Preliminary Notes

Isolation and identification of 18-hydroxyoestrone from the urine of pregnant women

LOKE, WATSON AND MARRIAN[1] recently isolated a previously undescribed Kober chromogen (KC-6A) having the probable formula $C_{18}H_{22}O_3$ from the urine of pregnant women. The infrared spectrum of KC-6A showed no band at 1377 cm^{-1}, and on treatment with alkali it yielded 0.9 *M* equivalent of formaldehyde (chromotropic acid reaction) and a ketonic-phenolic product (I) which resembled oestrone in its chromatographic behaviour, but which, unlike the latter, was not a Kober chromogen. On the basis of this evidence KC-6A was provisionally identified as 18-hydroxyoestrone and the product (I) as an 18-noroestrone.

By similar methods to those used by LOKE *et al.*[1] a further batch of KC-6A has been obtained in a yield of 16 mg from 400 l of urine. After three crystallizations from methanol a product was obtained which showed a double melting point (about 220° and 248–257°) (uncorr.; evacuated sealed capillary).

KC-6A recovered from the mother liquors (11.2 mg) was treated with 1 *N* NaOH at room temperature for 5 h when the theoretical amount of formaldehyde as judged by the chromotropic acid reaction was formed. By acidification of the reaction mixture and extraction with chloroform, (I) was obtained as a crystalline solid in a yield of 10 mg. Distillation of the aqueous phase into Brady's reagent yielded a product (II) which after purification by chromatography on Al_2O_3 and crystallization from ethanol had m.p. 160–162.5° (uncorr.). The mixed m.p. of (II) with authentic formaldehyde 2:4-dinitrophenylhydrazone (m.p. 162–164°, uncorr.) was 161–163° (uncorr.).

I (9 mg of above-described preparation combined with 2 mg obtained from a previous batch of KC-6A) was methylated with dimethyl sulphate in 1 *N* NaOH for 2 h at 37°. Ether extraction gave the crude methyl ether of I in a yield of 8.5 mg. After chromatography on Al_2O_3 (benzene–hexane) and two crystallizations from methanol, 1.3 mg of a product (m.p. 143–147°, uncorr.) were obtained. Mixed with the synthetic methyl ether described below (m.p. 145–147°, uncorr., Edinburgh determination) the m.p. was 142–147°.

Authentic 18-noroestrone-3-methyl ether was prepared by total synthesis as follows. The 155° (β) isomer of *dl*-1-oxo-8-methoxy-1,2,3,4,4a,4b,5,6,10b,11,12,12a-dodecahydrochrysene, known to have the natural configuration by its conversion to oestrone[2], was condensed with furfuraldehyde to give the 2-furfurylidene derivative (m.p. 170–172° corr.), which in turn was degraded by ozonization to *dl*-β-(2-carboxy-7-methoxy-1,2,3,4,9,10,11,12-octahydro-1-phenanthrene)-propionic acid (m.p. 215–215.5° corr.). The dimethyl ester (m.p. 81–82°), produced by the action of diazomethane on the diacid, was cyclized with potassium *t*-butoxide[3], and the resulting keto ester treated with hydrochloric and acetic acids to effect hydrolysis and decarboxylation. The product was a mixture consisting of two isomers, one melting at 120–121° corr. and the other at 149–150° corr. (Wisconsin determination). One of these is clearly *dl*-18-noroestrone 3-methyl ether, and the other the 13-iso epimer. The solution (chloroform) infrared spectrum of the higher melting isomer was identical with that of the product derived from KC-6A. Whether this material has the natural or 13-iso configuration is still uncertain, and this matter is receiving further attention. In any case the identity of the synthetic and naturally derived products proves unequivocally that KC-6A is indeed correctly formulated as 18-hydroxyoestrone.

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[1] K. H. LOKE, E. J. D. WATSON AND G. F. MARRIAN, *Biochim. Biophys. Acta*, 26 (1957) 230.
[2] W. S. JOHNSON, D. K. BANERJEE, W. P. SCHNEIDER, C. D. GUTSCHE, W. E. SHELBERG AND L. J. CHINN, *J. Am. Chem. Soc.*, 74 (1952) 2832.
[3] W. S. JOHNSON, B. BANNISTER AND R. PAPPO, *J. Am. Chem. Soc.*, 78 (1956) 6331.

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