Structure of Mimengosides A and B, New Triterpenoid Glycosides from Buddlejae Flos Produced in China

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Two new triterpenoid glycosides, named mimengosides A (1) and B (2), along with acteoside (3) were isolated from the Buddlejae Flos (flower and bud of *Buddleja officinalis*). The structures of 1 and 2 were determined as 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranoside of 16-dehydroxysaikogenin G and that of 3,23,28-trihydroxy-11-methoxy-olean-12-ene, respectively, by spectral and chemical methods.

Keywords Buddlejae Flos; *Buddleja officinalis*; Buddlejaceae; mimengoside A, B; 16-dehydroxysaikogenin G

Buddlejae Flos (Chinese name: Mi Meng Hua), the flower and bud of *Buddleja officinalis* MAXIM. are a Chinese crude drug used for antiinflammation.[1] With regard to the ingredient of this crude drug, the flavonoids such as linarin and acacetin are known.[1] We have now obtained two triterpenoid glycosides (**1** and **2**) together with acteoside (**3**). This paper deals with the structural characterization of these new triterpene glycosides, named mimengosides A (**1**) and B (**2**).

A methanolic extract of the crude drug was defatted with benzene and then repeatedly chromatographed to give compounds **1**, **2** and **3** in yields of 0.056, 0.051 and 1.03%, respectively.

Mimengoside A (**1**), a white powder, $[\alpha]_D$ +32.0°, showed a quasi ion peak $[M+H]^+$ at *m/z* 1073 in the positive ion fast atom bombardment mass spectrometry (FAB-MS) and absorption bands due to hydroxyl groups at 3428 cm^{-1} in the infrared (IR) spectrum. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I) of **1** exhibited fifty four carbon signals constituted of one disubstituted double bond (δ 131.6, 131.9), two hydroxymethyls (δ 64.4, 76.9), one oxygenated methine (δ 82.4), one oxygenated quarternary carbon (δ 84.7), six methyls, nine methylenes, three methines and six quarternary carbons in the aglycone moiety, and four anomeric carbons and two methyls in the sugar moiety. These signals due to the aglycone moiety were in good coincident with those of saikosaponin a[2] except for those due to C-14—17 carbons. Signals at δ 43.9 (s), 30.9 (t), 25.7 (t), and 41.5 (s) could be reasonably assigned to C-14—17, respectively, on the D-ring of the aglycone by comparing with those of 16-dehydroxysaikogenin G.[3] Acid hydrolysis with 1 N

