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Constituents of Chinese Crude Drug "Wujiapi." VIII.[1] On the Structures of New Oligosaccharides C_1, D_2, F_1 and F_2 of Bei-Wujiapi

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Four new type oligosaccharides C_1, D_2, F_1 and F_2 of Bei-Wujiapi (cortex of *Periploca sepium* Bge.) were elucidated to be 2-*O*-acetyl-β-D-digitalopyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-L-oleandronic acid-δ-lactone (Ia), 2-*O*-acetyl-β-D-digitalopyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-canaropyranosyl(1→4)-β-D-digitoxopyranosyl(1→4)-L-oleandronic acid-δ-lactone (IIa), β-D-digitalopyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-L-oleandronic acid-δ-lactone (IIIa) and β-D-digitalopyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-canaropyranosyl(1→4)-β-D-digitoxopyranosyl(1→4)-L-oleandronic acid-δ-lactone (IVa).

It should be noted that Ia, IIa, IIIa and IVa are the first oligosaccharides composed of 2,6-dideoxysugars and 2,6-dideoxyaldonic lactone.

Furthermore, it is interesting that the sugar sequences of these oligosaccharides are ruled by the regularity in cardiac and pregnane type glycosides of Asclepiadaceous plants.

Keywords——oligosaccharides; oligosaccharide C_1; oligosaccharide D_2; oligosaccharide F_1; oligosaccharide F_2; Bei-Wujiapi; *Periploca sepium* Bge; Asclepiadaceae; 2,6-dideoxy sugar; aldonic acid lactone

In our previous papers, we reported the isolation and structural elucidation of cardiac glycoside, periplocin,[3] and pregnane glycosides, namely glycoside E,[1] H_1[4] and K,[3] of the

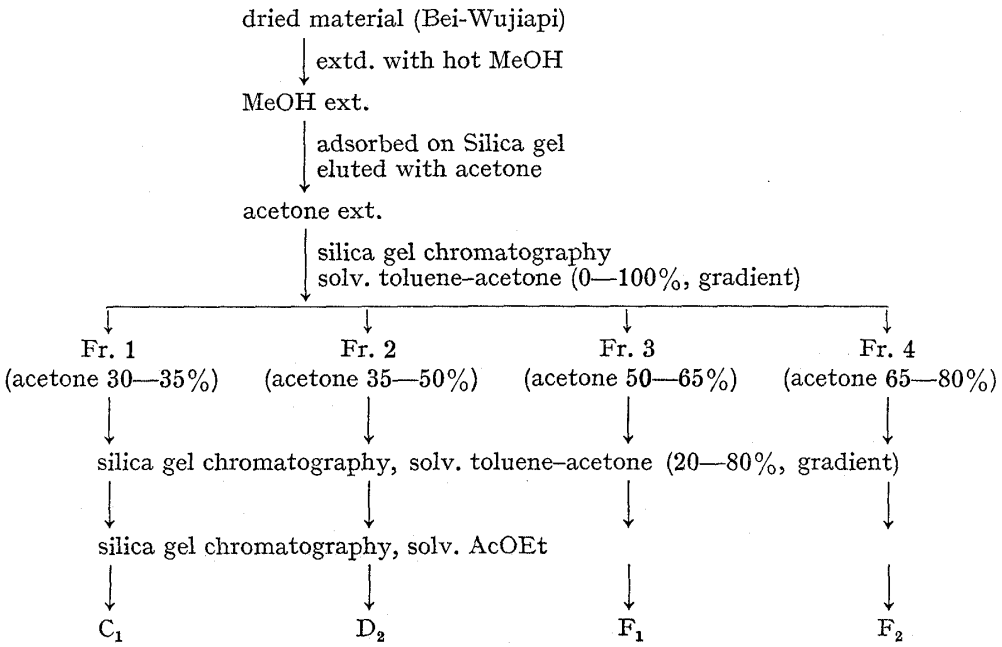


Chart 1. Extraction and Isolation of Oligosaccharides

1) Part VII: H. Ishizone, S. Sakuma, S. Kawanishi, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **20**, 2402 (1972).
2) Location: *Hatanodai, Shinagawa-ku, Tokyo*; *a*) Present address: *Institute of Pharmaceutical Sciences, Hiroshima University, School of Medicine, 1-2-3 Kasumi, Hiroshima-shi.*
3) S. Sakuma, H. Ishizone, R. Kasai, S. Kawanishi, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 52 (1971).
4) S. Kawanishi, S. Sakuma, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **20**, 469 (1972).

chinese crude drug, "Bei-Wujiapi" (cortex of *Periploca sepium* Bge., Asclepiadaceae). The present paper deals with the study on the structural elucidations of new oligosaccharides named oligosaccharide C_1(Ia), D_2(IIa), F_1(IIIa) and F_2(IVa).

