



Original Article

## Flavone C-glycosides and Megastigmanes from the Leaves of *Tinospora Sinensis*

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**Abstract:** Using various chromatographic methods, five compounds, including three flavone C-glycosides: i) Isoviolanthin; ii) Isoorientin; iii) Isovitexin; iv) Two megastigmanes excoecarioside A; and v) Corchoionoside C were isolated from the leaves of *Tinospora sinensis* (Lour.) Merr. Their structures were identified based on spectroscopic methods and comparisons with those reported in the literature.

**Keywords:** *Tinospora sinensis*, flavone C-glycoside, megastigmane.

### 1. Introduction

*Tinospora sinensis* (Lour.) Merr belongs to the family Menispermaceae. It is distributed in South and Southeast Asian countries, such as India, China, Myanmar, Thailand, Cambodia, and Vietnam. In Vietnam, it is distributed in Lao Cai, Ninh Binh, Ha Noi, Quang Nam, Da Nang, Phu Tho. *T. sinensis* has been traditionally used to treat bone fractures, chronic rheumatism, ulcerated wounds, diabetes, and gastritis [1-3]. Several phytochemical studies on *T. sinensis* have led to the isolation of steroids, flavonoids, alkaloids, phenolics [3], lignans [4], and diterpenoids [5-7]. In addition, biological studies on this plant have shown that they have

anti-inflammatory and antitumor activities [1]. In this study, we reported the isolation and structure elucidation of three flavone C-glycosides and two megastigmanes from the methanol extract of the leaves of *T. sinensis*.

### 2. Methodology

#### 2.1. Plant Materials

The leaves of *Tinospora sinensis* (Lour.) Merr was collected at Phuc Yen, Vinh Phuc, Vietnam, in May 2020 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (NCCT-TSL93) was deposited at The Institute of Ecology and Biological Resources, VAST.

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## 2.2. General Experimental Procedures

All NMR spectra, including  $^1\text{H-NMR}$  (500 MHz),  $^{13}\text{C}$  (125 MHz), HSQC, and HMBC were recorded on a Bruker AM500 FT-NMR spectrometer, and TMS was used as an internal standard. Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. Column chromatography (CC) was performed using silica gel (Kieselgel 60, 70-230 mesh, and 230-400 mesh, Merck) or RP-18 resins (30-50  $\mu\text{m}$ , Fuji Silysia Chemical Ltd.). Thin layer chromatography (TLC) was done using pre-coated silica gel 60 F<sub>254</sub> (0.25 mm, Merck) and RP-18 F<sub>254s</sub> plates (0.25 mm, Merck).

## 2.3. Extraction and Isolation

The dried powder of *T. sinensis* leaves (3.0 kg) was sonicated three times with hot methanol (MeOH) and then filtered through filter paper, removed solvent under reduced pressure to yield 150 g of MeOH extract. The MeOH extract was suspended in water and successively partitioned with *n*-hexane, dichloromethane, and ethyl acetate to give *n*-hexane (L1, 100 g), dichloromethane (L2, 2.7 g), ethyl acetate extracts (L3, 2.6 g), and water layer (L4). The L1 extract was subjected to a silica gel column chromatography (CC) eluting with a gradient solvent of *n*-hexane/acetone (30/1, 19/1, 9/1, 5/1, 2.5/1, v/v) to give five sub-fractions, L1A–L1E. The L1E was chromatographed on an RP-18 CC eluting with acetone/water (1/2, v/v) to yield three fractions, L1E1–L1E3. The L1E3 was further chromatographed on a silica gel CC eluting with  $\text{CH}_2\text{Cl}_2$ /acetone/water (1/1.25/0.07, v/v/v) to give two fractions, L1E3A and L1E3B. The L1E3A was purified by an HPLC using 22% ACN in water to yield compound **3** (9.0 mg). The L1E3B was purified by an HPLC using 15% ACN in water to yield compound **2** (10.8 mg). The water layer was chromatographed on a Diaion HP-20 CC eluting with water to remove polar components such as sugar and amino acid, then increase the concentration of MeOH in water (25 and 100%) to obtain two fractions, L4A (2.0 g) and L4B

