The Isolation of a Δ^5 -androstene-3 (β), 16, 17-triol from the Urine of Normal Human Males and Females

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In 1934 one of the authors isolated from the neutral ether-soluble fraction of acid-hydrolyzed human pregnancy urine a new solid alcohol melting at c. 263° . The alcoholic nature of the compound was demonstrated by the preparation of an acetate, but owing to the small amount available, little could be done to characterize it further. Subsequently, a systematic search for this new compound revealed its presence in small amounts in nearly all specimens of urine from normal men and normal non-pregnant women which were examined. Although the average yield from such urines was only about 0.1 mg./l., sufficient material was accumulated to permit a re-investigation of its nature.

The new substance was very sparingly soluble in benzene, ethyl acetate, light petroleum and chloroform; rather more soluble in acetone, methanol and ethanol, and still more soluble in dioxan and glacial acetic acid. The analytical specimen was obtained in fine white needles melting at 262 – 263° after one crystallization from methanol-benzene and two from methanol. Tests for halogen, nitrogen, sulphur and phosphorus were negative. The possible steroid nature of the substance was indicated by a positive Liebermann-Burchard reaction in which the chloroform layer showed a transient purple colour. Tests with tetranitromethane and with the Rosenmund-Kuhnnehn bromine reagent showed that it was unsaturated.

An acetate, m.p. 189 – 191° , a chloroacetate, m.p. 161 – 163° , a hydrogenation product, m.p. 257 – 5 – 259°

and an acetate and chloroacetate of the latter melting at 174 – 176° and 180 – 182° respectively were prepared. Analyses of these derivatives gave figures, however, which could not be reconciled with any of the possible multiples of the empirical formula the analysis of the original compound seemed to indicate.

On treatment of the compound with lead tetraacetate, an oxidation product, m.p. 199 – 201° , was obtained in small yield. This indicated that the compound might have two hydroxyl groups on adjacent carbon atoms, but again the analysis of the product could not be reconciled with any of the formulae which were then being considered for the original compound.

It was hoped that further amounts of the compound could be prepared so that a fuller investigation of its nature could be made. However, owing to other claims on the time of the authors during the past few years this has not been possible.

The reinvestigation of this compound now no longer seems to be necessary, since it appears to be identical with a steroid which has recently been isolated from the urine of a boy with an adenocarcinoma of the adrenal cortex by Hirschmann (1943) and satisfactorily characterized as a Δ^5 -androstene-3 (β), 16, 17-triol. In Table 1 the analytical figures and melting-points for the compound isolated by the present authors and those of certain of its derivatives are compared with the corresponding figures for Hirschmann's androstenetriol and its derivatives.

Table 1. Comparison of the steroid isolated from the urines of normal men and women (X) with Hirschmann's Δ^5 -androstene-3 (β), 16, 17-triol

	X			Δ^5 -Androstene-3 (β), 16, 17-triol						
	m.p.	% C	% H		m.p.	% C	% H	Calc. for	% C	% H
X	262–263°	73.05	9.65	Androstenetriol	265–270°	74.54	9.87	$C_{19}H_{30}O_3$	74.47	9.87
				Androstenetriol from hydrolysis of acetate and monoacetate	—	72.72	9.95	$C_{19}H_{30}O_3 + \frac{1}{2}CH_3OH$	72.63	10.00
					268–271°	72.97	10.35			
				266–270°	72.78	10.06				
X acetate	189–191°	69.43	8.33	Androstenetriol triacetate	187–188.5°	69.23	8.43	$C_{25}H_{36}O_6$	69.42	8.39
Hydrogenated X	257.5–259°	72.44	10.20	Androstanetriol	256–260°	73.92	10.38	$C_{19}H_{32}O_3$	73.98	10.46
								$C_{19}H_{32}O_3 + \frac{1}{2}CH_3OH$	72.16	10.57
Hydrogenated X acetate	174–176°	68.82	9.15	Androstanetriol triacetate	175.5–176.5°	68.44	8.91	$C_{25}H_{36}O_6$	69.09	8.81
						68.99	8.27			

