Two New Lignans from the Bark of *Zanthoxylum planispinum*

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Zanthoxylum planispinum Sieb. et Zucc. has been widely used as a folk medicine for clearing away cold, preventing toothache and expelling roundworms.[1] Chemical constituents of *Zanthoxylum* have been studied extensively. Previous phytochemical investigations on this species revealed that benzophenanthridine alkaloids,[2] coumarins,[3] amides[4] and lignans[5] are largely represented in this genus. Chemical studies of the bark of this plant have never been conducted previously. As continuation of our chemical studies of ethnomedicinal plants,[6] we have investigated the bark of *Z. planispinum* and reported herein the isolation and structure elucidation of two new furofuran lignans planispine A (**1**) and B (**2**). Their structures were established on the basis of various spectroscopic analyses including 1D-(^{1}H, ^{13}C NMR and DEPT) and 2D-NMR (HSQC, HMBC and ROESY) techniques and by comparison of their spectral data with those of related compounds.

Compound **1** was isolated as colorless oil. Its molecular formular was established as $C_{25}H_{30}O_6$ (*m/z* 449.1942 $[M+Na]^+$, calcd for 449.1940) on the basis of its HR-ESI-MS analyses. The ^{1}H NMR spectrum showed two CH group at δ_H 2.84 (1H, m) and 3.39 (1H, m), two benzylic OCH moieties at δ_H 4.38 (1H, d, J = 7.2 Hz) and 4.83 (1H, d, J = 5.7 Hz), two oxygenated CH_2 group at δ_H 4.11 (1H, d, J = 9.3 Hz) and 3.82 (1H, m), 3.78 (1H, m) and 3.24 (1H, dd, J = 8.7, 8.7 Hz), six aromatic protons as two ABX systems at δ_H 6.79 - 6.95 indicating the presence of two 1,2,4-trisubstituted benzene rings, in addition to two methoxy groups at δ_H 3.80, 3.83 (each 3H, s), one hydroxy group at δ_H 7.53 (1H, s), and one prenyloxy group at δ_H 4.53 (2H, d, J = 6.7 Hz), 5.47 (1H, t, J = 6.7 Hz), 1.72 (3H, s), 1.75 (3H, s). Accordingly, compound **1** was assigned to be a lignan of the furofuran type bearing one hydroxy, two methoxy and a prenyloxy group.[5b,7] This assumption was further supported by the ^{13}C NMR signal including 2 × CH (δ_C 55.3 and 50.5), 2 × benzylic OCH (δ_C 88.0 and 82.3), 2 × oxygenated CH_2 (δ_C 71.1 and 69.8), 6 × aromatic C and 6 × aromatic CH. The ^{1}H NMR and ^{13}C NMR data of compound **1** was similar to that of epipinoresinol,[8] suggesting one hydroxy of epipinoresinol was replaced by a prenyloxy group in **1** which was supported by MS fragments at *m/z* 358 ($M-C_5H_8$) and 69 (C_5H_9). In HMBC spectrum, the correlations of δ_H 7.53 (s, OH)/C-3′ (δ_C 147.7), C-4′ (δ_C 145.9) and C-5′ (δ_C 113.9), δ_H 3.80 (s, OCH_3)/C-3 (δ_C 150.4), δ_H 3.83 (s, OCH_3)/C-3′ and δ_H H_2-1″/C-4 (δ_C 148.5) indicated that two methoxy, one hydroxy and one prenyloxy groups were located at C-3, C-3′, C-4′ and C-4 respectively. Furthermore, the ROESY spectrum allowed us to confirm the position of the methoxy group, showing that both methoxyl signal at δ_H 3.80 and 3.83 can correlate with H-2 and H-2′ respectively. The relative configuration of **1** was determined on the basis of a ROESY experiment (see Fig. 2). Although compound **1** exhibited the same NMR data as (+)-epipinoresinol[8a] ($[\alpha]_D$ = +79 (c 0.1, MeOH)), which has the absolute configuration *7S, 7′R, 8R, 8′R*, its optical rotation was opposite. Accordingly, the absolute configuration was assigned as *7R, 7′S, 8S, 8′S*. Thus, the structure of **1** was established as (-)-(7β, 7′α, 8β, 8′β)-3,3′-dimethoxy-4-prenyloxy -7, 9′ : 7′, 9-diepoxylignan-4′-ol with

