Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2021, **19**(10): 796-800
doi: 10.1016/S1875-5364(21)60075-4
Chinese Journal of Natural Medicines

•Reaearch article•

Bioactive sesquineolignans from the twigs of *Litsea cubeba*

XIA Huan[1, 2], XIA Gui-Yang[1, 2], WANG Ling-Yan[2], WANG Min[3], WANG Ya-Nan[2], LIN Peng-Cheng[3*], LIN Sheng[1, 2*]

[1] *Key Laboratory of Chinese Internal Medicine of Ministry of Education and Beijing, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing 100700, China;*
[2] *State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China;*
[3] *College of Pharmaceutical Sciences, Qinghai University for Nationalities, Xining 810000, China*

Available online 20 Aug., 2021

[ABSTRACT] In a continuing search for biological natural products with structure diversity from traditional Chinese herbs, five new sesquineolignans (**1**−**5**) were isolated from an ethyl acetate extract of the twigs of *Litsea cubeba*. Their structures were elucidated based on MS, 1D and 2D NMR spectroscopic data, as well as experimental electronic circular dichroism (ECD) spectra. Compounds **1**−**5** showed moderate inhibitory effects against LPS-induced NO production in RAW264.7 macrophages, with IC_{50} values of 16.2, 20.2, 22.1, 15.1, and 16.6 μmol·L^{-1}, respectively.

[KEY WORDS] *Litsea cubeba*; Sesquineolignans; NO production inhibitory activity
[CLC Number] R284 **[Document code]** A **[Article ID]** 2095-6975(2021)10-0796-05

Introduction

Plants from the *Litsea* species (Lauraceae) are rich in lignans with a broad range of biological activities [1-4]. The dried fruits and roots of *L. cubeba*, called "Bi-cheng-qie" and "Dou-chi-jiang" in Chinese, were recorded in *Chinese Pharmacopoeia* and *Chinese Materia Medica* as two important traditional Chinese herbs, which can promote qi circulation to relieve pain, dispel wind, and eliminate dampness [5, 6]. Previous chemical investigation of the fruits and roots of *L. cubeba* revealed that aporphine-type alkaloids and lignans are the major active components of *L. cubeba* with respect to their anti-thrombotic, anti-inflammatory, and anti-nociceptive properties [3, 4, 7-11].

Our previous investigation of the twigs of *L. cubeba* found 55 aromatic compounds including 30 lignans from the H_2O- and EtOAc-soluble fraction of ethanolic extract. Notably, some lignans showed potent hepatoprotective, HDAC1 inhibitory, and anti-inflammatory activities [12-14]. As part of an ongoing search for biological natural products with structure diversity from *L. cubeba* [15-20], we report herein the isolation, structure elucidation, and biological assays of five new sesquineolignans (**1**−**5**) (Fig. 1) from the remaining EtOAc-soluble fractions.

Results and Discussion

Compound **1** was isolated as white amorphous powder. The IR spectrum displayed absorptions of hydroxy (3422 cm^{-1}), ester (1680 cm^{-1}), and aromatic (1612 cm^{-1} and 1516 cm^{-1}) functionalities. Its molecular formula $C_{30}H_{32}O_9$, with fifteen degrees of unsaturation, was assigned by HR-ESI-MS ion peak at *m*/*z* 559.1936 (Calcd. for $C_{30}H_{32}O_9Na$, 559.1939) combined with NMR data (Table 1). The NMR data of **1** showed resonances attributed to a 1, 2, 4-substituted benzene ring [δ_H 7.07 (1H, d, *J* = 1.5 Hz, H-2), 6.82 (1H, d, *J* = 8.0 Hz, H-5), and 6.91 (1H, dd, *J* = 8.0, 1.5 Hz, H-6); δ_C 133.7 (C-1), 110.6 (C-2), 148.4 (C-3), 147.5 (C-4), 115.7 (C-5), and 119.9 (C-6)] and a 1, 2, 3, 5-substituted benzene ring [δ_H 6.77 (1H, brs, H-2′) and 6.78 (1H, brs, H-6′); δ_C 136.7 (C-1′), 114.2 (C-2′), 145.0 (C-3′), 147.3 (C-4′), 128.6 (C-5′), and 117.3 (C-6′)]. Additionally, ^1H and ^{13}C NMR data revealed the presence of two methines [δ_H 5.51 (1H, d, *J* = 7.5 Hz, H-

[Received on] 26-Feb.-2021
[Research funding] This work was supported by the National Natural Science Foundation of China (Nos. 81903489 and 81522050), the Science and Technology Program of Qinghai (No. 2018-ZJ-739), the Innovation Platform for the Development and Construction of Special Project of Key Laboratory for Tibet Plateau Phytochemistry of Qinghai Province (No. 2017-ZJ-Y19), and the Fundamental Research Funds for the Central Universities (No. 3332019080).
[*]**Corresponding author]** E-mails: qhlpc@126.com (LIN Peng-Cheng); lsznn@126.com (LIN Sheng)
These authors have no conflict of interest to declare.