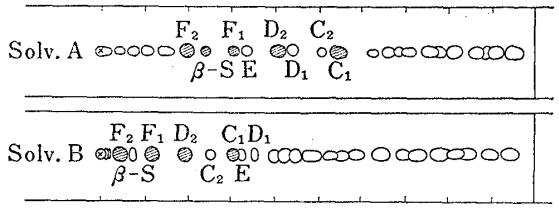


Fig. 1. Thin-Layer Chromatograms of Acetone Extract of "Bei-Wujiapi"

Plate: Kieselgel H.
Solv.: A) toluene: acetone (1:1),
B) AcOEt.
Color reag. : 10 % H_2SO_4.
β-S: β-sitosterol-β-D-glucoside.
E: glycoside E[1].

The isolation of Ia, IIa, IIIa and IVa from the acetone soluble fraction of methanol extract of Bei-Wujiapi was carried out as shown in Chart 1. The thin-layer chromatograms (TLC) of acetone extract are shown in Fig. 1. The yield of each Ia, IIa and IIIa was 0.001% and that of IVa was 0.0005% on the dried crude drug basis.

The general properties of Ia, IIa, IIIa and IVa are given in Table I. All of these oligosaccharides show positive reaction with xanthohydrol reagent, but they have not any reducing property.

On refluxing with 0.05 N sulfuric acid, Ia, IIa, IIIa and IVa afforded the same lactonic compound (Va) and the sugars shown in Table II.

Table I

Oligo-saccharides	Properties	mp (°C)	$[\alpha]_D$ (°C)	Formula	IR cm^{-1}	NMR
C_1 (Ia)	Colorless needles (EtOH)	221	+59.3°(26) (c=0.74 in $CHCl_3$)	$C_{30}H_{50}O_{15}$	3600—3200(OH) 1745(δ-lactone and ester)[a]	4 CH-C$\underline{H}_3$[c] 1 COC$\underline{H}_3$ 4 OC$\underline{H}_3$
D_2 (IIa)	Colorless needles (MeOH)	252	+40.2°(26) (c=2.0 in $CHCl_3$)	$C_{35}H_{58}O_{18}$	3600—3200(OH) 1745(δ-lactone and ester)[a]	5 CH-C$\underline{H}_3$[c] 1 COC$\underline{H}_3$ 3 OC$\underline{H}_3$
F_1 (IIIa)	Colorless needles (MeOH)	238	+40.1°(25) (c=0.67 in EtOH)	$C_{23}H_{43}O_{14}$	3600—3200(OH) 1755(lactone)[b]	4 CH-C$\underline{H}_3$[c] 4 OC$\underline{H}_3$
F_2 (IVa)	Colorless needles (acetone)	259	+32.7°(18) (c=1.07 in dioxane)	$C_{33}H_{56}O_{17}$	3600—3200(OH) 1755(lactone)[b]	5 CH-C$\underline{H}_3$[d] 3 OC$\underline{H}_3$

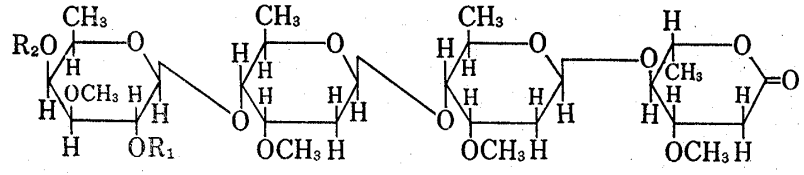
a) $CHCl_3$. b) KBr. c) $CDCl_3$. d) Pyridine-d_5.

Table II.

Oligosaccharides	The sugar components of oligosaccharides
C_1 (Ia)	D-Cymarose, acetyl biose (VIa)[a]
D_2 (IIa)	D-Digitoxose, D-canarose, acetyl biose (VIa)[a]
F_1 (IIIa)	D-Cymarose, biose (VIb)[b]
F_2 (IVa)	D-Digitoxose, D-canarose, biose (VIb)[b]

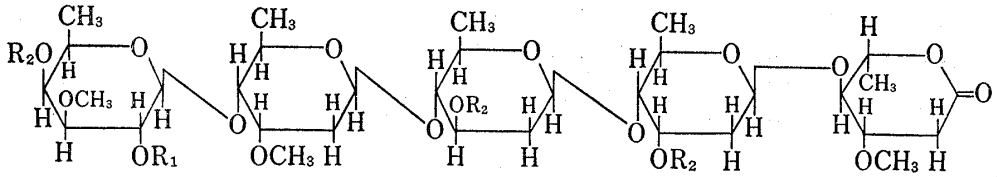
a) VIa=4-*O*-(2-*O*-acetyl-β-D-digitalopyranosyl)-β-D-cymaropyranose[5].
b) VIb=4-*O*-(β-D-digitalopyranosyl)-β-D-cymaropyranose.

