Bioorganic Chemistry 103 (2020) 104147

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Sesquineolignan and neolignan enantiomers from *Triadica sebifera*

Min Yu[1], Yong-Li Zhang[1], Jin-Long Liu, Su-Juan Wang, Gui-Jie Zhang*

College of Pharmacy, Guilin Medical University, Guilin 541199, People's Republic of China

ARTICLE INFO

Keywords:
Triadica sebifera
Sesquineolignan ennatiomers
Neolignan enantiomers
NO inhibitors

ABSTRACT

Two pairs of new sesquineolignan enantiomers (**1a/1b** and **1c/1d**), two pair of new 4′,7-epoxy-8,3′-neolignan enantiomers (**2a/2b** and **3a/3b**), and a pair of new 3′,7-epoxy-8,4′-oxyneolignan enantiomers (**4a/4b**), along with two pairs of known 4′,7-epoxy-8,3′-neolignan enantiomers (**5a/5b** and **6a/6b**), were obtained from the stems and leaves of *Triadica sebifera*. The structures of the enantiomers were elucidated by spectroscopic analyses, and their absolute configurations were assigned by the experimental ECD spectra. Among them, compounds **5b**, **6a** and **6b** showed inhibitory activities against NO production in activated microglial BV-2 cells, with IC_{50} values of 14.3, 23.2 and 33.3 μM, respectively.

1. Introduction

Triadica sebifera, a tree belonging to the Euphorbiaceae family, is distributed widely in southern provinces of China [1]. The roots and bark of this plant have been commonly used in folk medicine to treat dermatitis, eczema, snakebite, and beriberi [2]. Pharmacological researches have illustrated that the leaves of *T. sebifera* have anti-inflammatory, analgesic, and antihypertensive activities [3,4]. Previous phytochemical studies on this plant have led to the isolation of a few diterpenes [5], triterpenes [6], flavonoids [7], phenols [7], and coumarins [8]. Over the course of our continuing search for antineuroinflammatory agents from the genus *Triadica* [9–11], the stems and leaves of *T. sebifera* were investigated. This work led to the isolation of two pairs of new sesquineolignan enantiomers (**1a/1b** and **1c/1d**), two pair of new 4′,7-epoxy-8,3′-neolignan enantiomers (**2a/2b** and **3a/3b**), and a pair of new 3′,7-epoxy-8,4′-oxyneolignan enantiomers (**4a/4b**), along with two pair of known 4′,7-epoxy-8,3′-neolignan enantiomers (**5a/5b** and **6a/6b**). More and more enantiomers have been reported in natural products [12,13], and the activities of some enantiomers are drastically different [12,14]. Hence, it is mandatory to resolve them and provide pure stereoisomers. Details of the isolation, structural elucidation, and antineuroinflammatory evaluation of these enantiomers are reported herein (see Fig. 1).

2. Materials and methods

2.1. Plant material

The stems and leaves of *Triadica sebifera* were collected in August 2016 in Guilin, Guangxi Province, People's Republic of China. The plant material was identified by one of the authors (G.-J. Zhang), a voucher specimen (No. TS-201608) was deposited at the College of Pharmacy, Guilin Medical University.

2.2. General experimental procedures

UV absorption spectra were recorded on a PerkinElmer 650 spectrophotometer and the IR spectra were acquired on a PerkinElmer Spectrum Two FT-IR spectrometer. NMR spectra were recorded on a 600 or 400 MHz Bruker AVANCE. HRESIMS were carried out on an Agilent 6545 Q-TOF LC-MS spectrometer. Experimental ECD spectra were determined on a JASCO J-1500 spectrometer. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., People's Republic of China), RP-C18 silica gel (50 μm, YMC, Japan), MCI gel (CHP20, 75–150 μm, Mitsubishi Chemical Ltd., Japan), and Sephadex LH-20 gel (Pharmacia Biotech, Sweden). TLC analyses were carried out on the precoated silica gel GF254 plates (Qingdao Marine Chemical Ltd.). Semipreparative reversed-phase HPLC (RP-HPLC) was performed using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm) on an LC3000 instrument (Chuang Xin Tong Heng Science and Technology Ltd., Beijing, People's Republic of China) equipped with a UV3000 detector (Chuang Xin Tong Heng Science and Technology). Chiral separation was performed using a Chiralpak AD-H (5 μm, 10 × 250 mm) or ID column (5 μm, 4.6 × 250 mm).

* Corresponding author.
E-mail address: zhangguijie001@126.com (G.-J. Zhang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bioorg.2020.104147
Received 13 March 2020; Received in revised form 19 May 2020; Accepted 25 July 2020
Available online 28 July 2020

