New Iridoid and Secoiridoid Glucosides from the Roots of *Gentiana manshurica*

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One new iridoid glucoside, 4″-*O*-*β*-D-glucosyl-6′-*O*-(4-*O*-*β*-D-glucosylcaffeoyl)linearoside (**1**), and two new secoiridoid glucosides, 6′-*O*-acetylsweroside (**2**) and 6′-*O*-acetyl-3′-*O*-[3-(*β*-D-glucopyranosyloxy)-2-hydroxybenzoyl]sweroside (**3**), were isolated from the dried roots of *Gentiana manshurica* (Gentianaceae), together with 11 known ones, including one iridoid glucoside, five secoiridoid glucosides, and five triterpenes. The structures of the new compounds were determined on the basis of detailed spectroscopic analyses and acidic hydrolysis.

Introduction. – In the *Chinese Pharmacopoeia*, the dried roots and rhizomes of four species, namely *Gentiana manshurica* KITAG., *G. scabra* BUNGE, *G. triflora* PALL., and *G. rigescens* FRANCH., are used as the raw materials of *Gentianae Radix et Rhizoma* ('Long dan' in Chinese) [1] for the treatment of hepatitis, rheumatism, and cholecystitis. Iridoid and secoiridoid glucosides, which showed antibacterial, antifungal, anticancer, anti-inflammatory, and hepatoprotective activities [2] were reported as the main constituents in *Gentianae Radix et Rhizoma* [3–5]. However, the data on the chemical constituents of *G. manshurica*, one of the raw materials of *Gentianae Radix et Rhizoma*, are scarce. As a part of ongoing search for active iridoid and secoiridoid glucosides from the genus *Gentiana*, a detailed phytochemical investigation on *G. manshurica* was carried out. This led to the isolation of one new iridoid glucoside **1**, two new secoiridoid glucosides **2** and **3**, and 11 known compounds, including one iridoid glucoside, five secoiridoid glucosides, and five triterpenes, *i.e.*, **4**–**14** (*Fig. 1*).

Results and Discussion. – The dried roots of *G. manshurica* were extracted three times with MeOH under reflux. After removal of the organic solvent, the extract was suspended in H_2O and extracted with AcOEt and BuOH, successively. The BuOH fraction was subjected to column chromatography (CC; *MCI-gel CHP20P*, SiO_2, and *Daisogel ODS-AP*), to afford compounds **1**–**4** and **7**–**9**. The AcOEt fraction was also subjected to CC (SiO_2, *MCI-gel CHP20P*, and *Daisogel ODS-AP*) to furnish compounds **5**, **6**, and **10**–**14**. Compounds **4**–**14** were known, and identified as 6′-*O*-[3-(*β*-D-glucopyranosyloxy)-2-hydroxybenzoyl]sweroside (**4**) [6], trifloroside (**5**) [7],