(23.0 g). The L4B was chromatographed on a silica gel CC eluting with dichloromethane/MeOH (30/1, 19/1, 9/1, 5/1, 2.5/1, v/v) to give five sub-fractions, L4B1–L4B5. The L4B3 was chromatographed on an RP-18 CC eluting with MeOH/water (1/3, v/v) to give three smaller fractions, L4B3A–L4B3C. The L4B3A fraction was chromatographed on a silica gel CC eluting with  $\text{CH}_2\text{Cl}_2$ /MeOH/water (6.5/1/0.05, v/v/v) to give four fractions, L4B3A1–L4B3A4. The L4B3A3 was further chromatographed on an HPLC using ACN in  $\text{H}_2\text{O}$  (15%, v/v) to yield compound **5** (16.0 mg). Compound **4** (5.1 mg) was obtained from the L4B3A4 on an HPLC using the above condition. The L4B3B fraction was chromatographed on a silica gel CC eluting with  $\text{CH}_2\text{Cl}_2$ /acetone/water (1/1.7/0.09, v/v/v) to give three fractions, L4B3B1–L4B3B3. The L4B5 was chromatographed on an RP-18 column eluting with methanol/water (1/3, v/v) to give four smaller fractions, L4B5A–L4B5D. The L4B5B fraction was run on a silica gel CC eluting with EtOAc/MeOH/water (5/1/0.5, v/v/v) to provide three fractions, L4B5B1–L4B5B3. Finally, compound **1** (10.8 mg) was obtained from the L4B5B1 by an HPLC using 15% ACN in water.

**Isoviolanthin (1):** Yellow amorphous powder; ESI-MS  $m/z$  579  $[\text{M}+\text{H}]^+$ ,  $\text{C}_{27}\text{H}_{30}\text{O}_{14}$ ;  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ ): see Table 1.

**Isoorientin (2):** Yellow amorphous powder; ESI-MS  $m/z$  449  $[\text{M}+\text{H}]^+$ ,  $\text{C}_{21}\text{H}_{20}\text{O}_{11}$ ;  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ ): see Table 1.

**Isovitexin (3):** Yellow amorphous powder; ESI-MS  $m/z$  433  $[\text{M}+\text{H}]^+$ ,  $\text{C}_{21}\text{H}_{20}\text{O}_{10}$ ;  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ ): see Table 1.

**Excoecarioside A (4):** Colorless amorphous powder; ESI-MS  $m/z$  387  $[\text{M}-\text{H}]^-$ ,  $\text{C}_{19}\text{H}_{32}\text{O}_8$ ;  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ ): See Table 2.

**Corchoionoside C (5):** Colorless amorphous solid; ESI-MS  $m/z$  409  $[\text{M}+\text{Na}]^+$ ,  $\text{C}_{19}\text{H}_{30}\text{O}_8$ ;  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ ): See Table 2.

## 3. Results and Discussion

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula was

predicted to be  $C_{27}H_{30}O_{14}$  based on a quasi-molecular ion peak at  $m/z$  579  $[M+H]^+$  in the ESI-MS and  $^{13}C$ -NMR data. The  $^1H$ -NMR spectrum of compound **1** confirmed the presence of a flavone skeleton characterized by two doublet signals at  $\delta_H$  6.92 (d,  $J = 7.2$  Hz) and 7.94 (d,  $J = 7.2$  Hz) assigned to H-3', H-5' and H-2', H-6', respectively, and a singlet at  $\delta_H$  6.58 (H-3) (Table 1). In addition, the absence of signals for H-6 and H-8, the presence of two anomeric protons at  $\delta_H$  5.04 (d,  $J = 9.6$  Hz) and 5.17 (s), and the chemical shift of anomeric carbons at ( $\delta_C$  75.3 and 77.3) indicated a 6,8-di-C-glycosyl apigenin structure. In the HMBC spectrum of **1**, correlations were observed between H-1'' ( $\delta_H$  5.04) and C-5 ( $\delta_C$  157.2)/C-6