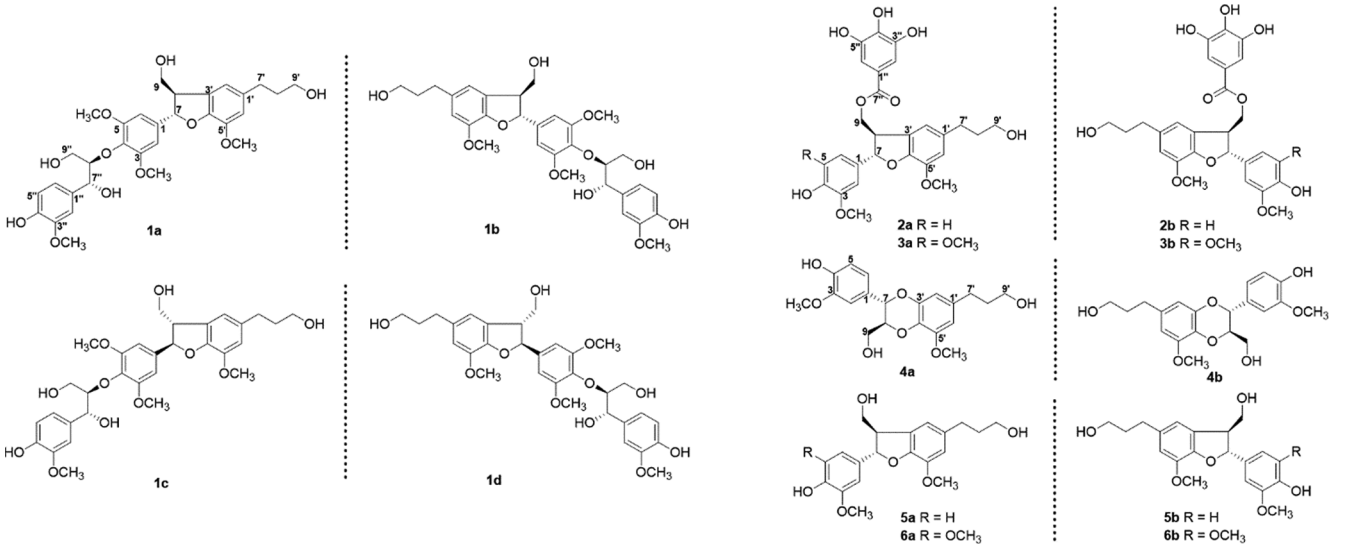

Fig. 1. The structures of enantiomers.

2.3. Extraction and isolation

The air-dried stems and leaves of *T. sebifera* (18 kg) were extracted with 95% aqueous EtOH (3 × 100 L, each 3 h) under reflux. The filtrate was evaporated under reduced pressure, and the residue (0.78 kg) was dispersed in H_2O, and then sequentially partitioned with petroleum ether, EtOAc, and *n*-BuOH.

The EtOAc fraction (257 g) was subjected to silica gel (200–300 mesh) CC, eluting with CH_2Cl_2/MeOH (100:3 to 1:1) to yield seven fractions (A − K). Fraction H (41 g) was separated into ten subfractions (H1 − H10) via MCI CC, eluting with a gradient of MeOH/H_2O (20:80 to 100:0). Subfraction H3 (5.8 g) was separated by RP-C18 CC (MeOH/H_2O, 20:80 to 80:20) to yield 18 subfractions (H3a − H3r). H3k (762 mg) was subjected to Sephadex LH-20 CC (MeOH) and then purified by semi-preparative RP-HPLC (CH_3CN/H_2O, 20:80, 8 mL/min) to yield **5** (17.5 mg, t_R 65.4 min) and **6** (20.0 mg, t_R 59.2 min). Compound **5** was separated by Daicel Chiralpak AD-H (*n*-hexane/EtOH, 80:20, 3 mL/min) to yield **5a** (3 mg, t_R 20.9 min) and **5b** (1.5 mg, t_R 24.8 min). Compounds **6a** (1.5 mg, t_R 24.8 min) and **6b** (3.0 mg, t_R 20.9 min) were obtained by Daicel Chiralpak ID CC (*n*-hexane/isopropanol, 65:35, 0.7 mL/min). Subfraction H4 (3.9 g) was separated by RP-C18 CC (MeOH/H_2O, 40:60 to 80:20) to yield 14 subfractions (H4a − H4n). Subfraction H4g (427 mg) was subjected to Sephadex LH-20 CC (MeOH) and then purified by semi-preparative RP-HPLC with CH_3CN/H_2O (28:72, 8 mL/min) to yield **4** (3 mg, t_R 23.0 min). Compounds **4a** (1.5 mg, t_R 23.5 min) and **4b** (1.1 mg, t_R 14.5 min) were obtained by chiral HPLC using a Daicel Chiralpak AD-H eluting with *n*-hexane/isopropanol (75:25, 3 mL/min). Subfraction H4h (443 mg) was purified by semi-preparative RP-HPLC with CH_3CN/H_2O (28:72, 8 mL/min) to yield **1** (10 mg, t_R 27.0 min). Compound **1** was separated by Daicel Chiralpak AD-H (*n*-hexane/EtOH, 80:20, 3 mL/min) to yield **1b** (2 mg, t_R 43.0 min) and **1d** (2 mg, t_R 48.0 min), and then eluted with *n*-hexane/isopropanol (70:30, 3 mL/min) to yield **1a** (2 mg, t_R 20.5 min) and **1c** (2 mg, t_R 17.5 min). Subfraction H4j (175 mg) was purified by semi-preparative RP-HPLC with CH_3CN/H_2O (35:65, 8 mL/min) to yield **2** (3.0 mg, t_R 19.0 min) and **3** (4.0 mg, t_R 17.0 min). Compounds **2a** (1.5 mg, t_R 17.0 min) and **2b** (1.1 mg, t_R 30.5 min) were obtained using a Daicel Chiralpak AD-H eluting with *n*-hexane/isopropanol (65:35, 3 mL/min). Compound **3** was separated by a Daicel Chiralpak AD-H eluting with *n*-hexane/isopropanol (65:35, 3 mL/min) to yield **3a** (1.8 mg, t_R 24.3 min) and **3b** (1.3 mg, t_R 40.0 min).

4″-hydroxy-3,3″,5,5′-tetramethoxy-4′,7-epoxy-4,8″-oxy-8,3′-sesquineoligna-7″,9,9′,9″-tetrol ***(1)***: white powder; $[\alpha]_D^{20}$ − 7.7 (*c* 0.1, MeOH), UV (MeOH) λ_{max} (log ε) 204 (4.69), 236 (4.04), 280 (3.44) nm; IR (KBr) ν_{max} 3399, 2924, 2850, 1600, 1502, 1462, 1423, 1329, 1275, 1214, 1123, 1029, 826 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+) HRESIMS *m/z* 609.2291 $[M + Na]^+$, calcd for $C_{31}H_{38}O_{11}Na$, 609.2306.

*(7S,8R,7″R,8″R)-**1a***: $[\alpha]_D^{20}$ − 40.5 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 240 (−1.91), 295 (+0.41) nm.

*(7R,8S,7″S,8″S)-**1b***: $[\alpha]_D^{20}$ + 41.2 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 240 (+2.38), 295 (−0.55) nm.

*(7R,8S,7″R,8″R)-**1c***: $[\alpha]_D^{20}$ − 11.5 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 214 (−7.40), 240 (−3.78), 295 (−0.82) nm.

