Two New Iridoid Glycosides from the Tibetan Folk Medicine *Swertia franchetiana*

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Two new iridoid glycosides designated as senburiside III (2) and senburiside IV (3), together with one known iridoid glycoside senburiside I (1) and three known secoiridoid glucosides swertiamarin (4), gentiopicroside (5) and sweroside (6), were isolated from the whole plant of *Swertia franchetiana*. The structures of the two new compounds were elucidated by spectroscopic methods.

Key words *Swertia franchetiana*; Gentianaceae; iridoid glycoside; senburiside

The plant *Swertia franchetiana* H. SMITH (Gentianaceae), distributed in southwestern China, is a traditional Tibetan medicine used as a remedy for hepatitis and cholecystitis. Previous phytochemical investigations using this plant have led to the isolation of various poly-oxygenated xanthones, xanthone glycosides, and flavonoid glucosides.[1—4] In this paper, we report the isolation and structure elucidation of two new iridoid glycosides senburiside III (**2**) and senburiside IV (**3**), from this plant.

The *n*-butanol extract of the whole plant of *S. franchetiana* on repeated column chromatography and preparative HPLC yielded two new compounds, **2** and **3**, in addition to four known compounds, **1**, **4**—**6**, as described in the Experimental. Compound **1** was obtained as a white amorphous powder. Its UV (237 nm) and IR (1703, 1634 cm^{-1}) absorptions were typical of an iridoidic enol ether system conjugated with a carbonyl group. The NMR spectral data of **1** (shown in Tables 1, 2) agreed with those of senburiside I previously isolated from the Japanese folk herb *S. japonica*.[5]

Senburiside III (**2**) was obtained as a white amorphous powder. Its UV and IR absorptions were similar to those of senburiside I. The negative atmospheric pressure chemical ionization mass spectrum (APCI-MS) gave a quasimolecular ion at *m*/*z* 983 $[M-H]^-$, corresponding to the molecular formula $C_{47}H_{52}O_{23}$, supported by elemental analysis (Experimental). In the ^{1}H-NMR spectrum of **2**, the signals at δ 7.52 (1H, s) and δ 5.68 (1H, br s) assigned to H-3 and H-1 respectively, were typical of an iridoid nucleus; the signals at δ 3.09 (1H, m, H-5), 2.62 (1H, m, H-6), 2.18 (3H, m, H-9, H-8, H-6), and 1.41 (3H, d, J=6.4 Hz, H-10) were almost identical with those of senburiside I, indicating that **1** and **2** bear similar iridoid nucleus; the proton signals at δ 7.76 (1H, d, J=16.0 Hz), δ 6.50 (1H, d, J=16.0 Hz), δ 7.06 (2H, s) and δ 4.05 (6H, s) were attributed to the *trans*-sinapoyl moiety. The ^{13}C-NMR data of **2** were quite similar to those of **1**, except for the signals arising from an additional glucose moiety and an *m*-substituted benzoyl group. The correlation between H-1‴ (δ 5.23) and C-22 (δ 159.5) in the heteronuclear multiple bond correlation (HMBC) spectrum indicated the linkage of the glucose unit to *m*-hydroxyl of the benzoyl group. It was observed that the signals of C-15 in **2** were shifted upfield to δ 152.7 when compared with compound **1**, in which it resonated at δ 158.9. This indicated that the *m*-hydroxybenzoyl moiety at the C-7 position was benzoylated in **2**. The correlation in HMBC between the carbonyl signal of the sinapoyl moiety at δ 168.3 (C-9″) and the proton signal at δ 5.00 which was assigned to H-2′ according to its correlations with H-1′ (δ 5.07) in ^{1}H–^{1}H correlated spectroscopy (COSY) and with C-1′ (δ 98.3) in HMBC, proved the attachment of the sinapoyl moiety to the C-2′ position of the glucose. These linkage positions were also confirmed by fragmental ion peaks in the APCI-MS spectrum of **2** (at *m*/*z* 495, 419, 299, 289, 223 and *m*/*z* 369 in negative and positive mode, respectively), shown in Fig. 3. The relative stereochemical configuration of **2** was determined by comparison of spectral data with those in the literature.[6,7] Based on the above mentioned analysis, the structure of senburiside III was determined as shown in **2** (Fig. 1).

