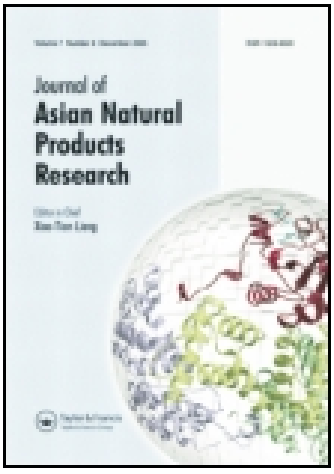

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Two novel secoiridoid glucosides from *Tripterospermum chinense*

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Two novel secoiridoid glucosides, tripterospermumcins C (**1**) and D (**2**), were isolated from the aerial parts of *Tripterospermum chinense*, along with four known compounds, tripterospermumcin B (**3**), sweroside (**4**), loganic acid (**5**), and 8-epi-kingiside (**6**). Their structures were determined by analysis of 1D and 2D NMR data, as well as by comparison with model compounds. Compound **1** was a rare iridoid tetramer with four glucosides.

Keywords: tripterospermumcin C; tripterospermumcin D; *Tripterospermum chinense*

1. Introduction

Tripterospermum chinense (Migo) H. Smith is widely distributed in South China, traditionally used for the treatment of cough, hemoptysis, and pulmonary disease by local inhabitants [1]. The constituents of this plant have been previously investigated and shown to contain iridoid glucosides and xanthones [2,3]. As a part of our continuing study of the constituents of the genus *Tripterospermum* (Gentianaceae), we have conducted a phytochemical screening of the aerial parts of *T. chinense* and isolated two new secoiridoid glycosides, tripterospermumcins C and D (Figure 1), together with four known iridoid glucosides. In this paper, we report the structural determination of these compounds on the basis of extensive spectroscopic analysis, including 2D NMR data, as well as by comparison with model compounds. To the best of our knowledge, **1** was a rare iridoid tetramer with four glucosides.

2. Results and discussion

Compound **1**, a white amorphous powder, showed IR absorption bands at 3425 cm^{-1} for the hydroxyl groups and at 1698 and 1634 cm^{-1} for the α,β-unsaturated ester carbonyl groups. The positive ESI-MS exhibited a pseudo molecular ion peak at *m/z* 1497 $[M + Na]^+$, and the high-resolution HR-ESI-MS gave the peak at *m/z* 1497.5057 $[M + Na]^+$, corresponding to the formula $C_{66}H_{90}O_{37}$. Acid hydrolysis of **1** gave D-glucose. The ^1H NMR spectrum of **1** indicated the presence of olefinic proton signals of the enol at δ 7.50 (1H, s), 7.46 (2H, s), and 7.41 (1H, s). The ^{13}C NMR spectrum of **1** showed signals due to four α,β-unsaturated ester carbonyl groups at δ 166.6 × 2, 165.9 and 165.7, and 152.5, 152.0 × 2, 150.8, 109.6 × 2, 108.7, and 107.1 for double bonds. In the ^{13}C NMR spectrum, four anomeric carbon signals at δ 98.7 × 3 and 98.3 were assigned readily. Four sets of terminal carbon–carbon double bond signals at δ 134.6, 134.5, 134.2, 133.9, and 119.0 × 2,