*(7S,8R,7″S,8″S)-**1d***: $[\alpha]_D^{20}$ + 13.2 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 214 (+5.22), 240 (+4.15), 295 (+0.93) nm.

4-hydroxy-3,5′-dimethoxy-9-(3,4,5-trihydroxy)benzoyloxy-4′,7-epoxy-8,3′-neoligna-9′-ol ***(2)***: white powder; $[\alpha]_D^{20}$ + 1.5 (*c* 0.3, MeOH), UV (MeOH) λ_{max} (log ε) 207 (4.56), 281 (3.81) nm; IR (KBr) ν_{max} 3432, 2924, 1610, 1515, 1460, 1212, 1124, 1032, 579 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+) HRESIMS *m/z* 513.1749 $[M + H]^+$, calcd for $C_{27}H_{29}O_{10}$, 513.1755.

*(7S,8R)-**2a***: $[\alpha]_D^{20}$ + 47.3 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 212 (+6.04), 290 (+1.73) nm.

*(7R,8S)-**2b***: $[\alpha]_D^{20}$ − 48.5 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 212 (−3.39), 290 (−0.81) nm.

4-hydroxy-3,5,5′-trimethoxy-9-(3,4,5-trihydroxy)benzoyloxy-4′,7-epoxy-8,3′-neoligna-9′-ol ***(3)***: white powder; $[\alpha]_D^{20}$ + 3.4 (*c* 0.3, MeOH), UV (MeOH) λ_{max} (log ε) 208 (4.67), 281 (3.73) nm; IR (KBr) ν_{max} 3294, 2922, 1612, 1463, 1323, 1214, 1114 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+) HRESIMS *m/z* 543.1869 $[M + H]^+$, calcd for $C_{28}H_{31}O_{11}$, 543.1861.

*(7S,8R)-**3a***: $[\alpha]_D^{20}$ + 28.3 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 212 (+7.79), 290 (+2.36) nm.

*(7R,8S)-**3b***: $[\alpha]_D^{20}$ − 30.8 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 212 (−3.39), 290 (−1.73) nm.

4-hydroxy-3,5′-dimethoxy-3′,7-epoxy-8,4′-oxyneoligna-9,9′-diol ***(4)***: white powder; $[\alpha]_D^{20}$ − 10.0 (*c* 0.1, MeOH), UV (MeOH) λ_{max} (log ε) 207 (4.06), 232 (3.53), 279 (2.94) nm; IR (KBr) ν_{max} 3708, 2920, 2851, 1734, 1466, 1362, 1119 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+) HRESIMS *m/z* 377.1593 $[M + H]^+$, calcd for $C_{20}H_{25}O_7$, 377.1595.

*(7S,8S)-**4a***: $[\alpha]_D^{20}$ + 18.2 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 233 (+4.18) nm.

*(7R,8R)-**4b***: $[\alpha]_D^{20}$ − 20.8 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 233 (−3.47) nm.

4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-ol ***(5)***: colorless oil; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+) HRESIMS *m/z*

Table 1
^{1}H NMR Data for Compounds **1–6** (δ in ppm, J in Hz).

no.	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]	**6**[b]
2	6.73 s	6.88 d (1.8)	6.60 s	6.99 d (1.8)	6.93 d (1.6)	6.65 s
5		6.75 d (7.8)		6.83 d (8.1)	6.85 d (8.4)	
6	6.73 s	6.82 dd (7.8, 1.8)	6.60 s	6.88 dd (8.1, 1.8)	6.90 dd (8.4, 1.6)	6.65 s
7	5.55 d (6.0)	5.55 d (6.6)	5.57 d (6.0)	4.86 d (8.4)	5.53 d (7.2)	5.53 d (7.6)
8	3.44 m	3.78 m	3.79 m	3.99 m	3.59 m	3.60 m
9a	3.87 m	4.57 dd (10.8, 5.4)	4.62 dd (10.8, 4.8)	3.69 dd (12.6, 2.4)	3.95 dd (11.6, 6.0)	3.97 dd (11.2, 2.0)
9b	3.77 m	4.47 dd (10.8, 7.8)	4.46 dd (10.8, 8.4)	3.48 dd (12.6, 4.8)	3.90 m	3.90 m
2′	6.72 br.s	6.76 br.s	6.765 br.s	6.42 d (1.8)	6.67 br.s	6.67 br.s
6′	6.74 br.s	6.76 br.s	6.771 br.s	6.47 d (1.8)	6.67 br.s	6.67 br.s
7′	2.62 t (7.8)	2.63 t (7.8)	2.64 t (7.8)	2.58 t (7.2)	2.66 t (7.6)	2.67 t (7.6)
8′	1.81 m	1.81 m	1.81 m	1.81 m	1.88 m	1.88 m
9′	3.56 t (6.6)	3.56 t (6.6)	3.56 t (6.6)	3.56 t (6.6)	3.68 t (6.4)	3.69 t (6.4)
1″						
2″	6.99 d (2.4)	7.04 s	7.06 s			
5″	6.74 d (8.4)					
6″	6.86 dd (8.4, 2.4)	7.04 s	7.06 s			
7″	4.98 d (7.2)					
8″	4.06 m					
9″	3.75 m					
	3.32 m					
OMe-3	3.82 s	3.72 s	3.71 s	3.87 s	3.85 s	3.85 s
OMe-5	3.82 s		3.71 s			3.85 s
OMe-5′	3.82 s	3.87 s	3.88 s	3.86 s	3.87 s	3.88 s
OMe-3″	3.87 s					

[a] Data were recorded at 600 MHz in MeOH-d_4.
[b] Data were recorded at 400 MHz in $CDCl_3$.

