METABOLISM OF 1-DEHYDROANDROSTANES IN MAN.

I. Metabolism of 17β-hydroxyandrosta-1,4-dien-3-one, 17β-cyclopent-1'-enyloxyandrosta-1,4-dien-3-one (quinbolone) and androsta-1,4-diene-3,17-dione (1)

Ferdinando Galletti and Rinaldo Gardi

Warner-Vister Steroid Research Institute
Casatenovo (Como), Italy

Dedicated to Professor Alberto Ercoli on his 65th Birthday.

Received 3/2/71

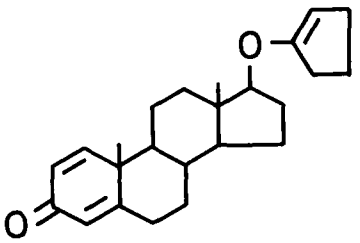
ABSTRACT

The excretion of urinary metabolites in healthy subjects after oral administration of 17β-hydroxyandrosta-1,4-dien-3-one (II) and its 17-cyclopentenyl ether (quinbolone, I), an orally active anabolic agent, has been comparatively investigated. The excretion pattern proved to be very similar for the two compounds, thus confirming the lability in vivo of the ether linkage of I to give II. A similar catabolite excretion has been observed also after ingestion of androsta-1,4-diene-3,17-dione (III).

The Δ^1-double bond has been found to survive metabolism to a great extent. The $\Delta^{1,4}$-dien-3-one group was still unchanged in about 40% of the metabolites excreted and most of the remaining ones were Δ^1-3-keto- or Δ^1-3α-hydroxy-5β-compounds. Moreover, a remarkable amount of 6β-hydroxy-$\Delta^{1,4}$-3-ketones has been identified. The most interesting feature of the metabolism of I, II and III was the high excretion of 17β-hydroxysteroids.

Within a research program on labile ethers of 17β-hydroxysteroids (2-5), 17β-cyclopent-1'-enyloxyandrosta-1,4-dien-3-one (quinbolone, I) was synthetized in our laboratories and found to be an orally effective anabolic agent devoid of hepatotoxic effects (6-8).

The chemical reversibility of the enol ether linkage to give free 17β-hydroxyandrosta-1,4-dien-3-one (1-dehydrotestosterone, II) prompted us to comparatively investigate the metabolism of 1-dehydrotestosterone and quinbolone in human subjects. In order to better understand the results obtained after the administration of the above compounds, our investigation has been extended to the metabolism of androsta-1,4-diene-3,17-dione (androstadienedione, III). Although a lot of work has been devoted to the metabolism of some 1-dehydroandrostanes (9-16), that of 1-dehydrotestosterone and androstadienedione has never been studied.



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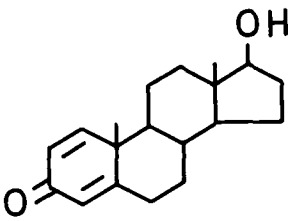
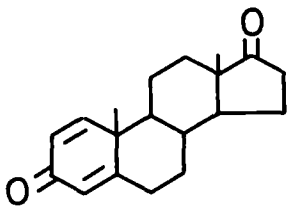


II



III

EXPERIMENTAL PROCEDURE

Single oral doses of 100-mg amounts of quinbolone, 1-dehydrotestosterone and androstadienedione, dissolved in 3 ml sesame oil, were given separately to adult normal men. Two weeks at least were allowed to elapse between the experiments. Urine was collected for the next 24 hours.

Urine samples were extracted with dichloromethane/ethyl ether (40 : 60 by vol.) to separate free steroids. The "glucuronide fraction" was obtained by extraction with the same solvent mixture after incubation at pH 4.5 with beef liver β-glucuronidase. The "sulfate fraction" was obtained after solvolysis according to Burstein and Lieberman (17). Both glucuronide and sulfate extracts were separated into ketonic and non ketonic fractions by treatment with Girard's P reagent (17) in 2% methanolic acetic acid at room temperature (18).

