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Neolignans and flavonoids from the root bark of *Illicium henryi*

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ABSTRACT

Two new neolignans (**1** and **2**) were isolated from the root bark of *Illicium henryi*, along with four known neolignans and seven known flavonoids (**3**–**13**). Their structures were elucidated on the basis of spectroscopic and chemical methods. The absolute configurations of compounds **1** and **2** were determined by the CD spectrum.

1. Introduction

Illicium henryi belongs to Family Illiciaceae, and distributes mainly in the south of China. Most of Illicium species are considered to be toxic plant. The root bark of *I. henryi* is locally taken to dispel wind and cold and relieve pain in traditional Chinese medicine. Currently, the water-soluble extract from the root bark of *I. henryi* has been applied to clinical use as an analgesic agent by intramuscular injection in China. It has been reported that three sesquiterpene lactones were isolated from the fruits of *I. henryi*, one of them exhibits high toxicity in mice [1]. However, there are no previous reports concerning the phytochemistry of the root bark of *I. henryi* so far. For the purpose of finding analgesic compounds, we carried out systematic studies on the chemical constituents of the root bark of *I. henryi*. Two new neolignans, together with four known lignans, seco-isolariciresinol-*O*-α-L-rhamnoside (**3**) [2], 4-*O*-(2-hydroxymethylethyl)-dihydroconigeryl alcohol 6-(4′-hydroxy-3′-methoxyphenyl)-glucoside (**5**) [3], icariside E4 (**6**) [4], and massonianoside B (**8**) [5] and seven known flavonoids, (2R,3R) taxifolin 3-*O*-β-D-xyloside (**4**) [6], (2 S,3 S) taxifolin 3-*O*-β-D-xyloside (**7**) [6], quercetin (**9**) [7], (2R,3R) catechin (**10**) [8], (2R,3R) taxifolin 3-*O*-β-D-glucoside (**11**) [9], (2 S,3 S) taxifolin 3-*O*-β-D-glucoside (**12**) [9], and quercitrin (**13**) [8], have been isolated from the root bark of *I. henryi*. The absolute configuration of **1** and **2** were deduced in the CD spectra.

2. Experimental

2.1. Generals

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR data were recorded on a Nicolet FIIR 750 spetrophotometer. The CD spectra were recorded in MeOH on JASCO J-810 spectropolarimeter. ^{1}H, ^{13}C and 2D NMR data spectra were taken with a Bruker AMX 400 instrument. Chemical shifts are recorded in δ (ppm). ESIMS were obtained on a Bruker Esquire 3000 Plus Spectrometer. HRESIMS were determined on a Micromass Q-Tif Global mass spectrometer. GC experiments were run on a GC-MS-QP5050A instrument (Shimadzu), using a db-1 column (0.25 mm i.d.×30 m; column temperature, 200 °C; injection temperature 250 °C; carrier gas N_2 at flow of 32.2 mL/min; detector, EI-MS). Open column chromatography (CC) was performed with MCI gel

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CHP 20P (high porous polymer, 75–150 μm, Mitsubishi chemical Ind.), silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., China), RP-18 (20–45 μm, Fuji Silysia Chemical Ltd.,), and sephadex LH-20 (20–100 μm, Pharmacia). TLC was carried out with GF-254 silica gel plates (Yantai Huiyou Inc., China). All solvents used were of chemical grade and purchased from the Shanghai Chemical plant, Shanghai, PR China.

2.2. Plant material

The root bark of *I. henryi* were collected in Kaihua County, Zhejiang Province, PR China in December 2009 and authenticated by Prof. Lihong Hu. A vocher sample of the plant (2009012003) was deposited at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, PR China.