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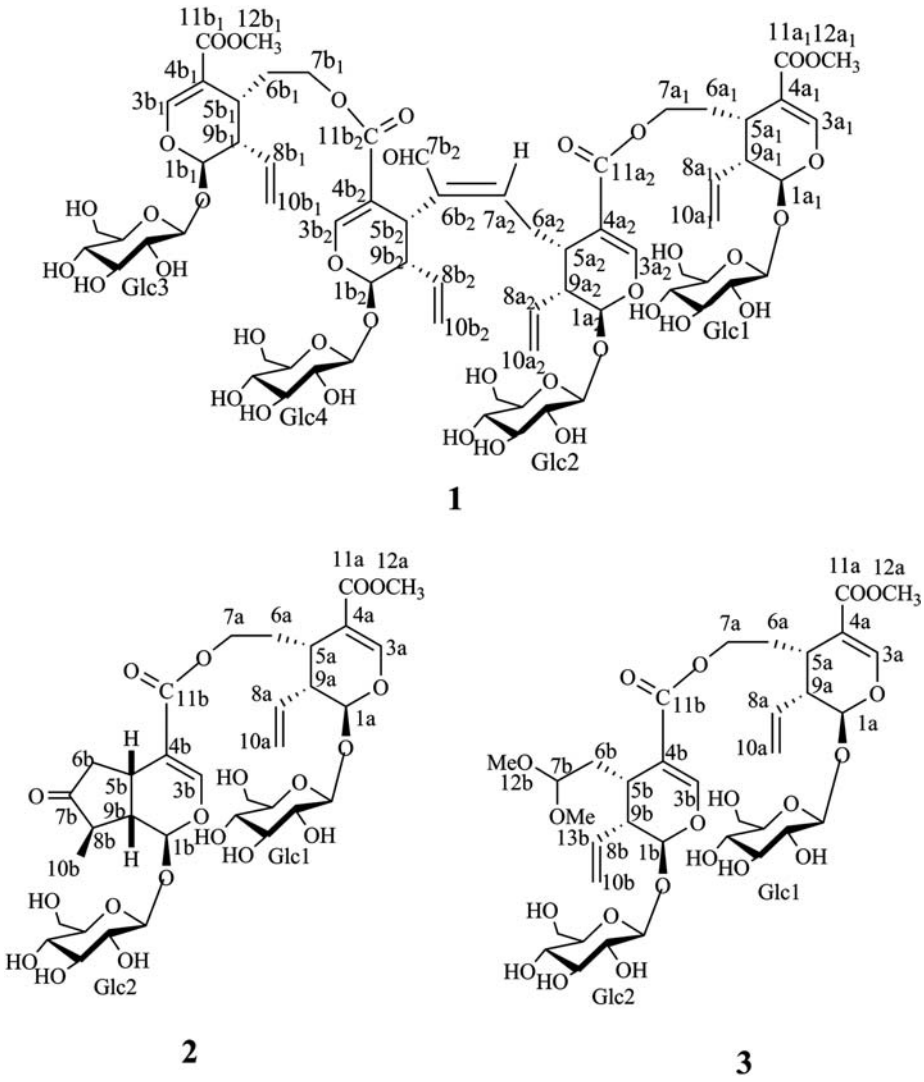

Figure 1. The structures of compounds **1**–**3**.

118.6, 119.8 were characteristic for four secoiridoid glucoside units. These data indicated clearly that **1** is an iridoid tetramer consisting of four secoiridoid glucoside units (units A and B) [4].

The NMR spectroscopic data of units A and B were closely related to those of the known compound **3**, respectively. The NMR spectra exhibited signals of an aldehyde [δ_H 9.18 (s), δ_C 195.0] and a trisubstituted carbon–carbon double bond [δ_H 6.64 (br. s), δ_C 140.7, 155.8], indicating that units A and B were linked, as shown in Figure 1 [4,5]. The linkage was also ascertained by long-range correlations between H-7a_2 at δ_H 6.64 and C-7b_2 at δ_C 195.0, H-7b_2 at δ_H 9.18, and C-7a_2 at δ_C 155.8 in the HMBC spectrum (Figure 2). The stereochemistry of the double bond conjugated to the aldehyde was assigned with NOESY experiment. In the NOESY spectrum, the correlations between H-7a_2 and H-7b_2 gave evidence for *Z*-configuration. Almost identical