Girard hydrazones were acid hydrolyzed in the presence of dichloromethane with continuous swirling. These conditions were chosen to prevent acid catalyzed epimerization of allylic Δ^1-3α-hydroxyl and other side reactions. In previous experiments carried out according to Pincus and Pearlman (19), only 40% of 3α-hydroxy-5β-androst-1-en-17-one present was recovered unchanged, together with 35% of the 3β-epimer and 25% of acetates or dehydration products. Under the conditions employed the $\Delta^{1,4}$-3-keto group reacts poorly with Girard's P reagent, therefore 1-dehydrotestosterone and 6β,17β-dihydroxyandrosta-1,4-ene-3-one were mainly recovered in the non ketonic fraction.

The non ketonic fractions were treated with digitonin (20) to separate 3β-hydroxy-Δ^5- and 3β-hydroxy-5α-derivatives from other steroids.

The single fractions were chromatographed on thin layer according to already described procedures (21). One dimensional ascending multiple chromatography was carried out using glass plates coated with layer of either Silica gel G (Merck AG, No.7731), or neutral Alumina type T (Merck AG, No.1101), both containing 2% inorganic phosphor (G5 grün/1S, Leuchtstoffwerke, Heidelberg, Germany). The following solvent systems were used.

A	-	Benzene : ethyl ether	1 : 1
CA	-	Chloroform : acetone	9 : 1
D	-	Benzene : ethanol	9 : 1

Ketonic fractions were chromatographed on alumina in system A. When necessary, eluted fractions were further resolved by a second chromatography on silica gel in system CA or D. Non ketonic fractions were chromatographed on silica gel in system CA. When necessary, eluted fractions were further resolved by a second chromatography on silica gel in system A or D.

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) (22), 10% phosphomolybdic acid in ethanol, 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.

Oxidation of steroid alcohols was carried out with chromic acid according to Ismail and Harkness (23). Oxidation products were chromatographed on silica gel in solvent system A.

Ultraviolet absorption spectra were determined on the CF_4 (Optica, Milan) grating spectrophotometer. Infrared absorption spectra were obtained on a Perkin Elmer Model 21 spectrometer. We are indebted to Dr. C. Pedrali for the IR spectra.

Steroids were quantitated by a micro-Zimmermann reaction (23) using dehydroepiandrosterone as a standard. 17-Hydroxy compounds were quantitated after oxidation to 17-ketones, against their own standards similarly treated.

Unknown reference compounds were prepared in our laboratories (24).

RESULTS

After administration of the three compounds the following steroids were identified in both the subjects besides the endogenous metabolites.

Compound No.	Systematic name
II	17β-hydroxyandrosta-1,4-dien-3-one
III	androsta-1,4-diene-3,17-dione
IV	5β-androst-1-ene-3,17-dione
V	17β-hydroxy-5β-androst-1-en-3-one
VI	3α-hydroxy-5β-androst-1-en-17-one
VII	5β-androst-1-ene-3α,17β-diol
VIII	6β-hydroxyandrosta-1,4-diene-3,17-dione
IX	6β,17β-dihydroxyandrosta-1,4-dien-3-one

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

II - 17β-Hydroxyandrosta-1,4-dien-3-one. - On the plate, UV absorption, weak Zimmermann reaction, and orange color after spraying with ethanolic sulfuric acid and heating. On the eluate, UV maximum at 243-244 mμ and IR maxima (CCl_4) at 3675 and 3500 (free and associate hydroxyl), 1670 (3-ketone), 1632 (Δ^4-double bond), and 1607 cm^{-1} (Δ^1-double bond). After chromic oxidation it gave III, identified as below.

III - Androsta-1,4-diene-3,17-dione. - On the plate, UV absorption, typical Zimmermann reaction, and salmon pink color after spraying with ethanolic sulfuric acid and heating. On the eluate, UV maximum at 242-243 mμ and IR maxima ($CHCl_3$) at 1740 (17-ketone), 1662 (3-ketone), 1624 (Δ^4-double bond), and 1606 cm^{-1} (Δ^1-double bond).

IV - 5β-Androst-1-ene-3,17-dione. - On the plate, UV absorption, typical Zimmermann reaction, and violet color after spraying with ethanolic sulfuric acid and heating. On the eluate, UV maximum at 229-231 mμ and IR maxima (CCl_4) at 1748 (17-ketone), 1685 (3-ketone), and 1615 cm^{-1} (Δ^1-double bond).

