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Studies of Oligosaccharides. XIV.[1] Structure-Activity Relationship of Variations in the Sugar Moiety of Digitoxin

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Digitoxigenin oligosaccharides of the 1,6-β-linked D-glucose series and 1,4-β-linked D-digitoxose series were obtained by the Königs-Knorr condensation of digitoxigenin with gentio oligosaccharides and by the partial hydrolysis of digitoxin, followed by chromatographic fractionation of products, respectively. Cardiac activity as well as toxicity increased and decreased with the increasing number of unit monosaccharides for glycosides of the D-glucose and the D-digitoxose series, respectively. There was observed a parallelism between the activity as well as toxicity and the lipophilicity of these glycosides.

Among a number of studies on structure-activity relationship of glycosides, discussions on sugar moieties have been relatively few, though the important role of sugar moieties may be anticipated from the fact that most glycosides lose or diminish their activities, whereas increase their toxicities, on the elimination of sugars from their molecules. In a previous paper of this series we reported the syntheses of ester, ether and ester–ether type glycosides of glycyrrhetinic acid by coupling its carboxyl and/or hydroxyl groups to gentio oligosaccharides, and discussed on the hydrophilizing effect of sugars in these synthetic analogues of glycyrrhezin.[3] Our effort to elucidate the role of sugar moieties in glycosides have been extended to a physical and pharmacological study of digitoxin analogues. In this paper we describe the synthetic substitution of the sugar moiety in this glycoside with gentio oligosaccharides, and present comparative data on cardiac activity as well as toxicity of these synthetic analogues of the 1,6β-linked D-glucose series, along with glycosides of 1,4β-linked D-digitoxose series which were prepared by partial hydrolysis of digitoxin. This biological activity as well as toxicity are discussed in relation to the hydrophilicity of these glycosides.

Digitoxigenin Glycosides of the 1,6β-Linked D-Glucose Series

The Königs-Knorr condensation of digitoxigenin (I, 3β,14-dihydroxy-5β-card-20(22)-enolide) with acetobromo sugars is suitable for the syntheses of C-3 glycosylated modifications of digitoxin (VII), since this condensation reaction has been proven to be specific to the secondary hydroxyl group of this aglycone.[4] The less reactive tertiary hydroxyl group at C-14 has been noted to remain not glycosylated during the condensation. Instead, this hydroxyl group is extremely sensitive to desiccating agents and the aglycone tends to undergo dehydration forming anhydrodigitoxigenin derivatives. For this reason the use of desiccating agents such as dried magnesium sulfate and Drierite should be avoided, and azeotropic distillation[5] was employed, with satisfactory results, to facilitate the condensation reaction by eliminating the water formed during condensation from the benzene medium. Thus, the acetates of

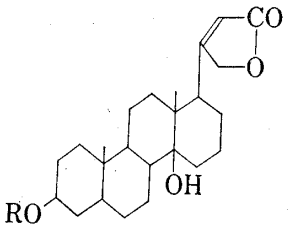
1) Part XIII: K. Takiura, M. Yamamoto, H. Murata, H. Takai, S. Honda, and H. Yuki, *Yakugaku Zasshi*, **94**, 998 (1974).
2) Location: *6-1-1 Toneyama, Toyonaka, Osaka.*
3) K. Takiura, S. Honda, M. Yamamoto, H. Takai, M. Kii, and H. Yuki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1618 (1974).
4) R.C. Elderfield, F.C. Uhle, and J. Fried, *J. Amer. Chem. Soc.*, **69**, 2235 (1947).
5) Ch. Meystre and K. Miescher, *Helv. Chim. Acta*, **27**, 231 (1944).

digitoxigenin 3β-O-D-glucopyranoside (II), -gentiobioside (III) and -gentiotrioside (IV) were obtained in fairly good yields minimizing the dehydration reaction, by condensation of I with respective acetobromo sugars in benzene in the presence of silver carbonate, followed by chromatographic purification of the products.

