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Constituents of Chinese Crude Drug "Wujiapi." V.[1] On the Structure of Glycoside H_1 of Bei-Wujiapi

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The chemical structure of glycoside H_1 (I), $C_{56}H_{92}O_{24}$, mp 182°, $[\alpha]_D^{24}$ −22.83° (EtOH), which was isolated from Bei-Wujiapi (cortex of *Periploca sepium* Bge.), was established to be Δ^5-pregnene-3β,20α-diol(3)-[2-O-acetyl-β-D-digitalopyranosyl($1_{dig}\rightarrow4_{cym}$)-$\beta$-D-cymaropyranoside](20)-[β-D-glucopyranosyl($1_{glu}\rightarrow6_{glu}$)-$\beta$-D-glucopyranosyl($1_{glu}\rightarrow2_{dig}$)-$\beta$-D-digitalopyranoside].

It should be noted that glycoside H_1 is the first example of the pregnane type glycoside whose sugar moiety links to both C-3 and C-20 hydroxyl groups of the aglycone and that the sugar sequences of Asclepiadaceous glycosides are ruled by certain regularity, such as aglycone-(2,6-dideoxysugar or its 3-O-methyl derivative)$_{0-l}$-(6-deoxysugar or its 3-O-methyl derivatives)$_{0-m}$-(glucose)$_{0-n}$.

In our previous paper,[3] it has been reported that the *n*-butanol soluble fraction of methanol extract of Chinese crude drug, Bei-Wujiapi (cortex of *Periploca sepium* Bge., Asclepiadaceae) was examined by thin-layer chromatography (TLC) and revealed to contain many glycosidic substances (A—N). Two crystalline glycosides, glycoside G and glycoside K, were isolated and established to be periplocin and Δ^5-pregnene-3β, 20α-diol(20)-β-D-glucopyranosyl-($1_{glu}\rightarrow6_{glu}$)-$\beta$-D-glucopyranosyl($1_{glu}\rightarrow2_{dig}$)-$\beta$-D-digitalopyranoside, respectively.

The present paper described the isolation and structure elucidation of glycoside H_1 which leads to the assignment of the structure I.

According to the previous paper,[3] the *n*-butanol soluble fraction of methanol extract of the crude drug was purified by column chromatography on silica gel eluted with ethyl acetate saturated with water and then with the same solvent containing 10—25% methanol. The fractions containing glycoside H, I, and J were repeatedly chromatographed on neutral alumina (Woelm) with chloroform:methanol:water=65:35:10 (lower phase) and finally glycoside H was obtained as a colorless powder. This substance was revealed to be the mixture of two glycosides, tentatively named glycoside H_1(I) and H_2, by paper partition chromatography (PPC) on Toyo Roshi No. 52 impregnated with formamide, developed with chloroform:tetrahydrofuran:pyridine/formamide=10: 10: 2/4[4] and antimony trichloride solution as a color reagent. Repeated recrystallization from methanol–ethyl acetate saturated with water gave glycoside H_1(I) as colorless needles (yield: 0.07% from dried crude drug).

The nuclear magnetic resonance (NMR) spectra and infrared (IR) spectra of I, $C_{56}H_{92}O_{24}$, mp 182°, $[\alpha]_D^{24}$ −22.83° (ethanol), showed the presence of one O-acetyl group, three O-methyl groups, two *tertiary* C-methyl groups, four *secondary* C-methyl groups and many hydroxyl groups.

On acid hydrolysis with 3N sulfuric acid–50% methanol for a half hour, I gave Δ^5-pregnene-3β,20α-diol(II), D-glucose and D-digitalose. The identification of II with an authentic sample was carried out by mixed fusion and comparison of TLC and IR spectra. The sugar components were detected by TLC, PPC and gas liquid chromatography (GLC).

1) Part IV: S. Kawanishi, S. Sakuma, H. Okino and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **20**, 93 (1972).
2) Location: *Hatanodai, Shinagawa-ku, Tokyo.*
3) S. Sakuma, H. Ishizone, R. Kasai, S. Kawanishi and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 52 (1971).
4) R. Tschesche and G. Wulff, *Chem. Ber.*, **94**, 2019 (1961).

Partial hydrolysis of I with 0.05N sulfuric acid–50% methanol refluxing for a half hour gave three main products (III, IV, V). The reaction mixture was treated as usual and extracted with chloroform. The aqueous phase was neutralized with Amberlite IR-4B, and then extracted with *n*-butanol. The chloroform soluble fraction was purified by column chromatography on silica gel with ethyl acetate. The first product (III), $C_{17}H_{30}O_9$, mp 171°, colorless needles from ethyl acetate, $[\alpha]_D^{23}$+23.5°(H_2O), IR ν_{max}^{KBr} cm^{-1}: 3550, 1750, 1240. NMR $\delta_{TMS}^{CDCl_3}$: 1.20 3H (d, J=6.4 cps), 1.38 3H (d, J=6.4 cps), 2.00 3H(s), 1.5—2.3 2H(m), 3.45 3H(s), 3.48 3H(s)×2, 4.42 1H(d, J=8.0 cps), 4.68 1H(q, J_1=9.6 cps, J_2=2.2 cps), 5.15 1H

