Secoiridoid Glycosides from *Swertia mileensis*

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From the MeOH extract of the aerial parts of *Swertia mileensis*, four new secoiridoid glycosides were isolated, 4′-*O*-[(*E*)-caffeoyl]swertiamarin (**1**), 4′-*O*-[(*Z*)-coumaroyl]swertiamarin (**7**), 6′-*O*-[(*E*)-coumaroyl]swertiamarin (**8**), and 6′-*O*-[(*Z*)-coumaroyl]swertiamarin (**9**), together with five known compounds. Their structures were elucidated by NMR spectroscopy and tandem mass spectrometry. Detailed HPLC/MS analyses and MS/MS fragmentation pathways are discussed for the identification of the swertiamarin-derived (*E*)/(*Z*) isomers **6**/**7** and **8**/**9**.

Introduction. – *Swertia mileensis* T. N. HE et SHI, traditionally known as '*Qing-Ye-Dan*', is a species of *Swertia*, belonging to the family Gentianaceae. This plant has long been used as a folk medicine in Yunnan Province, China, to treat virus-induced hepatitis [1–3]. Previous phytochemical investigations of this plant revealed the presence of secoiridoid glucosides and acyl secoridoid glucosides [3–5], of which swertiamarin and sweroside, which display antihepatotoxic and antitumor activities, are the main components [6]. Other compounds identified in this plant include xanthones, triterpenoids, and monoterpenoid glycosides [7–9].

The continuous investigation and careful examination of *S. mileensis* led to the isolation of nine compounds, **1**–**9**, including four new secoiridoid glycosides, **1** and **7**–**9**, and of five known compounds, **2**–**6**. This paper deals with the isolation and structure elucidation of the new compounds, and the application of HPLC-UV-MS methods for the discrimination of swertiamarins with (*E*)- *vs.* (*Z*)-configured coumaroyl substituents on their glycones (compounds **6**–**9**).

Results and Discussion. – The MeOH extract from the aerial parts of *S. mileensis* was investigated by liquid chromatography with photodiode-array and electrospray-ionization-mass-spectrometric detection (HPLC/PDA/ESI-MS). A total of nine well-separated signals were observed, and the corresponding compounds isolated (*Table 1*). Comparison of the HPLC profiles of the pure secoiridoid glycosides with the original crude MeOH extract showed that all compounds are naturally occurring.

Compound **1** was obtained as a colorless, amorphous powder. Its molecular formula was determined as $C_{25}H_{28}O_{13}$ by HR-FAB-MS (*m/z* 535.1528 ($[M-H]^-$; calc. 535.1452)). Its IR spectrum indicated the presence of OH groups (3107, 3474 cm^{-1}) and of a conjugated C=O group (1697, 1613 cm^{-1}). The structure of **1** was established by analysis of ^1H- and ^{13}C-NMR (see *Tables 2* and *3* in the *Exper. Part*, resp.), DEPT, HSQC, HMBC, and tandem-MS experiments.

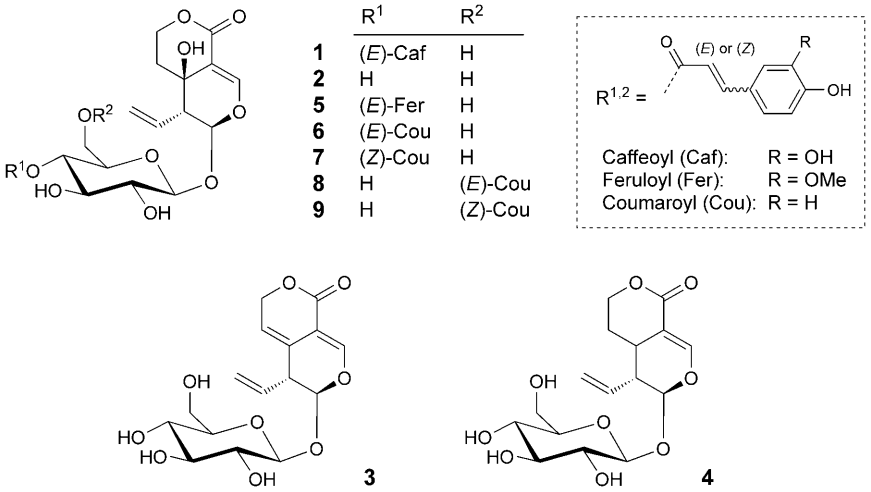

Table 1. *HPLC Retention Times, UV Data, and Selected MS Characteristics of Compounds* **1**–**9** *in the Extract of* S. mileensis

