The Isolation of 3β -Hydroxy- Δ^5 -Steroids from the Urine of Normal Men

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Dehydroepiandrosterone (3β -hydroxyandrost-5-en-17-one) was the first steroid containing the 5-en- 3β -ol group to be isolated from urine (Butenandt & Dannenbaum, 1934). During the succeeding twenty-two years only one other steroid of this type, namely androst-5-ene- 3β :16 α :17 β -triol (Marrian & Butler, 1944), was isolated from the urine of normal subjects, although a number of steroids of this type were isolated from the urine of patients with an adrenal cortical tumour. More recently, Fotherby, Colas, Atherden & Marrian (1957) obtained from extracts of the urine of normal males a previously undescribed substance which was characterized as 16 α -hydroxydehydroepiandrosterone (3β :16 α -dihydroxyandrost-5-en-17-one). This compound was liberated by boiling the urine without adjustment of the pH, a procedure which appears to be specific for the hydrolysis of the 3β -sulphates of 3β -hydroxy- Δ^5 -steroids (cf. Roy, 1956). By use of this method the extracts obtained were less contaminated with non-steroid material or with other steroids than were extracts of urine hydrolysed in other ways and the transformations which the 3β -hydroxy- Δ^5 -steroids undergo during acid hydrolysis were avoided. By analogy with dehydroepiandrosterone, which appears to be present in urine as the 3β -sulphate and 16 α -hydroxydehydroepiandrosterone, it seemed likely that other 3β -hydroxy- Δ^5 -steroids (if present in urine) would also be conjugated with sulphuric acid and should therefore be present in extracts of boiled urine. Accordingly, a search for 3β -hydroxy- Δ^5 -steroids in the urine of normal males was initiated.

RESULTS

Isolation of crystalline compounds from urine extracts

In the present work a large volume of urine from normal males was hydrolysed by boiling for 2 hr. without adjustment of pH. It would appear from the work of Dingemanse, Huis in't Veld & Hartogh-Katz (1952), Teich, Rogers, Lieberman, Engel & Davis (1953) and Fotherby *et al.* (1957) that the major reaction product resulting from boiling a solution of the sulphate of a 3β -hydroxy- Δ^5 -

steroid at neutral pH is the 3:5-*cyclo*-6 β -hydroxy steroid accompanied by a small amount of the 3β -hydroxy- Δ^5 -steroid. For this reason the residue obtained by evaporation of chloroform extracts of boiled urine was dissolved in aqueous ethanol and the solution acidified to pH 1 in order to convert the 3:5-*cyclo*-6 β -hydroxy steroids into 3β -hydroxy- Δ^5 -steroids. The concentrate thus obtained was separated into a non-ketonic fraction and a ketonic fraction by means of the Girard reaction carried out at room temperature and each fraction subjected to countercurrent distribution.

Examination of the non-ketonic fraction

This fraction was first distributed for 200 cycles by using the solvent system ethyl acetate-*cyclo*-hexane-ethanol-water (2:8:3:7, by vol.) described by Engel, Alexander, Carter, Elliott & Webster (1954). The modified Pettenkofer reaction described by Munson, Jones, McCall & Gallagher (1948), which has been shown to be fairly specific for 3β -hydroxy- Δ^5 -steroids, has been used throughout the examination of the non-ketonic fraction to follow the degree of resolution obtained during the countercurrent distributions and chromatographic separations. This preliminary distribution separated the non-ketonic fraction into a less 'polar' fraction containing those substances with a partition coefficient greater than one and a more 'polar' fraction containing those substances with a partition coefficient less than one. The less 'polar' fraction after distribution in a different solvent system for 300 cycles was almost completely separated into two components. The faster-moving component was crystallized from methanol and shown to be identical with androst-5-ene- 3β :17 β -diol. The slower-moving component, also after crystallization from methanol, melted at 213–217°, which suggested that the compound might be pregn-5-ene- 3β :17 α :20 α -triol; this suggestion was proved to be correct by comparison of the acetate of the isolated compound with authentic 3β :20 α -diacetoxy pregn-5-en-17 α -ol.