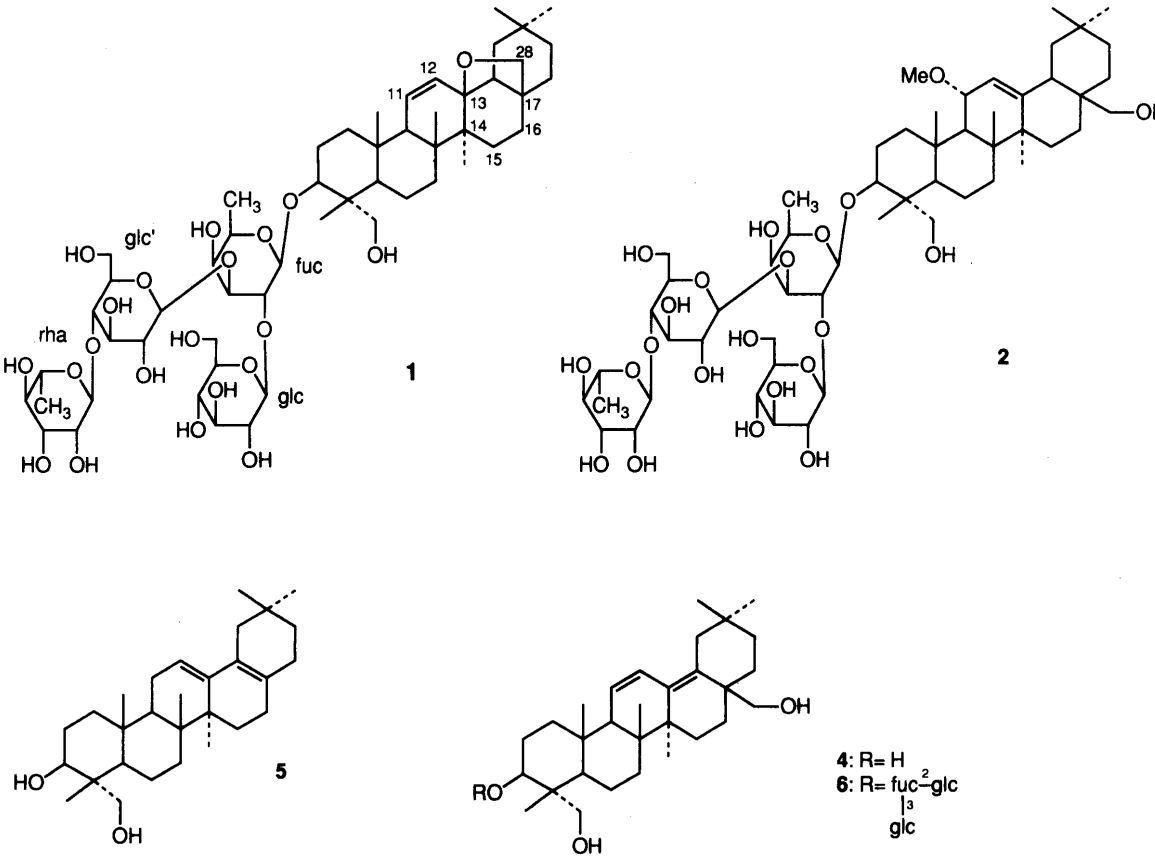

Chart 1

TABLE I. ^{13}C-NMR Data for **1**, **2**, **4**, **5** and **6** in Pyridine-d_5

	1	2	4	5	6		1	2	6
C-1	38.4	39.9	38.5	39.1	38.6	fuc			
C-2	25.5	26.1	27.6	27.7[a]	26.0	C-1	103.9	103.9	104.0
C-3	82.4	82.7	73.1	73.3	82.6	C-2	77.0	77.0	78.4
C-4	43.6	43.4	42.5	41.3	42.5	C-3	84.5	84.5	84.8
C-5	47.6	48.0	48.2	47.7	47.7	C-4	72.0	71.9	71.5
C-6	17.5	18.3	18.6	18.6	18.3	C-5	70.3	70.3	70.5
C-7	31.3	31.6	32.4	33.8	32.5	C-6	17.1	17.1	16.9
C-8	41.8	41.9	40.5	38.8	40.5	glc			
C-9	53.5	53.8	54.7	48.8	54.8	C-1	104.8	104.8	105.1
C-10	36.3	37.1	36.8	37.1	36.5	C-2	75.3	75.4	75.3
C-11	131.9	76.2	126.4	23.8	126.4	C-3	78.6	78.6	78.5
C-12	131.6	122.4	125.8	117.3	125.8	C-4	71.9	71.9	71.9
C-13	84.7	149.3	136.3[a]	128.9	136.0	C-5	77.0	77.0	77.1
C-14	43.9	43.8	43.0	42.7	43.8	C-6	61.1	61.1	62.5
C-15	30.9	33.0	32.4	29.4[b]	32.4	glc'			
C-16	25.7	22.7	24.6	27.4[a]	24.6	C-1	103.8	103.8	104.1
C-17	41.5	38.0	40.4	140.2	40.4	C-2	76.1	76.1	76.2
C-18	51.3	42.1	136.1[a]	125.7	135.0	C-3	77.3	77.4	78.8
C-19	37.2	46.8	38.3	39.1	38.3	C-4	78.1	78.1	72.8
C-20	31.6	31.2	33.0	29.5	33.0	C-5	76.2	75.9	77.5
C-21	31.6	31.2	33.0[b]	29.5[b]	33.0	C-6	62.9	62.9	63.0
C-22	25.8	26.3	29.2[b]	28.6[b]	29.2	rha			
C-23	64.4	64.8	67.4	67.8	64.5	C-1	102.6	102.6	
C-24	12.6	13.2	12.6	13.1	12.8	C-2	72.6	72.6	
C-25	18.4	17.7	18.6	16.6	18.7	C-3	72.4	72.4	
C-26	19.4	18.4	17.0	17.1	17.2	C-4	73.7	73.8	
C-27	19.7	25.2	20.7	21.2	20.7	C-5	70.2	70.2	
C-28	76.9	68.6	62.1		63.1	C-6	18.5	18.3	
C-29	33.5	33.3	32.5	28.7	32.5				
C-30	23.5	23.7	24.6	28.7	24.6				
OMe		52.6							