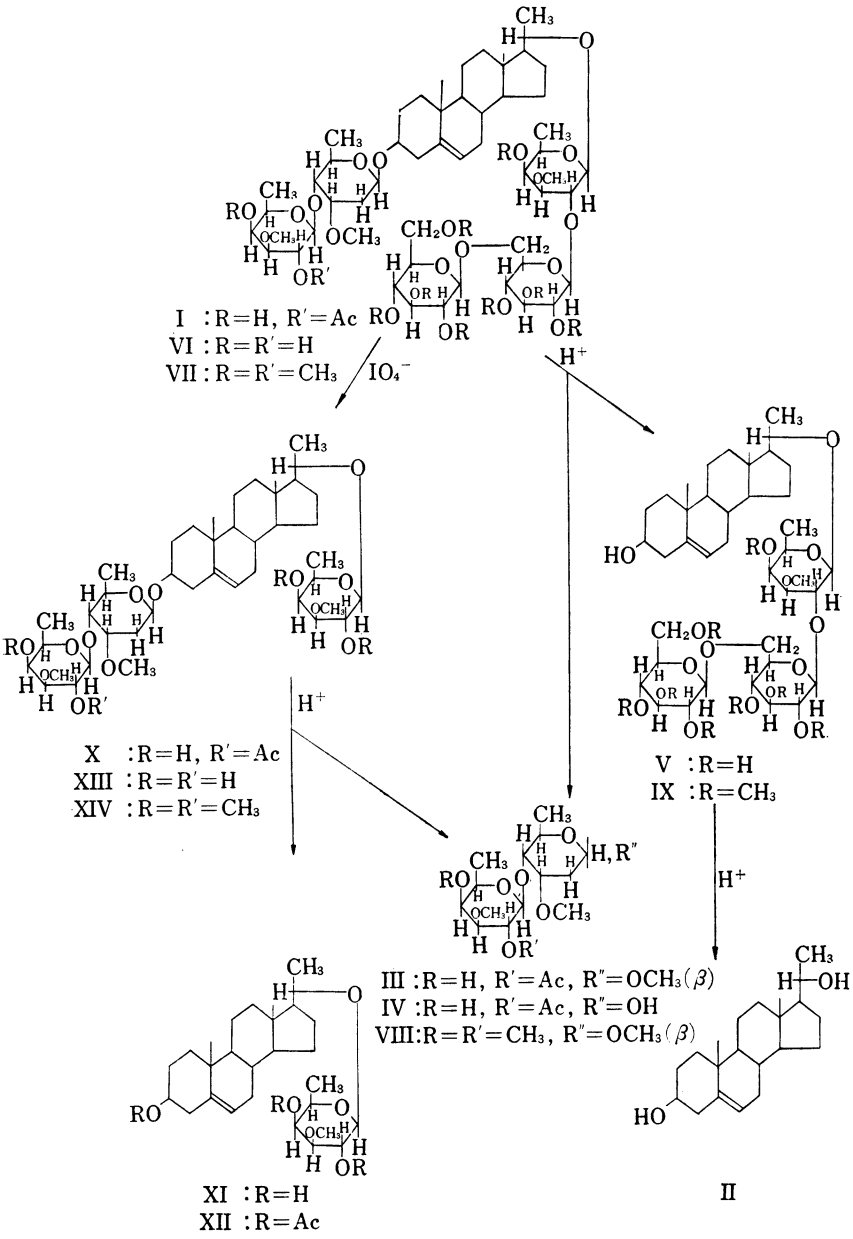

Fig. 1

(q, J_1=9.8 cps, J_2=8 cps), was deacetylated with 0.4N sodium hydroxide under nitrogen atmosphere. A deacetyl product of III was hydrolysed with 0.05N sulfuric acid–50% methanol to yield D-cymarose and D-digitalose which were detected by TLC and GLC of the reaction mixture. The product III was identified with an authentic sample of methyl 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-β-D-cymaropyranoside[1] by mixed fusion, TLC, NMR, and IR spectra.

The second product (IV), $C_{16}H_{28}O_9$, mp 177°, colorless needles from *n*-hexane–ethyl acetate, $[\alpha]_D^{24}$+64.5°(H_2O), IR ν_{max}^{KBr} cm^{-1}: 3400, 1745, 1240, was also identified with an authentic sample of 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-D-cymarose[1] by mixed fusion, TLC and IR spectra.

The third product (V), which was obtained from the *n*-butanol extract of the foregoing hydrolysate, $C_{40}H_{66}O_{16}$, mp 240—241°, colorless needles from methanol–ethyl acetate saturated with water, $[\alpha]_D^{25}$—27.58° (methanol), IR ν_{max}^{KBr} cm^{-1}: 3400(OH, broad), NMR δ_{TMS}^{Py}: 0.68 3H(s), 1.00 (s), 1.45 3H(d, J=6 cps)×2, 3.48 3H(s), was hydrolysed with 3N sulfuric acid–50% methanol for a half hour to give Δ^5-pregnene-3β,20α-diol(II), D-glucose and D-digitalose. Nona-O-methylether of V, $C_{49}H_{84}O_{16}$, which was obtained by methylation (Hakomori's method[5]) of V, was hydrolysed with refluxing 2N sulfuric acid in 50% methanol to give 3-O-methyl-Δ^5-pregnene-3β,20α-diol, 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4-tri-O-methyl-D-glucose and 4-O-methyl-D-digitalose which were characterised by TLC, mixed fusion and IR spectra.

