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# Constituents of Chinese Crude Drug "Wujiapi." III.[1] On the Structure of Glycoside G and K of Bei-Wujiapi[2]

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The chemical structure of glycoside G(I), $C_{36}H_{56}O_{13}$, mp 232—233°, $[\alpha]_D^{19}$ +30.2° (EtOH) and glycoside K(V), $C_{40}H_{66}O_{16}$, mp 240—241°, $[\alpha]_D^{20}$ −27.58° (MeOH) were isolated from Bei-Wujiapi (cortex of *Periploca sepium* Bge.) and established to be periplocin and $\Delta^5$-pregnene-3$\beta$,20$\alpha$-diol(20)-$\beta$-D-glucopyranosyl ($1_{glu}\rightarrow6_{glu}$)-$\beta$-D-glucopyranosyl ($1_{glu}\rightarrow2_{dig}$)-$\beta$-D-digitalopyranoside.

It should be noted that glycoside K is the first example of the pregnane type glycoside whose sugar moiety links to the hydroxyl group other than $C_3$-hydroxyl group of the steroidal aglycone.

In previous papers,[2,4] it has been shown on thin-layer chromatography (TLC) of *n*-BuOH soluble fraction of MeOH extract of Chinese crude drug, Bei-Wujiapi (cortex of *Periploca sepium* Bge.; Asclepiadaceae) to contain many glycosidic substances (A–N) (Fig. 1).


Fig. 1. Thin-Layer Chromatogram of *n*-BuOH Soluble Fraction of Bei-Wujiapi

plate: Kieselgel H
solvent: $CHCl_3$: MeOH: $H_2O$=65: 35:10 (lower phase)
color Reag: 10% $H_2SO_4$

The isolation of these glycosidic substances were somewhat difficult, but repeated purification by column chromatography afforded two crystalline glycosides, tentatively named glycoside G (0.02% from dried material) and glycoside K (0.005%). The present paper describes the isolation and the elucidation of chemical structures of two glycosides.

As we reported in previous paper,[4] the *n*-BuOH soluble fraction of MeOH extract of the crude drug was purified by column chromatography on silica gel with ethyl acetate saturated with water and then with the same solvent containing 10—25% MeOH. The separation of glycosidic components are summarized in Table I. The fractions (No. 41—62) and fractions (No. 110—128) were repeatedly column-chromatographed on neutral alumina (Woelm) with $CHCl_3$:MeOH:$H_2O$=65:35:10 (lower phase) and finally I from the former and V from the later were obtained in crystalline state.

I, $C_{36}H_{56}O_{13}$, mp 232—233°, colorless needles, $[\alpha]_D^{19}$ +30.2° (EtOH) gave a tetraacetate (II), $C_{44}H_{64}O_{17}$, on acetylation with acetic anhydride and pyridine. The absorption bands at 1620, 1735, 1790 (weak) $cm^{-1}$ in the infrared (IR) spectrum and the absorption maximum at

1) Part II: S. Sakuma, S. Kawanishi, and J. Shoji, *Syoyakugaku Zasshi*, **22**, 23 (1968).
2) S. Sakuma, H. Ishizone, R. Kasai, S. Kawanishi, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **17**, 2183 (1969).
3) Location: *Hatanodai, Shinagawa-ku, Tokyo.*
4) S. Sakuma, S. Kawanishi, J. Shoji, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **16**, 326 (1968).

Table I. Chromatographic Separation of Glycosides

| Fraction (100 ml) | Solvent | Glycoside |
|---|---|---|
| 1— 6 | AcOEt ($H_2O$ Satrd.)–MeOH ( 9:1) | A,B,C,D,E |
| 7— 40 | AcOEt ($H_2O$ Satrd.)–MeOH ( 9:1) | E,F,G |
| 41— 62 | AcOEt ($H_2O$ Satrd.)–MeOH ( 9:1) | G,H |
| 63— 90 | AcOEt ($H_2O$ Satrd.)–MeOH (17:3) | H,I,J |
| 91—109 | AcOEt ($H_2O$ Satrd.)–MeOH ( 4:1) | I,J,K |
| 110—128 | AcOEt ($H_2O$ Satrd.)–MeOH ( 4:1) | J,K,L |
| 129—160 | AcOEt ($H_2O$ Satrd.)–MeOH ( 4:1) | M,N |

217 m$\mu$ in the ultraviolet (UV) spectrum of I revealed the presence of cardinolidic structure in this glycoside.

Hydrolysis of I with both Kiliani mixture[5] and 0.05N $H_2SO_4$ afforded periplogenin (III),[6] D-cymarose and D-glucose. The identification of periplogenin was carried out by mixed fusion and comparison of IR spectra and TLC with an authentic sample. D-Cymarose and D-glucose were identified by TLC (plate: Kieselgel H, solvent: $CHCl_3$: MeOH: $H_2O$=7:3:1).[1]

Enzymatic hydrolysis of I with takadiastase-A gave glucose and glycoside GE (IV), $C_{30}H_{46}O_8$, mp 146°/208° (double melting point), colorless needles, $[\alpha]_D^{19}$ +26.41° (in EtOH). On hydrolysis with 0.05N $H_2SO_4$ in 50% MeOH, IV gave periplogenin and D-cymarose. From this fact and the comparison of physical constants, IV was assumed to be periplocymarin[6b] and the identity was proved by the mixed fusion, TLC (plate: Kieselgel H, solvent: AcOEt) and the infrared comparison with the authentic sample which was given us from Dr. T. Reichstein.

