Two New Secoiridoid Glycosides from *Tripterospermum chinense*

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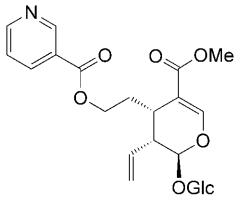
Two new secoiridoid glycosides, tripterospermumcin A (**1**) and B (**2**), were isolated from the EtOH extract of the aerial parts of *Tripterospermum chinense* (MIGO) H. SMITH, along with three known compounds, secologanin, secologanol, and sweroside. Their structures were established on the basis of UV, IR, MS, and extensive 1D- and 2D-NMR analyses, as well as by literature comparison of spectroscopic data.

Introduction. – The genus *Tripterospermum* (*Gentianaceae*) comprises more than ten species in China [1]. *Tripterospermum* plants are known to be rich in xanthones, flavonoids, iridoids, and triterpenes [2][3]. Many biological activities were reported for their secondary metabolites, including inhibition of *1*) angiotensin-I-converting enzyme [4], *2*) *Moloney* murine leukemia virus reverse-transcriptase [5], *3*) cutaneous plasma extravasation [6], and *4*) formylmethionyl-leucyl-phenylalanine-induced respiratory burst [7]. *T. chinense* (MIGO) H. SMITH, widely distributed in Southeast China, is traditionally used for the treatment of cough, haemoptysis, and pulmonary disease by local inhabitants [8].

In the present study, we report two new secoiridoid glycosides, tripterospermumcin A (**1**) and tripterospermumcin B (**2**), from *T. chinense*, which were obtained together with the known compounds secologanin, secologanol, and sweroside.



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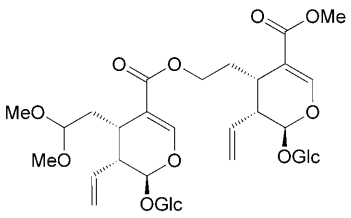

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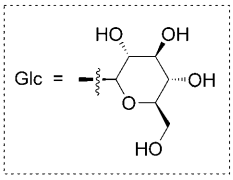

Results and Discussion. – Compound **1**, a colorless, amorphous powder, had the molecular formula $C_{23}H_{29}NO_{11}$, as determined by HR-ESI-MS (*m*/*z* 518.1634 ($[M+Na]^+$)). The IR absorptions at 1726 and 1705 cm^{-1}, and the ^{13}C-NMR signals at δ(C) 166.5 (C(6′)) and 169.4 (C(11)) (*Table*) indicated the presence of two α,β-unsatu-

rated C=O groups[1]). The ^{1}H,^{1}H-COSY correlations H–C(7)/CH_2(6), CH_2(6)/H–C(5), H–C(5)/H–C(9), H–C(9)/H–C(1), and the signals at δ(C) 65.6 (C(7)), 30.5 (C(6)), 32.0 (C(5)), 45.7 (C(9)), and 98.1 (C(1)), assigned from HSQC data, suggested the presence of a CH_2–CH_2–CH–CH–CH unit (*Fig. 1*). A CH_2=CH–CH moiety was inferred from the ^{1}H,^{1}H-COSY cross-peaks for CH_2(10)/H–C(8) and H–C(8)/H–C(9), and from HMBC correlations for H–C(9)/C(10) and H–C(1)/C(8). The structure of the dihydropyran ring in **1** was deduced from HMBC correlations between C(4) and both CH_2(6) and H–C(9), and between C(3) and both H–C(1) and H–C(5). The MeOOC group was established by HMBC correlation between C(11) and H–C(12), and attached at C(4), based on an HMBC cross-peak between C(11) and H–C(3).

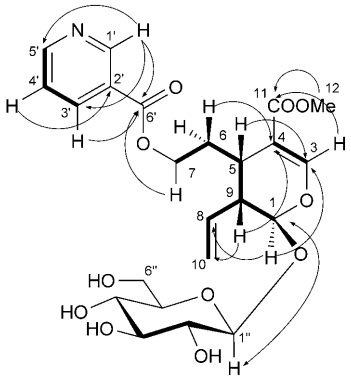

Fig. 1. *^{1}H,^{1}H-COSY* (▬) *and key HMBC* (→) *correlations of* **1**

Acid hydrolysis of **1** afforded glucose (Glc), as identified by comparative TLC (SiO_2; $CHCl_3$/MeOH/H_2O 18:10:1; R_f 0.30) and preparative TLC (AcOEt/pyridine/H_2O 2:1:2; R_f 0.28) with an authentic sample. The HMBC correlation of the anomeric resonance at δ(H) 4.71 (*d*, *J* = 7.9 Hz, H–C(1″)) with C(1) indicated the attachment of β-Glc at C(1).