2.3. Extraction and isolation

The air-dried root bark of *I. henryi* (1.0 kg) were extracted three times with 95% EtOH under reflux for 2 h. The extract was concentrated under reduced pressure to obtain a crude extract, which was suspended in H_2O, and then partitioned with petroleum ether, EtOAc and *n*-BuOH, respectively. The EtOAc layer residue (69.0 g) was subjected to silica gel CC with $CHCl_3$–MeOH gradient to give four fractions. Fr. 1 (1.227 g) was repeatedly applied to silica gel CC and eluted with $CHCl_3$–MeOH and further purified by sephadex LH-20 using MeOH to give **1** (15 mg). Fr. 2 (4.415 g) was fractionated on silica gel CC with $CHCl_3$–MeOH to give two subfractions. Fr. 2.1 was subjected to sephadex LH-20 CC using 1:1 MeOH–H_2O and further chromatographyed on RP-18 with 35:65 MeOH–H_2O to yield **2** (11 mg), **3** (98 mg) and **4** (190 mg). The separation of Fr. 2.2 by sephadex LH-20 using 1:1 MeOH–H_2O and RP-18 was eluted with 15:85 MeOH–H_2O to afford **5** (20 mg) and **6** (13 mg). Fr. 3 (1.321 g) was separated by sephadex LH-20 column using MeOH, silica gel CC with a $CHCl_3$–MeOH gradient and further purified by sephadex LH-20 CC eluted with 1:1 MeOH–H_2O to yield **7** (134 mg). Further purification of Fr. 4 (2.074 g) was achieved by sephadex LH-20 using 1:1 MeOH–H_2O to give **8** (32 mg) and **9** (221 mg). The crude n-BuOH extract (128.6 g) was subjected to MCI CC and eluted with MeOH–H_2O gradient to give three fractions, Fr. 5, 6 and 7. Fr. 5 (7.180 g) was successively purified on silica gel with $CHCl_3$–MeOH gradient and RP-18 eluted with 15:85 MeOH–H_2O to afford **10** (48 mg). Fr. 6 (14.1 g) was submitted to CC: silica gel, sephadex LH-20 and RP-18 to yield **11** (40 mg) and **12** (15 mg). Fr. 7 (52.5 g) was shown the presence of one main compound signal by TLC analysis. A little portion of Fr. 7 (400 mg) was further purified by sephadex LH-20 and then RP-18 CC to give **13** (55 mg).

2.4. Acid hydrolysis of compound **1**

Compound **1** (5 mg) was heated at 80 °C in 10% HCl–dioxane (1:1, 1 mL) for 4 h in water bath. The reaction mixtures were neutralized with $NaHCO_3$ and then evaporated to dryness. The residue was partitioned between CH_2Cl_2 and H_2O for three times. The aqueous phase was evaporated and passed through a sephdex LH-20 column eluted with MeOH–H_2O (1:1) to get a sugar fraction. The sugar portion was detected by TLC with authentic samples.

2.5. Determination of sugar components

The absolute configuration of xylose was determined according to a reported procedure [10]. The sugar portion and authentic sugar sample were derivatized with leucine reagent, and the leucine derivatives were subjected to GC (column temperature 200 °C; injection temperature 250 °C; carrier gas N_2 at flow rate of 32.2 mL/min; derivatives D-xylose: 8.23 min).

Compound **1**, white, amorphous power; $[\alpha]_D^{20}$ +13 (*c* 0.11, MeOH); IR (KBr) ν_{max} 3421, 2929, 1602, 1517, 1457, 1274, 1035, 819, and 603 cm^{-1}; CD (MeOH) λ_{max} (Δε) 212 (7.58), 235 (−8.75), and 274 (2.69) nm. ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 501.2 $[M+Na]^+$; HRESIMS *m/z* 501.1733 $[M+Na]^+$ (calcd for $C_{24}H_{30}NaO_{10}$, 501.1737).

Compound **2**, white, amorphous power; $[\alpha]_D^{20}$ 0 (*c* 0.11, MeOH); IR (KBr) ν_{max} 3396, 2927, 2879, 1700, 1608, 1511, 1452, 1263, 1135, and 1031 cm^{-1}; CD (MeOH) λ_{max} (Δε) 227 (3.58), 242 (−0.10), and 261 (0.91) nm. ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 443.2 $[M+Na]^+$; HRESIMS *m/z* 443.1686 $[M+Na]^+$ (calcd for $C_{22}H_{28}NaO_8$, 443.1682).

3. Results and discussion

Compound **1**, obtained as a white amorphous powder, has a molecular formular of $C_{24}H_{30}O_{10}$ on the basis of a positive-ion HRESIMS at *m/z* 501.1733 (cald. for $C_{24}H_{30}O_{10}Na$,

Table 1
^1H (400 MHz) and ^{13}C NMR (100 MHz) data of compounds **1** and **2** (in CD_3OD).