Table II

| Compound | Formula | mp (°C) | $[\alpha]_D$ |
|---|---|---|---|
| I | $C_{36}H_{56}O_{13}$ | 232—233 | +30.2 |
| Periplocin | $C_{36}H_{56}O_{13}$ | 224 | +23.0 |
| II | $C_{44}H_{64}O_{17}$ | 198 | +17.8 |
| Periplocin tetraacetate | $C_{44}H_{64}O_{17}$ | 195 | +20.0 |
| IV | $C_{30}H_{46}O_8$ | 146/208 | +26.4 |
| Periplocymarin | $C_{30}H_{46}O_8$ | 139/207 | +29.0 |
| III | $C_{23}H_{34}O_5$ | 140/238 | +27.0 |

Table III

| | | |
|---|---|---|
| Glucose | NMR anomer H $\delta$=5.14 (d) $J$=7 cps | $\beta$ |
| | $[M]_{D\cdot I}$—$[M]_{D\cdot IV}$ + 69° | $\beta$ |
| | methyl-$\alpha$-D-glucopyranoside $[M]_D$ +307° | |
| | methyl-$\beta$-D-glucopyranoside $[M]_D$ − 63° | |
| Cymarose | NMR anomer H $\delta$=4.88 (q) $J_1$=3, $J_2$=9 cps | $\beta$ |
| | $[M]_{D\cdot IV}$—$[M]_{D\cdot III}$ + 36° | $\beta$ |
| | methyl-$\alpha$-D-cyaropyranoside $[M]_D$ +370° | |
| | methyl-$\beta$-D-cyaropyranoside $[M]_D$ + 40° | |

From the foregoing observations I was deduecd to be identical with periplocin. The physical constants of I were also in good agreement with the reported values[7] as shown in Table II.

5) H. Kiliani, *Chem. Ber.*, **63**, 2866 (1930).
6) *a*) E. Ruppol and I. Irukovic, *J. Pharm. Belg.*, **10**, 221 (1955); *b*) P. Brauchil, O. Schindler, and T. Reichstein, *Helv. Chim. Acta*, **44**, 904 (1961).
7) A. Stoll and J. Renz, *Helv. Chim. Acta*, **22**, 1193 (1939).

The configuration of sugar components was assigned to be all $\beta$-form from the chemical shift and the coupling constants of the anomeric protons and the comparison of molecular optical rotation of I, IV and periplogenin. The results were summerised in Table III. The direct comparison of I with the authentic periplocin has not yet been done, but the chemical and physical evidences about I suggested that the I might be identical with periplocin.

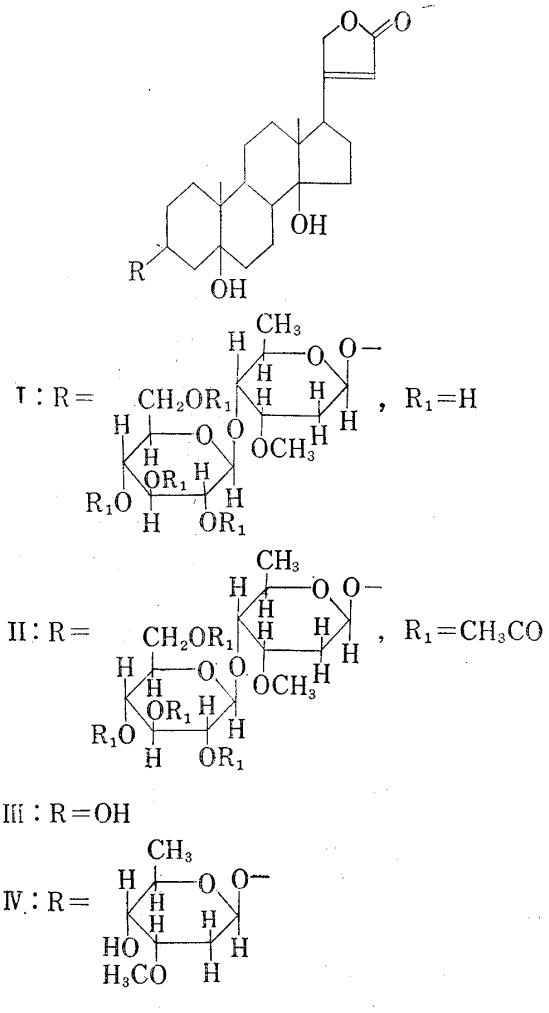


Fig. 2

The second crystalline glycoside, V, $C_{40}H_{66}O_{16}$, mp 240—241°, colorless needles, $[\alpha]_D^{20}$ −27.58° (in MeOH) gave a nonaacetate (VI), $C_{58}H_{84}O_{25}$, on acetylation with acetic anhydride and pyridine and a nona-O-methyl derivative (VII), $C_{49}H_{84}O_{16}$, on methylation by Hakomori's method.[8]

The nuclear magnetic resonance (NMR) signals of V, $\delta_{TMS}^{Py}$: 0.68 3H (s), 1.00 3H (s), 1.45 3H×2 (d, $J$=6 cps), 3.48 3H (s), 5.80 1H (m) revealed the presence of two (–$\overset{|}{C}$–$CH_3$), two (–$\overset{|}{C}$H–$CH_3$), one (–$OCH_3$) and one double bond.

