The reaction mixture was treated in the usual manner and the crystalline product (75 mg) thus obtained was sublimed to give a pure sample of V as colorless prisms, mp 161—165°, $[\alpha]_D^{23}$ −5.8° (c=0.791). *Anal.* Calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 11.00. Found: C, 75.76; H, 11.01. IR cm^{-1}: ν_{max} 3635 ($CHCl_3$). NMR τ: 9.12 (3H, d, J=5 cps, C_{10}-C<u>H</u>$_3$), 9.02 (3H, d, J=7 cps, C_4-C<u>H</u>$_3$), 8.82 and 8.63 (each 3H, s, C_{11}-(C<u>H</u>$_3$)$_2$), 5.53 (1H, broad d, J=6 cps, >C<u>H</u>-OH).

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Studies on Steroid Conjugates. V. Synthesis of 16-Epiestriol 3-Glucuronide[1]

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As a part of our program dealing with the studies on steroid conjugates we reported previously the synthesis of 16-epiestriol (estra-1,3,5(10)-triene-3,16β,17β-triol) 16- and 17-glucuronides.[3] Three possible monoglucuronides have become requisite for us to explore the estrogen conjugates in pregnancy urine and examine the multiplicity of the transferase which catalyzes the formation of the glucuronoside linkage. In this paper we wish to report the preparation of remaining 16-epiestriol 3-monoglucuronide.

First, 16-epiestriol (IIIa) was led to the acetonide to protect the 16,17-*cis*-glycol structure. However, the attempt to introduce the glucuronyl moiety employing Koenigs–Knorr reaction[4] resulted in failure.

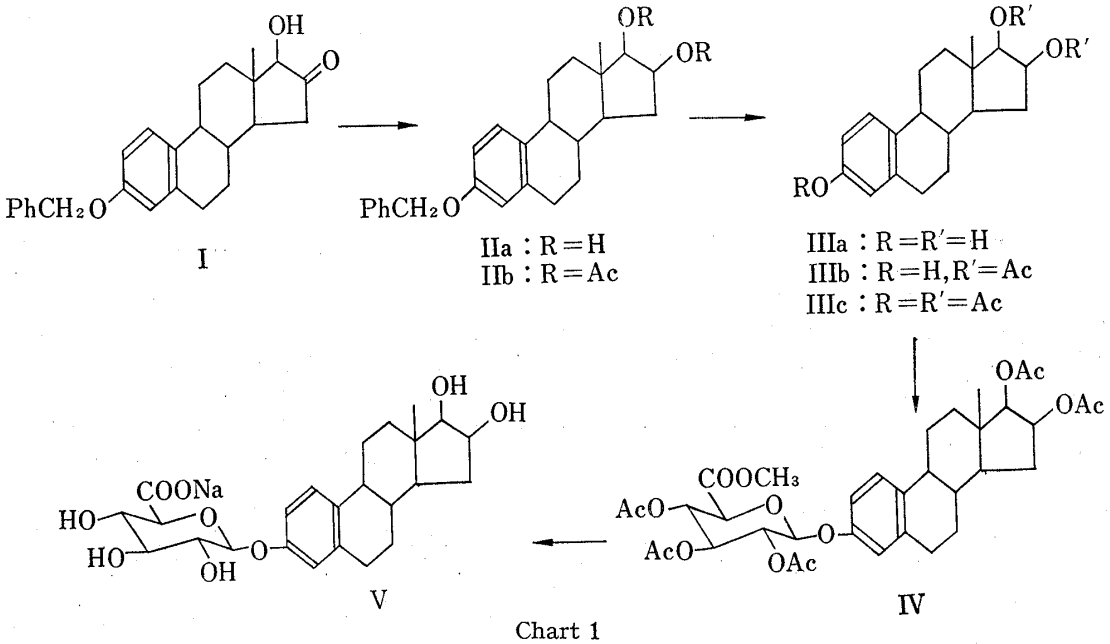

Chart 1

1) This paper constitutes Part XLIV of the series entitled, "Analytical Chemical Studies on Steroids"; Part XLIII: T. Nambara, H. Hosoda, M. Usui, and T. Anjyo, *Chem. Pharm. Bull.* (Tokyo), **19**, 612 (1971).
2) Location: *Aobayama, Sendai.*
3) T. Nambara, Y. Matsuki, and T. Chiba, *Chem. Pharm. Bull.* (Tokyo), **17**, 1636 (1969).
4) H.H. Wotiz, E. Smakula, N.N. Lichtin, and J.H. Leftin, *J. Am. Chem. Soc.*, **81**, 1704 (1959).