( $\delta_C$  105.5)/C-7 ( $\delta_C$  164.5) and between H-1''' ( $\delta_H$  5.17) and C-7 ( $\delta_C$  164.5)/C-8 ( $\delta_C$  107.9)/C-9 ( $\delta_C$  158.9); thus, the two sugar moieties were attached to C-6 and C-8, respectively. The NMR data obtained for **1** showed that glucose was bound to C-8. For the second hexose residue, the anomeric proton at [ $\delta_H$  5.17 (s),  $\delta_C$  75.3] and the secondary methyl group at [ $\delta_H$  1.40 (d,  $J = 5.4$  Hz),  $\delta_C$  18.4] are characteristic of an  $\alpha$ -L-rhamnopyranosyl moiety. Consequently, the structure of **1** was determined as isoviolanthin, a flavone-C-glycoside previously isolated from the leaves of *Microcos paniculata* [8]. Its NMR data were similar to the corresponding data in the literature (Table 1) [8].

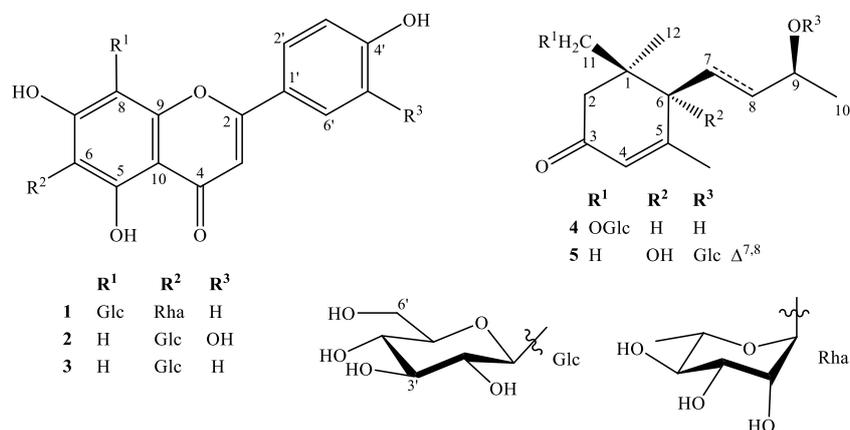


Figure 1. Chemical structures of compounds **1-5** from *T. sinensis*.

Table 1. The  $^1H$ - and  $^{13}C$ -NMR data for compounds **1-3**

C	1			2			3		
	# $\delta_C$	a,b $\delta_C$	a,c $\delta_H$ (mult., $J$ in Hz)	$\delta_C$	a,b $\delta_C$	a,c $\delta_H$ (mult., $J$ in Hz)	$\delta_C$	a,b $\delta_C$	a,c $\delta_H$ (mult., $J$ in Hz)
2	164.1	166.6	-	163.6	163.3	-	165.9	166.1	-
3	102.4	103.4	6.58 (s)	102.6	102.8	6.47 (s)	103.8	103.8	6.56 (s)
4	182.2	184.1	-	181.7	181.9	-	183.9	184.0	-
5	157.2	158.9	-	160.6	160.7	-	161.5	162.0	-
6	107.1	107.9	-	108.9	108.9	-	109.1	109.1	-
7	161.2	164.5	-	163.1	163.7	-	164.6	164.8	-
8	105.0	105.5	-	93.2	93.5	6.66 (s)	95.3	95.3	6.48 (s)
9	155.1	157.2	-	156.1	156.2	-	158.4	158.6	-
10	103.2	104.8	-	103.4	103.4	-	105.1	105.2	-
1'	121.6	123.3	-	121.4	121.4	-	123.0	123.0	-
2'	129.0	130.1	7.94 (d, 7.2)	113.1	113.3	7.41 (d, 2.0)	129.3	129.4	7.81 (d, 8.5)