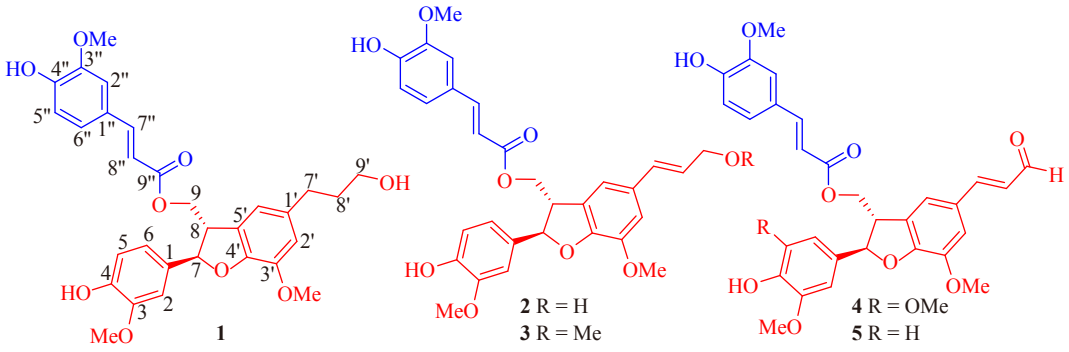

Fig. 1 Structures of compounds 1–5

7) and 3.83 (1H, m, H-8); δ_C 88.9 (C-7) and 51.6 (C-8)], four methylenes [δ_H 4.55 (1H, dd, J = 11.5, 5.5 Hz, H-9a), 4.41 (1H, dd, J = 11.5, 8.0 Hz, H-9b), 2.64 (2H, t, J = 7.5 Hz, H_2-7′), 1.79 (2H, m, H_2-8′) and 3.56 (2H, td, J = 6.5, 4.5 Hz, H_2-9′); δ_C 66.0 (C-9), 32.6 (C-7′), 35.9 (C-8′), and 61.7 (C-9′)], and two aromatic methoxy groups [δ_H 3.79, 3.84 (each 3H, s, 3-and 3′-OMe); δ_C 56.4 and 56.3 (3-and 3′-OMe)]. The above NMR data were consistent with the aglycone portion of a lignan glycoside isolated from the title plant by our group [18], suggesting a 4, 9, 9′-trihydroxy-3′, 3-dimethoxy-4′, 7-epoxy-8, 5′-neoligan moiety in **1**. This deduction was confirmed by 2D NMR COSY and HMBC correlations as depicted in Fig. 2. Furthermore, an *E*-feruloyl moiety was readily assigned by the remaining characteristic NMR signals (Table 1) [13]. Therefore, **1** was a sesquineolignan containing a 4, 9, 9′-trihydroxy-3, 3′-dimethoxy-4′, 7-epoxy-8, 5′-neoligan and an *E*-feruloyl moiety. Finally, the *E*-feruloyl moiety was linked to C-9 to form an ester bond, thereby completing the planar structure of **1**, according to the key HMBC correlation from H_2-9 to C-9". The coupling constant of H-7 (J = 7.5 Hz) required a *trans* relationship of H-7/H-8 as previously reported for 4′, 7-epoxy-8, 3′-neolignans [2, 21]. NOESY correlations of H-7 with H_2-9 and H-8 with H-2(6) were in full support of the *trans*-arrangement between H-7 and H-8. The absolute configuration of **1** was established as 7*R*, 8*S* by the negative Cotton effects at 212 nm and 297 nm in its ECD spectrum (Fig. S10, Supporting Information), showing an ECD curve trend similar to that of (7*R*, 8*S*)-3′-*O*-methylcedrusin 9-*p*-coumarate [22] and litsecol B, the latter of which was a known sesquineolignan obtained from the roots and stems of *L. cubeba* [2]. Thus, the structure of **1** was assigned as (−)-(7*R*, 8*S*)-9-*O*-(*E*)-feruloyl-4, 9, 9′-trihydroxy-3, 3′-dimethoxy-4′, 7-epoxy-8, 5′-neolignan.

