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Studies on the Constituents of *Panacis japonici Rhizoma*. III.[1] The Structure of Chikusetsusaponin. III[2]

Noriko Kondo, Kyoko Aoki, Harumi Ogawa, Ryoji Kasai, and Junzo Shoji

School of Pharmaceutical Sciences, Showa University[3]

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The chemical structure of chikusetsusaponin III, $C_{40}H_{80}O_{17}\cdot 2H_2O$, mp 196—197°, $[\alpha]_D^{19}$ +1.5° (MeOH), a crystalline saponin isolated from the rhizome of *Panax japonicum* was established to be 20(*S*)-protopanaxadiol-3-[β-D-glucopyranosyl(1→2)]-[β-D-xylopyranosyl(1→6)]-β-D-glucopyranoside as represented by formula (I).

It is very remarkable that the rhizome of *Panax japonicum* which has been used as the substitute drug of "Ginseng" in Japan, contains the homologous saponin of the ginseng.

In our previous papers,[1,4] it has been reported that three kinds of saponin were isolated in crystalline state from a crude drug, "Chikusetsu-Ninjin" (rhizome of *Panax japonicum* C.A. Meyer; Araliaceae) and the chemical structure of chikusetsusaponin IV agreed with a tonic saponin, araloside A which was isolated from *Aralia manschurica* (Araliaceae) by N.K. Kochetkov and others.[5] In the present paper, the structure elucidation of chikusetsusaponin III which leads to the assignment of the structure I is described.

Chikusetsusaponin III (I), $C_{47}H_{80}O_{17}\cdot 2H_2O$, colourless prisms, mp 196—197°, $[\alpha]_D^{19}$ +1.5° (MeOH), has been isolated from methanol extract of the crude drug by repeated column chromatography and recrystallization from methanol–ethyl acetate saturated with water.

On acetylation with acetic anhydride and pyridine, I gave decaacetate (II), $C_{67}H_{100}O_{27}$, $[\alpha]_D^{19}$ −20.26° ($CHCl_3$). The infrared spectrum (IR) of II showed a hydrogen bonding hydroxyl absorption band at 3545 cm^{-1} in CCl_4 solution.

As we reported in previous paper, I, on acidic hydrolysis, afforded as an aglycone, panaxadiol (VI),[6] and D-glucose and D-xylose as the sugar components. Acidic hydrolysis of hydrogenated I (IV) gave dihydroprotopanaxadiol (VIII)[7] which was identified with the authentic sample by thin-layer chromatography (TLC) and the mixed fusion.

To confirm the genuine sapogenin of I, further experiment was carried out. Degradation of I by oxidation with sodium metaperiodate followed by treatment with dilute potassium hydroxide[8] gave a genuine sapogenin, 20(*S*)-protopanaxadiol (VII),[9] mp 198°, colourless needles from benzene, which was identified by the mixed fusion, gas liquid chromatography (GLC), TLC, and the comparison of IR spectra with the authentic sample which was kindly given us from Prof. S. Shibata.

1) Part II: N, Kondo, J. Shoji, N. Nagumo, and N. Komatsu, *Yakugaku Zasshi*, **89**, 846 (1969).
2) This work was presented at the Annual meeting of the Pharmacognostical Society of Japan, Tokyo, September 23, 1969, Abstracts of paper, p. 38.
3) Location: *Hatanodai, Shinagawa-ku, Tokyo.*
4) N. Kondo and J. Shoji, *Yakugaku Zasshi*, **88**, 325 (1968).
5) N.K. Kochetkov, A.J. Khorlin, and V.E. Vaskovsky, *Tetrahedron Letters*, **1962**, 713; N.K. Kochetkov, A.J. Khorlin, and V.E. Vaskovsky *Izv. Akad. Nauk SSSR Ser Khim.*, **1963**, 1398, 1409.
6) S. Shibata, M. Fujita, S. Itokawa, O. Tanaka, and T. Ishii, *Chem. Pharm. Bull.* (Tokyo), **11**, 759 (1963).
7) S. Shibata, O. Tanaka, T. Ando, M. Sado, S. Tsushima, and T. Ohsawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 595 (1966).
8) J.J. Dugan and P. de. Mayo, *Can. J. Chem.*, **43**, 2033 (1965); Y. Shimizu, and S.W. Pelletier, *J. Am. Chem. Soc.*, **88**, 1544 (1966).
9) Y. Iida, O. Tanaka, and S. Shibata, *Tetrahedron Letters*, **1968**, 5449.