Table 2
^{13}C NMR Data for Compounds **1–6.**

no.	**1**[a]	**2**[a]	**3**[a]	**4**[a]	5[b]	**6**[b]
1	139.8	134.2	133.5	129.6	133.1	132.2
2	103.8	110.3	103.7	112.0	108.8	103.1
3	154.3	149.1	149.4	149.2	146.6	147.1
4	136.7	147.6	136.3	148.3	145.6	134.6
5	154.3	116.2	149.4	116.2	114.3	147.1
6	103.8	119.5	103.7	121.6	119.4	103.1
7	88.5	89.3	89.5	77.6	87.9	88.1
8	55.8	52.5	52.7	79.9	53.8	53.8
9	65.0	66.9	67.1	62.2	63.9	63.7
1′	137.2	137.2	137.3	135.7	135.4	135.5
2′	117.9	117.8	117.8	110.5	116.0	115.9
3′	129.5	128.6	128.5	145.6	127.8	127.7
4′	147.4	147.6	147.6	132.5	146.5	146.5
5′	145.3	145.3	145.3	149.9	144.2	144.2
6′	114.1	114.4	114.5	106.1	112.4	112.4
7′	32.9	32.8	32.9	32.9	32.0	32.0
8′	35.8	35.7	35.8	35.5	34.6	34.6
9′	62.2	62.2	62.2	62.2	62.3	62.2
1″	133.4	121.1	121.2			
2″	111.6	110.2	110.2			
3″	148.7	146.6	146.6			
4″	147.1	140.1	140.1			
5″	115.8	146.6	146.6			
6″	120.8	110.2	110.2			
7″	74.5	168.2	168.1			
8″	88.9					
9″	61.7					
OMe-3	56.6	56.2	56.6	56.4	56.0	56.3
OMe-5	56.6		56.6			56.3
OMe-5′	56.3	56.7	56.8	56.6	56.0	56.0
OMe-3″	56.8					

[a] Data were recorded at 151 MHz in MeOH-d_4.
[b] Data were recorded at 101 MHz in $CDCl_3$.

361.1647 $[M + H]^+$, calcd for $C_{20}H_{25}O_6$, 361.1646.

*(7S,8R)-**5a***: $[\alpha]_D^{20}$ + 4.8 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 210 (+6.07), 290 (+0.74) nm.

*(7R,8S)-**5b***: $[\alpha]_D^{20}$ − 4.7 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 210 (−5.53), 290 (−0.85) nm.

4-hydroxy-3,5,5′-trimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-ol ***(6)***: *white powder*; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; (+) HRESIMS *m/z* 413.1573 $[M + Na]^+$, calcd for $C_{21}H_{26}O_7Na$, 413.1571.

*(7S,8R)-**6a***: $[\alpha]_D^{20}$ − 10.3 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 217 (+2.18), 293 (+0.48) nm.

*(7R,8S)-**6b***: $[\alpha]_D^{20}$ + 8.7 (*c* 0.05, MeOH); ECD (MeOH) λmax (Δε) 217 (−0.78), 293 (−0.45) nm.

2.4. NO production measurement and cell viability assay

The accumulation of nitrite (NO_2^-) in the culture medium supernatants was measured using the Griess reaction [9,10]. BV2 cells were plated in 96-well microtiter plates and treated with each compound at various concentrations (1, 10, 25, and 50 μM) in the presence of LPS (100 ng/mL) for 24 h. The absorbance was measured on a plate reader (Bio-Tek, Winooski, VT, USA) at 540 nm. And cell viability was assessed by the MTT assay as previously reported [15].

3. Results and discussion

Compound **1** was obtained as a white powder. Its molecular formula, $C_{31}H_{38}O_{11}$, was established by the positive-ion HRESIMS data (*m/z* 609.2291 $[M + Na]^+$, calcd 609.2306) and the ^{13}C NMR data (Table 2) of this compound. The presence of hydroxy (3399 cm^{-1}) and aromatic ring (1600, 1502, 1462, 1423 cm^{-1}) groups was determined by their IR absorption bands. Analysis of the low-field region of its ^{1}H NMR spectrum indicated the presence of a symmetric 1,3,4,5-tetrasubstituted phenyl [δ_H 6.73 (2H, s)], an asymmetric 1,3,4,5-tetrasubstituted phenyl [δ_H 6.74 (1H, br.s) and 6.72 (1H, br.s)], and a 1,3,4-trisubstituted phenyl [δ_H 6.99 (1H, d, J = 2.4 Hz), 6.86 (1H, dd, J = 8.4, 2.4 Hz), and 6.74 (1H, d, J = 2.4 Hz)]. The ^{13}C NMR and HSQC spectra showed 31 carbon resonances, including 18 aromatic carbons, four methoxy carbons, five methylenes (three oxygenated), and four methines (three oxygenated). The above data suggested that **1** was a sesquineolignane [16,17]. The HMBC correlations (Fig. 2) from H-2 (H-6) (δ_H 6.73) to C-4/C-7, from H-7 (δ_H 5.55) to C-9/C-3′/C-4′, from H-8 (δ_H 3.44) to C-1/C-2′/C-4′, from H-7′ (δ_H 2.62) to C-2′/C-6′/C-9′, from H-8′ (δ_H 1.81) to C-1′, from O*Me*-3,5 (δ_H 3.82) to C-3 (C-5), and

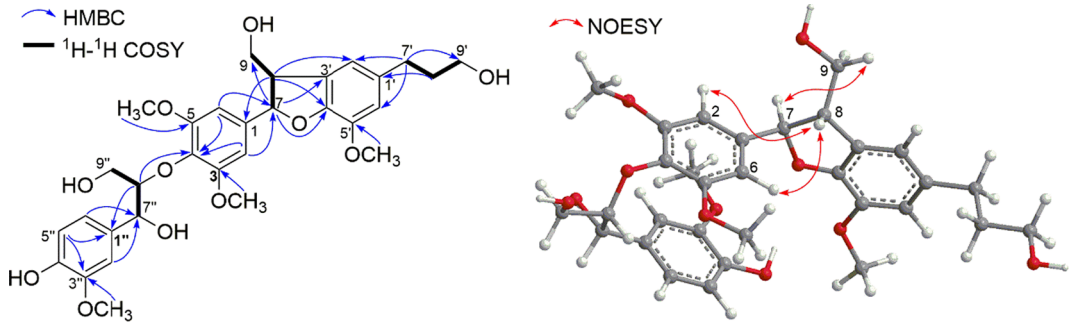

Fig. 2. Key ^{1}H–^{1}H COSY, HMBC and NOESY correlations of **1**.