From these experimental results, the compound (V) was deduced to be Δ^5-pregnene-3β,-20α-diol(20)-β-D-glucopyranosyl($1_{glu}\rightarrow6_{glu}$)-β-D-glucopyranosyl($1_{glu}\rightarrow2_{dig}$)-β-D-digitalopyranoside.[3] The identification with an authentic sample was carried out by mixed fusion and the comparison of TLC, NMR and IR spectra.

From the foregoing observations, I was suggested to be constituted from Δ^5-pregnene-3β,20α-diol-(20)-β-D-glucopyranosyl($1_{glu}\rightarrow6_{glu}$)-β-D-glucopyranosyl($1_{glu}\rightarrow2_{dig}$)-β-D-digitalopyranoside(V) and 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-D-cymaropyranose(IV).

To confirm the point of attachment of IV to V, I was deacetylated with 0.4N sodium hydroxide under nitrogen flow to give VI, $C_{50}H_{90}O_{23}$, mp 175—176°, colorless needles, $[\alpha]_D^{24}$ —26.08°(ethanol). Upon permethylation by Hakomori's method, VI afforded VII, $C_{64}H_{110}O_{23}$, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: OH(nil.). On methanolysis with refluxing 0.05N hydrogen chloride–methanol, VII gave two products, namely VIII and IX. The one compound, VIII, $C_{17}H_{32}O_8$, mp 106°, colorless needles, was identified with an authentic sample of methyl 4-O-(2,4-di-O-methyl-β-D-digitalopyranosyl)-β-D-cymaropyranoside. Another product, IX, $C_{48}H_{82}O_{16}$, colorless crystalline powder, NMR $\delta_{TMS}^{CDCl_3}$: 0.67 3H(s), 1.01 3H(s), 1.21—1.30 3H (d, J=6 cps) ×2, 3.44—3.65 3H(s)×9, was hydrolysed with 2N hydrogen chloride to give Δ^5-pregnene-3β,20α-diol(II) from the chloroform extract of the hydrolysate and 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4-tri-O-methyl-D-glucose and 4-O-methyl-D-digitalose from the aqueous layer. The occurence of Δ^5-pregnene-3β,20α-diol(II) in the hydrolysate suggested that the sugar moieties are combined to each hydroxyl groups of this compound.

From the results of the foregoing experiments, it was deduced that the structure of glycoside H_1 would be established as formula I.

To confirm the configuration of D-cymarose, oxidative cleavage of two glucoses in I was investigated. According to the degradation method which was described in the previous paper,[3] I was oxidized with sodium metaperiodate and then the product was reduced with sodium borohydride. The reaction mixture was extracted with chloroform and the extract was hydrolysed with 0.05N hydrogen chloride–methanol(3: 20) to give colorless crystalline powder, X, $C_{44}H_{72}O_{14}$, $[\alpha]_D^{23}$—20.30°(ethanol). Acid hydrolysis of X with refluxing 0.05N sulfuric acid–50% methanol gave III, IV, and XI, $C_{28}H_{46}O_6$, colorless needles, mp 234—236°,

5) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

$[\alpha]_D^{21}$—38.61°(ethanol), which on acetylation with pyridine and acetic anhydride gave triacetate(XII), $C_{34}H_{52}O_9$, mp 227—228°.

Based on the result of the acid hydrolysis of XI giving Δ^5-pregnene-3β,20α-diol(II) and D-digitalose, the structure of XI was assigned to be Δ^5-pregnene-3β,20α-diol(20)-β-D-digitalopyranoside. Thus the structure of X was established to be Δ^5-pregnene-3β,20α-diol-(3)-[2-acetyl-β-D-digitalopyranosyl($1_{dig}\rightarrow4_{cym}$)-D-cymaropyranoside](20)-β-D-digitalopyranoside.

Deacetylation of X with 0.4N sodium hydroxide gave colorless crystalline powder, XIII, $C_{42}H_{70}O_{13}$, $[\alpha]_D^{22}$—22.01°(ethanol), which was permethylated by Hakomori's method to give per-O-methylether(XIV), $C_{46}H_{78}O_{13}$, colorless needles, mp 204—206°, NMR $\delta_{TMS}^{CDCl_3}$: 0.67 3H(s), 0.99 3H(s), 1.29 3H(d, J=6 cps)×4, 3.43 3H(s), 3.53 3H(s)×2, 3.59 3H(s)×4, 4.24 1H(d, J=8 cps)×2, 4.89 1H(q, J_1=9 cps, J_2=2 cps), 5.35 1H(broad). The configration of D-cymarose in XIV was investigated by NMR analysis. The quartet signal of one proton at δ=4.89 ppm(J_1=9 cps, J_2=2 cps) was assigned to the anomeric proton of D-cymarose, so that the configuration of D-cymarose was assigned to be β form its coupling constants.