Compound	t_R [min]	λ_{max} [nm] (log ε)	MS Data [m/z]		
			$[M+Na]^+$	MS/MS	MS/MS/MS
1	12.6	233 (5.10)	559	417	347
2	15.0	236 (3.92)	397	255	
3	16.5	242, 274 (4.22, 4.06)	379	217, 199	199, 172
4	17.3	244 (3.29)	381	219, 149	166, 149
5	23.3	235, 271 (5.15, 4.87)	573	431, 361	361, 167
6	25.3	314 (5.30)	543	401	331
7	27.4	311 (4.49)	543	401	331
8	25.8	312 (5.23)	543	401	331
9	26.1	308 (4.57)	543	401	331

The ^{1}H-NMR spectrum of **1** showed the presence of a *trans*-caffeoyl[1]) moiety, which was evident from three aromatic H-atoms forming an *ABX* system [$\delta(A)$ 7.02, $\delta(B)$ 7.01, $\delta(X)$ 6.76; $J(AB)=J(AX)\approx 0$, $J(BX)=8.2$ Hz], and two H-atoms of an (*E*)-configured C=C bond at δ(H) 6.25, 7.48 (2*d*, $J=15.9$ Hz each). Furthermore, the signals at δ(H) 7.52 (*s*, 1 H), 5.38 (*ddd*, $J=16.1$, 9.0, 7.8 Hz, 1 H), 5.25 (*ddd*, $J=9.0$, 6.2, 2.5 Hz, 1 H), and 5.31 (*dd*, $J=16.0$, 6.2 Hz, 1 H), were assigned to H–C(3), H–C(8), and CH_2(10), respectively, of the presumed secoiridoid skeleton. In addition, an anomeric H-atom was observed at δ(H) 4.60 (*d*, $J=8.1$ Hz), which combined with the ^{13}C-NMR signals at δ(C) 98.2, 75.1, 73.3, 73.0, 71.0, and 60.6, typical for a β-glucopyranosyl moiety. This was further corroborated by TLC comparison of the hydrolysis product of **1** with an authentic glucose sample.

In the HMBC spectrum of **1** (*Fig. 1*), the anomeric H–C(1′) at δ(H) 4.60 showed a correlation with C(1) at δ(C) 96.6; and a correlation of H–C(4′) at δ(H) 4.66 with C=O at δ(C) 165.8 was also observed. This pointed to a 4′-*O*-(*E*)-caffeoyl-swertiamarin, as further confirmed by tandem-mass-spectrometric

[1]) Caffeoyl = (*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl.

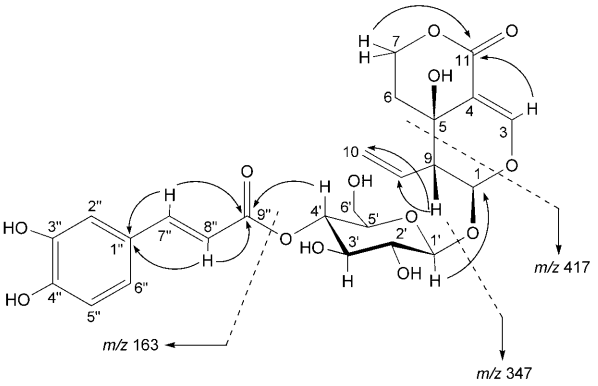

Fig. 1. *Key HMBC correlations and MS fragmentation for* **1**

analysis. The ESI-MS spectrum of **1** showed the pseudomolecular ion at *m/z* 559 ($[M+Na]^+$), and tandem MS showed fragment ions at *m/z* 417 ($[M+Na-142]^+$) and 347 ($[M+Na-212]^+$), corresponding to a *retro-Diels–Alder* cleavage within the aglycone and to the loss of the aglycone unit, respectively (*Fig. 1*).

Compounds **6** and **7** exhibited interesting spectroscopic features due to stereoisomerism. In HPLC-UV/MS experiments (*Fig. 2,a*), the UV spectroscopic characteristics of compounds **6** ($\lambda_{max}=314$ nm ($\log\varepsilon=5.30$)) and **7** ($\lambda_{max}=311$ nm ($\log\varepsilon=4.49$)) could reasonably be attributed to (*E*)- and (*Z*)-configured *p*-coumaroyl[2]) units [10]. Furthermore, both **6** and **7** showed intense $[M+Na]^+$ and $[M-H]^-$ ion peaks in positive and negative-mode mass spectra, respectively, in accord with identical molecular weights of 520 g/mol. The observed frament ion at *m/z* 147 is characteristic of a *p*-coumaroyl group [10].

