Steroidal Saponins from the Rhizomes of *Polygonatum sibiricum*†

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Four new steroidal saponins, named neosibiricosides A−D (**1**−**4**), were isolated from the rhizomes of *Polygonatum sibiricum*, along with two known spirostanol glycosides. The structures of the new glycosides were elucidated by spectroscopic methods and acid hydrolysis as (23*S*,24*R*,25*R*)-1-*O*-acetylspirost-5-ene-1*β*,3*β*,23,24-tetrol 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-*β*-D-fucopyranoside (**1**), (25*S*)-1-*O*-acetylspirost-5-ene-1*β*,3*β*-diol 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**2**), (25*S*)-spirost-5-en-3*β*-ol 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-2-*O*-acetyl-*β*-D-galactopyranoside (**3**), and (25*R*,*S*)-spirost-5-en-3*β*-ol 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**4**). The cytotoxic activity of the isolated compounds was evaluated with human MCF-7 breast cancer cells.

The rhizomes of *Polygonatum* species have been used traditionally as a tonic in Asia,[1] and recently, several tea products produced from these medicinal plants are being widely consumed in Korea. It has been reported that the methanol extracts of *P. odoratum* (Mill.) Druce, *P. falcatum* A. Gray, and *P. sibiricum* Redouté show a hypoglycemic activity and that of *P. sibiricum* exhibits cardiotonic activity.[2−4] Moreover, a steroidal saponin has been reported to be responsible for the hypoglycemic activity.[5]

In a preliminary phytochemical study on *Polygonatum* species, it was found that the amounts of some steroidal saponins are higher in *P. sibiricum* than in *P. odoratum*.[6] In addition, the HPLC-MS profile of this species showed that there are several glycosides not reported previously. Therefore, a further phytochemical analysis has been carried out on the rhizome of *P. sibiricum* with particular attention to the steroidal glycoside constituents. This study resulted in the isolation of four new spirostanol glycosides (**1**−**4**) and two known compounds. In this paper, the isolation and structure determination of the new steroidal saponins are reported.

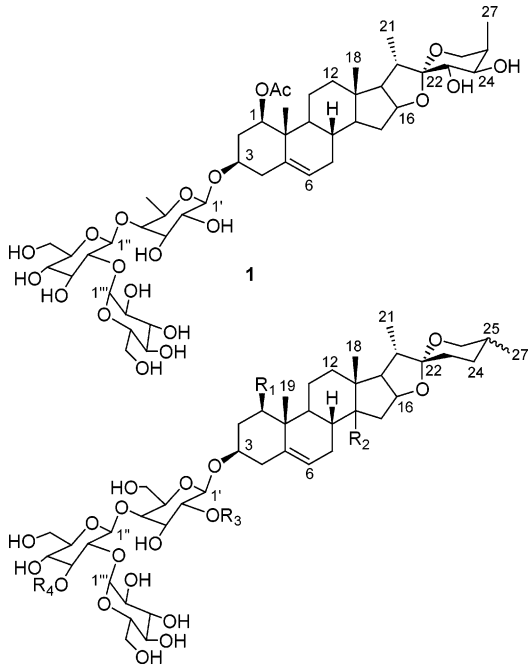
Results and Discussion

The concentrated MeOH extract of the rhizomes of *P. sibiricum* was partitioned between *n*-BuOH and water. The *n*-BuOH-soluble fraction was chromatographed on silica gel and octadecylsilanized (ODS) silica gel to give compounds **1**−**4**, along with two known steroidal saponins identified as PO-2 and PO-3.[7,8]

Neosibiricoside A (**1**), obtained as an amorphous powder, showed a positive dark green color with the Lieberman-Burchard reagent and a negative reaction to Ehrlich's reagent, indicating that **1** is a spirostane derivative, rather than having a furostane skeleton.[9] In the positive-ion ESIMS of **1**, a quasimolecular ion peak at *m*/*z* 997 $[M + Na]^+$ was observed, and HRFABMS analysis revealed the molecular formula to be $C_{47}H_{74}O_{21}$.

