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A Facile O-Glycosidation using Stannic Chloride

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Using stannic chloride, O-glycosidation, especially O-glucuronidation of *p*-nitrophenol, estradiol 17-acetate, estriol 16,17-diacetate, *p*-nitrobenzyl alcohol, trichloroethanol was carried out. This method is available for the preparation of some alkyl α- and β-glucuronosides and aryl β-glucuronosides, especially estrogen derivatives which are obtained in comparatively low yield by the Koenigs-Knorr method. Spectral differences of α- and β-anomers obtained were examined.

O-Glycosides are widely distributed in Nature, particularly in plants, and foreign substances are likely to be excreted from the body of animals in the form of glucuronosides. Moreover, recently it has been reported that glucose conjugates and xylose conjugates are also observed in either urine or the bile of some animals.[2] The most important synthetic method for the formation of aglycone-glycosyl linkages is the Koenigs-Knorr reaction in which phenols or alcohols react with protected 1-halosugars in the presence of a suitable catalyst such as Ag_2CO_3 or $CdCO_3$. The product of the reaction is predominantly the β-anomer. The condensation usually takes place also by the Helferich method and its modifications. However, this method sometimes gives an anomeric mixture of α- and β-anomers.[3]

Recently, Niedballa and Vorbruggen have reported the syntheses of pyrimidine nucleosides under mild conditions using Friedel-Craft catalyst, $SnCl_4$, $AlCl_3$, BF_3 *etc.*[4] The syntheses of C-ribosides using similar methods have been reported by Kalvoda and by Ohrui, *et al.*[5] Lemieux, *et al.*[6] in 1953, prepared methyl glucoside and phenyl glucoside using $SnCl_4$. Therefore, we have focussed our attention on a facile O-glycosidation, especially on a glucuronidation under similar conditions.

We have attempted the preparation of O-riboside, O-glucosides and O-glucuronosides of some phenols and alcohols. The O-ribosylation of *p*-nitrophenol has been carried out using 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (I) in the presence of $SnCl_4$ in dichloroethane at room temperature. After alkaline hydrolysis of the acyl product (II), the anomeric configuration of *p*-nitrophenyl-D-ribofuranoside (III) was determined to be the β-form on the basis of the nuclear magnetic resonance (NMR) spectrum in which the C-1 proton was observed at 5.70 ppm as a singlet according to the rule of Karplus.[7] O-Glucosidation of *p*-nitrophenol with β-D-glucose pentaacetate (IV) by the same procedure afforded the anomeric *p*-nitrophenyl-2,3,4,6-tetra-O-acetyl-D-glucopyranosides, (Vα and Vβ). After alkaline hydrolysis of Vα and Vβ, *p*-nitrophenyl-α- and β-D-glucopyranosides (VIα and VIβ) were individually obtained.

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2) D.G. Williamson, D.C. Collins, D.S. Layne, R.B. Conrow and S. Bernstein, *Biochemistry*, **8**, 4299 (1969); J. Fevery, G.P. Van Hees, P. Leroy, F. Compernolle, and K.P.M. Heirwegh, *Biochem. J.*, **125**, 803 (1971).
3) *a*) J. Stanek, M. Cerney, J. Kocourek, J. Pacak, "The Monosaccharides," Academic Press, New York, 1963, p. 255; *b*) W.G. Overend, "The Carbohydrate," Vol. IA, 1972, p. 292
4) U. Niedballa and H. Vorbruggen, *Angew. Chem. Intern. Ed. Engl.*, **9**, 461 (1970); *idem*, *J. Org. Chem.*, **39**, 3660 (1974).
5) L. Kalvoda, *Collection Czech. Chem. Commun.*, **38**, 1679 (1973); H. Ohrui, H. Kuzuhara, and S. Emoto, *Agr. Biol. Chem.*, **36**, 1651 (1972).
6) R.U. Lemieux and W.P. Shyluk, *Can. J. Chem.*, **31**, 528 (1953).
7) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).