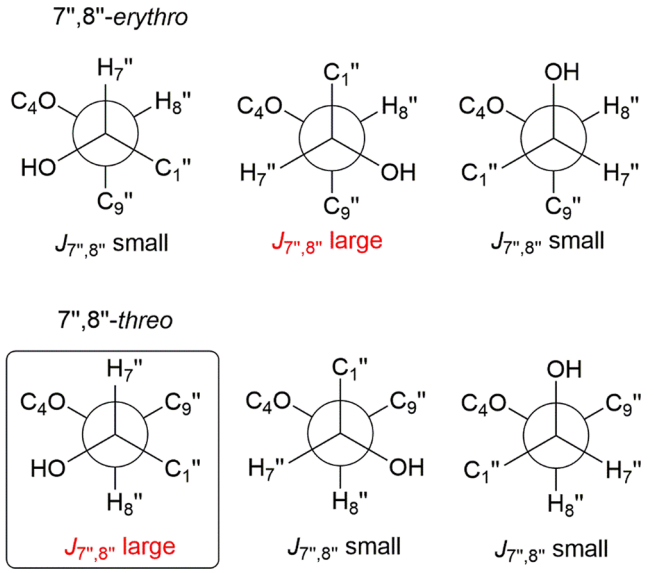

Fig. 3. Stereochemistry of H-7″ and H-8″ in **1**. Box indicates conformation that agrees with measured data.

from O*Me*-5′ (δ_H 3.82) to C-5′, together with the ^{1}H–^{1}H COSY correlations (Fig. 2) of H-7/H-8/H_2-9 and H_2-7′/H_2-8′/H_2-9′ revealed the presence of a 4-substituted 3,5,5′-trimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-diol moiety. In addition, the HMBC correlations (Fig. 2) from H-2″ (δ_H 6.99)/H-6″ (δ_H 6.86) to C-7″, from H-5″ (δ_H 6.74) to C-1″/C-3″, from H-8″ (δ_H 4.06) to C-1″, from O*Me*-3″ (δ_H 3.87) to C-3″, combined with the ^{1}H–^{1}H COSY correlations (Fig. 2) of H-7″/H-8″/H_2-9′ indicated the presence of an arylglycerol unit. Furthermore, the above two units were connected through C-4 and C-8″, which was confirmed by the HMBC correlation (Fig. 2) from H-8″ to C-4. The NOESY correlations (Fig. 2) of H-7/H-9 (δ_H 3.77) and H-8/H-2 (H-6), combined with the coupling constant ($J_{7,8}$ = 6.0 Hz) indicated a *trans* configuration between H-7 and H-8 [17]. The large coupling constant between H-7″ and H-8″ (J = 7.2 Hz) verified the 7″,8″-*threo* configuration for **1** [12,18] (Fig. 3). Thus, compound **1** was established as 7,8-*trans*-7″,8″-*threo*-4″-hydroxy-3,3″,5,5′-tetramethoxy-4′,7-epoxy-4,8″-oxy-8,3′-sesquineoligna-7″,9,9′,9″-tetrol.

As more and more lignans were reported as partial racemates in natural products [12,13], compound **1** was subsequently analyzed by chiral HPLC using a Daicel Chiralpak AD-H column, yielding two pairs of enantiomers (**1a**/**1b** and **1c**/**1d**). Compounds **1a** and **1b** showed mirror image-like ECD curves (Fig. 4A) and specific rotations (**1a**: $[\alpha]_D^{20}$ − 40.5; **1b**: $[\alpha]_D^{20}$ + 41.2), and similarly **1c** and **1d** displayed opposite ECD curves (Fig. 4A) and specific rotations (**1c**: $[\alpha]_D^{20}$ − 11.5, **1d**: $[\alpha]_D^{20}$ + 13.2). On the basis of the reversed helicity rule [18,19], the positive Cotton effect at 295 nm indicated a 7*S*,8*R* configuration for **1a** and **1d**, while the negative Cotton effect at 295 nm of **1b** and **1c**, in turn, indicated a 7*R*,8*S* configuration for these two stereoisomers. In addition, the positive Cotton effect around 240 nm in the ECD spectra (Fig. 4A) revealed an 8″*S* configuration for **1b** and **1d**, meanwhile, the 8″*R* configuration for **1a** and **1c** was supported by the negative Cotton effect around 240 nm [12,20]. The 7″,8″-*threo* relative configuration allowed to assign the 7″*S* and 7″*R* absolute configuration for **1b**/**1d** and **1a**/**1c**, respectively. Therefore, the two pairs of enantiomers were assigned as follows: (−)-(7*S*,8*R*,7″*R*,8″*R*)-4″-hydroxy-3,3″,5,5′-tetramethoxy-4′,7-epoxy-4,8″-oxy-8,3′-sesquineoligna-7″,9,9′,9″-tetrol (**1a**), (+)-(7*R*,8*S*,7″*S*,8″*S*)-4″-hydroxy-3,3″,5,5′-tetramethoxy-4′,7-epoxy-4,8″-oxy-8,3′-sesquineoligna-7″,9,9′,9″-tetrol (**1b**), (−)-(7*R*,8*S*,7″*R*,8″*R*)-4″-hydroxy-3,3″,5,5′-tetramethoxy-4′,7-epoxy-4,8″-oxy-8,3′-sesquineoligna-7″,9,9′,9″-tetrol (**1c**), and (+)-(7*S*,8*R*,7″*S*,8″*S*)-4″-hydroxy-3,3″,5,5′-tetramethoxy-4′,7-epoxy-4,8″-oxy-8,3′-sesquineoligna-7″,9,9′,9″-tetrol (**1d**).