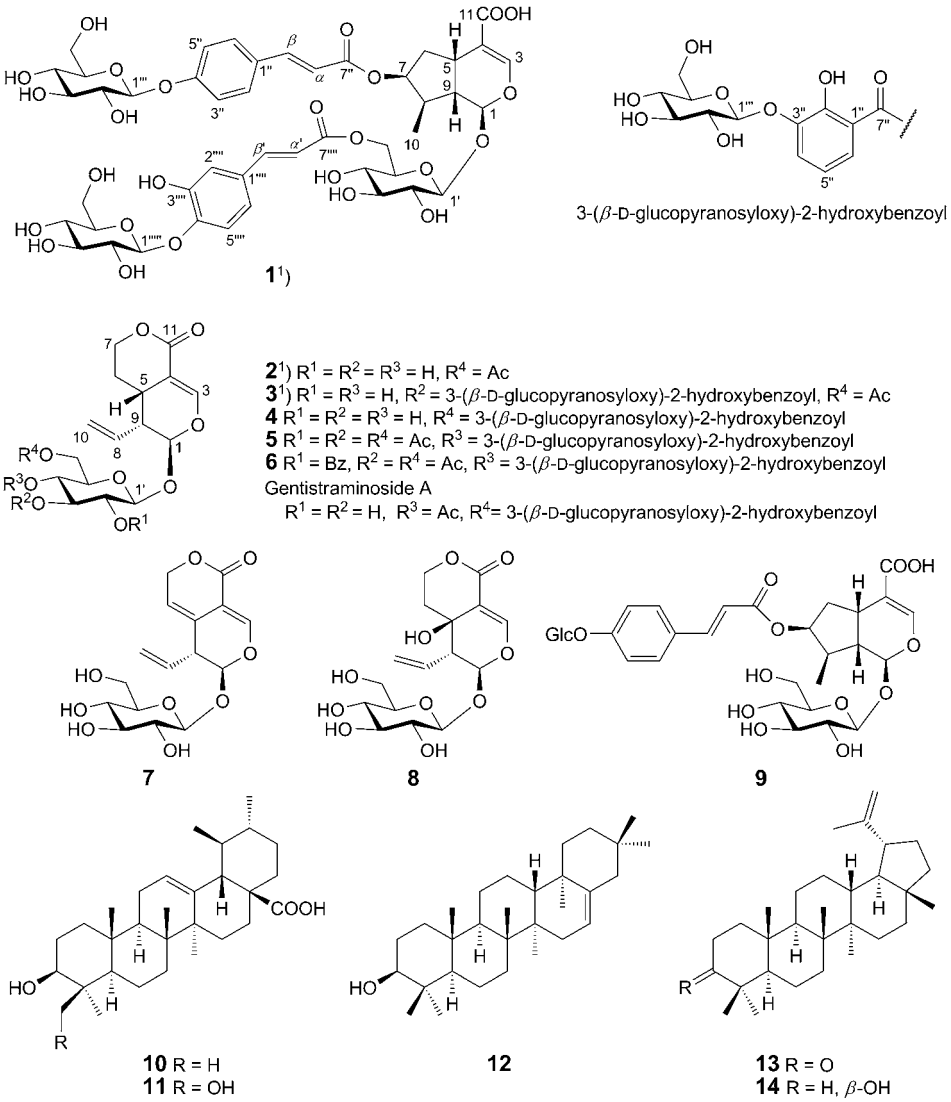

Fig. 1. *Chemical constituents from* G. manshurica

scabraside (**6**) [8], gentiopicroside (**7**), swertimarin (**8**), 4″-*O*-β-D-glucopyranosyllinearoside (**9**) [9], ursolic acid (**10**), 3,24-dihydroxyurs-12-en-28-oic acid (**11**) [10], chiratenol (**12**) [11], lup-20(29)-en-3-one (**13**) [12], and lupeol (**14**) [13] by comparison of their spectroscopic data with those reported previously in the literature or by direct comparison with authentic compounds. Compounds **1**–**3** were new iridoid and

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

secoiridoid glucosides and identified as 4″-*O*-*β*-D-glucosyl-6′-*O*-[(4-*O*-*β*-D-glucosyl)-caffeoyl]linearoside[1]) (**1**), 6′-*O*-acetylsweroside[1]) (**2**) and 6′-*O*-acetyl-3′-*O*-[3-(*β*-D-glucopyranosyloxy)-2-hydroxybenzoyl]sweroside[1]) (**3**) (sweroside = (4a*S*,5*R*,6*S*)-5-ethenyl-6-(*β*-D-glycopyranosyloxy)-4,4a,5,6-tetrahydro-1*H*,3*H*-pyrano[3,4-*c*]pyran-1-one; linearoside = (1*S*,4a*S*,6*S*,7*R*,7a*S*)-1-(*β*-D-glucopyranoxsyloxy)-1,4a,5,6,7,7a-hexahydro-6-{[(2*E*)-3-(4-hydroxyphenyl)-1-oxoprop-2-en-1-yl]oxy}-7-methylcyclopenta[*c*]-pyran-4-carboxylic acid). Their structures were established as follows.

Compound **1** was obtained as a white amorphous powder. Its molecular formula $C_{46}H_{56}O_{25}$ was determined on the basis of the HR-ESI-MS (*m*/*z* 1007.3023 ($[M-H]^-$)). The IR spectrum showed absorptions at 3404, 1693, 1637, and 1605 cm^{-1}, suggesting the presence of OH and C=O groups. The ^{1}H- and ^{13}C-NMR data (*Table 1*) exhibited the signals arising from two benzene rings (*δ*(H) 7.32 and 6.96, and 7.12, 6.96, and 6.92, resp.), two C=O groups at *δ*(C) 168.9 (C(7″)) and 168.7 (C(7‴)), two sets of olefinic protons at two *trans*-C=C bonds (*δ*(H) 7.43 and 6.25, and 7.35 and 6.06, resp., *J* = 16.0 Hz), and three *β*-glucopyranosyl moieties (anomeric protons at *δ*(H) 4.59 (*d*, *J* = 8.0 Hz, H–C(1′)), 4.80 (*d*, *J* = 7.5 Hz, H–C(1⁗)), and 4.87 (*d*, *J* = 7.0 Hz, H–C(1‴)), resp.). These signals indicated the presence of *p*-coumaroyl (= 3-(4-hydroxyphenyl)-1-oxoprop-2-en-1-yl) and caffeoyl (= 3-(3,4-dihydroxyphenyl)-1-oxo-