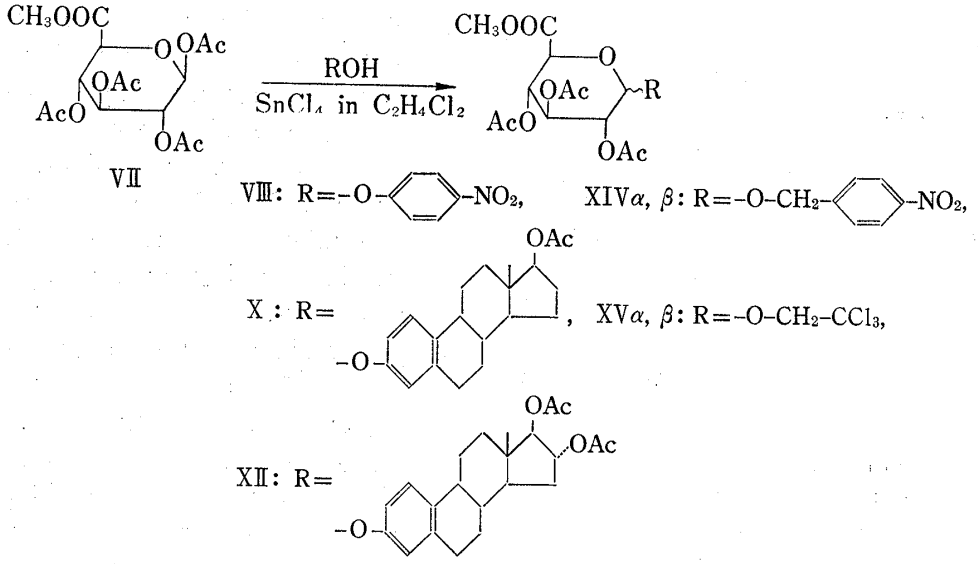


Chart 1

They were treated with β-glucosidase from sweet almonds, which hydrolyzed only VIβ to give free *p*-nitrophenol. O-Glucuronidation of *p*-nitrophenol with methyl-1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronate (VII) was accomplished in the same manner to afford methyl (*p*-nitrophenyl-2,3,4-tri-O-acetyl-β-D-glucopyranosid) uronate (VIII). *p*-Nitrophenyl glucosiduronic acid (IX) obtained by alkaline hydrolysis of VIII was enzymatically hydrolyzed by beef liver β-glucuronidase. O-Glucuronidation of estradiol 17-acetate with methyluronate (VII) was carried out in the presence of $SnCl_4$ in dichloroethane at 10° to give a fair yield of the β-anomer of estradiol 3-glucuronoside derivative (X). The analogous reaction of estriol 16,17-diacetate with methyluronate (VII) also gave the β-anomer of estriol 3-glucuronoside derivative (XIIβ). The α-anomer (XIIα) was separated from the mother liquor of XIIβ in a small amount. Sodium 3-glucuronosides of estrogen (XI and XIIIβ) isolated by alkaline hydrolysis were treated with beef liver β-glucuronidase to liberate estradiol and estriol, respectively. On the analogous glucuronidation of *p*-nitrobenzyl alcohol and trichloroethanol with methyluronate (VII) at room temperature (25—30°), the α-anomers of methyl (*p*-nitrobenzyl-2,3,4-tri-O-acetyl-D-glucopyranosid) uronate (XIVα) and methyl (trichloroethyl-2,3,4-tri-O-acetyl-D-glucopyranosid) uronate (XVα) were isolated, while on the reaction under cooling (at 0—5°) the corresponding β-anomers (XIVβ, XVβ) were obtained, respectively.

The α- and β-anomers of glucuronosides prepared by the method described above were compared in the following ways. The melting points of the β-anomers of acyl derivatives usually showed a higher value than that of α-anomers. The $[\alpha]_D$ value of the α-anomer was more positive than that of the β-anomer in all compounds prepared. This results are coincident with Hudson's rule.[8] On the optical rotatory dispersion (ORD) spectra[9] the α-anomers exhibited a positive curve with a fair distinct peak in the region of about 280 to 340 nm except that the glucuronoside derivative of trichloroethanol (XVα) showed a positive plane curve. On the contrary, the β-anomers exhibited a negative curve with a trough in the region of about 280 to 380 nm except for XVβ which showed a negative plane curve (Fig. 1). These results indicate that the C-1 configuration of glucuronosides can be easily determined on the basis of ORD spectrum.

It is well known that analysis of the NMR spectra of acylated glycosides can distinguish between the α- and β-anomers from the C-1 proton signals. However, when its signal is obscured

8) J. Stanek, M. Cerney, J. Kocourek, J. Pacak, "The Monosaccharides," Academic Press, New York, 1963, p. 50.
9) I. Listowsky, G. Avigad, and S. Englard, *Carbohyd. Res.*, **8**, 205 (1968).

