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Structural determination of neolignans from the stems of *Tinospora sinensis*

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***Abstract*—The stems of *Tinospora sinensis* have long been used in the folk medicinal remedies to treat inflammatory conditions, especial rheumatism. Chemical constituents of this plant, therefore, received much attention by medicinal researchers. In this report, five neolignans were isolated from the stems of *T. sinensis* using chromatographic techniques. Their structures were determined by extensive analysis of ^{1}D- and ^{2}D-NMR spectral data as well as comparison with those reported in the literature.**

Keywords*—*Tinospora sinensis; neolignan; isolation; structural elucidation.

I. INTRODUCTION

Tinospora sinensis is a liana shrub, and popularly distributes in the south and southeast Asia countries such as India, China, Myanmar, Thailand, and Vietnam [1]. The stems of *T. sinensis* have long been used in traditional medicines to treat rheumatism and lumber muscles strains [2]. Additionally, the anti-inflammatory, anti-diabetic, immunomodulatory, and cytotoxic activities of extracts from *T. sinensis* have been demonstrated in recent reports [3]. Previous phytochemical studies on *T. sinensis* revealed the presence of norditerpenoids and phenolics [1, 3]. With the aim to clarify chemical constituents of the stems of *T. sinensis*, herein, we describe the isolation and structural determination of five neolignans. The lignans are known to have potential antioxidant, neurodegenerative, antiviral, and anti-inflammatory activities [4]. Therefore, the finding of lignans in the stems of *T. sinnesis* would be supported for explanation of pharmacological properties of this plant.

II. MATERIALS AND METHODS

A. General experiment procedures

Optical rotations were recorded on a P2000 polarimeter (Jasco, Kyoto, Japan). 1D- and 2D-NMR spectra were recorded on a Bruker 500 MHz (Bruker BioSpin, Ettlingen, Germany). Thin layer chromatography was performed on pre-coated plates. The compounds were visualized by irradiation under UV light (254 and 365 nm) and then by spraying with sulfuric acid solution (5%), heating on a hotplate. Column chromatography was performed using silica gel (Merck KGaA, Darmstadt, Germany), reversed phase C-18 (YMC Ltd., Kyoto, Japan), and diaion HP-20 (Merck KGaA, Darmstadt, Germany) as adsorbents. Preparative HPLC was performed on an Agilent 1100 system (Agilent Technology, Santa Clara, California, United States), including quaternary pump, autosampler, DAD detector, and equipped with YMC J'sphere ODS-H80 (20×250 mm, 4 µm).

B. Plant materials

The plant samples were collected in May 2020 at Vinh Phuc province, Vietnam. Its scientific name was determined to be *Tinospora sinensis* L. Merr. By botanist Nguyen The Cuong, Institute of Ecology and Biological Resources, Hanoi, Vietnam. Voucher specimen (NCCT-P93) is kept at the Institute of Ecology and Biological Resources.

C. Extraction and isolation

The stems of *T. sinensis* were dried, powdered, and ultrasonically extracted with methanol for three times (each 12 L of MeOH, 3 h, at room temperature). After removal of MeOH, the methanol extract (320 g) was suspended in water and separated in turn with n-hexane, dichloromethane, and ethyl acetate to give corresponding soluble fractions and water layer. The dichloromethane fraction (17 g) was fractionated into five fractions (TSD1-TSD5) by silica gel column chromatography using gradient solvent system of n-hexane/acetone (50/1, 25/1, 10/1, 5/1, and 2.5/1, v/v). Fraction TSD4 (4.7 g) was continuously chromatographed on a reversed phase C-18 column, eluting with acetone/water (1/2, v/v) to give four subfractions TSD4A-TSD4D. Fraction TSD4A was first separated on a silica gel column, eluting with dichloromethane/methanol (35/1, v/v) and then further purified by HPLC using isocratic acetonitrile/water (1/3, v/v) to give compounds **1** (6.3 mg), **2** (6.1 mg), and **3** (22.0 mg). The water layer was loaded on a diaion HP20 column, washed with water, and then eluted with methanol/water (1/3, 1/1, 3/1, 1/0, v/v) to give four fractions, TSW1-TSW4. Fraction TSW3 was chromatographed on a silica gel column, eluting with gradient of dichloromethane/methanol (40/1, 20/1, 10/1, 5/1, 2.5/1, v/v) to give five fractions TSW3A-TSW3E. Fraction TSW3D was chromatographed on reversed phase C-18 column, eluting with acetone/water (1/4, v/v) to give three smaller fractions, TSW3D1- TSW3D3. Fraction TSW3D3 was purified by HPLC using isocratic 16% acetonitrile in water to give compounds **4** (20.3 mg) and **5** (4.9 mg).