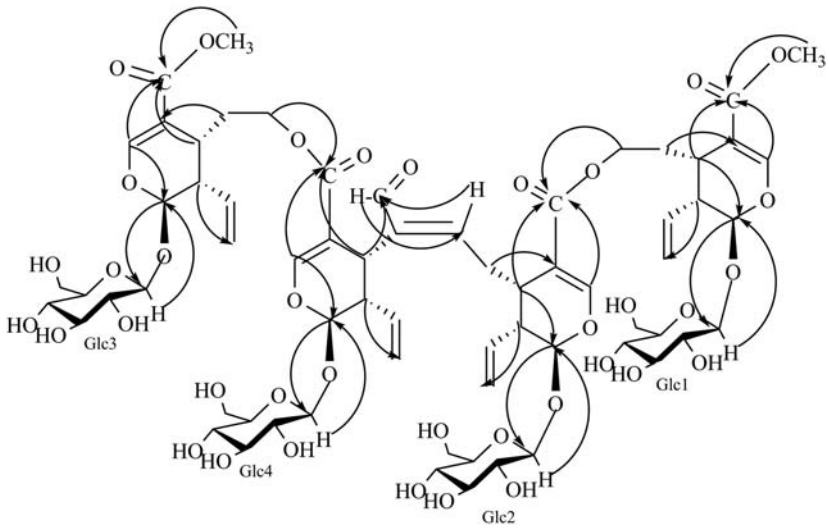

Figure 2. Key HMBC correlations for compound **1**.

NMR spectroscopic data indicated that units A and B, and **3** should be of the same configuration. NOESY supported the correctness of the above configuration. In the NOESY spectrum, the correlations between H-5a$_1$ and H-9a$_1$, between H-6a$_{1\beta}$, H-8a$_1$, and H-1a$_1$, suggested that H-5a$_1$ should be *cis* to H-9a$_1$, and H-6a$_{1\beta}$ and H-1a$_1$ should be *trans* to H-9a$_1$. The same relative configuration was also determined for unit A$_2$, unit B$_1$, and unit B$_2$. Thus, the structure of **1** was determined as shown in Figure 1, named tripterospermumcin C.

Compound **2** was obtained as a white amorphous powder. It showed IR absorption bands due to the hydroxyl groups (3423 cm^{-1}) and α,β-unsaturated ester carbonyl groups (1700, 1634 cm^{-1}). The molecular formula was determined to be $C_{33}H_{46}O_{19}$ by the positive-ion HR-ESI-MS at *m/z* 769.2543 $[M + Na]^+$. Acid hydrolysis of **2** gave D-glucose. The ^{1}H and ^{13}C NMR spectroscopic data for **2** showed characteristic peaks of bis-iridoid glucosides. Unit A, a secoiridoid moiety, had proton and carbon signals almost identical to those of unit A of **3**. For unit B, the ^{1}H and ^{13}C NMR spectroscopic data were almost identical to those of 7-ketologanin [6], except for the methoxy group. Consequently, units A and B are linked by an ester bond, which was also confirmed by HMBC analysis. In the HMBC spectrum, long-range correlations were observed between H-7a of unit A at δ 4.06 and 4.01 and C-11b of unit B at δ 165.9. The stereochemistry of unit B portion in **2** was determined by NOESY analysis. NOESY cross-peaks between H-5b, H-9b, and CH$_3$-10b revealed that H-9b should be *cis* to H-5b and CH$_3$-10b. Thus, compound **2**, named tripterospermumcin D, was elucidated as shown in Figure 1.

The four known compounds were identified as tripterospermumcin B, sweroside, loganic acid, and 8-epi-kingiside by comparing their spectroscopic data with those reported in the literature [2,7–9].