Compound **2** was obtained as white powder with the molecular formula $C_{30}H_{30}O_9$ established by HR-ESI-MS ion peak at *m*/*z* 557.1794 (Calcd. for $C_{30}H_{30}O_9Na$, 557.1782), 2 Da less than **1**. The presence of an *E*-disubstituted double bond [δ_H 6.54 (1H, d, J = 15.5 Hz, H-7′) and 6.27 (1H, dt, J = 15.5, 6.0 Hz, H-8′; δ_C 130.2 (C-7′) and 128.8 (C-8′)] were observed in the ^{1}H and ^{13}C NMR spectra of **2**. These data indicated that **2** was the *E* $\Delta^{7'(8')}$ double bond analogue of **1**, which was evident from the 2D NMR experiments of **2** (Fig. 2). The absolute configuration of **2** was defined as 7*R*, 8*S* by comparing the ECD data with those of **1** and litsecol B (Fig. S20, Supporting Information). [2] Thus, the structure of **2** was determined as (−)-(7*R*, 8*S*, 7′*E*)-9-*O*-(*E*)-feruloyl-4, 9, 9′-trihydroxy-3, 3′-dimethoxy-4′, 7-epoxy-8, 5′-neolignan-7′-ene.

The molecular formula $C_{31}H_{32}O_9$ of **3** had a CH_2 more than that of **2**. Comparison of the NMR data between **2** and **3** showed that **3** was the 9′-*O*-methyl derivative of **2** because an *O*-methyl signal [δ_H 3.29 (3H, s); δ_C 57.9] appeared in the ^{1}H and ^{13}C NMR spectra of **3** and the resonance of C-9′ was significantly shifted from δ_C 63.3 of **2** to δ_C 73.7 of **3**. Therefore, the structure of **3** was determined as (–)-(7*R*, 8*S*, 7′*E*)-9-*O*-(*E*)-feruloyl-4, 9-dihydroxy-3, 3′, 9′-trimethoxy-4′, 7-epoxy-8, 5′-neolignan-7′-ene.

Compound **4**, obtained as white power, was assigned the molecular formula $C_{31}H_{30}O_{10}$ by the HR-ESI-MS ion peak at *m*/*z* 585.1753 $[M + Na]^+$ (Calcd. for $C_{31}H_{30}O_{10}$, 585.1731). The ^{1}H and ^{13}C NMR spectra of **4** were very similar to those of litsecol B [2]. However, the data for **4** contained NMR signals for a conjugated aldehyde group [δ_H 9.64 (1H, d, J = 7.8 Hz, H-9′); δ_C 193.9] in place of the signals for the oxymethylene observed in litsecol B. These observations, coupled with the HMBC cross-peaks from H-9′ to C-7′ and C-8′ verified that **4** was the CHO-9 analogue of litsecol B (Fig. 2). Similar to **1** and **2**, comparing the ECD spectra with those of (7*R*, 8*S*)-3′-*O*-methylcedrusin 9-*p*-coumarate and litsecol B (Fig. S32, Supporting Information) [2, 22], revealed that the absolute configuration of **4** was 7*R*, 8*S*. Hence, the structure of **4** was elucidated as (–)-(7*R*, 8*S*, 7′*E*)-9-*O*-(*E*)-feruloyl-4, 9-dihydroxy-3, 5, 3′-trimethoxy-4′, 7-epoxy-8, 5′-neolignan-7′-ene-9′-al.

It was obvious that compound **5** was the 5-demethxoy derivative of **4** because the molecular formula of **5** had a CH_2O less than that of **4** and the NMR resonances for the symmetrically tetrasubstituted aromatic ring with two methoxy groups of **4** were replaced by resonances attributed to a 1, 2, 4-trisubstituted benzene moiety and one methoxy group of **5** (Table 1). This deduction was confirmed by the 2D COSY, HSQC, HMBC, and NOESY NMR experiments of **5**. Therefore, the structure of **5** was established as (–)-(7*R*, 8*S*, 7′*E*)-9-*O*-(*E*)-feruloyl-4, 9-dihydroxy-3, 3′-dimethoxy-4′, 7-epoxy-8, 5′-neolignan-7′-ene-9′-al.

