[Chem. Pharm. Bull. 32(4)1365—1372(1984)]

Five Steroidal Components from the Rhizomes of *Polygonatum odoratum* var. *pluriflorum*

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(Received July 26, 1983)

Five steroidal components (PO-a(**1**)-PO-e(**5**)) were obtained from the methanolic extract or its partial hydrolysate of the fresh rhizomes of *Polygonatum odoratum* var. *pluriflorum*, and their chemical structures were characterized as (25 *R* and *S*)-spirost-5-en-3β,14α-diol, 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranoxyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl (=β-lycotetraosyl)-(25 *R* and *S*)-spirost-5-en-3β,14α-diol, 3-*O*-β-lycotetraosyl-22-methoxy-(25 *R* and *S*)-furost-5-en-3β,14α,26-triol 26-*O*-β-D-glucopyranoside, 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-1(→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl-22-methoxy-(25 *R* and *S*)-furost-5-en-3β,14α,26-triol 26-*O*-β-D-glucopyranoside and 3-*O*-β-lycotetraosyl yamogenin, respectively. Moreover, (25 *S*)-spirost-5-en-3β,14α-diol (**7**) was isolated from a mixture of the (25 *R*) and (*S*) derivatives of PO-a (**1**) and it was designated as neoprazerigenin A.

Keywords——*Polygonatum odoratum* var. *pluriflorum*; Liliaceae; neoprazerigenin A; spirostanol glycoside; furostanol glycoside

Polygonatum odoratum var. *pluriflorum* (Liliaceae) grows wild in Japan, the Korean peninsula and China, and its rhizomes have been used as a nutritious tonic. As regards its constituents, quercitol,[1] azetidine 2-carboxylic acid,[2] mucous polysaccharides,[3] vitamin A, mucilage[4] and starch have been identified and diosgenin was also detected[5] in the hydrolysate of the benzene–methanol extract.

Our investigation on this plant has resulted in the isolation of five steroidal components and their structural characterization. The MeOH extraction of the rhizomes of this plant was subjected to Amberlite XAD-2 and silica gel column chromatographies to afford five new steroidal ingredients, tentatively designated as PO-a (**1**), PO-b (**2**), PO-c (**3**), PO-d (**4**) and PO-e (**5**).

PO-a (**1**), colorless needles, mp 215—216 °C, $[\alpha]_D$ −86.1° ($CHCl_3$), showed the molecular ion at *m/z* 430.3054, giving the molecular formula $C_{27}H_{42}O_4$, and a characteristic prominent peak[6] at *m/z* 139 ($C_9H_{15}O^+$) deriving from the spiroketal side chain of the steroidal sapogenol in the mass spectrum. The absorptions[7] at 980, 960, 918, 890 and 840 cm^{-1} in the infrared (IR) spectrum also indicated that PO-a (**1**) is a spirostanol derivative. The proton nuclear magnetic resonance (^1H NMR) spectrum of PO-a (**1**) showed two singlet signals at δ 0.94 and 1.05 due to C-18-Me and C-19-Me and two doublet signals (both J= 7 Hz) at δ 1.01 and 1.08 ascribable to C-27-Me and C-21-Me. Moreover, the signals at δ 3.30 (br d, J=11 Hz) and 3.95 (dd, J=2.5, 11 Hz) could be assigned to the C-26-axial and -equatorial protons, respectively, indicating that the spirostanol derivative **1** possesses (25 *S*) configuration.[8] The signals at δ 3.48, 4.64 and 5.37 were ascribable to C-3-H, C-16-H and C-6-H, respectively. However, besides the above signals two low-intensity doublet signals at δ 0.79 and 0.99 were detected, and these were assumed to be due to C-27-Me and C-21-Me of the sapogenol with (25 *R*) configuration present as a minor contaminent. The C-26-methylene (25 *R*) signals were hidden by the C-3-H signal. Next, the carbon-13 nuclear magnetic resonance (^{13}C NMR) spectrum of PO-a (**1**) exhibited four carbons bearing a hydroxyl group