The proton magnetic resonance (PMR) spectrum of II acetate gave a doublet of the H-1′ proton at τ 5.45 with a spacing of 7.8 Hz, indicating that the anomeric configuration is β. Comparison of its specific rotation (−8.2°) with the predicted values for α- (+77°) and β-(−1°) linked glycosides, calculated according to the isorotation rule[6] also supported this anomeric assignment. The anomeric configurations of the sugar-aglycone likages in the acetates of III and IV were determined to be also β from similar considerations.

When these condensates were subjected to normal deacetylation conditions, the aglycone part underwent isomerization to isodigitoxigenin. However, the use of a weak alkali such as 0.004M methanolic barium methoxide depressed this side reaction, and glycosides II, III and IV were obtained with the aglycone intact. All mass spectra of these glycosides as well as the aglycone gave an intense peak at *m/e* 374 which was ascribable to the molecular ion of I. At identical conditions the dehydration product obtained by acidification of I gave an intense peak at *m/e* 356, whereas no peak at *m/e* 374. Thin-layer chromatography (TLC) and paper chromatography (PC) examinations indicated that almond β-glucosidase was active to only interglycosidic linkages, giving II from III as well as IV, whereas inactive to the newly formed sugar-aglycone linkages.

Digitoxigenin Glycosides of the 1,4β-Linked D-Digitoxose Series



I : (digitoxigenin) R=H
II : R=β-D-Glu
III : R=β-D-Glu-(1→6)-β-D-Glu
IV : R=β-D-Glu-(1→6)-β-D-Glu-(1→6)-β-D-Glu
V : R=β-D-Dig
VI : R=β-D-Dig-(1→4)-β-D-Dig
VII : (digitoxin) R=β-D-Dig-(1→4)-β-D-Dig-(1→4)-β-D-Dig
Glu: glucopyranose
Dig: digitoxopyranose

Chart 1

Digitoxigenin glycosides of D-digitoxose oligosaccharides are of structural and pharmacological interests, since D-digitoxose is a characteristic 2,6-dideoxy sugar, the distribution in nature of which is limited to digitalis glycosides. The synthesis of 3β-O-β-D-digitoxoside has been reported,[7] whereas the 1,4β-linked bis-D-digitoxoside has been supplied only by partial degradation of VII (the 1,4β-linked tri-D-digitoxoside),[8,9] though synthetic studies are in progress in our laboratory. In this study the monoside (V) and the bioside (VI), along with I, were prepared by partial hydrolysis of VII in dilute aqueous methanolic sulfuric acid according to the literature,[8] followed by our simplified method of fractionation of the hydrolysate on a silica gel column.

Hydrophilicity

Partition coefficients of digitoxin analogues between water and *n*-butanol were determined by ultraviolet (UV) absorption spectroscopy. As seen from Table I glycosides of the D-glucose series gave stronger hydrophilicity than those of the D-digitoxose series. In the D-glucose series the hydrophilicity increased with the increasing number of the unit monosaccharide, whereas a reverse relationship was observed for the D-digitoxose series.

6) T. Reichstein, *Angew. Chem.*, **63**, 412 (1951). These values were obtained from the following specific rotations: digitoxigenin, +19.1°; methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside, +130.5°; methyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside, −18.2°.
7) W.W. Zorbach, N. Henderson, and S. Saeki, *J. Org. Chem.*, **29**, 2016 (1964).
8) F. Kaiser, E. Haack, and H. Spingler, *Ann.*, **603**, 75 (1957).
9) D. Satoh and K. Aoyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 94 (1970).