Compound **2** was obtained as a white powder. Its molecular formula was determined to be $C_{27}H_{28}O_{10}$ by the HRESIMS ion at *m/z* 513.1749 $[M + H]^+$ (calcd 513.1755). The ^{1}H NMR spectrum displayed a 1,3,4-trisubstituted [δ_H 6.88 (1H, d, J = 1.8 Hz), 6.82 (1H, dd, J = 7.8, 1.8 Hz), and 6.75 (1H, d, J = 7.8 Hz)] and two 1,3,4,5-tetrasubstituted [δ_H 7.04 (2H, s), 6.76 (2H, br.s)] aromatic rings. The ^{13}C NMR and HSQC spectra displayed 27 carbons, ascribed to one carbonyl, 18 aromatic carbons, two methoxy groups, four methylenes (two oxygenated), and two methines (one oxygenated). According to the above data, together with the 2D NMR analysis, the structure of **2** was similar to that of 4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-ol (**5**) [21,22], except for the presence of a 3,4,5-trihydroxybenzoyl at C-9 in **2**. The above deduction was confirmed by the HMBC correlation from H_2-9 (δ_H 4.57, 4.47) to C-7″ (δ_C 168.2). The coupling constant of $^3J_{7,8}$ (6.6 Hz) along with the NOESY correlations of H-7/H-9 (δ_H 4.47) and H-8/H-2 (H-6) indicated the 7,8-*trans* configuration for **2**. Thus, compound **2** was determined to be 7,8-*trans*-4-hydroxy-3,5′-dimethoxy-9-(3,4,5-trihydroxy)benzoyloxy −4′,7-epoxy-8,3′-neoligna-9′-ol. Compound **2** was also obtained as a racemate, and the chiral HPLC separation with a Daicel Chiralpak AD-H column afforded a pair of enantiomers **2a** ($[\alpha]_D^{20}$ + 47.3) and **2b** ($[\alpha]_D^{20}$ − 48.5). The two enantiomers showed typical antipodal ECD curves (Fig. 4B). The positive Cotton effect at 290 nm in the ECD spectrum of **2a** suggested that it had a 7*S*,8*R* configuration, on the contrary, its enantiomer **2b** had a 7*R*,8*S* configuration [18,19]. Therefore, compounds **2a** and **2b** were established as (+)-(7*S*,8*R*)-4-hydroxy-3,5′-dimethoxy-9-(3,4,5-trihydroxy) benzoyloxy-4′,7-epoxy-8,3′-neoligna-9′-ol (**2a**) and (−)-(7*R*,8*S*) −4-hydroxy-3,5′-dimethoxy-9-(3,4,5-trihydroxy)benzoyloxy-4′,7-epoxy-8,3′-neoligna-9′-ol (**2b**), respectively.

Compound **3** was isolated as a white powder. The HRESIMS ion at *m/z* 543.1869 $[M + H]^+$ suggested that the molecular formula was $C_{28}H_{30}O_{11}$. The ^{1}H and ^{13}C NMR data of **3** (Tables 1 and 2) showed that it was an analogue of **2**, except for the presence of an additional methoxy group at C-5. The ^{13}C NMR chemical shift of C-5 in **3** (δ_C 149.4; $\Delta\delta_C$ + 33.2) was deshielded compared to the same position in **2**, and the HMBC correlation from O*Me* (6H, δ_H 3.71) to C-3/C-5 also demonstrated the above deduction. Subsequent chiral separation of **3** afforded a pair of enantiomers **3a** and **3b**, which showed opposite ECD