Table 1. *^{1}H- and ^{13}C-NMR Data* (CD_3OD, 500 and 125 MHz, resp.) *of Compound* **1**[1]). *δ* in ppm, *J* in Hz.

Position	*δ*(H)	*δ*(C)	Position	*δ*(H)	*δ*(C)
H–C(1)	5.09 (*d*, *J* = 5.0)	98.0	H–C(1‴)	4.87 (*d*, *J* = 7.0)	101.8
H–C(3)	7.18 (*s*)	150.0	H–C(2‴)	3.42 – 3.40 (*m*)	75.1
C(4)		[a])	H–C(3‴)	3.67 – 3.65 (*m*)	75.9
H–C(5)	3.14 (*t*, *J* = 8.5)	33.9	H–C(4‴)	3.32 – 3.29 (*m*)	72.4
CH_2(6)	2.36 – 2.33, 1.73 – 1.68 (2*m*)	41.0	H–C(5‴)	3.22 – 3.20 (*m*)	78.6
H–C(7)	5.15 – 5.13 (*m*)	79.2	CH_2(6‴)	3.83 – 3.79 (*m*),	63.1
H–C(8)	2.11 – 2.08 (*m*)	41.4		3.58 (*dd*, *J* = 12.0, 5.0)	
H–C(9)	1.99 – 1.96 (*m*)	47.7	C(1⁗)		131.2
Me(10)	0.98 (*d*, *J* = 6.5)	14.4	H–C(2⁗)	6.96 (br. *s*)	116.3
C(11)		[a])	C(3⁗)		149.0
H–C(1′)	4.59 (*d*, *J* = 8.0)	100.5	C(4⁗)		149.5
H–C(2′)	3.14 (*t*, *J* = 8.0)	75.0	H–C(5⁗)	7.12 (*d*, *J* = 8.0)	118.4
H–C(3′)	3.31 – 3.28 (*m*)	78.3	H–C(6⁗)	6.92 (br. *d*, *J* = 8.0)	122.4
H–C(4′)	3.21 – 3.19 (*m*)	71.6	C(7⁗)		168.7
H–C(5′)	3.67 – 3.65 (*m*)	75.9	H–C(1⁗)	4.80 (*d*, *J* = 7.5)	104.0
CH_2(6′)	4.76 (br. *d*, *J* = 12.0),	65.0	H–C(2⁗)	3.45 (*t*, *J* = 7.0)	75.1
	4.28 (*dd*, *J* = 12.0, 8.0)		H–C(3⁗)	3.22 – 3.20 (*m*)	78.6
C(1″)		130.2	H–C(4⁗)	3.21 – 3.19 (*m*)	71.9
H–C(2″,6″)	7.32 (*d*, *J* = 8.5)	131.0	H–C(5⁗)	3.46 – 3.43 (*m*)	77.7
H–C(3″,5″)	6.96 (*d*, *J* = 8.5)	118.4	CH_2(6⁗)	3.83 – 3.79 (*m*),	62.8
C(4″)		160.7		3.64 (*dd*, *J* = 12.0, 6.0)	
C(7″)		168.9	H–C(*α*)	6.06 (*d*, *J* = 16.0)	117.7
			H–C(*β*)	7.35 (*d*, *J* = 16.0)	145.6
			H–C(*α*′)	6.25 (*d*, *J* = 16.0)	117.4
			H–C(*β*′)	7.43 (*d*, *J* = 16.0)	146.7

[a]) The signals of C(4) and C(11) were not detected in the ^{13}C-NMR spectrum.