Compound **6** could be obtained in pure form, but **7** rapidly isomerized to a mixture of the two isomers. By comparison with published spectroscopic data, **6** was identified as 4′-*O*-[(*E*)-coumaroyl]swertiamarin, which has been isolated before [3]. The chemical shifts corresponding to the coumaroyl moieties of **6** and **7** at δ(H) 5–8 ppm indicated structural differences between the two compounds. The olefinic H-atoms of **6** at δ(H) 6.39 and 7.51 (2*d*, $J=16$ Hz each, 2×1 H) were shifted upfield to δ(H) 5.76 and 6.92 (2*d*, $J=12$ Hz each) in the case of **7**. Also, the four aromatic H-atoms of **7** at δ(H) 6.78 and 7.68 (2*d*, $J=8.4$ Hz each, 2×2 H) established the presence of a (*Z*)-coumaroyl moiety [11]. Subsequently, a detailed 2D-NMR analysis was performed that corroborated the proposed structure of **7**. Briefly, ^{1}H- and ^{13}C-NMR assignments were confirmed by long-range C,H correlations (*Fig. 3,a*). The positions of glycosidation were obvious from the cross-peaks in the HMBC spectrum, which showed interactions of H–C(1′) at δ(H) 4.58 with C(1) at δ(C) 96.6, and of H–C(4′) at δ(H) 4.61 with C(9″) at δ(C)165.9. Thus, the structure of **7** was elucidated as 4′-*O*-[(Z)-coumaroyl]swertiamarin. Finally, tandem mass spectrometry was used to elucidate the structures of **6** and **7**. In MS/MS experiments with the precursor ion at *m/z* 543 ($[M+Na]^+$), an intense ion at *m/z* 401 was observed due to *retro-Diels–Alder* cleavage in the secoiridoid aglycone. In the MS/MS/MS spectrum, the fragment ion at *m/z* 401 produced a further ion at *m/z* 331 (see *Table 1* and *Fig. 3,a*).

Compounds **8** and **9**, eluting at 10.62 and 11.26 min, respectively, showed UV maxima at 312 ($\log\varepsilon=5.23$) and 308 nm (4.47) (*Fig. 2,b*). Both compounds showed peaks at

[2]) Coumaroyl = (*E*)-3-(4-hydroxyphenyl)prop-2-enoyl.

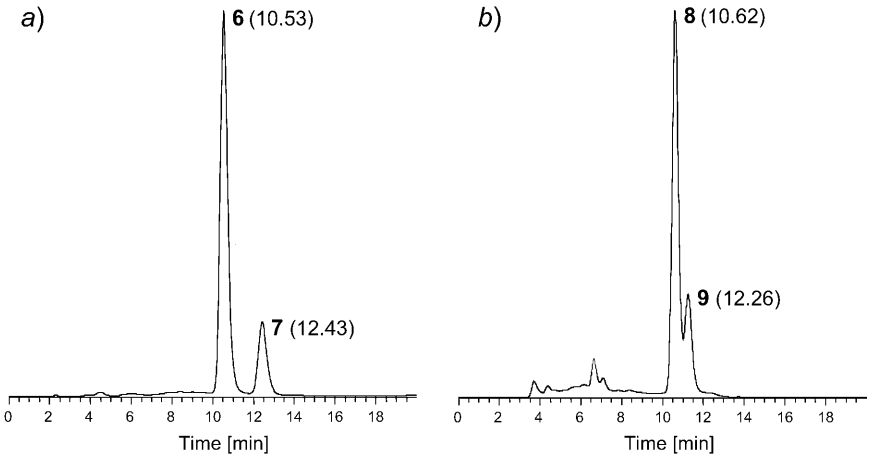

Fig. 2. *HPLC Profiles of the mixtures of* **6**/**7** (a) *and* **8**/**9** (b). For details, see *Exper. Part.*

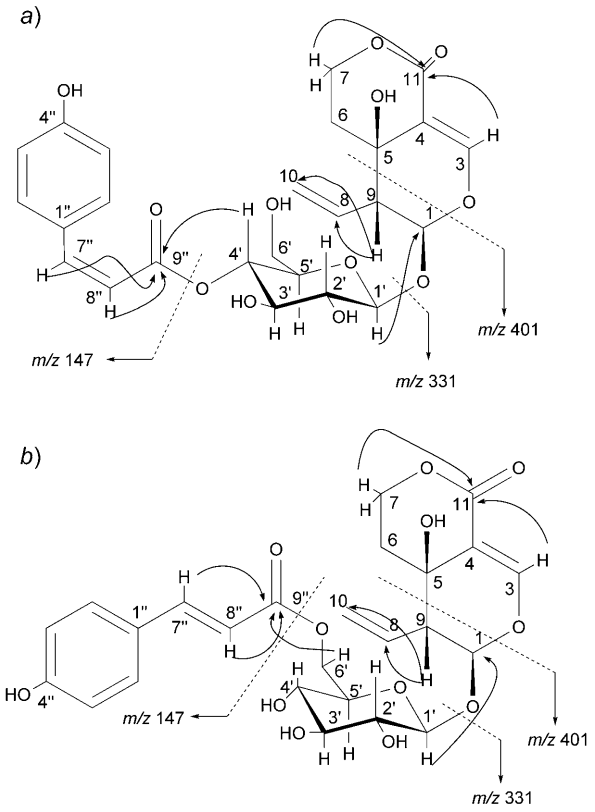

Fig. 3. *Key HMBC correlations and MS fragmentations for* **6** and **7**. Similar data were obtained for the congeners **8** and **9**, resp.

