New Lignan Glucosides from the Stems of *Tinospora sinensis*

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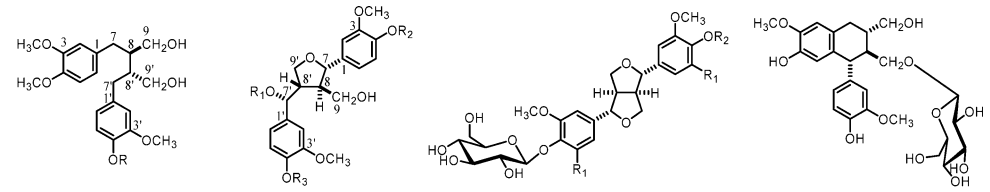
Two new lignan glucosides, tinosposides A and B (1 and 2), were isolated from the stems of *Tinospora sinensis* collected in Hainan Island, China. Their structures were elucidated on the basis of spectroscopic analysis and chemical evidence.

Key words *Tinospora sinensis*; Menispermaceae; lignan; tinosposide

The stems of *Tinospora sinensis* MERR. (Menispermaceae), commonly called "Shen Jin Teng" in Chinese, have been used in China as a folk medicine in the treatment of rheumatism, bruises with pain and lumbar muscle strain. Also, pharmacological studies on this plant have demonstrated anti-inflammatory,[1] immunomodulatory,[2] and antidiabetic[3] activities. In the previous chemical studies, it was reported the isolation of two dinorditerpene glucosides, tinosinesides A (**11**) and B (**12**), and a phenylpropanoid diglycoside, tinosinen (**10**).[3,4] As part of our current interest in the medicinal plant in Hainan Island, and the medicinal uses of the stems of *T. sinensis*, we also carried out a phytochemical investigation on the stems of *T. sinensis*, which resulted in two new lignan glucosides, tinosposides A and B (**1** and **2**), together with eight known compounds. This paper deals with the isolation and structural elucidation of the new constituents on the basis of spectroscopic analysis and chemical evidence.

The stems of *T. sinensis* collected in Hainan Island, China, were extracted with ethanol. The ethanolic extract was partitioned between ethyl acetate and water. The water layer was passed through a Diaion HP-20 column, and washed with 30% and 100% methanol. The methanolic eluate fraction was evaporated and applied to an ODS column chromatography. Further purification by reverse-phase HPLC gave ten compounds. The known compounds **3**—**10** were determined to be tanegoside (**3**),[5] (+)-pinoresinol *O*-β-D-glucopyranoside (**4**),[6] (+)-pinoresinol monomethyl ether *O*-β-D-glucopyranoside (**5**),[6] (+)-syringaresinol *O*-β-D-glucopyranoside (**6**),[7] (−)-isolariciresinol 3α-*O*-β-D-glucopyranoside (**7**),[8] 4-allyl-2-methoxyphenyl 6-*O*-β-D-apiofuranosyl (1→6)-β-D-glucopyranoside (**8**),[9] icariside D1 (**9**)[10] and tinosinen (**10**)[3] by analysis of the physical and spectroscopic evidence, and confirmed by comparing with literature data.

Tinosposide A (**1**) was isolated as an amorphous powder. Its molecular formula was determined to be $C_{27}H_{38}O_{11}$ by high-resolution (HR)-FAB-MS. The ^{1}H-NMR spectrum of **1** (Table 1) revealed two sets of ABC type trisubstituted aromatic proton signals at δ 6.63 (1H, d, J=1.9 Hz), 6.64 (1H, dd, J=8.7, 1.9 Hz) and 6.80 (d, J=8.7 Hz), and δ 6.62 (1H, dd, J=8.3, 1.8 Hz), 6.68 (1H, d, J=1.8 Hz) and 7.01 (d, J=8.3 Hz), three methoxy proton signals at δ 3.72, 3.74 and 3.79, and a set of β-glucopyranose proton signals, with the resonance for the anomeric proton at δ 4.82 (1H, d, J=7.5 Hz). The ^{1}H-NMR spectrum also exhibited signals attributable to two aliphatic methines at δ 1.89 and 1.91, two benzylic methylenes at δ 2.56, 2.68, and δ 2.58, 2.70, and two hydroxymethyls at δ 3.58, which were characteristic of H-8, 8′, 7, 7′, 9 and 9′ of a secoisolariciresinol-type lignan.[11] The signals due to methoxy protons could be assigned as 3-OCH_3 at δ 3.72, 4-OCH_3 at δ 3.79 and 3′-OCH_3 at δ 3.74, based on the cross peaks between δ 3.72 and 6.63 (H-2), δ 3.79 and 6.80 (H-5), and δ 3.74 and 6.68 (H-2′) in phase-sensitive two-dimensional nuclear Overhauser effect spectroscopy (2D-NOESY). These locations were further confirmed by analysis of the heteronuclear multiple bond connectivity (HMBC) experiment data as shown in Fig. 1. Furthermore, enzymatic hydrolysis by naringinase gave the aglycon **1a**, which was identified as (−)-secoisolariciresinol