Deca-O-methylchikusetsusaponin III (III), $C_{57}H_{102}O_7$, mp 88—89°, $[\alpha]_D^{25}$ −34.8° ($CHCl_3$), IR $\nu_{max}^{CCl_4}$ 3388 cm^{-1} (hydrogenbonding OH), prepared by repeated methylation by the Hakomori's method[10] was catalytically reduced on PtO_2. The deca-O-methyldihydrochikusetsusaponin III (V) gave, on hydrolysis with concentrated HCl at room temperature for 7.5 hours, an aglycone, 12-O-methyldihydroprotopanaxadiol (IX)[11] and a mixture of methylated sugars.

The above results suggested the partial structure of I to be constructed with 20(*S*)-protopanaxadiol as a genuine sapogenin and sugar moiety which was combined with the C_3-hydroxyl group of the genin. The lack of sugar moiety at the C_{20}-hydroxyl group of the genin was deduced from the IR spectra of II and IV which showed a hydrogen bonding hydroxyl absorption band at 3543 cm^{-1} [7] in the former and 3388 cm^{-1} [11] in the latter.

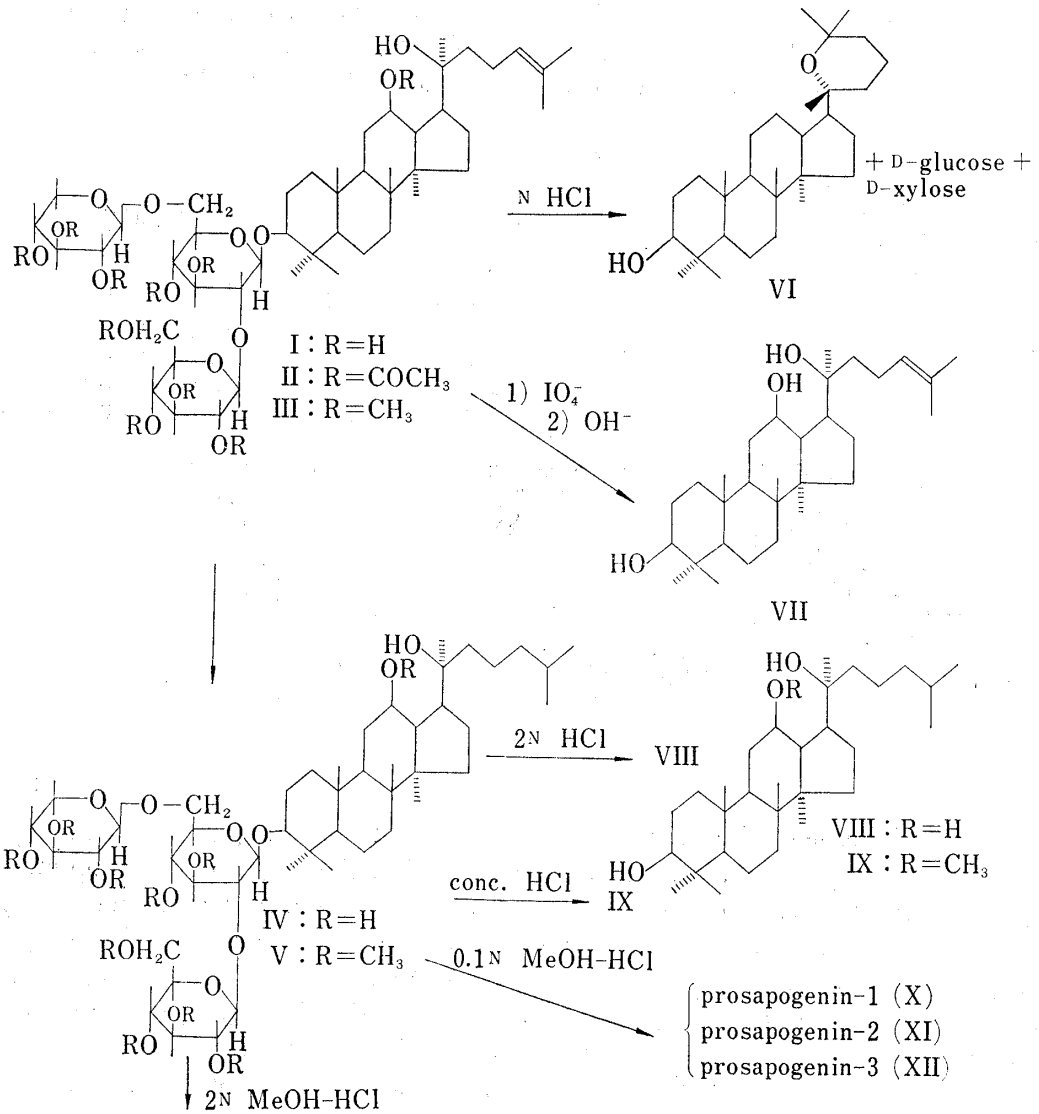


Chart 1

10) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
11) S. Shibata, T. Ando, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 1157 (1966).

The sugar portions of the methanolysate of III with 2N HCl in dried methanol refluxing 4.5 hours were identified to be methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, methyl 2,3,4-tri-O-methyl-D-xylopyranoside and methyl 3,4-di-O-methyl-D-glucopyranoside by TLC and GLC. Besides these methylated sugars, a partial methylated biose was isolated by preparative TLC. The permethylated biose prepared by repeated methylation by the Hakomori's method gave, on methanolysis, methyl 3,4,6-tri-O-methyl-D-glucopyranoside and methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside.

