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Further Studies on Metabolites of 3-Desoxyestrone in Rabbit Urine

In the preceding publication¹⁾ the authors reported the isolation of 17 α -estradiol and 2-hydroxy-3-desoxyestrone²⁾ as the urinary metabolites of 3-desoxyestrone (I)³⁾ administered to a rabbit. The present communication deals with the characterization of additional two phenolic and three neutral metabolites separated from the rabbit urine. In a typical run, a single dose of a suspension of I (350 mg) in Tween 80 was orally given to an adult male rabbit weighing about 2.0 kg. The urine collected for 48 hours after the administration was processed in the same manner as described in the previous paper.¹⁾ When the crude extract was chromatographed on alumina, the non-polar metabolites were initially eluted with benzene before the phenolic substances. Thin-layer chromatography indicated that this fraction would consist of three neutral metabolites besides the unchanged steroid. These substances showed the negative reaction with Folin-Ciocalteu reagent and the yellow coloration with *conc.* sulfuric acid.

- 1) T. Nambara and M. Numazawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 373 (1968).
- 2) These metabolites have been designated as A and B, respectively.¹⁾
- 3) A.H. Goldkamp, W.M. Hoehn, R.A. Mikulec, E.F. Nutting, and D.L. Cook, *J. Med. Chem.*, **8**, 409 (1965).

Of the neutral metabolites⁴⁾ the most polar one, A', mp 225—228°, was obtained as colorless plates (8 mg) by recrystallization of the eluate from acetone. The infrared spectrum ($\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1725, 1404) and Zimmermann reaction were indicative of the presence of oxo group in ring D. On the mass spectrum the strong peaks appeared at m/e 270 (M⁺), 252 (M⁺-H₂O) and 141 (benztropylium ion) suggesting the existence of hydroxyl group at C-6.⁵⁾ The oxidation product with Jones reagent exhibited the characteristic infrared ($\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1730, 1680) and ultraviolet absorptions ($\lambda_{\text{max}}^{\text{MeOH}}$ m μ (ϵ); 252 (17000), 294 (2200)), which also justified putting the second oxygen function at C-6. In actuality this diketone was found identical with the authentic estra-1,3,5(10)-triene-6,17-dione (II), mp 181.5—182°, prepared from I by chromium trioxide oxidation in acetic acid.⁶⁾ Inspection of the nuclear magnetic

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resonance spectra⁷⁾ (5.15 τ , 1H, broad, half-band width 7.8 cps, $-\overset{\text{OH}}{\underset{|}{\text{CH}}}-\text{CH}_2-$) of the metabolite A' supported the assignment of α -configuration rather than β to the hydroxyl group at C-6. Accordingly the project was focused to the preparation of the authentic sample.

It has already been established that on borohydride reduction the 6-ketone of ring A-aromatic steroid is transformed solely into the 6 α -hydroxy compound.⁸⁾ Reaction of the 6,17-diketone with sodium borohydride gave the 6 α ,17 β -dihydroxy derivative (III), mp 148—150°, as a single product. Subsequent treatment with benzoyl chloride in pyridine furnished a mixture of the 6-monobenzoate (IV), mp 178—180°, and the dibenzoate (V), mp 157.5—159.5°, whose separation was resolved by means of thin-layer chromatography. The monobenzoate was then led to 6 α -benzoyloxyestra-1,3,5(10)-trien-17-one (VI), mp 200—204°, by Jones oxidation, which on hydrolysis with methanolic potassium hydroxide was converted to the desired 6 α -hydroxyestra-1,3,5(10)-trien-17-one (VII), mp 124°. However, the metabolite A' proved not identical with the synthetic specimen and hence 6 β -epimer.

The preparative thin-layer chromatography of the non-polar fraction gave 3 mg of the metabolite B', mp 116—117°, as colorless needles (from acetone-hexane). Based upon the physical and chemical properties as well as chromatographic behaviors it seemed very likely to be estra-1,3,5(10)-trien-17 β -ol (VIII). On mixed melting point measurement and infrared spectra comparison with the authentic sample⁹⁾ these two were found completely identical.

In the mother liquor after separation of B', an additional monohydroxy compound, the metabolite C', could be detected by thin-layer chromatography. The difficulties were encountered to isolate it in pure state because of close similarity of the physical properties to those of B', and of very small amounts available. Upon trimethylsilylation, however, these two could be distinctly separated with success on the non-selective phases, 1.5% SE-30 (t_R : C', 0.26; B', 0.30) and 2% OV-17 (t_R : C', 0.29; B', 0.34).¹⁰⁾ Thus the structure of C' was supposed to be 3-desoxy-17 α -estradiol (IX). In fact the identity of the metabolite and the authentic sample (mp 158—161°) was demonstrated by the usual criteria. It should be now emphasized that transformation of 3-desoxysteroid *in vivo* takes place at C-6 and C-17 despite of lacking hydroxyl group on ring A.

4) The neutral metabolites are represented as A', B' and C' according to the order of decreasing polarity.

5) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, 1964, p. 59.

6) B. Longwell and O. Wintersteiner, *J. Biol. Chem.*, **133**, 219 (1940).

7) The nuclear magnetic resonance spectra were obtained on Hitachi Model H-60 spectrometer at 60 Mc in CDCl₃.

8) O. Wintersteiner and M. Moore, *J. Am. Chem. Soc.*, **81**, 422 (1959).