Table I. Water–Oil Distribution of Digitoxin Analogues

Compound	Water/*n*-BuOH partition coefficient (37°)	Compound	Water/*n*-BuOH partition coefficient (37°)
I	0	V	2.61×10^{-2}
II	4.65×10^{-2}	VI	1.82×10^{-2}
III	25.2×10^{-2}	VII	1.37×10^{-2}
IV	128×10^{-2}		

Inotropic Effect

Digitalis and its preparations are known to have positive inotropic effect on heart muscles, and evaluation of these drugs as cardiacs has been performed by measuring mechanical responses of isolated hearts which are suspended in drug solutions. As summarized in Table II positive inotropic action on isolated guinea pig atria[10] was observed for the aglycone I and glycosides of the D-digitoxose series (V—VII) at drug concentrations higher than 2×10^{-8}M. The minimal effective concentrations for glycosides of the D-glucose series increased from this level (2×10^{-7}M) for the monoside II to 2×10^{-5}M for the trioside IV with the increasing number of D-glucose residues. From these observations it was indicated that the coupling of both types of monosaccharides to the aglycone brought about no particular change of inotropic action, whereas the introduction of gentio oligosaccharides exerted depressive effect on this positive inotropic activity. The structures of unit monosaccharides are also considered to have influence on this biological action, since there were differences of minimal effective concentrations between the glycosides of both series having the same degree of polimerization (III and VI; IV and VII). D-Digitoxose which is the constituent of the natural glycoside VII appeared to be more effective than D-glucose.

Table II. Positive Inotropic Effect of Digitoxin Analogues on Guinea Pig Atria

Compound	Positive inotropic effect[a] Drug concentration (M)			
	2×10^{-8}	2×10^{-7}	2×10^{-6}	2×10^{-5}
I	−	+	+	+
II	−	+	+	+
III	−	−	±	+
IV	−	−	−	+
V	−	+	+	+
VI	−	+	+	+
VII	−	+	+	+

a) The signs, + and −, indicate that the drugs gave positive and no inotropic effect (average of 4 experiments for each concentration), respectively.

Toxicity

Table III summarizes the mice intravenous median lethal doses (LD_{50}'s) during 24 hr of digitoxin analogues. With D-glucoside II only one death was observed for a dose of 310 nanomoles/10 g body weight, and gentiobioside III and gentiotrioside IV did not produce any deaths even for doses of 780 and 730 nanomoles, respectively. From these data it was shown that the LD_{50} values for glycosides of the D-glucose series (II—IV) were at least several times as high as those of glycosides of the D-digitoxose series (V—VII). On the other hand the toxicity of glycosides of the D-digitoxose series appeared to increase with the increasing

10) B.F. Roth-Schechter, G.T. Okita, D. Anderson, and F. Richardson, *J. Pharmacol. Exptl. Therap.*, 171, 249 (1970).

number of D-digitoxose residues. This observation accorded with the results reported by Large and Spratt.[11]

Occurrence of convulsions during 24 hr after injection was a measure of central toxicities of these drugs. Among the seven drugs tested the aglycone I gave the most prominent convulsions even for doses lower than its LD_{50}, and this central sign always preceeded the onset of deaths. With glycosides of the D-digitoxose series, the monoside V and the bioside VI caused convulsions for doses slightly higher than their LD_{50} levels, whereas this central sign was observed only in a few cases at approximately its LD_{50} level for the trioside VII. Glycosides of the D-glucose series did not produce convulsions for any doses injected. Convulsion is considered to be a typical sign resulted from direct toxicity to the central nervous system, and hence the occurrence of this sign appears to be related to the affinity of drugs to this strongly lipophilic organ. Since this affinity should vary inversely as the hydrophilicity of these drugs, it may be rationalized based on the data in Table I, that glycosides of the D-digitoxose series which are less hydrophilic were more toxic than glycosides of the D-glucose series. It is also reasonable that the introduction of gentio oligosaccharides with higher degree of polymerization should give rise to weaker binding strength to the site of this biological action, exerting smaller central toxicities, and the relationship between the degree of polymerization and toxicity was reversed for glycosides of the D-digitoxose series.

