† III, a potent oral andro-anabolic agent[3], in comparison with that of its parent steroid II.

EXPERIMENTAL PROCEDURE

Single oral doses of 100 mg amounts of I, II and III, dissolved in sesame oil, were given separately to normal adult men. Urine was collected for the next 24 h. Basal values of steroid excretion were estimated on urine collected for 3 days before the treatment.

A portion of the urine collection was processed according to the conventional procedures as outlined in previous papers[1, 2].

Single metabolites were separated by thin layer chromatography on neutral alumina or silica gel with the solvent system benzene: ethyl ether 1 : 1. When necessary, eluted fractions were oxidized with chromic acid and further resolved by a second chromatography on silica gel. Steroids were detected on the plates by inspection under ultraviolet light and after spraying with Zimmermann reagent and with 75% sulfuric acid in ethanol. Physical determinations and chemical reactions were performed on microamounts of metabolites obtained by preparative chromatography on 2,000 μm thick layers.

Steroids were evaluated by the Zimmermann reaction. 17-Hydroxy compounds were measured after oxidation to 17-ketones, against their corresponding standards similarly treated.

Reference compounds were prepared in our laboratories[4].

RESULTS

After administration of compounds I, II and III the following steroids were identified in all cases, irrespectively to the compound given, besides the endogenous metabolites:

5α-androst-1-ene-3,17-dione
3α-hydroxy-5α-androst-1-en-17-one
17β-hydroxy-5α-androst-1-en-3-one
5α-androst-1-ene-3α,17β-diol

After all compounds, excretion of androsterone was significantly higher than the pre-treatment values even considering the physiological and analytical variations.

Each metabolite was identified by comparison to the corresponding reference compound of its chromatographic mobility on silica gel and alumina in different solvent systems and of the following properties:

5α-Androst-1-ene-3,17-dione. On the plate: UV absorption, typical Zimmermann reaction, violet color after spraying with ethanolic sulfuric acid and heating. On eluate: UV maximum at 228–230 nm and IR maxima (CCl_4) at 1735 (17-ketone), 1670 (3-ketone), and 1605 cm^{-1} (C_1-C_2-double bond).

3α-Hydroxy-5α-androst-1-en-17-one. On the plate: no UV absorption, typical Zimmermann reaction, pink color after spraying with ethanolic sulfuric acid changing to blue-green with heating. On eluate: IR maxima (CCl_4) at 3530 (hydroxyl) and 1735 cm^{-1} (17-ketone). Chromic oxidation gave 5α-androst-1-ene-3,17-dione, identified as above.

†Proposed as international non-proprietary name.

17β-Hydroxy-5α-androst-1-en-3-one. On the plate: UV absorption, pale blue color with Zimmermann reagent, violet color after spraying with ethanolic sulfuric acid and heating. On the eluate: UV maximum at 228–230 nm and IR maxima (CCl_4) at 3540 and 3420 (free and associate hydroxyl), 1660 (3-ketone), 1600 cm^{-1} (C_1-C_2-double bond). Chromic oxidation gave 5α-androst-1-ene-3,17-dione, identified as above.

5α-Androst-1-ene-3α,17β-diol. On the plate: no UV absorption, no Zimmermann reaction, pink color after spraying with ethanolic sulfuric acid changing to blue–green with heating. No sufficient material was isolated to allow IR determination. Chromic oxidation gave 5α-androst-1-ene-3,17-dione, identified as above.

In addition to the above metabolites, unidentified compounds giving the Zimmermann reaction were eluted from the thin layer plate together with the group of 11-oxygenated 17-ketosteroids and estimated as the difference over the average pre-treatment values of the latter ones. After administration of compound I the extra-excretion of this fraction significantly exceeded the upper limits of the physiological and analytical variations. This polar fraction was not investigated further.

The excretion values of the exogenous metabolites in the urine of the first 24 h are reported in Table 1. The above metabolites were found almost completely in the glucuronoside fraction.

Taking into account the molecular weight of compound III, the total amount of metabolites detected in urine was 20·8% of the administered dose.

DISCUSSION

After ingestion of 5α-androst-1-ene-3,17-dione (I), and 17β-hydroxy-5α-androst-1-en-3-one (II) the amount of 17β-hydroxy compounds excreted in urine ranged from 14 to 24% of the total metabolites. These figures are significantly higher than those observed after testosterone [5], but also significantly lower than those reported by us after the related 1,4-dienones [1] and 5β-1-enones [2].