or ring oxide at δ 65.1 (t), 71.3 (d), 82.0 (d) and 86.5 (s), representing chemical shifts due to (25 *S*) spirostanol (those of the (25 *R*) compound were at δ 66.9 (t), 71.3 (d), 81.9 (d), 86.4 (s)). The former three signals could be assigned to C-26, C-3 and C-16 by comparison with those in the ^{13}C NMR spectra of diosgenin and yamogenin.[9] The remaining carbon was considering to be a quaternary one carrying a hydroxyl, and it might be at C-8, -9, -14, -17 or -25. C-25 was eliminated because of the appearance of a peak at *m/z* 139 derived from the spiroketal side chain in the mass spectrum and of a doublet signal (J=7 Hz) due to C-27-Me in the ^{1}H NMR spectrum. C-17 was also ruled out since PO-a (**1**) was not identical with pennogenin. Among the remaining positions C-8, -9 and -14, C-14 seemed most likely since the observed chemical shifts (δ 0.94 and 1.05) of C-18-Me and C-19-Me were nearly coincident with the calculated values[10] (δ 0.91 and 1.03, respectively). Consequently, PO-a (**1**) was represented as a 3β,14α-dihydroxy-spirost-5-ene. The signals in the ^{13}C NMR spectrum of PO-a (**1**) were tentatively assigned as shown in Table I; the intensity ratio of the signals due to C-22 of the (25 *S*) and (25 *R*) derivatives was approximately 2.3 : 1. Chromatographic separation of the corresponding monoacetate (**6**) of PO-a (**1**) followed by saponification afforded a sapogenol (**7**), which appeared to be composed only of the 25 *S* compound, based on the ^{1}H NMR spectrum. An example of a sapogenol having the hydroxyl at C-14, prazerigenin A (3β,14α-dihydroxy-(25 *R*)-spirost-5-ene),[11] is known. Therefore, the new sapogenol (**7**) should be designated as neoprazerigenin A.

PO-b (**2**), colorless needles, mp 261—265 °C, $[\alpha]_D$ −60.0° (pyridine), was negative to the Ehrlich reagent[12] and showed characteristic absorptions (912, 885, 835 cm^{-1}) due to the spiroketal side chain along with strong hydroxyl group absorptions (3600—3300 cm^{-1}), suggesting that PO-b (**2**) is a spirostanol derivative. PO-b (**2**), on acid hydrolysis, yielded glucose, galactose and xylose as sugar components, together with unidentified artifactual sapogenols derived from the genuine aglycone. The field desorption mass (FD-MS) spectrum[13] exhibited [M+Na$^+$] at *m/z* 1071, [M+Na$^+$−pentose] at *m/z* 939 and [M+Na$^+$−pentose] at *m/z* 909. The dodecamethyl ether (**8**) of PO-b (**2**), prepared by Hakomori's method,[14] showed terminal permethylated-hexosyl, -pentosyl and -pentosyl dihexosyl cations at *m/z* 219, 175 and 583, respectively, in the mass spectrum. In the ^{1}H NMR spectrum of **8**, four doublet signals with J=8 Hz at δ 4.94, 4.89, 4.68, 4.27 ascribable to the anomeric protons were detected, indicating that all glycosidic linkages are β.[15] The methyl ether, upon methanolysis, afforded methyl 2,3,4-tri-*O*-methyl α- and β-D-xylopyranoside, methyl 2,3,4,6-tetra-*O*-methyl α-D-glucopyranoside, methyl 2,3,6-tri-*O*-methyl α- and β-D-galactopyranoside and methyl 4,6-di-*O*-methyl α- and β-D-glucopyranoside. The above evidence suggested that the sugar moiety might be β-lycotetraose. Therefore, the ^{13}C NMR spectrum of PO-b (**2**) was compared with those of PO-a (**1**) and desgalactotigonin.[16] The signals of the aglycone were superimposable on those of PO-a (**1**) except for those of C-2, -3 and -4, which were somewhat different due to glycosylation shifts[17] caused by sugar bonding to C-3-OH of the aglycone, and the signals due to the sugar were coincident with those of β-lycotetraoside, the sugar moiety of desgalactotigonin. Moreover, the ratio of the signal intensities at δ 110.0 and 109.5 due to C-22 of the (25 *S*) and (25 *R*) derivatives was approximately 6 : 5. The attempted hydrolysis of PO-b (**2**) with a mixture of crude hesperidinase and β-glucosidase liberated a small amount of a genuine sapogenol, PO-a (**1**). Consequently, the structure of PO-b (**2**) was assigned as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl-(25 *R* and *S*)-spirost-5-en-3β,14α-diol.