On acidic hydrolysis with refluxing 3N $H_2SO_4$–50% MeOH for a half hour, V gave $\Delta^5$-pregnene-3$\beta$,20$\alpha$-diol (VIII),[4] D-glucose and D-digitalose. The identification of VIII with an authentic sample was carried out by mixed fusion and comparison of TLC and IR spectra. The sugar components were detected by TLC, partition paper chromatography (PPC) and gas liquid chromatography (GLC).

The NMR spectrum of VII showed ten O-methyl signals at $\delta_{TMS}$ 3.48—3.64 in $CDCl_3$ solution while the hydroxyl absorption band of IR spectrum of V was disappeared. On acidic hydrolysis with refluxing 2N $H_2SO_4$ in 50% MeOH, VII gave mono-O-methyl derivative of $\Delta^5$-pregnene-3$\beta$,20$\alpha$-diol (IX), $C_{22}H_{36}O_2$, mp 132—133°, colorless needles, which was isolated from the $CHCl_3$ extract of the reaction mixture.

To confirm the position of O-methyl group, IX was oxidized with Jones' reagent[9] in acetone at 0° and gave colorless needles, $C_{22}H_{34}O_2$ (X), mp 124°. The IR spectrum of X showed carbonyl absorption band at 1700 $cm^{-1}$ and hydroxyl band which was presented at 3500 $cm^{-1}$ in IX was disappeared. The comparison of NMR spectra of IX ($\delta_{TMS}^{CDCl_3}$ 1.20 3H (d, $J$=9 cps)) and X ($\delta_{TMS}^{CDCl_3}$ 2.11 3H (s)) revealed that the H–$\overset{|}{\underset{OH}{C}}$–$CH_3$ system in IX was converted into –CO–$CH_3$. The identification of X with the authentic sample of 3$\beta$-O-methyl-$\Delta^5$-pregnene-20-one,[10] which was synthesized from pregnenolone according to A. Butenandt, *et al.*,[10] was carried out by the mixed fusion and the comparison of IR, TLC, NMR and optical rotatory

8) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
9) C. Djerassi, R.R. Engle, and A. Boers, *J. Org. Chem.*, **21**, 1547 (1956).
10) A. Butenandt and W. Grosse, *Chem. Ber.*, **70**, 1446 (1937).

dispersion (ORD). From these facts it will be deduced that the compound IX is 3$\beta$-O-methyl-$\Delta^5$-pregnene-20$\alpha$-ol[11] and the sugar moiety of V is combined to the $C_{20}$-hydroxyl group of VIII.

The aqueous layer of hydrolysate of VII was neutralized, and 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4-tri-O-methyl-D-glucose and 4-O-methyl-D-digitalose were detected and identified by PPC and GLC.

To clarify the sugar sequence of two glucose and one digitalose in V, enzymatic hydrolysis with takadiastase-A was examined. The hydrolysis mixture was extracted with *n*-BuOH and purified by column chromatography on Kieselgel with $CHCl_3$:MeOH:$H_2O$=65:35:10 (lower phase). The sugar component of the aqueous layer of hydrolysate was examined and determined to be D-glucose. The main product in BuOH extract was recrystallized from MeOH–AcOEt saturated with water to give colorless needles, mp 268—270°, $C_{34}H_{56}O_{11}$ (XI), $[\alpha]_D^{21}$ −29.16° (in MeOH) which gave a hexaacetate (XII), colorless needles, $C_{46}H_{68}O_{17}$, mp 210° on acetylation with acetic anhydride in pyridine. On hydrolysis with 3N $H_2SO_4$ in 50% MeOH, XI gave VIII, glucose and digitalose. The $\Delta^5$-pregnene-3$\beta$,20$\alpha$-diol-bioside (XI) was methylated by Hakomori's method, and then methanolysed with 2N HCl in MeOH. The reaction mixture was examined and 3$\beta$-O-methyl-$\Delta^5$-pregnene-20$\alpha$-ol (IX) was detected in $CHCl_3$ extract by TLC and 2,3,4,6-tetra-O-methyl-D-glucose and 4-O-methyl-D-digitalose were identified by PPC and GLC from the mother liquor.

Partial hydrolysis of V and XI with dilute acid to obtain monoglycoside of VIII was unsuccessful, then the oxidative cleavage of V was investigated. According to the degradation method of digitoxide to bis- and monodigitoxide reported by D. Satoh, *et al.*[12] V was oxidised with sodium metaperiodate and then the product was reduced with sodium borohydride. The reaction mixture was extracted with $CHCl_3$ and hydrolysed with 0.05N HCl–50% MeOH to give $\Delta^5$-pregnene-3$\beta$,20$\alpha$-diol-monodigitaloside, $C_{28}H_{46}O_6$, (XIV), mp 234—236°, $[\alpha]_D^{24}$ −38.21° (in EtOH).