Countercurrent distribution of the more 'polar' non-ketonic fraction for 100 cycles with the solvent system *cyclo*hexane-ethyl acetate-ethanol-water (80:20:45:45, by vol.) gave a distribution pattern which suggested the presence of at least two components. By recycling the upper phases for a

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further 400 transfers, these two components were completely separated and the slower-moving component shown to be androst-5-ene- 3β : 16α : 17β -triol. After chromatography of the faster-moving component on a magnesium silicate-Celite column to remove interfering pigments, and crystallization from methanol, 9 mg. of material was obtained which melted at $259-262^\circ$ and did not depress the melting point of authentic androst-5-ene- 3β : 16β : 17β -triol. A mixture of the isolated compound and androst-5-ene- 3β : 16α : 17β -triol melted at $235-240^\circ$. The melting point of the acetate of the isolated compound was not depressed by admixture with authentic 3β : 16β : 17β -triacetoxandrost-5-ene and the spectra of solutions of the acetates in concentrated sulphuric acid were identical.

Examination of the ketonic fraction

This was subjected to countercurrent distribution for 180 cycles in the solvent system ethyl acetate-cyclohexane-ethanol-water (2:8:3:7, by vol.) and alternate fractions were analysed for 3β -hydroxy- Δ^5 -steroids by the modified Pettenkofer reaction, for oxo steroids by the Zimmermann reaction and for α -ketolic compounds by the 'blue tetrazolium' reaction. The distribution pattern showed the presence of two peaks giving a positive Pettenkofer reaction. The faster-moving of these was shown to be dehydroepiandrosterone. The slower-moving peak was a mixture of at least two compounds: one reacted positively in the blue-tetrazolium reaction and negatively in the Zimmermann reaction and had a partition coefficient of 0.52; the second component reacted negatively in the blue-tetrazolium reaction and positively in the Zimmermann reaction and had a partition coefficient of 0.65. By partition chromatography on a Celite column the blue-tetrazolium-reactive material was further separated from the material giving a positive Zimmermann reaction and shown to be identical with 16α -hydroxydehydroepiandrosterone. Owing to lack of material the compound reacting positively in the Zimmermann test has not yet been identified. However, if the compound is a steroid it is possible that it has not previously been isolated from urine, as its melting point ($219-220^\circ$) does not correspond with that of any 3β -hydroxy- Δ^5 -steroid so far isolated from natural sources.

EXPERIMENTAL

Methods

Modified Pettenkofer reaction. This was performed as described by Munson *et al.* (1948), except that only 0.2 ml. of acetic acid, 0.8 ml. of furfuraldehyde soln. and 3 ml. of H_2SO_4 were used.

Blue-tetrazolium reaction. The reaction described by Mader & Buck (1952) was slightly modified. To the dry

residue to be analysed were added 0.25 ml. of 1% (w/v) tetramethylammonium hydroxide in 95% ethanol and 0.35 ml. of 0.07% (w/v) blue tetrazolium [3:3'-dianisolebis-4:4'-(3:5-diphenyl)tetrazolium chloride] in 95% ethanol. After incubation for 1 hr. at 25° in the dark the mixture was diluted with 4 ml. of 1% (v/v) acetic acid in 50% ethanol and the optical density of the soln. measured at $510 m\mu$.

Zimmermann reaction. A scaled-down version of the reaction described by Callow, Callow & Emmens (1938) has been used. To the material to be analysed 0.24 ml. of a mixture of 2 vol. of 1% (w/v) *m*-dinitrobenzene in ethanol and 1 vol. of 2.5% (w/v) potassium hydroxide in ethanol were added. After 1 hr. at 25° the soln. was diluted with 4 ml. of ethanol and the optical densities were measured in a Unicam spectrophotometer SP. 600 at 440, 490, 520, 540 and 600 $m\mu$. The corrected optical density at 520 $m\mu$ was calculated from the readings at 440, 520 and 600 $m\mu$ as recommended by Allen (1950), and indicated the presence of 17-oxo steroids. Similarly, the corrected optical density at 490 $m\mu$ (the wavelength at which 20-oxo steroids show maximum absorption) was calculated from the readings at 440, 490 and 540 $m\mu$.