m/z 543 ($[M+Na]^+$) and at 519 ($[M-H]^-$) in positive- and negative-mode mass spectra, consistent with identical molecular weights of 520 g/mol. Their tandem mass spectra of the $[M+Na]^+$ ions showed a fragmentation pattern very similar to those of **6** and **7**. Specifically, a *retro-Diels–Alder* cleavage in the secoiridoid aglycone gave rise to ions at *m/z* 401 in MS/MS spectra, and cleavage of the glucosidic bond formed the ion at *m/z* 331 in MS/MS/MS spectra. According to its ^{1}H- and ^{13}C-NMR spectra, **8** possessed a swertiamarin aglycone and an (*E*)-coumaroyl moiety, and the only difference between **8** and **6** laid in the glucosidic part. In the ^{13}C-NMR spectrum of **8**, C(6′) of the β-glucopyranosyl (Glc) residue was shifted downfield to δ(C) 63.2 ($\Delta\delta = +2.6$ ppm), which suggested that the Glc moiety was esterified at C(6′) [12]. Additionally, in the HMBC spectrum, a correlation of H–C(6′) at δ(H) 4.37 with C(9″) at δ(C) 165.9 was observed. Hence, the coumaroyl moiety was attached to C(6′) of Glc, and compound **8** was identified as 6′-*O*-[(*E*)-coumaroyl]swertiamarin, with **9** being the corresponding (*Z*)-isomer.

By comparison with authentic samples, the remaining isolates were identified as swertiamarin (**2**) [13], gentiopicroside (**3**) [14], sweroside (**4**) [13], and angustiamarin (**5**) [13][15]. The ^{1}H- and ^{13}C-NMR spectroscopic data of the new compounds **1** and **7**–**9**, as well as those of **6**, are collected in *Tables 1* and *2*, respectively, in the *Exper. Part*.

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Experimental Part

General. Column chromatography (CC): Silica gel (200–300 mesh; *Qingdao Marine Chemical Group, Co.*), *Lobar LiChroprep RP-18* (40–63 μm; *Merck*), *Lobar LiChroprep Si-60* (40–63 μm; *Merck*), or *Sephadex LH-20* (*Pharmacia*). Optical rotation: *Perkin-Elmer 341* polarimeter. IR Spectra: *Perkin-Elmer Spectrum One* FT-IR spectrometer; in cm^{-1}. NMR Spectra: *Bruker AM-400* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-FAB-MS: *VG AutoSpec-3000*. HPLC/ESI-MS and tandem MS: *Finnigan LCQ^{DECA}*; in *m/z*.

Plant Material. The aerial parts of *S. mileensis* were collected in 1999 from Chuxiong, Yunnan Province, China, and identified by Prof. *Zuo-Cheng Zhao*. A voucher specimen was deposited at the Herbarium of the Chengdu Institute of Biology, Chinese Academy of Sciences.

Extraction and Isolation. The dried and powdered aerial parts of *S. mileensis* (9 kg) were extracted with EtOH at r.t. (3×8 d), filtered, and evaporated. The resulting extract (1.6 kg) was suspended in H_2O and re-extracted with $CHCl_3$, AcOEt, and *t*-BuOH in this order. The AcOEt-soluble part (63 g) was subjected to CC (SiO_2; $CHCl_3$/Me_2CO 10:0, 9:1, 8:2, 7:3, 5:5, 0:10): *Fr. 1–10*. *Fr. 3* was subjected to CC (*RP-18*; MeOH/H_2O 6:1, 3:1, 2:1) to afford *Fr. 3.1–3.4*, which were individually purified by CC (*RP-18*) to afford **5** (115 mg), **6** (75 mg), and mixtures of **6** and **7** (60 mg), and **8** and **9** (176 mg). *Fr. 4* was purified by CC (1. *Sephadex LH-20*, MeOH; 2. *Si-60*, AcOEt/MeOH mixtures of increasing polarity, starting at 15:1): *Fr. 4.1–4.7*. *Fr. 4.2* was purified by CC (*RP-18*; MeOH/H_2O 5:1, 2:1, 1:1) to yield **1** (120 mg). *Fr. 4.3* was submitted to CC (*RP-18*) to give **2** (98 mg). *Fr. 4.5* was purified by CC (*RP-18*; MeOH/H_2O 1:3) to provide **3** (160 mg) and **4** (700 mg).