The ^{1}H NMR spectrum of **1** showed signals for two tertiary methyl groups at *δ* 1.03 and 1.02 (each 3H, s), three secondary methyl groups at *δ* 1.60 (d, *J* = 6.4 Hz), 1.37 (d, *J* = 7.0 Hz), and 1.19 (d, *J* = 7.0 Hz), and an olefinic proton at *δ* 5.44 (m). The signal at *δ* 1.60 was due to the methyl group of a 6-deoxyhexopyranose. These ^{1}H NMR data and the chemical shift of a quaternary carbon signal at *δ* 113.9 (C-22) supported the fact that **1** has a spirostene steroidal skeleton.[10]

An α-hydroxy group at C-23 in ring F was expected from the downfield shifts of C-22 (+4.1 ppm) and C-23 (+43.5 ppm), and an upfield shift of C-20 (−5.5 ppm) from the *γ*-gauche effect.[10,11] The proton coupling constants of H-24 [*δ* 4.47 (dd, *J* = 9.6, 3.8 Hz)] suggested a *β*-orientation for the C-24 hydroxy group. The 25*R*-configuration of **1** was deduced from the ^{1}H NMR parameters of the H-26 methylene protons at *δ* 4.11 (dd, $J_{26ax,26eq}$ = 10.5 Hz, $J_{26ax,25eq}$ = 2.4 Hz, H-26$_{ax}$) and 3.48 (d, $J_{26ax,26eq}$ = 10.5 Hz, H-26$_{eq}$). The 23*S*-, 24*R*-, and 25*R*-configurations were confirmed by the clear NOE correlations of H-23 (*δ* 4.03)/Me-27 (*δ* 1.37), H-24 (*δ* 4.47)/H-25 (*δ* 2.23), H-25 (*δ* 2.23)/H-26$_{ax}$ (*δ* 4.11), and H-26$_{eq}$ (*δ* 3.48)/Me-27 (*δ* 1.37) in the NOESY spectrum (Figure 1).[12] These findings, and the ^{13}C NMR spectra of **1**, suggested that the aglycon of neosibiricoside A is analogous to (23*S*,24*S*,25*S*)-23,24-dihydroxyruscogenin, except for the carbon signals corresponding to the rings A and F.[13,14]



2 R_1 = OAc, R_2, R_3 = H, R_4 = β-D-xyl (25*S*)

3 R_1 = H, R_2 = H, R_3 = Ac, R_4 = β-D-xyl (25*S*)

4 R_1, R_2, R_3, R_4 = H (25*R*,*S*)

PO-2 R_1 = H, R_2 = OH, R_3 = H, R_4 = β-D-xyl (25*R*,*S*)

PO-3 R_1, R_2, R_3 = H, R_4 = β-D-xyl (25*R*,*S*)

† Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on biologically active natural products.

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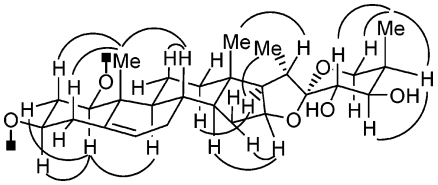

Figure 1. NOE correlations of the aglycon moiety of neosibiricoside A (**1**).

In turn, a peak due to the loss of an acetyl moiety was detected at *m*/*z* 937 $[M + Na - CH_3COOH]^+$ in the MS^2 spectrum of the $[M + Na]^+$ ion. In addition, the presence of the acetyl group in the aglycon was revealed by IR (1725 cm^{-1}), ^1H NMR [δ 2.03 (3H, s)], and ^{13}C NMR [δ 170.6, (C═O) and 22.0 (Me)] spectra. The correlation peaks were observed from the H-1 proton (δ 4.87) of the aglycon to the acetyl carbonyl carbon (δ 170.6) and C-9 (δ 50.5) and C-19 (δ 14.9) of the aglycon in the HMBC spectrum. Also, the H-1 signal was coupled to H-2_{ax} (δ 1.95) with a large *J* value of 11.8 Hz and to H-2_{eq} (δ 2.57) with $J = 4.3$ Hz in the $^1H-^1H$ COSY experiment. These findings with NOE correlations of H-1/H-2_{eq} and H-1/H-9 indicated that the acetyl moiety was attached to the C-1 equatorial position of the aglycon in β-orientation. From the above results, the structure of the sapogenin was established as (23*S*,24*R*,25*R*)-23,24-dihydroxyruscogenin 1-acetate, a new sapogenin.