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a PerkinElmer 343 polarimeter (PerkinElmer, Waltham, MA, USA). IR spectra were recorded on the Bio-Rad FTS-65A spectrometer (Bio-Rad, Richmond, VA,

USA). UV spectra were recorded on Shimadzu UV-2501 PC (Shimadzu, Kyoto, Japan). The NMR spectra were recorded with a Varian ^{UNITY}INOVA 600 (Varian, Palo Alto, CA, USA) and JNM-ECA-400 (JEOL, Tokyo, Japan), and the chemical shifts were given on δ (ppm) scale with tetramethylsilane as an internal standard. The HR-ESI-MS was recorded on a 9.4-T Q-FT-MS Apex Qe (Bruker Co., Billerica, MA, USA). ESI-MS was recorded on Thermo Finnigan Advantage MAX HPLC-MS (Thermo Electron, Pittsburgh, PA, USA). Macroporous resin AB-8 (Nan Kai College Chemical Inc., Tianjin, China), silica gel (Qingdao Marine Chemistry Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), and ODS silica gel (120 Å, 50 μm, YMC, Kyoto, Japan) were used for column chromatography. HPLC was carried out using Waters 600E system (Waters, Milford, MA, USA): an analytical column, ODS (5 μm, 4.6 × 250 mm, Hanbon Science & Technology Co., Ltd, Huaian, China); preparative column, a YMC C_{18} (5 μm, 20.0 × 250 mm, YMC, Kyoto, Japan); detector, Knauer RID (refractive index detector, Knauer, Berlin, Germany) and Alltech ELSD (evaporative light-scattering detector, Alltech, Los Angeles, CA, USA) 2000ES. Gas chromatographic analysis was carried out with an Aglient 6890 Series gas chromatograph equipped with an H_2 flameionization detector. The column was a HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent, Santa Clara, CA, USA).

3.2 Plant material

The aerial parts of *T. chinense* were collected from Yongshun region of Hunan Province, China, in April 2010. The plant was identified by Prof. Bin Li (Beijing Institute of Radiation Medicine), and a voucher specimen (No. 100403) is deposited in the herbarium of Beijing Institute of Radiation Medicine, Beijing.

3.3 Extraction and isolation

The aerial parts of *T. chinense* (60 kg) were refluxed three times with EtOH–H_2O (3:2). The combined extract was concentrated under reduced pressure to furnish a dark brown residue (1800 g), which was suspended in H_2O and partitioned in turn with $CHCl_3$ and *n*-BuOH. The *n*-BuOH extract was evaporated under reduced pressure to yield a residue (386 g). The latter was separated chromatographically on macroporous resin AB-8 (15 × 100 cm) and eluted with a gradient mixture of EtOH–H_2O (1:5, 1:1, and 9:1; 60,000 ml each) to give five fractions (A–E). Fraction C (134 g) was separated chromatographically on silica gel column (12 × 50 cm) with a gradient mixture of $CHCl_3$–MeOH (20:1, 10:1, 5:1, 5:2, and 1:1) as eluent, and a total of 160 tubes (1000 ml each) were collected. Tubes 70–75 (11 g) and 118–127 (4 g) were individually subjected to a series of purification steps using Sephadex LH-20 column chromatography (MeOH), ODS silica gel column chromatography (3 × 50 cm; MeOH–H_2O, 40:60 and 70:30), and finally purified by preparative HPLC (MeOH–H_2O, 55:45, flow rate: 8.0 ml/min) to afford **1** (46 mg, t_R: 21.63 min), **2** (26 mg, t_R: 9.03 min), and **3** (100 mg, t_R: 18.48 min). Fraction B (86 g) was subjected to a series of purification steps using silica gel column chromatography ($CHCl_3$–MeOH, 100:1, 60:1, 30:1, 15:1, 7:1, 3:1, and 1:1), Sephadex LH-20 column chromatography (MeOH) and preparative HPLC (MeOH–H_2O, 35:65, flow rate: 8.0 ml/min) were carried out to afford **4** (450 mg), **5** (100 mg), and **6** (40 mg, t_R: 10.23 min).

3.3.1 *Tripterospermumcin C (**1**)*

White amorphous powder from MeOH–H_2O (55:45), 46 mg. $[\alpha]_D^{20}$ −116.4 (*c* 0.084, MeOH). UV (MeOH) λ_{max}: 234 nm. IR (KBr) ν_{max}: 3425, 2923, 1698, 1634, 1293, and 1075 cm^{-1}. For ^1H and

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1**[a] (δ in DMSO-d_6).