From these experimental data, the structure of I was established to be Δ^5-pregnene-3β,20α-diol (3)-[2-O-acetyl-β-D-digitalopyranosyl($1_{dig}\rightarrow4_{cym}$)-$\beta$-D-cymaropyranoside](20)-[β-D-glucopyranosyl($1_{glu}\rightarrow6_{glu}$)-$\beta$-D-glucopyranosyl($1_{glu}\rightarrow2_{dig}$)-$\beta$-D-digitalopyranoside].

It should be noted that I is the first example of the pregnane type glycoside whose sugar moieties combined to both C_3-hydroxyl group and C_{20}-hydroxyl group.

Furthermore, the sugar components and their sequences in glycoside H_1 are very interesting, because this glycoside is assumed to be one of the biogenetical intermediate of cardiac glycoside. As we pointed out in the previous paper,[1] the sugar sequences of these glycosides are ruled by certain regularity. In the cardiac and pregnane type glycosides of Asclepiadaceous plants, the sugar components, namely 2,6-dideoxysugar, 6-deoxysugar and glucose, are attached to the aglycone in the order, such as aglycone-(2,6-dideoxysugar)$_{0-l}$-(6-deoxysugar)$_{0-m}$-(glucose)$_{0-n}$. Although not so many examples are available, besides glycoside H_1, condurangoglycoside-A, -A_1, -C, -C_1 from *Marsdenia cundurango* REICHENBACH fil.,[6] drebys-

TABLE I. The List of Asclepiadaceous Glycosides

Glycoside	Genin	Sugar sequence	Origin	Reference
Condurango glycoside-A	condurangogenin A	←Cym←Ole←Ole←3-O-Me-6deo-all	*Marsdenia condurango* REICHENBACH fil.	6)
-A_1		←Cym←Cym←Ole←3-O-Me-6deo-all ←Glu←Glu		
-C	condurangogenin C	←Cym←Ole←Ole←3-O-Me-6deo-all		
-C_1		←Cym←Cym←Ole←3-O-Me-6deo-all ←Glu←Glu		
Drebyssoside-1	drevogenin A	←Cym←Ole←3-O-Me-6deo-all	*Dregea abyssinica* (HOCHST) K.SCHUM.	7)
-2	drebyssogenin F	←Cym←Cym←3-O-Me-6deo-all		
-3		←Cym←Ole←3-O-Me-6deo-all		
Glydoside-G	sarmentogenin	←Dgx←Cym←Cym	*Gongronema gazense* (S.MOORE) BULLOK	8*a*,*b*)
-L	periplogenin	←Dgx←Cym		
-M	sarmentogenin	←Dgx←Cym		
-R		←Dgx←Dgx←Ole		
Glycoside-K	Δ^5-pregnene-3β,20α-diol	←Dig←Glu←Glu	*Periploca sepium* BGE.	3)
-H_1		←Dig←Glu←Glu ←Cym←2-O-Ac-dig		

Abb. Cym: cymarose, Ole: oleandrose, 3-O-Me-6deo-all:3OMethyl-6-deoxy-allose, Glu: gucose, Dgx: digitoxose, Dig: digitalose, 2-O-Ac-Dig: 2-O-Acetyl digitalose

6) R. Tshesche and H. Kohl, *Tetrahedron*, **24**, 4359 (1968).
7) A. Bhatnagar, W. Stöcklin and T. Reichstein, *Helv. Chim. Acta*, **51**, 133 (1968).
8) *a*) M.L. Lewbart, W. Wehrli and T. Reichstein, *Helv. Chim. Acta*, **46**, 505 (1963); *b*) M.L. Lewbart, W. Wehrli, H. Kaufmann and T. Reichstein, *ibid.*, **46**, 517 (1963).

soside-1, -2, -3 from *Dregea abyssinica*(Hochst) K. Schum.[7] and glycoside-G, -L, -M, -R from *Gongronema gazense*(S. Moore) Bullock[8a,b] are all ruled by the same regularity. Further investigation on other glycosides of "Bei-Wujiapi" are now in progress.

Experimental

All melting points were determined on a Yanagimoto Micro Melting point apparatus and uncorrected. IR absorption spectra were measured in a Hitachi Model EPI-2. NMR spectra were measured in a Japan electron Co. JNM.4H-100 spectrometer and a Hitachi Model R-20 High Resolution NMR spectrometer with tetramethylsilane as an internal standard. The chemical shifts are given as δ values and the solvent used are indicated. Gas chromatograph used was a Hitachi Model K-53 with hydrogen flame ionization detecter. The *Rf* values were determined by thin-layer chromatography on Kiesel gel H using (A) the lower phase of $CHCl_3$–MeOH–H_2O (7 : 3 : 1) for sugars (B) AcOEt for aglycones and 10% H_2SO_4 (spraying followed by heating) as a color reagent. *Rf* values of O-methylated sugars were taken on paper chromatograms (Toyo Roshi No. 51, solvent; the upper layer of *n*-BuOH–AcOH–H_2O, (A) 4 : 1 : 2, (B) 4 : 1 : 5, spray reagent; aniline hydrogen phthalate.