Compounds **1**–**5** were evaluated for their inhibitory activity against NO production in LPS-induced RAW264.7 macrophage cells. Compounds **1**−**5** showed moderate inhibit-

Table 1 NMR spectroscopic data (δ) of compounds 1−5 in acetone-d_6

No.	**1** [a]		**2** [a]		**3** [a]		**4** [b]		**5** [b]	
	δ_H (mult. *J*, Hz)	δ_C	δ_H (mult. *J*, Hz)	δ_C	δ_H (mult. *J*, Hz)	δ_C	δ_H (mult. *J*, Hz)	δ_C	δ_H (mult. *J*, Hz)	δ_C
1		133.7		133.5		133.6		131.8		133.0
2	7.07 d (1.5)	110.6	7.07 d (1.5)	110.7	7.08 d (2.0)	110.8	6.82 s	104.9	7.11d (1.8)	111.0
3		148.4		148.4		148.6		148.3		148.6
4		147.5		147.6		147.7		137.1		147.8
5	6.82 d (8.0)	115.7	6.83 d (8.5)	115.8	6.84 d (8.0)	115.9		148.3	6.85 d (7.8)	115.9
6	6.91 dd (8.0, 1.5)	119.9	6.92 dd (8.5, 1.5)	119.9	6.93 dd (8.0, 2.0)	120.1	6.82 s	104.9	6.95 dd (7.8, 1.8)	120.2
7	5.51 d (7.5)	88.9	5.56 d (7.5)	89.2	5.57 d (7.5)	89.4	5.67 d (7.8)	90.5	5.68 d (7.8)	90.2
8	3.83 m	51.6	3.84 m	51.4	3.85 m	51.5	3.92 m	51.0	3.94 m	51.1
9	4.55 dd (11.5, 5.5) 4.41 dd (11.5, 8.0)	66.0	4.56 dd (11.0, 5.5) 4.45 dd (11.0, 4.0)	65.9	4.58 dd (11.0, 5.5) 4.45 dd (11.0, 7.5)	66.0	4.64 dd (11.4, 5.4) 4.51 dd (11.4, 7.8)	65.7	4.62 dd (11.4, 5.4) 4.51 dd (11.4, 7.8)	65.8
1′		136.7		132.3		132.0		129.4		129.5
2′	6.77 brs	114.2	7.00 brs	111.9	7.03 brs	112.2	7.35 d (1.2)	113.9	7.35 d (1.8)	114.0
3′		145.0		145.3		145.5		145.7		145.9
4′		147.3		148.8		149.2		152.2		152.4
5′		128.6		129.1		129.3		129.9		130.1
6′	6.78 brs	117.3	7.03 brs	115.8	7.07 brs	116.1	7.39 d (1.2)	119.3	7.40 d (1.8)	119.5
7′	2.64 t (7.5)	32.6	6.54 d (15.5)	130.2	6.56 d (16.0)	130.7	7.61 d (15.6)	153.9	7.62 d (16.2)	154.0
8′	1.79 m	35.9	6.27 dt (15.5, 6.0)	128.8	6.21 dt (16.0, 6.0)	129.3	6.71 dd (15.6, 7.8)	127.4	6.71 dd (16.2, 7,8)	127.4
9′	3.56 td (6.5, 4.5)	61.7	4.19 dd (6.0, 5.0)	63.3	4.01 dd (6.0, 1.5)	73.7	9.64 d (7.8)	193.9	9.64 d (7.8)	193.9
1″		127.3		127.3		127.4		127.2		127.6
2″	7.29 d (1.5)	111.3	7.29 d (1.5)	111.3	7.30 d (2.0)	111.4	7.28 d (1.2)	111.3	7.30 d (1.8)	111.5
3″		148.8		148.8		148.9		148.8		148.9
4″		150.2		150.2		150.3		150.3		150.3
5″	6.84 d (8.5)	116.1	6.86 d (8.5)	116.1	6.87 d (8.0)	116.2	6.86 d (8.4)	116.1	6.87 d (8.4)	116.2
6″	7.11 dd (8.5, 1.5)	124.0	7.11 dd (8.5, 1.5)	124.1	7.12 dd (8.0, 2.0)	124.2	7.12 dd (8.4, 1.2)	124.0	7.12 dd (8.4, 1.8)	124.2
7″	7.52 d (15.5)	146.2	7.52 d (16.0)	146.2	7.53 d (16.0)	146.4	7.52 d (15.6)	146.3	7.53 d (15.6)	146.5
8″	6.37 d (15.5)	115.4	6.54 d (16.0)	114.8	6.38 d (16.0)	115.4	6.38 d (15.6)	115.2	6.39 d (15.6)	115.4
9″		167.3		167.3		167.4		167.3		167.4
OMe-3	3.79 s	56.4	3.81 s	56.3	3.82 s	56.4	3.80 s	56.7	3.83 s	56.6
OMe-5							3.80 s	56.7		
OMe-3′	3.84 s	56.3	3.87 s	56.3	3.88 s	56.5	3.95 s	56.5	3.94 s	56.5
OMe-3″	3.91 s	56.2	3.91 s	56.3	3.92 s	56.5	3.93 s	56.4	3.92 s	56.4
OMe-9′					3.29 s	57.9				