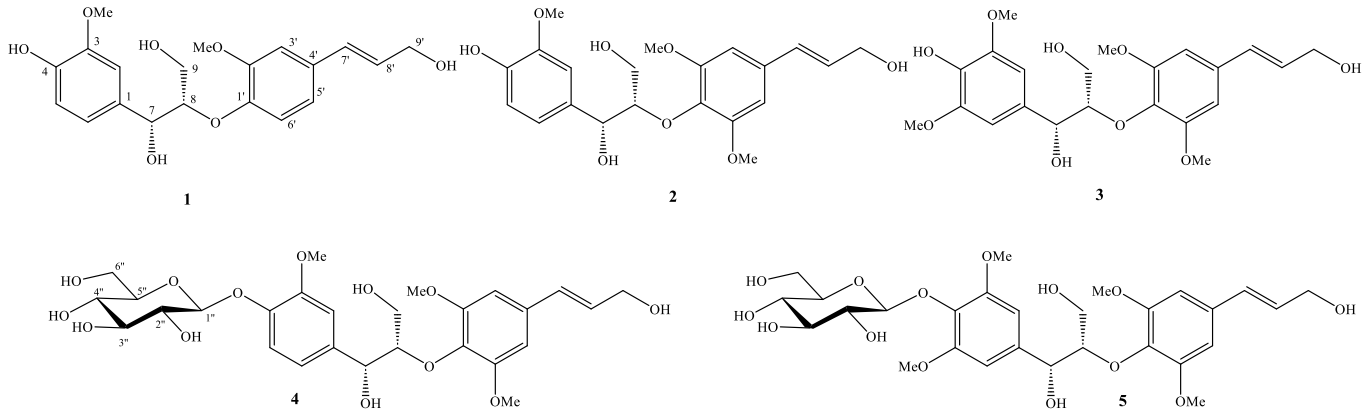

Fig. 1. *Chemical structure of compounds **1–5***

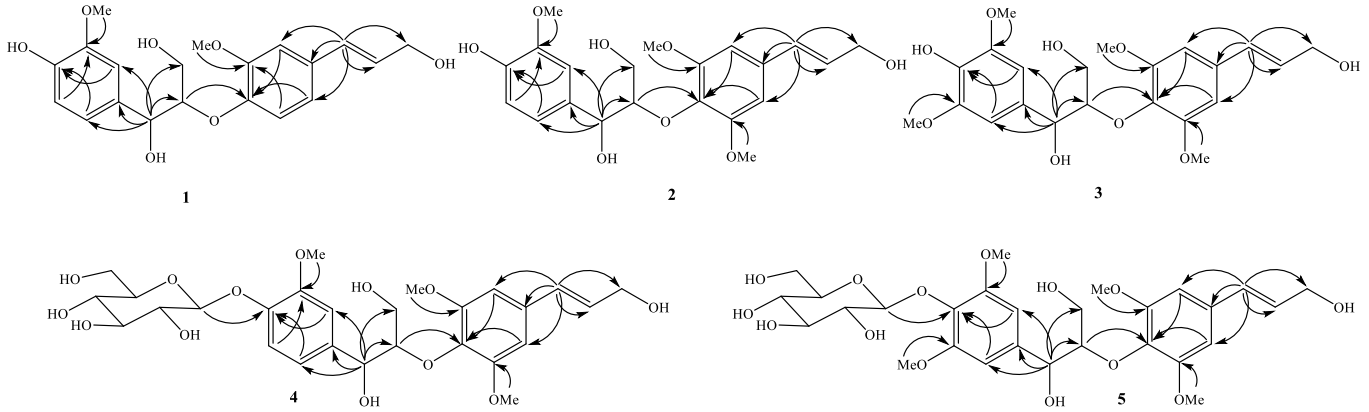

Fig. 2. *Key HMBC correlations (H→C) for compounds **1–5***

- **Compound 1**: Molecular formula: $C_{20}H_{24}O_7$; Yellow amorphous powder; $[\alpha]_D^{30}$: −14 (*c* = 0.1, MeOH); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1.