	^{1}H		^{1}H		^{13}C		^{13}C
$1a_1$	5.46–5.50 o[b]	$1a_2$	5.46–5.50 o	$1a_1$	95.6	$1a_2$	95.6
$3a_1$	7.46 s	$3a_2$	7.50 s	$3a_1$	152.0	$3a_2$	152.5
$4a_1$		$4a_2$		$4a_1$	109.6	$4a_2$	108.7
$5a_1$	2.77 m	$5a_2$	2.73 m	$5a_1$	30.3	$5a_2$	30.0
$6a_1$	1.77 m, 1.83 m	$6a_2$	1.67 m, 1.77 m	$6a_1$	28.9	$6a_2$	28.9
$7a_1$	3.95–4.00 o, 4.09 m	$7a_2$	6.64 br. s	$7a_1$	62.2	$7a_2$	155.8
$8a_1$	5.62–5.72 o	$8a_2$	5.62–5.72 o	$8a_1$	134.6	$8a_2$	133.9
$9a_1$	2.55–2.58 o	$9a_2$	2.43 m	$9a_1$	43.1	$9a_2$	44.0
$10a_1$	5.25 m, 5.30 m	$10a_2$	5.25 m, 5.30 m	$10a_1$	119.0	$10a_2$	119.8
$11a_1$		$11a_2$		$11a_1$	166.6	$11a_2$	165.9
$12a_1$	3.59 s			$12a_1$	51.1		
Glc1		Glc2		Glc1		Glc2	
1	4.50–4.55 o	1	4.50–4.55 o	1	98.7	1	98.7
2	2.95–3.00 o	2	2.95–3.00 o	2	73.0	2	73.0
3	3.15–3.16 o	3	3.15–3.16 o	3	77.2	3	77.2
4	3.03 o	4	3.03 o	4	70.0	4	70.0
5	3.15–3.16 o	5	3.15–3.16 o	5	76.6	5	76.6
6	3.65–3.69 o, 3.43 o	6	3.65–3.69 o, 3.43 o	6	61.1	6	61.1
$1b_1$	5.46–5.50 o	$1b_2$	5.46–5.50 o	$1b_1$	95.6	$1b_2$	95.4
$3b_1$	7.46 s	$3b_2$	7.41 s	$3b_1$	152.0	$3b_2$	150.8
$4b_1$		$4b_2$		$4b_1$	109.6	$4b_2$	107.1
$5b_1$	2.73 m	$5b_2$	3.78 m	$5b_1$	30.3	$5b_2$	29.7
$6b_1$	1.67 m, 1.77 m	$6b_2$		$6b_1$	28.9	$6b_2$	140.7
$7b_1$	3.95–4.00 o, 3.84 m	$7b_2$	9.18 s	$7b_1$	61.8	$7b_2$	195.0
$8b_1$	5.62–5.72 o	$8b_2$	5.62–5.72 o	$8b_1$	134.5	$8b_2$	134.2
$9b_1$	2.55–2.58 o	$9b_2$	2.73 m	$9b_1$	43.1	$9b_2$	43.2
$10b_1$	5.25 m, 5.30 m	$10b_2$	5.00–5.09 o	$10b_1$	119.0	$10b_2$	118.6
$11b_1$		$11b_2$		$11b_1$	166.6	$11b_2$	165.7
$12b_1$	3.59 s			$12b_1$	51.1		
Glc3		Glc4		Glc3		Glc4	
1	4.50–4.55 o	1	4.50–4.55 o	1	98.7	1	98.3
2	2.95–3.00 o	2	2.95–3.00 o	2	73.0	2	73.0
3	3.15–3.16 o	3	3.15–3.16 o	3	77.2	3	77.1
4	3.03 o	4	3.03 o	4	70.0	4	70.0
5	3.15–3.16 o	5	3.15–3.16 o	5	76.6	5	76.6
6	3.65–3.69 o 3.43 o	6	3.65–3.69 o, 3.43 o	6	61.1	6	61.1

[a] Recorded at 600 MHz.
[b] Overlapped with other signals.