PO-c (**3**), a white powder (mp 208—210 °C), $[\alpha]_D$ −49.0° (MeOH) showed absorptions due to hydroxyls, but no spiroketal side chain absorptions in the IR spectrum, and was positive to the Ehrlich reagent[12] suggesting that PO-c (**3**) is a furostanol glycoside. On enzymic hydrolysis with β-glucosidase, PO-c (**3**) liberated a glycoside, colorless needles, mp

TABLE I. ^{13}C NMR Chemical Shifts of PO-a (**1**), PO-b (**2**), Compound **9** and PO-e (**5**)

		PO-a (**1**) 25*S* (25*R*)	PO-b (**2**) 25*S* (25*R*)	Compound **9** 25*S* (25*R*)	PO-e (**5**)
Aglycone	1	38.1	37.7	37.7	37.5
	2	32.7	31.9	32.0	30.1
	3	71.3	78.6	78.6	78.7
	4	43.5	42.5	42.5	39.3
	5	141.6	140.5	140.5	141.1
	6	121.6	122.1	122.2	121.6
	7	26.3	29.9	30.2	32.3
	8	35.7	35.6	35.6	31.7
	9	43.7	43.6	43.6	50.3
	10	37.4	37.3	37.4	37.1
	11	20.5	20.4	20.4	21.1
	12	32.1	31.9	32.0	39.9
	13	45.1	45.1	45.1	40.4
	14	86.5 (86.4)	86.4	86.4	56.6
	15	39.9	39.2	39.0	32.2
	16	82.0 (81.9)	81.9 (81.8)	82.0 (81.9)	81.2
	17	59.8 (59.9)	59.9 (59.7)	59.7 (59.9)	62.7
	18	20.1	20.5	20.0	16.3
	19	19.5	19.3	19.3	19.4
	20	42.6 (42.1)	42.5 (42.0)	42.0	42.5
	21	15.2 (15.4)	15.2 (15.3)	15.2 (15.3)	14.8
	22	110.1 (109.6)	110.0 (109.5)	110.0 (109.6)	109.2
	23	26.7 (30.7)	26.6 (30.1)	26.7 (30.2)	26.4
	24	26.6 (29.4)	26.3 (29.3)	26.3 (29.3)	26.2
	25	27.6 (30.0)	27.5 (30.6)	27.6 (30.6)	27.5
	26	65.1 (66.9)	65.1 (66.8)	65.1 (66.8)	65.1
	27	16.4 (17.3)	16.3 (17.3)	16.4 (17.3)	16.3
C-3 Sugars					
Galactose	1′		102.6	102.7	102.8
	2′		73.1	73.1	73.1
	3′		75.0	75.2	75.3
	4′		79.8	80.1	79.8
	5′		76.1	76.1	76.2
	6′		60.5	60.5	60.6
Glucose	1″		104.7	104.5	104.8
(Inner)	2″		81.2	81.4	81.3
	3″		86.9	88.4	86.9
	4″		70.7	70.8	70.7
	5″		78.6	78.2	78.2[a]
	6″		62.4	62.3	62.5
Glucose	1‴		104.9	104.8	104.9
	2‴		75.2	75.2	75.0
	3‴		78.2	78.2	78.7
	4‴		71.0	70.9	71.7[b]
	5‴		77.7	77.8	77.6
	6‴		62.9	63.0	63.0
Xylose	1⁗		105.0		105.1
	2⁗		75.5		75.6
	3⁗		77.5		77.8[a]
	4⁗		70.4		70.5[b]
	5⁗		67.3		65.1
Glucose	1⁗			105.0	
	2⁗			75.5	
	3⁗			78.6	
	4⁗			71.6	
	5⁗			77.5	
	6⁗			63.0	

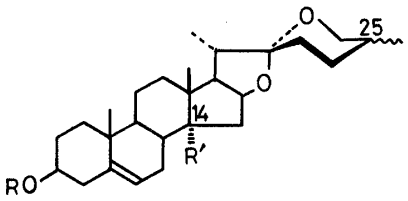
Assignments marked *a*, *b*) in each column may be reversed.

262—265 °C, $[\alpha]_D$ −62.0° (pyridine), and D-glucose. The former was identical with PO-b (**2**) in terms of physical data and ^{13}C NMR spectrum. Therefore, PO-c (**3**) was deduced to be a furostanol glycoside corresponding to PO-b (**2**), 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl 22-methoxy-(25 *R* and *S*)-furost-5-en-3β,14α,26-triol 26-*O*-β-D-glucopyranoside.