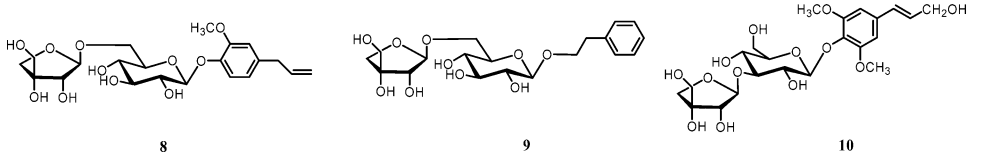


1 R=β-D-glucopyranose
1a R=H

2 R_1=H, R_2=β-D-glucopyranose, R_3=CH_3
3 R_1=β-D-glucopyranose, R_2=R_3=H
3a R_1=R_2=R_3=H

4 R_1=R_2=H
5 R_1=H, R_2=CH_3
6 R_1=OCH_3, R_2=H

7



8 **9** **10**

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Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Spectral Data of **1** and **2** in Methanol-d_4

Position	**1**		**2**	
	δ ^{1}H (mult, J in Hz)	δ ^{13}C	δ ^{1}H (mult, J in Hz)	δ ^{13}C
1		135.4		138.6
2	6.63 (d, 1.9)	114.0	7.01 (d, 1.6)	111.9
3		150.4		150.9
4		148.6		147.5
5	6.80 (d, 8.7)	113.0	7.13 (d, 8.2)	118.0
6	6.64 (dd, 8.7, 1.9)	122.6	6.91 (dd, 8.2, 1.6)	120.1
7	2.56 (dd, 14.2, 7.3)	36.1	4.67 (d, 7.1)	84.8
	2.68 (dd, 14.2, 7.3)			
8	1.89 (m)	44.2	1.89 (m)	53.8
9	3.58[a)]	62.1	3.25 (dd, 11.0, 6.0)	62.4
			3.32 (dd, 11.0, 6.0)	
1′		137.5		137.6
2′	6.68 (d, 1.8)	114.5	6.90 (d, 1.6)	111.8
3′		150.6		150.6
4′		146.2		150.1
5′	7.01 (d, 8.3)	117.9	6.88 (d, 8.2)	112.8
6′	6.62 (dd, 8.3, 1.8)	122.8	6.81 (dd, 8.2, 1.6)	120.7
7′	2.58 (dd, 14.2, 7.5)	36.1	4.49 (d, 8.5)	76.4
	2.70 (dd, 14.2, 7.5)			
8′	1.91 (m)	44.1	2.54 (m)	50.9
9′	3.58[a)]	62.1	3.95 (dd, 8.9, 7.3)	71.6
			4.26 (dd, 8.9, 4.4)	
3-OCH$_3$	3.72 (s)	56.4	3.86 (s)	56.9
4-OCH$_3$	3.79 (s)	56.6		
3′-OCH$_3$	3.74 (s)	56.6	3.80 (s)	56.6
4′-OCH$_3$			3.80 (s)	56.6
Glc-1″	4.82 (d, 7.5)	103.2	4.88 (d, 7.6)	103.1
2″	3.48 (dd, 8.7, 7.5)	75.0	3.50 (dd, 9.1, 7.6)	75.0
3″	3.46 (t, 8.7)	77.9	3.46 (t, 9.1)	78.0
4″	3.39[a)]	71.4	3.40[a)]	71.5
5″	3.39 (m)	78.2	3.40 (m)	78.2
6″	3.69 (dd, 11.7, 5.2)	62.6	3.69 (dd, 10.5, 5.1)	62.6
	3.86 (dd, 11.7, 2.3)		3.86 (dd, 10.5, 2.3)	

a) Overlapped signals.