[a] NMR data (δ) were measured at 500 MHz for ^{1}H and 125 MHz for ^{13}C. [b] NMR data (δ) were measured at 600 MHz for ^{1}H and 150 MHz for ^{13}C. Proton coupling constants (*J*) in Hz are given in parentheses. The assignments were based on ^{1}H-^{1}H COSY, HSQC and HMBC experiments.

ory effects against LPS-induced NO production in RAW264.7 macrophages, with IC_{50} values of 16.2, 20.2, 22.1, 15.1, and 16.6 μmol·L^{-1}, respectively. Dexamethasone was used as the positive control with an IC_{50} value of 7.5 μmol·L^{-1}. At concentrations up to 100 μmol·L^{-1}, compounds **1**–**5** were not cytotoxic to LPS-induced RAW264.7 cells. Compounds **1**−**5** were also evaluated for their inhibitory activities against TNF-*α* secretion in mouse peritoneal macrophages [23], PTP1B (protein tyrosine phosphatase 1B) [15], acetaminophen-induced HepG2 cell injury [15], and cytotoxic properties toward HCT-8 colon, A2780 ovary, BGC-823 stomach, Bel-7402 hepatoma, and A549 lung cell lines [24], but inactive at 10 μmol·L^{-1}.

Experimental

General experimental procedures

Optical rotation values were measured with a JASCO P-2000 automatic digital polarimeter (JASCO, Tokyo, Japan).

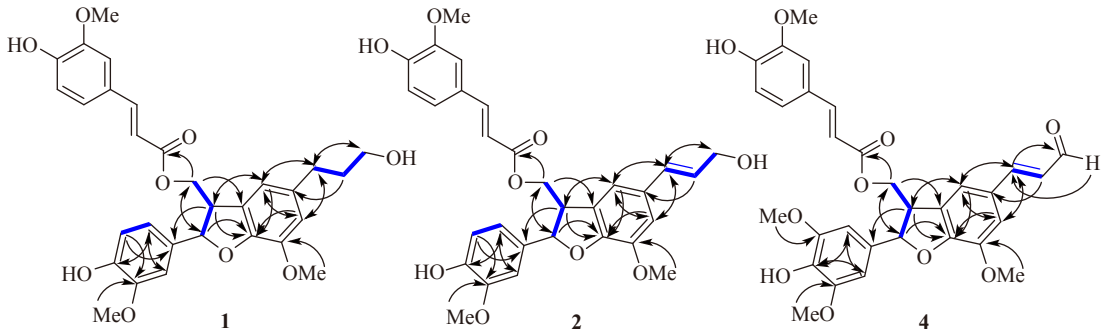

Fig. 2 Key ^{1}H-^{1}H COSY (blue bold lines) and HMBC (arrows) correlations of compounds 1, 2, and 4

UV spectra were recorded on a JASCO V-650 spectrometer (JASCO, Tokyo, Japan). CD spectra were recorded on a JASCO J-815 spectropolarimeter (JASCO, Tokyo, Japan). IR (4000−400 cm^{-1}) spectra (KBr pellets) were recorded on a Nicolet 5700 FT-IR spectrophotometer (Thermo Electron Corporation, Madison, WI, USA). NMR spectra were obtained on a Bruker spectrometer (600 MHz for ^{1}H, 150 MHz for ^{13}C) or a Varian INOVA spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C) equipped with an inverse detection probe (Bruker Corp., Karlsruhe, Germany). Chemical shifts were given in δ (ppm) using the peak signals of the solvent acetone-d_6 as references from Cambridge Isotope Laboratories, and coupling constants are reported in Hz. ESI-MS and HR-ESI-MS data were measured using an AccuToFCS JMS-T100CS spectrometer (Agilent Technologies, Ltd., Santa Clara, CA, USA). Silica gel GF254 prepared for TLC and silica gel (200 to 300 mesh) for column chromatography were obtained from Qingdao Marine Chemical Factory (Qingdao, China). Sephadex LH-20 was a product of Pharmacia Biotech AB (Uppsala, Sweden). HPLC analyses were performed on a Waters HPLC instrument (Waters, USA) equipped with a Waters 600 pump, a Waters 2487 dual absorbance detector, and a Grace (250 mm × 10 mm) semipreparative column packed with C_{18} (5 μm). All the reagents were of HPLC or analytical grade and purchased from Beijing Chemical Works. TLC was carried out with precoated glass silica gel GF_{254} plates (Qingdao Marine Chemical Factory, Qingdao, China). Spots were visualized under UV light or by spraying with 10% H_2SO_4 in EtOH followed by heating.