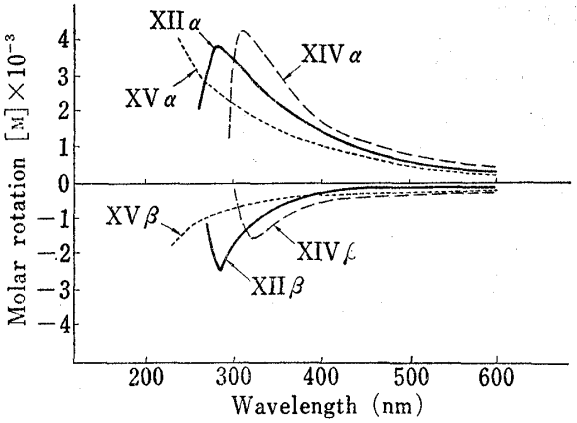
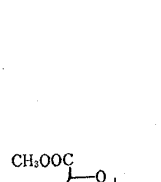
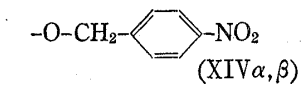


Fig. 1. Optical Rotatory Dispersion Curves for Some D-Glucosiduronate Derivatives

——: estriol 3-glucosiduronate (XIIα, XIIβ)
----: *p*-nitrobenzyl (XIVα, XIVβ)
-----: trichloroethyl (XVα, XVβ)

by overlapping with other proton signals, it may be difficult to distinguish the C-1 proton. The following procedure is a useful method for distinguishing between α- and β-anomers of acylated glucuronosides. As shown in Table I, the C-5 proton signal appeared as a doublet in the range of 4.30—4.40 ppm and its coupling constant was 10 Hz in the α-anomers. On the other hand, the C-5 proton signal of the β-anomers was observed as a doublet-doublet or a multiplet in the higher field, 4.10—4.20 ppm. This phenomenon assumed that the long range coupling of the C-5 proton with the C-3 proton occured in the β-anomers. Since the C-5 proton signal of acylated glucuronosides usually appears in the higher field apart from the C-1, C-2, C-3, and C-4 protons in the both anomers, it is easy to assign it. Moreover, the C-1, C-2, C-3 and C-4 protons appeared in the range of about 1.0 ppm in the α-anomer, while the β-anomer gave those signals within a narrow range of about 0.50—0.75 ppm. These results

Table I. The NMR Prameters for D-Glucosiduronate Derivatives (δ)

Aglycone (R)	α-Anomer H-1～H-4	α-Anomer H-5	β-Anomer H-1～H-4	β-Anomer H-5
 (XIIα,β)	4.87—5.93 1.06 (ppm)	4.41 (d, J=10)	4.88—5.48 0.60	4.18 m[a]
 (XIVα,β)	4.85—5.76 0.91	4.35 (d, J=10)	4.64—5.40 0.76	4.10 dd (J=7 and 3)
-O-CH_2-CCl_3 (XVα,β)	4.75—5.80 1.05	4.38 (d, J=10)	4.89—5.40 0.51	4.10 m[a]

a) The total distance between the strong outer lines was 10 Hz.

give additional evidence to the data described by Matsui, *et al.*[10] Infrared (IR) spectra showed a different pattern between the α- and β-anomers of glucosiduronate derivatives. As reported by Nitta, *et al.*,[11] the α-anomers had strong absorption bands in the region of 1200—1100 cm^{-1} and of 1000—900 cm^{-1}, while the β-anomers had only weak absorption bands.

Attempts of anomerization were carried out. The α- and β-anomers of acetylated *p*-nitrophenyl glucoside (Vα, Vβ) were individually treated with $SnCl_4$ in dichloroethane at room temperature for 24 hr. However, the convertion into the other was not observed by means of thin-layer chromatographic (TLC) analysis. These data under the above conditions show no evidence that the first stage of the $SnCl_4$ catalyzed reaction gives the β-anomer of glycosyl derivative, which are anomerized to the stable isomer in a subsequent step.

10) M. Matsui and M. Okada, *Chem. Pharm. Bull.* (Tokyo), **18**, 2129 (1970).
11) Y. Nitta, Y. Nakazima, M. Kuranari, A. Momose, and J. Ide, *Yakugaku Zasshi*, **81**, 1160 (1961).

The ratio of the α- and β-anomers was affected by temperature. A higher temperature on the reaction favored the production of the α-glycoside. This is especially true for the aliphatic alcohols rather than the phenols.

Attempts to synthesize the α-D-derivatives of glucuronoside were very unsatisfactory also by the Helferich method with the use of $ZnCl_2$.[3a] The glycosidation using $SnCl_4$ is an available method for the preparation of some alkyl α- and β-glucuronosides and aryl β-glucuronosides, especially estrogen derivatives, which are obtained in comparatively low yield by the Koenigs-Knorr method.