HPLC-UV Analysis. Anal. HPLC was performed with a *TSP* HPLC system (*Thermo Quest*, Tokyo) equipped with an *AS-3000* autosampler, two *P4000* gradient pumps, and a *UV-6000* photodiode-array detector (200–400 nm range; 5-nm bandwidth). Separations were performed on an *Inertsil ODS-3* (250×4.6 mm; 5 μm) C_{18} column, eluting at a flow rate of 1.0 ml/min with MeOH/H_2O gradients: 1. 10–30% MeOH from 0 to 10 min; 2. 30–50% MeOH from 10 to 20 min; 3. 50–100% MeOH from 20 to 30 min; 4. 100% MeOH for 10 min. For purification of **6**/**7** and **8**/**9** by anal. HPLC, MeOH/H_2O 50:50 was used at a flow rate of 1 ml/min, with detection at 236 nm.

HPLC/MS Analysis. The following conditions were used for ESI-MS: capillary temperature, 350°; spray voltage, 4.5 kV; capillary voltage, 5.0 V (const.); full scan at *m/z* 100–1000, with 500-ms collection time, summing three micro scans; N_2 gas flow, setting the divert valve to the mass spectrometer from 2–40 min. MS/MS and MS/MS/MS Spectra were recorded under the same conditions as for HPLC/MS analyses, with optimized relative collision energies of 30 and 40%, resp. The HPLC effluent was split such that *ca.* 250 µl/min of effluent entered the ESI source.

*4′-O-[(*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoyl]swertiamarin* (**1**). Yield: 120 mg. Yellow, amorphous powder. M.p. 144.0–145.5°. UV: see *Table 1*. $[\alpha]_D^{15.8} = -168$ ($c = 0.79$, MeOH). IR (KBr): 3474, 3107, 2921, 1697, 1613. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*, resp. ESI-MS (pos.): 559 ($[M+Na]^+$). ESI-MS (neg.): 535 ($[M-H]^-$). HR-FAB-MS: 535.1528 (($[M-H]^-$, $C_{25}H_{27}O_{13}^-$; calc. 535.1452). Tandem MS: see *Table 1*.

Swertiamarin (**2**) [13]. Yield: 98 mg. Colorless, amorphous powder. M.p. 104–106°. UV: see *Table 1*. ^{1}H-NMR (400 MHz, C_5D_5N): 7.89 (br. *s*, H–C(3)); 5.92 (br. *s*, H–C(1)); 5.28 (*d*, *J* = 8.0, H–C(1′)); 5.03–5.22 (*m*, H–C(8), CH_2(10)); 4.26–4.31, 4.73–4.79 (*m*, CH_2(7)); 2.99 (*d*, *J* = 9.9, H–C(9)); 1.66–1.79 (*m*, CH_2(6)). ^{13}C-NMR (100 MHz, C_5D_5N): 165.0 (C=O); 152.2 C(3); 132.7 (C(8)); 120.5 (C(10)); 109.7 (C(4)); 99.0 (C(1′)); 97.5 (C(1)); 79.2 (C(5′)); 78.4 (C(3′)); 74.6 (C(2′)); 71.3 (C(4′)); 64.5 (C(7)); 64.0 (C(5)); 62.4 (C(6′)); 50.7 (C(9)); 32.8 (C(6)). ESI-MS (pos): 397 ($[M+Na]^+$). Tandem MS: see *Table 1*.

Gentiopicroside (**3**) [14]. Yield: 160 mg. Colorless, amorphous powder. M.p. 99–102°. UV: see *Table 1*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 7.40 (*s*, H–C(3)); 5.67–5.71 (*m*, H–C(8)); 5.60–5.66 (*m*, H–C(6)); 5.58 (*d*, *J* = 3.2, H–C(1)); 5.27 (br. *d*, *J* = 15.7, H_a–C(10)); 5.20 (br. *d*, *J* = 9.0, H_b–C(10)); 5.03–4.95 (*m*, CH_2(7)); 4.49 (*d*, *J* = 8.0, H–C(1′)); 3.10–3.14 (*m*, H–(9)). ^{13}C-NMR (100 MHz, (D_6)DMSO): 172.7 (C(11)); 148.8 (C(3)); 134.0 (C(8)); 125.0 (C(5)); 117.9 (C(10)); 116.1 (C(6)); 103.3 (C(4)); 98.8 (C(1′)); 96.5 (C(1)); 77.3 (C(5′)); 76.6 (C(3′)); 72.8 (C(2′)); 70.0 (C(4′)); 69.1 (C(7)); 61.1 (C(6′)); 44.4 (C(9)). ESI-MS (pos.): 379 ($[M+Na]^+$). ESI-MS (neg.): 355 ($[M-H]^-$). Tandem MS: see *Table 1*.