Senburiside IV (**3**) was obtained as a white amorphous powder. Its molecular formula $C_{36}H_{42}O_{16}$ was deduced from its (−)APCI-MS spectrum (the quasimolecular ion peak at

Table 1. ^{13}C-NMR (100 MHz, CD_3OD) Spectral Data[a)] of Compounds **1**—**3**

C	1	2	3	C	1	2	3
1	96.0 d	95.8 d	96.5 d	25		125.4 d	125.4 d
3	151.3 d	151.5 d	152.1 d	1′	98.4 d	98.3 d	100.4 d
4	113.9 s	113.6 s	113.6 s	2′	75.0 d	75.0 d	75.0 d
5	32.5 d	32.7 d	33.0 d	3′	76.2 d	76.2 d	78.2 d
6	38.0 t	37.8 t	38.5 t	4′	71.9 d	71.9 d	71.8 d
7	83.6 d	84.1 d	84.0 d	5′	78.8 d	78.8 d	78.6 d
8	42.9 d	43.1 d	43.3 d	6′	62.9 t	62.9 t	63.0 t
9	49.0 d[b)]	49.0 d[b)]	49.0 d[b)]	1″	127.2 s	127.1 s	102.7 d
10	18.1 q	18.3 q	18.6 q	2″	107.2 d	107.2 d	75.2 d
11	170.8 s	170.5 s	167.2 s	3″	149.6 s	149.6 s	78.2 d
12	168.1 s	167.1 s	166.4 s	4″	139.7 s	139.6 s	71.6 d
13	133.0 s	132.0 s	132.1 s	5″	149.6 s	149.6 s	78.6 d
14	117.2 d	124.1 d	124.2 d	6″	107.2 d	107.2 d	62.8 t
15	158.9 s	152.7 s	152.7 s	7″	147.6 d	147.6 d	
16	121.5 d	127.9 d	128.0 d	8″	116.0 d	116.0 d	
17	130.8 d	131.0 d	131.1 d	9″	168.3 s	168.3 s	
18	121.9 d	127.9 d	128.4 d	OCH_3 (×2)	57.1 q	57.1 q	
19		166.3 s	166.4 s	1‴		102.6 d	
20		133.4 s	133.5 s	2‴		75.2 d	
21		119.5 d	119.6 d	3‴		78.2 d	
22		159.5 s	159.6 s	4‴		71.6 d	
23		123.7 d	123.8 d	5‴		78.5 d	
24		131.3 d	131.3 d	6‴		62.7 t	

a) Assignments were confirmed by HMQC and HMBC experiments. *b*) Overlapped with the methanol-d_4 carbon signals.

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Table 2. ^{1}H-NMR (400 MHz, CD_3OD) Spectral Data[a,b] of Compounds **1**—**3**