Experimental

General Procedure——All melting points were determined on a H_2SO_4 bath without correction. $[\alpha]_D$ values were determined on a Yanagimoto OR 50, and ORD spectra were recorded on a Jasco IRA-1 spectrometer. NMR spectra were recorded on a Hitachi R-22 apparatus using tetramethylsilane (TMS) as a internal standard. Silica gel (100 mesh) for column chromatography was obtained from Kanto Chemical Co., Inc. and TLC was performed on Silica gel HF254 with benzene–MeOH (100: 2) (A) and with $CHCl_3$–MeOH (100: 2) (B). β-Glucosidase from sweet almonds was obtained from Seikagaku Kogyo Co., Ltd. and beef liver β-glucuronidase was obtained from Tokyo Zoki Kagaku Co., Ltd.

***p*-Nitrophenyl-2,3,5-tri-O-benzoyl-β-D-ribofuranoside (II)**——Stannic chloride (2 ml) was added to a solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (I)[12] (3.5 g, 7 mmoles) and *p*-nitrophenol (2.0 g, 14.4 mmoles) in dichloroethane (20 ml) and the mixture was stirred at room temperature for 2 hr. The solution was diluted with dichloroethane and washed with water for the decomposition of $SnCl_4$. The organic layer was then washed with 1N NaOH and water, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was crystallized from acetone–isopropanol (1: 9, v/v) to afford II as colorless needles (2.1 g, 52%), mp 125—126°. $[\alpha]_D^{19}$ −55° (c=1.19, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{25}O_{10}N$: C, 65.86; H, 4.32; N, 2.40. Found: C, 65.57; H, 4.41; N, 2.58.

***p*-Nitrophenyl-β-D-ribofuranoside (III)**——A solution of II (1.17 g, 2 mmoles) in methanol (30 ml) was saturated with NH_3 in the usual manner, sealed completely and allowed to stand for 5 days at room temperature. The reaction mixture was concentrated and the residue was dissolved in water and washed with $CHCl_3$. After evaporation of the aqueous layer, the residue was crystallized from aqueous ethanol to affored III as somewhat yellowish needles (400 mg, 74%), mp 152—153°. $[\alpha]_D^{20}$ −121° (c=0.62, H_2O). ORD $[\alpha]_{331}^{20}$ −590° (c=0.3, H_2O) (trough) UV λ_{max}^{EtOH} 302 nm ($\varepsilon=1.16\times10^4$). NMR ($CD_3OD$) δ: 3.40—3.87 (2H, m, H-5,5′), 4.00—4.40 (3H, m, H-2, H-3, H-4), 5.70 (1H, s, H-1), 7.21 (2H, d, J=9 Hz, aromatic H), 8.20 (2H, d, J=9 Hz, aromatic H). *Anal.* Calcd. for $C_{11}H_{13}O_7N$: C, 48.71; H, 4.83; N, 5.16. Found: C, 48.56; H, 4.73; N, 5.35.

***p*-Nitrophenyl-2,3,4,6-tetra-O-acetyl-D-glucopyranoside (Vα, Vβ)**——A solution of β-D-pentaacetylglucose (IV)[13] (3.9 g, 10 mmoles) and *p*-nitrophenol (1.39 g, 10 mmoles) in dichloroethane (15 ml) was treated with $SnCl_4$ (1.5 ml) and stirred at room temperature for 3 hr. The reaction mixture was diluted with dichloroethane and washed with water. The organic layer was dried over Na_2SO_4 and evaporated under diminished pressure. The residue was crystallized from isopropanol to give Vβ as colorless needles (460 mg, 10%). The melting point and mixed melting point with authentic compound was 175—176°. $[\alpha]_D^{20}$ −32° (c=2.0, $CHCl_3$), (lit.[14] mp 174—175°, $[\alpha]_D$ −41°). ORD $[\alpha]_{360}^{20}$ −210° (c=0.1, $CHCl_3$) (trough). NMR ($CDCl_3$) δ: 2.0 (12H, 4×CH_3CO), 3.90 (1H, m, H-5), 4.15 (2H, m, H-6, 6′) 4.95—5.30 (4H, m, H-1, H-2, H-3, H-4), 6.99, 8.10 (4H, d, J=9 Hz aromatic H) *Anal.* Calcd. for $C_{20}H_{23}O_{12}N$: C, 51.18; H, 4.94; N, 2.98. Found: C, 51.26; H, 5.04; N, 3.19.