Spectra of solutions of compounds in concentrated sulphuric acid. The method described by Zaffaroni (1950) has been used. Between 70 and 100 μg . of steroid was dissolved in 4 ml. of conc. H_2SO_4 and after the soln. had stood for 2 hr. at 25° the optical density over the wavelength range 220-600 $m\mu$ was determined. The figures recorded below in the sections describing the isolation of steroids from urine show the wavelengths of the maxima and minima in the absorption spectra, the $E_{1\%}^{1\text{cm}}$ value at these wavelengths being given in parentheses.

Miscellaneous. Melting points were determined on a microscope hot-stage and are uncorrected for emergent stem.

Samples for C and H analysis and for optical-rotation measurements were dried to constant weight at 80° *in vacuo* over P_2O_5 .

The solvents and Celite (Celite 535, Johns-Manville Co. Ltd., London) used were purified by the methods employed by Watson & Marrian (1956). Magnesium trisilicate (British Drug Houses Ltd.) was activated by drying overnight at 110° . Celite columns for partition chromatography were prepared as described by Bauld (1955).

For the countercurrent distributions a 100-tube all-glass fully automatic apparatus of the type described by Craig, Hausmann, Ahrens & Harfenist (1951) was used. Upper- and lower-phase volumes each of 40 ml. have been used throughout the investigation.

Preparation of urine extracts for countercurrent distribution

Urine from normal men was collected in batches of 20 l. without preservative and concentrated *in vacuo* to approx. 2 l. The concentrated urine was boiled for 2 hr. without adjustment of pH and extracted twice with an equal vol. of $CHCl_3$. The combined $CHCl_3$ extracts were washed twice with 0.2 vol. of 0.1N-NaOH and twice with 0.2 vol. of water, dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue thus obtained was dissolved in 28 ml. of methanol, the soln. diluted with 12 ml. of water and extracted once with 40 ml. of hexane. The hexane extract was back-extracted with 40 ml. of 70% (v/v) aqueous methanol and the combined aqueous

methanol phases were evaporated under reduced pressure to about 20 ml. After dilution to about 60 ml. with water the aqueous solution was extracted twice with 100 ml. of ether, the combined ether extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness.

The residues were stored in the dry state at -20° until 250 l. of urine had been processed and the combined residues then separated into a non-ketonic fraction and a ketonic fraction by use of Girard's reagent T according to the procedure described by Fotherby *et al.* (1957). Each fraction was then dissolved in 200 ml. of a water-ethanol (9:1, v/v) mixture and acidified to pH 1 with 5 N- H_2SO_4 and after 1 hr. the mixture was extracted twice with 2 vol. of freshly distilled ether. The ether extracts were washed twice with 0.2 vol. of 0.1 N- NaOH and twice with 0.2 vol. of water, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residues representing the ketonic and non-ketonic fractions were stored at -20° in the dry state until 2000 l. of urine had been processed.

Examination of the non-ketonic fraction

The dry residue of the non-ketonic fraction (2.5 g.) was distributed by means of the solvent system cyclohexane-ethyl acetate-ethanol-water (8:2:3:7, by vol.). After completion of the fundamental procedure 100 fractions were collected by single withdrawal. Because of a mechanical fault it was necessary to combine the material in tubes 0-90 of the apparatus (the more 'polar' fraction) and to combine the material in tubes 91-99 with those fractions collected by single withdrawal (the less 'polar' fraction). Analysis of tubes 0-100 of the apparatus before combination of the contents showed that by the above division no compound giving a positive Pettenkofer reaction would appear in both fractions.

Less 'polar' non-ketonic fraction. This fraction was placed in the first two tubes of the countercurrent apparatus and distributed by means of the solvent system cyclohexane-ethyl acetate-ethanol-water (10:90:45:55, by vol.). After completion of the fundamental procedure tubes 38 to 99 were emptied to dispose of the main bulk of pigmented impurities. The tubes were then refilled with lower phase, the fundamental procedure again completed and the upper phases recycled for a further 200 cycles. Two peaks of material reacting positively in the Pettenkofer reaction were detected, one occupying tubes 19-40 and the second occupying tubes 41-91.