The ^1H and ^{13}C NMR spectra of **1** also showed signals for three anomeric protons at δ 4.80 (H-1′), 5.10 (H-1″), and 5.28 (H-1‴) and three anomeric carbons at δ 103.1 (C-1′), 105.3 (C-1″), and 107.3 (C-1‴). These results indicated the presence of three monosaccharide moieties. The monosaccharides obtained from the acidic hydrolysis of **1** were identified as D-fucose and D-glucose with the molar ratio of 1:2 by GC of their respective trimethylsilyl L-cysteine derivatives.[15,16] The β-orientations of the anomeric centers of the three monosaccharide units were supported by the relatively large *J* values of their anomeric protons ($J = 7.7$, 7.7, and 7.5 Hz for H-1′, H-1″, and H-1‴, respectively).[17] The combined use of HSQC, 1D-selective TOCSY, and HMBC experiments allowed the sequential assignments of all resonances for each monosaccharide, starting from the anomeric protons (Table 1). The sequence and interglycosidic linkages among the three sugar moieties and the aglycon were revealed by the HMBC spectrum. The H-3 proton of the aglycon (δ 3.98) showed a correlation with the anomeric carbon of fucose (δ 103.1). The anomeric proton of the inner glucose (δ 5.10) was correlated with C-4′ of the fucose (δ 84.6), and that of the outer glucose (δ 5.28) was correlated with C-2″ of the inner glucose (δ 86.8). On the basis of the obtained data, the structure of neosibiricoside A (**1**) was assigned as (23*S*,24*R*,25*R*)-1-*O*-acetylspirost-5-ene-1β,3β,23,24-tetrol 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-fucopyranoside.

The ^1H and ^{13}C NMR spectroscopic properties for the aglycon of neosibiricoside B (**2**) were similar to those of **1**, except for the peaks related to the F-ring. The chemical shifts of C-22 (δ 110.2) and the other carbons of the F-ring revealed that there is no hydroxylated carbon in this ring. The 25*S*-configuration of **2** was inferred from the H-26 methylene proton signals at δ 4.03 (dd, $J_{26ax,26eq} = 11.2$ Hz, $J_{25eq,26ax} = 2.6$ Hz, H-26_{ax}) and 3.34 (d, $J_{26ax,26eq} = 11.2$ Hz, H-26_{eq}) in the ^1H NMR spectrum.[18] These spectroscopic data revealed that the aglycon of **2** is (25*S*)-ruscogenin 1-acetate, a new sapogenin.

Additionally, neosibiricoside B (**2**) exhibited the $[M + Na]^+$ ion peak at *m*/*z* 1113 in the ESIMS, which differed from that of **1** by 116 amu, corresponding to a pentose moiety (+132 amu) and one less hydroxy group (−16 amu). Four monosaccharides were inferred from the signals of four anomeric protons at δ 4.87, 5.15, 5.60, and 5.23 and their corresponding anomeric carbons at δ 103.3, 105.3, 105.4, and 105.7, respectively, in the ^1H and ^{13}C NMR and HSQC spectra. Acid hydrolysis of **2** and GC analysis of chiral derivatives of the hydrolysate yielded D-galactose, D-glucose, and D-xylose in a ratio of 1:2:1. The interglycosidic linkages of the four sugar units were determined by the HMBC correlations between the galactosyl H-1′ (δ 4.87) and C-3 (δ 74.7) of the aglycon moiety, the inner glucosyl H-1″ (δ 5.15) and the galactosyl C-4′ (δ 80.4), the outer glucosyl H-1‴ (δ 5.60) and the inner glucosyl C-2″ (δ 81.8), and the xylosyl H-1⁗ (δ 5.23) and the inner glucosyl C-3″ (δ 87.2). Consequently, the structure of **2** was established as (25*S*)-1-*O*-acetylspirost-5-ene-1β,3β-diol 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

The positive-ion ESIMS of neosibiricoside C (**3**) displayed the molecular ion at *m*/*z* 1097 $[M + Na]^+$, differing from that of PO-3, a known steroidal saponin,[8] by 42 amu, corresponding to an additional acetyl group. The ^1H and ^{13}C NMR spectral properties of the aglycon were in good agreement with those of PO-3, except for the configuration at the C-25 position. The 25*S*-configuration of **3** was deduced from the ^1H NMR parameters of the H-26 methylene protons at δ 4.03 (br d, H-26_{ax}) and 3.33 (d, $J = 10.4$ Hz, H-26_{eq}).[18]