TABLE III. Mice Intravenous LD_{50}'s of Digitoxin Analogues

Compound	LD_{50} (nanomole/10 g body weight)	Compound	LD_{50} (nanomole/10 g body weight)
I	36	V	208
II	>310	VI	126
III	>780	VII	85
IV	>730		

From the preceding observations it is not likely that the sugar moieties in digitoxin analogues could act as the active centers of these cardiacs. However, their auxiliary function that regulates the affinity of these drugs to various organs by controling hydrophilicity–lipophilicity balance may be suggested. Hydrophilic sugars such as gentio oligosaccharides bring about less affinity to hearts as well as the central nervous system than lipophilic sugars of the D-digitoxose series. Although the introduction of such hydrophilic sugars to the aglycone reduces its cardiac activity, the decrease in toxicity will lead to the development of safer drugs.

Experimental[12]

3β-O-β-D-Glucopyranosyl-14-hydroxy-5β-card-20(22)-enolide (II)——This glycoside was synthesized by

11) G.L. Large and J.L. Spratt, *J. Pharmacol. Exptl. Therap.*, **152**, 501 (1966).

12) All evaporations were carried out below 40° under diminished pressure. Melting points were determined on a hot stage with a Yanagimoto micro melting point apparatus and are uncorrected. Specific rotations were measured in a 0.5-dm tube photoelectrically with a Yanagimoto OR-20 polarimeter. UV and infrared (IR) absorption spectra were obtained with Shimazu UV-200 and Hitachi EPI-G2 spectrophotometers, respectively. PMR spectra were observed at 90 MHz with a Hitachi R-22 spectrometer using tetramethylsilane as the internal standard. Mass spectra were obtained with a Hitachi RUM-6L instrument at an ionization potential of 70 eV. TLC was performed on glass plates (5×20 cm) coated with Wakogel B-5. Detection of spots were effected by spraying with concentrated sulfuric acid, followed by heating in an oven. Ascending PC was carried out on Whatman No. 1 filter paper with 6:4:3 *n*-BuOH–pyridine–water. Spots were visualized with alkaline silver nitrate.[13]

13) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature*, **166**, 444 (1950).

a modification of the literature.[14] The mixture of digitoxigenin, (I,160 mg, 0.43 mmole), freshly prepared silver carbonate (160 mg, 0.58 mmole) and benzene (30 ml) was heated with constant stirring, shielding from the light and the atmospheric moisture, until one third of benzene was distilled off. Subsequently a benzene solution (50 ml) containing acetobromoglucose (320 mg, 0.78 mmole) was added dropwise to the reaction mixture, during 5 hr, while the distillation speed was regulated so as to keep the volume of the reaction mixture constant. TLC (2: 1 $CHCl_3$–AcOEt) examination indicated the disappearance of the starting materials and the appearance of two main spots along with three faster moving minor spots. The reaction mixture was filtered through Celite (Hyflo-super-cel) and the filtrate was evaporated to dryness to give a thick syrup, which was fractionated on a silica gel column (Wakogel C-200, 50 g) with 9: 1 $CHCl_3$–AcOEt. The main component having higher mobility on TLC eluted first, followed by the second main component which was less mobile on TLC. The latter fraction was further separated on another column (Wakogel C-200, 50 g) with 3: 1 ether–petroleum ether into two chromatographically homogeneous fractions. The compound obtained from the first fraction of the second column was crystallized from ether to give needles, mp 125—127°. *Anal.* Calcd. for $C_{14}H_{20}O_{10}$ (D-glucose tetraacetate): C, 48.27; H, 5.79. Found: C, 47.99; H, 5.68.