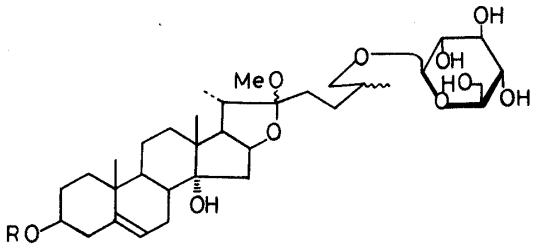
PO-d (**4**), a white powder (mp 208—213 °C), $[\alpha]_D$ −48.1° (MeOH) was also considered to be a furostanol glycoside from the IR and pink colorization with the Ehrlich reagent. On enzymic hydrolysis in the same way as for PO-c (**3**), PO-d (**4**) gave a spirostanol glycoside (**9**), colorless needles, mp 224—225 °C, $[\alpha]_D$ −69.0° (pyridine), along with D-glucose. The IR spectrum of **9** showed approximately equally intense absorptions at 915 and 885 cm^{-1}, suggesting that **9** is a mixture of (25 *R*) and (25 *S*) derivatives. The FD-MS spectrum exhibited peaks due to $[M+Na^+-\text{hexose}]$ at *m/z* 939 and $[M+Na^+-2\times\text{hexose}]$ at *m/z* 777. Acid hydrolysis of **9** with 2 N HCl–MeOH yielded methyl α-D-glucopyranoside and methyl α-D-galactopyranoside. Moreover, the ^{13}C NMR spectrum of **9** showed aglycone signals superimposable on those of PO-b (**2**) as listed in Table I. Therefore, the aglycone of **9** was concluded to be identical with that of PO-a (**1**). The C-22 signal intensities due to (25 *S*) and (25 *R*) glycosides were in a ratio of about 2.6 : 1. The presence of four anomeric carbons at δ 102.7, 104.5, 104.8 and 105.0 indicated **9** to be a tetraglycoside consisting of glucose and galactose. The tridecamethyl ether (**10**) prepared from **9** by Hakomori's method showed a terminal permethylated trihexosyl cation at *m/z* 627 and a terminal permethylated hexosyl cation at *m/z* 219 in its mass spectrum and four doublet signals (all $J=8$ Hz) at δ 4.28, 4.69, 4.88 and 4.99 ascribable to sugar anomeric protons in the ^1H NMR spectrum, suggesting all the hexose moieties to be β-linked. Methanolysis of this methylated glycoside (**10**) afforded methyl 2,3,4,6-tetra-*O*-methyl α-D-glucopyranoside, methyl 2,3,6-tri-*O*-methyl α- and β-D-galactopyranoside and methyl 4,6-di-*O*-methyl α- and β-D-glucopyranoside as methylated sugars. The appearance of the trihexosyl cation (*m/z* 627) in the mass spectrum suggested the structure aglycone^3←galactose^4←glucose$^2_3$←glucose for **9**. The ^{13}C NMR spectrum of **9** was
↖glucose
compared with that of SN-4,[18] which was isolated from *Solanum nigrum* and characterized as tigogenin 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside. The signals due to the sugar moieties of both substances were identical. The sugar moiety in **9** was concluded to be bound with C-3-OH of PO-a (**1**), since the signals due to the aglycone part of **9** in the ^{13}C NMR spectrum were superimposable on those of PO-b (**2**). Therefore, **9** could be represented as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl (25 *R* and *S*)-spirost-5-en-3β,14α-diol. Since PO-d (**4**) was a furostanol glycoside corresponding to **9**, it was deduced to be 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl 22-methoxy-(25 *R* and *S*)-furost-5-en-3β,14α,26-triol 26-*O*-β-D-glucopyranoside.

PO-e (**5**), colorless needles, mp 280—283 °C, $[\alpha]_D$ −63.0° (pyridine), was obtained by separation of the β-glucosidase hydrolysis of the original methanol extract. The mass spectrum of PO-e (**5**) showed a base peak at *m/z* 139 originating from the spiroketal side chain besides a peak at *m/z* 414 (aglycone molecular ion). PO-e (**5**) on acid hydrolysis gave yamogenin, glucose, galactose and xylose. The FD-MS spectrum showed peaks due to $[M+Na^+]$ at *m/z* 1055, $[M+Na^+-\text{pentose}]$ at *m/z* 923, $[M+Na^+-\text{hexose}]$ at *m/z* 893 and $[M+Na^+-\text{pentose}-\text{hexose}]$ at *m/z* 762, indicating that PO-e (**5**) is a yamogenin tetraglycoside constituted from 3 mol of hexose and 1 mol of xylose, and xylose and hexose are terminal moieties. Since the ^{13}C NMR spectrum revealed that the signals assigned to the sugar moiety were superimposable on those of PO-b (**2**), it was concluded that the oligoglucoside moiety of PO-e (**5**) was β-lycotetraoside. Consequently, the structure of PO-e (**5**) could be

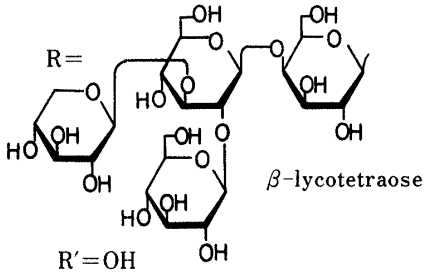
assigned as 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranosyl yamogenin.