The presence of an acetyl group was supported by the IR (1738 cm^{-1}), ^1H NMR [δ 2.04 (3H, s)], and ^{13}C NMR [δ 170.6 (C═O), 21.7 (Me)] spectra. However, this acetyl group, different from that in **1** and **2**, was found to be linked at the galactose C-2′ hydroxy position. In the HMBC spectrum, there were correlation peaks from the galactose H-2′ proton (δ 5.78) to the acetyl carbonyl carbon (δ 170.6), C-1′ (δ 101.1), and C-3′ (δ 73.7). From these results, the structure of **3** was determined as (25*S*)-spirost-5-en-3β-ol 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-2-*O*-acetyl-β-D-galactopyranoside.

The molecular formula ($C_{45}H_{72}O_{18}$) of neosibiricoside D (**4**) was established by the positive-ion HRFABMS (*m*/*z* 923.4617 $[M + Na]^+$) and supported by the ^{13}C NMR spectrum. By comparison of the proton and carbon chemical shifts of **4** with those of PO-3, the structure of **4** was found to have an aglycon part identical to that of PO-3. However, the ^1H NMR spectrum of **4** showed only three anomeric proton signals, at δ 4.92, 5.12, and 5.20. The MS data (*m*/*z* 923 $[M + Na]^+$), differing from that of PO-3 by 132 amu, corresponding to a pentose unit, and acid hydrolysis verified the absence of the terminal xylose moiety of PO-3. The ^{13}C signals ascribable to ring F appeared as pairs of signals, indicating that **4** was obtained as a C-25 epimeric mixture. Therefore, the structure of **4** was elucidated by spectroscopic analysis as (25*R*,*S*)-spirost-5-en-3β-ol 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compounds **1** and **2** are distinctive in carrying an acetyl group at the C-1β position of a steroidal aglycon, and this is the first report of a steroidal glycoside, **3**, with an acetylated sugar moiety from the genus *Polygonatum*.

All the isolated compounds were tested for cytotoxic activity against cultured human MCF-7 breast cancer cells. The new spirostanol saponins, **3** and **4**, showed moderate cytotoxic activity (IC_{50} values of 20.9 and 24.3 μM, respectively) when compared with the two known saponins, PO-2 and PO-3 (IC_{50} values of 17.6 and 15.2 μM, respectively).[19] On the other hand, cytotoxic activity was not found for compounds **1** and **2** (IC_{50} values of >100 and 71.4 μM, respectively).

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a JASCO DIP-1000 digital polarimeter. FT-IR spectra were recorded with a JASCO FT/IR-300 spectrophotometer. ^1H and ^{13}C NMR spectra were taken on a Bruker Avance-600 or a Bruker DMX-600 spectrometer at 600 and 150 MHz, respectively. HRFABMS and ESIMS were obtained on a JEOL JMS-AX505505WA and a Finnigan MAT LCQ ion-trap mass spectrometer, respectively. The experimental conditions for ESIMS were as follows: sheath gas flow rate 80 au, auxiliary gas

Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**4** (600 MHz, in pyridine-d_5)