prop-2-en-1-yl) moieties. In the up-field region, the HSQC experiments demonstrated the presence of one Me (δ(C) 14.4 (C(10))), one CH_2 (δ(C) 41.0 (C(6))), and three CH groups (δ(C) 47.7 (C(9)), 41.4 (C(8)), and 33.9 (C(5))). These NMR characteristics resembled those of **9** [9]. However, compared with **9**, **1** had an additional caffeoyl and an additional glucosyl moiety. Acidic hydrolysis of **1** gave D-glucose as the sugar moiety. In the HMBC spectrum of **1**, the correlations H–C(1′) (δ(H) 4.59)/C(1) (δ(C) 98.0), CH_2(6′) (δ(H) 4.76 and 4.28) and H–C(α') (δ(H) 6.25)/C(7‴′) (δ(C) 168.7), and H–C(β') (δ(H) 7.43)/C(1‴′) (δ(C)131.2) and C(6‴′) (δ(C) 122.4) demonstrated that a caffeoyloxy moiety was attached to C(6′), and the glycosyloxy group C(1′) was attached to C(1) of the aglycone. The HMBCs H–C(1‴) (δ(H) 4.87)/C(4″) (δ(C) 160.7) and H–C(α) (δ(H) 6.06)/C(7″) (δ(C) 168.9) indicated that a glucosyloxy group was attached to C(4″) of the *p*-coumaroyl unit. In addition, the HMBCs H–C(1⁗′) (δ(H) 4.80), H–C(2⁗′) (δ(H) 6.96), and H–C(6⁗′) (δ(H) 6.92)/C(4⁗′) (δ(C) 149.5), and H–C(2⁗′) (δ(H) 6.96) and H–C(5⁗′) (δ(H) 7.12)/C(3⁗′) (δ(C) 149.0) were also observed, indicating that the glucosyloxy residue C(1⁗′) was at C(4⁗′) of the caffeoyl unit. Other HMBCs (*Fig. 2*) further confirmed the structure of **1**. Accordingly, compound **1** was established to be 4″-*O*-β-D-glucosyl-6′-*O*-(4-*O*-β-D-glucosylcaffeoyl)-linearoside.

Compound **2** was a white amorphous powder. The molecular formula $C_{18}H_{24}O_{10}$ was deduced from the HR-ESI-MS (*m*/*z* 423.1270 ($[M+Na]^+$)). The ^{1}H- and ^{13}C-NMR data of **2** (*Table 2*) revealed the presence of an Ac group (δ(H) 2.05 (*s*, 3 H); (δ(C)

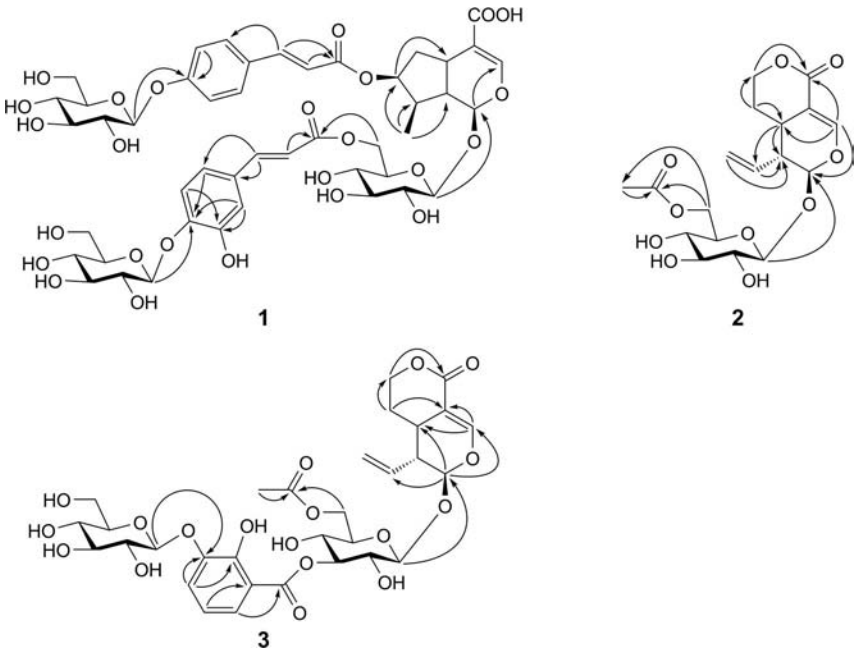

Fig. 2. *Key HMBC* (H → C) *features of compounds* **1**–**3**

Table 2. *^{1}H- and ^{13}C-NMR Data* (CD_3OD, 500 and 125 MHz, resp.) *of Compounds* **2** *and* **3**. δ in ppm, J in Hz.