In addition, five aromatic ^{13}C-NMR resonances were observed at δ(C) 151.5, 128.4, 139.2, 125.5, and 154.3. The ^{1}H,^{1}H-COSY correlations for H–C(3′)/H–C(4′) and H–C(4′)/H–C(5′), and the HMBC cross-peaks between C(2′) and H–C(4′), and between H–C(1′) and both C(3′) and C(5′) suggested the presence of a pyridine ring in **1**. This ring was part of a nicotinate, the ester C=O group being determined by HMBC correlations between C(6′) and both H–C(1′) and H–C(3′). Finally, an HMBC correlation between C(6′) and H–C(7) helped us to connect the nicotinate moiety with the secologanol part of the molecule.

The relative configuration of **1** was determined by analysis of NMR coupling constants and with the aid of NOESY experiments. A *J*(1,9) value of 6.7 Hz suggested a *trans*-diaxial orientation for H–C(1) and H–C(9) [9–12], and NOESY cross-peaks between H–C(5) and H–C(9), as well as between H_β–C(6) and H–C(9), revealed that they were on the same side of the molecular plane (β). The absolute configuration of the aglycone of **1** was not determined.

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[1]) Arbitrary C-atom numbering (see *Fig. 1*). For systematic names, see the *Exper. Part*.

Table. *^{1}H- and ^{13}C-NMR Data of* **1** and **2**. At 400/100 MHz, resp., in CD_3OD; δ in ppm, J in Hz. Assignments based on ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments. Arbitrary atom numbering.

Pos.	**1**		Pos.	**2**	
	δ(C)	δ(H)		δ(C)	δ(H)
1	98.1 (*d*)	5.60 (*d*, *J*=6.7)	1	98.3 (*d*)	5.53 (*d*, *J*=5.1)
3	154.1 (*d*)	7.50 (*s*)	3	153.6 (*d*)	7.48 (*s*)
4	111.7 (*s*)		4	112.1 (*s*)	
5	32.0 (*d*)	2.99 (*dd*, *J*=12.3, 6.4)	5	29.8 (*d*)	2.91–2.99 (*m*)
6	30.5 (*t*)	1.95–2.02 (*m*),	6	33.3 (*t*)	1.60–1.70 (*m*),
		2.08–2.17 (*m*)			2.09–2.19 (*m*)
7	65.6 (*t*)	4.35–4.45 (*m*)	7	104.1 (*d*)	4.58 (*dd*, *J*=7.0, 4.8)
8	136.0 (*d*)	5.85 (*ddd*, *J*=18.9, 10.4, 8.5)	8	136.1 (*d*)	5.70–5.87 (*m*)
9	45.7 (*d*)	2.66–2.72 (*m*)	9	45.6 (*d*)	2.68–2.76 (*m*)
10	119.3 (*t*)	5.32 (*d*, *J*=17.3),	10	120.1 (*t*)	5.32–5.40 (*m*)
		5.26 (*d*, *J*=10.5)	11	169.0 (*s*)	
11	169.4 (*s*)		12	52.9 (*q*)	3.27 (*s*)
12	52.0 (*q*)	3.60 (*s*)	13	54.3 (*q*)	3.29 (*s*)
1′	151.5 (*d*)	9.10 (br. *s*)	1′	98.1 (*d*)	5.59 (*d*, *J*=5.9)
2′	128.4 (*s*)		3′	154.0 (*d*)	7.52 (*s*)
3′	139.2 (*d*)	8.40 (*ddd*, *J*=7.9, 1.9, 1.9)	4′	111.7 (*s*)	
4′	125.5 (*d*)	7.55 (*dd*, *J*=8.0, 4.9)	5′	31.3 (*d*)	2.90–2.99 (*m*)
5′	154.3 (*d*)	8.75 (*dd*, *J*=4.9, 1.9)	6′	30.1 (*t*)	2.08–2.19 (*m*),
6′	166.5 (*s*)				1.77–1.88 (*m*)
			7′	63.8 (*t*)	4.21–4.30 (*m*),
					4.10–4.18 (*m*)
			8′	135.9 (*d*)	5.70–5.87 (*m*)
			9′	45.6 (*d*)	2.68–2.76 (*m*)
			10′	120.3 (*t*)	5.28–5.33 (*m*)
			11′	169.6 (*s*)	
			12′	52.3 (*q*)	3.67 (*s*)
1″	100.5 (*d*)	4.71 (*d*, *J*=7.9)	1a	104.7 (*d*)	4.76 (*d*, *J*=7.8)
2″	74.9 (*d*)	3.18 (*dd*, *J*=9.0, 7.9)	2a	74.9 (*d*)	3.20–3.30 (*m*)
3″	78.7 (*d*)	3.22–3.37 (*m*)	3a	78.7 (*d*)	3.35–3.40 (*m*)
4″	71.9 (*d*)	3.22–3.37 (*m*)	4a	71.9 (*d*)	3.20–3.30 (*m*)
5″	78.3 (*d*)	3.22–3.37 (*m*)	5a	78.3 (*d*)	3.45–3.50 (*m*)
6″	63.1 (*t*)	3.65 (*dd*, *J*=11.9, 5.9),	6a	61.3 (*t*)	3.67–3.75 (*m*),
		3.89 (*dd*, *J*=11.9, 1.9)			3.91–3.98 (*m*)
			1′a	104.7 (*d*)	4.77 (*d*, *J*=8.2)
			2′a	75.0 (*d*)	3.20–3.30 (*m*)
			3′a	78.6 (*d*)	3.35–3.40 (*m*)
			4′a	71.9 (*d*)	3.20–3.30 (*m*)
			5′a	78.1 (*d*)	3.45–3.50 (*m*)
			6′a	63.1 (*t*)	3.67–3.75 (*m*),
					3.91–3.98 (*m*)