Plant Material

The twigs of *L. cubeba* were collected in Zhaotong (Yunnan Province, China) in May 2013, and identified by Professor LI Gan-Peng in Yunnan Minzu University. A herbarium specimen was deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica (Beijing , China and herbarium No. 2013-05-10).

Extraction and isolation

The air-dried twigs of *L. cubeba* (12 kg) were ground and extracted using 30.0 L of 95% EtOH under ambient temperature for 3 × 48 h. The EtOH extract was concentrated in vacuo to afford an EtOAc extract (300 g) and a H_2O extract (380 g). The EtOAc fraction was chromatographed over silica gel (1500 g), eluting with a gradient of acetone (0%−100%) in petroleum ether, and 13 fractions (F_1–F_{13}) was obtained with the aid of TLC analysis.

The fraction F_8 (12.0 g) was subjected to reversed-phase flash chromatography over C_{18} silica gel, eluting with a step gradient from 20% to 95% MeOH in H_2O, to give 10 fractions ($F_{8\text{-}1}$−$F_{8\text{-}10}$). Fraction $F_{8\text{-}4}$ (1.5 g) was separated on Sephadex LH-20 eluting with $CHCl_3$−MeOH−PE (5 : 5 : 1, *V/V*) to give four subfractions, and the second subfraction was purified by reversed-phase semipreparative (RP_{18}, 5 μm, 230 nm, MeOH−H_2O, 68 : 32, 1.5 mL·min^{-1}) to afford **1** (5.2 mg, t_R 29.8 min). Using the same HPLC system, the third subfraction afforded **4** (7.3 mg, t_R 36.9 min) and **5** (12 mg, t_R 34.1 min). Fraction $F_{8\text{-}5}$ (0.8 g) was chromatographed over Sephadex LH-20 eluting with $CHCl_3$−MeOH−PE (5 : 5 : 1, *V/V*) to give three subfractions, and the second subfraction was purified by reversed-phase semipreparative HPLC (RP_{18}, 5 μm, 230 nm, MeOH−H_2O, 67 : 33, 2.5 mL·min^{-1}) to give **2** (6.5 mg, t_R 15.9 min) and **3** (8.2 mg, t_R 21.3 min).

*(−)-(7R, 8S)-9-O-(E)-feruloyl-4, 9, 9′-trihydroxy-3, 3′-dimethoxy-4′, 7-epoxy-8, 5′-neolignan (**1**)*

White amorphous powder; $[\alpha]_D^{25}$ −12.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 207 (1.43), 234 (0.52), 287 (0.30), 327 (0.32) nm; ECD (MeOH) λ_{max} (Δε) 212 (−4.75), 297 (1.86) nm; IR (KBr) ν_{max} 3422, 2920, 2851, 1680, 1632, 1604, 1516, 1464, 1430, 1385, 1273, 1207, 1143, 1034, 846, 821, 723 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 1; HR-ESI-MS *m/z* 559.1936 [M + Na]$^+$ (Calcd. for $C_{30}H_{32}O_9Na$, 559.1939).

*(−)-(7R, 8S, 7′E)-9-O-(E)-feruloyl-4, 9, 9′-trihydroxy-3, 3′-dimethoxy-4′, 7-epoxy-8, 5′-neolignan-7′-ene (**2**)*

White amorphous powder; $[\alpha]_D^{25}$ −28.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 205 (1.10), 230 (0.53), 286 (0.39), 328 (0.51) nm; ECD (MeOH) λ_{max} (Δε) 220 (−1.04), 240 (0.75), 297 (−1.55) nm; IR (KBr) ν_{max} 3418, 2934, 2849, 1702, 1667, 1632, 1599, 1516, 1462, 1431, 1384, 1331, 1272, 1215, 1154, 1032, 974, 852, 821, 776, 737 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 1; HR-ESI-MS *m/z* 557.1794 [M + Na]$^+$ (Calcd. for $C_{30}H_{30}O_9Na$, 557.1782).