The other fraction of the second column gave the crystalline acetate of the glucoside (II acetate, 119 mg, 39%), mp 181—184°; $[\alpha]_D^{15}$ −8.2° (c=0.69, $CHCl_3$). Lit.[4] mp 163—168°; $[\alpha]_D^{25}$ −8.6° (c=0.35, EtOH). *Anal.* Calcd. for $C_{37}H_{52}O_{13}$: C, 63.05; H, 7.44. Found: C, 63.00; H, 7.35. UV λ_{max}^{EtOH} nm(ε): 218 (15400). IR ν_{max}^{Nujol} cm^{-1}: 1625 (C=C), 1720, 1780 (CO of the lactone), 1760 (CO of the acetyl group), 3400 (OH). PMR ($CDCl_3$) τ: 4.13 (1H, s-like, H-22), 5.05 (1H, s-like, H-21), 5.12 (1H, s-like, H-21), 5.45 (1H, d, H-1′, $J_{1',2'}$= 7.8 Hz), 5.96 (1H, t-like, H-3), 4.6—6.5 (6H, m, sugar ring protons), 7.23 (1H, d-like, H-17), 7.5—9.2 (24H, m, steroid ring protons and OH), 7.93 (3H, s, $COCH_3$), 7.97 (9H, s, 3×$COCH_3$), 9.08 (3H, s, 3×H-19), 9.12 (3H, s, 3×H-18).

A part of II acetate (88 mg) was dissolved in MeOH (13 ml), and to this solution was added 0.25M methanolic barium methoxide (0.20 ml). After 3 hr the reaction solution was deionized by stirring with Amberlite IR-120 (H^+) and IRA-410 (CO_3^{2-}) resins, and the deionized solution was evaporated to dryness. The resultant solid mass was crystallized from EtOH–ether to give chromatographically homogeneous II in nearly quantitative yield, mp 233—235°. Lit.[4] 242—246°. UV λ_{max}^{EtOH} nm(ε): 218 (12500). IR ν_{max}^{Nujol} cm^{-1}: 1620 (C=C), 1720, 1750 (CO of the lactone), 3400 (OH). Mass spectrum m/e: 374 (the parent peak of I). Glycoside II (1 mg) was dissolved in an aqueous solution (0.1 ml) containing β-glucosidase (1 mg, Sigma Chemicals Co. Ltd., lot 125B 0100, prepared from almond), and the solution was incubated at 35° for 3 hr. TLC (4: 1 $CHCl_3$–MeOH) examination indicated the presence of only one spot, *Rf* 0.70. Glycoside II gave *Rf* 0.70.

From the first fraction of the first column a crystalline compound was obtained. Recrystallization from EtOH–ether afforded needles, mp 226—228°; $[\alpha]_D^{15}$ −7.7° (c=1.04, $CHCl_3$). *Anal.* Calcd. for $C_{37}H_{50}O_{12}$ (dehydrated derivative of II acetate): C, 64.70; H, 7.34. Found: C, 64.35; H, 7.17. UV λ_{max}^{EtOH} nm(ε): 213 (19000). IR ν_{max}^{Nujol} cm^{-1}: 1625 (C=C), 1750 (CO of the acetyl group), 1780 (CO of the lactone).

3β-O-β-Gentiobiosyl-14-hydroxy-5β-card-20(22)-enolide (III)——The reaction mixture obtained from the reaction of I (200 mg, 0.54 mmole), silver carbonate (400 mg, 1.45 mmole) and acetobromogentiobiose (700 mg, 1.00 mmole) in a similar manner as described for the synthesis of II acetate gave three main spots on TLC (2: 1 $CHCl_3$–AcOEt). The reaction mixture was worked up similarly and chromatographed on a silica gel column (Wakogel C-200, 100 g) with 4: 1 $CHCl_3$–AcOEt. The component with the highest TLC mobility eluted first in homogeneous state. Subsequently the other two components eluted with incomplete separation. This fraction was rechromatographed on another column (Wakogel C-200, 100 g) with 6: 1 ether–petroleum ether with good resolution of components. The first component crystallized from CH_2Cl_2–ether–petroleum ether to give needles, mp 179—180°. *Anal.* Calcd. for $C_{26}H_{36}O_{18}$ (gentiobiose heptaacetate): C, 49.06; H, 5.70. Found: C, 49.10; H, 5.66. IR ν_{max}^{Nujol} cm^{-1}: 1740 (CO of the acetyl group), 3450 (OH).