				4	
position	**1**	**2**	**3**	*R*	*S*
1	4.87 dd (11.8, 4.3)	4.82 dd (11.7, 4.6)	1.62, 0.90	1.65 br d, 0.93	
2ax	1.95 m	1.89 br d (11.7)	1.69	1.72 m	
2eq	2.57 m	2.49 m	2.02	2.07 br d	
3	3.98 m	3.95 m	3.79 m	3.90 m	
4a	2.63 dd (11.9, 4.1)	2.61 dd (11.6, 4.3)	2.53 dd (11.4, 4.1)	2.64 dd (11.6, 4.2)	
4b	2.42 br t	2.40 t (11.6)	2.32 m	2.40 br t	
6	5.44 m	5.44 m	5.33 m	5.26 m	
7a, 7b	1.72 m, 1.44 m	1.75, 1.45	1.76, 1.45	1.80 m, 1.45 m	
8	1.48 m	1.44	1.50 m	1.46 m	
9	1.13 m	1.08	0.83	0.91	
11	1.27 m	1.37	1.25	1.42	
12a,12b	1.65 m, 1.08 m	1.65, 1.03	1.66, 1.03	1.67, 1.06	
14	1.01 m	0.98 m	1.02	1.01	
15a,15b	1.99 m, 1.40 m	1.93 m, 1.38	1.96, 1.38	1.96, 1.37	
16	4.58 q-like (7.0)	4.48 m	4.51 m	4.49 m	
17	1.79 dd (8.4, 7.2)	1.71 dd (8.5, 7.5)	1.75 m	1.74 m	
18	1.02 s	0.81 s	0.80	0.79 s	
19	1.03 s	1.04 s	0.93	0.84 s	
20	2.98 q	1.87	1.87	1.88 m	
21	1.19 d (7.0)	1.18 d (7.0)	1.12 d (6.9)	1.11 d (6.7)	1.12 d (6.7)
23	4.03 m	1.45, 1.33	1.44, 1.34	1.63, 1.32	1.94, 1.37
24	4.47 dd (9.6, 3.8)	2.17, 1.87	1.87	1.64, 1.54	1.64
25	2.23 m	1.55 m	1.54 m	1.55	1.53
26ax	4.11 dd (10.5, 2.4)	4.03 dd (11.2, 2.6)	4.03 br d	3.48 t (10.8)	4.19 br d
26eq	3.48 d (10.5)	3.34 d (11.2)	3.33 d (10.4)	3.56 br d	3.34 d (10.9)
27	1.37 d (7.0)	1.05 d (6.9)	1.04 d (7.1)	0.66 d (5.1)	1.04 d (7.0)
-OC*H*$_3$	2.03 s	2.03 s	2.04 s		
Fuc/Gal					
1′	4.80 d (7.7)	4.87 d (7.6)	4.84 d (8.0)	4.89 d (7.7)	
2′	4.51 dd (9.1, 7.7)	4.40 dd (8.7,7.6)	5.78 dd (9.7, 8.0)	4.46 dd (8.1, 7.7)	
3′	3.98	4.08 dd (9.1, 8.7)	4.20	4.05 dd (8.1, 8.1)	
4′	3.95	4.56	4.33	4.20 dd (8.1,8.1)	
5′	3.70 m	3.95	3.96	3.66 m	
6′	1.60 d (6.4)	4.60	4.58 m	4.35 br d	
		4.16	4.18 m	4.24 dd (10.5, 4.9)	
Glc					
1″	5.10 d (7.7)	5.15 d (7.9)	5.18 d (7.9)	5.12 d (7.8)	
2″	4.10 dd (8.4, 7.7)	4.43 dd (8.8, 7.9)	4.26 dd (9.0, 7.9)	4.13 dd (8.7, 7.8)	
3″	4.21 dd (8.8, 8.4)	4.15 dd (9.0, 8.8)	4.33 dd (9.0, 9.0)	4.29 dd (8.7, 8.7)	
4″	4.26 dd (9.1, 8.8)	3.79 dd (9.5, 9.0)	4.16 dd (9.0, 8.8)	3.94 dd (8.7, 8.7)	
5″	3.82 m	3.86 ddd (9.5, 6.8, 3.1)	3.89 m	3.91 m	
6″a	4.46 br d	4.50 dd (11.6, 1.7)	4.53 br d	4.60 br d	
6″b	4.30 br d	4.02 dd (11.6, 6.8)	4.38 br d	4.07 dd (11.4, 6.2)	
Glc					
1‴	5.28 d (7.5)	5.60 d (7.5)	5.69 d (7.8)	5.20 d (7.6)	
2‴	4.06 dd (9.1, 7.5)	4.14 dd (9.1, 7.5)	4.21 dd (9.1, 7.8)	4.02 dd (9.1, 7.6)	
3‴	4.14 dd (9.3, 9.1)	4.21 dd (9.1, 8.8)	4.25 dd (9.1, 8.8)	4.09 dd (9.1, 9.0)	
4‴	4.22 dd (9.1, 9.1)	4.08 distorted t	4.49 dd (8.8, 8.8)	4.20 dd (9.1, 9.0)	
5‴	3.77 m	3.92 (m)	3.94 m	3.76 m	
6‴a	4.52 br d	4.56 br d	4.57 dd (12.0, 4.5)	4.56 br d	
6‴b	4.38 br d	4.35 dd (11.6, 1.8)	4.52 br d	4.37 br d	
Xyl					
1⁗		5.23 d (8.1)	5.26 d (7.8)		
2⁗		3.95 dd (8.7, 8.0)	3.93 dd (9.0, 7.8)		
3⁗		4.06 dd (8.9, 8.7)	4.40 dd (8.9, 7.8)		
4⁗		4.10 ddd (11.3, 8.9, 4.4)	4.19 dd (9.0, 8.9)		
5⁗a		4.21 dd (11.3, 4.9)	4.15 dd (12.0, 5.9)		
5⁗b		3.66 dd (11.3, 10.2)	3.89 m		