Position	**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	5.38 (*d*, *J* = 2.0)	98.6	5.29 (br. *s*)	98.7
H–C(3)	7.58 (*s*)	154.1	7.50 (*s*)	154.1
C(4)		106.4		106.4
H–C(5)	3.15 – 3.11 (*m*)	28.7	3.06 – 3.03 (*m*)	28.7
CH_2(6)	1.79 – 1.76 (*m*),	26.2	1.70 – 1.66 (*m*),	26.2
	1.70 (*dt*, *J* = 12.5, 4.0)		1.60 (*dd*, J = 12.5, 5.0)	
CH_2(7)	4.40 – 4.33 (*m*)	70.0	4.37 – 4.34 (*m*)	70.0
H–C(8)	5.55 (*ddd*, *J* = 10.0, 10.0, 3.0)	133.6	5.49 – 5.42 (*m*)	133.6
H–C(9)	2.71 – 2.68 (*m*)	44.2	2.62 – 2.60 (*m*)	44.2
CH_2(10)	5.30 (*dd*, *J* = 25.5, 2.0),	121.2	5.23 (br. *d*, *J* = 17.0),	120.4
	5.27 (*dd*, *J* = 18.5, 1.5)		5.19 (br. *d*, *J* = 18.0)	
C(11)		168.7		168.7
H–C(1′)	4.68 (*d*, *J* = 7.5)	100.2	4.59 (*d*, *J* = 8.0)	100.2
H–C(2′)	3.18 (*t*, *J* = 7.5)	74.9	3.10 (*t*, *J* = 8.0)	74.9
H–C(3′)	3.39 – 3.36 (*m*)	75.9	3.29 (*t*, *J* = 9.0)	78.0
H–C(4′)	3.36 – 3.33 (*m*)	71.7	3.24 – 3.21 (*m*)	71.7
H–C(5′)	3.51 – 3.49 (*m*)	78.0	3.44 – 3.41 (*m*)	75.9
CH_2(6′)	4.47 – 4.44 (*m*),	64.8	4.36 – 4.34 (*m*),	64.9
	4.23 (*dd*, *J* = 12.0, 6.0)		4.13 (*dd*, *J* = 12.0, 5.5)	
Me–*C*=O		173.0		173.0
Me–C=O	2.05 (*s*)	21.0	1.96 (*s*)	21.0
C(1″)				115.0
C(2″)				153.2
C(3″)				147.5
H–C(4″)			7.31 (*d*, *J* = 8.0)	124.0
H–C(5″)			6.77 (*t*, *J* = 8.0)	121.2
H–C(6″)			7.50 (*dd*, *J* = 8.0, 2.5)	124.9
C(7″)				170.6
H–C(1‴)			4.80 (*d*, *J* = 7.5)	103.4
H–C(2‴)			3.44 – 3.41 (*m*)	75.0
H–C(3‴)			3.23 – 3.21 (*m*)	71.7
H–C(4‴)			3.39 – 3.36 (*m*)	78.0
H–C(5‴)			3.32 – 3.29 (*m*)	78.6
CH_2(6‴)			3.78 (br. *d*, *J* = 12.5),	62.8
			3.59 (*dd*, *J* = 12.5, 4.5)	

173.0 and 21.0), a β-glucopyranosyl moiety (anomeric H-atom at δ(H) 4.68 (*d*, *J* = 7.5 Hz), and C-atoms at δ(C) 100.2 (C(1′)), 74.9 (C(2′)), 75.9 (C(3′)), 71.7 (C(4′)), 78.0 (C(5′)), and 64.8 (C(6′))). The NMR characteristics were very similar to those of 3′-*O*-acetylsweroside [14]. However, ^{1}H,^{1}H-COSY and HMBC data (*Fig. 2*) demonstrated that the AcO group was attached to C(6′) of the glucosyl moiety. Acidic hydrolysis of **2** gave D-glucose as the sugar moiety. Based on the above evidences, **2** was determined to be 6′-*O*-acetylsweroside.

