The Isolation of Pregn-5-ene-3 β :16 α :20 α -triol from the Urine of Normal Males

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Relatively large amounts of dehydroepiandrosterone (3 β -hydroxyandrost-5-en-17-one) are present in the urine from cases of adrenal tumour (see Mason & Engstrom, 1950). In addition to this 3 β -hydroxy- Δ^5 -steroid, Hirschmann & Hirschmann (1950*b*) have isolated six other steroids containing this grouping from the urine of a boy with an adrenal tumour. Before 1956 only two steroids of this type, namely dehydroepiandrosterone (Butenandt & Dannenbaum, 1934) and androst-5-ene-3 β :16 α :17 β -triol (Marrian & Butler, 1944), had been isolated from the urine of normal people, but recently 16 α -hydroxydehydroepiandrosterone (Fotherby, Colas, Atherden & Marrian, 1957) and 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 (Fotherby, 1958) have been isolated from this source. The present paper describes the isolation of another 3 β -hydroxy- Δ^5 -steroid, pregn-5-ene-3 β :16 α :20 α -triol, from the urine of normal males.

RESULTS

The procedures used in the extraction of the urine and purification of the extract have already been described in detail (Fotherby, 1958). Briefly they consisted in boiling urine without adjustment of the pH, a procedure which appears to be specific for the hydrolysis of the 3 β -sulphates of 3 β -hydroxy- Δ^5 -steroids and separation and isolation of the 3 β -hydroxy- Δ^5 -steroids present in the extract by countercurrent distribution and chromatography. The starting material for the present isolation was the residue from the mother liquors from the crystallization of androst-5-ene-3 β :16 β :17 β -triol. This residue was semicrystalline, and by two further crystallizations additional amounts of androst-5-ene-3 β :16 β :17 β -triol were obtained. The mother liquors remaining from the final crystallization were chromatographed and the elution pattern obtained by analysis of successive fractions suggested the presence of two components which were incompletely separated. Those fractions consisting mainly of the faster-moving component were rechromatographed, and by crystallization a compound was obtained which melted at 244–246°. The melting point indicated that this compound might be pregn-5-ene-3 β :16 α :20 α -triol, and this was proved

to be so by comparing the acetate of the isolated material with authentic 3 β :16 α :20 α -triacetoxypregn-5-ene.

EXPERIMENTAL

Details of the extraction of the urine, purification of the urine extract and the methods used have been published (Fotherby, 1958).

Isolation of pregn-5-ene-3 β :16 α :20 α -triol. By successive crystallizations of the mother liquors from the isolation of androst-5-ene-3 β :16 β :17 β -triol two further yields of this compound were obtained.

The residue from the mother liquors from the final crystallization was chromatographed on a column (24 cm. $\times$ 2 cm.) of 50 g. of Celite with the solvent system benzene-methanol-water (5:4:1, by vol.) and successive 10 ml. fractions were collected and analysed by the modified Pettenkofer reaction (Munson, Jones, McCall & Gallagher, 1948). Fractions 18–32 reacted positively and the elution pattern indicated the presence of two components which were incompletely separated. Those fractions (18–23) consisting mainly of the faster-moving component were therefore rechromatographed on a Celite column similar to that described above. Analysis of successive 5 ml. fractions showed that a single peak of material was eluted in fractions 51–64. These fractions were combined, but on attempting to crystallize the material from methanol only an oil was obtained. The methanol was removed *in vacuo* and the dry residue leached twice with a small volume of cold ether. The ether-insoluble material was crystallized from methanol to give 2 mg. of crystals which had m.p. 244–246°. [Hirschmann & Hirschmann (1950*a*) report 245–247° for pregn-5-ene-3 β :16 α :20 α -triol.] The compound formed an acetate which after crystallization from methanol had m.p. 177–179°, unchanged by admixture with authentic 3 β :16 α :20 α -triacetoxypregn-5-ene. The absorption spectra of solutions of the isolated and authentic acetates in conc. H₂SO₄ were identical both after 2 hr. and 24 hr. at 25°. [After 2 hr. max. 390 m μ ($E_{1\%}^{1\text{cm}}$ 200), 475 m μ (194); min. 265 m μ (40), 432 m μ (104); inflexions 235 m μ (50), 320 m μ (120), 355 m μ (155), 510 m μ (111), 540 m μ (60). After 24 hr. max. 310 m μ (130), 385 m μ (188), 475 m μ (212); min. 260 m μ (50), 335 m μ (92), 430 m μ (139); inflexion 530 m μ (79).]