3'	115.8	117.0	6.92 (d, 7.2)	145.8	145.8	-	116.9	117.0	6.92 (d, 8.5)
4'	161.3	162.9	-	149.7	149.7	-	162.5	162.7	-
5'	115.8	117.0	6.92 (d, 7.2)	115.7	116.1	6.89 (d, 8.0)	116.9	117.0	6.92 (d, 8.5)
6'	129.0	130.1	7.94 (d, 7.2)	118.7	118.9	7.41 (dd, 8.0, 2.0)	129.3	129.4	7.81 (d, 8.5)
6-C-Rha			6-C-Glc			6-C-Glc			
1''	77.4	77.3	5.17 (s)	72.8	73.1	4.59 (d, 10.0)	75.3	75.3	4.92 (d, 10.0)
2''	72.2	73.4	4.08 (br s)	69.9	70.6	4.04 (t, 9.0)	72.7	72.6	4.20 (t, 8.0)
3''	74.0	75.8	3.62 (m)	78.7	78.9	3.42 (m)	80.1	80.1	3.50 (m)
4''	71.7	73.8	3.54 (m)	70.4	70.2	3.43 (t, 9.0)	71.8	71.8	3.51 (m)
5''	77.3	78.9	3.46 (m)	81.4	81.5	3.42 (m)	82.5	82.6	3.46 (m)
6''	18.2	18.4	1.40 (d, 5.4)	61.2	61.5	3.68 (d, 11.5) 3.89 (dd, 11.5, 5.5)	62.9	62.9	3.77 (dd, 5.0, 12.0) 3.91 (dd, 2.0, 12.0)
8-C-Glc									
1'''	73.3	75.3	5.04 (d, 9.6)						
2'''	70.8	72.9	4.14 (dd, 9.6, 9.0)						
3'''	78.7	80.3	3.55 (t, 9.0)						
4'''	70.6	72.4	3.68 (t, 9.0)						
5'''	81.9	82.9	3.48 (m)						
6'''	61.4	63.2	3.80 (dd, 5.4, 11.4) 3.96 (br d, 11.4)						

Recorded in <sup>a</sup>)CD<sub>3</sub>OD, <sup>b</sup>)150 MHz, <sup>c</sup>)600 MHz, <sup>#</sup>δ<sub>C</sub> of isoviolanthin in DMSO-d<sub>6</sub> [8],  
<sup>%</sup>δ<sub>C</sub> of isoorientin in DMSO-d<sub>6</sub> [9], <sup>\$</sup>δ<sub>C</sub> of isovitexin in DMSO-d<sub>6</sub> [11].

Compound **2** was isolated as a yellow amorphous powder. Its molecular formula was predicted to be C<sub>21</sub>H<sub>20</sub>O<sub>10</sub> based on a quasi-molecular ion peak at *m/z* 449 [M+H]<sup>+</sup> in the ESI-MS and <sup>13</sup>C-NMR data. The <sup>1</sup>H-NMR spectrum of **2** showed signals of ABX spin systems of a tri-substituted benzene ring at [δ<sub>H</sub> 6.89 (d, *J* = 8.0 Hz), 7.41 (d, *J* = 2.0 Hz), 7.41 (dd, *J* = 8.0, 2.0 Hz)]; two singlet aromatic proton signals at δ<sub>H</sub> 6.47 and 6.66; and one anomeric proton at δ<sub>H</sub> 4.59 (d, *J* = 10.0 Hz). The <sup>13</sup>C-NMR and HSQC spectrum of **2** showed the signals of 21 carbons, including one carbonyl group at δ<sub>C</sub> 181.9, fourteen aromatic carbons at δ<sub>C</sub> 93.5 to 163.3, and six carbons of a sugar moiety. In addition, the signals of C-glucopyranoside was proved based on the appearance of the characteristic signals on the <sup>1</sup>H-NMR spectrum at δ<sub>H</sub> 4.59 (d, *J* = 10.0 Hz), hydroxymethylene at δ<sub>H</sub> 3.68 (d, *J* = 11.5 Hz) and 3.89 (dd, *J* = 11.5, 5.5 Hz), and the <sup>13</sup>C-NMR spectra at δ<sub>C</sub> 61.5, 70.2, 70.6, 73.1, 78.9, and 81.5 ppm. The β-configuration for the anomeric carbon of glucose was suggested by the large coupling constant (10.0 Hz) of H-1' (δ<sub>H</sub> 4.59).