monomethyl ether.[12)] Also, on acid hydrolysis, **1** afforded D-glucose as a component sugar, which was identified by GLC analysis of its trimethylsilyl thiazolidine derivative.[13)] The glycosidic linkage of **1** was determined to be at the C-4′ hydroxyl group by the correlations observed between δ 4.82 (Glc-H-1″) and δ 7.01 (H-5′) in 2D-NOESY, and between δ_H 4.82 (Glc-H-1″) and δ_C 146.2 (H-4′) in HMBC experiments. Thus, the structure of tinosposide A (**1**) was elucidated as (−)-secoisolariciresinol monomethyl ether 4′-*O*-β-D-glucopyranoside.

Tinosposide B (**2**) was isolated as an amorphous powder. Its molecular formula was determined to be $C_{27}H_{36}O_{12}$ by HR-FAB-MS. On acid hydrolysis, **2** afforded D-glucose as a component sugar. In the ^{1}H- and ^{13}C-NMR spectra of **2** (Table 1), besides the signals due to two sets of ABC type trisubstituted aromatic ring, three methoxy groups, and a set of β-glucopyranose, it also showed the signals due to two three-carbon units including a benzylic oxymethylene, a methane and an oxymethyl (C-7, 7′, 8, 8′, 9 and 9′), which were connected at the methine carbons (C-8 and 8′) from an extensive 2D-NMR study including double quantum filter correlation spectroscopy (DQF-COSY) and ^{1}H-detected multiple quantum coherence (HMQC) experiments. In comparison of the ^{1}H- and ^{13}C-NMR data of **2** and tanegoside (**3**), the proton signals pattern and chemical shifts of carbon signals

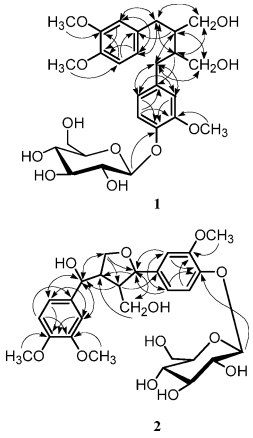

Fig. 1. Key ^{1}H–^{13}C Long-Range Correlations by HMBC Spectra of **1** and **2**

due to C-7, 8, 8′, 9 were superimposable, except that a downfield shift was observed at C-7′ in **2** by 5.6 ppm than that of **3**, suggesting **2** is a tanegool monomethyl ether β-D-glucopyranoside. The three methoxy groups could be assigned to be 3-OCH$_3$, 3′-OCH$_3$ and 4′-OCH$_3$, and the β-glucopyranose was connected to C-4 from correlations in the HMBC spectrum as shown in Fig. 1. The absolute configuration of **2** was determined as 7-*S*, 7′-*S*, 8-*R*, and 8′-*S* from the same Cotton effects in the circular dichroism (CD) spectra of **2**, **3**, and tanegool (**3a**), which was obtained by enzymatic hydrolysis of **3**. Thus, the structure of **2** was determined as tanegool monomethyl ether 4-*O*-β-D-glucopyranoside.

Experimental

General Experimental Procedures The UV spectra were obtained with a Shimadzu UV-160 spectrophotometer, whereas the IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5-dm cell, while the CD spectra were recorded on a JASCO J-720W spectropolarimeter. The ESIMSs were taken on an LCQ mass analyzer. The HR-FAB-MS was taken on a JEOL Mstation spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL ECP-500 spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). For HPLC, a JASCO PU-1580 HPLC system, equipped with a Shodex RI-71 Differential Refractometer detector, was used. Reversed-phase column chromatography (CC) was accomplished with RP-C$_{18}$ silica gel (Chromatotex DM1020T ODS, Fuji Silysia Chemical Ltd.). Silica gel CC was carried out using Kieselgel 60 (E. Merck). TLC was conducted in Kieselgel 60 F$_{254}$ plates (E. Merck). GLC was carried out on a PerkinElmer Clarus 500 GC-MS instrument.