Sweroside (**4**) [13]. Yield: 700 mg. Colorless, amorphous powder. M.p. 98–100°. UV: see *Table 1*. ^{1}H-NMR (400 MHz, C_5D_5N): 7.90 (*s*, H–C(3); 5.78 (br. *s*, H–C(1)); 5.29–5.33 (*m*, H–C(8)); 5.29 (*d*, *J* = 8.0, H–C(1′)); 5.07 (br. *d*, *J* = 15, H_a–C(10)); 5.01 (br. *d*, *J* = 9.0, H_b–C(10)); 4.74–4.77, 4.25–4.29 (2*m*, CH_2(7)); 2.97–2.30 (*m*, H–C(5)); 2.63 (*d*, *J* = 9.0, H–C(9)); 1.65–1.78 (*m*, CH_2(6)). ^{13}C-NMR (100 MHz, C_5D_5N): 165.1 (C(11)); 152.5 (C(3)); 132.5 (C(8)); 120.0 (C(10)); 105.2 (C(4)); 100.5 (C(1′)); 97.3 (C(1)); 78.9 (C(5′)); 78.4 (C(3′)); 74.9 (C(2′)); 71.4 (C(4′)); 67.8 (C(7)); 62.5 (C(6′)); 42.9 (C(9)); 27.7 (C(5)); 25.0 (C(6)). ESI-MS (pos.): 381 ($[M+Na]^+$). ESI-MS (neg.): 357 ($[M-H]^-$). Tandem MS: see *Table 1*.

Angustiamarin (**5**) [13][15]. Yield: 115 mg. Yellow, amorphous powder. M.p. 115–119°. UV: see *Table 1*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 7.54 (*d*, *J* = 16.0, H–C(7″)); 7.51 (*s*, H–C(3)); 7.33 (*d*, *J* = 2.4, H–C(2″)); 7.01 (*dd*, *J* = 8.0, 2.4, H–C(6′)); 6.78 (*d*, *J* = 8.0, H–C(5″)); 6.28 (*d*, *J* = 16.0, H–C(8″)); 5.60 (br. *s*, H–C(1)); 5.36–5.40 (*m*, H–C(8)); 5.29–5.32 (*m*, H_a–C(10)); 5.18–5.21 (*m*, H_b–C(10)); 4.57–4.62, 4.26–4.30 (2*m*, CH_2(7)); 4.58 (*d*, *J* = 8.0, H–C(1′)); 3.81 (*s*, MeO); 2.88 (*d*, *J* = 0.7, H–C(9)); 1.65–1.74 (*m*, CH_2(6)). ^{13}C-NMR (100 MHz, (D_6)DMSO): 165.9 (C(9″)); 164.4 (C(11)); 151.9 (C(3)); 149.4 (C(3″)); 147.9 (C(4″)); 145.4 (C(7″)); 132.8 (C(8)); 125.5 (C(1″)); 123.3 (C(6″)); 120.4 (C(10)); 115.5 (C(5″)); 114.3 (C(8″)); 111.1 (C(2″)); 108.2 (C(4)); 98.2 (C(1′)); 96.6 (C(1)); 75.1(C(5′)); 73.4 (C(3′)); 73.0 (C(2′)); 71.0 (C(4′)); 64.1 (C(7)); 62.4 (C(5)); 60.8 (C(6′)); 55.7 (MeO); 49.9 (C(9)); 32.1 (C(6)). ESI-MS: 573 ($[M+Na]^+$); 549 ($[M-H]^-$). Tandem MS: see *Table 1*.

*4′-O-[(*E*)-3-(4-Hydroxyphenyl)prop-2-enoyl]swertiamarin* (**6**) [3]. Yield: 75 mg. Colorless, amorphous powder. M.p. 118–122°. UV: see *Table 1*. $[\alpha]_D^{25} = -102$ ($c = 0.24$, MeOH). IR (KBr): 3600–3100, 1720, 1700, 1620, 1515. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*, resp. ESI-MS (pos.): 543 ($[M+Na]^+$). ESI-MS (neg.): 519 ($[M-H]^-$). Tandem MS: see *Table 1*.

*4′-O-[(*Z*)-3-(4-Hydroxyphenyl)prop-2-enoyl]swertiamarin* (**7**). Yield: 60 mg. Colorless, amorphous powder (mixture with **6**; see text). M.p. 114–122°. UV: see *Table 1*. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*, resp. ESI-MS (pos.): 543 ($[M+Na]^+$). ESI-MS (neg.): 519 ($[M-H]^-$). Tandem MS: see *Table 1*.

*6′-O-[(*E*)-3-(4-Hydroxyphenyl)prop-2-enoyl]-* (**8**) *and 6′-O-[(*Z*)-3-(4-Hydroxyphenyl)prop-2-enoyl]swertiamarin* (**9**). Colorless, amorphous powder. Yield: 176 mg. M.p. 126–131°. UV: see *Table 1*.