Isolation and Properties of Glycoside H_1 (I)——As we reported in previous paper,[3] the crude glycoside fraction (100 g) was submitted to column chromatography on silica gel (500 g) eluted with AcOEt saturated with H_2O and then with the same solvent containing 10—25% MeOH, and each fraction (100 ml) was collected. Fractions No. 63—90 which were eluted with the same solvent containing 15% MeOH were combined and repeatedly purified by column chromatography on neutral alumina (Woelm) with $CHCl_3$: MeOH : H_2O=65:35:10 (lower layer) and finally I was isolated.

I was recrystallized from MeOH–AcOEt saturated with H_2O (1 : 25—30) to give colorless needles (yield: 0.07% from dried crude drug), mp 182°, $[\alpha]_D^{24}$ −22.83° (c=1.84, EtOH). *Anal.* Calcd. for $C_{56}H_{92}O_{24}$: C, 58.49; H, 8.07. Found: C, 58.21; H, 8.23. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH, broad), 1750, 1240 (ester). NMR δ_{TMS}^{PY}: 0.61 3H (s) -C(CH$_3$)-CH$_3$, 0.92 3H (s) -C(CH$_3$)-CH$_3$, 1.40—1.52 3H (d, J=6 cps) × 4 -CH(CH$_3$)-CH$_3$, 2.10 3H (s) -OCOCH$_3$, 3.39 3H (s) OCH$_3$, 3.49 3H (s) × 2 OCH$_3$.

Hydrolysis of I with 3N H_2SO_4——Sixty mg of I was dissolved in 5 ml of MeOH and refluxed for 30 min with 5 ml of 6N H_2SO_4 on a water bath. The reaction mixture was diluted with 10 ml of water and MeOH was evaporated *in vacuo* at room temperature. The residue was extracted with $CHCl_3$, washed with water and dried over anhyd. Na_2SO_4. After removal of the solvent, the residue was recrystallized from AcOEt to give prisms (12 mg), mp 182°, which were identified with an authentic sample of Δ^5-pregnene-3β,20α-diol by mixed fusion, TLC (b) and IR.

The aqueous layer of the reaction mixture was neutralized with Amberlite IR-4B and then concentrated *in vacuo*. The residue was examined by PPC (B, *Rf* 0.17 D-glucose, 0.43 D-cymarose) and GLC (column SE-52 on chromosorb W, 6 mm × 1 m, column temp. 155°, injection temp. 200°, carrier gas N_2, 45 ml/min, trimethylsilylated sugar t_R (min) 4.1, 4.5, 5.3 D-digitalose, 18.1, 29.0 D-glucose).

Hydrolysis of I with 0.05N H_2SO_4–50% MeOH——Six hundred mg of I was refluxed with 0.05N H_2SO_4–50% MeOH (20 ml) for 30 min on a water-bath. The reaction mixture was diluted with 10 ml of water and MeOH was evaporated *in vacuo* at room temperature. The residue was extracted with $CHCl_3$, washed with water and dried over anhyd. Na_2SO_4. After removal of the solvent, the residue (180 mg) was purified by column chromatography on silica gel (60 g) with AcOEt (20 ml/fraction). The product III was detected in fractions No. 21—30 (74 mg) and IV was detected in fractions No. 32—43 (82 mg). The aqueous layer was neutralized with Amberlite IR-4B and extracted with *n*-BuOH. The extract was evaporated *in vacuo* to give product V as a white powder (400 mg).

Properties of III——Product III was recrystallized from AcOEt to form colorless needles, mp 171°, $[\alpha]_D^{23}$ +23.5° (c=1.00, H_2O). *Anal.* Calcd. for $C_{17}H_{30}O_9$: C, 53.95; H, 7.99. Found: C, 53.72; H, 7.78. IR ν_{max}^{KBr} cm^{-1}: 3550 (OH), 1750, 1240 (ester). NMR $\delta_{TMS}^{CDCl_3}$: 1.20 3H (d, J=6.4 cps), -CH(CH$_3$)-CH$_3$, 1.38 3H (d, J=6.4 cps), -CH(CH$_3$)-CH$_3$, 2.00 3H (s) -O-COCH$_3$, 1.5—2.3 2H (m), 3.45 3H (s) -OCH$_3$, 3.48 3H (s) × 2 -OCH$_3$, 4.42 1H (d, J=8.0 cps), anomeric H, 4.68 1H (q, J_1=9.6 cps, J_2=2.2 cps), anomeric H, 5.15 1H (q, J_1=9.8 cps, J_2=8 cps), -CH-OCOCH$_3$. III was identified with an authentic sample of methyl 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-β-D-cymaropyranoside by mixed fusion, TLC and IR spectra.