Isolation of androst-5-ene-3 β :17 β -diol. The contents of tubes 41-91 were combined and crystallized three times from methanol to give 50 mg. of white crystals, which had m.p. $170-173^\circ$. The mixed m.p. with an authentic sample of androst-5-ene-3 β :17 β -diol (m.p. $176-179^\circ$) was $175-178^\circ$. A portion of the isolated compound (15 mg.) was acetylated with acetic anhydride and pyridine at room temperature overnight; the acetate, after two crystallizations from methanol, had m.p. $153-157^\circ$, unchanged by admixture with an authentic sample of 3 β :17 β -diacetoxyandrost-5-ene (Found C, 73.9; H, 9.4. Calc. for $\text{C}_{25}\text{H}_{38}\text{O}_4$: C, 73.8; H, 9.2%). The spectra of solutions of the isolated and authentic compounds in conc. H_2SO_4 were identical [max. $310\text{ m}\mu$ ($E_{1\text{cm}}^{1\%}$ 145), $350\text{ m}\mu$ (135), $410\text{ m}\mu$ (280); min. $260\text{ m}\mu$ (50), $332\text{ m}\mu$ (128), $370\text{ m}\mu$ (118); inflexion $480\text{ m}\mu$ (115)].

Isolation of pregn-5-ene-3 β :17 α :20 α -triol. The contents of tubes 19-40 were combined and crystallized three times

from methanol, to give 70 mg. of crystals which had m.p. $213-217^\circ$; $[\alpha]_D^{25} -82^\circ$ in ethanol (c, 0.488) (Found: C, 75.3; H, 10.3. Calc. for $\text{C}_{27}\text{H}_{44}\text{O}_3$: C, 75.4; H, 10.2%). On drying a sample of this compound at 80° *in vacuo* for 3 hr. it lost 2.2% of its weight but regained it rapidly (within 10 min.) on exposure to the atmosphere (see also Hirschmann & Hirschmann, 1950b). The compound formed an acetate which had m.p. $198-200^\circ$, unchanged by admixture with an authentic sample of 3 β :20 α -diacetoxypregn-5-en-17 α -ol, and the spectra of solutions of the acetates of the isolated and authentic compounds in conc. H_2SO_4 were identical [max. $344\text{ m}\mu$ ($E_{1\text{cm}}^{1\%}$ 180), $412\text{ m}\mu$ (300), $442\text{ m}\mu$ (250), $500\text{ m}\mu$ (220); min. $260\text{ m}\mu$ (71), $370\text{ m}\mu$ (130), $430\text{ m}\mu$ (245), $471\text{ m}\mu$ (198)].

More 'polar' non-ketonic fraction. This fraction was placed in the first two tubes of the countercurrent apparatus and distributed by means of the solvent system cyclohexane-ethyl acetate-ethanol-water (80:20:45:55, by vol.). After completion of the fundamental procedure the upper phases were recycled for 400 transfers. The distribution pattern showed two peaks of material reacting positively in the Pettenkofer reaction, one occupying tubes 0-32 and the second occupying tubes 33-80.

Isolation of androst-5-ene-3 β :16 β :17 β -triol. The contents of tubes 33-80 were combined and the deeply pigmented residue obtained was chromatographed on a column (28 cm. $\times$ 2 cm.) containing 60 g. of a mixture of magnesium trisilicate and Celite (2:1 by wt.) prepared in light petroleum (boiling range $80-100^\circ$). Elution was commenced with 10 ml. of light petroleum from a reservoir containing 400 ml. of light petroleum and continued with a mixture of light petroleum and ethanol, the ethanol concentration being increased in a stepwise discontinuous gradient by adding 1 ml. of ethanol to the reservoir for every 10 ml. of liquid removed from it. Material reacting positively in the Pettenkofer reaction appeared in the eluate when 350 ml. of eluent had passed through the column and was then completely eluted from the column with 200 ml. of 5% ethanol in light petroleum and 50 ml. of 20% ethanol in light petroleum. The material was crystallized twice from methanol, to give 9 mg. of crystals which had m.p. $259-262^\circ$ unchanged by admixture with authentic androst-5-ene-3 β :16 β :17 β -triol. A mixture of the isolated compound and androst-5-ene-3 β :16 α :17 β -triol had m.p. $235-240^\circ$. The compound formed an acetate which after crystallization from methanol had m.p. $206-209^\circ$ unchanged by admixture with authentic 3 β :16 β :17 β -triacetoxandrost-5-ene (m.p. $208-211^\circ$) (Found: C, 69.3; H, 8.3. Calc. for $\text{C}_{25}\text{H}_{38}\text{O}_5$: C, 69.4; H, 8.3). The compound had $[\alpha]_D^{25} -35^\circ$ in ethanol (c, 0.2) and the spectra of solutions of the isolated and authentic acetates in conc. H_2SO_4 were identical [max. $410\text{ m}\mu$ ($E_{1\text{cm}}^{1\%}$ 280), $475\text{ m}\mu$ (183); min. $265\text{ m}\mu$ (37), $450\text{ m}\mu$ (70); inflexion $350\text{ m}\mu$ (120)].