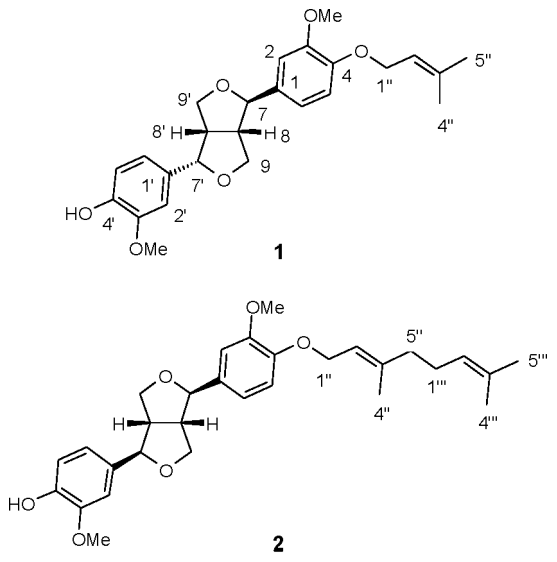

Figure 1. Their structures of compounds **1** and **2**.

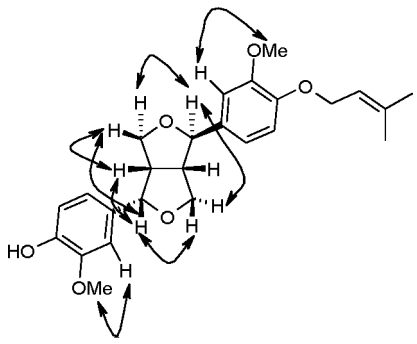

Figure 2. Key NOE correlations for compound **1**.

(*7R, 7'S, 8S, 8'S*), named as planispine A.

Compound **2** was obtained as colorless oil, and had a molecular formular $C_{30}H_{38}O_6$ from the positive HR-ESI-MS (*m/z* 517.2556 $[M+Na]^+$, calcd for 517.2566). Most of the ^1H NMR and ^{13}C NMR signals of **2** were similar to those of **1**. The major spectroscopic differences between them as followed: *i*) the presence of a geranyloxy group in **2**, instead of prenyloxy group in the former; *ii*) more downfield shifted for C-7' (δ_C 86.1), C-8' (δ_C 54.9), C-9' (δ_C 71.9) and C-1' (δ_C 135.1); *iii*) Small coupling constants were observed for H-7 (J = 4.5 Hz) and H-7' (J = 4.2 Hz). All over the data suggested the structure of compound **2** was similar to that of pinoresinol,[9] except one hydroxy of pinoresinol replaced by a geranyloxy group in **2** which was supported by MS fragments at *m/z* 358 ($M-C_{10}H_{16}$) and 137 ($C_{10}H_{17}$). The substitued pattern was unambiguously confirmed by HMBC data. Correlations were oberved between δ_H 7.58 (s, OH)/C-3' (δ_C 147.9), C-4' (δ_C 146.5) and C-5' (δ_C 115.2), δ_H 3.81 (s, OCH_3)/C-3 (δ_C 150.4), δ_H 3.84 (s, OCH_3)/C-3' and H_2-1''/C-4 (δ_C 148.4), confirming two methoxy, one hydroxy and one geranyloxy groups were located at C-3, C-3', C-4' and C-4 respectively. Furthermore, the ROESY spectrum allowed us to confirm the position of the methoxy group, showing that both methoxyl signal at δ_H 3.81 and 3.84 can correlate with H-2 and H-2' respectively. The relative configuration of **2** was determined on the basis of a ROESY experiment (see Fig. 3). On the basis of levorotatory nature and relative configuration, the absolute configuration of **2** must be the same as that of (-)-pinoresinol monomethyl ether[8a] ($[\alpha]_D$ = -58 (c 0.05, EtOH)). Therefore, the structure of **2** was established as (-)-(7β, 7'β, 8β, 8'β)-3, 3'-dimethoxy-4-geranyloxy-7, 9' : 7', 9-diepoxylignan-4'-ol with (*7R, 7'R, 8S, 8'S*), named as planispine B.