The lactonic compound(Va) is a syrup, $[\alpha]_D^{22}$ +8.9°, and the infrared (IR) spectrum of Va reveals the presence of γ-lactone(1790 cm^{-1}), while the nuclear magnetic resonance(NMR) spectrum shows the presence of one of each secondary methyl group, hydroxyl group, methylene group adjacent to a carbonyl group, methoxyl group and three methine groups bearing an oxygen atom. Va was acetylated with acetic anhydride and pyridine to afford the monoacetate(Vb). $C_9H_{14}O_5$, mp 106°, $[\alpha]_D^{24}$ +12.8°. The structure of Vb was examined by the



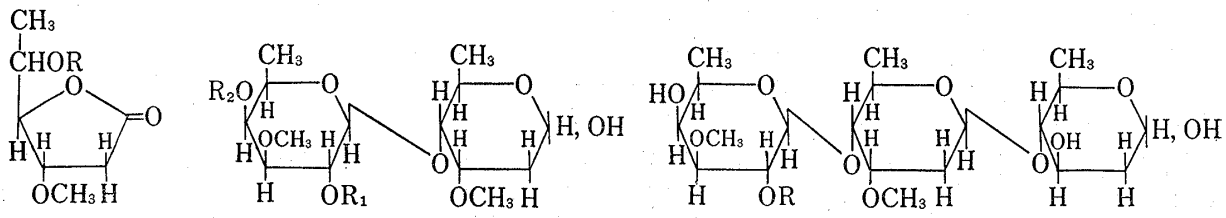
(D-digitalose) (D-cymarose) (D-cymarose) (L-oleandronic acid-δ-lactone)

Ia : $R_1=COCH_3$, $R_2=H$, Ib=IIIb : $R_1=R_2=COCH_3$, Ic : $R_1=COCH_3$, $R_2=CH_3$,
IIIa : $R_1=R_2=H$



(D-digitalose) (D-cymarose) (D-canarose) (D-digitoxose) (L-oleandronic acid-δ-lactone)

IIa : $R_1=COCH_3$, $R_2=H$, IIb=IVb : $R_1=R_2=COCH_3$, IIc : $R_1=COCH_3$, $R_2=CH_3$,
IVa : $R_1=R_2=H$



(D-digitalose) (D-cymarose) (D-digitalose) (D-cymarose) (D-canarose)

Va : R=H
Vb : R=$COCH_3$

VIa : $R_1=COCH_3$, $R_2=H$
VIb : $R_1=R_2=H$
VIc : $R_1=COCH_3$, $R_2=CH_3$

VII : R=$COCH_3$

Chart 2

application of spin–spin decoupling technique and it was deduced to be 5-*O*-acetyl-2,6-dideoxy-3-*O*-methylaldonic acid-γ-lactone(Fig. 2).

To confirm the structure, Va was treated with phenylhydrazine and a product was identified with an authentic sample of L-oleandronic acid phenylhydrazide[5] by comparing TLC and by a mixed fusion. The foregoing experiments have established the identity of Va with L-oleandronic acid-γ-lactone[6a,b] but the absorption band at 1745 cm^{-1} in the IR spectra of Ia, IIa, IIIa diacetate (IIIb) and IVa tetraacetate (IVb) shows that the lactonic compound of each oligosaccharide is assumed to be δ-lactone.

On acetylation with acetic anhydride and pyridine, Ia, IIa, IIIa and IVa afforded Ib, IIb, IIIb and IVb, respectively. The properties of these acetates are summarized in Table III. By a mixed fusion and by comparing TLC, optical rotation, IR and NMR spectra, Ib and IIb were proved to be identical with IIIb and IVb, respectively.