flow 20 au, source voltage 4.5 kV, capillary voltage 36.5 V, inter-octapole lens voltage 10 V, and capillary temperature 300 °C. GC was conducted on a GC353B-FSL gas chromatograph (GL Sciences) with a flame ionization detector (FID). HPLC separations were performed with a Hitachi HPLC system (L-6200 pump, L-4000 UV−vis detector) on an ODS C_{18} column (4 μm, 250 × 10 mm, YMC), with detection at 204 nm. TLC was carried out on silica gel-precoated plates (Art. No. 5715, Merck). The following reagents were obtained from the indicated companies: RPMI 1640 medium (Gibco, Gland Island, NY); FBS (Bio-Whittaker, Walkersville, MD); 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma, St. Louis, MO); penicillin and streptomycin (Meiji-Seika, Tokyo, Japan). All other chemicals used in bioassay were of biochemical reagent grade.

Plant Material. The rhizomes of *Polygonatum sibiricum* Redouté (Liliaceae) were collected in Kangwon Province of Korea, in May 2002. This plant was identified by Prof. Jong Hee Park of the College of Pharmacy, Pusan National University, Korea. A voucher specimen (SNUPH-0322) is deposited in the Herbarium of the College of Pharmacy, Seoul National University.

Extraction and Isolation. The fresh rhizomes (30 kg) of *P. sibiricum* were extracted twice with 100% MeOH (40 L) and evaporated in vacuo. The MeOH extract (4.5 kg) was dissolved in water and partitioned with *n*-butanol. The *n*-butanol layer (83 g) was concentrated in vacuo and divided into five fractions (Fr. 1−Fr. 5) on silica gel column chromatography (Merck, 230−400 mesh, 1 kg) using $CHCl_3$−MeOH−H_2O mixtures of increasing polarity (30:5:1 (Fr. 1), 15:5:1 (Fr. 2), 10:5:1 (Fr. 3, Fr. 4), 6:5:1 (Fr. 5), 3 L of each). Fraction 5 (14 g) was then chromatographed on ODS silica gel (MeOH−H_2O, 20:80, 50:50, 80:20, 400 mL of each) to give five subfractions. Compound **1** (6 mg) was purified from subfraction 2 by HPLC (MeCN−H_2O, 25:75 → 50:

Table 2. ^{13}C NMR Data of Compounds **1**−**4** (150 MHz, in pyridine-d_5)

				4	
position	**1**	**2**	**3**	*R*	*S*
1	79.9	79.8	37.8	37.4	
2	37.3	37.0	30.4	30.2	
3	74.6	74.7	79.2	78.2	
4	39.8	39.7	39.8	39.2	
5	137.8	137.7	141.3	140.9	
6	126.7	126.6	122.3	121.6	
7	32.4	32.3	32.7	32.2	
8	32.7	32.7	32.1	31.6	
9	50.5	50.3	50.7	50.2	
10	42.8	42.6	37.4	37.0	
11	24.2	24.1	23.4	21.0	
12	40.9	40.4	40.3	39.8	
13	41.0	40.4	40.9	40.4	
14	56.9	56.7	57.1	56.6	
15	33.0	33.0	32.6	32.1	
16	82.5	81.5	81.6	81.0	
17	62.5	63.2	63.1	62.8	
18	17.2	16.9	16.8	16.3	
19	14.9	14.8	20.0	19.3	
20	37.1	42.9	42.9	42.4	
21	15.0	15.3	15.3	15.0	14.8
22	113.9	110.2	110.2	109.2	109.7
23	70.0	26.8	26.8	30.5	26.3
24	72.6	26.7	26.7	29.6	26.1
25	37.7	28.0	28.0	31.8	27.5
26	64.4	65.5	65.5	66.8	65.0
27	11.9	16.7	16.7	17.3	16.2
$-COCH_3$	22.0	21.9	21.7		
$-COCH_3$	170.6	170.5	170.6		
1′	103.1	103.3	101.1	102.6	
2′	73.2	73.6	75.1	73.2	
3′	76.4	75.5	73.7	75.1	
4′	84.6	80.4	79.2	80.9	
5′	70.8	76.7	76.1	75.1	
6′	18.6	60.9	61.1	60.3	
1″	105.3	105.3	104.6	105.2	
2″	86.8	81.8	80.6	86.1	
3″	78.8	87.2	87.6	78.9	
4″	71.4	71.4	71.2	71.8	
5″	79.0	79.3	78.7	78.4	
6″	63.2	62.9	63.2	63.2	
1‴	107.3	105.4	104.9	106.9	
2‴	77.1	76.0	75.7	76.7	
3‴	78.4	78.2	78.8	78.0	
4‴	71.1	71.2	71.3	70.2	
5‴	79.4	78.1	76.4	77.6	
6‴	62.1	63.5	63.5	61.4	
1⁗		105.7	105.5		
2⁗		75.8	76.2		
3⁗		79.1	78.4		
4⁗		71.0	70.6		
5⁗		67.8	66.5		