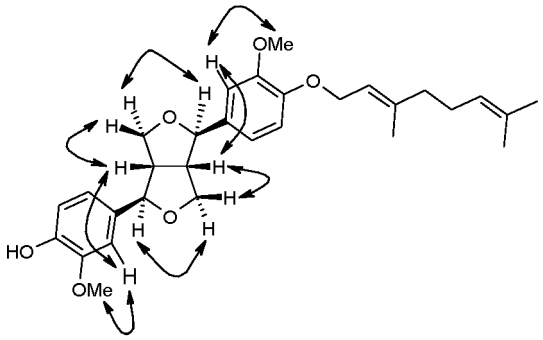

Figure 3. Key NOE correlations for compound **2**.

Experimental Section

Reagent and equipment. Thin-layer chromatography (TLC): Pre-coated silica gel GF_{254} plates (*Qingdao Haiyang Chemical*

Table 1. ^1H, ^{13}C NMR and HMBC data of compound 1 and **2** in acetone

position	**1**			**2**		
	^1H-NMR	^{13}C-NMR	HMBC	^1H-NMR	^{13}C-NMR	HMBC
1		135.2 (s)			133.7 (s)	
2	6.99 (d, 2.4)	110.5 (d)	C-1, 3, 4, 6, 7	6.98 (d, 1.5)	110.2 (d)	
3		150.4 (s)			150.4 (s)	
4		148.5 (s)			148.4 (s)	
5	6.79-6.85 (m)	115.1 (d)		6.85 (m)	114.0 (d)	
6	6.82-6.92 (m)	118.7 (d)		6.84 (m)	118.6 (d)	
7	4.38 (d, 7.2)	88.0 (d)	C-1, 2, 6, 8, 9	4.67 (d, 4.5)	86.2 (d)	C-2, 6, 8, 9
8	2.84 (m)	55.3 (d)	C-1, 7, 7', 8', 9'	3.09 (m)	54.9 (d)	
9a	3.82 (m)	71.1 (t)		3.80 (m)	71.8 (t)	C-7, 8
9b	4.11 (d, 9.3)			4.20 (m)		
1'		130.9 (s)			135.1 (s)	
2'	7.00 (d, 2.4)	109.7 (d)	C-1', 3', 4', 6', 7'	6.98(d, 1.5)	110.7 (d)	
3'		147.7 (s)			147.9 (s)	
4'		145.9 (s)			146.5 (d)	
5'	6.90-6.95 (m)	113.9 (d)		6.79 (m)	115.2 (d)	
6'	6.82-6.92 (m)	118.7 (d)		6.82 (m)	119.2 (d)	
7'	4.83 (d, 5.7)	82.3 (d)	C-1', 2', 6', 8', 9'	4.70 (d, 4.2)	86.1 (d)	C-2', 6', 8', 9'
8'	3.39 (m)	50.5 (d)	C-9	3.09 (m)	54.9 (d)	
9'a	3.78 (m)	69.8 (t)	C-7', 8'	3.80 (m)	71.9 (t)	C-7', 8'
9'b	3.24 (dd, 8.7, 8.7)			4.20 (m)		
1''	4.53 (d, 6.7)	65.8 (t)	C-4, 2'', 3''	4.56 (d, 6.1)	65.9 (t)	C-4, 2'', 3''
2''	5.47 (t, 6.7)	121.0 (d)	C-4'', C-5''	5.49 (t, 6.1)	120.9 (d)	C-4'', 5''
3''		137.1 (s)			140.4 (s)	
4''	1.72 (s)	17.7 (q)	C-2'', 3''	1.72 (s)	16.2 (q)	C-2'', 3'', 5''
5''	1.75 (s)	25.4 (q)	C-2'', 3''	2.05 (m)	39.8 (t)	C-2'', 3'', 1''', 2'''
1'''				2.10 (m)	26.7 (t)	C-2''', 3''', 5''
2'''				5.11 (m)	124.4 (d)	C-4''', 5'''
3'''					131.7 (s)	
4'''				1.65 (s)	25.5 (q)	C-2''', 3'''
5'''				1.59 (s)	17.3 (q)	C-2''', 3'''
3-OCH_3	3.80 (s)	55.6 (q)	C-3	3.81 (s)	55.7 (q)	C-3
3'-OCH_3	3.83 (s)	55.8 (q)	C-3'	3.84 (s)	55.8 (q)	C-3'
4'-OH	7.53 (s)		C- 3', 4', 5'	7.58 (s)		C- 3', 4', 5'