Purification of the second component of the second column by precipitation from EtOH–H_2O afforded the acetate of the bioside (III acetate, 240 mg, 44%) as amorphous powder, mp 128—134°; $[\alpha]_D^{15}$ −9.7° (c=1.64, $CHCl_3$). *Anal.* Calcd. for $C_{49}H_{68}O_{21}$: C, 59.26; H, 6.90. Found: C, 59.31; H, 6.96. UV λ_{max}^{EtOH} nm(ε): 218 (13300). IR ν_{max}^{Nujol} cm^{-1}: 1625 (C=C), 1720, 1780 (CO of the lactone), 1750 (CO of the acetyl group), 3400 (OH). PMR ($CDCl_3$) τ: 4.14 (1H, s-like, H-22), 5.45 (1H, d, H-1′, $J_{1',2'}$=7—8 Hz), 5.97 (1H, t-like, H-3), 4.7—6.3 (15H, m, 2×H-21 and sugar ring protons), 7.26 (1H, d-like, H-17), 7.5—9.2 (24H, m, steroid ring protons and OH), 7.93—8.03 (21H, m, 7×$COCH_3$), 9.10 (3H, s, 3×H-19), 9.13 (3H, s, 3×H-18).

Deacetylation of III acetate in a similar manner as described for the deacetylation of II acetate afforded amorphous glycoside III in nearly quantitative yield. UV λ_{max}^{EtOH} nm(ε): 219 (12800). IR ν_{max}^{Nujol} cm^{-1}: 1620 (C=C), 1740, 1780 (CO of the lactone), 3400 (OH). Mass Spectrum m/e: 374 (the parent peak of I). Incubation of glycoside III with the β-glucosidase solution in a similar manner as described for glycoside II gave

14) K. Reyle, K. Meyer, and T. Reichstein, *Helv. Chim. Acta*, 33, 1541 (1950).

two spots on TLC, *Rf* 0.70 and 0.03. Glycoside II and D-glucose gave *Rf* 0.70 and 0.03, respectively. D-Glucose was also detected on PC.

From the first fraction of the first column was obtained another glycoside acetate, mp 190—196°; $[\alpha]_D^{15}$ −14.7° (c=1.18, $CHCl_3$). *Anal.* Calcd. for $C_{49}H_{66}O_{20}$ (dehydrated derivative of III acetate): C, 60.36; H, 6.82. Found: C, 60.03; H, 6.67. UV λ_{max}^{EtOH} nm(ε): 211 (18500). IR ν_{max}^{Nujol} cm^{-1}: 1625 (C=C), 1750 (CO of the acetyl group), 1780 (CO of the lactone).

3β-O-β-Gentiotriosyl-14-hydroxy-5β-card-20(22)-enolide (IV)——The reaction mixture obtained from the reaction of I (200 mg, 0.54 mmole), silver carbonate (200 mg, 0.72 mmole) and acetobromogentiotriose[15] (1.00 mmole) in a similar manner as described for the synthesis of II acetate, except that a minimum amount of CH_2Cl_2 was added to the benzene medium to effect complete solubilization of acetobromogentiotriose, gave a main spots on TLC (1:1 $CHCl_3$–AcOEt) along with three minor spots. The reaction mixture was worked up similarly and fractionated on a silica gel column (Wakogel C-200, 100 g) with 1:1 $CHCl_3$–AcOEt. The main fraction was rechromatographed on another column (Wakogel C-200, 100 g) with 9:1 ether–petroleum ether to give well resolved two fractions. The first fraction was crystallized and recrystallized from CH_2Cl_2–ether–petroleum ether to give needles, mp 163—167°. *Anal.* Calcd. for $C_{38}H_{52}O_{26}$ (gentiotriose decaacetate): C, 49.35; H, 5.67. Found: C, 49.51; H, 5.63. IR ν_{max}^{Nujol} cm^{-1}: 1740 (CO of the acetyl group), 3450 (OH).