Isolation of androst-5-ene-3 β :16 α :17 β -triol. The contents of tubes 0-32 were combined and crystallization from methanol-benzene yielded 11 mg. of crystals which had m.p. $258-262^\circ$ unchanged by admixture with authentic androst-5-ene-3 β :16 α :17 β -triol. It formed an acetate which after crystallization from methanol had m.p. $178-181^\circ$ unchanged by admixture with authentic 3 β :16 α :17 β -triacetoxandrost-5-ene. The spectra of solutions of the isolated and authentic acetates in conc. H_2SO_4 were identical [max. $360\text{ m}\mu$ ($E_{1\text{cm}}^{1\%}$ 136), $410\text{ m}\mu$ (342), $470\text{ m}\mu$ (166); min. $245\text{ m}\mu$ (30), $368\text{ m}\mu$ (133), $443\text{ m}\mu$ (142)].

Examination of the ketonic fraction

The dry residue of the ketonic fraction (1.6 g.) was distributed by means of the solvent system cyclohexane-ethyl acetate-ethanol-water (8:2:3:7, by vol.). After completion of the fundamental procedure 80 fractions were collected by single withdrawal and at the end of the distribution alternate fractions were analysed by the Pettenkofer, Zimmermann and blue-tetrazolium reactions. The distribution pattern showed two peaks of material reacting positively in the Pettenkofer reaction.

Isolation of dehydroepiandrosterone. The material comprising one peak (partition coefficient 30) also reacted positively in the Zimmermann reaction and after crystallization from methanol had m.p. 135–137°, 147–149°, unchanged by admixture with authentic dehydroepiandrosterone.

Isolation of 16 α -hydroxydehydroepiandrosterone. The second peak of material reacting positively in the Pettenkofer reaction extended from tube 43 to tube 90. Analyses by the Zimmermann and blue-tetrazolium reactions showed the peak to consist of two components. One of these, which occupied tubes 43–81 (partition coefficient 0.52), reduced blue tetrazolium but did not react positively in the Zimmermann reaction. The second component, which occupied tubes 52–90 (partition coefficient 0.65), reacted positively in the Zimmermann reaction but did not reduce blue tetrazolium. The contents of tubes 43–90 were combined and chromatographed on a column (80 cm. $\times$ 2 cm.) of 120 g. of Celite with the solvent system benzene-hexane-methanol-water (7:3:8:2, by vol.). Successive 25 ml. fractions were collected and analysed. The Zimmermann-reactive material appeared in fractions 26–42 and the blue-tetrazolium-reducing material in fractions 35–48. Fractions 40–48 were combined and chromatographed on a column (28 cm. $\times$ 2 cm.) of 60 g. of Celite with the solvent system methanol-water (80%, v/v)-benzene. The blue-tetrazolium-reducing material, eluted by 200–320 ml. of solvent, was free of the Zimmermann-reactive material, and after two crystallizations from methanol it had m.p. 175–179°, unchanged by admixture with authentic 16 α -hydroxydehydroepiandrosterone.