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

HCl of **1** yielded D-glucose, D-fucose and L-rhamnose as sugar components and compounds **4** and **5** as aglycone. Compound **4** showed a molecular ion peak at *m/z* 456 in the electron impact mass spectrometry (EI-MS), and ultraviolet (UV) absorption bands at 245, 254 and 263 nm due to conjugated double bonds. In the ^{13}C-NMR spectrum (Table I) exhibited thirty carbon signals due to six methyls, two hydroxymethyls, one oxygenated methine, two double bonds, six quartenary carbons, nine methylenes and two methines. Compound **4** was therefore identified with 3,23,28-trihydroxyoleane-11,13(18)-diene, isolated from *Scrophularia smithii* W.[4] On the other hand, compound **5** showed a molecular ion peak at *m/z* 426 in the EI-MS and UV absorption band at 242 nm. The ^{13}C-NMR spectrum (Table I) exhibited twenty nine carbon signals due to six methyls, one hydroxymethyl, one oxygenated methine, two double bonds, five quarternary carbons, ten methylenes and two methines. Compound **5** was therefore identified to 3,23-dihydroxy-28-nor-oleane-12,17-diene.[4] Compounds **4** and **5** were recognized as artifact compounds from **1**. Partial acid hydrolysis with 2% trifluoroacetic acid (TFA) of **1** yielded L-rhamnose and a prosapogenin (**6**), $[\alpha]_D$ −13.4°, which exhibited a $[M+H]^+$ at *m/z* 927, and on acid hydrolysis provided **4** and **5** as aglycone part, and glucose and fucose as sugar component. The prosapogenin (**6**) showed signals due to two terminal β-glucopyranosyl residues and one β-fucopyranosyl residue shifted at C-2 (+5.4 ppm) and C-3 (+9.3 ppm) by comparing with those of β-D-fucopyranoside[5] as listed in Table I. Therefore, the structure of **6** was elucidated as 3-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→2)-β-D-fucopyranosyl 3,23,28-trihydroxyoleane-11,13(18)-diene. The location of the glycosyl bond of rhamnose in **1** was determined by the ^{13}C-NMR and ^{1}H–^{13}C long range (5 Hz) correlation spectroscopy (COSY) spectra. As listed in Table I, the shifts at glucosyl C-3, C-4 and C-5 in **1** were observed by −1.5, +5.3 and −1.3 ppm, respectively, in comparing with those of **6**, and the cross peaks in the ^{1}H–^{13}C long range COSY were observed between δ 84.5 (fuc C-3) and δ 5.24 (glc' H-1); and δ 78.1 (glc' C-4) and δ 5.81 (rha H-1), indicating that the rhamnosyl moiety attached to the C-4 of fucosyl-3-*O*-glucosyl moiety. Consequently, the structure of mimengoside A (**1**) was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranosyl 16-dehydroxy-saikogenin G.

Mimengoside B (**2**), a white powder, $[\alpha]_D$ +1.5°, showed a quasi ion peak due to $[M+Na]^+$ at *m/z* 1127 and a fragment ion peak at *m/z* 1073 $[M-MeOH]^+$. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1**, except for signals at δ 52.6 (q), 122.4 (d), 149.3 (s) and 68.6 (t) assignable to methoxy, trisubstituted double bond function and hydroxymethyl groups, instead of signal at δ 131.9 (d), 131.6 (d), 84.7 (s) and 76.9 (t) originated from C-11—13 and C-28 in **1**. These signals could be reasonably assigned to C-11 methoxy, C-12, C-13, and C-28 on the C-ring of the aglycone, by comparison with those of saikosaponin b_3.[2] Treatment of **1** with *p*-toluenesulfonic acid (*p*-TsOH) in MeOH afforded a product identical with **2**. The solution of **2** in acetate buffer (pH 4.2) was heated at 37 °C for one day to yield **1**. Therefore the structure of **2** was concluded to be as shown in the Chart. Compound **2** might be changed from **1** during extraction and chromatographic procedure.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The optical rotations were measured with a JASCO DIP-360 digital polarimeter. The IR spectra were recorded with a HITACHI 270-30 type spectrometer. The UV spectra were recorded with a Hitachi U-3200 type spectrometer. The MS were measured with a JEOL JMS DX-303HF. The NMR spectra were recorded with a JEOL JNM GX-400 spectrometer; chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. Column chromatography was carried out with MCI gel CHP-20P (75—150 μ, Mitsubishi Chemical Industries Co., Ltd.), Kieselgel 60 (230—400 mesh, Merck), Bondapak C_{18} (Waters Associates) and Sephadex LH-20 (25—100 μ Pharmacia Co., Ltd.). Thin-layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm, Merck) using $CHCl_3$–MeOH–H_2O system as the developing solvent for the free compounds and detection was achieved by spraying 20% H_2SO_4 reagent followed by heating.