Analysis of <sup>1</sup>H- and <sup>13</sup>C-NMR data indicated that **2** was an 8-C-glycosyl luteolin structure (Table 1). Comparing 1D NMR data of **2** with those published in the literature [9] led to identifying **2** as isoorientin, a compound previously found in some plants such as *Aspalathus linearis*, *Aloe vera*, *Annona muricata*, ... [10].

Compound **3** was obtained as a yellow amorphous powder. It possessed a molecular formula of C<sub>21</sub>H<sub>20</sub>O<sub>10</sub> as demonstrated from the ESI-MS spectrum at *m/z* 433 [M+H]<sup>+</sup> and <sup>13</sup>C-NMR data. The <sup>1</sup>H- and <sup>13</sup>C-NMR data of **3** were similar to those of **1**, except for the absence of one sugar unit in **3**. These implied that **3** was also a C-glycosyl apigenin structure. In addition that a sugar moiety in **3** was proved as glucopyranose based on the appearance of the characteristic signals on the <sup>1</sup>H-NMR spectrum at δ<sub>H</sub> 4.92 (d, *J* = 10.0 Hz), 4.20 (t, *J* = 8.0 Hz), 3.46 (m), 3.50 (m), 3.51 (m), 3.77 (dd, *J* = 12.0, 5.0 Hz), 3.91 (dd, *J* = 12.0, 2.0 Hz) and the <sup>13</sup>C-NMR spectra at δ<sub>C</sub> 75.3, 72.6, 80.1, 71.8, 82.6, 62.9 ppm. The β-configuration for the anomeric carbon of glucose was suggested

by the large coupling constant (10.0 Hz) of H-1' ( $\delta_{\text{H}}$  4.92). Moreover, the NMR data of **3** agreed with those reported (Table 1) [11]. From the above evidence, the structure of **3** was elucidated as isovitexin.

Compound **4** was isolated as a colorless amorphous powder. It gave a molecular formula of  $\text{C}_{19}\text{H}_{32}\text{O}_8$  at  $m/z$  387  $[\text{M}-\text{H}]^-$  in the ESI-MS and  $^{13}\text{C}$ -NMR data. The  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR (Table 2), DEPT, and HSQC spectra of **4** showed the presence of 13 carbons of a megastigmane skeleton, including one carbonyl group at  $\delta_{\text{C}}$  201.4; two olefinic carbons at  $\delta_{\text{C}}$  126.0 and 169.7; an  $\text{sp}^3$  hybridized quaternary carbon at  $\delta_{\text{C}}$  41.4; two methines at  $\delta_{\text{C}}$  48.2 and 69.0; four methylenes at  $\delta_{\text{C}}$  43.6, 27.2, 38.7, 73.3; and three methyl groups at  $\delta_{\text{C}}$  23.5, 24.5, and 25.1; and six carbons of  $\beta$ -D-glucopyranose [ $\delta_{\text{C}}$  104.7, 75.1, 78.2, 71.7, 78.0, and 62.8; anomeric proton at  $\delta_{\text{H}}$  4.29 (d,  $J = 8.0$  Hz)]. The COSY cross-peaks of H-6 ( $\delta_{\text{H}}$  4.96)/H-7 ( $\delta_{\text{H}}$  5.32)/H-8 ( $\delta_{\text{H}}$  2.26 and 1.84)/H-9 ( $\delta_{\text{H}}$  4.90)/H-10 ( $\delta_{\text{H}}$  1.77) confirmed an acyclic side chain H-6/H-7/H-8/H-9/H-10. The