The mother liquor of the first crop of the β-anomer (Vβ) was chromatographed on a silica gel column (2×30 cm) using benzene–methanol (100: 1.5) as the eluting solvent. The fractions corresponding to Rf 0.53 on TLC with the same solvent were combined, evaporated under diminished pressure. The residue was crystallized from acetone–isopropanol (1: 9) to afford the α-anomer (Vα) (770 mg, 16%), mp 112—113°. $[\alpha]_D^{20}$ +191° (c=2.0, $CHCl_3$), (lit[14] mp 113°, $[\alpha]_D$ +200°). ORD $[\alpha]_{330}^{20}$ +1370° (c=0.1, $CHCl_3$) (trough). NMR ($CDCl_3$) δ: 2.0 (12H, 4×CH_3CO), 3.92 (1H, m, H-5), 4.10 (2H, m, H-6,6′), 4.90—5.24 (2H, m, H-2, H-4), 5.65 (1H, t, J=10 Hz, H-3), 5.80 (1H, d, J=4 Hz, H-1), 7.15 (2H, d, J=9 Hz, aromatic H), 8.14 (2H, d, J=9 Hz, aromatic H). *Anal.* Calcd. for $C_{20}H_{23}O_{12}N$: C, 51.18; H, 4.94; N, 2.98. Found: C, 51.22; H, 5.19; N, 3.00.

***p*-Nitrophenyl-α-D-glucopyranoside (VIα)**——A solution of Vα (300 mg, 0.64 mmole) in methanol (20 ml) was saturated with NH_3 in the usual manner and allowed to stand overnight at 4°. The solution was concentrated *in vacuo*, and the residue was crystallized from methanol to give VIα as colorless needles (150 mg,

12) E.F. Recond and H. Rinderknecht, *Helv. Chim. Acta*, **42**, 1171 (1959).
13) M.L. Wolfrom and A. Thompson, Method in Carbohydraye Chemistry, Vol. II, (Academic Press Inc., New York and London, 1963), p. 211.
14) E.M. Montgomery, N.K. Richtmyer, and C.S. Hudson, *J. Am. Chem. Soc.*, **64**, 690 (1942).

78%), mp 212—213° (decomp.). ORD $[\alpha]_{589}^{20}$ +264° (c=0.14, H_2O), (lit.[14] mp 216°, $[\alpha]_D$ +215°) *Anal.* Calcd. for $C_{12}H_{15}O_8N$: C, 47.84; H, 5.02; N, 4.65. Found: C, 47.88; H, 4.72; N, 4.71.

***p*-Nitrophenyl-β-D-glucopyranoside (VIβ)**——A solution of Vβ (300 mg, 0.64 mmole) in methanol (20 ml) saturated with NH_3 was allowed to stand overnight at 4°. After evaporation of the mixture the crystalline residue was obtained. This was recrystallized from EtOAc–MeOH as colorless needles (140 mg, 71%), mp 163—164°. ORD $[\alpha]_{589}^{20}$ −126° (c=0.1, H_2O). (lit.[14] mp 164°, $[\alpha]_D$ −103°). *Anal.* Calcd. for $C_{12}H_{15}O_8N \cdot 1/2\ H_2O$: C, 46.45; H, 5.20; N, 4.51. Found: C, 46.66; H, 5.25; N, 4.75.

Methyl (*p*-Nitrophenyl-2,3,4-tri-O-acetyl-β-D-glucopyranosid) uranate (VIII)——A mixture of methyl-1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronate[15] (VII) (1.88 g, 5 mmoles) and *p*-nitrophenol (1.39 g, 10 mmoles) in dichloroethane (20 ml) was treated with $SnCl_4$ (2.0 ml) and stirred at room temperature for 3 hr. The reaction mixture was diluted with dichloroethane, washed with water, then with 1N NaOH, finally with water. The organic layer was dried over Na_2SO_4 and evaporated under diminished pressure. The residue was crystallized from methanol to afford VIII as colorless needles (630 mg, 28%), mp 152—153°. $[\alpha]_D^{20}$ −30° (c=0.8, $CHCl_3$). (lit.[16] mp 151—152°, $[\alpha]_D$ −48°). ORD $[\alpha]_{360}^{20}$ −280° (c=0.1, $CHCl_3$) (trough). NMR ($CDCl_3$) δ: 2.0 (9H, s, 3×CH_3CO), 3.65 (3H, s, $COOCH_3$), 4.20 (1H, m, H-5), 5.13—5.43 (4H, m, H-1, H-2, H-3, H-4), 7.02 (2H, d, J=9 Hz, aromatic H), 8.13 (2H, d, J=9 Hz, aromatic H). *Anal.* Calcd. for $C_{19}H_{21}O_{12}N$: C, 50.11; H, 4.65; N, 3.08. Found: C, 49.80; H, 4.46; N, 3.23.