*(−)-(7R, 8S, 7′E)-9-O-(E)-feruloyl-4, 9-dihydroxy-3, 3′, 9′-trimethoxy-4′, 7-epoxy-8, 5′-neolignan-7′-ene (**3**)*

White amorphous powder; $[\alpha]_D^{25}$ −25.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max}(log ε) 205 (1.10), 230 (0.53), 286 (0.39), 328 (0.51) nm; IR (KBr) ν_{max} 3418, 2934, 2849, 1702, 1667, 1632, 1599, 1516, 1462, 1431, 1384, 1331, 1272, 1215, 1154,

1032, 974, 852, 821, 776, 737 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 1; HR-ESI-MS *m/z* 571.1927 $[M + Na]^+$ (Calcd. for $C_{31}H_{32}O_9Na$, 571.1939).

(−)-(7R, 8S, 7'E)-9-O-(E)-feruloyl-4, 9-dihydroxy-3, 5, 3'-trimethoxy-4', 7-epoxy-8, 5'-neolignan-7'-ene-9'-al (**4**)

White amorphous powder; $[\alpha]_D^{25}$ −18.0 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 216 (−0.13), 235 (0.24), 314 (−0.32) nm; ^1H NMR (acetone-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 1; HR-ESI-MS *m/z* 585.1753 $[M + Na]^+$ (Calcd. for $C_{31}H_{30}O_{10}Na$, 585.1731).

(−)-(7R, 8S, 7'E)-9-O-(E)-feruloyl-4, 9-dihydroxy-3, 3'-dimethoxy-4', 7-epoxy-8, 5'-neolignan-7'-ene-9'-al (**5**)

White amorphous powder; $[\alpha]_D^{25}$ −20.0 (*c* 0.1, MeOH); ^1H NMR (acetone-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 1; HR-ESI-MS *m/z* 533.1780 $[M + H]^+$ (Calcd. for $C_{30}H_{29}O_9$, 533.1806).

Nitric Oxide (NO) production in RAW264.7 macrophages

RAW 264.7 macrophages were cultured in RPMI 1640 medium (Hyclone, Logan, UT) containing 10% FBS. The compounds were dissolved in DMSO and further diluted in medium to produce different concentrations. The cell mixture and culture medium were dispensed into 96-well plates (2×10^5 cells/well) and maintained at 37 °C under an atmosphere of 5% CO_2. After preincubation for 24 h, serial dilutions of the test compounds were added into the cells, up to the maximum concentration of 25 $\mu mol \cdot L^{-1}$, followed by addition of LPS to a concentration of 1 $\mu g \cdot mL^{-1}$ before incubation for 18 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW264.7 macrophage supernatants with Griess reagent. Aliqueots of supernatants (100 μL) were incubated, in sequence, with 50 μL 1% sulphanilamide and 50 μL 1% naphthylethylenediamine in 2.5% phosphoric acid solution. The sample absorbance was measured at 570 nm by a 2104 Envision Multilabel Plate Reader. Dexamethasone was used as a positive control.

Cytotoxicity

The cytotoxicity of compounds **1**−**5** were tested *in vitro* using human HCT-8 colon, A2780 ovary, BGC-823 stomach, and Bel-7402 hepatoma, and A549 lung cell lines. MTT assay was used as previously described[23].

References

[1] Guo Q, Zeng KW, Gao XL, *et al*. Chemical constituents with NO production inhibitory and cytotoxic activities from *Litsea cubeba* [J]. *J Nat Med*, 2015, **69**(1): 94-99.

[2] Guo Q, Bai RF, Su GZ, *et al*. Chemical constituents from the roots and stems of *Litsea cubeba* [J]. *J Asian Nat Prod Res*, 2016, **18**(1): 51-58.

[3] Zhang SY, Guo Q, Gao XL, *et al*. A phytochemical and pharmacological advance on medicinal plant *Litsea cubeba* (Lauraceae) [J]. *Chin J Chin Mater Med*, 2014, **39**(5): 769-776.

[4] Yan XH, Zhang FX, Xie HH, *et al*. A review of the studies on chemcial constituents from *Litsea Lam* [J]. *J Trop Subtrop Bot*, 2000, **8**: 171-176.

[5] *Chinese Pharmacopoeia* [M]. China Medical Science and Technology Press: Beijing, 2015: 235.