9) K. Sakakibara, M. Sawai, Y. Suzuki, and K. Chuma, Japan. Patent 4071 (1963) [*C.A.*, **59**, 11607 c (1963)].

10) Gas chromatography was run under the following conditions: Shimadzu Gas Chromatograph Model GC-1C equipped with hydrogen flame ionization detector, column 1.5% SE-30 or 2% OV-17 (1.875 m $\times$ 3 mm *i.d.*), column temperature 230°, injection chamber temperature 240°, N₂ flow rate 35 (SE-30) or 60 (OV-17) ml/min. Retention times relative to cholestane (16 min (SE-30); 7.5 min (OV-17)), t_R , are given.

Further elution with benzene from the alumina column and rechromatography of the eluate gave 8 mg of the phenolic metabolite, D, mp 183—184.5°, as colorless needles (from aqueous methanol). Both nuclear magnetic resonance (7.97 τ , 3H, singlet, $\text{CH}_3\text{CO}-$; 5.15 τ , 1H, doublet, $J=6$ cps, AcOCH) and infrared spectra ($\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1705, 1214) were indicative of the presence of acetyl group. The hydrolyzate produced with methanolic potassium hydroxide seemed to be 17 α -estradiol with respect to thin-layer and gas chromatographic constants. On the other hand usual acetylation with acetic anhydride and pyridine gave 17 α -estradiol diacetate. All these chemical and spectroscopic evidences together led to the assumption that the structure of 17 α -estradiol 17-monoacetate would be assigned to the metabolite D.

The synthetic route to the authentic sample involved the following reaction sequence. Reduction of estrone 3-benzyl ether (X)¹¹⁾ with sodium borohydride gave estradiol 3-benzyl ether (XI), mp 96—97°, which in turn was converted to the 17 β -tosylate (XII), mp 116—117°. Inversion of the C-17-substituent was undertaken employing the method of Henbest, *et al.*¹²⁾ Treatment with tetrabutylammonium acetate in N-methylpyrrolidone yielded 3-benzyl oxy-estra-1,3,5(10)-trien-17 α -ol acetate (XIII), mp 99—100°, in satisfactory yield. Hydrogenolysis over 5% palladium-on-charcoal gave 17 α -estradiol 17-acetate (XIV), mp 188—190°, as colorless needles (from aqueous methanol). The natural product proved to be identical with the authentic specimen as judged by infrared spectra comparison and the mixed melting point measurement.

It has already been reported that the steroid acetates are sometimes artificially formed during the processing of isolation.¹³⁾ The authors examined carefully whether the acetate might be produced in the step of hydrolysis of the urinary conjugates. In consequence, it was revealed that O-acetylation seemed unlikely under the conditions employed, and actually I was metabolized into D *in vivo*. Recently, Schubert, *et al.*¹⁴⁾ and YoungLai, *et al.*¹⁵⁾ isolated 3 β -acetoxyandrost-5-ene-7,17-dione and 16 α -acetoxy-3 α -hydroxyandrost-5-en-17-one from the human urine respectively, which were considered to be the naturally occurring products. The biochemical introduction of O-acetyl group appears to be a novel transformation in the animal kingdom and is of particular interest.

In addition, the presence of the fourth phenolic metabolite, C, was also suggested. This substance showed almost the same chromatographic behaviors as those of 2-hydroxy-3-desoxyestrone, B. However, distinct separation was attained by gas chromatography on 1.5% SE-30 (t_R : C, 0.56; B, 0.50) or 2% OV-17 (t_R : C, 1.02; B, 0.90) columns, when converted to the trimethylsilyl derivatives. The results of color reactions with Zimmermann and Folin-Ciocalteu reagents, and of chromatographic constants together led to the conclusion that the metabolite C should be estrone (XV).

Further studies on the quantitative examination of the metabolites are being in progress and the details will be reported in near future.

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15) E. YoungLai and S. Solomon, *Biochemistry*, **6**, 2040 (1967).

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Synthesis of 8,5'-Anhydro-8-mercaptadenosine

Since the first purine cyclonucleoside has been reported from our laboratory,¹⁾ the possible cyclonucleosides derived from adenosine, *e.g.* 8,2-²⁾ 8,3'-³⁾ and 8,5'-O-⁴⁾ as well as 8,2'-⁵⁾ and 8,3'-S-⁵⁾ cyclonucleosides were synthesized and characterized in their chemical and physical nature. Especially in optical rotatory dispersion study,⁶⁾ these cyclonucleosides have been shown to have large positive Cotton curves around major absorption band.

Now, we wish to report on the synthesis of 8,5'-anhydro-8-mercaptadenosine (I), which is the last possible cyclonucleoside derivable from adenosine.⁷⁾ In the study of 5'-

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- 5) M. Ikehara and H. Tada, *Chem. Pharm. Bull. (Tokyo)*, **15**, 94 (1967).
- 6) M. Ikehara, M. Kaneko, K. Muneyama, and H. Tanaka, *Tetrahedron Letters*, **1967**, 3977.
- 7) Although cyclonucleoside bearing 8,3'-O-linkage had been synthesized from 2'-deoxyadenosine³⁾ 8,3'-O-cyclization in adenosine was recently achieved in our laboratory.⁸⁾
- 8) M. Kaneko and K. Tominoto, unpublished experiments.