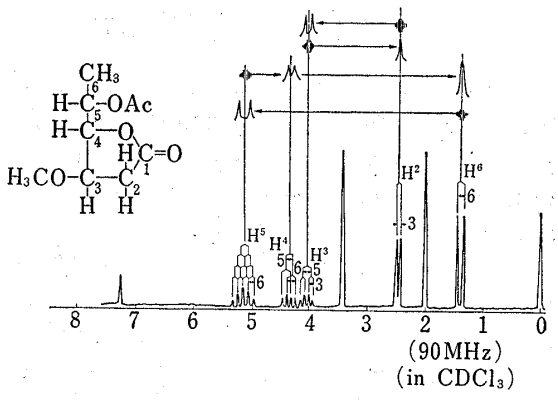


Fig. 2. NMR Spectrum of Vb

5) The sample was given by Prof. T. Reichstein, Universitat Basel.
6) *a*) H. Allgeier, *Helv. Chim. Acta*, **51**, 311, 668 (1968); *b*) A. Sauer and H. Allgeier, *ibid.*, **52**, 1655 (1969).

On methylation by the Kuhn's method,[7] Ia gave mono-*O*-methyloligosaccharide C_1(Ic), $C_{31}H_{52}O_{15}$, while IIa gave tri-*O*-methyloligosaccharide D_2(IIc), $C_{38}H_{64}O_{18}$. Hydrolysis of Ic with 0.05 N sulfuric acid gave Va, D-cymarose and 4-*O*-(2-*O*-acetyl-4-*O*-methyl-*β*-D-digitalopyranosyl)-*β*-D-cymaropyranose(VIc),[8] so that the terminal sugar of Ic is established to be D-digitalose. The intensity of all proton signals which assigned to D-cymarose in NMR spectrum of Ia indicates the presence of two moles of D-cymarose in Ia, and the chemical shift(δ=4.77 ppm) and the coupling constants (J_1=9, J_2=2 Hz) suggest that each cymarose must be *β*-pyranosyl form. Based on the experimental data, the structures of Ia and IIIa have been elucidated to be 2-*O*-acetyl-*β*-D-digitalopyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-L-oleandronic acid-*δ*-lactone and *β*-D-digitalopyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-L-oleandronic acid-*δ*-lactone, respectively.

The structures of IIa and IVa were deduced as follows. The terminal sugar of IIa was determined to be 2-*O*-acetyl-D-digitalose by the hydrolysis of IIc with 0.05 N sulfuric acid to afford Va, 3-*O*-methyl-D-digitoxose(=D-cymarose), 3-*O*-methyl-D-canarose(=D-oleandrose) and VIc. The partial hydrolysis of IIa with 0.001 N sulfuric acid gave Va, D-digitoxose and a new triose (VII), mp 250°, which was further hydrolyzed with 0.05 N sulfuric acid to give VIa and D-canarose. Consequently, the sugar linked to the lactonic compound was deduced to be D-digitoxose.

According to Allgeier[6a] the chemical shifts and the coupling constants of each anomeric proton of 2,6-dideoxysugars are :methyl *α*- and *β*-L-canaropyranoside δ=4.68, q, J_1=4, J_2=1 Hz; δ=4.36, q, J_1=10, J_2=2 Hz, methyl 4-*O*-D-thevetosyl-*β*-D-cymaropyranoside δ=4.66, q, J_1=9, J_2=2 Hz, methyl L-canarofuranoside δ=5.17, t, J=4 Hz, D-digitoxonic acid-*δ*-lactone (4)-*β*-D-cymaropyranoside δ=4.64, q. Present investigations have also revealed that the signals of anomeric protons of methyl 2,6-dideoxypyranosides appear at δ=4.3—4.9 ppm with the coupling constants of J_1=9—10 and J_2=1—3 Hz, while the chemical shifts of methyl 2,6-dideoxyfuranoside are lower than δ=5.0 ppm and the coupling constants are J_1=4—5, J_2=1—3 Hz(Table III). Accordingly, the configurations of D-cymarose, D-canarose and D-digitoxose of IIa are assumed to be all *β*-pyranosyl type.

Table III. *Rf* Value and Assignment of NMR Spectrum of Methyl 2, 6-Dideoxysugar

Methyl 2, 6-dideoxysugars	TLC *Rf*	NMR (in $CDCl_3$) anomeric proton
Methyl *β*-D-oleandropyranoside	0.65[a]	δ=4.84, q, J_1=10, J_2=2 Hz
Methyl *β*-D-digitoxopyranoside	0.41[a]	δ=4.75, q, J_1=9, J_2=2 Hz
Methyl D-oleandrofuranoside	0.70[a]	δ=5.39, q, J_1=4, J_2=1 Hz
Methyl D-digitoxofuranoside	0.36[a]	δ=5.11, q, J_1=4, J_2=1 Hz
Methyl *β*-D-cymarofuranoside	0.21[b]	δ=5.16, q, J_1=5, J_2=3 Hz
Methyl *α*-D-cymarofuranoside	0.31[b]	δ=5.08, q, J_1=4, J_2=2 Hz

a) Solv. toluene: acetone=1:2.
b) Solv. toluene: acetone=3:1.

