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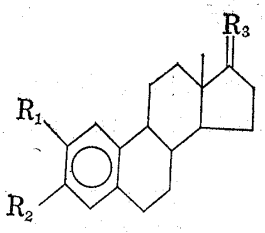
Metabolites of 3-Desoxyestrone in Rabbit Urine

3-Desoxyestrone (estra-1,3,5(10)-trien-17-one) (I) is now used for clinical states associated with hypercholesterolemia as a lipid-shifting drug.[1] However, the metabolic fate of this drug has not yet been clarified. In this paper we wish to report the isolation and characterization of the urinary metabolites of 3-desoxyestrone administered to a rabbit.

A single dose of a suspension of the steroid (350 mg) in Tween 80 was orally given to an adult male rabbit weighing about 2.0 kg. The urine was collected for the following 48 hours, and hydrolysis of glucuronides was processed in the usual manner employing beef-liver β-glucuronidase. The hydrolyzate was extracted with ethyl acetate, and the extract was in turn subjected to solvolysis.[2]

A gummy substance thus obtained as the nonconjugated steroid fraction was dissolved in benzene and chromatographed on alumina for the preliminary purification. Elution with benzene to benzene-ether (4:1) gave a mixture of several metabolites, which were indicated by the thin-layer chromatography. The eluate was then submitted to rechromatography on alumina. Elution with hexane-benzene (5:8) and recrystallization of the eluate from aqueous methanol provided 20 mg of a metabolite, A, mp 221—222°, as colorless needles. This substance exhibited positive reaction with Folin-Ciocalteu reagent and gave the acetate, 140—141°, by usual acetylation. Oxidation with Jones reagent[3] provided an oxosteroid, whose chromatographic behaviors[4] and color reactions were identical with those of estrone (t_R 0.51; TL-I 0.73, II 0.51, III 0.34). Subsequent reduction of this product with sodium borohydride yielded again a dihydroxy compound (t_R 0.64; TL-I 0.52, II 0.36, III 0.24), which could be readily distinguished from A (t_R 0.58; TL-I 0.57, II 0.38, III 0.26). These results led unequivocally to the conclusion that the metabolite would be 17α-estradiol (II). In actuality, A proved to be entirely identical with the authentic specimen by the usual criteria.



I : $R_1=R_2=H$, $R_3=O$

II : $R_1=H$, $R_2=OH$, $R_3=$ 

III : $R_1=OH$, $R_2=H$, $R_3=$ 

IV : $R_1=OH$, $R_2=H$, $R_3=O$

From the mother liquor after separating A, 4 mg of the more nonpolar metabolite, B, mp 201—203°, was obtained as colorless prisms (from ether) by the preparative thin-layer chromatography. This substance gave positive reaction with Folin-Ciocalteu and Zimmermann reagents, and was distinctly different from estrone. Reduction with sodium borohydride gave a crystalline product, mp 217—221° (t_R 0.60; TL-I 0.44, II 0.31, III 0.18), which was

1) A.H. Goldkamp, W.M. Hoehn, R.A. Mikulec, E.F. Nutting, and D.L. Cook, *J. Med. Chem.*, **8**, 409 (1965).
2) S. Burstein and S. Lieberman, *J. Biol. Chem.*, **233**, 331 (1958).
3) K. Bowden, I.M. Heilbron, E.R.H. Jones, and B.C.I. Weedon, *J. Chem. Soc.*, **1946**, 39.
4) Thin-layer chromatography was carried out on Silica Gel G by the following systems: TL-I=benzene-ether (1:1); TL-II=hexane-ethyl acetate (3:2); TL-III=hexane-ethyl acetate (2:1), and *Rf* values are given. Gas-liquid chromatography was run under the following condition: Shimadzu Gas Chromatograph Model GC-1C with 1.5% SE-30 column (1.875 m×3 mm *i.d.*), column temperature 215°, flash heater temperature 250°, and detector temperature 250°, carrier gas N_2 flow rate 50 ml/min. Retention times relative to cholestane (17.4 min), t_R, are given.

found to be identical with the authentic 2-hydroxy-3-desoxyestradiol (III) (reported mp 222—224°[5]) by mixed melting point and chromatographic comparison. All these results together permitted the assignment of the sturcture 2-hydroxy-3-desoxyestrone (IV) (reported mp 202—204°[5]) to B. Indeed, comparison of the metabolite with the authentic sample showed identity in every respect, *i.e.* Zimmermann reaction and chromatographic constants (t_R 0.52; TL-I 0.76, II 0.52, III 0.38).

It should be now emphasized that hydroxylation *in vivo* takes place at C-2 and C-3 on aromatic A-ring. A problem whether these biotransformation products may have the lipid-shifting and/or estrogenic activities seems to be of particular interest. Further studies on the characterization of other metabolites including the conjugates are being conducted in this laboratory and the details will be reported in near future.

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5) J. Fishman and M. Tomasz, *J. Org. Chem.*, **27**, 365 (1962).

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Stimulation of Protein Synthesis in Mouse Liver by Insect-Moulting Steroids

Recently it has become generally recognized that insect-moulting steroids are widely distributed in the plant kingdom: isolation of the ponasterones, ecdysterone, inokosterone, pterosterone, and cyasterone, has been reported.[1]

In this communication, we will report that the administration of various moulting steroids to mice can elevate the protein synthetic activity in livers as well as does 4-chlorotestosterone, a potent anabolic steroid.

Male mice of *dd*-strain weighing 18—22 g were used throughout the experiments. Insect-moulting steroids dissolved in 0.9% saline solution were administered intraperitoneally or orally in a dose of 0.05 mg or 0.5 mg per 100 g of body weight. 4-Chlorotestostrone was also suspended in saline and injected in a dose of 1 mg per 100 g body weight. The mice were decapitated at the indicated time after treatment. Livers were removed rapidly and rinsed in an ice-cold 1.15% KCl, weighed and homogenized with 1.5 volumes of Medium K_1[2] by Potter-Elvehjem Teflon homogenizer. The homogenate was centrifuged at $20000 \times g$ for 15 minutes. The supernatant fluid (S-20 fluid) was used as enzyme source for the measurement of ^{14}C-amino acid incorporation *in vitro*. The protein synthetic activity *in vivo* was assayed as follows: 1 μC of ^{14}C-chlorella hydrolysate was injected 15 minutes before sacrifice to mice

1) *cf.* T. Takemoto, Y. Hikino, A. Arai, M. Kawahara, C. Konno, S. Arihara, and H. Hikino, *Chem. Pharm. Bull.* (Tokyo), **15**, 1816 (1967).
2) K. Koike, T. Otaka, and S. Okui, *J. Biochem.*, **59**, 201 (1966).