Table 2. *^{1}H-NMR Data of Compounds* **1** *and* **6**–**9**. At 400 MHz in (D_6)DMSO; δ in ppm, J in Hz. Arbitrary atom numbering.

	1	**6**	**7**	**8**	**9**
H–C(1)	5.60 (*d*, *J* = 1.5 H)	5.59 (*d*, *J* = 1.5)	5.59 (*d*, *J* = 1.5)	5.38 (*d*, *J* = 1.5)	5.38 (*d*, *J* = 1.5)
H–C(3)	7.52 (*s*)	7.50 (*s*)	7.50 (*s*)	7.50 (*s*)	7.50 (*s*)
CH_2(6)	1.67 (*ddd*, *J* = 14.0, 3.0, 1.5), 1.72 (*ddd*, *J* = 14.0, 12.7, 5.3)	1.66 (*ddd*, *J* = 14.0, 3.2, 1.5), 1.73 (*ddd*, *J* = 14.0, 13.1, 5.1)	1.66 (*ddd*, *J* = 14.0, 3.2, 1.5), 1.73 (*ddd*, *J* = 14.0, 13.1, 5.1)	1.66 (*ddd*, *J* = 13.9, 3.0, 1.3), 1.71 (*ddd*, *J* = 13.9, 12.8, 5.3)	1.66 (*ddd*, *J* = 13.9, 3.0, 1.3), 1.71 (*ddd*, *J* = 13.9, 12.8, 5.3)
CH_2(7)	4.28 (*ddd*, *J* = 11.0, 5.0, 1.4), 4.63 (*ddd*, *J* = 12.5, 11, 3.0)	4.27 (*ddd*, *J* = 11.0, 5.2, 1.5), 4.58 (*ddd*, *J* = 12.5, 10.9, 3.3)	4.27 (*ddd*, *J* = 11.0, 5.2, 1.5), 4.58 (*ddd*, *J* = 12.5, 10.9, 3.3)	4.24–4.28 (*m*), 4.58 (*ddd*, *J* = 12.7, 10.6, 3.2)	4.24–4.28 (*m*), 4.58 (*ddd*, *J* = 12.7, 10.6, 3.2)
H–C(8)	5.38 (*ddd*, *J* = 16.1, 9.0, 7.8)	5.40 (*ddd*, *J* = 16.0, 8.2, 7.6)	5.40 (*ddd*, *J* = 16.0, 8.2, 7.6)	5.38 (*ddd*, *J* = 16.0, 7.8, 7.6)	5.38 (*ddd*, *J* = 16.0, 7.8, 7.6)
H–C(9)	2.88 (*ddd*, *J* = 8.0, 1.2, 1.5)	2.86 (*dd*, *J* = 9.3, 1.5)	2.86 (*d*, *J* = 9.3, 1.5)	2.85 (*d*, *J* = 9.0, 1.3)	2.85 (*d*, *J* = 9.0, 1.3)
CH_2(10)	5.25 (*ddd*, *J* = 9.0, 6.2, 2.5), 5.31 (*dd*, *J* = 16.0, 6.2)	5.25 (*ddd*, *J* = 9.5, 6.3, 2.4), 5.31 (*dd*, *J* = 16.1, 6.3)	5.25 (*ddd*, *J* = 9.5, 6.3, 2.4), 5.31 (*dd*, *J* = 16.1, 6.3)	5.12 (*ddd*, *J* = 9.0, 6.1, 2.5), 5.30 (*dd*, *J* = 16.0, 6.1)	5.12 (*ddd*, *J* = 9.0, 6.1, 2.5), 5.30 (*dd*, *J* = 16.0, 6.1)
H–C(1′)	4.60 (*d*, *J* = 8.1)	4.58 (*d*, *J* = 8.0)	4.58 (*d*, *J* = 8.0)	4.56 (*d*, *J* = 8.0)	4.56 (*d*, *J* = 8.0)
H–C(2′)	3.41 (*dd*, *J* = 9.8, 8.1)	3.37 (*dd*, *J* = 9.3, 8.0)	3.37 (*dd*, *J* = 9.3, 8.0)	3.07 (*dd*, *J* = 9.1, 8.0)	3.07 (*dd*, *J* = 9.1, 8.0)
H–C(3′)	3.86 (*dd*, *J* = 9.8, 9.8)	3.83 (*dd*, *J* = 9.3, 9.3)	3.83 (*dd*, *J* = 9.3, 9.3)	3.34 (*t*, *J* = 9.1)	3.34 (*t*, *J* = 9.1)
H–C(4′)	4.66 (*dd*, *J* = 9.5, 9.5)	4.61 (*dd*, *J* = 9.3, 9.3)	4.61 (*dd*, *J* = 9.3, 9.3)	3.19 (*t*, *J* = 9.1)	3.19 (*t*, *J* = 9.1)
H–C(5′)	3.53–3.57 (*m*)	3.55–3.59 (*m*)	3.55–3.59 (*m*)	3.46–3.51 (*m*)	3.46–3.51 (*m*)
CH_2(6′)	3.68 (*dd*, *J* = 12.2, 1.8), 3.55 (*dd*, *J* = 12.2, 5.9)	3.65 (*dd*, *J* = 12.0, 2.0), 3.55 (*dd*, *J* = 12.0, 5.5)	3.65 (*dd*, *J* = 12.0, 2.0), 3.55 (*dd*, *J* = 12.0, 5.5)	4.37 (*dd*, *J* = 11.9, 1.9), 4.22 (*dd*, *J* = 11.9, 5.6)	4.37 (*dd*, *J* = 11.9, 1.9), 4.22 (*dd*, *J* = 11.9, 5.6)
H–C(2″)	7.02 (*s*)	7.50 (*d*, *J* = 8.4)	7.68 (*d*, *J* = 8.4)	7.53 (*d*, *J* = 8.4)	7.65 (*d*, *J* = 8.5)
H–C(3″)		6.78 (*d*, *J* = 8.4)	6.78 (*d*, *J* = 8.4)	6.78 (*d*, *J* = 8.4)	6.78 (*d*, *J* = 8.5)
H–C(5″)	6.76 (*d*, *J* = 8.2)	6.78 (*d*, *J* = 8.4)	6.78 (*d*, *J* = 8.4)	6.78 (*d*, *J* = 8.4)	6.78 (*d*, *J* = 8.5)
H–C(6″)	7.01 (*d*, *J* = 8.2)	7.50 (*d*, *J* = 8.4)	7.68 (*d*, *J* = 8.4)	7.53 (*d*, *J* = 8.4)	7.65 (*d*, *J* = 8.5)
H–C(7″)	7.48 (*d*, *J* = 15.9)	7.51 (*d*, *J* = 16.0)	6.92 (*d*, *J* = 12.0)	7.55 (*d*, *J* = 16.1)	6.88 (*d*, *J* = 13.2)
H–C(8″)	6.25 (*d*, *J* = 15.9)	6.39 (*d*, *J* = 16.0)	5.76 (*d*, *J* = 12.0)	6.40 (*d*, *J* = 16.1)	5.75 (*d*, *J* = 13.2)

