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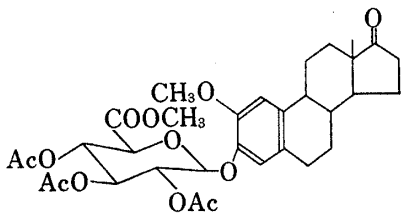
2-Methoxyestrone 3-Glucuronide: A New Biliary Metabolite in the Rat

The physiological role of the estrogen conjugate in the biliary excretion is of particular interest, because estrogen metabolism is characteristic of the enterohepatic circulation.[1] However, the conjugated metabolites excreted in the bile have not yet fully been elucidated. In this paper we report the isolation and characterization of two biliary metabolites in the rat administered with estrone.

A suspension of estone-4-^{14}C (50 mg, 1 μCi) in Tween 80 was orally given to each of nine male rats (Wistar strain, body weight *ca.* 350 g) with cannulation to the bile duct, and the bile was collected for the following 24 hr. The pooled bile (360 ml) was filtered and percholated through a column of Amberlite XAD-2 resin. After washing with water the conjugated steroid was eluted with methanol. The eluate was dissolved in 80% methanol and subjected to the gel filtration on Sephadex LH-20. The radioactive fraction exhibiting the positive naphthoresorcinol test was collected and chromatographed on Sephadex G-25 using *n*-butanol/*tert*-butanol/2N NH_4OH (200:200:133) as eluent.[2] Further purification by chromatography on Sephadex LH-20 followed by fractional crystallization from methanol-isopropanol afforded a new conjugated metabolite (20 mg), mp 211—215°.

This substance exhibited the signals at 3.82 (3H, singlet, $-OCH_3$) and 6.90 ppm (2H, singlet, aromatic ring proton) in the nuclear magnetic resonance (NMR) spectrum (60 Mc in CD_3OD) and showed a strong absorption at 1738 cm^{-1} (five-membered ring C=O) in the infrared (IR) spectrum (KBr). Enzymatic hydrolysis with beef-liver β-glucuronidase yielded 2-methoxyestrone,[3] mp 191—192°, which could be characterized by direct comparison with the authentic sample prepared in these laboratories.[4] These findings prompted us to synthesize 2-methoxyestrone 3-glucuronide and its derivative. The Koenigs-Knorr reaction of 2-methoxyestrone with methyl acetobromoglucuronate in the presence of silver carbonate gave methyl (2-methoxy-17-oxoestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (I) as colorless fibers (from methanol), mp 215—217°. *Anal.* Calcd. for $C_{32}H_{40}O_{12}$: C, 62.32; H, 6.54. Found: C, 62.33; H, 6.47. The formation of the β-glucuronoside structure was further confirmed by mass (*m/e*): 616 (M^+), 317, 300, and NMR spectra (ppm): 5.01 (1H, doublet, J=8 cps, C-1'-H). Simultaneous removal of the protecting groups by the alkaline hydrolysis gave the desired sodium 3-glucosiduronate (II), mp 213—219°. *Anal.* Calcd. for $C_{25}H_{31}O_9Na \cdot 2\frac{1}{2}H_2O$: C, 55.24; H, 6.68. Found: C, 55.29; H, 6.13. Identity of the biliary metabolite with II was determined by usual criteria. The metabolite was transformed into the acetate-methyl ester by treatment with diazomethane and then with acetic



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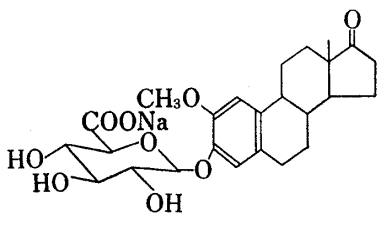

II

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anhydride-pyridine. Indeed the derivatized metabolite proved to be identical with I by mixed melting point, IR, NMR, and mass spectral measurements.

Treatment of the mother liquor provided the second metabolite (7 mg), mp>300°, which was similarly led to the acetate-methyl ester, mp 227°, mass spectrum (*m/e*): 586 (M^+), 317, 270. This derivative proved to be identical in all respects with the synthetic sample, methyl (17-oxoestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate,[5] mp 226.5—227.5°. Thus the structure of estrone 3-glucuronide was unequivocally assignable to this metabolite.

Further studies on the biliary estrogen metabolites which may possibly be involved in the enterohepatic circulation are being conducted and the details will be reported in the near future.

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Components of *Metaplexis japonica* MAKINO

The structures of a number of polyoxypregnane derivatives isolated from *Metaplexis japonica* MAKINO (Japanese name: Gagaimo), a plant of Asclepiadaceae family, have been reported.[1-4] In this communication, we wish to describe the isolation and the structure of a new aglycone, ester A (1), from the same source and the structure of dibenzoyl gagaimol (III).[2]

The aglycone mixture, obtained after the mild hydrolysis of the crude glycoside,[1] was worked up by alumina column chromatography. This procedure yielded two new aglycones, ester A (I), and dibenzoylgagaimol (III). Ester A (1) shows the following data: mp 166—169° from acetone, bluish green colour with Liebermann-Burchard reaction, violet with $SbCl_3$, positive with Dragendorff reagent, negative Keller–Kiliani reaction, $[\alpha]_D^{21}=+116.2°$ ($c=1.6$, in EtOH), *Anal.* Calcd. for $C_{36}H_{43}O_8N$: C, 69.99; H, 7.02; N, 2.27. Found: C, 69.88; H, 7.09; N, 2.30), ultraviolet (UV) absorption λ_{max}^{EtOH} mμ (log ε): 280 (4.33), infrared (IR) absorption ν_{Max}^{Nujol} cm^{-1}: 3500, 1720, 1640, 1600, nuclear magnetic resonance (NMR) ($CDCl_3$) δ: 1.10 (19-CH_3), 1.35 (21-CH_3, d, $J=6$ Hz), 1.60 (18-CH_3), 3.50 (1H, broad m, 3α-proton), 4.85 (2H, m, 12α and 20-protons), 5.35 (1H, broad t, 6-vinylproton), 6.03 (1H, d, $J=16$ Hz, olefinic proton),

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