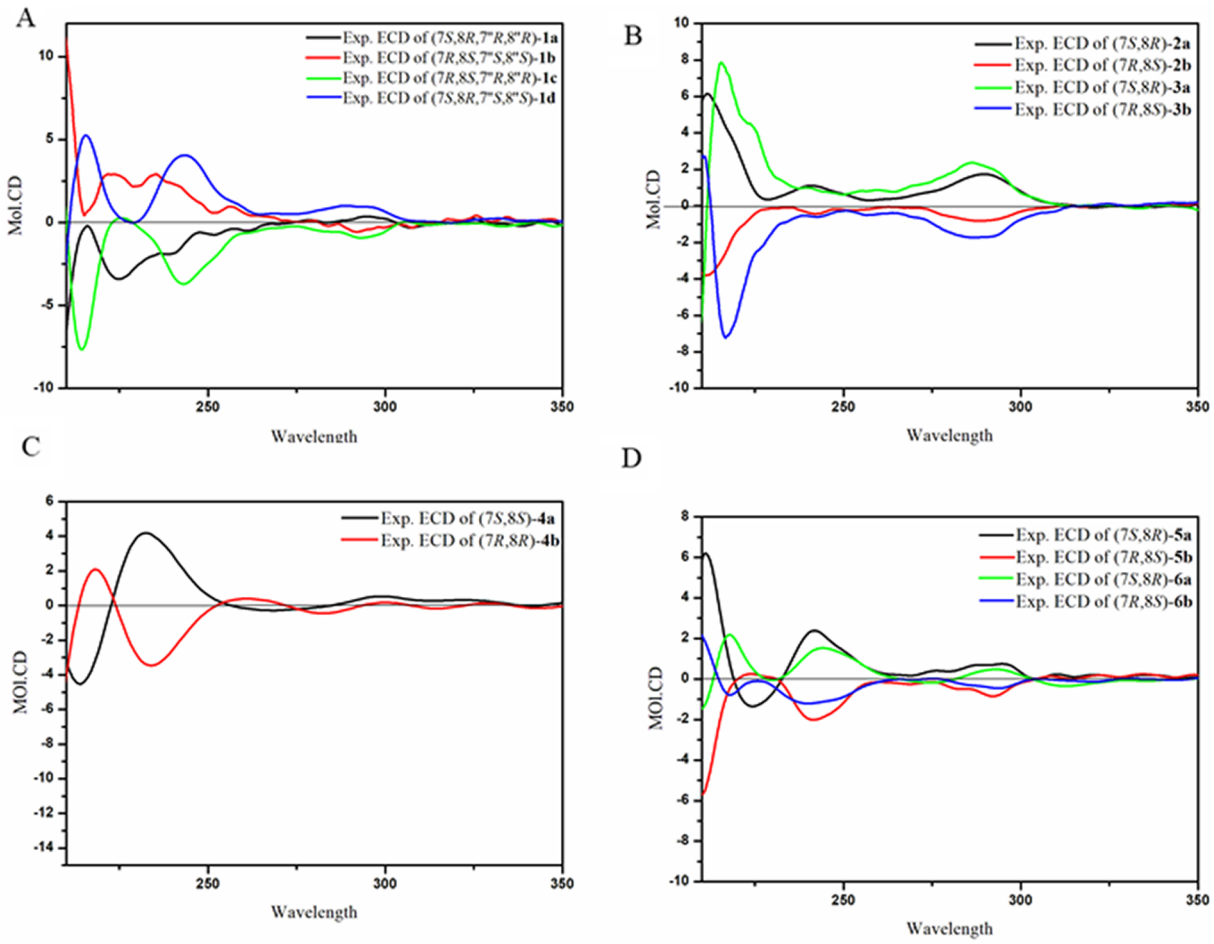

Fig. 4. Experimental ECD spectra of **1a**/**1b** − **4a**/**4b** and **1c**/**1d**.

curves (Fig. 4B) and optical rotations (**3a**: $[\alpha]_D^{20}$ + 28.3, **3b**: $[\alpha]_D^{20}$ − 30.8). In the same way, the absolute configurations of **3a** and **3b** were determined as 7*S*,8*R* and 7*R*,8*S* according to the reversed helicity rule [18,19]. Thus, compounds **3a** and **3b** were defined as (+)-(7*S*,8*R*)-4-hydroxy-3,5,5′-trimethoxy-9-(3,4,5-trihydroxy)benzoyloxy-4′,7-epoxy-8,3′-neoligna-9′-ol (**3a**) and (−)-(7*R*,8*S*)-4-hydroxy-3,5,5′-trimethoxy-9-(3,4,5-trihydroxy)benzoyloxy-4′,7-epoxy-8,3′-neoligna-9′-ol (**3b**), respectively.

Compound **4**, a white powder, showed the molecular formula $C_{20}H_{24}O_7$ as determined by the HRESIMS ion at *m/z* 377.1593 $[M + H]^+$ (calcd 377.1595), requiring nine degrees of unsaturation. The ^{1}H NMR data (Table 1) showed signals of a 1,3,4-trisubstituted aromatic ring [δ_H 6.99 (1H, d, J = 1.8 Hz), 6.88 (1H, dd, J = 8.1, 1.8 Hz), and 6.83 (1H, d, J = 8.1 Hz)] and a 1,3,4,5-tetrasubstituted aromatic ring [δ_H 6.47 (1H, d, J = 1.8 Hz) and 6.42 (1H, d, J = 1.8 Hz)]. The ^{13}C NMR and HSQC spectra revealed the existence of 12 aromatic carbons, four methylenes (two oxygenated), two oxygenated methines and two methoxy groups. The above information suggested **4** was a lignan [12,18]. The ^{13}C NMR chemical shifts of C-7 (δ_C 77.6) and C-8 (δ_C 79.9), along with the degrees of unsaturation implied the presence of a 1,4-dioxane ring in **4** [9,10]. Furthermore, the HMBC correlations from H-2′ (δ_H 6.42) to C-3′ (δ_C 145.6)/C-4′ (δ_C 132.5)/C-6′ (δ_C 106.1), from H-6′ (δ_H 6.47) to C-2′ (δ_C 110.5)/C-4′/C-5′ (δ_C 149.9), and from H-7 (δ_H 4.86) to C-3′ indicated that **4** was a 3′,7-epoxy-8,4′-oxyneolignane [23]. The NMR data of **4** showed distinct similarity to (7*S*,8*S*)-3-methoxy-3′,7-epoxy-8,4′-oxyneolignan-4,9,9′-triol [23], differing only by the presence of signals for a methoxy group [δ_C 56.6, δ_H 3.86 (s)] at C-5′, which was confirmed by the HMBC correlation from O*Me* (δ_H 3.86) to C-5′. In the NOESY spectrum, the correlations of H-7/H-9 (δ_H 3.48) and H-8 (δ_H 3.99)/H-2 (H-6), combined with the coupling constant $^3J_{7,8}$ (8.4 Hz) indicated the 7,8-*trans* configuration for **4**. The chiral HPLC purification of **4** afforded a pair of enantiomers **4a** and **4b**. The enantiomers also displayed typical antipodal ECD curves (Fig. 4C) and opposite optical rotations (**4a**: $[\alpha]_D^{20}$ + 18.2, **4b**: $[\alpha]_D^{20}$ − 20.8). The absolute configurations of **4a** and **4b** were assigned as 7*S*,8*S* and 7*R*,8*R*, respectively, by analyzing the Cotton effect at 233 nm of the benzodioxane system [20,23]. Therefore, compounds **4a** and **4b** were assigned as (+)-(7*S*,8*S*)-4-hydroxy-3,5′-dimethoxy-3′,7-epoxy-8,4′-oxyneoligna-9,9′-diol (**4a**) and (−)-(7*R*,8*R*)-4-hydroxy-3,5′-dimethoxy-3′,7-epoxy-8,4′-oxyneoligna-9,9′-diol (**4b**), respectively.

The known compounds **5** and **6** were also obtained as enantiomers. These enantiomers were identified as (7*S*,8*R*)-4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-ol (**5a**) [21], (7*R*,8*S*)-4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-ol (**5b**) [22], (7*S*,8*R*)-4-hydroxy-3,5,5′-trimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-ol (**6a**) [24] and (7*R*,8*S*)-4-hydroxy-3,5,5′-trimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-ol (**6b**) [25] by comparing NMR data, specific rotations and ECD data with values in the literature.

All the isolated enantiomers were tested for their antineuroinflammatory activities by an NO inhibition assay in LPS-induced BV-2 microglial cells using the Griess reaction [9,10]. The results displayed that **5b**, **6a** and **6b** showed inhibitory activities with IC_{50} values of 14.3 ± 0.3, 23.2 ± 1.4, and 33.3 ± 2.5 μM, respectively, while the positive control minocycline gave the IC_{50} values of 13.5 ± 1.1 μM. Interestingly, compound **5b** showed inhibitory effects, however, its enantiomer **5a** was inactive.