50, t_R 13.8 min). Compounds **2** (10 mg, t_R 19.4 min) and **4** (18 mg, t_R 16.5 min) were obtained through HPLC separation (MeCN−H_2O, 40:60 → 55:45) from subfractions 4 and 5, respectively. Fraction 2 (6 g) was separated by HPLC (MeCN−H_2O, 40:60 → 55:45, t_R 21.4 min) to give compound **3** (45 mg). The known compounds PO-2 (45 mg) and PO-3 (52 mg) were isolated from fraction 4 (12 g) by chromatography on ODS silica gel (MeOH−H_2O, 20:80, 50:50, 80:20, 500 mL of each), followed by precipitation (in 80% MeOH).

Analysis of Sugar Components of 1−4. Compounds (1−3 mg) were dissolved in 1 N HCl (dioxane−H_2O, 1:1, 1 mL) and then heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven out under N_2 gas overnight. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2 gas. The residue was dissolved in 0.1 mL of dry pyridine, and then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL) was added to the solution. The reaction mixture was heated at 60 °C for 2 h, and 0.1 mL of trimethylsilylimidazole solution was added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 mL, each), and the organic layer was analyzed by gas liquid chromatography (GC): column BPX50 (0.25 mm × 30 m); detector FID, column temp 210 °C, injector temp 270 °C, detector temp 300 °C, carrier gas He (2.0 kg/cm^2). Under these conditions, standard sugars gave peaks at t_R (min) 9.17 for D-fucose, 13.70 and 15.24 for D- and L-galactose, 12.22 and 13.70 for D- and L-glucose, and 8.22 and 9.05 for D- and L-xylose, respectively.

Compound 1: amorphous powder; $[\alpha]^{20}_D$ −31.5 (*c* 0.24, pyridine−MeOH); IR (KBr) ν_{max} 3433 (OH), 1725 (ester), 1377, 1069, 982, 895, 839 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive mode) *m*/*z* 997 $[M + Na]^+$; HRFABMS *m*/*z* 997.4631 (calcd for $C_{47}H_{74}O_{21}Na$, 997.4620).

Compound 2: white amorphous powder; $[\alpha]^{20}_D$ −36.3 (*c* 0.14, pyridine−MeOH); IR (KBr) ν_{max} 3431 (OH), 1733 (ester), 1631, 1376, 1069, 984, 920, 898, 857 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive mode) *m*/*z* 1113 $[M + Na]^+$; HRFABMS *m*/*z* 1113.5078 (calcd for $C_{52}H_{82}O_{24}Na$, 1113.5094).

Compound 3: white amorphous powder; $[\alpha]^{20}_D$ −76.4 (*c* 0.09, pyridine−MeOH); IR (KBr) ν_{max} 3433 (OH), 1739 (ester), 1631, 1375, 986, 919, 898, 847 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive mode) *m*/*z* 1097 $[M + Na]^+$; HRFABMS *m*/*z* 1097.5135 (calcd for $C_{52}H_{82}O_{23}Na$, 1097.5145).