From the above data, the structure of compound **1** was elucidated as 2-[(2*S**,3*R**,4*S**)-3-ethenyl-2-(β-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2*H*-pyran-4-yl]ethyl pyridine-3-carboxylate, and named *tripterospermumcin A*.

Compound **2**, a colorless, amorphous powder, had the molecular formula $C_{35}H_{52}O_{20}$, as determined by HR-ESI-MS (*m/z* 815.2950 ($[M+Na]^+$)). The IR absorptions at 1703 and 1702 cm^{-1}, in combination with the ^{13}C-NMR signals at δ(C) 169.0 (C(11)) and 169.6 (C(11′)) indicated the presence of two α,β-unsaturated C=O groups. The ^1H- and ^{13}C-NMR data of **2** (*Table*) showed two sets of major signals, both similar to those of secologanol. In the ^1H-NMR spectrum, there were double peaks each for H–C(1,1′), H–C(3,3′), H–C(5,5′), CH_2(6,6′), H–C(8,8′) H–C(9,9′), and H–C(10,10′), with the corresponding ^{13}C-NMR signals being assigned by HSQC experiments. This clearly demonstrated that **2** was a dimeric iridoid glycoside. Based on $^1H,^1H$-COSY, HSQC, and HMBC experiments, each structural unit of **2** had similar correlations to the secologanol moiety of **1** (*Fig. 2*), and they were linked *via* an ester function, O=C(11)–O–C(7′), as deduced from the HMBC correlation between C(11) and CH_2(7′). A clear difference was observed for the signal pairs at δ(H) 4.58 (*dd*, $J=7.0$, 4.8 Hz, H–C(7)) and δ(H) 4.10–4.18, 4.21–4.30 (2*m*, CH_2(7′)), with δ(C) 104.1 (C(7)) and 63.8 (C(7′)). Furthermore, compound **2** was lacking any nicotinate signals; instead, two MeO groups were observed at δ(C) 52.9 and 54.3, and δ(H) 3.27 and 3.29, respectively, which were involved in HMBC correlations with C(7).

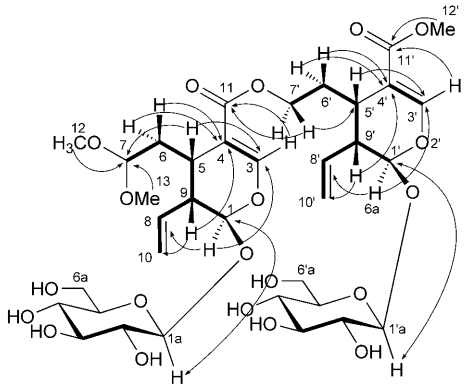

Fig. 2. *$^1H,^1H$-COSY* (▬) *and key HMBC* (→) *correlations of* **2**

Acid hydrolysis of **2** afforded Glc, as identified by co-TLC, which were attached to C(1) and C(1′), based on HMBC correlations with the anomeric H-atoms at δ(H) 4.76 (*d*, $J=7.8$ Hz, H–C(1a)) and 4.77 (*d*, $J=8.2$ Hz, H–C(1′a)], respectively.

From a J(1,9) value of 5.1 Hz in the ^1H-NMR spectrum of **2**, a *trans*-diaxial orientation was inferred for H–C(1) and H–C(9) [9–12]. NOESY Cross-peaks between H–C(9) and both H–C(5) and H_β–C(6) revealed their β-orientation. The same relative configuration was also determined for the second structurally related unit (primed atoms). The absolute configuration of the aglycone of **2** was not determined. From the above data, the structure of compound **2** was identified as 2-[(2*S**,3*R**,4*S**)-3-ethenyl-2-(β-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2*H*-pyran-4-yl]ethyl (2*S**,3*R**,4*S**)-4-(2,2-dimethoxyethyl)-3-ethenyl-2-(β-D-glucopyranosyloxy)-3,4-dihydro-2*H*-pyran-5-carboxylate, and named *tripterospermumcin B*.