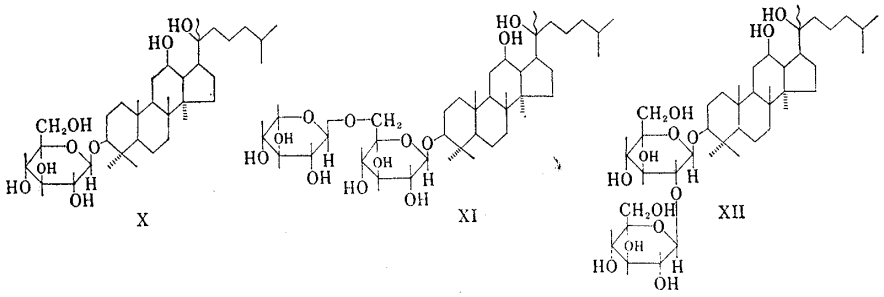
Therefore the trisaccharide portion of I should have a branched chain structure and represented by [D-glucopyranosyl (1→2)]-[D-xylopyranosyl (1→6)]-D-glucopyranoside. From the methanolysis experiments, the partially methylated biose was deduced to be methyl hexa-O-methyl-sophorose, but further examination was not performed.

Partial methanolysis of IV with 0.1N HCl in dried methanol gave prosapogenin-1, $C_{36}H_{62}O_8$, prosapogenin-2, $C_{41}H_{72}O_{12}$ and prosapogenin-3, $C_{42}H_{74}O_{13}$, which were isolated from the methanolysate by repeated column chromatography and characterised (Table I).

Table I

Prosapogenin	Molecular formula	mp	$[\alpha]_D$	Sugar	NMR $\delta^{CDCl_3}_{TMS}$ ppm
Prosapogenin-1	$C_{36}H_{62}O_8$	181—182°	+0.72° (in MeOH c=1.38)	glucose	4.83 (1H (d) J=6 cps)
Prosapogenin-2	$C_{41}H_{72}O_{12}$	174—175°	−7.9° (in MeOH c=1.26)	glucose +xylose	4.78 (1H (d) J=6 cps) 5.21 (1H (d) J=6 cps)
Prosapogenin-3	$C_{42}H_{74}O_{13}$	198—200°	+1.02° (in MeOH c=0.98)	glucose +glucose	4.83 (1H (d) J=6 cps) 5.21 (1H (d) J=8 cps)

prosapogenin-1 prosapogenin-2 prosapogenin-3



X XI XII

The application of Klyne's rule[12] for the determination of the configuration of the glycoside linkages was not favourable, because it was assumed that the disproportional epimerisation at C_{20}-hydroxyl group of each prosapogenins would be occured in the partial methanolysis reaction. The chemical shifts and the coupling constants of the anomeric proton signals of these prosapogenins revealed the configuration of the three sugars of I were all β-form.