DISCUSSION

Since the work of Ronzoni (1952), Dingemans *et al.* (1952) and Cohen & Oneson (1953) dehydroepiandrosterone has been recognized as one of the major constituents of the 17-oxo steroid fraction of the urine of normal people. However, the possibility that other 3β -hydroxy- Δ^5 -steroids might be present in the urine of normal people has not previously been systematically investigated. Thus, of the compounds whose isolation is reported here, androst-5-ene-3 β :17 β -diol and pregn-5-ene-3 β :17 α :20 α -triol have not previously been isolated from this source, and the isolation of androst-5-ene-3 β :16 β :17 β -triol from natural sources is reported for the first time. Large amounts of 3β -hydroxy- Δ^5 -steroids have been found in the urine of subjects with an adrenal-cortical tumour by numerous investigators, the most thorough studies being those

of Hirschmann & Hirschmann (1945, 1947, 1950*a, b*) and Hirschmann (1943), who isolated from the urine of a boy with this condition seven 3β -hydroxy- Δ^5 -steroids, namely dehydroepiandrosterone, androst-5-ene-3 β :17 β -diol, androst-5-ene-3 β :16 α :17 β -triol, pregn-5-ene-3 β :20 α -diol, pregn-5-ene-3 β :16 α :20 α -triol, 3 β :17 α -dihydroxypregn-5-en-20-one and pregn-5-ene-3 β :17 α :20 α -triol. Of these, pregn-5-ene-3 β :20 α -diol, pregn-5-ene-3 β :16 α :20 α -triol and 3 β :17 α -dihydroxypregn-5-en-20-one have not been isolated in the present work.

The isolation of androst-5-ene-3 β :17 β -diol and androst-5-ene-3 β :16 β :17 β -triol is of interest in that it extends the analogy between the metabolism of dehydroepiandrosterone and that of oestrone [3-hydroxyoestra-1:3:5(10)-trien-17-one]. Hirschmann (1943) and Marrian & Butler (1944) have suggested that androst-5-ene-3 β :16 α :17 β -triol arises from dehydroepiandrosterone by a metabolic route similar to that by which oestriol [oestra-1:3:5(10)-triene-3:16 α :17 β -triol] is formed from oestrone. The conversion of dehydroepiandrosterone into androst-5-ene-3 β :16 α :17 β -triol has been demonstrated both in man (Mason & Kepler, 1947) and with rabbit-liver slices (Schneider & Mason, 1948). Fotherby *et al.* (1957) have suggested that 16 α -hydroxydehydroepiandrosterone may be an intermediate in this transformation and have shown that 16 α -hydroxydehydroepiandrosterone is a metabolic product of dehydroepiandrosterone in man. This metabolic pathway is similar to that suggested by Marrian, Watson & Panattoni (1957*b*) and Marrian, Loke, Watson & Panattoni (1957*a*) for the conversion of oestrone into oestriol via 16 α -hydroxyoestrone [3:16 α -dihydroxyoestra-1:3:5(10)-trien-17-one], which they have isolated from the urine of pregnant women. Marrian *et al.* (1957*b*) have further suggested that 16 β -hydroxyoestrone (3:16 β -dihydroxyoestra-1:3:5(10)-trien-17-one) might be the intermediate in the conversion of oestrone into 16-epioestriol [oestra-1:3:5(10)-triene-3:16 β :17 β -triol], which has been isolated from pregnancy urine by Marrian & Bauld (1955). Similarly, the androst-5-ene-3 β :16 β :17 β -triol isolated in the present work might arise from dehydroepiandrosterone via 16 β -hydroxydehydroepiandrosterone.

The interconvertibility of oestrone and oestra-diol-17 β [oestra-1:3:5(10)-triene-3:17 β -diol] in humans is well known, and the analogous reactions between dehydroepiandrosterone and androst-5-ene-3 β :17 β -diol have also been demonstrated. Thus Mason & Kepler (1945) have isolated androst-5-ene-3 β :17 β -diol from the urine of a man with pituitary insufficiency who was receiving dehydroepiandrosterone, and Ungar, Miller & Dorfman (1954) have isolated dehydroepiandrosterone from the urine of a man to whom androst-5-ene-3 β :17 β -diol had been

administered. There is, however, one difference between the metabolism of oestrone and that of dehydroepiandrosterone. The conversion of oestrone into the other oestrogens discussed above has been assumed to be the major metabolic pathway for oestrone, whereas it is known that for dehydroepiandrosterone the major metabolic route is conversion into 3 α -hydroxy-5 α -androstan-17-one and 3 α -hydroxy-5 β -androstan-17-one and metabolism to compounds retaining the 5-en-3 β -ol group is a minor metabolic pathway.