Properties of IV——Product IV was recrystallized from AcOEt–*n*-hexane to form colorless needles, mp 177°, $[\alpha]_D^{24}$ +64.5° (c=1.64, H_2O). *Anal.* Calcd. for $C_{16}H_{28}O_9$: C, 52.74; H, 7.75. Found: C, 52.65; H, 7.71. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1745, 1240 (ester). IV was identified with an authentic sample of 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-D-cymarose by mixed fusion, TLC and IR spectra.

Properties of V——Product V was recrystallized from MeOH–AcOEt saturated with water to give colorless needles, mp 240—241°, $[\alpha]_D^{20}$ −27.58° (c=1.16, MeOH). *Anal.* Calcd. for $C_{40}H_{66}O_{16}$: C, 59.82; H, 8.26. Found: C, 59.53; H, 8.21. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH, broad), NMR δ_{TMS}^{PY}: 0.68 3H (s) -C(CH$_3$)-CH$_3$, 1.00 3H (s) -C(CH$_3$)-CH$_3$, 1.45 3H (d, J=6 cps) O-CH(CH$_3$)-CH$_3$ × 2, 3.48 3H (s) -OCH$_3$. V was identified with an authentic

sample of Δ^5-pregnene-3β,20α-diol(20)-β-D-glucopyranosyl(1$_{glu}\rightarrow$6$_{glu}$)-β-D-glucopyranosyl(1$_{glu}\rightarrow$2$_{dig}$)-β-D-digitalopyranoside by mixed fusion, TLC and IR spectra.

Deacetylation of III with 0.4N NaOH——The solution of III (40 mg) in 0.4N NaOH (3 ml) was warmed at 50° for 2 hr with stirring under N_2 gas flow. The reaction mixture was neutralized with Amberlite IR-200 and concentrated *in vacuo*. The residue was recrystallized from AcOEt–*n*-hexane to give colorless needles, mp 116°, $[\alpha]_D^{23}$ +12.4° (c=1.61, H_2O), *Anal.* Calcd. for $C_{15}H_{28}O_8$: C, 53.56; H, 8.39. Found: C, 53.50; H, 8.23. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH).

Hydrolysis of Deacetyl-III with 0.05N H_2SO_4–50% MeOH——Twenty mg of deacetyl-III was refluxed with 0.05N H_2SO_4–50% MeOH (10 ml) for a half hour and treated by the usual manner. D-Cymarose and D-digitalose were detected by TLC (A): *Rf* 0.60 (D-cymarose), 0.24 (D-digitalose) and GLC: column SE-52 3% on chromosorb W, 6 mm×2 m, column temp. 110°, injection temp. 200°, carrier gas N_2 45 ml/min, TMS-sugar t_R(min) 11.0, 12.2 (D-cymarose), 29.0, 38.7 (D-digitalose).

Hydrolysis of V with 3N H_2SO_4–50% MeOH——Forty five mg of V was refluxed with 3N H_2SO_4–50% MeOH for a half hour and treated as usual. Δ^5-Pregnene-3β,20α-diol (II) (14 mg), D-glucose and D-digitalose were obtained from the reaction mixture and identified.

Permethylation of V——According to the previous report[3] 160 mg of V was methylated by Hakomori's method to afford nona-O-methylether of V, colorless needles from *n*-hexane, mp 161—162°, *Anal.* Calcd. for $C_{49}H_{84}O_{16}$: C, 63.33; H, 9.11. Found: C, 63.49; H, 9.03. IR $\nu_{max}^{CHCl_3}$: OH (nil). NMR $\delta_{TMS}^{CDCl_3}$: 0.66 3H (s). -$\overset{|}{C}$-C$\underline{H}_3$, 1.00 3H (s) -$\overset{|}{C}$-C$\underline{H}_3$, 1.25 3H (d, J=6 cps) -$\dot{C}$H-C$\underline{H}_3$, 1.34 3H (d, J=6 cps) -$\dot{C}$H-C$\underline{H}_3$, 3.48—3.64 3H (s) OC$\underline{H}_3$×10, 4.18 1H (d, J=9 cps) anomeric H, 4.38 1H (d, J=8 cps) anomeric H, 4.67 1H (d, J=8 cps) anomeric H, 5.40 1H (broad) $>$C=C$<^{\underline{H}}$.

Hydrolysis of Nona-O-methylether of V——One hundred mg of nona-O-methylether of V was hydrolysed with 2N HCl–MeOH (4 ml) as reported in the previous paper.[3] Evaporation of the $CHCl_3$ extract *in vacuo* gave a white powder which was recrystallized from *n*-hexane to give colorless needles (41 mg), mp 132—133°, which was identified with an authentic sample of Δ^5-pregnene-3β,20α-diol-3-methylether by mixed fusion, TLC and IR spectra. The aqueous layer was neutralized with Amberlite IR-4B and then concentrated *in vacuo*. The residue was examined by GLC and 4-O-methyl-D-digitalose, 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose were identified. GLC: column 3% SE-30 on chromosorb W, 3 mm×1 m, column temp. 120°, injection temp. 200°, carrier gas N_2 28 ml/min, TMS sugars t_R(min) 4.3, 5.0 (4-O-methyl-D-digitalose), 5.3 (2,3,4,6-tetra-O-methyl-D-glucose), 8.1, 8.9 (2,3,4-tri-O-methyl-D-glucose).