Compound **3** was isolated as a white amorphous powder. The molecular formula was determined to be $C_{31}H_{38}O_{18}$ based on the HR-ESI-MS data (*m*/*z* 721.1976 ([*M* +

Na]$^+$)). The IR spectrum showed absorptions at 3421, 1734, 1684, and 1617 cm^{-1}, suggesting the presence of OH and C=O groups. The ^{1}H- and ^{13}C-NMR data (*Table 2*) indicated the presence of a 1,2,3-trisubstituted benzene ring (δ(H) 7.50 (*dd*, $J = 8.0$ and 2.5 Hz), 7.31 (*d*, $J = 8.0$ Hz), and 6.77 (*t*, $J = 8.0$ Hz)), two C=O groups (δ(C) 173.0 and 170.6), and two β-glucosyl moieties (anomeric protons at δ(H) 4.80 (*d*, $J = 7.5$ Hz), and 4.59 (*d*, $J = 8.0$ Hz)), which were further confirmed to derive from D-glucose by acidic hydrolysis. The above and other NMR characteristics resembled those of gentistraminoside A [15]. However, in contrast to gentistraminoside A, the HMBCs (*Fig. 2*) of CH_2(6′) (δ(H) 4.36–4.34, 4.13) and *Me*CO (δ(H) 1.96)/Me*C*O (δ(C) 173.0), revealed that the Ac group was attached to C(6′). In addition, the C(3′) signal of **3** was shifted downfield ($\Delta\delta = 2.6$ ppm), while the C(4′) signal was shifted upfield ($\Delta\delta = 3.1$ ppm), suggesting that C(3′) was esterified with the 3-(β-D-glucopyranosyloxy)-2-hydroxybenzoyl residue. The HMBCs, H–C(6″) (δ(H) 7.50)/C(7″) (δ(C) 170.6), and H–C(4″) (δ(H) 7.31) and H–C(1‴) (δ(H) 4.80)/C(3″) (δ(C) 147.5) were also observed. Thus, the structure of **3** was established as 6′-*O*-acetyl-3′-*O*-[3-(β-D-glucopyranosyloxy)-2-hydroxybenzoyl]sweroside.

In the present work, we investigated the chemical constituents of *G. manshurica* in detail, which led to the isolation of one new iridoid glucoside, 4″-*O*-β-D-glucosyl-6′-*O*-[(4-*O*-β-D-glucosyl)-caffeoyl]linearoside (**1**), two new secoiridoid glucosides, 6′-*O*-acetylsweroside (**2**) and 6′-O-aceyl-3′-*O*-[2-(β-D-glucopyranosyloxy)-2-hydroxybenzoyl]sweroside (**3**), and 11 known ones, including one iridoid glucoside, five secoiridoid glucosides, and five triterpenes. Similar to other raw materials of *Gentianae Radix et Rhizoma*, iridoid and secoiridoid glucosides were the main constituents in the title plant [3–5]. Moreover, a number of other *O*-acylsecoiridoids (see **4**–**6**) were also isolated. The results were in agreement with the use of *G. manshurica* as *Gentianae Radix et Rhizoma*. Triterpenes **10**–**14** were isolated for the first time from *G. manshurica*. However, in contrast to other sources of *Gentianae Radix et Rhizoma* [16], no dammarane-type triterpenes were obtained in the present investigation.

Expertimental Part

General. TLC: *HSGF$_{254}$* plates (*Yantai Jiangyou Silica Gel Development Co., Ltd.*), eluent $CHCl_3$/MeOH/H_2O 8:2:0.2; spots were detected by spraying with 10% H_2SO_4 soln. followed by heating. Column chromatography (CC): *MCI* gel *CHP20P* (*Mitsubishi Chemical Co.*), silica gel (SiO_2; 200–300 mesh; *Qingdao Makall Group Co., Ltd.*), and *Daisogel ODS-AP* (40–60 μm, *Daiso Co., Ltd.*). GC: *Thermo-TR-5 MS* spectrometer. Optical rotations: *Krüss-P800-T* polarimeter. UV Spectra: *Jasco-J-180* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-380* spectrometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-DRX-500* instrument at 500 (^{1}H) and 125 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Waters-UPLC-Premior-QTOF* spectrometer; in *m*/*z*.

Plant Material. The dried roots of *G. manshurica* were collected in August 2009 in Taikang City, Heilongjiang, China, and authenticated by Dr. *Li-Hong Wu*, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine. A voucher specimen (No. LD-090808) was deposited with the Herbarium of the Shanghai R&D Center for Standardization of Chinese Medicines.