$\alpha,\beta$ -unsaturated ketone was assigned at C-3, C-4, C-5 due to the HMBC correlations between H-2 ( $\delta_{\text{H}}$  2.01 and 2.52) and C-3 ( $\delta_{\text{C}}$  201.4)/C-4 ( $\delta_{\text{C}}$  120.6), between H-4 ( $\delta_{\text{H}}$  5.85) and C-2 ( $\delta_{\text{C}}$  43.6)/C-3/C-5 ( $\delta_{\text{C}}$  169.7)/C-6 ( $\delta_{\text{C}}$  48.2)/C-13 ( $\delta_{\text{C}}$  25.1), and between H-6 ( $\delta_{\text{H}}$  2.35) and C-4/C-5/C-13. In addition, the HMBC observations from H-12 ( $\delta_{\text{H}}$  1.12) to C-1 ( $\delta_{\text{C}}$  41.4)/C-2 ( $\delta_{\text{C}}$  43.6)/C-6 ( $\delta_{\text{C}}$  48.2)/C-11 ( $\delta_{\text{C}}$  76.3); H-10 ( $\delta_{\text{H}}$  1.18) to C-8 ( $\delta_{\text{C}}$  38.7)/C-9 ( $\delta_{\text{C}}$  69.0); H-13 ( $\delta_{\text{H}}$  2.06) to C-4 ( $\delta_{\text{C}}$  126.0)/C-5 ( $\delta_{\text{C}}$  169.7)/C-6 ( $\delta_{\text{C}}$  48.2) indicated the presence of three methyl groups at C-1, C-5, and C-9, respectively. On the other hand, a  $\beta$ -D-glucopyranose was located at C-11 by the HMBC correlations from proton anomeric H-1' ( $\delta_{\text{H}}$  4.29) to C-12 ( $\delta_{\text{C}}$  24.5) and from H-12 ( $\delta_{\text{H}}$  1.12) to C-1' ( $\delta_{\text{C}}$  104.7). Consequently, the structure of **4** was identified as excoecarioside A, a megastigmane previously isolated from *Excoecaria cochinchinensis* [12]. Its NMR data agreed with those reported data (Table 2) [12].

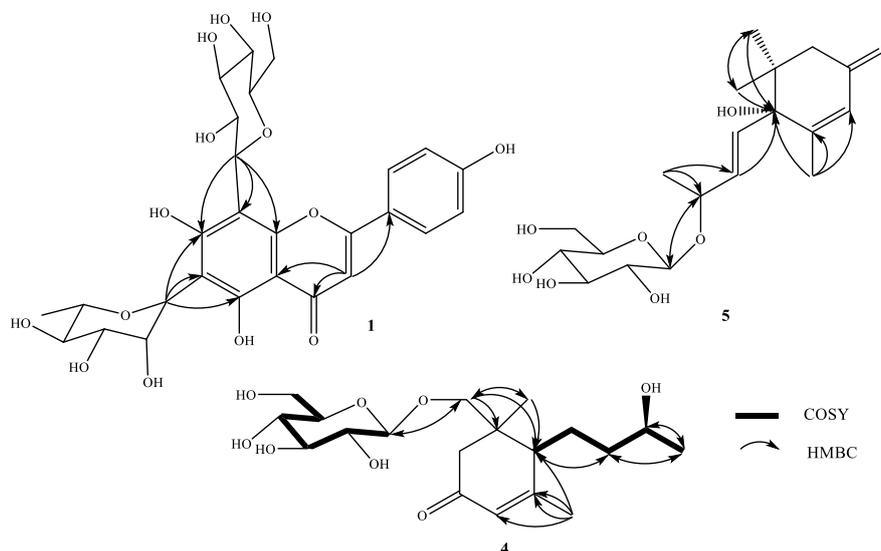


Figure 2. The key HMBC, COSY correlations of compounds **1**, **4-5**.

Compound **5** was isolated as an amorphous colorless solid. Its molecular formula was predicted as  $\text{C}_{19}\text{H}_{30}\text{O}_8$  from the ESI-MS ( $m/z$  409  $[\text{M}+\text{Na}]^+$ ) and NMR data. The  $^1\text{H}$ - and

$^{13}\text{C}$ -NMR spectra (Table 2) of **5**, which were assigned by 2D experiments (HSQC and HMBC), showed the presence of a  $\beta$ -D-glucopyranosyl unit and an aglycone moiety

consisting of 13 carbon atoms. Of the signals attributed to the aglycone, signals were consistent for the existence of four methyls, one methylene, five methine, and three quaternary carbons. In the  $^1\text{H-NMR}$  spectrum, four methyl signals, two being singlets (1.04 and 1.06), one doublet ( $\delta_{\text{H}}$  1.31,  $J = 6.0$  Hz), and vinylic methyl ( $\delta_{\text{H}}$  1.96, d,  $J = 6.0$  Hz), were observed. An olefinic proton at the  $\alpha$ -carbon of an  $\alpha,\beta$ -unsaturated ketone  $\delta_{\text{H}}$  5.89 (brs, H-4), two