H	1	2	3	H	1	2	3
1	5.68 d (1.9)	5.68 br s	5.69 d (3.6)	3′	3.84 m[c]	3.83 m[c]	3.50
3	7.53 s	7.52 s	7.62 s	4′	3.61 m[c]	3.60 m[c]	|
5	3.09 m	3.09 m	3.12 m	5′	3.61 m[c]	3.60 m[c]	|
6	2.66 m	2.62 m	2.75 m	6′	4.12 m	4.10 m[c]	
	2.18 m[c]	2.18 m[c]	2.19 m[c]		3.91 m[c]	3.92 m[c]	4.11
7	5.07 m	5.03 m	5.15 m	1″			5.20 d (7.2)
8	2.18 m[c]	2.18 m[c]	2.19 m[c]	2″	7.08 s	7.06 s	3.50
9	2.18 m[c]	2.18 m[c]	2.19 m[c]	3″			|
10	1.40 d (6.3)	1.41 d (6.4)	1.43 d (6.8)	4″			|
14	7.53 s	7.96 s	8.02 s	5″			
16	7.16 d (8.0)	7.66 d (7.6)	7.73 d (8.0)	6″	7.08 s	7.06 s	4.11
17	7.41 dd (8.0, 8.0)	7.69 dd (7.6, 7.6)	7.76 dd (7.6, 8.0)	7″	7.78 d (16.0)	7.76 d (16.0)	
18	7.58 d (8.0)	8.03 d (7.6)	8.11 d (7.6)	8″	6.51 d (16.0)	6.50 d (16.0)	
21		8.08 s	8.10 s	OMe×2	4.08 s	4.05 s	
23		7.62 d (7.6)	7.65 d (8.0)	1‴		5.23 d (6.8)	
24		7.63 dd (7.6, 7.6)	7.69 dd (7.6, 8.0)	2‴		3.70 m[c]	
25		8.06 d (7.6)	8.05 d (7.6)	3‴		3.70 m[c]	
1′	5.10 m[c]	5.07 m[c]	4.87 d (8.0)	4‴		3.67 m[c]	
2′	5.03 m[c]	5.00 m[c]	3.38 t (8.0)	5‴		3.60 m[c]	
				6‴		4.10 m[c]	
						3.92 m[c]	

a) Assignments were confirmed by ^{1}H–^{1}H COSY, HMQC and HMBC experiments. *b*) Chemical shifts in ppm, *J* values in parentheses were recorded in Hz. *c*) Overlapped signals.