From the foregoing observations, the structures of IIa and IVa have elucidated to be 2-*O*-acetyl-*β*-D-digitalopyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-*β*-D-canaropyranosyl(1→4)-*β*-D-digitoxopyranosyl(1→4)-L-oleandronic acid-*δ*-lactone and *β*-D-digitalopyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-*β*-D-canaropyranosyl(1→4)-*β*-D-digitoxopyranosyl(1→4)-L-oleandronic acid-*δ*-lactone, respectively.

The cardiac glycosides and pregnane type glycosides composed of 2,6-dideoxysugars are well known, but it should be noted that Ia, IIa, IIIa, and IVa are the first examples of oligosaccharides composed of 2,6-dideoxyaldonic lactone and 2,6-dideoxysugars.

Furthermore, it is interesting that the sugar sequences of these oligosaccharides are ruled by the regularity in cardiac and pregnane type glycosides of Asclepiadaceous plants.[9]

7) R. Kuhn, *Angew. Chem.*, **67**, 32 (1955).
8) S. Kawanishi, S. Sakuma, H. Okino, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **20**, 93 (1972).
9) S. Kawanishi, S. Sakuma, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **20**, 469 (1972).

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. IR absorption spectra were obtained with a Hitachi Model EPI-2. NMR spectra were measured with a Hitachi Model R-22 High Resolution NMR spectrometer (90 MHz). Gas chromatograph was run on a Hitachi Model K-53 with hydrogen flame ionization detector (3% SE-52, on Chromosorb W, 4 mm×2 m) and all sugars were injected as their tetramethylsilyl ether (TMS) derivatives. The *Rf* values were determined by thin-layer chromatography on Kieselgel H using the solvents: (A) toluene: acetone (1: 1), (B) AcOEt, (C) toluene: acetone (2: 1), (D) $CHCl_3$: MeOH: H_2O (7: 3: 1, lower phase) and 10% H_2SO_4 (Spraying followed by heating) as a staining agent.

Isolation of Oligosaccharide C_1(Ia), D_2(IIa), F_1(IIIa) and F_2(IVa)——The crushed material (3 kg) was extracted with hot MeOH. The MeOH extract (800 g) was adsorbed on silica gel and eluted with acetone. After evaporation of the solvent, the syrupy brown residue (230 g) was submitted to column chromatography on silica gel by the technique of gradient elution using toluene containing 0—100% acetone. The oligosaccharide-rich fraction was rechromatographed on silica gel using toluene containing 20—80% acetone. Finally pure Ia, IIa, IIIa and IVa were isolated by silica gel column chromatography using AcOEt.

Oligosaccharide C_1(Ia)——*Anal.* Calcd. for $C_{30}H_{50}O_{15}\cdot H_2O$: C, 53.88; H, 7.84. Found: C, 54.02; H, 7.94. NMR $\delta_{TMS}^{CDCl_3}$ ppm: 1.17 (3H×2,d, J=6 Hz, CH-C$\underline{H}_3$×2), 1.23 (3H, d, J=6 Hz, CH-C$\underline{H}_3$), 1.42 (3H, d, J=6 Hz, CH-C$\underline{H}_3$), 2.04 (3H, s, $COCH_3$), 2.70 (2H, d, J=3 Hz, COC$\underline{H}_2$-CH), 3.35 (3H, s, OCH_3), 3.42 (3H×3, s, OCH_3), 4.36 (1H, d, J=8 Hz digitalose-C_1H), 4.78 (1H×2, q, J_1=9, J_2=2 Hz, cymarose-C_1H), 5.10 (1H, q, J_1=10, J_2=8 Hz, digitalose-C_2H).

Oligosaccharide D_2(IIa)——*Anal.* Calcd. for $C_{35}H_{58}O_{18}\cdot H_2O$: C, 53.56; H, 7.71. Found: C, 53.42; H, 7.69. NMR $\delta_{TMS}^{CDCl_3}$ ppm: 1.2—1.5 (3H×5, d, CH-C$\underline{H}_3$×5), 2.07 (3H, s, $COCH_3$), 2.73 (2H, d, J=3 Hz, COC$\underline{H}_2$-CH), 3.40 (3H, s, OCH_3), 3.43 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 4.41 (1H, d, J=8 Hz, digitalose-C_1H), 4.57 (1H, q, J_1=10, J_2=2 Hz, canarose-C_1H), 4.77 (1H×2, q, J_1=9, J_2=2 Hz, cymarose-C_1 and digitoxose-C_1H), 5.07 (1H, q, J_1=10, J_2=8 Hz, digitalose-C_2H).