^{13}C NMR spectral data, see Table 1. HR-ESI-MS (pos.): *m/z* 1497.5057 $[M + Na]^+$ (calcd for $C_{66}H_{90}O_{37}Na$, 1497.5053).

3.3.2 *Tripterospermumcin D (**2**)*

White amorphous powder from MeOH–H_2O (55:45), 26 mg. $[\alpha]_D^{20}$ −96.2 (*c* 0.110, MeOH). UV (MeOH) λ_{max}: 234 nm. IR (KBr) ν_{max}: 3423, 2927, 1700, 1634, 1290, and 1075 cm^{-1}. For ^{1}H and ^{13}C NMR spectral data, see Table 2. HR-ESI-MS (pos.): *m/z* 769.2543 $[M + Na]^+$ (calcd for $C_{33}H_{46}O_{19}Na$, 769.2526).

3.4 *Acid hydrolysis and sugar analysis*

Compounds **1** and **2** (2.0 mg each) were individually hydrolyzed in 2 M CF_3COOH (2 ml) at 95°C for 3 h. The reaction mixture was extracted with CH_2Cl_2 (5 ml) three times. The aqueous layer was repeatedly evaporated to dryness with EtOH until neutral. In the monosaccharide mixture,

Table 2. ^{1}H and ^{13}C NMR spectral data of compound **2**[a] (δ in DMSO-d_6).

	^{1}H	^{13}C		^{1}H	^{13}C
1a	5.47 d (4.8)	95.6	1b	5.55 d (3.0)	93.2
3a	7.46 s	152.0	3b	7.41 s	151.5
4a		109.6	4b		109.1
5a	2.77 m	30.2	5b	3.10 m	26.4
6a	1.75 m, 1.85 m	28.9	6b	2.60 d (10.8), 2.44 br s	42.0
7a	4.01 m, 4.06 m	62.1	7b		217.7
8a	5.70 m	134.6	8b	1.94 m	42.7
9a	2.57 m	43.1	9b	2.30 m	44.2
10a	5.30 d (17.4), 5.22 br d (10.2)	118.9	10b	1.05 d (16.2)	13.1
11a		166.6	11b		165.9
12a	3.58 s	51.0			
Glc1			Glc2		
1	4.52 d (8.4)	98.7	1	4.51 d (8.4)	98.6
2	2.97 m	73.0	2	2.97 m	73.0
3	3.10 o[b]	77.3	3	3.10 o	77.3
4	2.99 m	70.0	4	2.99 m	70.0
5	3.10 o	76.6	5	3.10 o	76.6
6	3.74 m, 3.40 m	61.1	6	3.74 m, 3.40 m	61.1

[a] Recorded at 600 MHz.
[b] Overlapped with other signals.

glucose was detected by TLC on a silica gel [using *n*-BuOH–EtOAc–C_5H_5N–H_2O (6:1:5:4) as development] by comparison with authentic sample: glucose (R_f 0.46). The residue of the sugars was dissolved in anhydrous pyridine (2 ml), 12 mg of L-cysteine methyl ester hydrochloride was added, and the mixture was stirred at 60°C for 1 h. Then, the mixture hexamethyldisilazane–trimethylchlorosilane (2:1; 0.6 ml) was added, and the mixture was kept at 60°C for 0.5 h [10]. The supernatant was analyzed by GC under the following conditions: column temperature: 180°C/250°C; programmed increase, 15°C/min; carrier gas: N_2 (1 ml/min); injection and detector temperature: 250°C; injection volume: 4.0 µl, and split ratio: 1/50. The derivative of D-glucose was detected with t_R values of 17.91 min.

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