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METABOLISM OF 1-DEHYDROANDROSTANES IN MAN

II—METABOLISM OF 5β-ANDROST-1-ENES*

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SUMMARY

In order to better understand the results of a previous study on the metabolism of 3-keto-1,4-androstadienes, the excretion of urinary metabolites in healthy subjects after oral administration of 5β-androst-1-ene-3,17-dione (I), 17β-hydroxy-5β-androst-1-en-3-one (II), 3α-hydroxy-5β-androst-1-ene-17-one (III) and 5β-androst-1-ene-3α,17β-diol (IV) have been investigated.

The main features of the metabolism of these compounds were the large survival of C_1–C_2 double bond in the metabolites, reduction to etiocholanolone being relatively important only after IV, and the significant excretion of 17β-hydroxysteroids, chiefly after I and II.

IN THE previous paper[1] we reported the results of our investigations on the metabolism of 17β-hydroxy-1,4-androstadien-3-one (1-dehydrotestosterone) and 1,4-androstadiene-3,17-dione in man. The most interesting feature of the metabolism of these compounds was the high excretion of 17β-hydroxysteroids, strikingly different from that observed after administration of the corresponding 4-ene-3-ones, testosterone and 4-androstene-3,17-dione, which are metabolized to 17β-hydroxyderivatives only to a very small extent[2–5].

In order to further elucidate the role of 1,2 double bond, oxigenated functions at C-3, and of stereochemistry at C-5 in affecting the metabolic transformations at C-17, we have extended our researches to the metabolism of related 1-dehydroandrostane compounds.

This paper will deal with the metabolism of the following 1-dehydro-5β-androstanes, all metabolites of 1-dehydrotestosterone: 5β-androst-1-ene-3,17-dione (I), 17β-hydroxy-5β-androst-1-en-3-one (II), 3α-hydroxy-5β-androst-1-en-17-one (III), and 5β-androst-1-ene-3α,17β-diol (IV).

No data are available in the literature on the metabolism of these compounds in man.

EXPERIMENTAL PROCEDURE

Single oral doses of 100 mg amounts of I, dissolved in sesame oil, and of II, III, and IV, as microcrystals, were given separately to normal adult men. At least two weeks were allowed to elapse between the experiments in the same subject. Urine was collected for the next 24 h.

A 200 ml sample of the urine collection after extraction of free steroids was treated with beef liver β-glucuronidase and thereafter the sulfates were solvolyzed by the method of Burstein and Lieberman[6]. Both glucuronoside and sulfate

*The results of this paper were partially presented at The Third Meeting of the International Study Group for Steroid Hormones (Rome, December 1967). R. Gardi and F. Galletti, *Research on Steroids*, (Editor C. Cassano), North Holland, Amsterdam, (1968) p. 67.

extracts were separated into ketonic and non ketonic fraction by treatment with Girard's P reagent. The non ketonic fraction was further separated by precipitation with digitonin. Each fraction was chromatographed on thin layer according to already described procedures[7], using glass plates coated with layer of either Silica gel G, or neutral Alumina type T, both containing 2% inorganic phosphor. Plates were developed in benzene: ethyl ether 1 : 1 (solvent system A).

Ketonic fractions were chromatographed on alumina. When necessary, eluted fractions were further resolved by a second chromatography on silica gel. Non ketonic fractions were chromatographed on silica gel. When necessary, eluted fractions were oxidized with chromic acid according to Ismail and Harkness[8] and further resolved by a second chromatography on silica gel.

Steroids were detected on the plates by inspection under ultraviolet light and by the following reagent sprays: m-dinitrobenzene and N-benzyltrimethylammonium methoxide (Zimmermann reaction)[9], 75% sulfuric acid in ethanol. Sufficient amounts of metabolites for physical determinations and chemical reactions were obtained by preparative chromatography on 2,000 μ thick layers.

RESULTS

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

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

Each metabolite was identified by comparison with the corresponding reference compound for its chromatographic mobility and for the following properties:

5β-Androst-1-ene-3,17-dione. On chromatogram: U.V. absorption, typical Zimmermann reaction, violet color after spraying with ethanolic sulfuric acid and heating. On eluate: U.V. maximum at 229–231 nm and IR maxima (CCl_4) at 1747 (17-ketone), 1685 (3-ketone), and 1615 cm^{-1} (C_1–C_2-double bond).

17β-Hydroxy-5β-androst-1-en-3-one. On chromatogram: U.V. absorption, pale blue color with Zimmermann reagent, violet color after spraying with ethanolic sulfuric acid and heating. On eluate: U.V. maximum 229–231 mμ and IR maxima (CCl_4) at 3670 and 3500 (free and associate hydroxy), 1690 (3-ketone), 1615 cm^{-1} (C_1–C_2-double bond). Chromic oxidation gave 5β-androst-1-ene-3,17-dione.