Position	1		2	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	130.1		138.6	
2	112.9	7.12 d (2.1)	119.2	7.08 d (1.5)
3	149.5		152.4	
4	148.6		148.8	
5	116.7	6.87 d (8.4)	111.6	7.10 d (8.1)
6	122.0	6.97 dd (8.4, 2.1)	119.8	6.98 dd (8.1, 1.5)
7	77.7	5.07 d (8.4)	88.7	5.57 d (5.7)
8	79.1	4.16 m	56.4	3.46 m
9	69.8	4.04 dd (11.4, 2.1); 3.39 dd (11.4, 2.1)	65.6	3.75 m; 3.84 m
1′	137.0		137.3	
2′	122.8	6.72 dd (8.1,2.4)	117.5	6.60 br, s
3′	118.1	6.85 d (8.1)	142.4	
4′	143.3		146.9	
5′	145.5		130.0	
6′	118.2	6.77 d (1.8)	117.1	6.62 br, s
7′	32.8	2.60 t (7.2)	33.1	2.58 t (6.6)
8′	36.0	1.81 m	36.2	1.81 m
9′	62.6	3.58 t (6.6)	62.7	3.58 t (6.6)
1″	106.0	4.08 d (7.8)	83.4	4.24 m
2″	75.3	3.23 m	62.4	3.74 m
3″	78.2	3.28 m	62.4	3.74 m
4″	71.6	3.46 m		
5″	67.4	3.11 m; 3.79 m		
OCH_3	57.1	3.88 s	56.9	3.81 s

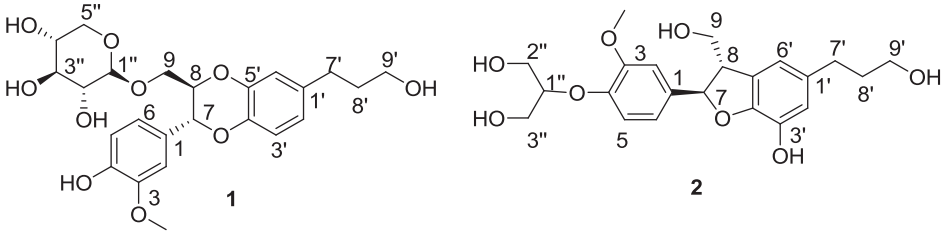

Fig. 1. The key HMBC correlations of compounds **1**–**2**.

501.1737). The ^{1}H NMR spectrum exhibited signals of a 1,3,4-trisubstituted aromatic ring [δ 7.12 (1H, d, J = 2.1 Hz, H-2), δ 6.87 (1H, d, J = 8.4 Hz, H-5), δ 6.97 (1H, dd, J = 8.4, 2.1 Hz, H-6)], a 1′,4′,5′-trisubstituted aromatic ring [δ 6.72 (1H, dd, J = 8.1, 2.4 Hz, H-2′), δ 6.85 (1H, d, J = 8.1 Hz, H-3′), δ 6.77 (1H, d, J = 2.4 Hz, H-6′)], a hydroxypropyl group [δ 2.60 (2H, t, J = 7.2 Hz, H_2-7′), δ 1.81 (2H, m, CH_2, H_2-8′), δ 3.58 (2H, t, J = 6.6 Hz, H_2-9′)], a moiety of (Ph)**CH**(O)-**CH**(O)-**CH_2**O- [δ 5.07 (1H, d, J = 8.4 Hz, H-7), δ 4.16 (1H, m, H-8), δ 4.04 (1H, dd, J = 11.4, 2.1 Hz, H-9a), δ 3.39 (1H, dd, J = 11.4, 2.1 Hz, H-9b)], and a methoxyl group [δ 3.88 (3H, s)]. The carbon signals in the ^{13}C NMR spectrum of **1** further confirmed the above units. The 1D and 2D NMR spectra showed the presence of a xylopyranosyl moiety. The anomeric proton signal appeared as a doublet at δ 4.08 (1H, J = 7.8 Hz) and permitted assignment of a β configuration of the xylose. On acid hydrolysis and GC analysis, the sugar part was determined to be a β-D-xylopyranosyl group. Significant HMBC correlations were observed between H-6/C-7, H-7/C-6, H-8/C-7, H-8/C-9, and OCH_3/C-3 (Fig. 1). Thus the skeleton could be concluded as 3-methoxyl-4′:7,5′:8-diepoxyneolignan-4,9,9′-triol. The β-D-xylopyranosyl group could be assigned at C-9 from the correlation between H-9 and C-1″ of xyl. This could be further confirmed by a downfield methylene signal at δ 69.8 (C-9) in the ^{13}C NMR spectrum. The relative configuration of H-7 and H-8 was trans from the $J_{7,8}$ value of 8.4 Hz in the ^{1}H NMR. The absolute stereochemistries of C-7 and C-8 positions were determined by the CD spectrum (Fig. 2) with the comparison of model compounds. A negative cotton effect at the peak of 235 nm for the benzodioxane moiety in the CD spectrum indicated assignment of 7R,8R absolute configuration for **1** according to the study of a related benzodioxane system [11–14]. Therefore the structure of **1** was elucidated as (7R,8R)-3-methoxyl-9-*O*-β-D-xylopyranosyl-4′:7,5′:8-diepoxyneolignan-4, 9′-diol.