The configurations of three sugars were assigned by the comparison of chemical shifts and the coupling constants of each anomeric protons of V, XI and XIV and by the application of Klyne's rule[13] to compare the molecular optical rotation of each glycosides. The results were summarized in Table IV.

Table IV

| | | | |
|---|---|---|---|
| Glucose → Glucose | NMR anomer H $\delta$=4.38 $J$=8 cps | | $\beta$ |
| | $[M]_{D.V}$—$[M]_{D.XI}$ | − 35° | $\beta$ |
| Glucose → Digitalose | NMR anomer H $\delta$=4.18 $J$=9 cps | | $\beta$ |
| | $[M]_{D.XI}$—$[M]_{D.XIV}$ | − 4° | $\beta$ |
| Digitalose → Genin | NMR anomer H $\delta$=4.67 $J$=8 cps | | $\beta$ |
| | $[M]_{D.XIV}$—$[M]_{D.VIII}$ | + 37° | $\beta$ |
| | methyl-$\alpha$-D-glucopyranoside | $[M]_D$ +307° | |
| | methyl-$\beta$-D-glucopyranoside | $[M]_D$ − 63° | |
| | methyl-$\alpha$-D-digitalopyranoside | $[M]_D$ +240° | |
| | methyl-$\beta$-D-digitalopyranoside | $[M]_D$ −100° | |

From these experimental data, the structure of V was established to be $\Delta^5$-pregnene-3$\beta$, 20$\alpha$-diol(20)-$\beta$-D-glucopyranosyl($1_{glu}\rightarrow 6_{glu}$)-$\beta$-D-glucopyranosyl($1_{glu}\rightarrow 2_{dig}$)-$\beta$-D-digitalopyranoside.

It should be noted that V is the first example of the pregnane type glycoside whose sugar moiety links to the $C_{20}$-hydroxyl group other than $C_3$-hydroxyl group of the aglycone.

11) M.N. Huffman and J.W. Sadler, *J. Org. Chem.*, **18**, 919 (1953).
12) D. Satoh and K. Aoyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 94 (1970).
13) W. Klyne, *Biochem. J.*, **47**, xli (1950).

The structural study of other glycosides and the pharmacological investigation of these glycosides are now being in progress.