3α-Hydroxy-5β-androst-1-en-17-one. On chromatogram: no u.v. 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 3607 (hydroxyl) and 1740 cm^{-1} (17-ketone). Chromic oxidation gave 5β-androst-1-ene-3,17-dione.

5β-Androst-1-ene-3α,17β-diol. On chromatogram: no U.V. absorption, no Zimmermann reaction, pink color after spraying with ethanolic sulfuric acid

changing to blue-green with heating. On eluate: no typical IR spectrum. Chromic oxidation gave 5β-androst-1-ene-3,17-dione.

Excretion of etiocholanolone significantly higher than average basal values was evident only after II, III and IV.

Unidentified compounds showing on thin layer plate the mobility of trioxygenated steroids and giving a typical Zimmermann reaction were recovered after administration of compounds I, II and III. At least one of the unidentified metabolites showed U.V. absorption on thin layer and very likely it retained the 1-en-3-one group.

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 except 5β-androst-1-ene-3,17-dione present mainly as free compound and in trace amount in the sulfate fraction.

DISCUSSION

The total excretion of exogenous steroid metabolites in urine after administration of I, II and III ranged between 34 and 55 per cent of the ingested amount, thus being in the same order of magnitude as the recovery reported after testosterone and 4-androstene-3,17-dione[2, 10, 11] and comparable with that observed by us after 1-dehydrotestosterone and 1,4-androstadiene-3,17-dione[1]. The excretion was much lower after compound IV, likely owing to a poor gastrointestinal absorption. This, however, should not affect the ratios among its individual metabolites.

Etiocholanolone is the only saturated metabolite identified in the urine. It was absent after I but present in substantial amount after II, III and IV, being in the last case about 34 per cent of the total excretion. The saturation of 1,2 double bond takes place at a higher rate on 17β-hydroxysteroids (II and IV) and this might suggest that the 17β-hydroxyl pathway to etiocholanolone is more important in the metabolism of these compounds than in that of testosterone[5, 12].

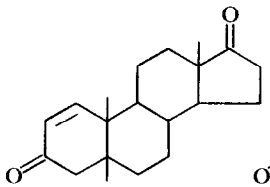
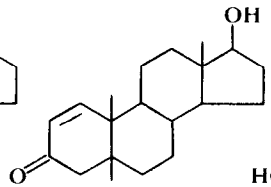
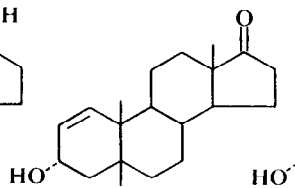
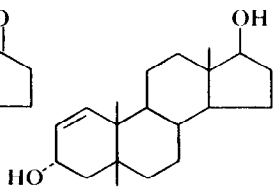
As could be foreseen on the basis of the isolation of substantial amount of 1-dehydro-5β-compounds after administration of 1,4-dien-3-ones, the 1,2 double bond proved to be scarcely prone to reduction by body enzymes also in the absence of the 4,5 double bond and independently from the nature of the oxygenated function at C-3. Thus 3-hydroxy-1-ene group survives largely the metabolic processes.

The excretion of 17β-hydroxy metabolites after I and II was remarkable, ranging from 27 to 43 per cent of the total excretion. It is therefore undoubted that in 5β-series 1-en-3-one group affects the 17-keto/17-alcohol redox potential or the conjugation and clearance rate of 17β-alcohols nearly as the 1,4-dien-3-one.

After III and IV the excretion of 17β-hydroxy metabolites was lower, ranging from 13 to 24 per cent of the total excretion. Moreover a remarkable oxidation of 1-en-3-ol to 1-en-3-one was observed. Therefore it is difficult to state whether the 3α-hydroxy-1-ene group affects *per se* metabolic processes at C-17 or only after its oxidation to 1-en-3-one.

Finally it should be noted that the identification of the until now unidentified more polar metabolites, recovered in substantial amount particularly after I, might partially modify the above deductions.

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

Urinary metabolites†	Administered compounds			
	 I	 II	 III	 IV
Trioxygenated-17-ketosteroids‡§	16·0	5·0	1·4	—
5β-Androst-1-ene-3α,17β-diol	2·2	3·0	0·9	0·3
Etiocholanolone‡	—	4·9	1·9	2·3
3α-Hydroxy-5β-androst-1-en-17-one	11·0	8·4	23·9	2·2
17β-Hydroxy-5β-androst-1-en-3-one	12·9	12·0	3·7	1·3
5β-Androst-1-ene-3,17-dione	12·9	1·3	2·2	0·5
Total recovery	55·0	34·6	34·0	6·6

*Single doses of 100 mg were administered. All figures are in mg.
†Only the exogenous metabolites are considered.
‡Extra excretion over the average basal values.
§Tentative attribution.

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