***p*-Nitrophenyl-β-D-glucopyranosiduronic Acid (IX)**——A solution of VIII in acetone (5 ml) and 1N NaOH (5 ml) was stirred at room temperature for 0.5 hr, then percolated through a column of Dowex 50 (×8) H^+ (200—400 mesh) (4 ml) for a removal of Na^+, and the column was washed with water. The whole filtrate was evaporated under reduced pressure. The residue was crystallized by the method described by Kato *et al.*,[16] namely, the residue was dissolved in a small amount of EtOAc saturated with water, and to which ether was added untill the solution became turbid and crystallized to give IX as colorless crystals (190 mg, 60%), mp 135—137°. ORD $[\alpha]_{589}^{20}$ −97° (c=0.35, H_2O). (lit.[16] mp 137—139°, $[\alpha]_D$ −108°). IR ν_{max}^{KBr} 1740 cm^{-1} (COOH). *Anal.* Calcd. for $C_{12}H_{13}O_9N$: C, 47.72; H, 4.16; N, 4.44. Found; C, 46.12; H, 4.42; N, 4.15.

Methyl (17β-Acetoxyestra-1,3,5(10)-triene-3-yl-2,3,4-tri-O-actyl-β-D-glucopyranosid) uronate (X)——Stannic chloride (1.5 ml) was added to a solution of VII (750 mg, 2 mmoles) and estra-1,3,5(10)triene-3,17β-diol 17-acetate (estradiol 17-acetate) (630 mg, 2 mmoles) in dichloroethane (30 ml). The mixture was stirred at 10° for 2 hr, washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to give a crystalline residue. This was recrystallized from acetone–methanol to afford X as colorless needles (620 mg, 49%), mp 222—224°. ORD $[\alpha]_{589}^{20}$ +16°, $[\alpha]_{285}^{20}$ −224° (trough) (c=0.25, $CHCl_3$) NMR ($CDCl_3$) δ: 0.82 (3H, s, 18–CH_3) 2.04 (12H, s, 4×CH_3CO) 3.75 (3H, s, $COOCH_3$), 4.19 (1H, m, H-5), 5.07—5.50 (4H, m, H-1, H-2, H-3, H-4), 6.73—7.35 (3H, m, aromatic H). *Anal.* Calcd. for $C_{33}H_{42}O_{12}$: C, 62.84; H, 6.71. Found: C, 62.70; H, 6.74.

Sodium (17β-Hydroxyestra-1,3,5(10)-triene-3yl-β-D-glucopyranosid) uronate (XI)——Methanolic 1N NaOH (2 ml) was added to a solution of X (200 mg, 0.32 mmole) in acetone (15 ml) and methanol (20 ml). The mixture was allowed to stand at room temperature for 24 hr and then concentrated to about 10 ml under reduced pressure to afford the sodium salt (XI) as colorless needles (130 mg, 87%). This was recrystallized from methanol. mp 244—245° (decomp.) (lit.[17] mp 256—260°). ORD $[\alpha]_{589}^{20}$ −249° (trough) (c=0.19, H_2O). IR ν_{max}^{KBr} 1610 cm^{-1} (COONa). *Anal.* Calcd. for $C_{24}H_{31}O_8Na \cdot 2H_2O$: C, 56.91; H, 6.97. Found: C, 57.33; H, 7.05.

Methyl (16α,17β-Diacetoxyestra-1,3,5(10)-triene-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (XII)——A solution of VII (600 mg, 1.6 mmoles) and estra-1,3,5(10)-triene-3,16α,17β-triol 16,17-diacetate (500 mg, 1.35 mmoles) in dichloroethane (20 ml) was treated with $SnCl_4$ (1.5 ml) at 10° for 2 hr. The reaction mixture was diluted with dichloroethane, washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was crystallized from methanol to afford XIIβ as colorless needles (420 mg, 45%), mp 191—193°. (lit.[17] mp 192—194°, $[\alpha]_D$ −31°). ORD $[\alpha]_{589}^{20}$ −20°, $[\alpha]_{285}^{20}$ −356° (trough) (c=0.25, $CHCl_3$). NMR ($CDCl_3$) δ: 0.85 (3H, s, 18–CH_3), 2.04 (15H, CH_3CO), 3.73 (3H, s, $COOCH_3$), 4.18 (1H, m, H-5), 4.88—5.48 (6H, m, H-1, H-2, H-3, H-4, 16β-H, 17α-H), 6.63—7.27 (3H, m, aromatic-H). *Anal.* Calcd. for $C_{35}H_{44}O_{14}$: C, 61.04; H, 6.44. Found: C, 61.27; H, 6.43.