The total structure of chikusetsusaponin III was suggested to be 20(*S*)-protopanaxadiol-3-[β-D-glucopyranosyl(1→2)]-[β-D-xylopyranosyl(1→6)]-β-D-glucopyranoside.

It is very remarkable that panacis japonici rhizoma which has been used as the substitute drug of ginseng radix in Japan, contains the dammarane type saponin having the similar structure to those of ginseng radix. From this point the pharmacological investigation of I are now studying.

Experimental

All melting points were determined on Yanagimoto Micro Melting Point apparatus and uncorrected. Infrared absorption spectra were measured in $CHCl_3$ or CCl_4 solution with Hitachi Model EPI-2. Nuclear

12) W. Klyne, *Biochem. J.*, **47**, XLI (1950).

magnetic resonance spectra were measured with Japan Electron Co. JNM. 4H-100 spectrometer and Hitachi Model R-20 High Resolution NMR Spectrometer with tetramethylsilane as a internal standard. The chemical shifts are reported in δ and the solvents used are indicated. Gas chromatograph used was Hitachi Model K-53 with hydrogen flame ionization detecter. Molecular weight was determined using a Hitachi Perkin-Elmer Molecular Weight apparatus Model 115.

Chikusetsusaponin III (I)——As we reported in previous paper, I was obtained from the crude saponin fraction of the rhizome of *Panax japonicum* by repeated column chromatography and recrystallized from MeOH–AcOEt saturated with water to colorless needles, mp 196—197°, $[\alpha]_D^{19}$ +1.5° (c=1.69, MeOH). *Anal.* Calcd. for $C_{47}H_{80}O_{17}\cdot 2H_2O$: C, 59.24; H, 8.80. Found: C, 59.01; H, 9.04. IR ν_{max}^{Nujol} cm^{-1}: 3300—3400 (OH), 1160 (C–O).

Chikusetsusaponin III-deca-O-acetate (II)——I (1 g) was dissolved in pyridine (20 ml), and Ac_2O (20 ml) was added and allowed to stand for 48 hr at room temperature. The product was worked up as usual and reprecipitated from MeOH–H_2O to give colorless powder. mp 126—129°, $[\alpha]_D^{19}$ −20.26° (c=1.67, $CHCl_3$). *Anal.* Calcd. for $C_{67}H_{100}O_{27}$: C, 60.17; H, 7.47. Mol. Wt. 1336. Found: C, 60.42; H, 7.61. Mol. Wt. 1379 (Osmotic vapour pressure method in MeOH). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3543 (OH), 1760 (C=O), NMR $\delta_{TMS}^{CDCl_3}$: 1.6—1.7 $\left(3H,\ s\times 2,\ =C\langle^{CH_3}_{CH_3}\right)$; 2.0—2.2 (3H, s×10, $-OCOCH_3$).

Catalytic Reduction of I (Preparation of Dihydrochikusetsusaponin III (IV)) and it's Hydrolysis——I (300 mg) was catalytically reduced on PtO_2 (50 mg) in EtOH (20 ml) added a small amount of AcOH, and the product (IV) was hydrolysed with 2N HCl–50% EtOH under reflux for 4 hr. The reaction mixture was treated in same manner as reported in previous paper. The isolated colorless powder was recrystallized from MeOH to give colorless needles, mp 249—251°, which was identified as dihydroprotopanaxadiol (VIII) by comparing with the authentic sample by mixed mp, TLC and IR spectra.