The melting-points agree so closely in the case of the acetate, the hydrogenation product, and the acetate of the latter, that the identity of the two series of compounds with one another can hardly be doubted. Furthermore the melting-points of the acetate of the compound and of the acetate of its hydrogenation product were not depressed by admixture with androstenetriol triacetate and androstanetriol triacetate respectively, specimens of which were most kindly supplied by Dr Hirschmann. The melting-point recorded for the androstenetriol itself is, however, appreciably higher than any observed by the present authors for their product, which would seem to indicate that the latter was never obtained in a state of complete purity. This is hardly surprising since at no time were large enough amounts of the substance on hand to allow of a really rigorous purification.

The carbon and hydrogen contents of the acetate and of the acetate of the hydrogenated substance agree excellently with the theoretical figures for the acetates of an androstenetriol and of an androstanetriol respectively. In the cases of the original steroid and its hydrogenation product, the agreement with the theoretical figures for an androstenetriol and an androstanetriol is less satisfactory. These discrepancies, however, can be explained in a not unreasonable manner. By saponification of the diacetate and monoacetate of the triol, Hirschmann obtained products which showed the same melting-point as that of the original substance and whose identity with the latter was clearly shown by mixed melting-points and by reacetylation to the triacetate. However, these products gave analytical figures which suggested that half a molecule of methanol of crystallization had been retained even though they were dried *in vacuo* at temperatures as high as 135°. While it was not made clear why the androstenetriol prepared by saponification of the acetates should retain methanol of crystallization while that isolated directly from urine did not, Hirschmann's figures suggest that this was the case with the particular samples which were analyzed. It seems reasonable to assume, therefore, that the discrepancies in the carbon and hydrogen figures observed by the present authors, which are referred to above, might be explained in a similar manner by supposing that methanol of crystallization was retained by the substance itself and by its hydrogenation product.

Hirschmann has raised the interesting possibility that this androstenetriol may be formed from dehydroisoandrosterone by a metabolic reaction similar to that by which oestrone is converted to oestriol in the body (Pearlman & Pincus, 1943). The demonstration that the same androstenetriol is present in the urines of normal men and women, in both of which dehydroisoandrosterone is known to

occur (Butenandt & Tscherning, 1934; Callow & Callow, 1939), lends indirect support to the theory that the latter may be the precursor of the triol. What is more important, however, is that it is now clearly shown that the metabolic path by which the androstenetriol is formed is a normal one.

EXPERIMENTAL

Isolation of Δ^5 -androsterone-3 (β), 16, 17-triol from urine. The urine was collected in toluene, evaporated under reduced pressure to one-tenth of its original volume, and after acidification to pH 1.5 with HCl it was heated for 2 hr. in a boiling water-bath. The concentrated hydrolyzed urine was extracted four times with one-quarter volumes of ether, and the ethereal extract after washing thoroughly with 5% Na_2CO_3 solution, 0.1N-NaOH solution, and water, was evaporated to dryness.

The subsequent steps by which the crude triol was isolated from the neutral ether-soluble fraction and by which it was purified were varied considerably from batch to batch, depending on the amount of triol present and the degree of contamination with impurities. In some cases good yields of crude material were deposited on chilling a benzene solution of the neutral fraction. It was found in other cases to be advantageous to give the neutral fraction a preliminary leaching with light petroleum to remove waxy impurities which seemed to interfere with the crystallization from benzene. A preliminary saponification of the neutral fraction with 5% ethanolic KOH and subsequent ether extraction of the non-saponifiable material was also found to be useful as a preliminary to the benzene crystallization. With certain batches of urine good yields of crude crystalline triol were obtained by dissolving the neutral fraction in a small volume of ethyl acetate, adding light petroleum until a faint cloudiness appeared, and then chilling for several days.