Table 3. *^{13}C-NMR Data of Compounds* **1** *and* **6**–**9**. At 100 MHz in (D_6)DMSO; δ in ppm. Arbitrary atom numbering.

Position	**1**	**6**	**7**	**8**	**9**
1	96.6	96.6	96.6	96.9	96.9
3	151.9	151.8	151.8	151.8	151.8
4	108.2	108.2	108.2	108.1	108.1
5	62.5	62.4	62.4	62.4	62.4
6	32.1	32.1	32.1	32.0	32.0
7	64.1	64.1	64.1	64.1	64.1
8	132.8	132.8	132.8	132.8	132.8
9	49.9	49.9	49.9	49.9	49.9
10	120.4	120.4	120.4	120.2	120.2
11	164.4	164.4	164.4	164.3	164.3
1′	98.2	98.2	98.2	98.6	98.6
2′	73.0	73.0	73.0	72.7	72.7
3′	73.3	73.4	73.2	75.7	75.7
4′	71.0	71.0	70.8	69.9	69.9
5′	75.1	75.1	74.9	74.0	74.1
6′	60.6	60.6	60.2	63.2	63.0
1″	125.5	125.0	125.3	125.0	125.4
2″	115.8	130.3	132.7	130.3	132.6
3″	145.5	115.7	114.8	115.7	114.9
4″	148.4	159.8	158.8	159.8	158.8
5″	115.8	115.7	114.8	115.7	114.9
6″	121.3	130.3	132.7	130.3	132.6
7″	145.5	145.1	143.7	144.9	143.6
8″	114.9	114.0	115.1	113.9	115.0
9″	165.8	165.9	165.9	165.9	165.9

IR (KBr): 3590–3100, 2921, 1715, 1700, 1520. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*, resp. ESI-MS (pos.): 543 ($[M+Na]^+$). ESI-MS (neg.): 519 ($[M-H]^-$). Tandem MS: see *Table 1*. HR-FAB-MS: 519.1476 ($[M-H]^-$, $C_{25}H_{28}O_{12}^-$; calc. 519.1503).

Acid Hydrolysis. Compounds **1** and **6**–**9** were each applied to a TLC plate, and then hydrolyzed under HCl vapor at 60° for 40 min. After removal of excess HCl, β-D-glucose (Glc) was applied to the same plate as reference compound. The TLC plate was developed with $CHCl_3$/MeOH/H_2O/AcOH 16:9:2:2, sprayed with aniline-phthalic acid, and heated. All hydrolyzed compounds showed identical purple spots, with R_f values of 0.42.

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