4. Conclusion

In conclusion, seven pairs of lignan enantiomers (**1a**/**1b** − **6a**/**6b** and **1c**/**1d**) were isolated from the stems and leaves of *T. sebifera*. The structures of the enantiomers were elucidated by spectroscopic analysis, and their absolute configurations were determined by the experimental ECD spectra. Detailed analysis of the ECD data of compounds **1a**/**1b**, **1c**/**1d** and **4a**/**4b**, together with literature surveys [12,18,20] indicated that the Cotton effects in the 230–260 nm region could determine the absolute configuration of C-8″ in the arylglycerol unit and C-8 in the 1,4-benzodioxane system. The Cotton effect around 290 nm allowed to define the absolute configuration of C-7 (positive for 7*S* and negative for 7*R*) for the benzofuran neolignans on the basis of the reversed helicity rule [18,19]. In addition, all the enantiomers were screened for their NO inhibitory effects in LPS-induced microglial BV-2 cells, and compounds **5b**, **6a**, and **6b** showed significant activities with IC_{50} values of 14.3–33.3 μM. This study also suggested that we should pay attention to enantiomers from natural products, especially lignans, which may exhibit different activities [12,14].

Declaration of Competing Interest

The authors declare no competing financial interest.

Acknowledgments

This work was supported partially by the National Natural Science Foundation of China (21562017 and 21967007) and the Natural Science Foundation of Guangxi (2018GXNSFAA138051)

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.104147.

References

[1] Z.Y. Wu, X.Q. Chen, Flora Republicae Popularis Sinicae (Zhongguo Zhiwu Zhi), Science Press, Beijing, 1997, p. 14.
[2] X.Y. Qin, J.Y. Luo, Z.G. Gao, Yao Ethnic Medicinals in China (Zhongguo yaoyao Xue), Nationalities Publishing House, Beijing, 2002, p. 403.
[3] F.L. Hsu, Y.Y. Lee, J. Nat. Prod. 57 (1994) 308–312.
[4] R. Fu, Y.T. Zhang, Y.R. Guo, Q.L. Huang, T. Peng, Y. Xu, L. Tang, F. Chen, J. Ethnopharmacol. 147 (2013) 517–524.
[5] L. Gao, Y. Liu, J.L. Gu, H. Tian, L.J. Yang, J. Zhang, Y.F. Wang, J. Asian Nat. Prod. Res. 21 (2019), https://doi.org/10.1080/10286020.2019.1666822.
[6] B.P. Pradhan, S. De, A. Nath, J.N. Shoolery, Phytochemistry 23 (1984) 2593–2595.
[7] L. Gao, H. Tian, P.J. Lv, J.P. Wang, Y.F. Wang, Chin. J. Chin. Mater. Med. 40 (2015) 1518–1522.
[8] P. Yang, A.D. Kinghorn, J. Nat. Prod. 48 (1985) 486–488.
[9] G.J. Zhang, Q.M. Pan, Y.L. Zhang, H.B. Liao, Y.Q. Yang, Y. Hou, D. Liang, J. Nat. Prod. 81 (2018) 2251–2258.
[10] Y.L. Zhang, Q.M. Pan, H.B. Liao, J.K. Qin, N. Li, D. Liang, G.J. Zhang, Fitoterapia 133 (2019) 17–22.
[11] Y.L. Zhang, Q.M. Pan, G.J. Zhang, D. Liang, Chin. J. Chin. Mater. Med. 44 (2019) 3738–3744.
[12] Y.Y. Lu, Y.B. Xue, J.J. Liu, G.M. Yao, D.Y. Li, B. Sun, J.W. Zhang, Y.F. Liu, C.X. Qi, M. Xiang, Z.W. Luo, G. Du, Y.H. Zhang, J. Nat. Prod. 78 (2015) 2205–2214.
[13] C. Jiang, P. Luo, Y. Zhao, J.L. Hong, S.L. Morris-Natschke, J. Xu, C.H. Chen, K.H. Lee, Q. Gu, J. Nat. Prod. 79 (2016) 578–583.
[14] I. Agranat, H. Caner, J. Caldwell, Nat. Rev. Drug Discovery 1 (2002) 753–768.
[15] N. Li, Z.J. Ma, M.J. Li, Y.C. Xing, Y. Hou, J. Ethnopharmacol. 152 (2014) 508–521.
[16] L. Li, N.P. Seeram, J. Agric. Food Chem. 59 (2011) 7708–7716.
[17] T. Morikawa, J. Tao, K. Ueda, H. Matsuda, M. Yoshikawa, Chem. Pharm. Bull. 51 (2003) 62–67.
[18] L. Xiong, C.G. Zhu, Y.R. Li, Y. Tian, S. Lin, S.P. Yuan, J.F. Hu, Q. Hou, N.H. Chen, Y.C. Yang, J.G. Shi, J. Nat. Prod. 74 (2011) 1188–1200.
[19] S. Antus, T. Kurtan, L. Juhasz, L. Kiss, M. Hollosi, Z.S. Majer, Chirality 13 (2001) 493–506.
[20] L.I. Pilkington, D. Barker, J. Org. Chem. 77 (2012) 8156–8166.
[21] T. Deyama, T. Ikawa, S. Kitagawa, S. Nishibe, Chem. Pharm. Bull. 35 (1987) 1785–1789.
[22] Y. Fukuyama, M. Nakahara, H. Minami, M. Kodama, Chem. Pharm. Bull. 44 (1996) 1418–1420.
[23] J.M. Fang, C.K. Lee, Y.S. Cheng, Phytochemistry 31 (1992) 3659–3661.
[24] Q.B. Liu, X.X. Huang, X.J. Yan, M. Bai, L.H. Yu, C. Hu, T. Zhu, L.Z. Li, S.J. Song, Biochem. Syst. Ecol. 55 (2014) 236–240.
[25] Y.W. Chin, H.B. Chai, W.J. Keller, A.D. Kinghorn, J. Agric. Food Chem. 56 (2008) 7759–7764.