The second fraction gave the crystalline acetate of the triose (IV acetate, 260 mg, 38%) from EtOH–H_2O. Recrystallization from the same solvent system afforded needles, mp 168—169°; $[\alpha]_D^{15}$ −14.1° (c=0.95, $CHCl_3$). *Anal.* Calcd. for $C_{61}H_{84}O_{28}$: C, 57.17; H, 6.61. Found: C, 57.18; H, 6.45. UV λ_{max}^{EtOH} nm(ε): 218 (14000). IR ν_{max}^{Nujol} cm^{-1}: 1625 (C=C), 1720, 1780 (CO of the lactone), 1750 (CO of the acetyl group). PMR ($CDCl_3$) τ: 4.16 (1H, s-like, H-22), 5.45 (1H, d, H-1′, $J_{1'\,2'}$=7—8 Hz), 5.97 (1H, t-like, H-3), 4.7—6.5 (22H, m, 2×H-21 and sugar ring protons), 7.25 (1H, d-like, H-17), 7.5—9.2 (24H, m, steroid ring protons and O<u>H</u>), 7.93—8.03 (30H, m, 10×COC<u>H</u>$_3$), 9.10 (3H, s, 3×H-19), 9.13 (3H, s, 3×H-18).

Deacetylation of IV acetate in a similar manner as described for the deacetylation of II acetate, except that a minimum amount of CH_2Cl_2 was added to the reaction mixture to effect complete solubilization, afforded amorphous glycoside IV in nearly quantitative yield. UV λ_{max}^{EtOH} nm(ε): 218 (12400). IR ν_{max}^{Nujol} cm^{-1}: 1620 (C=C), 1740, 1780 (CO of the lactone), 3400 (OH). Mass Spectrum m/e: 374 (the parent peak of I). Incubation of glycoside IV with the β-glucosidase solution in a similar manner as described for II gave two spots on TLC, *Rf* 0.70 and 0.03. Glycoside II and D-glucose gave *Rf* 0.70 and 0.03, respectively. D-Glucose was also detected on PC.

Partial Hydrolysis of Digitoxin (VII)——Hydrolysis condition was the same as described in the literature.[8] To a methanolic solution (670 ml) containing VII (1.00 g) was added 0.025M H_2SO_4. The solution was heated at 37° for 45 min. After rapid cooling the reaction solution was deionized by stirring with Amberlite IRA-410 (CO_3^{2-}) resin, and concentrated to 50 ml. The solid material separated during concentration was collected and washed with water. This material was dissolved in $CHCl_3$ and to this solution was added a small amount of silica gel. The mixture was gently evaporated by a rotary evaporator, and the resultant gel was introduced to a silica gel column (Wakogel C-200, 100 g). Fractionation with 2:2:3 ether–tetrahydrofuran–petroleum ether afforded digitoxigenin (I), the mono-D-digitoxoside (V) and bis-D-digitoxoside (VI) in this order in chromatographically homogeneous states.

I: Crystallization and recrystallization from AcOEt–petroleum ether afforded needles (89 mg, 16%), mp 241—243°; $[\alpha]_D^{18}$ +17.6° (c=0.62, EtOH). Lit.[16] mp 250°; $[\alpha]_D^{17}$ +19.1° (c=1.36, EtOH). *Anal.* Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.60; H, 9.09. UV λ_{max}^{EtOH} nm(ε): 218 (16500). IR ν_{max}^{Nujol} cm^{-1}: 1620 (C=C), 1740, 1780 (CO), 3500 (OH). Mass Spectrum m/e: 374 (the parent peak).