Extraction and Isolation. The dried roots of *G. manshurica* (4.5 kg) were extracted three times with MeOH (20 l) under reflux (each 2 h). After evaporation of the org. solvent, the extract was suspended in H_2O (5 l) and extracted four times with AcOEt (5 l) and BuOH (5 l), successively, to give an AcOEt fraction (135 g) and a BuOH fraction (240 g). The BuOH fraction (150 g) was subjected to CC (*MCI* gel *CHP20P*, MeOH/H_2O 0:1 → 8:2): *Fractions 1–4*. Further CC (SiO_2, $CHCl_3$/MeOH 15:1 → 3:2, then

Daisogel ODS-AP, MeOH/H_2O 1:9 → 5:5) afforded **1** (9 mg), **2** (89 mg), **3** (53 mg), **4** (110 mg), and **9** (152 mg) from *Frs. 3* (9.5 g) and *4* (3.1 g). CC (*MCI* gel *CHP20P*, MeOH/H_2O 0:1 → 5:5, then SiO_2, AcOEt/MeOH/H_2O 15:1:0.5) gave **8** (88 mg) from *Fr. 1* (8.2 g). CC (*MCI* gel *CHP20P*, MeOH/H_2O 0:1 → 4:6) gave **7** (8.8 g) from *Fr. 2* (10.3 g). The AcOEt fraction (85 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 100:1 → 9:1): *Fractions 5–10*. Further CC (*MCI* gel *CHP20P*, MeOH/H_2O 4:6 → 9:1) afforded **5** (820 mg) and **6** (491 mg) from *Fr. 9* (5.8 g). CC (SiO_2, petroleum/AcOEt 9:1 → 6:4, then *Daisogel ODS-AP*, MeOH/H_2O 7:3 → 1:0) gave **10** (76 mg), **11** (41 mg), and **12** (62 mg) from *Fr. 7* (9.8 g). Compounds **13** (490 mg) and **14** (630 mg) were obtained from *Fr. 5* (7.7 g) by CC (SiO_2, petroleum/AcOEt 100:1 → 25:1) and recrystallization in $CHCl_3$/MeOH 1:1.

*4″-*O*-β-*D*-Glucosyl-6′-*O*-(4-*O*-β-*D*-glucosylcaffeoyl)linearoside* (= *(1*S*,4a*S*,6*S*,7*R*,7a*S*)-1-{{6-*O*-{(2*E*)-3-[4-(β-*D*-Glucopyranosyloxy)-3-hydroxyphenyl]-1-oxoprop-2-en-1-yl}-β-*D*-glucopyranosyl}oxy}-6-{{(2*E*)-3-[4-(β-*D*-glucopyranosyloxy)phenyl]-1-oxoprop-2-en-1-yl}oxy}-1,4a,5,6,7,7a-hexahydro-7-methylcyclopenta[*c*]pyran-4-carboxylic acid*; **1**): White amorphous powder. $[\alpha]_D^{25} = -67.0$ ($c = 0.12$, MeOH). UV (MeOH): 289 (4.60), 204 (4.59), 192 (4.35). IR (KBr): 3404, 1693, 1637, 1605, 1509, 1074, 621. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 1007.3023 ($[M-H]^-$, $C_{46}H_{55}O_{25}^-$; calc. 1007.3032).

*6′-*O*-Acetylsweroside* (= *(4a*S*,5*R*,6*S*)-6-[(6-*O*-Acetyl-β-*D*-glucopyranoysl)oxy]-5-ethenyl-4,4a,5,6-tetrahydro-1*H*,3*H*-pyrano[3,4-*c*]pyran-1-one*; **2**): White amorphous powder. $[\alpha]_D^{25} = -225.5$ ($c = 0.11$, MeOH). UV (MeOH): 241 (4.06), 203 (4.00). IR (KBr): 3413, 1739, 1693, 1617, 1268, 1075. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 423.1270 ($[M+Na]^+$, $C_{18}H_{24}O_{10}Na^+$; calc. 423.1267).

*6′-*O*-Acetyl-3′-*O*-[3-(β-*D*-glucopyranosyloxy)-2-hydroxybenzoyl]sweroside* (= *(4a*S*,5*R*,6*S*)-6-{{6-*O*-Acetyl-3-*O*-[3-(β-*D*-glucopyranosyloxy)-2-hydroxybenzoyl]-β-*D*-glucopyranosyl}oxy}-5-ethenyl-4,4a,5,6-tetrahydro-1*H*,3*H*-pyrano[3,4-*c*]pyran-1-one*; **3**): White amorphous powder. $[\alpha]_D^{25} = -142.0$ ($c = 0.10$, MeOH). UV (MeOH): 245 (4.23), 208 (4.58). IR (KBr): 3421, 1684, 1617, 1251, 1073, 592. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 721.1976 ($[M+Na]^+$, $C_{31}H_{38}O_{18}Na^+$; calc. 721.1956).