Compound 4: amorphous solid; $[\alpha]^{20}_D$ −53.2 (*c* 0.1, pyridine−MeOH); IR (KBr) ν_{max} 3432 (OH), 1635, 1068, 982, 916, 896 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive mode) *m*/*z* 923 $[M + Na]^+$; HRFABMS *m*/*z* 923.4617 (calcd for $C_{45}H_{72}O_{18}Na$, 923.4616).

Cytotoxicity Assay. MCF-7 cells were purchased from Korean Cell Line Bank (KCLB). The cells (1×10^4 cells/well) were seeded in 96-well tissue culture plates (NUNC, Roskilde, Denmark). Cells were incubated for 12 h with/without each sample. After incubation, the spent medium was discarded and cells were washed once with RPMI 1640 medium and resuspended in the fresh medium (200 μL/well). MTT (0.1 mg/well) was added to cells followed by incubation for 4 h at 37 °C in 5% CO_2 environment. The formazan crystals formed were solubilized by incubating the cells with DMSO for 1 h. The absorbance of the solution was measured at 570 nm, using a microplate reader (E-Max, Molecular Devices, Sunnyvale, CA).

Test samples used in each assay were dissolved in dimethyl sulfoxide and then diluted with H_2O. The final concentration of DMSO did not exceed 0.1%, a concentration that was nontoxic to the cells. IC_{50} values were calculated from the mean values from four wells.

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References and Notes

(1) Jiang Su New Medical College Ed. *Dictionary of Traditional Chinese Crude Drugs*; Shanghai Scientific and Technological Press: Shanghai, 1978; Vol. 2, pp 2041−2044.
(2) Kato, A.; Miura, T. *Planta Med.* **1994**, *60*, 201−203.
(3) Miura, T.; Kato, A.; Usami, M.; Kadowaki, S.; Seino, Y. *Biol. Pharm. Bull.* **1995**, *18*, 624−625.
(4) Hirai, N.; Miura, T.; Moriyasu, M.; Ichimaru, M.; Nishiyama, Y.; Ogura, K.; Kato, A. *Biol. Pharm. Bull.* **1997**, *20*, 1271−1273.
(5) Kato, A.; Miura, T. *Biol. Pharm. Bull.* **1993**, *16*, 1118−1120.
(6) Ahn, M.-J.; Kim, J. *Arch. Pharm. Res.* **2005**, *28*, 592−597.
(7) Son, K. H.; Do, J. C. *J. Nat. Prod.* **1990**, *53*, 333−339.
(8) Ono, M.; Shoyama, K.; Nohara, T. *Shoyakugaku Zasshi* **1988**, *42*, 135−142.
(9) Kiyosawa, S.; Hutoh, M.; Komori, T.; Nohara, T.; Hosokawa, I.; Kawasaki, T. *Chem. Pharm. Bull.* **1968**, *16*, 1162−1164.
(10) Agrawal, P. K.; Jain, D. C.; Pathak, A. K. *Magn. Reson. Chem.* **1995**, *33*, 923−953.
(11) Chakravarty, A. K.; Pakrashi, S. C.; Uzawa, J. *Can. J. Chem.* **1981**, *59*, 1328−1330.
(12) Mimaki, Y.; Kuroda, M.; Nakamura, O.; Sashida, Y. *J. Nat. Prod.* **1997**, *60*, 592−597.

(13) Asano, T.; Murayama, T.; Hirai, Y.; Shoji, J. *Chem. Pharm. Bull.* **1993**, *41*, 566−570.
(14) Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479−2496.
(15) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501−507.
(16) Honbu, T.; Ikeda, T.; Zhu, X.-H.; Yoshihara, O.; Okawa, M.; Nafady, A. M.; Nohara, T. *J. Nat. Prod.* **2002**, *65*, 1918−1920.
(17) Duus, J. O.; Gotfredsen, C. H.; Bock, K. *Chem. Rev.* **2000**, *100*, 4589−4614.
(18) Agrawal, P. K. *Magn. Reson. Chem.* **2003**, *41*, 965−968.
(19) Dance A.-M.; Ralton, L.; Fuller, Z.; Milne, L.; Duthie, S.; Bestwick, C. S.; Lin, P. K. T. *Biochem. Pharmacol.* **2005**, *69*, 19−27.

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