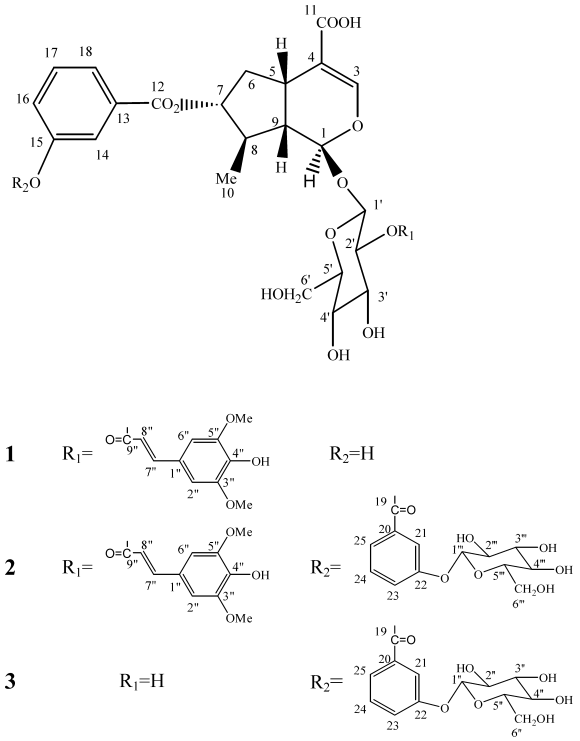

Fig. 1. The Structures of Compounds **1**—**3**

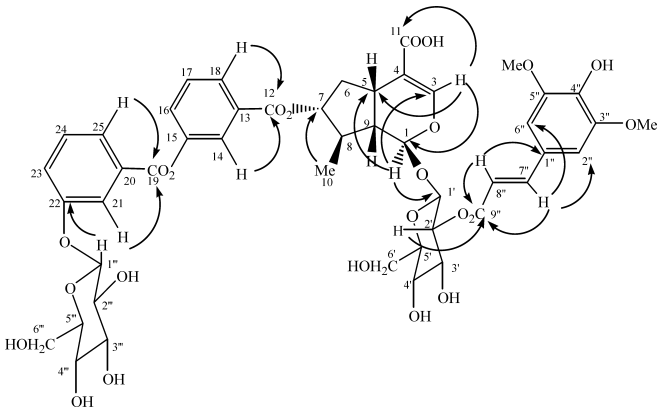

Fig. 2. Selected HMBC Correlations of Compound **2**

m/*z* 777 $[M-H]^-$) and elemental analysis (Experimental). Its UV (234 nm) and IR (1715, 1645 cm^{-1}) absorptions were similar to those of **1** and **2**. In the ^{1}H-NMR spectrum of **3**, the signals at δ 5.69 (1H, d, J=3.6 Hz, H-1) and δ 7.62 (1H, s, H-3) were typical of an iridoid nucleus. The ^{1}H- and ^{13}C-NMR data of **3** were quite similar to those of **2** except for the lack of a *trans*-sinapoyl group. The chemical shifts of C-1′, C-2′ and C-3′ of compound **3** at δ 100.4, 75.0, and 78.2 respectively, were different from those of **2**, in which they were at δ 98.3, 75.0, and 76.2 respectively. This indicated that there was no group attached at the C-2′ position in compound **3**. The fragment ion peaks at *m*/*z* 495 and *m*/*z* 289 in (−)APCI-MS and the absence of a base peak at *m*/*z* 369 in (+)APCI-MS also confirmed the structural difference between **3** and **2** (Experimental). Thus, the structure of senburiside IV was determined as shown in **3** (Fig. 1).

The structures of **4**—**6** were identified as swertiamarin, gentiopicroside, and sweroside by comparing their UV, IR, MS, ^{1}H- and ^{13}C-NMR data with those in the literature.[8]

Experimental

General Procedures Melting points were obtained by using an XT-4 melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 spectrophotometer. IR spectra were obtained on a Perkin-Elmer 983 G spectrometer. ^{1}H-, ^{13}C-NMR and 2D NMR were recorded on a Bruker DRX400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). APCI-MS were obtained with a Finnigan TSQ MS/MS spectrometer. Elemental analysis data were obtained with Elementar Vario EL III. Analytical HPLC was carried out on Waters 2690 with a Waters 996 PDA detector and Hypersil ODS-2 column (5 μm, 250 mm×4 mm I.D.). Column chromatography was performed on Polyamide (30—60 mesh, Shanghai Chemical Co.), Silica gel (200—300 mesh, Qingdao Marine Chemical Co.) and RP-18 (Varian). Preparative HPLC was carried out on a Waters Delta

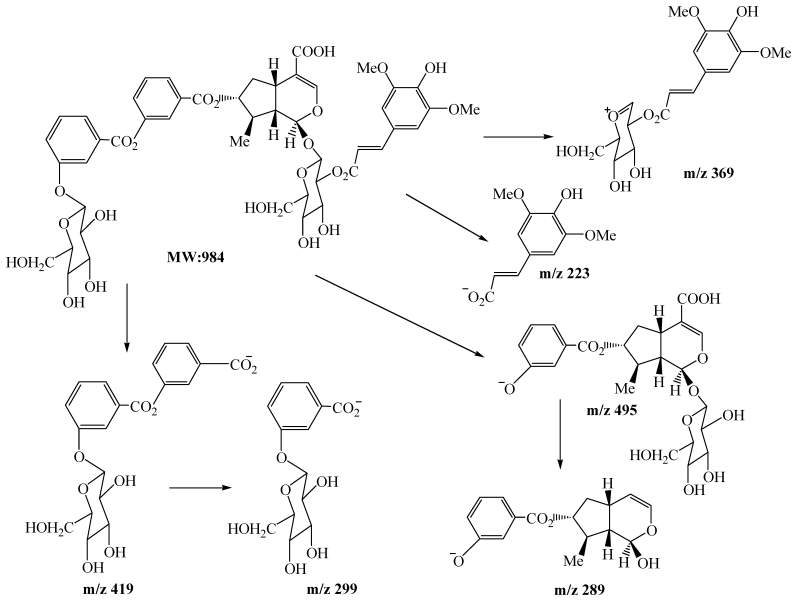

Fig. 3. The Key Fragment Ions in APCI-MS Spectra of Compound **2**

Prep 4000 with a Waters 996 PDA detector and a Superiorex ODS column (5 μm, 250 mm×20 mm I.D., Shiseido).