Purification of the crude crystalline material obtained in these ways could usually be effected by washing with ice-cold chloroform and crystallizing from ethyl acetate-methanol or benzene-methanol. The melting-points of preparations purified by one such crystallization were usually in the range 255–260°. In some cases in which crystallization was difficult, the crude material was acetylated, and the crude acetates sublimed at 165° in a high vacuum and subsequently saponified. The material so obtained was found to crystallize readily from ethyl acetate-methanol or benzene-methanol.

In Table 2 are shown the yields and melting-points of the once-crystallized material obtained from the urines of five non-pregnant women and of four men. Further quantities were obtained for chemical examination from large volumes of mixed urines from men.

A sample for analysis, m.p. 262–263°, was obtained by recrystallizing the combined crystals from several batches once from benzene-methanol, and twice from methanol.

4.490 mg. (dried at 80° *in vacuo* over P_2O_5) gave 12.020 mg. CO_2 and 3.870 mg. H_2O . (Found: C, 73.05; H, 9.65%. Calc. for $\text{C}_{19}\text{H}_{30}\text{O}_3 \cdot \frac{1}{2}\text{CH}_3\text{OH}$: C, 72.63; H, 10.00%.)

$[\alpha]_{\text{D}}^{25} = -88.5^\circ$ (0.56% in dioxan); $[\alpha]_{\text{D}}^{26} = -75.7^\circ$ (0.63% in 90% acetic acid).

Determinations of the iodine value by the method of Rosenmund & Kuhnnehn on 4.027 and 4.701 mg. gave values of 57.3 and 65.8 respectively. The calculated iodine value for $\text{C}_{19}\text{H}_{30}\text{O}_3 \cdot \frac{1}{2}\text{CH}_3\text{OH}$ (1⁷), is 78.6.

Table 2. *Yields of once-crystallized androstenetriol from urines of non-pregnant women and of men*

	Vol. of urine (l.)	Yield of crystals (mg.)	m.p. of crystals
Female	96.5	60.0	255-260°
	149.8	45.5	258-261°
	52.9	9.6	252-258°
	20.3	16.6	255-262°
	31.3	4.4	259-263°
Male	54.3	6.5	258-261°
	53.0	3.4	260-262°
	49.6	3.0	258-260°
	48.4	7.0	255-260°

Δ^5 -Androstene-3-(β), 16, 17-triol triacetate. 20 mg. androstenetriol (m.p. 258-260°) were heated at 100° for 2 hr. with 2 ml. of acetic anhydride and one drop of pyridine. After decomposition of the excess acetic anhydride with ice and water, the crude acetate was filtered off, washed, dried, and crystallized from ether-hexane. Hexagonal plates, m.p. 189-191°, were obtained.

3.621 mg. (dried at 80° *in vacuo* over P_2O_5) gave 9.200 mg. CO_2 and 2.690 mg. H_2O . (Found: C, 69.43; H, 8.33%. Calc. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39%.)

Δ^5 -Androstene-3 (β), 16, 17-triol tri(chloroacetate). 19.7 mg. of androstenetriol (m.p. 260-263°) were treated at 100° for 2 hr. with 4 ml. of chloroacetyl chloride. The excess chloroacetyl chloride was decomposed with cold water and the crude tri(chloroacetate) filtered off, washed with water and dried. Three crystallizations from 75% aqueous methanol yielded plates melting at 161-163°.

4.545 mg. (dried at 80° *in vacuo* over P_2O_5) gave 9.300 mg. CO_2 and 2.530 mg. H_2O . (Found: C, 55.83; H, 6.23%. Calc. for $C_{25}H_{35}O_6Cl_3$: C, 56.52; H, 6.14%.)

Δ^5 -Androstane-3 (β), 16, 17-triol. Hydrogenation of the androstenetriol was carried out in glacial acetic acid solution with electrolytic hydrogen and a platinum oxide catalyst. Five experiments on batches of weight varying between 10 and 17 mg. were carried out. In every case the measured uptake of hydrogen was about 150% of that required for the saturation of a single double bond in a substance of the formula $C_{19}H_{30}O_3 \cdot \frac{1}{2}CH_3OH$. Little significance is attached to this discrepancy, however, since the hydrogenation apparatus used was not well suited for accurate quantitative work.