Cleavage of I with Sodium Metaperiodate and Isolation of 20(*S*)-Protopanaxadiol——I (500 mg) was dissolved in MeOH (200 ml)–EtOH (33.3 ml)–H_2O (100 ml) and sodium metaperiodate (1.67 g) was added. The solution was stirred for 140 hr at 2° in the dark. The reaction mixture was distilled *in vacuo* to evaporate the alcohols. The residual aqueous solution was extracted with *n*-BuOH saturated with water. The BuOH extract was evaporated *in vacuo* and the residue was dissolved again in EtOH (2.5 ml)–MeOH (5 ml)–H_2O (17.5 ml) and then added solid potassium hydroxide (1.25 g). The solution was heated at 100° in N_2 gas flow under stirring for 1 hr, cooled and kept stirring for 16 hr at 2°. The reaction mixture was carefully neutralized with diluted sulfuric acid. Extraction with ether gave the crude sapogenin.[13]

Chromatography on silica gel with benzene–acetone (4:1) gave a crystalline sapogenin, colorless needles from benzene, mp 198°. IR ν_{max}^{KBr} cm^{-1}: 3300—3250 (OH), 1640—1630 (C=C). TLC: plate, Kieselgel H; solvent, benzene–acetone (4:1), *Rf*, 0.25; (20*R*-protopanaxadiol 0.27, 20*S*-protopanaxadiol 0.25). The sapogenin was identified as 20(*S*)-protopanaxadiol by comparing with the authentic sample which was kindly given us from Prof. S. Shibata by mixed mp, TLC, IR spectra and GLC (glass column, 1.5% SE-30 on DMCS-Chromosorb W, 3 mm×2 m; column temp. 280°; carrier gas, N_2 (1.5 kg/cm^2); t_R 5.2; 20(*S*)-protopanaxadiol t_R 5.2, 20(*R*)-protopanaxadiol t_R 3.8).

Deca-O-methyldihydrochikusetsusaponin III (V)——i) Methylation of I: According to the Hakomori's method, NaH (530 mg) was warmed with dimethylsulfoxide (50 ml) at 70° for 1 hr under stirring in N_2 gas flow. To this reagent I (1.06 g) in dimethylsulfoxide was added and the mixture was kept at 70° for 20 min under stirring in N_2 gas flow. CH_3I (10 ml) was added and the reaction mixture was allowed to stand at room temperature for 4 hr under stirring. After dilution with water, the mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and concentrated to dryness. The residue was methylated again under the same condition and the product was chromatographed on Kieselgel eluted with AcOEt to give deca-O-methyl ether of I (III) (829 mg), colorless powder from aqueous EtOH. NMR $\delta_{TMS}^{CDCl_3}$: 0.8—1.2 (3H, s×6, CH_3); 1.6—1.7 $\left(3H,\ s\times 2,\ =C\langle^{CH_3}_{CH_3}\right)$; 3.28—3.7 (3H, s×10, OCH_3).

ii) Catalytical hydrogenation of Deca-O-methylchikusetsusaponin III (V): III (774 mg) was catalytically reduced on PtO_2 (70 mg) in EtOH (50 ml) added a small amount of AcOH for 2 hr. The reaction mixture was evaporated *in vacuo* to dryness and the residue was reprecipitated from aqueous EtOH to give colorless powder (V), mp 88—89°, $[\alpha]_D^{25}$ −34.8° (c=0.66 in $CHCl_3$). *Anal.* Calcd. for $C_{57}H_{102}O_7$: C, 64.66; H, 9.63. Found: C, 64.58; H, 9.64. NMR $\delta_{TMS}^{CDCl_3}$: 0.7—1.4 (3H, s×8, $-CH_3$), 3.28—3.7 (3H, s×10, OCH_3). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3388 (hydrogen bonding OH).

Hydrolysis of Deca-O-methyldihydrochikusetsusaponin III (V)——V (500 mg) was hydrolysed with conc. HCl (20 ml) for 7.5 hr at room temperature. The reaction mixture was diluted with water and extracted with ether. The ether extract was chromatographed on silica gel with benzene–acetone (4:1) and crystallized from aqueous acetone affording 12-O-methyldihydroprotopanaxadiol (IX), colorless needles, mp 143—145°, which was identified with authentic sample kindly given us from Prof. S. Shibata by the mixed fusion and IR spectra. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3388 (hydrogen bonding OH), 3626 (free OH).