Deacetylation of I with 0.4N NaOH——The solution of I (480 mg) in 0.4N NaOH (8 ml) was warmed at 50° for 2 hr with stirring under N_2 gas flow. The reaction mixture was cooled and neutralized with Amberlite IR-200 and concentrated *in vacuo*. The residue was recrystallized from diluted EtOH–ether to give VI, colorless needles (462 mg), mp 175—176°, $[\alpha]_D^{24}$ −26.08° (c=2.14, EtOH). *Anal.* Calcd. for $C_{54}H_{90}O_{23}$: C, 58.55; H, 8.20. Found: C, 58.29; H, 8.14. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH). NMR δ_{TMS}^{Py}: 0.65 3H (s) -$\overset{|}{C}$-C$\underline{H}_3$, 0.94 3H (s) -$\overset{|}{C}$-C$\underline{H}_3$, 1.40—1.60 3H (d, J=6 cps)×4, 3.36 3H (s) -OC$\underline{H}_3$, 3.46 3H (s)×2, -OC$\underline{H}_3$.

Permethylation of VI——According to the Hakomori's method, NaH (290 mg) was warmed with dimethylsulfoxide (DMSO, 5 ml) at 65° for 1 hr with stirring under N_2 gas flow. To this reagent a solution of VI (340 mg) in DMSO (4 ml) was added and the mixture was kept at 65° for 15 min with stirring under N_2 gas flow. Then CH_3I (3 ml) was added and the mixture was allowed to stand at room temperature for 3 hr with stirring. After dilution with water, the mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and concentrated. The residue was reprecipitated from aqueous EtOH to give VII, colorless crystalline powder (315 mg), mp 109°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: OH (nil). *Anal.* Calcd. for $C_{61}H_{110}O_{23}$: C, 61.60; H, 8.89. Found: C, 61.35; H, 8.80. NMR $\delta_{TMS}^{CDCl_3}$: 0.66 3H (s) -$\overset{|}{C}$-C$\underline{H}_3$, 1.00 3H (s) -$\overset{|}{C}$-C$\underline{H}_3$, 1.19—1.39 3H (d, J=6 cps)×4 -$\dot{C}$H-C$\underline{H}_3$, 3.42—3.64 3H (s)×13 -OC$\underline{H}_3$, 5.40 1H (broad) $>$C=C$<^{\underline{H}}$.

Methanolysis of VII with 0.05N HCl–MeOH——VII (280 mg) was refluxed with 2N HCl–MeOH (8 ml) for a half hr. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* and the residue was subjected to column chromatography on silica gel (70 g) with benezne: acetone=2:1, 30 ml/fraction. The fractions No. 3—4 were combined and evaporated and the residue was recrystallized from acetone–*n*-hexane to afford colorless needles (36 mg), mp 106°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: OH (nil). *Anal.* Calcd. for $C_{17}H_{32}O_8$: C, 56.02; H, 8.85. Found: C, 56.17; H, 8.89, which was identified with an authentic sample of methyl 4-O-(2,4-di-O-methyl-β-D-digitalopyranosyl)-β-D-cymaropyranoside by mixed fusion, TLC and IR spectra. The fractions No. 7—10 were combined and evaporated and the residue was recrystallized from aq. acetone to give IX, colorless crystalline powder (210 mg), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH). *Anal.* Calcd. for $C_{48}H_{82}O_{16}$: C, 62.99; H, 9.03. Found: C, 62.73; H, 8.94. NMR $\delta_{TMS}^{CDCl_3}$: 0.67 3H (s) -$\overset{|}{C}$-C$\underline{H}_3$, 1.01 3H (s)-$\overset{|}{C}$-C$\underline{H}_3$, 1.21—1.30 3H (d, J=6 cps)×2 -$\dot{C}$H-C$\underline{H}_3$, 3.44—3.65 3H(s)×9 -OC$\underline{H}_3$.

Acid **Hydrolysis of IX with 2N HCl–MeOH**——Acid hydrolysis of IX (120 mg) with 2N HCl–MeOH (6 ml) was carried out according to the method described in the case of VI. Δ^5-Pregnene-3β,20α-diol (II) (46 mg), mp 182° obtained from the $CHCl_3$ layer of the reaction mixture and 2,3,4,6-tetra-O-methyl-D-glucose,

2,3,4-tri-O-methyl-D-glucose and 4-O-methyl-D-digitalose obtained from the aqueous layer were characterized respectively.