Oligosaccharide F_1(IIIa)——*Anal.* Calcd. for $C_{28}H_{48}O_{14}$: C, 55.25; H, 7.95. Found: C, 54.99; H, 8.06. NMR $\delta_{TMS}^{CDCl_3}$ ppm: 1.17 (3H, d, J=6 Hz, CH-C$\underline{H}_3$), 1.20 (3H×2, d, J=6 Hz, CH-C$\underline{H}_3$×2), 1.42 (3H, d, J=6 Hz, CH-C$\underline{H}_3$), 2.70 (2H, d, J=3 Hz, COC$\underline{H}_2$-CH), 3.32 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 3.44 (3H×2, s, OCH_3×2), 4.42 (1H, d, J=8 Hz), 4 .78 (1H×2, q, J_1=10, J_2=2 Hz).

Oligosaccharide F_2(IVa)——*Anal.* Calcd. for $C_{33}H_{56}O_{17}\cdot 1/2\ H_2O$: C, 54.01; H, 7.83. Found: C, 53.79; H, 7.57. NMR $\delta_{TMS}^{pyridine\text{-}d_5}$ ppm: 1.31 (3H, d, J=6 Hz, CH-C$\underline{H}_3$), 1.39 (3H×2, d, J=6 Hz, CH-C$\underline{H}_3$×2), 1.53 (3H×2, d, J=6 Hz, CH-C$\underline{H}_3$×2), 2.87 (2H, br., COC$\underline{H}_2$-CH), 3.38 (3H, s, OCH_3), 3.49 (3H, s, OCH_3), 3.53 (3H, s, OCH_3).

Acid Hydrolysis of Ia, IIa, IIIa and IVa——A solution of each sample (100 mg) in dioxane (2 ml) and 0.1 N H_2SO_4 (2 ml) was refluxed for 30 min on a water bath. The reaction mixture was cooled and extracted with AcOEt. The AcOEt extract was washed with water and evaporated *in vacuo* to give a powder. The aqueous layer was neutralized with Amberlite IR-4B and evaporated *in vacuo*.

Both AcOEt soluble fraction and water soluble fraction were examined by TLC using solvent A. The sugar components were shown in Table II. In AcOEt soluble fraction a lactonic compound (Va) (*Rf* 0.42), cymarose (*Rf* 0.33) and an acetylbiose (VIa) (*Rf* 0.22) were detected, while in water soluble fraction cymarose, VIa, digitoxose (*Rf* 0.18) were found. Each component was isolated by preparative TLC. D-Cymarose, D-digitoxose and D-canarose were identified with authentic samples by TLC, GLC and by comparison of the optical rotation. GLC: column temp. 120°, inj. temp. 210°, N_2 35 ml/min; t_R(min) cymarose 5.3, 6.3, 7.0, digitoxose 4.5, 7.8, 10.2, canarose 10.6, 13.6. Optical rotation: cymarose $[\alpha]_D^{22}$ +50.0° (c=2.40, H_2O), digitoxose $[\alpha]_D^{22}$ +54.2° (c=1.20, H_2O), canarose $[\alpha]_D^{22}$ +24.1° (c=1.76, H_2O).

VIa——VIa was crystallized from AcOEt-*n*-hexane to give colorless needles, mp 177°. *Anal.* Calcd. for $C_{16}H_{28}O_9$: C, 52.74; H, 7.75. Found: C, 52.84; H, 7.53. IR ν_{max}^{KBr} cm^{-1}: 3300—3500 (OH), 1730 (>C=O). VIa was identified with an authentic sample of 4-*O*-(2-*O*-acetyl-*β*-D-digitalopyranosyl)-*β*-D-cymaropyranose[8] by a mixed fusion and by comparing TLC, IR and NMR spectra.

Va——Va was obtained as a syrup, $[\alpha]_D^{22}$ +8.9° (c=2.82, acetone) (L-oleandronic acid lactone $[\alpha]_D$ +12.8° (c=1.0, acetone)[10]). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600—3400 (OH), 1790 (*γ*-lactone). NMR $\delta_{TMS}^{CDCl_3}$ ppm: 1.34 (3H, d, J=6 Hz, CH-C$\underline{H}_3$), 2.65 (1H, s, OH), 2.70 (2H, d, J=3 Hz, COC$\underline{H}_2$-CH), 3.38 (3H, s, OCH_3), 4.0—4.5 (1H×3, m, -C$\underline{H}$-O-×3).