While it is thus possible to fit the C₁₉ 3 β -hydroxy- Δ^5 -steroids isolated in the present work into a metabolic scheme the position of pregn-5-ene-3 β :17 α :20 α -triol is at present obscure. It could be the precursor of the urinary dehydroepiandrosterone, and Lieberman & Teich (1953) have suggested that this compound is a metabolite of an intermediate in the biosynthesis of the adrenal hormones.

SUMMARY

1. Androst-5-ene-3 β :17 β -diol, androst-5-ene-3 β :16 β :17 β -triol and pregn-5-ene-3 β :17 α :20 α -triol have been isolated from normal male urine which has been boiled for 2 hr. at neutral pH.

2. By analogy with dehydroepiandrosterone and 16 α -hydroxydehydroepiandrosterone it is likely that these steroids are present in urine as the 3 β -sulphates.

3. The metabolism of dehydroepiandrosterone is compared with that of oestrone.

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REFERENCES

- Allen, W. M. (1950). *J. clin. Endocrin. Metab.* **10**, 71.
Bauld, W. S. (1955). *Biochem. J.* **59**, 294.

- Butenandt, A. & Dannenbaum, H. (1934). *Hoppe-Seyl. Z.* **229**, 192.
Callow, N. H., Callow, R. K. & Emmens, C. W. (1938). *Biochem. J.* **32**, 1312.
Cohen, S. L. & Oneson, I. B. (1953). *J. biol. Chem.* **204**, 245.
Craig, L. C., Hausmann, W., Ahrens, E. H. & Harfenist, E. J. (1951). *Analyt. Chem.* **23**, 1236.
Dingemans, E., Huis in't Veld, L. G. & Hartogh-Katz, S. L. (1952). *J. clin. Endocrin. Metab.* **12**, 66.
Engel, L. L., Alexander, J., Carter, P., Elliott, J. & Webster, M. (1954). *Analyt. Chem.* **26**, 639.
Fotherby, K., Colas, A., Atherden, S. M. & Marrian, G. F. (1957). *Biochem. J.* **66**, 664.
Hirschmann, H. (1943). *J. biol. Chem.* **150**, 363.
Hirschmann, H. & Hirschmann, F. B. (1945). *J. biol. Chem.* **157**, 601.
Hirschmann, H. & Hirschmann, F. B. (1947). *J. biol. Chem.* **167**, 7.
Hirschmann, H. & Hirschmann, F. B. (1950a). *J. biol. Chem.* **184**, 259.
Hirschmann, H. & Hirschmann, F. B. (1950b). *J. biol. Chem.* **187**, 137.
Lieberman, S. & Teich, S. (1953). *Pharmacol. Rev.* **5**, 285.
Mader, W. J. & Buck, R. R. (1952). *Analyt. Chem.* **24**, 666.
Marrian, G. F. & Bauld, W. S. (1955). *Biochem. J.* **59**, 136.
Marrian, G. F. & Butler, G. C. (1944). *Biochem. J.* **38**, 322.
Marrian, G. F., Loke, K. H., Watson, E. J. D. & Panattoni, M. (1957a). *Biochem. J.* **66**, 60.
Marrian, G. F., Watson, E. J. D. & Panattoni, M. (1957b). *Biochem. J.* **65**, 12.
Mason, H. L. & Kepler, E. J. (1945). *J. biol. Chem.* **160**, 255.
Mason, H. L. & Kepler, E. J. (1947). *J. biol. Chem.* **167**, 73.
Munson, P. L., Jones, M. E., McCall, P. J. & Gallagher, T. F. (1948). *J. biol. Chem.* **176**, 73.
Ronzoni, E. (1952). *J. clin. Endocrin. Metab.* **12**, 527.
Roy, A. B. (1956). *Biochem. J.* **62**, 41.
Schneider, J. J. & Mason, H. L. (1948). *J. biol. Chem.* **172**, 771.
Teich, S., Rogers, J., Lieberman, S., Engel, L. L. & Davis, J. W. (1953). *J. Amer. chem. Soc.* **75**, 2523.
Ungar, F., Miller, A. M. & Dorfman, R. I. (1954). *J. biol. Chem.* **206**, 597.
Watson, E. J. D. & Marrian, G. F. (1956). *Biochem. J.* **63**, 64.
Zaffaroni, A. (1950). *J. Amer. chem. Soc.* **72**, 3828.