Isolation Buddlejae Flos (1.1 kg) was extracted with MeOH and its extract was evaporated under reduced pressure to afford a residue (222.3 g), which was shaken with benzene and water. The water layer (170.5) was filtered. After removal of the solvent of the filtrate, the residue (150.1 g) was obtained, which was subjected to column chromatographies of MCI gel CHP-20P (eluted with H_2O→40%→60%→80%→100% MeOH, gradiently), Sephadex LH-20 (eluted with 20% MeOH) and silica gel (eluted with $CHCl_3$: MeOH : H_2O = 7 : 2.5 : 0.3) to furnish acteoside (**3**, 11.4 g, 1.03%) and mimengosides A (**1**, 621 mg, 0.056%) and B (**2**, 561 mg, 0.051%).

Acteoside (3) A white powder, $[\alpha]_D^{26}$ −109.9° (*c* = 0.91, MeOH). Pos. FAB-MS *m/z*: 625 $[M+H]^+$. ^{13}C-NMR (CD_3OD) δ: 132.2, 117.1, 145.3, 146.7, 117.3, 122.0, 72.9, 37.2 (C-1—6, α, β), 104.8, 76.8, 82.4, 71.1, 76.6, 63.0 (glc C-1—6), 103.7, 72.8, 73.0, 74.5, 71.3, 19.2 (rha C-1—6), 128.3, 116.0, 147.5, 150.4, 117.9, 124.0, 115.4, 148.8, 169.0 (cafferoyl C-1—9).

Mimengoside A (1) A white powder, $[\alpha]_D^{24}$ +32.0° (*c* = 0.87, MeOH). Pos. FAB-MS *m/z*: 1095 $[M+Na]^+$, 1073 $[M+H]^+$, 911 $[M-hex]^+$,

457 [aglycone]$^+$, 439 [457$-H_2O$]$^+$. IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 2928 (CH), 1066 (C–O). ^{1}H-NMR (pyridine-d_5) δ: 1.05, 0.96, 0.96, 1.31, 0.92, 0.82 (each 3H s, H_3-24—27, -29, -30), 1.95 (1H, m, H-2), 2.03 (1H, br s, H-9), 2.23 (1H, br d, J=9.9 Hz, H-2), 3.32, 3.72 (each 1H, AB q, J=6.6 Hz, H_2-28), 3.72, 4.38 (each 1H, AB q, J=6.6 Hz, H_2-23), 4.13 (1H, m, H-3), 5.53 (1H, dd, J=10.3, 1.0 Hz, H-11), 5.94 (1H, d, J=10.3 Hz, H-12), 4.89 (1H, d, J=7.7 Hz, fuc H-1), 4.62 (1H, dd, J=7.7, 9.1 Hz, fuc H-2), 4.03 (1H, m, fuc H-3), 4.15, (1H, m, fuc H-4), 3.59 (1H, m, fuc H-5), 1.38 (3H, d, J=6.1 Hz, fuc H_3-6), 5.55 (1H, d, J=7.3 Hz glc H-1), 4.08 (1H, m, glc H-2), 4.18 (1H, m, glc H-3), 4.31 (1H, m, glc H-4), 3.62 (1H, m, glc H-5), 4.25 (1H, dd, J=4.8, 11.4 Hz, glc H-6), 4.30 (1H, m, glc H-6), 5.24 (1H, d, J=7.7 Hz, glc′ H-1), 3.91 (1H, dd, J=7.7, 9.5 Hz, glc′ H-2), 4.16 (1H, m, glc′ H-3), 4.37 (1H, t, J=9.5 Hz, glc′ H-4), 3.70 (1H, m, glc′ H-5), 4.05, 4.20 (each 1H, m glc′ H_2-6), 5.81 (1H, s, rha H-1), 4.85 (1H, br s, rha H-2), 4.51 (1H, br d, J=9.2 Hz, rha H-3), 4.34 (1H, m, rha H-4), 4.94 (1H, m, rha H-5), 1.70 (3H, d, J=6.2 Hz, rha H_3-6).