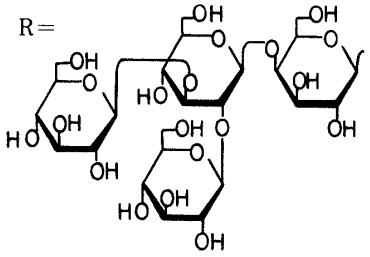
PO-a (**1**) : 25*R* and *S*, R=H, R′=OH
neoprazerigenin (**7**) : 25*S*, R=H, R′=OH
PO-b (**2**) : 25*R* and *S*,



PO-c (**3**) : 25*R* and *S*, R=*β*-lycotetraose
PO-d (**4**) : 25*R* and *S*,



PO-e (**5**) : 25*S*, R=*β*-lycotetraose, R′=H



Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (hot stage type) and are uncorrected. The optical rotations were measured with a Union PM-201 automatic digital polarimeter. The IR spectra were recorded with Hitachi 215 machine and NMR spectra with a JEOL PS-100 spectrometer and a JEOL FX-200 spectrometer (100 and 200 MHz for ^{1}H NMR and 50.1 MHz for ^{13}C NMR). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. The EI-MS and FD-MS spectra were recorded on a JEOL JMS-D 300 spectrometer. Thin-layer chromatography (TLC) was performed on precoated Kiesel gel plates (Merck) and detection was achieved by spraying 10% H_2SO_4 followed by heating. Gas liquid chromatography (GLC) was run on a Shimadzu GC-3BF machine with a flame ionization detector using a glass column (3 mm × 2 m) packed with 1.5% 1, 4 butanediol succinate polyester on Shimalite.

Extraction and Separation of PO-a (1), PO-b (2), PO-c (3), PO-d (4) and PO-e (5)——The sliced fresh rhizomes (11.5 kg) of *Polygonatum odoratum* var. *pluriflorum* (Miq.) Ohwi, collected at the Botanical Garden of this Faculty in July 1981, were soaked with MeOH (12 l) for one week. The methanolic extractive was mixed with MeOH (10 l) to give a resinous ppt. The supernatant was evaporated to give a residue (135 g), which was then passed through an Amberlite XAD-2 column with water followed by elution with MeOH to give a mixture of saponin components. This mixture was chromatographed over silica gel ($CHCl_3$–MeOH–H_2O=7:3:0.2→7:3:0.5) to yield PO-a (**1**), *Rf* 0.88, 20.4 mg, PO-b (**2**), *Rf* 0.37, 34 mg, PO-c (**3**), *Rf* 0.14, 580.5 mg, PO-d (**4**), *Rf* 0.08, 419.2 mg (*Rf* values on TLC, solv. $CHCl_3$–MeOH–H_2O=7:3:0.5). PO-c (**3**) and PO-d (**4**) were obtained by reprecipitation upon addition of acetone after refluxing with MeOH. PO-e (**5**) was obtained as follows; the methanolic extractive (91 g) from the fresh rhizomes of this plant (1.4 kg) was separated into the MeOH-soluble portion (14 g) and insol. portion, and the former was subjected to Amberlite XAD-2 column chromatography with water and then MeOH to give a mixture of saponins (3.2 g). It was then incubated with a mixture of *β*-glucosidase (320 mg) and crude hesperidinase (100 mg) at 38 °C for one week to afford the products, which were chromatographed over silica gel ($CHCl_3$–MeOH–H_2O= 8:2:0.2→7:3:0.5) to give PO-e (**5**), 28.6 mg, together with PO-a (**1**), 7.2 mg, PO-b (**2**), 75.3 mg, and compound **9**, 73.7 mg.

PO-a (1)——*Rf* 0.63 (solv. hexane–acetone=1:1), colorless needles from dil. MeOH, mp 215—216 °C, $[\alpha]_D^{24}$ −86.1° (*c*=0.72, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 980, 960, 918, 890, 840 (918>890, spiroketal). MS (*m/z*): 430.3054 (Calcd for $C_{27}H_{42}O_4$: 430.3085 (M^+)), 412 (M^+-H_2O), 394 (M^+-2H_2O), 379 ($M^+-2H_2O-CH_3$), 139 ($C_9H_{15}O^+$), 126 ($C_8H_{14}O^+$). ^{1}H NMR ($CDCl_3$) δ: 0.94 (3H, s, 18-CH_3), 1.05 (3H, s, 19-CH_3), 0.79, 1.01 (each d, *J*= 7 Hz, 27-CH_3 (25*R*) and (25*S*) derivatives, respectively)), 0.99, 1.08 (each d, *J*=7 Hz, 21-CH_3 (25*R*) and (25*S*) derivatives, respectively)), 3.30 (d, *J*=11 Hz, 26-ax(*α*)H(25*S*)), 3.95 (dd, *J*=2.5, 11 Hz, 26-eq(*β*)H(25*S*)), 3.48 (m, 3-