The three known compounds were identified as secologanin, secologanol, and sweroside by comparing their spectroscopic data with those reported in the literature [13–15].

Experimental Part

General. Column chromatography (CC): silica gel (160–200 and 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*), *Lobar LiChroprep RP-18* (40–60 μm; *Merck*), *Sephadex LH-20* (*Amersham*), and *MCI CHP20P* gel (75–150 μm; *Mitsubishi Chemical Industries, Ltd*). Thin-layer chromatography (TLC): silica gel GF_{254} (0–40 μm), activated at 110° for 2 h; visualization by spraying with 20% H_2SO_4 in EtOH. All solvents were distilled prior to use. Petroleum ether: b.p. 60–90°. Melting points (m.p.): *SGW X-4* melting-point apparatus (*Shanghai Precision & Scientific Instrument Co., Ltd.*); uncorrected. UV Spectra: *Shimadzu UV-2401PC* spectrophotometer; λ_{max} (log ε) in nm. Optical rotations: *Jasco DIP-370* polarimeter. IR Spectra: *Nicolet Manga-750* FT-IR spectrophotometer, KBr cells; in cm^{-1}. NMR Spectra: *Bruker AM-400* instrument; δ in ppm rel. to Me_4Si; *J* in Hz. ESI-MS: *Finnigan LCQ-DECA* instrument. HR-ESI-MS: *Micromass LCT* and *Mariner* spectrometers; in *m/z*.

Plant Material. Tripterospermum chinense was collected in Yongshun County, Hunan Province, P. R. China, in May 2004, and identified by Prof. *Jingui Shen*, Shanghai Institute of Materia Medica, Shanghai. A voucher specimen (TC-052004) was deposited at the Herbarium of Shanghai Institute of Materia Medica, Chinese Academic of Sciences, Shanghai.

Extraction and Isolation. The dried and powered aerial parts of *T. chinense* (10 kg) were extracted with 95% aq. EtOH (3×80 l, 2 d each) at ambient temperature. After solvent removal *in vacuo*, the resulting extract (950 g) was suspended in H_2O (1.5 l) and successively extracted with petroleum ether (PE; 4×5 l), AcOEt (4×5 l), and BuOH (4×5 l). The BuOH-soluble extract was concentrated *in vacuo*, and the residue (350 g) was subjected to CC (*MCI* gel; H_2O/MeOH 9:1, 7:3, 5:5, 3:7, 1:9, 0:10): six fractions (*Fr. 1–6*). *Fr. 2* (5.6 g) was separated by regular CC (SiO_2; $CHCl_3$/MeOH 9:2) and then by RP-CC (*RP-18*; MeOH/H_2O 35:65, 40:60, 45:55) to afford **1** (8 mg), secologanin (35 mg), and secologanol (63 mg). Separation of *Fr. 3* (4.1 g) by CC (*RP-18*; MeOH/H_2O 40:60) provided **2** (78 mg) and sweroside (1.82 g).

Tripterospermumcin A (=*2-[(2S*,3R*,4S*)-3-Ethenyl-2-(β-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2H-pyran-4-yl]ethyl Pyridine-3-carboxylate*; **1**). Colorless, amorphous powder. UV (MeOH): 227 (4.03). $[\alpha]_D^{20} = -147.6$ ($c = 0.25$, MeOH). IR (KBr): 3410, 2940, 1726, 1705, 1620, 1570, 1498, 1436, 1290, 1082. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 518.1634 ($[M+Na]^+$, $C_{23}H_{29}NNaO_{11}^+$; calc. 518.1638).

Tripterospermumcin B (=*2-[(2S*,3R*,4S*)-3-Ethenyl-2-(β-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2H-pyran-4-yl]ethyl (2S*,3R*,4S*)-4-(2,2-Dimethoxyethyl)-3-ethenyl-2-(β-D-glucopyranosyloxy)-3,4-dihydro-2H-pyran-5-carboxylate*; **2**). Colorless, amorphous powder. UV (MeOH): 234 (3.98). $[\alpha]_D^{20} = -126.0$ ($c = 0.25$, MeOH). IR (KBr): 3423, 2921, 1703, 1702, 1632, 1439, 1385, 1286, 1076. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 815.2932 ($[M+Na]^+$, $C_{35}H_{52}NaO_{20}^+$; calc. 815.2950).

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