The mother liquor of XIIβ was submitted to preparative TLC with solvent (B) and the α-anomer (XIIα) was separated in amorphous (50 mg). ORD $[\alpha]_{589}^{20}$ +50°, $[\alpha]_{285}^{20}$ +580° (peak) (c=0.2, $CHCl_3$). NMR ($CDCl_3$) δ: 0.83 (3H, s, 18–CH_3), 2.04 (15H, 5×CH_3CO), 3.71 (3H, s, $COOCH_3$), 4.41 (1H, d, J=10 Hz, H-5) 4.87—5.93 (6H, m, H-1, H-2, H-3, H-4, 16β-H, 17α-H), 6.72—7.28 (3H, m, aromatic H).

Sodium (16,α17β-Dihydroxyestra-1,3,5(10)-triene-3-yl-β-D-glucopyranosid) uronate (XIIIβ)——Methanolic 1N NaOH (2.2 ml) was added to a solution of XIIβ (200 mg, 0.29 mmole) in methanol (15 ml). The mixture was allowed to stand at room temperature for 24 hr and concentrated to about 5 ml under reduced

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pressure to afford the sodium salt (XIII) as colorless needles (120 mg, 79%), mp 247—249° (decomp.) (lit.[17] mp 272.5—281°). ORD $[\alpha]_{589}^{20}$ −33°, $[\alpha]_{284}^{20}$ −333° (trough) (c=0.12, H_2O). IR ν_{max}^{KBr} 1610 cm^{-1} (COONa). *Anal.* Calcd. for $C_{24}H_{31}O_9Na \cdot 2H_2O$: C, 55.17; H, 6.75. Found: C, 54.73; H, 6.83.

Methyl (*p*-Nitrobenzyl-2,3,4-tri-O-acetyl-α-D-glucopyranosid) uronate (XIVα)——A solution of VII (1.88 g, 5 mmoles) and *p*-nitrobenzyl alcohol (0.92 g, 6 mmoles) in dichloroethane (20 ml) was treated with $SnCl_4$ (2 ml) and stirred at room temperature (25—30°) for 2 hr. The mixture was diluted with dichloroethane and washed with water. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with chloroform as eluting solvent. The fractions (TLC *Rf* 0.33 with $CHCl_3$) were combined and evaporated under reduced pressure. The residue was crystallized from methanol to afford XIVα as colorless crystals (690 mg, 30%), mp 90—92°. ORD $[\alpha]_{589}^{20}$ +106°, $[\alpha]_{305}^{20}$ +930° (peak) (c=0.18, $CHCl_3$). UV λ_{max}^{EtOH} 267 nm (ε=1.3×10⁴). NMR ($CDCl_3$) δ: 2.02 (9H, 3×CH_3-CO), 3.77 (3H, s, $COOCH_3$), 4.35 (1H, d, J=10 Hz, H-5), 4.72, 4.92 (2H, each d, J=14 Hz, CH_2), 4.85—5.76 (4H, m, H-1, H-2, H-3, H-4), 7.58 (2H, d, J=9 Hz, aromatic H), 8.30 (2H, d, J=9 Hz, aromatic H). *Anal.* Calcd. for $C_{20}H_{23}O_{12}N$: C, 51.17; H, 4.94; N, 2.98. Found: C, 50.91; H, 4.77; N, 2.89.

Methyl (*p*-Nitrobenzyl-2,3,4-tri-O-acetyl-β-D-glucopyranosid) uronate (XIVβ)——A mixture of VII (2.6 g, 7 mmoles) and *p*-nitrobenzyl alcohol (1.3 g, 8 mmoles) in dichloroethane (50 ml) was treated with $SnCl_4$ (1.5 ml) and stirred under cooling at 0—5° for 2 hr. The solution was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was crystallized from methanol to afford XIVβ as colorless needles (490 mg, 15%), 176—177°. ORD $[\alpha]_{589}^{20}$ −57°, $[\alpha]_{315}^{20}$ −340° (trough) (c=0.21, $CHCl_3$). UV λ_{max}^{EtOH} 267 nm (ε=1.3×10⁴). NMR ($CDCl_3$) δ: 2.0 (9H, s, 3×CH_3CO), 3.77 (3H, s, $COOCH_3$), 4.10 (1H, dd, H-5), 4.75, 5.05 (2H, each d, J=12 Hz, CH_2), 4.65—5.40 (4H, m, H-1, H-2, H-3, H-4), 7.51 (2H, d, J=9 Hz, aromatic H) 8.22 (2H, d, J=9 Hz, aromatic H). *Anal.* Calcd. for $C_{20}H_{23}O_{12}N$: C, 51.17; H, 4.94; N, 2.98. Found: C, 51.27; H, 4.88; N, 3.23.