H, 26-H_2 (25*R*)), 4.64 (1H, m, 16-H), 5.37 (1H, m, 6-H). *Anal.* Calcd for $C_{27}H_{42}O_4$: C, 75.31; 9.83. Found: C, 75.02; H, 9.81.

Monoacetate (6) of PO-a (1)——PO-a (**1**), 7 mg, was acetylated with Ac_2O and pyridine (each 2 ml) in the usual manner to give the corresponding monoacetate, which was purified by silica gel column chromatography with hexane–AcOEt = 5 : 1 to yield colorless needles (5 mg), mp 149—151 °C, $[\alpha]_D^{23}$ −45.6° (*c* = 0.57, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 990, 965, 918, 898, 850, 815 (918 > 898, spiroketal). MS (*m/z*): 472 (M^+, $C_{29}H_{44}O_5^+$), 413 ($C_{27}H_{41}O_3^+$), 394 ($M^+ - AcOH - H_2O$), 379, 298, 280, 265, 251, 158, 139, 126. ^1H NMR ($CDCl_3$) δ: 0.94 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 1.08 (3H, d, *J* = 7.5 Hz, 21-CH_3), 1.02 (3H, d, *J* = 7 Hz, 27-CH_3), 2.03 (3H, s, OAc). *Anal.* Calcd for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.34; H, 9.42.

Neoprazerigenin A (7)——The monoacetate (**6**), 4 mg, was saponified with 3% KOH–MeOH (3 ml) at room temperature for 5 h giving the acetyl-free compound, which was purified by silica gel column chromatography (hexane–acetone = 4 : 1) to give neoprazerigenin A (**7**), colorless needles from MeOH, mp 211—213 °C. ^1H NMR ($CDCl_3$) δ: 0.94 (3H, s, 18-CH_3), 1.02 (3H, d, *J* = 7 Hz, 27-CH_3), 1.05 (3H, s, 19-CH_3), 1.09 (3H, d, *J* = 7 Hz, 21-CH_3), 3.31 (1H, d, *J* = 11.5 Hz, 26-ax(α)H), 3.50 (1H, m, 3-H), 3.95 (1H, dd, *J* = 2.4, 11.5 Hz, 26-eq(β)H), 4.64 (1H, ddd, *J* = 6.6, 7.5, 7.5 Hz, 16-H), 5.39 (1H, br d, 6-H). MS (*m/z*): 430 (M^+), 412 ($M^+ - H_2O$), 394 ($M^+ - 2H_2O$), 379 ($M^+ - 2H_2O - CH_3$), 139 ($C_9H_{15}O^+$), 126 ($C_8H_{14}O^+$).

PO-b (2)——Colorless needles from MeOH, mp 261—265 °C, $[\alpha]_D^{19}$ −60.0° (*c* = 0.50, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 912, 885, 835, 810 (spiroketal). FD-MS (*m/z*): 1071 [M + Na^+], 939 [M + Na^+ − pentose], 909 [M + Na^+ − hexose]. MS (*m/z*): 412, 394, 379, 282, 139, 126, 115. *Anal.* Calcd for $C_{50}H_{80}O_{23} \cdot H_2O$: C, 56.27; H, 7.75. Found: C, 56.01; H, 7.72.

Methanolysis of PO-b (2)——Acid hydrolysis of PO-b (**2**), 20 mg, with 3 N HCl–MeOH gave the aglycone and a mixture of methyl glycosides. The aglycone was collected by filtration and could not be characterized owing to its small amount and the presence of impurities. The filtrate was further hydrolyzed with 2 N H_2SO_4, and xylose, galactose and glucose were detected on silica gel TLC (*Rf* 0.52, 0.30 and 0.34, solv. $CHCl_3$–MeOH–acetone–H_2O = 3 : 3 : 3 : 1).