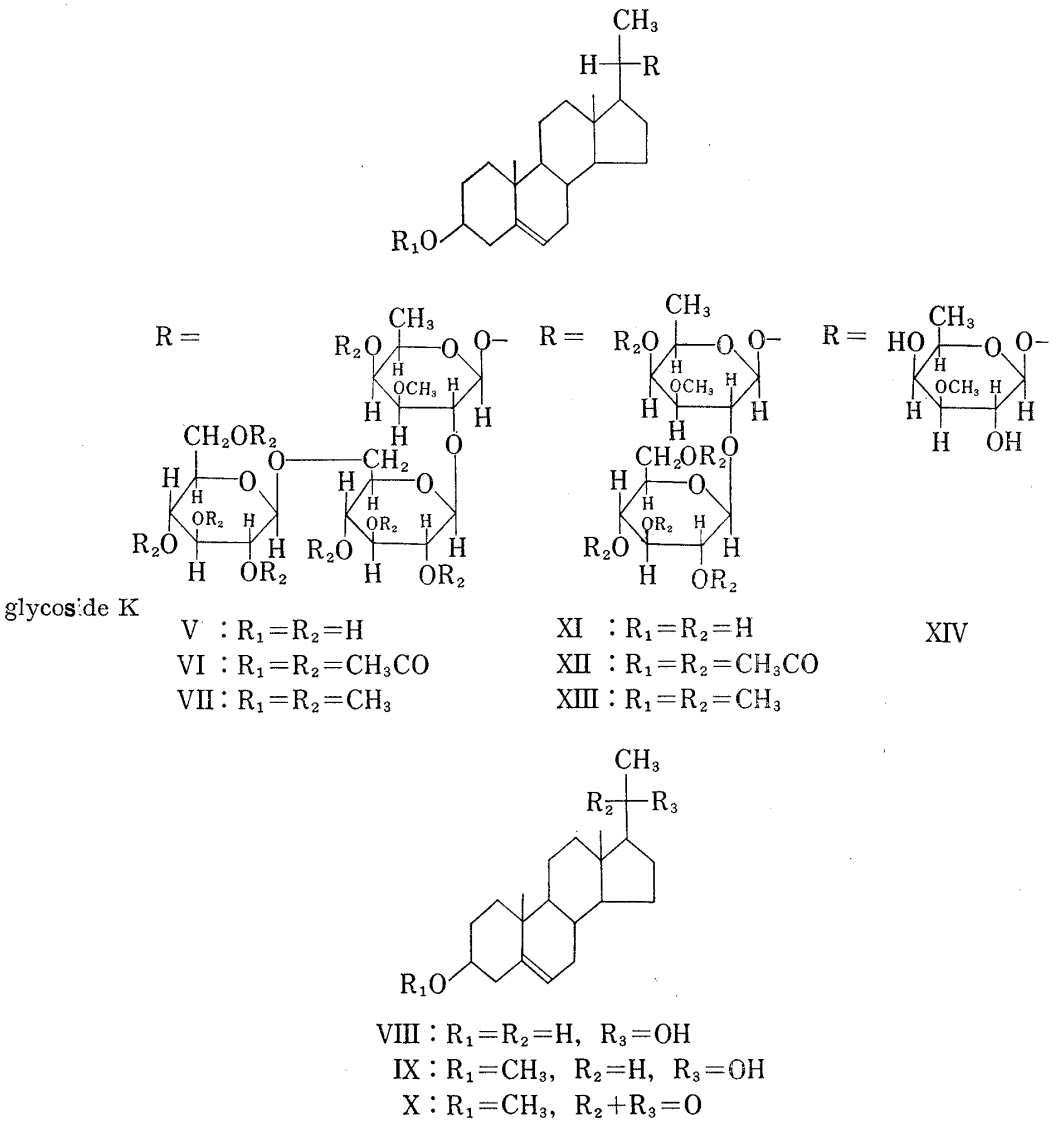


Fig. 3

## Experimental

All melting points were determined on Yanagimoto Micro Melting Point apparatus and uncorrected. IR absorption spectra were measured with Hitachi Model EPI-2. NMR spectra were measured with Japan Electron Co. JNM. 4H-100 spectrometer and Hitachi Model R-20 High Resolution NMR Spectrometer with tetramethylsilane as an internal standard. The chemical shifts are reported in $\delta$ and the solvent used are indicated. Gas chromatograph used was Hitachi Model K-53 with hydrogen flame ionization detecter. ORD curves were measured in solution using JASCO Optical Rotatory Dispersion Recorder Model ORD/UV-5. The *Rf* values were determined by thin-layer chromatography on Kieselgel H using (A) $CHCl_3$–MeOH–$H_2O$ (7:3:1) lower phase for sugars (B) AcOEt for aglycones and 10% $H_2SO_4$ (spraying followed by heating) as a color reagent. *Rf* values of methylated sugar were taken on paper chromatograms (Toyo Roshi No. 51, solvent; butanol–acetic acid–water, (A) 4:1:2, (B) 4:1:5 upper layer, spray reagent; aniline hydrogenphthalate.

**Isolation of I and V**——As we reported in previous paper, the crushed material was extracted with hot EtOH. After evaporation of the solvent under a reduced pressure, the syrupy brown residue was obtained and dissolved in water and extracted with benzene. The water layer which contained the benzene insoluble fraction was extracted with *n*-BuOH saturated with water. This crude glycosidic fraction was detected by TLC and revealed to contain more than fourteen glycosides (Fig. 1).

The crude glycoside fraction (100 g) was submitted to column chromatography on silica gel (500 g) with ethylacetate saturated with water and eluted with the same solvent containing 10—25% MeOH. The separation of glycosides were summerized in Table I.

Fraction No. 41—62 and fraction No. 110—128 were repeatedly purified by column chromatography on neutral alumina (Woelm) with $CHCl_3$–MeOH–$H_2O$ (65:35:10; lower phase) and finally I and V were obtained.

**I**——I was recrystallized from AcOEt saturated with water (yield: 0.02% from crude drug), colorless needles, mp 232—233°, $[\alpha]_D^{19}$ +30.2° ($c$=0.99, EtOH). *Anal.* Calcd. for $C_{36}H_{56}O_{13}\cdot 1/2H_2O$: C, 61.21; H, 8.09. Found: C, 61.02; H, 8.09. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3500, 1790 (weak), 1735, 1620. NMR $\delta_{TMS}^{Py}$: 1.00 3H(s), 1.06 3H(s), 1.61 3H(d, $J$=7 cps), 3.46 3H(s), 4.88 1H(q, $J_1$=3 cps, $J_2$=9 cps), 5.14 1H(d, $J$=7 cps). UV $\lambda_{max}^{EtOH}$ m$\mu$: 217 (log $\varepsilon$=4.16).

**II**——To the solution of I (20 mg) in pyridine (1 ml), $Ac_2O$ (1 ml) was added and allowed to stand for 48 hr at room temperature. The product was worked up as usual and recrystallized from EtOH–*n*-hexane to give colorless needles (19 mg), mp 198°, $[\alpha]_D^{19}$ +17.8° ($c$=0.34, EtOH). *Anal.* Calcd. for $C_{44}H_{64}O_{17}$: C, 61.11; H, 7.41. Found: C, 60.99, H, 7.49. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3500 (OH), 1750 (C=O, broad), 1235 (C–O). NMR $\delta_{TMS}^{CDCl_3}$: 0.85 3H(s), 0.92 3H(s), 1.13 3H(d, $J$=6 cps), 1.98 3H(s), 2.01 3H×2(s), 2.04 3H(s), 3.41 3H(s), 4.89 2H(d, $J$=7 cps), 5.86 1H(s).