Accordingly 3-benzyloxy-17β-hydroxyestra-1,3,5(10)-trien-16-one[5] (I) was taken as a starting compound. Reduction with borohydride gave solely 16-epiestriol 3-benzyl ether (IIa), which on usual acetylation was led to the diacetate (IIb) in reasonable yield. Upon hydrogenolysis over palladium–on–charcoal debenzylation occurred readily to furnish 16-epiestriol 16,17-diacetate (IIIb). This compound could be obtained by an alternative route with more advantage. On brief treatment with bicarbonate solution 16-epiestriol triacetate[6] (IIIc) underwent the partial hydrolysis yielding IIIb in satisfactory yield.

Condensation of methyl acetobromoglucuronate with IIIb in the presence of freshly prepared silver carbonate[4] followed by thin–layer chromatographic purification afforded methyl (16β,17β-diacetoxyestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid) uronate (IV) in 18% yield. Upon treatment with methanolic sodium hydroxide under mild conditions the simultaneous removal of the protecting groups in both steroid and sugar moieties proceeded to furnish desired sodium 16-epiestriol 3-glucosiduronate (V). The evidence for the β-glucuronoside linkage in V was demonstrated by characterizing 16-epiestriol after incubation with beef-liver β-glucuronidase.

It is hoped that the availability of three isomeric monoglucuronides[7] as authentic samples may serve for the biochemical problems associated with glucuronic acid conjugation of 16-epiestriol.

Experimental[8]

3-Benzyloxyestra-1,3,5(10)-triene-16β,17β-diol (IIa)——To a solution of 3-benzyloxy-17β-hydroxyestra-1,3,5(10)-trien-16-one (I) (1.8 g) in DMF (30 ml)–MeOH (20 ml) was added a solution of KBH_4 (250 mg) in 30% MeOH (6 ml), and the resulting solution was stirred at room temperature for 6.5 hr. After decomposition of excess KBH_4 with AcOH the reaction mixture was poured into ice–water. The precipitate was collected by filtration, washed with H_2O and then dried. Recrystallization from acetone–hexane gave IIa (1.5 g) as colorless needles. mp 194—197°. $[\alpha]_D^{16.7}$ +65.2° (c=0.12). *Anal.* Calcd. for $C_{25}H_{30}O_3$: C, 79.33; H, 7.99. Found: C, 79.55; H, 8.24.

3-Benzyloxyestra-1,3,5(10)-triene-16β,17β-diol Diacetate (IIb)——A solution of IIa (1.5 g) in pyridine (20 ml) and Ac_2O (10 ml) was heated at 70° for 10 hr. The resulting solution was diluted with ether, washed with 5% HCl, 5% $NaHCO_3$ and H_2O, successively and dried over anhydrous Na_2SO_4. After usual work–up recrystallization of crude product from MeOH gave IIb (0.96 g) as colorless needles. mp 123—124°. $[\alpha]_D^{15}$ +46.0° (c=0.34). *Anal.* Calcd. for $C_{29}H_{34}O_5$: C, 75.30; H, 7.41. Found: C, 75.57; H, 7.56. NMR (4% solution in $CDCl_3$) δ: 0.93 (3H, s, 18–CH_3), 2.04 (3H, s, 17β-OCOCH_3), 2.06 (3H, s, 16β-OCOCH_3), 4.58 (1H, d, 17α-H), 5.01 (2H, s, –$C\underline{H}_2C_6H_5$), 5.46 (1H, m, 16α–H).

Estra-1,3,5(10)-triene-3,16β,17β-triol 16,17-Diacetate (IIIb)——i) A solution of IIb (960 mg) in AcOEt (30 ml)–EtOH (100 ml) was shaken with 5% Pd/C (900 mg) under a current of H_2 for 24 hr at room temperature. After removal of catalyst by filtration the filtrate was concentrated to give the crystalline product. Recrystallization from MeOH gave IIIb (830 mg) as colorless needles. mp 225—226°. $[\alpha]_D^{27}$ +54.5° (c=0.17). *Anal.* Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58. Found: C, 70.93; H, 7.62. NMR (4% solution in $CDCl_3$) δ: 0.93 (3H, s, 18-CH_3), 2.03 (3H, s, 17β-OCOCH_3), 2.05 (3H, s, 16β-OCOCH_3), 4.58 (1H, d, 17α-H), 5.46 (1H, m, 16α-H).

ii) To a solution of estra-1,3,5(10)-triene-3,16β,17β-triol triacetate (IIIc) (26 mg) in acetone (1 ml)–MeOH (1 ml) was added 10% $KHCO_3$ (0.5 ml) and stirred at room temperature for 10 min. The resulting solution was diluted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. Recrystallization from MeOH gave IIIb (20 mg) as colorless needles. mp 222—223°. Mixed melting point on admixture with the sample obtained in i) showed no depression.