V - 17β-Hydroxy-5β-androst-1-en-3-one. - On the plate, UV absorption, pale blue color with Zimmermann reagent, and violet color after spraying with ethanolic sulfuric acid and heating. On the eluate, UV maximum at 229-231 mμ and IR maxima (CCl_4) at 3670 and 3500 (free and associate hydroxyl), 1690 (3-ketone), 1615 cm^{-1} (Δ^1-double bond). After chromic oxidation it gave IV, identified as above.

VI - 3α-Hydroxy-5β-androst-1-en-17-one. - On the plate, no UV absorption, typical Zimmermann reaction, and pink color after spraying with ethanolic sulfuric acid changing to blue-gree with heating. On the eluate, IR maxima (CCl_4) at 3608 (hydroxyl) and 1740 cm^{-1} (17-ketone). No maximum was observed in the double bond region. The reference compound gave a maximum at 1650 cm^{-1} in nujol, but this maximum could be hardly detected in CCl_4. After chromic oxidation the eluted material gave IV, identified as above.

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

VIII - 6β-Hydroxyandrosta-1,4-diene-3,17-dione. - On the plate, UV absorption, typical Zimmermann reaction, and brown color after spraying with ethanolic sulfuric acid and heating. On the eluate, IR maxima ($CHCl_3$) at 3600 (hydroxyl), 1730 (17-ketone), 1662 (3-ketone), 1620 (Δ^4-double bond) and 1602 cm^{-1} (Δ^1-double bond). The eluate gave no typical UV spectrum, unlike the reference compound. After chromic oxidation it gave a product identified as androsta-1,4-diene-3,6,17-trione, by comparison with the corresponding reference compound for its chromatographic mobility and for the following properties: on the plate, UV absorption, typical Zimmermann reaction, and yellow color after spraying with ethanolic sulfuric acid and heating; on the eluate, UV maximum at 249-250 mμ and IR maxima ($CHCl_3$) at 1728 (17-ketone), 1695 (6-ketone), 1655 (3-ketone), 1620 (Δ^4-double bond) and 1600 cm^{-1} (Δ^1-double bond).

IX - 6β,17β-Dihydroxyandrosta-1,4-dien-3-one. On the plate, UV absorption, weak Zimmermann reaction, and brown color after spraying with ethanolic sulfuric acid and heating. On the eluate, IR maxima ($CHCl_3$) at 3600 (hydroxyl), 1660 (3-ketone), 1618 (Δ^4-double bond) and a shoulder at 1600 cm^{-1} (Δ^1-double bond). The eluate gave no typical UV spectrum, unlike the reference compound. After chromic oxidation it gave androsta-1,4-diene-3,6,17-trione identified as above.

Compounds II, VI and VII were found almost completely in the glucuronide fraction. Compounds III, IV, VIII and IX were present in substantial amount in the free, glucuronide and sulfate fractions. The amounts of each compound in various fractions have been summed and the resulting figure has been reported in Table I.

Table I shows the excretion values of the exogenous metabolites in the urine of the first 24-hours.

The percentage of metabolites excreted after quinbolone (m. wt. 352.2), calculated on the corresponding molar amount of 1-dehydrotestosterone (m.wt.286.4) was 47.6% and 49.2% for subjects FG and FM, respectively.

TABLE I. - EXCRETION OF URINARY METABOLITES IN THE FIRST 24 HOURS AFTER ORAL ADMINISTRATION *

Urinary metabolites**	Subject F.G.			Subject F.M.		
	After 1-dehydro testosterone	After quinbolone	After androsta diendione	After 1-dehydro testosterone	After quinbolone	After androsta diendione
6β, 17β-Dihydroxyandrosta-1, 4-dien-3-one	1.0	2.4	3.7	3.7	2.2	2.8
6β-Hydroxyandrosta-1, 4-diene-3, 17-dione	2.4	3.1	5.2	5.2	4.3	12.3
Other trioxygenated 17-ketosteroids §	0.5	0.9	2.4	1.0	1.9	4.9
5β-Androst-1-ene-3α, 17β-diol	0.9	1.3	0.9	0.6	0.5	1.2
17β-Hydroxyandrosta-1, 4-dien-3-one	9.2	10.1	7.1	9.5	7.8	11.1
Etiocholanolone §	0.7	1.4	0.2	2.4	1.8	0.9
3α-Hydroxy-5β-androst-1-en-17-one	11.2	11.5	7.3	11.6	11.8	9.3
17β-Hydroxy-5β-androst-1-en-3-one	10.5	6.5	7.6	7.7	7.9	7.4
Androsta-1, 4-diene-3, 17-dione	--	--	0.2	--	0.4	1.0
5β-Androst-1-ene-3, 17-dione	1.2	1.5	2.1	1.3	1.4	2.5
Total:	37.1	38.7	36.7	43.0	40.0	53.4