Oxidative Degradation of I with $NaIO_4$——To a solution of I (1.2 g) in 95% EtOH (300 ml), a solution of $NaIO_4$ (1.5 g) in H_2O (40 ml) was added under stirring at room temperature for 1 hr. After removing the precipitate by filtration, EtOH was evaporated *in vacuo* below 50° and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was dissolved in 95% MeOH (45 ml) and then $NaBH_4$ (300 mg) was added by portions at room temperature with stirring. After stirring the mixture at the same temperature for 1 hr, the reaction mixture was neutralized with 5% AcOH, concentrated *in vacuo* below 50° and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The products were dissolved in MeOH (300 ml) and then 0.05N HCl (45 ml) was added. The solution was kept at room temperature for 8 days. The reaction mixture was neutralized with 0.1N $KHCO_3$ and, after an addition of H_2O, the solution was evaporated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give a crude product which was purified by column chromatography on silica gel (80 g) using benzene:acetone=7:3, 50 ml/fraction. The fraction No. 15—19 were combined and evaporated and the residue was reprecipitated from dil EtOH–ether to give X, colorless crystalline powder (670 mg), mp 128°. *Anal.* Calcd. for $C_{44}H_{72}O_{14}$: C, 64.05; H, 8.80. Found: C, 63.79; H, 8.87. $[\alpha]_D^{23}$ −20.30° (c=0.94, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1740, 1240 (ester). NMR $\delta_{TMS}^{CDCl_3}$: 0.67 3H (s) -Ç-C$\underline{H}_3$, 0.99 3H (s) -Ç-C$\underline{H}_3$ 1.37 3H (d, J=6 cps)-ĊH-C$\underline{H}_3$×4, 2.12 3H (s) -O-C$\underline{H}_3$, 3.51 3H (s) -OC$\underline{H}_3$, 3.56 3H (s) -OC$\underline{H}_3$, 3.61 3H (s) -OC$\underline{H}_3$, 5.11 1H (t, J_1=9 cps, J_2=8 cps), 5.50 1H (broad) >C=C<$\underline{H}$.

Acetylation of XI——Seventy mg of XI was dissolved in pyridine (2 ml) and Ac_2O (2 ml) was added and allowed to stand for 48 hr at room temperature. The product was worked up as usual and crystallized from EtOH to give XII, colorless needles, mp 227—228°. *Anal.* Calcd. for $C_{34}H_{52}O_9$: C, 67.52; H, 8.67. Found: C, 67.47; H, 8.59. IR ν_{max}^{KBr} cm^{-1} 1740, 1240 (ester). NMR $\delta_{TMS}^{CDCl_3}$: 0.63 3H (s)-Ç-C$\underline{H}_3$, 1.00 3H (s) -Ç-C$\underline{H}_3$, 1.17 3H (d, J=6 cps) -ĊH-C$\underline{H}_3$, 1.97 3H (s) -OCOC$\underline{H}_3$, 2.01 3H (s) -O-COC$\underline{H}_3$, 2.10 3H (s) -O-COC$\underline{H}_3$, 3.27 3H (s) -OC$\underline{H}_3$, 4.36 1H, (d, J=8 cps), 5.35 1H (broad) >C=C<$\underline{H}$.

Acid Hydrolysis of X——X was refluxed with 0.05N H_2SO_4–50% MeOH for 30 min. The reaction mixture was treated as usual and the resulting III, IV and V were characterized by TLC, IR spectra and mixed fusion.

Deacetylation of X with 0.4N NaOH——Deacetylation of X (450 mg) with 0.4N NaOH (4 ml) was carried out according to the method described in the case of I, and 410 mg of XIII was obtained as colorless plates, mp 136—138° from dil. acetone-EtOH, $[\alpha]_D^{22}$ −22.01°(c=1.04, EtOH), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH). *Anal.* Calcd. for $C_{42}H_{70}O_{13}$: C, 64.42; H, 9.01. Found: C, 64.24; H, 8.74. NMR δ_{TMS}^{Py}: 0.65 3H (s) -Ç-C$\underline{H}_3$, 0.99 3H (s) -Ç-C$\underline{H}_3$, 1.41—1.51 3H (d, J=6 cps)×4 -ĊH-C$\underline{H}_3$, 3.40 3H (s) -OC$\underline{H}_3$, 3.50 3H (s)×2 -OC$\underline{H}_3$, 5.5 1H (broad) C>=C<$\underline{H}$.

Permethylation of XIII——Permethylation of XIII (150 mg) was carried out according to Hakomori's method described in the case of V, and 124 mg of XIV was obtained, colorless needles, mp 204—206°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: OH (nil). *Anal.* Calcd. for $C_{46}H_{78}O_{13}$: C, 65.96; H, 9.36. Found: C, 65.78; H, 9.66. NMR $\delta_{TMS}^{CDCl_3}$: 0.67 3H (s) -Ç-C$\underline{H}_3$, 0.99 3H (s) -Ç-C$\underline{H}_3$, 1.29 3H (d, J=6 cps)×4 -ĊH-C$\underline{H}_3$, 3.43 3H (s) -OC$\underline{H}_3$, 3.53 3H (s)×2 -OC$\underline{H}_3$, 3.59 3H (s)×4 -OC$\underline{H}_3$, 4.24 1H (d, J=8 cps)×2 anomeric H, 4.89 1H (q, J_1=9 cps, J_2=2 cps), 5.35 1H, (broad) >C=C<$\underline{H}$.

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