Extraction and Isolation The stems of *T. sinensis* were collected in Hainan Island, China. Air-dried stems (3.0 kg) were extracted three times with 95% ethanol under reflux for 2 h each at 80 °C. The alcoholic extract was concentrated (171.8 g), suspended in water and then partitioned successively with EtOAc (1200 ml). The water layer was passed through a Diaion HP-20 column, and washed with water, 30% and 100% methanol. The 100% MeOH fraction (17.2 g) was chromatographed over silica gel and ODS column and preparative HPLC purification (20% CH$_3$CN) to afford ten compounds, **1** (12 mg), **2** (3 mg), **3** (15 mg), **4** (4 mg), **5** (3 mg), **6** (8 mg), **7** (4 mg), **8** (1 mg), **9** (3 mg) and **10** (50 mg).

Tinosposide A (**1**): Amorphous powder, $[\alpha]_D^{25}$ −39° (c=1.0, MeOH). UV (MeOH) λ_{max} (log ε): 226.8 (4.3), 277.8 (3.8). IR (KBr) ν_{max}: 3389, 2924, 1594, 1512, 1457, 1264, 1030. ^{1}H- (CD$_3$OD, 500 MHz) and ^{13}C-NMR (CD$_3$OD, 500 MHz): see Table 1. ESI-MS (positive) m/z 561.2 [M+Na]$^+$; HR-FAB-MS (positive) m/z 561.2312 [M+Na]$^+$ (Calcd for $C_{27}H_{38}O_{11}Na$ 561.2312).

Tinosposide B (**2**): Amorphous powder, $[\alpha]_D^{25}$ −10° (c=0.3, MeOH). UV (MeOH) λ_{max} (log ε): 228.4 (4.3), 277.2 (3.8). IR (KBr) ν_{max}: 3412, 2925, 1599, 1514, 1457, 1265, 1069. CD (c=1.76×10^{-4}, MeOH, 25 °C): $[\theta]_{237}$ 29633.9, $[\theta]_{285}$ 2244.6. ^{1}H- and ^{13}C-NMR: see Table 1. ESI-MS (positive)

m/z 575.2 $[M+Na]^+$; HR-FAB-MS (positive) *m/z* 575.2111 $[M+Na]^+$ (Calcd for $C_{27}H_{36}O_{12}Na$ 575.2105).

Acid Hydrolysis and Determination of the Absolute Configuration of Sugars in 1 and 2 Each solution of **1** and **2** (each 0.5 mg), in 1 M HCl (dioxane–H_2O, 1 : 1, 200 μl) was heated at 100 °C for 1 h under an Ar atmosphere. After dioxane was removed, the solution was extracted with EtOAc (1 ml×3) to remove the aglycon. The aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column, concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. The residue was dissolved in pyridine (1 ml), to which 0.1 M L-cysteine methyl ester hydrochloride in pyridine (2 ml) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.2 ml) for 2 h. The mixture was partitioned between hexane and H_2O (0.3 ml each) and the hexane extract was analyzed by gas-liquid chromatography (GLC) under the following conditions: capillary column, EQUITY™-1 (30 m×0.25 mm×0.25 μm, Supelco), column temperature, 230 °C; injection temperature, 250 °C; carrier N_2 gas. In the acid hydrolysate of **1** and **2**, D-glucose was confirmed by comparison of the retention times of their derivatives with those of D-glucose and L-glucose derivatives prepared in a similar way, which showed retention times of 11.79 and 11.33 min, respectively.

Enzymatic Hydrolysis of 1 and 3 A solution of **1** and **3** (each 5 mg) in 0.1 M acetate buffer (pH 4.0, 1.0 ml) was treated with naringinase (Sigma Chemical Co., 3 mg) and then the reaction mixture was stirred at 40 °C for 12 h. The reaction mixture was passed through a Sep-Pak C_{18} cartridge using H_2O and MeOH. The MeOH elute was further purified by silica gel column [$CHCl_3$–MeOH (95 : 5)] to give the aglycones **1a** (2.5 mg) and **3a** (2.9 mg). Compounds **1a** and **3a** were identified by comparison of physical data ($[\alpha]_D$, ^{1}H-, ^{13}C-NMR, MS) with reported values.[5,12]

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