* 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.

DISCUSSION

After ingestion of 1-dehydrotestosterone (II) and its 17-cyclopentenyl ether (quinbolone, I) the same urinary metabolites were identified. The amounts of the individual metabolites and of the total excretion were roughly similar, the differences observed in the same subject after administration of the two compounds being not higher than those observed after administration of the same compound to different subjects. Evidently the ether group at C-17 splits off entirely and rapidly in the human body just as in vitro under suitable conditions.

As to 1-dehydrotestosterone and androstadienedione, the total amount of metabolites recovered in the 24-hour urine after ingestion ranged from 37 to 53% of the administered dose. This recovery rate is not much lower than that observed after orally given testosterone and androst-4-ene-3,17-dione (25-27).

The excretion of etiocholanolone over the average basal values in most instances was beyond the experimental error. Even disregarding the possible inhibition of gonadal steroidogenesis by the administered compounds, a fraction of the excreted etiocholanolone should reasonably be of exogenous source.

Except for this small amount of etiocholanolone, all the identified metabolites were unsaturated compounds.

The presence of a large amount of unreduced ring A compounds is in agreement with the results obtained after administration of other $\Delta^{1,4}$-3-ketosteroids such as prednisone, prednisolone (28,29) and 1-dehydrotestololactone (10) and is due to the limited susceptibility of the dienone group to reduction by body enzymes. The persistence in the body of the conjugated ketone accounts for the formation of

remarkable amounts of 6β-hydroxy derivatives. 6β-Hydroxy-$\Delta^{1,4}$-3-ketones were also found as main metabolites of 17β-hydroxy-17α-methylandrosta-1,4-dien-3-one (11), triamcinolone (30) and betamethasone (31).

The olefinic bond in 1,2 position survives nearly completely the metabolic processes. However, unlike Δ^4-3-ketones, reduction by 3-keto-reductase is only partially hindered by the resonance energy of the conjugated Δ^1-3-ketone. As a result, both 5β-Δ^1-3-ketones and 5β-Δ^1-3α-alcohols are present in the urine in substantial amounts.

Similar results were observed in the metabolism of other $\Delta^{1,4}$-steroids (10,29). The selective and stereospecific reduction of 4,5 double bond to 5β-compounds is also in accordance with the chemical behaviour of $\Delta^{1,4}$-dienones (24, 32).

The most interesting feature of the metabolism of 1-dehydrotestosterone and androstadienedione is the large excretion of 17β-hydroxysteroids, mainly 1-dehydrotestosterone and 17β-hydroxy-5β-androst-1-en-3-one. After testosterone and androst-4-ene-3,17-dione the 17-hydroxy metabolites are excreted in small quantities (11, 33, 34).

There is no evidence to state whether the effect of the dienone group in ring A on the metabolic reactions at C-17 is due to a modification of the redox potential 17-keto/17-alcohol, or to an increase of the conjugation and clearance rate of the 17β-alcohols.

Inhibition of dehydrogenation of the 17β-alcohol has been observed also after administration of 17β-hydroxy-5α-androst-1-en-3-one and its 1-methylated homologue (12).

As to the conjugation form of the metabolites, the presence of 6β,17β-dihydroxyandrosta-1,4-dien-3-one and 6β-hydroxyandrosta-1,4-diene-3,17-dione in the free fraction and the presence of 5β-androst-1-ene-3,17-dione and androstadienedione in the conjugate fraction deserve further investigation. Evidences of conjugation of non-hydroxylated steroids have been already reported (14, 35, 36).

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