DISCUSSION

Pregn-5-ene-3 β :16 α :20 α -triol has not previously been isolated from the urine of normal people, although it was obtained by Hirschmann & Hirschmann (1950*a*) from the urine of a boy with an adrenal tumour.

Table 1. *Neutral 16-hydroxy steroids isolated from urine, and their precursors*

16-Hydroxy steroid	16-Deoxy precursor
Androst-5-ene-3 β :16 α :17 β -triol	Dehydroepiandrosterone
Androst-5-ene-3 β :16 β :17 β -triol	
16 α -Hydroxydehydroepiandrosterone	
5 α -Androstane-3 α :16 α :17 β -triol	3 α -Hydroxy-5 α -androstane-17-one (Androsterone)
5 β -Androstane-3 α :16 α :17 β -triol	3 α -Hydroxy-5 β -androstane-17-one (Etiocolanolone)
5 α -Pregnane-3 β :16 α :20 α -triol	3 β -Hydroxy-5 α -pregnan-20-one
Pregn-5-ene-3 β :16 α :20 α -triol	3 β -Hydroxypregn-5-en-20-one

Seven neutral steroids containing a hydroxyl group at position 16 have now been isolated from human urine. These are shown in Table 1 with the corresponding 16-deoxy precursors. 5 α -Pregnane-3 β :16 α :20 α -triol is possibly a metabolite of pregn-5-ene-3 β :16 α :20 α -triol. The C₁₉ 16-deoxy steroids shown in Table 1 are those which occur in the largest amount in urine and possibly therefore are present in relatively large amounts in the body. Since the amounts of the 16-hydroxysteroids found in the urine are small it would appear that this hydroxylation is one that does not proceed too readily. If this is the case also with the C₂₁ 16-hydroxy steroids it would suggest that large amounts of pregnenolone (3 β -hydroxypregn-5-en-20-one) or pregn-5-ene-3 β :20 α -diol are produced *in vivo*. Current views on the biosynthesis of the adrenocortical hormones (cf. Hechter & Pincus, 1954) suggest that pregnenolone is an intermediate in the conversion of cholesterol into progesterone (pregn-4-ene-3:20-dione), but pregnenolone has not so far been isolated from urine or from the adrenal. Whether the 16-hydroxy steroids have any unique biological function has yet to be determined.

SUMMARY

1. Pregn-5-ene-3 β :16 α :20 α -triol has been isolated from the urine of normal males which has

been boiled for 2 hr. without adjustment of the pH.

2. The 16-hydroxy steroids found in urine are considered and the possible significance of the present isolation is discussed.

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REFERENCES

- Butenandt, A. & Dannenbaum, H. (1934). *Hoppe-Seyl. Z.* **229**, 192.
 Fotherby, K. (1958). *Biochem. J.* **69**, 596.
 Fotherby, K., Colas, A., Atherden, S. M. & Marrian, G. F. (1957). *Biochem. J.* **66**, 664.
 Hechter, O. & Pincus, G. (1954). *Physiol. Rev.* **34**, 459.
 Hirschmann, H. & Hirschmann, F. B. (1950a). *J. biol. Chem.* **184**, 259.
 Hirschmann, H. & Hirschmann, F. B. (1950b). *J. biol. Chem.* **187**, 137.
 Marrian, G. F. & Butler, G. C. (1944). *Biochem. J.* **38**, 322.
 Mason, H. L. & Engstrom, W. W. (1950). *Physiol. Rev.* **30**, 355.
 Munson, P. L., Jones, M. E., McCall, P. J. & Gallagher, T. F. (1948). *J. biol. Chem.* **176**, 73.

Nitrogenous Compounds and Nitrogen Metabolism in the Liliaceae

5. THE METABOLISM OF AZETIDINE-2-CARBOXYLIC ACID*

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Studies on the nitrogenous constituents of members of the Liliaceae family of plants have been reported in earlier papers of this series (Fowden & Steward, 1957a, b; Zacharius, Cathey & Steward, 1957; Fowden & Bryant, 1958). Particular emphasis was

placed on the more recently discovered amino and imino acids, several of which are fairly widely distributed in species of the family. Azetidine-2-carboxylic acid (A-2-C), the lower imino acid analogue of proline, is practically confined to liliaceous plants; Fowden & Steward (1957a) showed it to be present in about one-quarter of the

* Part 4: Fowden & Bryant (1958).