Dodecamethyl Ether (8) of PO-b (2)——A solution of PO-b (**2**), 30 mg, in DMSO (3 ml) was added to a preheated (70 °C, 20 min) mixture of DMSO (3 ml) and NaH (200 mg) and the whole was kept at 70 °C for 20 min. Then CH_3I (5 ml) was added, and the reaction mixture was stirred at room temp. overnight then poured into water and extracted with ether. The organic layer was taken and evaporated *in vacuo* to give a residue, which was chromatographed on a silica gel column with hexane–acetone = 5 : 1 to afford PO-b dodecamethyl ether (**8**) as a colorless oil (12 mg). MS (*m/z*): 583 (terminal pentosyl 8 × CH_3), 395, 391, 219 (terminal hexosyl 4 × CH_3), 187 (*m/z* 219-MeOH), 175 (terminal pentosyl 3 × CH_3). ^1H NMR ($CDCl_3$) δ: 3.32—3.60 (12 × OCH_3), 4.27, 4.68, 4.89, 4.94 (each 1H, d, *J* = 8 Hz, 4 × anomeric protons).

Methanolysis of Dodecamethyl Ether (8)——The dodecamethyl ether (**8**), 10 mg, was methanolyzed with 3 N HCl–MeOH (2 ml), neutralized with 3% KOH–MeOH and subjected to Sephadex LH-20 column chromatography to give a mixture of methylated sugars, which was examined by TLC with hexane–AcOEt = 2 : 3 and GLC (column temp. 180 °C, N_2 1.20 kg/cm^2) to detect methyl 2,3,4,6-tetra-*O*-methyl α-D-glucopyranoside (*Rf* 0.33, t_R 1′29″), methyl 2,3,4-tri-*O*-methyl α- and β-D-xylopyranoside (*Rf* 0.44 (α), 0.33 (β), t_R 1′11″ (α), 1′40″ (β)), methyl 2,3,6-tri-*O*-methyl α- and β-D-galactopyranoside (*Rf* 0.10 (α), 0.07 (β), t_R 9′26″ (α), 12′35″ (β)), methyl 4,6-di-*O*-methyl α- and β-D-glucopyranoside (*Rf* 0.05 (α), 0.02 (β), t_R 25′30″ (α), 32′45″ (β)). These compounds were identical with authentic specimens.

Enzymic Hydrolysis of PO-b (2)——A mixture of PO-b (**2**), 16 mg, crude hesperidinase (78 mg, Tanabe Co., Ltd.) and β-glucosidase (21 mg, Sigma Co., Ltd.) in AcOH–NaOAc buffer (pH 4.5, 6 ml) was incubated at 37 °C for 6 d. Usual work-up yielded a small amount of a sapogenol (2 ml), mp 215—216 °C, which was identical with PO-a (**1**) on TLC, together of a large amount of unchanged PO-b (**2**).

PO-c (3)——A white powder (mp 208—210 °C, $[\alpha]_D^{26}$ −49.0° (*c* = 1.00, MeOH), was obtained by reprecipitation by adding acetone to a conc. MeOH solution of **3**. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH). MS (*m/z*): 412, 394 (*m/z* 412-H_2O), 379 (*m/z* 394-CH_3), 376 (*m/z* 394-H_2O), 361, 298, 283, 280, 269, 265, 251, 250, 139. *Anal.* Calcd for $C_{57}H_{94}O_{29} \cdot H_2O$: C, 54.27; H, 7.67. Found: C, 54.51; H, 7.73.

Enzymic Hydrolysis of PO-c (3)——A mixture of PO-c (**3**), 400 mg, β-glucosidase (50 mg) and dist. water (30 ml) was incubated at 37 °C for 3 h, then MeOH was added and the whole was evaporated *in vacuo* to give a residue, which was chromatographed over silica gel ($CHCl_3$–MeOH–H_2O = 7 : 3 : 0.3 → 7 : 3 : 0.4) to give a spirostanol glycoside (30 mg), colorless needles, mp 261—265 °C, $[\alpha]_D^{22}$ −62.0° (*c* = 0.60, pyridine), identical with PO-b (**2**) in terms of MS, and IR and ^{13}C NMR spectra, and D-glucose, $[\alpha]_D^{22}$ +43.8° (*c* = 0.72, H_2O), *Rf* 0.27 (on TLC, solv. $CHCl_3$–MeOH–H_2O = 7 : 3 : 0.5).

PO-d (4)——A white powder (mp 208—213 °C), $[\alpha]_D^{22}$ −48.1° (*c* = 1.06, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH).

Enzymic Hydrolysis of PO-d (4)——A mixture of PO-d (**4**), 400 mg, β-glucosidase (50 mg) and dist. water (35 ml) was incubated at 37 °C in the same way as described for PO-c (**3**) to give a spirostanol glycoside (**9**), 110 mg, colorless needles, mp 224—225 °C, $[\alpha]_D^{20}$ −69.0° (*c* = 1.00, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 915, 885, 830, 810 (spiroketal). FD-MS (*m/z*): 1117 [M + K^+], 1101 [M + Na^+], 1083 [*m/z* 1101-H_2O], 939 [*m/z* 1101-hexose], 777 [*m/z* 939-hexose].