- **Compound 2**: Molecular formula: $C_{21}H_{26}O_8$; Yellow amorphous powder; $[\alpha]_D^{30}$: −21 (*c* = 0.1, MeOH); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1.

- **Compound 3**: Molecular formula: $C_{22}H_{28}O_9$; Yellow amorphous powder; $[\alpha]_D^{30}$: −17 (*c* = 0.1, MeOH); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1.

- **Compound 4**: Molecular formula: $C_{27}H_{36}O_{13}$; Yellow amorphous powder; $[\alpha]_D^{30}$: -62 (*c* = 0.1, MeOH); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 2.

- **Compound 5**: Molecular formula: $C_{28}H_{38}O_{14}$; Yellow amorphous powder; $[\alpha]_D^{30}$: -55 (*c* = 0.1, MeOH); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 2.

III. Results and Discussion

The stems of *T. sinensis* was extracted with methanol. The crude extract was fractionated and purified by chromatographic methods to give five compounds (Fig. 1).

Compound **1** was obtained as a yellow amorphous powder. The ^{1}H-NMR spectrum of **1** showed signals of two ABX coupled systems [δ_H 7.07 (1H, d, *J* = 2.0 Hz), 6.75 (1H, d, *J* = 8.0 Hz), 6.86 (1H, dd, *J* = 2.0, 8.0 Hz), 7.05 (1H, d, *J* = 2.0 Hz), 7.01 (1H, d, *J* = 8.5 Hz), 6.93 (1H, dd, *J* = 8.5, 2.0 Hz)], a pair of *trans* coupled olefinic protons [δ_H 6.56 (1H, d, *J* = 1.5, 16.0 Hz) and 6.34 (1H, dt, *J* = 6.0, 16.0 Hz)], two oxymethine groups [δ_H 4.90 (1H, d, *J* = 5.0 Hz) and 4.30 (1H, m)], two oxymethylene groups [δ_H 4.23 (2H, d, *J* = 6.0 Hz) and 3.81 (1H, dd, *J* = 5.5, 12.0 Hz)/ 3.50 (1H, dd, *J* = 3.5, 12.0 Hz)], and two methoxy groups [δ_H 3.84 and 3.90 (each 3H, s)]. The ^{13}C-NMR and HSQC spectrum of **1** observed signal of 20 carbons including 6 non-protonated carbons, 10 methines, 2 methylenes, and 2 methyl groups. Analysis of HMBC spectrum of **1** revealed correlation of two C6-C3 patterns. Particularly, the HMBC correlations (Fig. 2) between H-7′ (δ_H 6.56) and C-3′ (δ_C 111.3)/ C-4′ (δ_C 133.2)/ C-5′ (δ_C 120.8)/ C-8′ (δ_C 131.4)/ C-9′ (δ_C 63.7), H-3′(δ_H 7.05)/ H-5′ (δ_H 6.93) and C-1′ (δ_C 149.3), H-3′/2′-OMe (δ_H 3.90) and C-2′ (δ_C 151.8) indicated the presence of 4-[(*E*)-3-hydroxy-1-propenyl]-2-methoxyphenyl group. The *E*-configuration of the double bond was confirmed by the large coupling constant values of vinyl protons (*J* = 16 Hz). The HMBC correlations between H-7 (δ_H 4.90) and C-1(δ_C 133.8)/ C-2 (δ_C 111.8)/ C-6 (δ_C 120.8)/ C-8 (δ_C 87.2)/ C-9 (δ_C 63.7), H-2 (δ_H 7.07)/ H-6 (δ_H 6.86) and C-4 (δ_C 147.2), H-5 (δ_H 6.75)/ 3-OMe (δ_H 3.84) and C-3 (δ_C 148.9) indicated the presence of 1-[4-hydroxy-3-methoxyphenyl] propane-1,2,3-triol moiety. A connection between abovementioned C6-C3 moieties to form neolignan skeleton was established by an ether linkage between C-8 and C-1′, confirming by HMBC correlation between H-8 (δ_H 4.30) and C-1′ (δ_C 149.3). Relative configuration at C-7 and C-8 was