Vb——Va was acetylated with Ac_2O in pyridine at room temperature for 48 hr. The reaction mixture was worked up as usual and the product was crystallized from AcOEt-*n*-hexane to give colorless needles, mp 106°, $[\alpha]_D^{24}$ +12.8° (c=0.51, EtOH). *Anal.* Calcd. for $C_9H_{14}O_5$: C, 53.46; H, 6.98. Found: C, 53.63; H, 6.81. IR $\nu_{max}^{CCl_4}$ cm^{-1}: OH (nil), 1790 (*γ*-lactone), 1740, 1230 (ester). NMR $\delta_{TMS}^{CDCl_3}$ ppm: 1.35 (3H, d, J=6 Hz, CH-C_6H_3), 2.03 (3H, s, OCOC$\underline{H}_3$), 2.63 (2H, d, J=3 Hz, $COC_2\underline{H}_2$-CH), 4.12 (1H, q, J_1=3, J_2=5 Hz, $C_3\underline{H}$-OCH_3), 4.39 (1H, q, J_1=5, J_2=6 Hz, CH-$C_4\langle^{O-}_{\underline{H}}$), 5.22 (1H, m, ABX$_3$ J_1=6, J_2=6 Hz, CH_3-

10) E. Vischer and T. Reichstein, *Helv. Chim. Acta*, **27**, 1332 (1944).

$C_5<^{OCOCH_3}_{H}$).

Phenylhydrazide of Va——To a solution of Va (19 mg) in EtOH (3 ml) was added phenylhydrazine (0.02 ml), and the mixture was evaporated *in vacuo* to dryness, and the residue was heated in a boiling water-bath for 30 min. After cooling, ether was added to the solution to form a flocculent precipitate. The precipitate was crystallized from mixed solvent of anhyd. EtOH–ether–*n*-hexane to give bundles of needles, mp 131°, which were identified with an authentic sample of L-oleandronic acid phenylhydrazide[5] by a mixed fusion and TLC (solvent A, *Rf* 0.13).

Acetylation of Ia, IIa, IIIa and IVa——A solution of each sample in pyridine and Ac_2O was allowed to stand for 48 hr at room temperature. The reaction mixture was worked up as usual and the product was crystallized.

Ib——Colorless needles from AcOEt–*n*-hexane, mp 179°, $[\alpha]_D^{26}$ +60.3° (c=1.16, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: OH (nil), 1745 (δ-lactone and ester), 1235 (ester). NMR $\delta_{TMS}^{CDCl_3}$ ppm: 1.17 (3H, d, J=6 Hz, CH–C<u>H</u>$_3$), 1.20 (3H×2, d, J=6 Hz, CH–C<u>H</u>$_3$), 1.42 (3H, d, J=6 Hz, CH–C<u>H</u>$_3$), 2.04 (3H, s, $COCH_3$), 2.12 (3H, s, $COCH_3$), 2.70 (2H, d, J=3 Hz, COC<u>H</u>$_2$–CH), 3.32 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 3.44 (3H×2, s, OCH_3×2), 4.16 (1H, d, J=8 Hz, digitalose-C_1H), 4.78 (1H×2, q, J_1=9, J_2=2 Hz, cymarose-C_1H), 5.10 (1H, q, J_1=10, J_2=8 Hz, digitalose-C_2H), 5.31 (1H, m, digitalose-C_4<u>H</u>–$OCOCH_3$). *Anal.* Calcd. for $C_{32}H_{52}O_{16}$: C, 55.48; H, 7.57. Found: C, 55.57; H, 7.28.