Compound **2** gave the molecular formula $C_{22}H_{28}O_8$ base on an $[M+Na]^+$ ion at 443.1686 in the HRESIMS. The ^{1}H NMR spectrum showed signals assigned to a 1,3,4-trisubstituted aromatic ring [δ 7.10 (1H, d, J = 8.1 Hz, H-5), δ 7.08 (1H, d, J = 1.5 Hz, H-2), δ 6.98 (1H, dd, J = 8.1, 1.5 Hz, H-6)] and a 1′,3′,4′,5′-tetrasubstituted aromatic ring [δ 6.62 (1H, br, s, H-6′), δ 6.60 (1H, br, s, H-2′)], in addition with a methoxyl group [δ 3.81 (3H, s, OCH_3)]. The ^{1}H NMR data also were in good agreement with a 1,2,3-propantriol moiety [δ 4.24 (1H, m, H-1″), δ 3.74 (4H, m, H-2″ and H-3″)], a propan-3-ol moiety [δ 2.58 (2H, t, J = 6.6 Hz, H-7′), δ 1.81 (2H, m, H-8′), δ 3.58 (2H, t, J = 6.6 Hz, H-9′] and a moiety of [(**CH**(O)-**CH**(Ph)-**CH_2**O)] [δ 5.57 (1H, d, J = 5.7 Hz, H-7), δ 3.46 (1H, m, H-8), δ 3.84 (1H, m, H-9a), δ 3.75 (1H, m, H-9b)]. The assignment of the ^{1}H NMR and ^{13}C NMR signals of **2** was based on its HSQC experiment. These units were assigned by the HMBC correlation from H-7 to C-6, H-6′ to C-7′, H-1″ to C-4 and OCH_3 to C-3. A trans configuration of **2** was confirmed by the $J_{7,8}$ value of 5.7 Hz in the ^{1}H NMR spectrum. It could be further determined on the basis of NOESY correlations between H-8 and H-2, H-6 protons; H-7 and H-9a protons. It has been reported that the configurations at C-7 and C-8 of the dihydrobenzofuran skeleton can be clearly distinguished from the 240–220 nm region. In the CD spectrum, a positive cotton effect at 227 nm indicated an 8S configuration [4,15,16]. Thus, the structure of **2** was determined to be 7R,8S-configuration as shown in Fig. 3. Therefore the structure of **2** was elucidated as (7R,8S)-3′,9,9′-trihydroxyl-3-methoxyl-4-*O*-glycerol-7,8-dihydrobenzofuran-1′-propanolneoligan.

The other four known neolignans were identified as seco-isolariciresinol-*O*-α-L-rhamnoside (**3**), 4-*O*-(2-hydroxymethylethyl)-dihydro-conigeryl alcohol 6-(4′-hydroxy-3′-

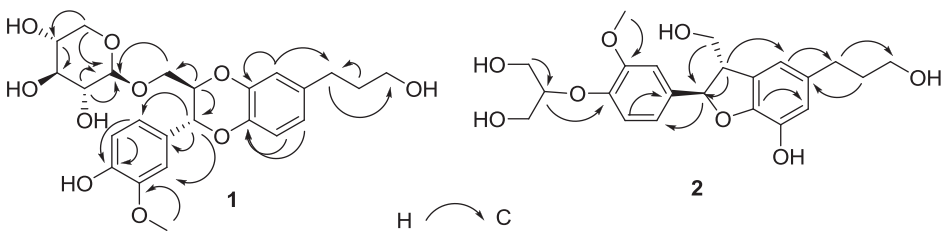

Fig. 2. The CD spectra of compounds **1** and **2**.