**Hydrolysis of I with 0.05N $H_2SO_4$**——I (10 mg) was dissolved in 1 ml of MeOH and refluxed for 30 min with 1 ml of 0.1N $H_2SO_4$ on a water bath. The reaction mixture was diluted with 2 ml of water and MeOH was evaporated *in vacuo* at room temperature and the residue was extracted with $CHCl_3$ (3 ml×3). The $CHCl_3$ layer was washed with water and dried over anhyd. $Na_2SO_4$. Removal of the solvent gave a powder. The product was recrystallized from MeOH to prisms (3 mg), mp 238° (softens from 140°) which was identified as periplogenin by mixed fusion, TLC (plate: Kieselgel H; solvent: $CHCl_3$: MeOH=95:5, *Rf* 0.21) and IR comparison with an authentic sample.

**Hydrolysis of I with Kiliani Mixture**——I (10 mg) was refluxed with 3 ml of Kiliani mixture ($CH_3COOH$: $H_2O$:conc. HCl=3.5:5.5:1) for one hour on a water bath. The reaction mixture was neutralized with IRA-410 ion exchange resin and then concentrated *in vacuo*. The residue was examined by TLC (A, *Rf* 0.02 and 0.60), and glucose and cymarose were identified by comparing with authentic samples.

**Hydrolysis of I with Takadiastase A**——Takadiastase A (200 mg) and toluene (0.5 ml) were added to the solution of I in water (30 ml) and stand for 280 hr at 34° under stirring. The reaction mixture was extracted with $CHCl_3$ (10 ml×4). The $CHCl_3$ layer was washed with water and dried over anhyd. $Na_2SO_4$. After removal of the solvent, the residue was purified by column chromatography on Kieselgel 0.05—0.02 mm (70 g) with $CHCl_3$. The main product was recrystallized from diluted EtOH to give colorless needles, IV, mp 144—146°/208—209° (double melting point), $[\alpha]_D^{19}$ +26.41° ($c$=0.92, 95% EtOH). *Anal.* Calcd. for $C_{30}H_{46}O_8$: C, 67.39; H, 8.68. Found: C, 67.38; H, 8.78. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 1750 (C=O). NMR $\delta_{TMS}^{CDCl_3}$: 0.87 3H(s), 0.93 3H(s), 1.25 3H(d, $J$=6 cps), 3.41 3H(s), 4.54 1H(q, $J_1$=3 cps, $J_2$=9 cps), 4.86 2H(d, $J$=7 cps), 5.85 1H(s). IV was identified as periplocymarin by mixed fusion, TLC (B, *Rf* 0.22) and IR comparison with an authentic sample.

The aqueous layer of reaction mixture was concentrated *in vacuo* and D-glucose was detected from the residue by PPC (A, *Rf* 0.19).

**Acidic Hydrolysis of IV**——IV (5 mg) was refluxed with 2 ml of 0.05N $H_2SO_4$–50% MeOH for 30 min on water bath. The reaction mixture was diluted with 2 ml of water and MeOH was evaporated *in vacuo* at room temperature and the residue was extracted with $CHCl_3$. The chloroform layer was washed with water, and dried over anhyd. $Na_2SO_4$. Removal of the solvent gave periplogenin (2 mg), which was identified by direct comparison with an authentic sample. The aqueous layer was neutralized with Amberlite IR-4B and then concentrated *in vacuo*. The residue was examined by TLC(A) and D-cymarose was identified by comparing with authentic sample (*Rf* 0.60).

**V**——V was recrystallized from MeOH–AcOEt saturated with $H_2O$ (yield: 0.005% from crude drug), 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, 59.64; H, 8.45. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH, broad). NMR $\delta_{TMS}^{Py}$: 0.68 3H(s), 1.00 3H(s), 1.45 3H×2 (d, $J$=6 cps), 3.48 3H(s), 5.80 1H(broad).

**VI**——To the solution of V in pyridine, $Ac_2O$ was added and allowed to stand for 48 hr at room temperature. The product, isolated by the usual working up, was reprecipitated from AcOEt–*n*-hexane to give colorless amorphous powder, mp 131°, $[\alpha]_D^{21.5}$ −41.5° ($c$=0.885, pyridine). *Anal.* Calcd. for $C_{58}H_{84}O_{25}$: C, 58.97; H, 7.16. Found: C, 58.61; H, 7.57. IR $\nu_{max}^{KBr}$ $cm^{-1}$: OH(nil), 1760, 1240 (–O–$COCH_3$).

**Hydrolysis of V with 3N $H_2SO_4$-50% MeOH**——V (20 mg) was refluxed with 3N $H_2SO_4$–50% MeOH (3 ml) for 30 min. The reaction mixture was diluted with 3 ml of water and MeOH was evaporated *in vacuo* at room temperature. The concentrated aqueous solution was extracted with $CHCl_3$ (3 ml×4). The $CHCl_3$ layer was washed with water, and dried over anhyd. $Na_2SO_4$. Removal of the solvent gave a powder. The product was recrystallized from AcOEt to give colorless leaflets (8 mg), mp 182° which was identified as VIII by mixed fusion, TLC(B) and IR comparison with an authentic sample.

The aqueous layer of hydrolysate was neutralized with Amberlite IR-4B and concentrated *in vacuo*. The residue was examined by TLC (A, *Rf* 0.02 D-glucose, 0.24 D-digitalose), PPC (B, *Rf* 0.17 D-glucose, 0.43

D-digitalose) 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).