Table 1. Excretion of urinary metabolites in the first 24 h after oral administration†

Urinary metabolites‡	Administered compounds I (Subject F.M.)	II (Subject C.F.)	III (Subject F.G.)
Unidentified 17-ketosteroids*	5·2	1·0	1·3
5α-Androst-1-ene-3α,17β-diol	1·8	0·8	0·3
3α-Hydroxy-5α-androst-1-en-17-one	6·1	5·9	4·8
Androsterone§	5·6	6·5	4·5
17β-Hydroxy-5α-androst-1-en-3-one	1·9	4·1	3·2
5α-Androst-1-ene-3,17-dione	5·9	1·7	0·8
Total recovery	26·5	20·0	14·9

†Single doses of 100 mg were administered. All figures are in mg.

‡Only the exogenous metabolites are considered.

*Extra-excretion over the following average pre-treatment values of 11-oxygenated 17-ketosteroids (mg/24 h): 2·26 ± 0·17 (F.M.); 3·06 ± 0·14 (C.F.); 2·44 ± 0·09 (F.G.).

§Extra-excretion over the following average pre-treatment values (mg/24 h): 2·74 ± 0·20 (F.M.); 3·54 ± 0·16 (C.F.); 1·95 ± 0·06 (F.G.).

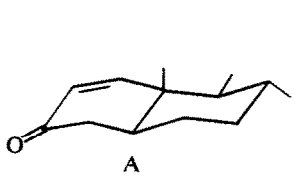
The C_1-C_2 double bond was still present in 66 to 73% of the total amount of identified urinary metabolites. When reduction of 3-ketone occurred, it followed the prevailing physiological steric course to 3α-alcohols. The recovery of the only identified saturated metabolite, androsterone, evaluated disregarding the possible inhibitory effect of the administered compounds on gonadal steroidogenesis, ranged from 21 to 32% of the total excretion.

The urinary metabolites of I and II therefore proved to be markedly different from that of the physiological C_4-C_5 unsaturated analogues. Also in the 5α-series the 1,2 double bond affects the 17-keto/17-alcohol redox potential or the conjugation and the clearance rate of 17β-alcohol. Moreover the double bond largely survived enzymic reduction.

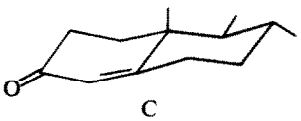
Our results are in disagreement with those of a previous paper by Ungar and Dorfman [6], who reported the recovery of only saturated 17-ketones after oral administration of 5α-androst-1-ene-3,17-dione (I).

The metabolism of 17β-hydroxy-5α-androst-1-en-3-one was studied by Langecker [7] and his findings have been confirmed by us, as to the recovery of substantial amounts of androsterone, unchanged II, and I. However Langecker failed to isolate 3α-hydroxy-5α-androst-1-enes, thus drawing the conclusion that C_1-C_2 double bond strongly inhibits reduction of 3-ketone. This statement is in contrast with the present results as well as with those of our previous papers [1, 2] on 5β-analogues, which shows that the effect of C_1-C_2 double bond on redox and conjugation processes at C_3 gives rise to urinary excretion of 3α-hydroxy-1-enes and 3-oxo-1-enes in comparable amounts.

The differences between the metabolism of 5α-1-en-3-ones and that of the natural compounds are not so striking as observed after 5β-1-en-3-ones or 1,4-dienones, which gave by far a larger excretion of 17β-hydroxy metabolites and a poor, if any, excretion of saturated metabolites. In this connection it is evident from inspection of models that a 5α-1-en-3-one (A) may fit better than the 5β-isomer (B) an enzymic substrate suitable for the physiological 4-en-3-ones (C).



A



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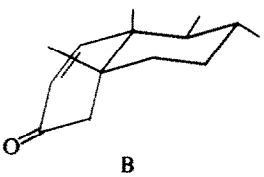

B

Mesabolone (I) gave rise to the same urinary metabolites as the parent 17β-hydroxy-5α-androst-1-en-3-one (II). Moreover the amounts of the single metabolites and the total excretion were comparable with those obtained after II. We may therefore assume that the ether group at C_{17} splits off completely *in vivo*, as could be foreseen on the basis of its behaviour *in vitro*.

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