MS (*m/z*): 412, 394, 379, 282, 251, 139, 126, 115, and D-glucose, $[\alpha]_D^{21}$ +48.2° (*c*=0.56, H_2O). *Anal.* Calcd for $C_{51}H_{82}O_{24}\cdot 2H_2O$: C, 54.92; H, 7.77. Found: C, 54.68; H, 7.68.

Methanolysis of 9——A solution of **9** (3 mg) in 3 N HCl–MeOH (2 ml) was refluxed for 1 h then neutralized with 3% KOH–MeOH to detect methyl α-D-glucopyranoside (*Rf* 0.59, solv. $CHCl_3$–MeOH–acetone–H_2O=3:3:3:1) and methyl α-D-galactopyranoside (*Rf* 0.54) on TLC.

Tridecamethyl Ether (10) of Compound 9——Compound **9** (30 mg) was methylated in the same manner (Hakomori's method) as described for PO-b (**2**) to give a tridecamethyl ether (**10**) as a syrup (16 mg), $[\alpha]_D^{22}$ −36.4° (*c*=1.32, $CHCl_3$). MS (*m/z*): 627 (terminal trihexosyl 10×CH_3), 563 (*m/z* 627-2×MeOH), 531 (*m/z* 563-MeOH), 499 (*m/z* 531-MeOH), 467 (*m/z* 499-MeOH), 435 (*m/z* 467-MeOH), 412, 395, 391, 377 (*m/z* 409-MeOH), 219 (terminal hexosyl 4×CH_3), 187 (*m/z* 219-MeOH), 155 (*m/z* 187-MeOH). ^{1}H NMR ($CDCl_3$) δ: 3.27—3.61 (all s, 13×OCH_3), 4.28, 4.69, 4.88, 4.99 (each 1H, d, *J*=8 Hz, hexosyl 4× anomeric protons), 5.35 (1H, m, 6-H).

Methanolysis of Tridecamethyl Ether (10)——A solution of tridecamethyl ether (**10**), 8 mg in 2 N HCl–MeOH (3 ml) was refluxed for 6 h and neutralized. Methyl 2,3,4,6-tetra-*O*-methyl glucopyranoside (*Rf* 0.33 (α)), methyl 2,3,6-tri-*O*-methyl galactopyranoside (*Rf* 0.10 (α), 0.07 (β)), and methyl 4,6-di-*O*-methyl glucopyranoside (*Rf* 0.05 (α), 0.02 (β)) were detected on TLC with hexane–AcOEt=2:3 as a solvent.

PO-e (5)——Colorless needles from dil. MeOH, mp 280—283 °C, $[\alpha]_D^{24}$ −63.0° (*c*=1.00, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 870, 840, 795 (spiroketal). MS (*m/z*): 576, 558, 414, 397, 342, 327, 300, 285, 282, 271, 253, 139, 115. FD-MS (*m/z*): 1071 [M+K$^+$], 1055 [M+Na$^+$], 923 [M+Na$^+$−xylose], 893 [M+Na$^+$−glucose], 762 [M+Na$^+$−xylose−glucose].

Methanolysis of PO-e (5)——A solution of PO-e (**5**), 11.7 mg, in 2 N HCl–MeOH (4 ml) was refluxed for 2 h, then diluted with water. The reaction mixture was shaken with $CHCl_3$ and the organic layer was taken and evaporated to dryness *in vacuo* to give a residue, which was chromatographed over silica gel (hexane–acetone=7:1) to give a sapogenol, colorless needles (3 mg) from dil. MeOH, mp 190—195 °C, MS (*m/z*): 414 (M$^+$), 342, 300, 285, 282, 271, 253, 139, 115. ^{1}H NMR ($CDCl_3$) δ: 0.72 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 3.23 (1H, d, *J*=11 Hz, 26-ax(α)H), 3.89 (1H, *J*=2, 7, 11 Hz, 26-eq (β) H), 4.35 (1H, m, 16-H), 5.23 (1H, br d, 6-H). The aqueous layer was heated for 3 h on a boiling water bath and neutralized by passage through Amberlite IRA-400, then evaporared *in vacuo* to give a mixture of sugars, xylose (*Rf* 0.53 on TLC, solv. $CHCl_3$–MeOH–acetone–H_2O=3:3:3:1), glucose (*Rf* 0.35) and galactose (*Rf* 0.31).

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