**Permethylation of V**——According to the Hakomori's method, NaH (180 mg) was warmed with dimethylsulfoxide (3 ml) at 65° for 1 hr under stirring in $N_2$ gas flow. To this reagent the solution of V (150 mg) in dimethylsulfoxide was added and the mixture was kept at 65° for 15 min under stirring in $N_2$ gas flow. Then $CH_3I$ (0.8 ml) was added and the reaction mixture was allowed to stand at room temperature for 3 hr under stirring. After dilution with 90 ml of water, the mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and concentrated. The residue was crystallized from *n*-hexane to give per-O-methyl-glycoside K(VII) (138 mg), colorless needles, mp 161—162°, $[\alpha]_D^{20}$ −31.34° ($c$=1.34, EtOH). *Anal.* Calcd. for $C_{49}H_{84}O_{16}$: C, 63.33; H, 9.11. Found: C, 63.28; H, 9.16. NMR $\delta_{TMS}^{CDCl_3}$: 0.66 3H(s), 1.00 3H(s); 1.25 3H(d, $J$=6 cps), 1.34 3H(d, $J$=6 cps), 3.48—3.64 3H×10(s), 4.18 1H(d, $J$=9 cps), 4.38 1H(d, $J$=8 cps), 4.67 1H(d, $J$=8 cps), 5.40 1H(broad).

**Acid Hydrolysis of VII**——VII (85 mg) was dissolved in MeOH (3 ml) and hydrolysed with 4N $H_2SO_4$ (3 ml) for 30 min under refluxing on water bath. The reaction mixture was diluted with water (4 ml) and MeOH was evaporated *in vacuo* at room temperature. The aqueous residue was extracted with $CHCl_3$ (5 ml×3). The $CHCl_3$ layer was washed with water and dried over anhyd. $Na_2SO_4$. The aqueous layer was neutralized with Amberlite IR-4B and concentrated *in vacuo*. The residue was examined by PPC (A, *Rf* 0.86 2,3,4,6-tetra-O-methyl-D-glucose, 0.67 2,3,4-tri-O-methyl-D-glucose, 0.45 4-O-methyl-D-digitalose) and 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; sugar sample was trimethylsilylated as usual manner, $t_R$ (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).

Removal of the solvent from $CHCl_3$ extract gave a colorless powder which was recrystallized from *n*-hexane to form colorless needles (IX, 34 mg), mp 132—133°, $[\alpha]_D^{20}$ −50.40° ($c$=1.04, 95% EtOH). *Anal.* Calcd. for $C_{22}H_{36}O_2$: C, 79.46; H, 10.92. Found: C, 79.50; H, 10.79. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3500 (OH). NMR $\delta_{TMS}^{CDCl_3}$: 0.68 3H(s), 1.00 3H(s), 1.20 3H(d, $J$=6 cps), 3.31 3H(s), 5.31 1H(broad).

**Oxidation of IX with Chromium Trioxide**——To a solution of IX (30 mg) in acetone (5 ml), 0.2 ml of the Jones reagent ($CrO_3$ 2 g, $H_2SO_4$ 3 g, $H_2O$ 15 ml) was added dropwise under stirring at 0° and then kept at room temperature for 25 min. To the reaction mixture 0.5 ml of MeOH was added and diluted with 50 ml of water and then extracted with benzene. The benzene layer was washed with 5% $NaHCO_3$ and water, and dried over anhyd. $Na_2SO_4$. Evaporation of the solvent *in vacuo* gave an colorless powder which was recrystallized from *n*-hexane to form colorless needles (24 mg) (X), mp 124°, $[\alpha]_D^{18}$ +16.95° ($c$=2.30, $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 80.08; H, 10.26. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1700 (C=O). NMR $\delta_{TMS}^{CDCl_3}$: 0.64 3H(s), 1.00 3H(s), 2.11 3H(s), 3.35 3H(s), 5.40 1H(broad). ORD ($c$=0.011, EtOH) [M]$^{19}$ (m$\mu$): −12217 (264) (trough), +6404 (307) (peak). X was identified as 3$\beta$-O-methyl-$\Delta^5$-pregnene-20-one by mixed fusion, TLC, ORD, NMR and IR spectra with authentic samples which was synthesized from pregnenolone according to A. Butenandt, *et al.*[10]

**Enzymatic Hydrolysis of V**——Takadiastase A (360 mg) and toluene (1 ml) were added to the solution of V (240 mg) in aqueous ethanol (EtOH 2 ml, $H_2O$ 100 ml), and kept at 34° for 9 days. The reaction mixture was extracted with *n*-BuOH (30 ml×4), and *n*-BuOH layer was concentrated *in vacuo*. The residue was purified by column chromatography on Kieselgel 0.05—0.20 (40 g) with $CHCl_3$:MeOH:$H_2O$=65:35:10 lower phase. Only one product, tentatively named XI (96 mg) was obtained and unchanged V was recovered (83 mg). The aqueous layer of reaction mixture was concentrated *in vacuo* and D-glucose was detected by PPC.