Methyl (Trichloroethyl-2,3,4-tri-O-acetyl-α-D-glucopyranosid) uronate (XVα)——A mixture of VII (750 mg, 2 mmoles) and trichloroethanol (600 mg, 4 mmoles) in dichloroethane (20 ml) was treated with $SnCl_4$ (1.5 ml) at room temperature (30°) for 2 hr. The solution was diluted with dichloroethane, washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was crystallized from methanol to give XVα as colorless needles (510 mg, 55%), mp 145—146°. ORD $[\alpha]_{589}^{20}$ +74°, a positive plane curve with no peak (c=0.19, $CHCl_3$). NMR ($CDCl_3$) δ: 2.03 (9H, 3×CH_3CO), 3.75 (3H, s, $COOCH_3$), 4.15, 4.26 (2H, d, J=11 Hz, CH_2), 4.38 (1H, d, J=10 Hz, H-5), 4.75—5.80 (4H, m, H-1, H-2, H-3, H-4). *Anal.* Calcd. for $C_{15}H_{19}O_{10}Cl_3$: C, 38.69; H, 4.11. Found: C, 38.81; H, 4.21.

Methyl (Trichloroethyl-2,3,4-tri-O-acetyl-β-D-glucopyranosid) uronate (XVβ)——A solution of VII (1.88 g, 5 mmoles) and trichloroethanol (0.89 g, 6 mmoles) in dichloroethane (20 ml) was treated with $SnCl_4$ (2 ml) under cooling at 0—5° for 2 hr, diluted with dichloroethane and washed with water. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was crystallized from methanol to give XVβ as colorless needles (1.05 g, 45%), mp 160—161°. (lit.[18] mp 157—158°). ORD $[\alpha]_{589}^{20}$ −53°, a negative plane curve with no trough (c=0.15, $CHCl_3$). NMR ($CDCl_3$) δ: 2.04 (9H, 3×CH_3CO), 3.78 (3H, s, $COOCH_3$), 4.10 (1H, m, H-5), 4.19, 4.45 (2H, d, J=12 Hz, CH_2), 4.89—5.40 (4H, m, H-1, H-2, H-3, H-4). *Anal.* Calcd. for $C_{15}H_{19}O_{10}Cl_3$: C, 38.69; H, 4.11. Found: C, 38.69; H, 4.13.

Enzymatic Hydrolysis of *p*-Nitrophenyl Glucopyranosides (VIα, VIβ) with β-Glucosidase from Sweet Almonds——VIα and VIβ (*ca.* 800 μg) were individually treated with β-glucosidase from sweet almonds (500 units) in 0.08M phosphate buffer (pH 6.8, 2.5 ml) containing glutathion (2.5 mg) at 37° for 3 hr. The incubated medium was boiled at 100° for 3 min and centrifuged at 2500×*g*. The supernatant was concentrated and submitted to paper chromatography on Whatmann No. 3 using aqueous 16% $(NH_4)_2CO_3$ together with VIα, VIβ, and *p*-nitrophenol for 17 hr. The incubated sample VIβ was hydrolyzed to give free *p*-nitrophenol, while that of VIα was not.

Enzymatic Hydrolysis of *p*-Nitrophenyl Glucuronoside (IX), Estradiol 3-Glucuronoside Na Salt (XI) and Estriol 3-Glucuronoside Na Salt (XIIIβ) by Beef Liver β-Glucuronidase——IX (*ca.* 500 μg) was treated with beef β-glucuronidase (4000 Fishman units) in 0.05M AcOH–NaOH buffer (pH 5.2, 2 ml) at 37° for 3 hr. The incubated fluid was boiled at 100° for 3 min and centrifuged at 2500×*g*. The supernatant was concentrated and submitted to paper chromatography on Whatmann No. 3 using *n*–BuOH–H_2O (86: 14) together with IX and *p*-nitrophenol. IX was hydrolyzed completely to give free *p*-nitrophenol. XI and XIIIβ (*ca.* 500 μg) were individually treated with β-glucuronidase by the same method described in hydrolysis of IX and submitted to TLC with $CHCl_3$–MeOH (20: 1) followed by spraying H_2SO_4. XI and XIIIβ were completely hydrolyzed to give free estradiol and estriol, respectively.

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