Acid Hydrolysis of 1 A solution of **1** (100.0 mg) in 1 N HCl (10 ml) was heated at 80 °C for 3 h on a hot bath, then it was poured into water and extracted with $CHCl_3$. Removal of the respective solvent furnished the water and $CHCl_3$ extracts. The water extract was purified by silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5) to furnish D-glucose (7.1 mg, $[\alpha]_D^{30}$ +46.3° (c=0.70, H_2O), Rf 0.43), D-fucose (1.2 mg, $[\alpha]_D^{29}$ +69.3° (c=0.03, H_2O), Rf 0.62) and L-rhamnose (3.0 mg, $[\alpha]_D^{30}$ +6.7° (c=0.30, H_2O), Rf 0.76) detected by TLC (impregnated 0.5 M NaH_2PO_4; solvent, 2-propanol : acetone : 0.1 M lactic acid = 4 : 4 : 2),[6] and the $CHCl_3$ extract was purified by silica gel column chromatography (benzene : acetone = 6 : 1) to give **4** (3.3 mg) and **5** (21.0 mg). **4**: Colorless needles (from MeOH–$CHCl_3$), mp 286—289 °C (dec.), $[\alpha]_D^{28}$ −64.8° (c=0.47, $CHCl_3$). EI-MS m/z: 456 [M$^+$]. UV λ_{max}^{MeOH} nm (log ε): 245 (4.55), 254 (4.58), 263 (4.41). ^{1}H-NMR (pyridine-d_5) δ: 1.05, 1.05, 0.96, 1.07, 0.89, 0.87 (each 3H, s, CH_3-24—27, -29, -30), 2.13 (1H, br s), 2.30 (1H, br d, J=10.6 Hz), 2.38 (1H, br d, J=10.6 Hz), 2.54 (1H, d, J=14.3 Hz), 3.75, 4.23 (each 1H, d, J=10.3 Hz, H_2-23), 3.75, 4.11 (each 1H, d, J=11.0 Hz, H_2-28), 4.29 (1H, dd, J=10.3, 5.3 Hz, H-3), 5.74 (1H, d, J=10.6 Hz, H-12), 5.86, 6.07, 6.39 (each 1H, br s, OH), 6.61 (1H, dd, J=10.6, 2.6 Hz, H-11). **5**: Colorless needles (MeOH–$CHCl_3$), mp 246—249 °C, $[\alpha]_D^{24}$ +126.3° (c=0.40, $CHCl_3$). EI-MS m/z: 426 [M]$^+$. UV λ_{max}^{MeOH} nm (log ε): 242 (4.18). ^{1}H-NMR (pyridine-d_5) δ: 1.08, 1.02, 0.95, 1.09, 0.93, 0.91 (each 3H, s, CH_3-24—28, -29, -30), 3.74, 4.19 (each 1H, d, J=9.9 Hz, H_2-23), 4.21 (1H, m, H-3), 5.80, 6.36 (each 1H, br s, OH), 5.63 (1H, br s, H-12).

Partialy Acid Hydrolysis of 1 A solution of **1** (98 mg) in 2% TFA–50% dioxane (10 ml) was heated at 37 °C for 12 h. After the reaction mixture was neutralized with dil. NaOH, it was then passed through a MCI gel CHP-20P column and washed with water, subsequently eluted with MeOH. The aqueous and methanol fractions were evaporated under reduced pressure to afford the respective residues, which were purified by silica gel ($CHCl_3$: MeOH : H_2O = 7 : 2.5 : 0.3) and Bondapak C_{18} (70% MeOH) column chromatographys to give L-rhamnose (2 mg), and prosapogenin **6** (6 mg). **6**: A white powder, $[\alpha]_D^{28}$ −13.4° (c=0.31, MeOH). Pos. FAB-MS m/z: 927 [M+H]$^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.97, 1.02, 0.96, 1.04, 0.87, 0.82 (each 3H, s, H_3-24—27, -29, -30), 6.55 (1H, dd, J=10.3, 2.5 Hz, H-11), 5.66 (1H, d, J=10.3 Hz, H-12), 4.84 (1H, d, J=8.1 Hz, fuc H-1), 1.40 (3H, d, J=6.6 Hz, fuc H_3-6), 5.49 (1H, d, J=8.1 Hz, glc H-1), 5.16 (1H, d, J=7.7 Hz, glc′ H-1).