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7) These positional isomers can be separated each other by paper and thin–layer chromatography (T. Nambara, Y. Matsuki, J. Igarashi, and Y. Kawarada, to be published).
8) All melting points were taken on a micro hot–stage apparatus and are uncorrected. Nuclear magnetic resonance spectra were run on Hitachi Model H–60 spectrometer at 60 Mc; the chemical shifts are quoted as ppm downfield from $(CH_3)_4Si$ used as an internal standard. Abbreviation used s=singlet, d=doublet, and m=multiplet.

Methyl (16β,17β-Diacetoxyestra-1,3,5 (10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid) uronate (IV)—To a solution of IIIb (200 mg) and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranosiduronate (400 mg) in anhydrous benzene (40 ml) was added freshly prepared Ag_2CO_3 (400 mg), and the suspended solution was stirred at room temperature for 24 hr. During the continuation of stirring additional amount of Ag_2CO_3 (500 mg) was added in several portions. The precipitate was filtered off and the filtrate was evaporated to dryness *in vacuo*. The crude product thus obtained was submitted to preparative thin-layer chromatography using hexane–AcOEt (2:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.43) and recrystallization of the eluate from EtOH gave IV (66 mg) as colorless needles. mp 218—220°. $[\alpha]_D^{20}$ +31.0° (c=0.19). *Anal.* Calcd. for $C_{35}H_{44}O_{14}\cdot{}^1/_2H_2O$: C, 60.25; H, 6.50. Found: C, 59.83, 59.89; H, 6.15, 6.11. NMR (5% solution in $CDCl_3$) δ: 0.93 (3H, s, 18-CH_3), 2.03 (6H, s, 16β-, 17β-$OCOCH_3$), 3.70 (3H, s, -$COOCH_3$), 4.58 (1H, d, 17α-H), 5.25 (4H, m, 16α-H, pyranose-C<u>H</u>-$OCOCH_3$).

Sodium (16β, 17β-Dihydroxyestra-1,3,5 (10)-trien-3-yl-β-D-glucopyranosid)uronate (V)——To a solution of IV (50 mg) in MeOH (6 ml) was added 1N NaOH (1.2 ml), and the resulting solution was allowed to stand at room temperature for 24 hr. The precipitated crystalline product was collected by filtration, washed with H_2O (1 ml) and dried. Recrystallization from MeOH gave V (24 mg) as colorless prisms. mp 272—276° (decomp.). $[\alpha]_D^{22}$ 0° (c=0.11, H_2O). *Anal.* Calcd. for $C_{24}H_{31}O_9Na\cdot H_2O$: C, 57.13; H, 6.59. C, 57.18; H, 6.97.

Enzymatic Hydrolysis of V with β-Glucuronidase——To an aq. solution (2 ml) of V (*ca.* 0.1 mg) were added 0.1M acetate buffer (pH 4.7, 5 ml) and beef-liver β-glucuronidase (Tokyo Zōkikagaku Co., Ltd.) (13000 Fishman U/ml, 2 ml), and the solution was incubated at 37° for 24 hr. The incubated fluid was saturated with NaCl and extracted with ether (30 ml ×2). The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4 and then concentrated *in vacuo*. A portion of the residue thus obtained was submitted to thin-layer chromatography employing Silica gel G (E. Merck AG) as adsorbent and ether–benzene (3:1) as developing solvent. The test sample exhibited a sport at *Rf* 0.43, which proved to be identical with that of the authentic sample (16-epiestriol).

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Isolation of Isonarthogenin from *Dioscorea quinqueloba* Thunb.[1]

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Although diosgenin (25D-spirost-5-en-3β-ol) and/or yamogenin (25L-spirost-5-en-3β-ol) are known to be contained in many species of the genus Dioscorea, other steroidal sapogenins have been isolated only from a few species of this genus. Kryptogenin, which was reported by Marker, *et al.*[3] to be contained in eleven Central American species, could not be found by other investigators[4] in these plant. Except kryptogenin, four sapogenins were isolated together with diosgenin and yamogenin from two Dioscorea species of the New World; gentrogenin (25D-spirost-5-en-3β-ol-12-one) and correllogenin (25L-epimer of gentrogenin) from

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