TABLE I. ^{1}H- AND ^{13}C-NMR SPECTROSCOPIC DATA OF COMPOUNDS **1** - **3** IN CD_3OD

No.	**1**		**2**		**3**	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
1	133.8	-	133.8	-	133.1	-
2	111.8	7.07 (d, 2.0)	111.5	7.08 (d, 2.0)	105.3	6.70 (s)
3	148.9	-	148.7	-	149.0	-
4	147.2	-	146.8	-	135.9	-
5	115.9	6.75 (d, 8.0)	115.8	6.75 (d, 8.0)	149.0	-
6	120.8	6.86 (dd, 2.0, 8.0)	120.6	6.81 (dd, 2.0, 8.0)	105.3	6.70 (s)
7	74.1	4.90 (d, 5.0)	74.0	4.95 (d, 5.0)	74.3	4.94 (d, 5.0)
8	87.2	4.30 (m)	87.6	4.26 (m)	87.6	4.26 (m)
9	63.7	3.81 (dd, 5.5, 12.0) 3.50 (dd, 3.5, 12.0)	61.5	3.91 (dd, 5.5, 12.0) 3.60 (dd, 3.5, 12.0)	61.6	3.92 (dd, 4.5, 12.0) 3.60 (dd, 2.0, 12.0)
1'	149.3	-	136.4	-	136.5	-
2'	151.8	-	154.6	-	154.6	-
3'	111.3	7.05 (d, 2.0)	105.0	6.76 (s)	105.0	6.76 (s)
4'	133.2	-	134.8	-	134.8	-
5'	120.8	6.93 (dd, 8.5, 2.0)	105.0	6.76 (s)	105.0	6.76 (s)
6'	118.9	7.01 (d, 8.5)	154.6	-	154.6	-
7'	128.6	6.56 (d, 16.0)	129.9	6.56 (d, 16.0)	129.9	6.57 (d, 16.0)
8'	131.4	6.34 (dd, 6.0, 16.0)	131.4	6.34 (dd, 6.0, 16.0)	131.4	6.34 (dd, 6.0, 16.0)
9'	63.7	4.23 (d, 6.0)	63.6	4.23 (d, 6.0)	63.6	4.24 (d, 6.5)
3-OCH_3	56.4	3.84 (s)	56.4	3.85 (s)	56.7	3.88 (s)
2'-OCH_3	56.6	3.90 (s)	56.7	3.85 (s)	56.8	3.90 (s)
6'-OCH_3	-	-	56.7	3.85 (s)	56.8	3.90 (s)
5-OCH_3	-	-	-	-	56.7	3.88 (s)

TABLE II. ^{1}H- AND ^{13}C-NMR SPECTROSCOPIC DATA OF COMPOUNDS **4** AND **5** IN CD_3OD

No.	**4**		**5**	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
1	137.5	-	139.5	-
2	112.5	7.10 (dd, 2.0, 5.0)	106.1	6.77 (s)
3	150.5	-	153.8	-
4	147.2	-	135.6	-
5	117.7	7.14 (d, 8.0)	153.8	-
6	120.8	6.93 (dd, 2.0, 8.0)	106.1	6.77 (s)
7	73.8	4.97 (d, 5.0)	74.1	4.94 (d, 5.5)
8	87.3	4.26 (m)	87.1	4.30 (m)
9	61.5	3.92 (dd, 5.5, 12.0) 3.58 (dd, 3.5, 12.0)	61.6	3.93 (dd, 4.5, 12.0) 3.62 (dd, 2.0, 12.0)
1'	134.8	-	134.7	-
2',6′	104.9	6.74 (s)	104.9	6.74 (s)
3',5′	154.5	-	154.5	-
4'	136.4	-	136.4	-
7'	131.4	6.56 (d, 16.0)	131.3	6.57 (d, 16.0)
8'	129.9	6.33 (dd, 6.5, 16.0)	129.9	6.33 (dd, 6.5, 16.0)
9'	63.6	4.23 (d, 6.5)	63.6	4.24 (d, 6.5)
1″	103.0	4.88 (d, 7.5)	105.7	4.81 (d, 7.5)
2″	74.9	3.50 (dd, 7.5, 9.0)	75.7	3.50 (dd, 7.5, 9.0)
3″	78.2	3.41 (t, 9.0)	78.4	3.43 (t, 9.0)
4″	71.4	3.42 (t, 9.0)	71.4	3.42 (t, 9.0)
5″	77.8	3.47 (m)	77.8	3.22 (m)
6″	62.5	3.86 (dd, 2.0, 12.0) 3.70 (dd, 5.0, 12.0)	62.6	3.80 (dd, 2.0, 12.0) 3.68 (dd, 5.0, 12.0)
3-OCH_3	56.7	3.84 (s)	57.0	3.83 (s)
2',6′-OCH_3	56.7	3.87 (s)	56.7	3.86 (s)
5-OCH_3	-	-	57.0	3.83 (s)