Acidic Hydrolysis of Compounds **1**–**3**. A soln. of **1**, **2** or **3** (each 2–3 mg) in 3M aq. CF_3COOH (3 ml) was heated for 2 h at 120°. Then, the mixture was cooled and concentrated. To this hydrolyzate were added the following solns.: (2*S*)-1-aminopropan-2-ol/anh. MeOH 1:8 (20 µl), AcOH/anh. MeOH 1:4 (17 µl), and 3% $NaBH_3CN$ in anh. MeOH (17 µl). The mixture was allowed to react for 2 h at 65°. After cooling, 3M aq. CF_3COOH was added dropwise until the pH dropped to 1–2. Then, the mixture was evaporated, and the residue was dried overnight in a desiccator. Pyridine/Ac_2O 1:1 (0.5 ml) was added, and the mixture was allowed to react at 100° for 1 h. After addition of 1 ml of $CHCl_3$, the mixture was washed three times with sat. Na_2CO_3 soln. and H_2O successively. The org. phase was dried (Na_2SO_4) and subjected to GC/MS (*Thermo TR-5 MS*, 60 m × 0.25 mm × 2.5 µm); carrier gas He, flow rate 1 ml/min; oven temp.: 180 → 220° (4°/min), 220° for 2 min, 220 → 270° (1°/min), and 270° for 1 min). By comparison with the retention times of authentic samples (t_R (D-glucose) 54.88 min, and t_R (L-glucose) 55.08 min), the configuration of the sugar moieties were determined to be D.

REFERENCES

[1] 'Pharmacopoeia of P. R. China', Editorial Board of Chinese Pharmacopoeia, Chinese Medical Science and Technology Press, Beijing, 2010, Vol. 1, p. 89.
[2] B. Dinda, D. R. Chowdhury, B. C. Mohanta, *Chem. Pharm. Bull.* **2009**, *57*, 765.
[3] R. Kakuda, T. Iijima, Y. Yaoita, K. Machida, M. Kikuchi, *J. Nat. Prod.* **2001**, *64*, 1574.
[4] M. Kikuchi, R. Kakuda, M. Kikuchi, Y. Yaoita, *J. Nat. Prod.* **2005**, *68*, 751.
[5] R.-W. Jiang, K.-L. Wong, Y.-M. Chan, H.-X. Xu, P. P.-H. But, P.-C. Shaw, *Phytochemistry* **2005**, *66*, 2674.
[6] G. Chen, S.-H. Wei, C.-Y. Yu, *Biochem. Syst. Ecol.* **2009**, *37*, 766.
[7] R.-X. Tan, J.-L. Wolfender, L. X. Zhang, W. G. Ma, N. Fuzzati, A. Marston, K. Hostettmann, *Phytochemistry* **1996**, *42*, 1305.
[8] J.-A. Kim, N. S. Son, J. K. Son, Y. Jahng, H. W. Chang, T. S. Jang, M. K. Na, S.-H. Lee, *Arch. Pharm. Res.* **2009**, *32*, 863.
[9] C. Bergeron, A. Marston, R. Gauthier, K. Hostettmann, *Phytochemistry* **1997**, *44*, 633.

[10] Y. Sashida, K. Ogawa, T. Yamanouchi, H. Tanaka, Y. Shoyama, I. Nishioka, *Phytochemistry* **1994**, *35*, 377.
[11] R. Kakuda, T. Iijima, Y. Yaoita, K. Machida, M. Kikuchi, *Phytochemistry* **2002**, *59*, 791.
[12] A. Hisham, G. J. Kumar, Y. Fujimoto, N. Hara, *Phytochemistry* **1995**, *40*, 1227.
[13] D. Burns, W. F. Reynolds, G. Buchanan, P. B. Reese, R. G. Enriquez, *Magn. Reson. Chem.* **2000**, *38*, 488.
[14] M.-I. Chung, C.-N. Lin, *J. Nat. Prod.* **1993**, *56*, 982.
[15] M. Xu, M. Zhang, Y.-J. Zhang, C.-R. Yang, *Helv. Chim. Acta* **2009**, *92*, 321.
[16] M. Xu, D. Wang, Y.-J. Zhang, C.-R. Yang, *J. Nat. Prod.* **2007**, *70*, 880.

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