After removal of the catalyst by filtration, the solution of hydrogenated material was evaporated to dryness under reduced pressure and the residue crystallized from benzene-methanol. After three crystallizations crystals melting sharply at 257.5-259° were obtained. An iodine value determination showed that the product was saturated.

3.556 mg. (dried at 80° *in vacuo* over P_2O_5) gave 9.445 mg. CO_2 and 3.240 mg. H_2O . (Found: C, 72.44; H, 10.20%. Calc. for $C_{19}H_{32}O_3 \cdot \frac{1}{2}CH_3OH$: C, 72.16; H, 10.57%.)

$[\alpha]_{D}^{25} = 0.0^\circ$ (0.50 and 0.52% in methanol).

The melting-point of the androstanetriol after storage

for long periods in the dry state became lower and less sharp. When such material was wetted and rapidly redried, the melting-point returned to its original value. This peculiar change in the melting-point after long storage could not be duplicated by drying at 80° in a high vacuum over P_2O_5 , sublimation at 155° and 0.05 mm. or by heating at 100° for 12 hr. in the presence of oxygen.

Δ^5 -Androstane-3 (β), 16, 17-triol triacetate. 13.2 mg. of the androstanetriol were acetylated in the manner previously described. The product, after crystallization once from 50% aqueous methanol and once from 50% aqueous acetone, melted at 174-176°.

3.057 mg. (dried at 80° *in vacuo* over P_2O_5) gave 7.715 mg. CO_2 and 2.500 mg. H_2O . (Found: C, 68.82; H, 9.15%. Calc. for $C_{25}H_{38}O_6$: C, 68.44; H, 8.91%.)

Δ^5 -Androstane-3 (β), 16, 17-triol tri(chloroacetate). A chloroacetylation was carried out with 15 mg. of the androstanetriol in the same way as for the androstenetriol. The crude product crystallized from 75% aqueous methanol in plates melting at 180-182°.

2.057 mg. (dried at 80° *in vacuo* over P_2O_5) gave 4.150 mg. CO_2 and 1.130 mg. H_2O . 5.423 mg. (dried as before) gave 3.975 mg. $AgCl$. (Found: C, 55.05; H, 6.15; Cl, 18.12%. Calc. for $C_{25}H_{35}O_6Cl_3$: C, 56.31; H, 6.48; Cl, 19.57%.)

Oxidation of Δ^5 -androstene-3 (β), 16, 17-triol with lead tetra-acetate. Two experiments were carried out in which 17.0 and 17.8 mg. amounts of somewhat impure androstenetriol were each allowed to react at room temperature for 20 hr. with 5 ml. of an 0.1N solution of lead tetra-acetate in glacial acetic acid. The reaction mixtures, after heavy dilution with water, were extracted with ether, and the extracts, after thorough washing with 5% Na_2CO_3 solution and with water, were evaporated to dryness. The residues on sublimation at 160-170° and 0.1 mm. yielded in each case a mixture of a small quantity of white needles and a larger quantity of glassy material. A total of only 3.5 mg. of the needles (m.p. 199-201°) was obtained.

2.074 mg. (dried at 80° *in vacuo* over P_2O_5) gave 5.720 mg. CO_2 and 1.805 mg. H_2O . (Found: C, 75.21; H, 9.74%. Calc. for $C_{19}H_{28}O_3$: C, 74.95; H, 9.27%.)

SUMMARY

1. A substance isolated from the urines of normal men and women in amounts of about 0.1 mg./l. has been identified with the Δ^5 -androstene-3 (β), 16, 17-triol which has been isolated in large amounts by Hirschmann from the urine of a boy with an adenocarcinoma of the adrenal cortex.

2. This finding indicates that the androstenetriol is a normal steroid metabolic product.

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