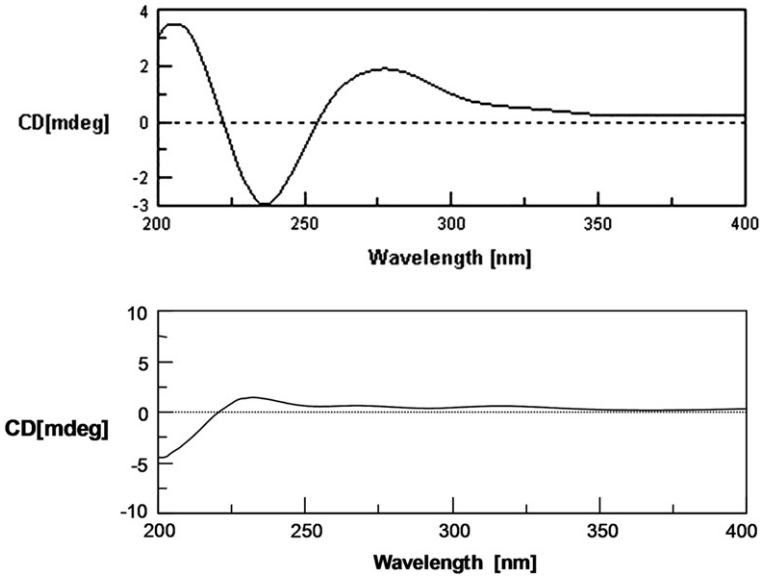

Fig. 3. Structures of compounds **1**–**2**.

methoxyphenyl)-glucoside (**5**), icariside E4 (**6**), and massonianoside B (**8**). The presence of neolignans was first reported in *I. henryi* and estimated as clue to the classification of genus *Illicium*. The flavonoids taxifolin 3-*O*-β-D-xyloside (**4**), (2S,3S) taxifolin 3-*O*-β-D-xyloside (**7**), quercetin (**9**), (2R,3R) catechin (**10**), (2R,3R) taxifolin 3-*O*-β-D-glucoside (**11**), (2S,3S) taxifolin 3-*O*-β-D-glucoside (**12**) and quercitrin (**13**) were also found in the water-soluble extract which was employed as a remedy against pain by intramuscular injection. Among them quercitrin (**13**) was the most abundant constituent by HPLC analysis. It has been demonstrated that some of flavonoids, such as quercetin, quercitrin and other quercetin glycosides showed pronounced antinociceptive properties [17–20]. The flavonoids isolated from the root bark of *I. henryi* were estimated as clue to analgesic activities, especially quercitrin appears to contribute for the antinociceptive property.

Acknowledgements

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References

[1] Liu JS, Zhou QR. Acta Pharmaceutica Sinica 1988;23:221.
[2] Vassilios A, Marinos ME, Williams T, Williams PJ. Phytochemistry 1992;31:4307.
[3] Kouno I, Jiang ZH, Yang CS, Tanaka T. Stud Plant Sci 1999;6:297.
[4] Nakanishi T, Iida N, Inatomi Y, Murata H, Inada A, Murata J, et al. Phytochemistry 2004;65:207.
[5] Shen YB, Kojima Y, Terazawa M. J Wood Sci 1999;45:326.
[6] Hosoi S, Shimizu E, Ohno K, Yokosawa R, Kuninaga S, Coskun M, et al. Phytochem Anal 2006;17:20.
[7] Mnawwar MA, Souleman AMA, Buddrus J. Phytochemstry 1984;23:2447.
[8] Yu DQ, Yang JS, Xie JX. Handbook of Analytical Chemistry (The Seventh Fascicule), 7; 1999. p. 838.
[9] Sakushima A, Ohno K, Coskun M, Seki KI, Ohkura K. Nat Prod Lett 2002;16:383.
[10] Xiang WJ, Guo CY, Ma L, Hu LH. Fitoterapia 2010;81:248.
[11] Kim NC, Graf TN, Sparacino CM, Wani MC, Wall ME. Org Biomol Chem 2003;1:1684.
[12] Sy-Cordero A, Graf TN, Nakanishi Y, Wani MC, Agarwal R, Kroll DJ. Oberlies NH. Planta Med 2010;76:644.
[13] Lin TC, Fang JM, Cheng YS. Phytochemistry 1999;51:793.
[14] Silva MS, Barbosa-Filho JM, Yoshida M, Gottlieb OR. Phytochemistry 1989;28:3477.
[15] Matsuda N, Sato H, Yaoita Y, Kikuchi M. Chem Pharm Bull 1996;44:1122.
[16] Lemiere G, Gao M, Groot AD, Dommisse R, Lepoivre J, Pieters L, et al. J Chem Soc Perkin Trans 1995;1:1775.
[17] Filho AW, Filho VC, Olinger L, de Souza MM. Arch Pharm Res 2008;31:713.
[18] Anjaneyulu M, Chopra K. Indian J Exp Biol 2004;42:766.
[19] Krogh R, Kroth R, Berti C, Madeira AO, Souza MM, Cechinel-Filho V, et al. Pharmazie 1999;54:464.
[20] Gadotti VM, Schmeling LO, Machado C, Liz FH, Filho VC, Meyre-Silva C, et al. J Pharm Pharmacol 2005;57:1345.