Plant Material The whole plant of *S. franchetiana* was collected from Qinghai Province, PR China, in September 2001. A voucher specimen is deposited in the Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China.

Extraction and Isolation Air-dried whole plants (12.5 kg) were ground and percolated with MeOH at room temperature to exhaustion. The combined extracts were concentrated *in vacuo* to yield 2.9 kg of residue, which was suspended in water and extracted successively with petroleum ether (60—90 °C), $CHCl_3$, EtOAc and *n*-BuOH. *n*-BuOH extracts (200 g) were subjected to a polyamide column and eluted with water, and then 20%, 40%, 60% and 95% EtOH. The 40% EtOH eluate (25.8 g) from polyamide CC was chromatographed over silica gel, eluted with $CHCl_3$–MeOH gradient (9 : 1—6 : 4) and monitored by HPLC to yield four fractions (I—IV). Fraction III (5.1 g) was subjected to a silica gel CC using $CHCl_3$–EtOH as the solvent system (gradient from 7 : 3—5 : 5) and five subfractions were obtained. The fourth subfraction was subjected to preparative HPLC with 45% MeOH–H_2O as a mobile phase at a flow rate of 35 ml·min^{-1} to furnish compounds **1** (18 mg) and **2** (26 mg). The 20% EtOH eluate (10.5 g) from polyamide CC was subjected to silica gel ($CHCl_3$–EtOH, 9 : 1—1 : 1) and ten fractions were obtained. The tenth fraction (1.0 g) was chromatographed on RP-18 CC using 70—90% MeOH as a solvent system to yield five fractions. The third and fourth fractions were combined and concentrated to give compound **3** (92 mg). The water eluate (25.0 g) from polyamide CC was subjected to RP-18 CC and eluted with 10%, 20%, 30%, 40% and 50% MeOH. The 30% MeOH eluate (500 mg) was chromatographed on preparative HPLC with a 10—35% MeOH gradient as a solvent system at a flow rate of 35 ml·min^{-1} to give compounds **4** (80 mg), **5** (15 mg) and **6** (45 mg).

Senburside III (**2**): White amorphous powder (MeOH–H_2O); $[\alpha]_D^{25}$ −60.0° (*c*=1.03, MeOH); UV λ_{max} (MeOH) nm: 234, 330; IR (KBr) cm^{-1}: 3420, 2925, 1715, 1635, 1516, 1456, 1262, 1074, 753; ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; (+)-APCI-MS *m*/*z*: 685, 369, 239, 207; (−)-APCI-MS *m*/*z*: 983, 701, 495, 419, 367, 299, 289, 223; *Anal.* Calcd for $C_{47}H_{52}O_{23}$ requires: C, 57.32; H, 5.32. Found: C, 57.28; H, 5.40.

Senburiside IV (**3**): White amorphous powder (MeOH–H_2O); $[\alpha]_D^{25}$ −60.2° (*c*=1.14, MeOH); UV λ_{max} (MeOH) nm: 234, 286; IR (KBr) cm^{-1}: 3419, 2927, 1715, 1645, 1540, 1488, 1263, 1074, 753; ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; (+)-APCI-MS *m*/*z*: 599, 315; (−)-APCI-MS *m*/*z*: 777, 495, 289; *Anal.* Calcd for $C_{36}H_{42}O_{19}$ requires: C, 55.53; H, 5.44. Found: C, 55.25; H, 5.62.

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