Co., Ltd., P. R. China). Column Chromatography (CC): Silica gel (200-300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*, P. R. China) and C_{18} reversed-phase silica gel (YMC *CO., LTD.*, Japan). HPLC: Ultimate 3000 HPLC system(Dionex Co. California, USA); Ultimate 3000 pump; Ultimate 3000 Variable Wavelength; column waters 5C_{18}-MS-II (10 × 250 mm). Optical rotation: *Perkin-Elmer 341* plolarimeter (Perkin Elmer Inc., Massachusetts, USA). ^{1}H- and ^{13}C-NMR spectral: *Bruker-AM-400* instrument (Bruker company, Massachusetts, USA): δ in ppm rel. to SiMe4 as internal standard (= 0 ppm), *J* in Hz. EI-MS: *Finnigan-MAT-95* mass spectrometer (Finnigan company; UK) (70 eV); in *m/z* (rel. %). ESI-MS and HR- ESI-MS data was obtained form Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Plant material. The barks of *Zanthoxylum planispinum* were collected from BaDong county, Hubei Province, PR China and identified by Prof. Dinrong Wan, College of Pharmacy, South Central University for Nationalities. The voucher specimen (07092701) was deposited in the Herbarium of College of Pharmacy, South Central University for Nationalities.

Extraction and isolation. The air-dried bark of *Zanthoxylum planispinum* (17 kg) were powdered and then extracted three times with MeOH at room temperature, and the methanolic extract (1.6 kg) was successively partitioned with petroleum ether, EtOAc and *n*-BuOH. The EtOAc extract (47 g) was chromatographed on a silica gel (800 g) column (8 × 50 cm) eluting with a gradient mixture of cyclohexane and acetone (cyclohexane-Me_2CO 95:5, 3 L; 9:1, 6 L; 8:2, 5 L; 7:3, 4 L; 1:1, 3 L, 0:1, 3 L) to afford 8 fractions (Fr1-Fr8). Fr6 was further chromatographed on a silica gel column (5 × 70 cm) with gradient mixture of cyclohexane and ethyl acetate (cyclohexane-EtOAc 9:1, 1.2 L; 8:2, 1.5 L; 7:3, 5 L; 1:1, 2.5 L; 0:1, 4 L) to give 6 subfractions (Fr6.1-Fr6.6). Fr6.3 was further purified by C18 reversed-phase silica gel column (2 × 50 cm) with gradient mixture of H_2O and MeOH (H_2O-MeOH 7:3, 1 L; 1:1, 2 L, 3:7, 6 L; 2:8, 2 L, 1:9, 1 L; 0:1, 2 L) to afford 7 subfractions (Fr6.3.1-Fr6.3.7). Fr6.3.3 was subjected to semiprepared HPLC (MeOH/H_2O 80:20, 3 mL/min; t_R 3.5 min) to afford compound **1** (20 mg). Fr6.3.6 were purified by semiprepared HPLC (MeOH/H_2O 70:30, 3 mL/min; t_R 46.7 min) to give compound **2** (40 mg).

Planispine A (**1**) colorless oil, $[\alpha]_D$ = -22.3 (c 0.35, acetone). EIMS *m/z* 358 (100), 205 (25), 163 (35), 151 (85), 137 (50), 131 (20), 69 (30), HR-ESI-MS *m/z* 449.1942 $[M+Na]^-$ (calculated for $C_{25}H_{30}O_6Na$, 449.1940), ^{1}H and ^{13}C-NMR: see Table 1.

Planispine B (**2**) colorless oil, $[\alpha]_D$ = -5.4 (c 0.95, acetone). EIMS *m/z* 388 (10), 358 (100), 327 (15), 205 (15), 163 (35), 151 (70), 137 (50), 131 (15), 124 (10), 69 (55), HR-ESI-MS *m/z* 517.2556 $[M+Na]^-$ (calculated for $C_{30}H_{38}O_6Na$, 517.2566) ^{1}H and ^{13}C-NMR: see Table 1.

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