V: Crystallization and recrystallization from AcOEt–ether–petroleum ether afforded needles (103 mg, 19%), mp 190—195° $[\alpha]_D^{18}$ +1.4° (c=0.52, MeOH). Lit.[8] mp 181—184°. Lit.[9] mp 197—200°; $[\alpha]_D^{28}$ −5.2° (c=0.33, MeOH). *Anal.* Calcd. for $C_{29}H_{44}O_7$: C, 69.02; H, 8.79. Found: C, 68.65; H, 8.82. UV λ_{max}^{EtOH} nm(ε): 218 (15000). Lit.[9] 218 (15090). IR ν_{max}^{Nujol} cm^{-1}: 1620 (C=C), 1750, 1785 (CO), 3450 (OH). Mass Spectrum m/e: 374 (the parent peak of I).

VI: Crystallization and recrystallization from AcOEt–ether–petroleum ether afforded needles (132 mg, 16%), mp 186—189°; $[\alpha]_D^{18}$ +6.9° (c=0.58, MeOH). Lit.[8] mp 187—190°. Lit.[9] mp 228—230°; $[\alpha]_D^{28}$ +7.3° (c=0.83, MeOH). *Anal.* Calcd. for $C_{35}H_{54}O_{10}$: C, 66.22; H, 8.57. Found: C, 66.25; H, 8.57. UV λ_{max}^{EtOH} nm(ε): 218 (14500). Lit.[9] 217.5 (14200). IR ν_{max}^{Nujol} cm^{-1}: 1620 (C=C), 1750, 1785 (CO), 3450 (OH). Mass Spectrum m/e: 374 (the parent peak of I).

Water/*n*-BuOH Partition Coefficients——Digitoxin analogues (1—2 mg) were dissolved in water-satd. *n*-BuOH (5.00 ml) and to these solutions were added *n*-BuOH-satd. water (5.00 ml). The mixtures were shaken constantly on a temperature-controlled water bath for 10 hr, and then allowed to stand for 24 hr. Aliquots of both phases (2.00 ml) were evaporated to dryness and the residues were dissolved in appropriate

15) K. Takiura, S. Honda, T. Endo, and K. Kakehi, *Chem. Pharm. Bull.* (Tokyo), **20**, 438 (1972).
16) A. Windaus and G. Stein, *Ber.*, **61**, 2436 (1928).

volumes of ethanol and the UV absorption was read at λ_{max}'s. Partition coefficients were obtained as the ratios of absorbancies of both phases.

Inotropic Activity——The method was based on the literature described by Roth-Schechter, *et al.*[10] Atria were removed from male albino guinea pigs weighing *ca.* 300 g, and placed in a muscle bath containing 25 ml of Tyrode buffer at 30° which was oxygenated by continuous flow of 95% O_2–5% CO_2. After equibration periods of 1 hr, samples of digitoxin analogues were added to the bath media in ethanolic solutions. Controls were provided by addition of equal volumes of EtOH without samples. For the bioside III and the trioside IV of the D-glucose series, which were soluble in water, aqueous solutions were used in stead of ethanolic solutions. Doses of four different levels shown in Table II were used for each sample. Contraction and quiescence of atria were expanded to about ten-fold scales and recorded during exposure to drugs for 30 min. The atria were washed with the stock solution of bath media for several times until contraction and quiescence were restored to the control levels, and allowed to stand in drug-free media for some periods before used for the next exposures.

Lethality——The method of Large and Spratt[11] was used for obtaning mice LD_{50} values. Each group of seven adult male DDY mice weighing 23.3 g on the average was fed with food and water *ad libitum* both before and after injection of drugs. Each animal was used only once. Injections were prepared by dissolving digitoxin analogues in appropriate volumes of 33.3% EtOH, and 0.05 ml of a preparation was injected to each animal *via* the tail vein. Control injections containing equal amount of EtOH caused no change during 24 hr. LD_{50} values were obtained from the number of deaths during 24 hr after injection.

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