deduced to be *erythro* by *J* coupling constant value between H-7 and H-8 ($J_{H\text{-}7/H\text{-}8}$ = 5.0 Hz) as previously described. Consequently, compound **1** was determined to be *erythro*-1-(4-hydroxy-3-methoxy phenyl)-2-[4-[(*E*)-3-hydroxy-1-propenyl]-2-methoxy phenoxy]-1,3-propanediol. The NMR data of **1** was well consisted with that reported in the literature [5]. This compounds was previously isolated from several plants such as *Anastatica hierochuntica* [6], *Broussonetia papyrifera* [7], *Eucommia ulmoides* [5], *Ehretia ovalifolia* [8] and known as *erythro*-guaiacylglycerol-8-*O*-4′-(coniferyl alcohol) ether.

Compound **2** was isolated as a yellow amorphous powder. The ^{1}H-NMR spectrum of **2** showed similar proton signals with **1**, except signals in the aromatic proton region. Compound **2** revealed signal of an ABX coupled system [δ_H 7.08 (1H, d, *J* = 2.0 Hz), 6.81 (1H, dd, *J* = 2.0, 8.0 Hz), 6.75 (1H, d, *J* = 8.0 Hz)] and a

symmetric 1,3,4,5-tetrasubstitedbenzen ring [δ_H 6.76 (2H, s)]. Moreover, the presence of an additional methoxy group in **2** compared to **1** was also recognized by total of 9 methoxy protons [δ_H 3.85 (9H, s)]. The ^{13}C-NMR spectrum of **2** clearly revealed signal of 21 carbons. Of these, signal corresponding to 1-[4-hydroxy-3-methoxyphenyl]propane-1,2,3-triol moiety (C-1 to C-9) were identical with those of **1** (Table 1), indicating the presence of this structural fragment in both **1** and **2**. The additional methoxy group, therefore, located at remaining C_6-C_3 moiety. The HMBC correlations between H-7′ (δ_H 6.56) and C-3′ (δ_C 105.0)/C-5′ (δ_C 105.0), H-3′ (δ_H 6.76)/H-5′ (δ_H 6.76) and C-2′ (δ_C 154.6)/ C-6′ (δ_C 154.6)/ C-1′ (δ_C 136.4) indicated three oxygenated groups at C-1′, C-2′, and C-6′. Because of symmetric structure, the methoxy groups were then assigned at C-2′ and C-6′. An ether bridge between C-8 and C-1′ was also confirmed by HMBC correlation between H-8 (δ_H 4.26) and C-1′ (δ_C 136.4). The relative *erythro* configurations at C-7 and C-8 was indicated by *J* coupling constant value between H-7 and H-8 ($J_{H\text{-}7/H\text{-}8}$ = 5.0 Hz) as previously described. Consequently, compound **2** was determined to be *erythro*-1-(4-hydroxy-3-methoxyphenyl)-2-[4-[(*E*)-3-hydroxy-1-propenyl]-2,6-dimethoxyphenoxy]-1,3-propanediol. The NMR data of **2** was well consisted with that reported in the literature [5]. This compounds was previously isolated from several plants such as *Eucommia ulmoides* [5], *Praxelis clematidea* [9], and *Wikstroemia hainanensis* [10], and known as *erythro*- syringylglycerol-8-*O*-4′-(sinapyl alcohol) ether.