(*E*)-oriented vinyl protons at  $\delta_{\text{H}}$  6.00 (d,  $J = 16.0$  Hz) and 5.75 (dd,  $J = 16.0$  and 7.5 Hz), an oxygenated methine proton  $\delta_{\text{H}}$  4.56 (qui,  $J = 6.5$  Hz) were the other signals observed. These spectroscopic data suggested that **5** has the structure of corchoionoside C which was first isolated from *Capparis spinosa* fruits [13]. Detailed analysis of HSQC and HMBC of **5**, as shown in Figure 2, undoubtedly confirmed the structure of **5**.

Table 2. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of compounds **4-5**

C	4			5		
	$^{\#}\delta_{\text{C}}$	$^{\text{a,c}}\delta_{\text{C}}$	$\delta_{\text{H}}^{\text{a,b}}$ (mult., $J$ in Hz)	$^*\delta_{\text{C}}$	$^{\text{a,c}}\delta_{\text{C}}$	$\delta_{\text{H}}^{\text{a,b}}$ (mult., $J$ in Hz)
1	41.3	41.4	-	40.9	42.2	-
2	44.1	43.6	2.01 (br d, 17.0) 2.52 (br d, 17.0)	49.3	50.8	2.20 (br d, 17.0) 2.63 (br d, 17.0)
3	201.4	201.4		197.2	201.3	
4	125.5	126.0	5.85 (br s)	125.5	127.1	5.89 (br s)
5	169.1	169.7	-	163.6	167.0	-
6	46.1	48.2	-	77.9	80.0	-
7	26.2	27.2	1.67 (m)/ 1.85 (m)	131.6	133.7	6.00 (d, 16.0)
8	39.2	38.7	1.55 (m)	131.4	133.8	5.75 (dd, 16.0, 7.5)
9	68.5	69.0	3.72 (m)	71.9	74.7	4.56 (qui, 6.5)
10	24.3	23.5	1.18 (d, 6.0)	22.0	22.2	1.31 (d, 6.0)
11	76.4	76.3	3.51 (m)/3.83 (m)	23.1	23.5	1.06 (s)
12	23.1	24.5	1.12 (s)	24.1	24.7	1.04 (s)
13	21.9	25.1	2.06 (s)	18.6	19.6	1.96 (d, 1.0)
<i>O</i> - $\beta$ -D-Glc						
1'	104.3	104.7	4.29 (d, 8.0)	99.9	101.3	4.29 (d, 8.0)
2'	74.8	75.1	3.23 (dd, 9.0, 8.0)	73.3	75.0	3.20 (dd, 9.0, 8.0)
3'	77.8	78.2	3.38 (t, 9.0)	77.0	78.2	3.16 (t, 9.0)
4'	71.3	71.7	3.30 (m)	70.0	71.7	3.26 (m)
5'	77.6	78.0	3.29 (m)	77.1	78.4	3.28 (m)
6'	62.2	62.8	3.69 (dd, 12.0, 6.5) 3.89 (dd, 12.0, 2.0)	61.0	62.8	3.65 (dd, 12.0, 6.5) 3.87 (dd, 12.0, 2.0)

Measured in <sup>a)</sup>  $\text{CD}_3\text{OD}$ , <sup>b)</sup> 125 MHz, <sup>c)</sup> 500 MHz, <sup>d)</sup>  $\delta_{\text{C}}$  of excoecarioside A in  $\text{DMSO-d}_6$  [12], <sup>e)</sup>  $\delta_{\text{C}}$  of corchoionoside C in  $\text{DMSO-d}_6$  [13].

#### 4. Conclusion

Three flavone C-glycosides: i) Violanthin; ii) Soorientin; iii) Isovitexin; iv) Two megastigmanes excoecarioside A; v) Corchoionoside C were isolated from the leaves of *Tinospora sinensis* (Lour.) Merr by using combined chromatographic methods.

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