Acid Hydrolysis of 6 A solution of **6** (2 mg) in 1 N HCl (1 ml) was treated as described for **1** to give glucose, fucose, **4** and **5**, which were detected by TLC.

Acetylation of 6 A solution of **6** (3 mg) in pyridine (300 μl) and Ac_2O (150 μl) was heated at 60 °C for 2 h. The reaction mixture was purified by silica gel column chromatography (n-hexane : acetone = 5 : 1) to give **6**-peracetate (1 mg). **6**-Peracetate: A white powder. ^{1}H-NMR ($CDCl_3$) δ: 0.71, 0.79, 0.80, 0.91, 2 × 0.96 (each s, H_3-24—27, -29, -30), 1.99, 2.00, 2.01, 2.02, 2.06, 2.08, 2.09, 2.10, 2.13, 2.14, 2.19 (each 3H s, Ac), 3.98, 4.18 (each 1H, AB q, J=10.8 Hz, H_2-28), 4.06, 4.22 (each 1H, AB q, J=10.2 Hz, H_2-23), 4.24 (1H, d, J=8.1 Hz, fuc H-1), 3.89 (1H, dd, J=8.1, 9.9 Hz, fuc H-2), 3.74 (1H, dd, J=3.3, 9.9 Hz, fuc H-3), 5.22 (1H, d, J=3.3 Hz, fuc H-4), 3.60 (1H, m, fuc H-5), 1.16 (3H, d, J=6.2 Hz, fuc H_3-6), 4.68 (2H, d, J=7.7 Hz, 2 × glc H-1), 4.95, 4.99 (each 1H, dd, J=7.7, 9.1 Hz, 2 × glc H-2), 5.13 (2H, t, J=9.1 Hz, 2 × glc H-3), 5.09 (2H, t, J=9.1 Hz, 2 × glc H-4), 3.62 (2H, m, 2 × glc H-5), 4.09 (2H, dd, J=3.7, 12.1 Hz, 2 × glc H-6), 4.33 (2H, m, 2 × glc H-6).

Mimengoside B (2) A white powder, $[\alpha]_D^{26}$ +1.5° (c=0.95, MeOH). Pos. FAB-MS m/z 1127 [M+Na]$^+$, 1105 [M+H]$^+$, 1073 [M−MeOH]$^+$, 911 [1073−hex]$^+$, 765 [911−deoxyhex]$^+$, 603 [765−hex]$^+$, 585 [603$-H_2O$]$^+$, 457 [603−deoxyhex]$^+$, 439 [457$-H_2O$]$^+$. IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 2944 (CH), 1074 (C–O). ^{1}H-NMR (pyridine-d_5) δ: 0.90, 2 × 0.97, 2 × 1.09, 1.31 (each s, H_3-24—27, -29, -30), 5.47 (1H, d, J=3.3 Hz, H-12), 3.21 (3H, s, OMe), 4.99 (1H, d, J=7.7 Hz, fuc H-1), 1.35 (3H, d, J=6.2 Hz, fuc H_3-6), 5.57 (1H, d, J=7.7 Hz, glc H-1), 5.25 (1H, d, J=7.7 Hz, glc′ H-1), 5.83 (1H, s, rha H-1), 1.72 (3H, d, J=6.2 Hz, rha H_3-6).

Acid Treatment of 1 A mixture of **1** (21 mg) and p-TsOH (1.2 mg) in MeOH (10 ml) was stirred for 2 h at 37 °C. After the reaction mixture was diluted with water, it was then passed through a MCI gel CHP-20P column and washed with water until it was neutral, subsequently eluted with MeOH. The methanolic fraction was evaporated under reduced pressure to afford a residue, which was purified by silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 7 : 2.5 : 0.3) to give **2** (7 mg).

Transformation of 2 into 1 A solution of **2** (50 mg) in acetate buffer (pH 4.2, 50 ml) was incubated at 37 °C for one day, then it was passed through a MCI gel CHP-20P column and washed with water until it was neutral, subsequently eluted with MeOH. Then the methanolic fraction was purified by a silica gel column chromatography to give **1** (23 mg).

Added in Proof (February 20, 1992) After this paper was submitted, mimengoside B (**2**) was crystallized as colorless needles, mp 279—282 °C, from dil. MeOH.

References and Notes

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