[6] Editorial Committee of Chinese Materia Medica, State Administration Bureau of Traditional Chinese Medicine. *Chinese Materia Medica* (*Zhonghua Bencao*) [M]. Shanghai Science & Technology Press: Shanghai, 1999, **7**: 73.

[7] Lee SS, Chen CK, Huang FM, *et al*. Two dibenzopyrrocoline alkaloids from *Litsea cubeba* [J]. *J Nat Prod*, 1996, **59**: 80-82.

[8] Wu YC, Liou JY, Duh CY, *et al*. Litebamine, a novel phenanthrene alkaloid from *Litsea cubeba* [J]. *Tetrahedron Lett*, 1991, **32**: 4169-4170.

[9] Feng T, Xu Y, Cai XH, *et al*. Antimicrobially active isoquinoline alkaloids from *Litsea cubeba* [J]. *Planta Med*, 2009, **75**(1): 76-79.

[10] Zhang SY, Guo Q, Cao Y, *et al*. Alkaloids from roots and stems of *Litsea cubeba* [J]. *Chin J Chin Mater Med*, 2014, **39**(20): 3964-3968.

[11] Zhang W, Hu JF, Lv WW, *et al*. Antibacterial, antifungal and cytotoxic isoquinoline alkaloids from *Litsea cubeba* [J]. *Molecules*, 2012, **17**(11): 12950-12960.

[12] Wang LY, Chen MH, Wu J, *et al*. Bioactive glycosides from the twigs of *Litsea cubeba* [J]. *J Nat Prod*, 2017, **80**(6): 1808-1818.

[13] Li XT, Xia H, Wang LY, *et al*. Lignans from the twigs of *Litsea cubeba* and their bioactivities [J]. *Molecules*, 2019, **24**(2): 306.

[14] Wang LY, Qu YH, Li YC, *et al*. Water soluble constituents from the twigs of *Litsea cubeba* [J]. *Chin J Chin Mater Med*, 2017, **42**(14): 2704-2713.

[15] Lin PC, Ji LL, Zhong XJ, *et al*. Taraxastane-type triterpenoids from the medicinal and edible plant *Cirsium setosum* [J]. *Chin J Nat Med*, 2019, **17**(1): 22-26.

[16] Liu YF, Wang XL, Chen MH, *et al*. Three pairs of alkaloid enantiomers from the root of *Isatis indigotica* [J]. *Acta Pharm Sin B*, 2016, **6**(2): 141-147.

[17] Guo QL, Xu CB, Chen MH, *et al*. Sulfur-enriched alkaloids from the root of *Isatis indigotica* [J]. *Acta Pharm Sin B*, 2018, **8**(6): 933-943.

[18] Wang LY, Qiu BL, Xia H, *et al*. Yanhusanines A−F, isoquinoline-derived alkaloid enantiomers from *Corydalis yanhusuo* and their biological activity [J]. *J Nat Prod*, 2020, **83**(2): 489-496.

[19] Li R, Zhang JF, Wu YZ, *et al*. Structures and biological evaluation of monoterpenoid glycosides from the roots of *Paeonia lactiflora* [J]. *J Nat Prod*, 2018, **81**(5): 1252-1259.

[20] Lin PC, Yao J, Wu J, *et al*. A new ureido-substituted amino acid from the tubers of *Gymnadenia conopsea* [J]. *Chin Chem Lett*, 2017, **28**: 257-259.

[21] Xiong L, Zhu CG, Li YR, *et al*. Lignans and neolignans from *Sinocalamus affinis* and their absolute configurations [J]. *J Nat Prod*, 2011, **74**(5): 1188-1200.

[22] Yang BH, Zhang WD, Liu RH, *et al*. Lignans from bark of *Larix olgensis* var. *koreana* [J]. *J Nat Prod*, 2005, **68**(8): 1175-1179.

[23] Lin S, Wang SJ, Liu MT, *et al*. Glycosides from the stem bark of *Fraxinus sieboldiana* [J]. *J Nat Prod*, 2007, **70**(5): 817-823.

[24] Lin S, Shen YH, Li HL, *et al*. Acylated iridoids with cytotoxicity from *Valeriana jatamansi* [J]. *J Nat Prod*, 2009, **72**(4): 650-655.

Cite this article as: XIA Huan, XIA Gui-Yang, WANG Ling-Yan, WANG Min, WANG Ya-Nan, LIN Peng-Cheng, LIN Sheng. Bioactive sesquineolignans from the twigs of *Litsea cubeba* [J]. *Chin J Nat Med*, 2021, **19**(10): 796-800.