Communications to the Editor

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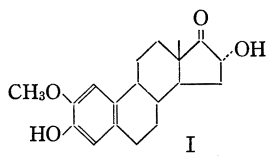
Isomeric 2,16α-Dihydroxyestrone Methyl Ethers: New Metabolites in Rat Bile

A particular interest in the enterohepatic circulation of estrogen prompted us further to explore the biliary metabolites in the rat administered with estrone.[1] In this paper we wish to report the isolation and characterization of two new metabolites, 2,16α-dihydroxyestrone 2- and 3-methyl ethers.

A suspension of estrone-4-^{14}C (25 mg, 1.5 μCi) in Tween 80 was orally given to each of four male rats (Wistar strain, body weight *ca.* 350 g) with cannulation to the bile duct. Similarly the nonlabeled steroid (25 mg) was administered to each of twenty rats. The pooled bile was combined (500 ml) and percholated through a column of Amberlite XAD-2 resin.[2] After washing with water the steroid conjugates were eluted with methanol. The eluate was dissolved in 80% methanol and subjected to gel filtration on Sephadex LH-20. The conjugated metabolites were hydrolyzed with beef-liver β-glucuronidase and the hydrolyzate was then submitted to solvolysis. The liberated steroids were separated by gradient elution chromatography with the system isooctane–ethyl acetate on ethylene glycol–impregnated Celite[3] into six fractions from A to F with the increasing polarity. Fraction D was then further purified by preparative thin-layer chromatography (TLC) using chloroform–methanol (97: 3) as solvent. Elution of the area corresponding to the radioactive spot (*Rf* 0.4) followed by fractional crystallization of the eluate from benzene gave a mixture of two metabolites. Separation of these two metabolites could be attained with success by multiple runs with chloroform on TLC. Recrystallization of the less polar one from benzene gave metabolite I (5 mg) as colorless needles. mp 199—201.5°.

This substance exhibited the signals at 0.98 (3H, s, 18-CH_3), 3.85 (3H, s, -OCH_3), 4.40 (1H, t, J=5 Hz, 16β-H), 6.64 (1H, s, aromatic 4-H), and 6.75 ppm (1H, s, aromatic 1-H) in the nuclear magnetic resonance (NMR) spectrum (60 MHz in $CDCl_3$)[4,5] and a carbonyl band at 1730 cm^{-1} in the infrared (IR) spectrum (KBr). On the mass spectra of the metabolite and its acetate the parent peak appeared at *m/e* 316 and 400, respectively. Unfortunately the more polar metabolite II could not be isolated in the crystalline state and therefore it was transformed into the acetate. mp 213—218°. As for the NMR spectrum of II the signals appeared at 0.98 (3H, s, 18-CH_3), 3.85 (3H, s, -OCH_3), 4.40 (1H, t, J=5 Hz, 16β-H), 6.57 (1H, s, aromatic 4-H), and 6.85 ppm (1H, s, aromatic 1-H). The mass spectra of metabolite II and its acetate showed the molecular ion peak at *m/e* 316 and 400, respectively. These data together permitted the assignment of the structures 2,16α-dihydroxyestrone 2- and 3-methyl ethers to two metabolites.



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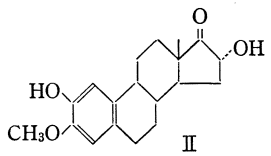

II

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The necessity for direct comparison prompted us to prepare the authentic samples by an unequivocal route. The desired compounds were synthesized from the isomeric 2-hydroxyestrone methyl ethers[4] according to the method worked out by Gallagher and his co-workers.[6] 2-Methoxyestrone 3-benzyl ether was transformed into the Δ^{16}-enol acetate with isopropenyl acetate and a catalytic amount of sulfuric acid. Treatment with *m*-chloroperbenzoic acid and cleavage of the epoxyacetate with sulfuric acid gave the 16α-hydroxy-17-ketone in a satisfactory yield. Removal of the benzyl group at C-3 by catalytic hydrogenolysis afforded the required 2-methoxy-16α-hydroxyestrone as colorless needles (from acetone–hexane). mp 201.5—204°. The synthetic sample proved to be identical with metabolite I by mixed melting point, IR, NMR and mass spectral measurements. 2,16α-Dihydroxyestrone 3-methyl ether was also prepared from 2-hydroxyestrone 3-methyl ether by the same reaction sequence as for its positional isomer. The desired compound was obtained in a reasonable yield as colorless prisms (from methanol). mp 195.5—198°. Subsequent acetylation in the usual manner provided the 2,16-diacetate as colorless prisms (from acetone–hexane). mp 219.5—222°. The identity of these synthetic samples and metabolite II and its 2,16-diacetate was justified by the usual criteria, *i.e.* IR, NMR and mass spectra and chromatographic behaviors.

To the best of our knowledge this is the first recorded instance of a bioconversion of estrone to catechol estrogen having the 16,17-ketol structure, though the isolation of 2-methoxyestrone has been reported.[7] It is also to be noted that *in vivo* O-methylation takes place at both hydroxyl groups in ring A to yield isomeric catechol estrogen monomethyl ethers. This finding is in good accord with the recent paper dealing with an *in vivo* conversion to 2-hydroxyestrone 3-methyl ether.[8]

Further studies on the biliary estrogen metabolism are being conducted and the details will be reported in the near future.

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Carboxylation of Cyclohexanone by Carbon Dioxide and Potassium Pyrrolidone in Aprotic Solvents

It has been known that biotin, sometimes called as vitamin H, is concerned with carboxylation and decarboxylation in living bodies.[1] This biological function by biotin has so far attracted the attention from a chemical point of view with regard to the capability of CO_2 donor-acceptor nature of biotin-like compounds.[2] As an extention of previous work,[3]

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