IIb——Colorless needles from EtOH, mp 178°, $[\alpha]_D^{26}$ +84.9° (c=0.48, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: OH (nil), 1745 (δ-lactone and ester), 1230 (ester). NMR $\delta_{TMS}^{CDCl_3}$ ppm: 1.1—1.3 (3H×4, d, CH–C<u>H</u>$_3$×4), 1.43 (3H, d, J=6 Hz, CH–C<u>H</u>$_3$), 2.03 (3H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), 2.10 (3H, s, $COCH_3$), 2.15 (3H, s, $COCH_3$), 2.72 (2H, d, J=3 Hz, COC<u>H</u>$_2$–CH), 3.33 (3H, s, OCH_3), 3.39 (3H, s, OCH_3), 3.43 (3H, s, OCH_3), 4.43 (1H, d, J=8 Hz, digitalose-C_1H), 4.53 (1H, q, J_1=10, J_2=2 Hz, canarose-C_1H), 4.63 (1H, q, J_1=9, J_2=2 Hz, cymarose-C_1H), 4.76 (1H, q, J_1=9, J_2=2 Hz, digitoxose-C_1H), 4.85 (1H, q, J_1=10, J_2=2 Hz, canarose-C_3<u>H</u>–$OCOCH_3$), 5.10 (1H, q, J_1=10, J_2=8 Hz, digitalose-C_2<u>H</u>–$OCOCH_3$), 5.33 (1H×2, m, digitalose-C_4<u>H</u>–$OCOCH_3$ and digitoxose-C<u>H</u>–$OCOCH_3$). *Anal.* Calcd. for $C_{41}H_{64}O_{21}$: C, 55.15; H, 7.22. Found: C, 55.32; H, 7.14.

IIIb——Colorless needles from AcOEt–*n*-hexane, mp 179°, $[\alpha]_D^{29}$ +66.8° (c=1.05, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{52}O_{16}$: C, 55.48; H, 7.57. Found: C, 55.77; H, 7.37. IIIb was identified with Ib by a mixed fusion and by comparing NMR spectra, IR spectra and optical rotations.

IVb——Colorless needles from EtOH, mp 178°, $[\alpha]_D^{23}$ +74.6° (c=0.52, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{64}O_{21}$: C, 55.15; H, 7.22. Found: C, 55.45; H, 7.03. IVb was identified with IIb by a mixed fusion and by comparing NMR spectra, IR spectra and optical rotations.

Permethylation of Ia and IIa——Ia and IIa were methylated by the Kuhn's method[7] for 120 hr at room temperature, respectively. After dilution with water, the reaction mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and concentrated to dryness. The residue was purified by preparative TLC and recrystallization. Ic was crystallized from EtOH to give colorless needles, mp 224°, $[\alpha]_D^{26}$ +72.0° (c=0.43, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{52}O_{15}$: C, 56.01; H, 7.89. Found: C, 55.56; H, 7.68. IR $\nu_{max}^{CCl_4}$ cm^{-1}: OH (nil). IIc was crystallized from AcOEt–*n*-hexane to give colorless fine needles, mp 202°. *Anal.* Calcd. for $C_{38}H_{64}O_{18}$: C, 56.42; H, 7.98. Found: C, 55.94; H, 7.62.

Acid Hydrolysis of Ic and IIc——Ic and IIc (20 mg) were refluxed with 0.1 N H_2SO_4 (1 ml) and dioxane (1 ml) for 30 min, respectively. The hydrolysates of Ic were Va, D-cymarose and VIc, which was further refluxed with methanolic 2 N HCl to give methyl 4-*O*-methyl-D-digitaloside. The identification of methyl 4-*O*-methyl-D-digitaloside with an authentic sample[8] was done by a mixed fusion (mp 105°) and by comparing TLC (solvent A: *Rf* 0.25, solvent B: 0.20) and GLC (column temp.: 150°, inj. temp.: 220°, N_2 35 ml/min t_R (min) 7.0). Furthermore, the hydrolysates of IIc were Va, 3-*O*-methyl-D-digitoxose (=D-cymarose), 3-*O*-methyl-D-canarose (=D-oleandrose) and VIc. D-Cymarose and D-oleandrose were identified with authentic samples by TLC (solvent A: *Rf* 0.33 (cymarose), 0.31 (oleandrose); solvent D: *Rf* 0.62 (cymarose), 0.45 (oleandrose)) and GLC (column temp.: 120°, inj. temp.: 210°, N_2 35 ml/min, t_R (min) cymarose: 5.3, 6.3, 7.0; oleandrose: 5.6, 6.3).

Partial Hydrolysis of IIa——A solution of IIa (50 mg) in dioxane (2 ml) was refluxed with 0.002 N H_2SO_4 (2 ml) for 30 min to give Va, D-digitoxose and a triose (VII) (5 mg), mp 250°, leaflets from AcOEt–*n*-hexane, which was refluxed with 0.05 N H_2SO_4 for 30 min to give VIa and D-canarose.

Preparation of Methyl 2,6-Dideoxypyranoside and Methyl 2,6-Dideoxyfuranoside for NMR Measurement——Each 2,6-dideoxyhexose was treated with methanolic 0.1 N HCl for 20 hr at room temperature and the product was purified by preparative TLC (solvent B).

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