Compound **3** was isolated as a yellow amorphous powder. Like **1** and **2**, the ^{1}H and ^{13}C-NMR spectra of **3** indicated it had similar neolignan skeleton as shown in compounds **1** and **2**. The difference in structure of **3** were the patterns of two symmetric 1,3,4,5-tetrasubstitued benzene rings which obviously revealed in the ^{1}H-NMR by two individual singlet aromatic protons [δ_H 6.76 and 6.70 (each 2H, s)]. Furthermore, the observation of total 12 methoxy protons [δ_H 3.84 and 3.85 (each 6H, s)] demonstrated the presence of four methoxy group. Because of symmetric pattern, the methoxy groups were respectively assigned at C-3, C-5, C-2′, and C-6′. Relative configuration at C-7 and C-8 was deduced to be *erythro* by *J* coupling constant value between H-7 and H-8 ($J_{H\text{-}7/H\text{-}8}$ = 5.0 Hz) as previously described. Consequently, compound **3** was determined to be *erythro*-1-(4-hydroxy-3,5-dimethoxyphenyl)-2-[4-[(*E*)-3-hydroxy-1-propenyl]-2,6-dimethoxy phenoxy]-1,3-propanediol. The NMR data of **3** was well consisted with that reported in the literature [11]. This compounds was previously isolated from several plants such as *Brassica fruticulosa* [12], *Eucommia ulmoides* [11], *Selaginella moellendorfii* [13], and known as *erythro*-guaiacylglycerol-8-*O*-4′-(sinapyl alcohol) ether.

Compound **4** was obtained as a yellow amorphous powder. The ^{1}H and ^{13}C-NMR spectra of **4** were recognized identical with those of **2**, except additional signal of sugar moiety. The signal of six carbons in sugar moiety were observed at δ_C 103.0, 74.9, 78.2, 71.4, 77.8, and 62.5 which suggested for glucopyranosyl group. Furthermore, the *J* coupling constant value at anomeric proton *J* = 7.5 Hz indicated for *β*-glucopyranosidic linkage. A HMBC correlation between anomeric proton H-1″ (δ_H 4.88) and C-4 (δ_C 147.2) indicated *O*-glycosylation at C-4. Therefore, compound 4 was determined to be *erythro*-1-(4-*O*-*β*-glucopyranosyl-3-methoxyphenyl)-2-[4-[(*E*)-3-hydroxy-1-propenyl]-2,6-dimethoxyphenoxy]-1,3-propanediol. The NMR data of **4** was well consisted with that reported in the literature [14]. This compound was previously isolated from several plants such as *Ailanthus altissima* [15], *Eucommia ulmoides* [16], *Ligularia veitchiana* [14], *Picrasma quassioides* [17], and known as citrusin B.

Compound **5** was obtained as a yellow amorphous powder. The ^{1}H and ^{13}C-NMR spectra of **5** were recognized identical with those of **3**, except additional signal of sugar moiety. The signal of six carbons in sugar moiety were observed at δ_C 105.7, 75.7, 78.4, 71.4, 77.8, and 62.6 which suggested for glucopyranosyl group. Furthermore, the *J* coupling constant value at anomeric proton *J* = 7.5 Hz indicated for *β*-glucopyranosidic linkage. A HMBC correlation between anomeric proton H-1″ (δ_H 4.81) and C-4 (δ_C 135.6) indicated *O*-glycosylation at C-4. Therefore, compound **5** was determined to be *erythro*-1-(4-*O*-*β*-glucopyranosyl-3,5-dimethoxy phenyl)-2-[4-[(*E*)-3-hydroxy-1-propenyl]-2,6-dimethoxy phenoxy]-1,3-propanediol. The NMR data of **5** was well consisted with that reported in the literature [18]. This compound was previously isolated from several plants such as *Picrasma quassioides* [17], *Polygonum perfoliatum* [19], *Saussurea stella* [20], *Selaginella moellendorfii* [13], and known as picraquassioside C

In summary, five neolignans (**1**-**5**) were isolated from the stems of *Tinospora sinensis*. Their chemical structures were determined based on 1D and 2D-NMR spectral data as well as comparison with those reported in the literature. Our study is the first report on the identification of neolignans **1**-**5** from *T. sinensis*.

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