**XI**——XI was recrystallized from MeOH–AcOEt saturated with water to form colorless needles, mp 268—270°, $[\alpha]_D^{21}$ −29.16° ($c$=1.20, MeOH). *Anal.* Calcd. for $C_{34}H_{56}O_{11}\cdot H_2O$: C, 61.98; H, 8.87. Found: C, 61.75; H, 9.01. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400 (OH). NMR $\delta_{TMS}^{Py}$: 0.67 3H(s), 1.02 3H(s), 1.48 3H(d, $J$=6 cps), 1.53 3H(d, $J$=6 cps), 3.50 3H(s), 4.19 1H(d, $J$=9 cps), 4.56 1H(d, $J$=8 cps), 5.41 1H(broad).

**XII**——To the solution of XI (52 mg) in pyridine (2 ml), $Ac_2O$ (2 ml) was added and allowed to stand for 48 hr at room temperature. The reaction mixture was worked up as usual and recrystallized from aqueous EtOH to give colorless needles (49 mg). mp 210°, $[\alpha]_D^{24}$ −35.22° ($c$=1.53, EtOH). *Anal.* Calcd. for $C_{46}H_{68}O_{17}$: C, 61.88; H, 7.62. Found: C, 62.07; H, 7.77. IR $\nu_{max}^{Nujol}$ cm$^{-1}$: OH(nil), 1740, 1230. NMR $\delta_{TMS}^{CDCl_3}$: 0.68 3H(s), 1.02 3H(s), 1.18 3H(d, $J$=6 cps), 1.29 3H(d, $J$=6 cps), 2.00 3H(s), 2.02 3H×3(s), 2.05 3H(s), 2.12 3H(s), 5.40 1H(broad).

**Acid Hydrolysis of XI**——XI (4 mg) was refluxed with 3N $H_2SO_4$–50% MeOH (2 ml) for 30 min. The reaction mixture was treated as usual and VIII was detected from $CHCl_3$ layer and digitalose and glucose from aqueous layer.

**Permethylation of XI**——The permethylation of XI (60 mg) was carried out by according Hakomori's method described in the case of V, and 53 mg of per-O-methyl-XI(XIII) was obtained, colorless amorphous powder, IR $\nu_{max}^{KBr}$ cm$^{-1}$: OH (nil). NMR $\delta_{TMS}^{CDCl_3}$: 0.68 3H(s), 1.00 3H(s), 1.28 3H×2(d, $J$=6 cps), 3.34—3.61 3H×7(s), 4.21 1H(d, $J$=8 cps), 4.68 1H(d, $J$=8 cps), 5.38 1H(broad).

**Methanolysis of XIII**——XIII (28 mg) was refluxed with 2N HCl–MeOH (4 ml) for 2 hr, and then 5 ml of 2N HCl was added to the reaction mixture and refluxed for 1 hr. The solution was diluted with 5 ml of

water and MeOH was evaporated *in vacuo* at room temperature. The concentrated solution was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and dried over anhyd. $Na_2SO_4$. Removal of the solvent gave IX which was identified by TLC (B, *Rf* 0.62). The sugar components of the aqueous layer of hydrolysate were examined by PPC and GLC, and 2,3,4,6-tetra-O-methyl-D-glucose and 4-O-methyl-D-digitalose were identified.

**Degradation of V with $NaIO_4$ (Formation of $\Delta^5$-Pregnene-3$\beta$,20$\alpha$-diol-(20)-$\beta$-D-digitalopyranoside (XIV))**——To a solution of V (300 mg) in 95% EtOH (45 ml), a solution of $NaIO_4$ (450 mg) in $H_2O$ (5 ml) was added under stirring at room temperature for 1 hr. After removing the precipitate by filtration, EtOH was evaporated *in vacuo* under 50° and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with $H_2O$, dried over $Na_2SO_4$ and evaporated *in vacuo*. The residue was dissolved in 95% MeOH (30 ml) and then added $NaBH_4$ (200 mg) in portionwise at room temperature under stirring. After the mixture was stirring at the same temperature for 1 hr, reaction mixture was neutralized with 5% AcOH, concentrated *in vacuo* under 50° and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with $H_2O$, dried over $Na_2SO_4$ and evaporated *in vacuo*. The products were dissolved in 0.05N HCl–50% MeOH (10 ml) and the solution was refluxed for 30 min. The reaction mixture was neutralized with 0.1N $KHCO_3$ and, after added $H_2O$ (5 ml), the solution was evaporated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution 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 using AcOEt saturated with $H_2O$. The separated monoglycoside was recrystallized from aqueous ethanol to give colorless needles, mp 234—236° (134 mg), $[\alpha]_D^{24}$ −38.21° (*c*=1.26, EtOH). *Anal.* Calcd. for $C_{28}H_{46}O_6$: C, 70.26; H, 9.69. Found: C, 70.26; H, 9.77. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH). NMR $\delta_{TMS}^{py}$: 0.71 3H(s), 1.03 3H(s), 1.54 3H×2(d, *J*=6 cps), 3.51 3H(s), 4.71 1H(d, *J*=8 cps), 5.4 1H(broad). On hydrolysis with 3N $H_2SO_4$–50% MeOH refluxing for 30 min, XIV gave VIII and digitalose.

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