13) This modified procedure was privately communicated from Prof. S. Shibata.

Methanolysis of Deca-O-methyldihydrochikusetsusaponin III (IV)——V (500 mg) was refluxed with 2N HCl methanol (40 ml) on a oil bath for 4.5 hr. The solution was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was chromatographed on silica gel with benzene–acetone (4:1). The isolated sugars were identified to be methyl 2,3,4-tri-O-methyl-D-xylopyranoside, methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 3,4-di-O-methyl-D-glucopyranoside by TLC and GLC comparing with the authentic samples.

Besides these methylated sugars, a partially methylated biose was isolated and characterised by methanolysis after permethylation by Hakomori's method affording methyl 3,4,6-tri-O-methyl-D-glucopyranoside and methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside.

TLC: i) plate, Kieselgel H; solvent, benzene–acetone (4:1). *Rf*, methyl 2,3,4-tri-O-methyl-D-xylopyranoside 0.32 (α), 0.42 (β); methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside 0.27 (α), 0.36 (β); methyl 3,4-di-O-methyl-D-glucopyranoside 0.04; biose 0.06. ii) solvent, benzene–acetone (1:1). *Rf*, methyl 3,4,6-tri-O-methyl-D-glucopyranoside 0.45.

GLC: i) column, 10% SE-30 on Chromosorb W (80—100 mesh) 3 mm × 1 m; column temperature, 100°; carrier gas, N_2 (1 kg/cm²); t_R, methyl 2,3,4-tri-O-methyl-D-xylopyranoside 5.0 (α), 4.3 (β); methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside 14.3 (α), 11.6 (β). ii) column, 3% SE-30 on Chromosorb W (80—100 mesh) 3 mm × 1m; column temperature, 110°; carrier gas, N_2 (0.8 kg/cm²); t_R, methyl 3,4,6-tri-O-methyl-D-glucopyranoside 14.4. iii) column, 3% SE-30 on Chromosorb W (80—100 mesh) 3 mm × 1 m; column temperature, 140°; carrier gas, N_2 (1 kg/cm²); t_R, methyl 3,4-di-O-methyl-D-glucopyranoside 4.1.

Partial Methanolysis of Dihydrochikusetsusaponin III (IV)——IV (760 mg) was refluxed with 0.1N HCl–MeOH (30 ml) for 1 hr. The solution was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (7:3:1;–lower phase). Each eluate was evapolated to dryness and examined by TLC. Three kinds of prosapogenins, tentatively named prosapogenin-1 (67.8 mg), prosapogenin-2 (120.2 mg) and prosapogenin-3 (154 mg) were obtained. The sugar components of each prosapogenins were determined by methanolysis with 2N HCl–MeOH and the results were summerised in Table I.

Prosapogenin-1 (X)——Prosapogenin-1 was further purified by chromatography on silica gel with benzene–acetone (2:1) and repeatedly precipitated from aqueous EtOH affording white powder mp 181—182°, $[\alpha]_D^{28}$ +0.721° (c=1.38, MeOH). *Anal.* Calcd. for $C_{36}H_{62}O_8 \cdot 1/2\ H_2O$: C, 68.24; H, 10.26. Found: C, 68.12; H, 10.21.

Prosapogenin-2 (XI)——The fraction of prosapogenin-2 was further purified by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (7:3:1; lower phase) and recrystallized from aqueous EtOH affording colorless needles, mp 174—175°, $[\alpha]_D^{28}$ −7.9° (c=1.26, MeOH). *Anal.* Calcd. for $C_{41}H_{72}O_{12} \cdot H_2O$: C, 63.56; H, 9.56. Found: C, 63.34; H, 9.60.

Prosapogenin-3 (XII)——The fraction of prosapogenin-3 was further purified by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (7:3:1; lower phase) and repeatedly precipitated from MeOH–acetone affording colorless powder, mp 198—200°, $[\alpha]_D^{28}$ +1.02° (c=0.98, MeOH). *Anal.* Calcd. for $C_{42}H_{74}O_{13} \cdot H_2O$: C, 60.00; H, 9.05. Found: C, 59.99; H, 9.40.

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