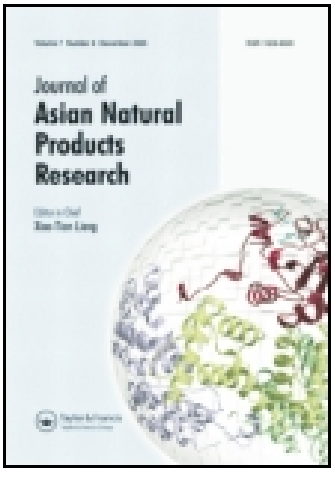

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

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Published online: 07 Nov 2014.

To cite this article: Cai-Yun Peng, Yan-Qun Liu, Yi-Hui Deng, Yue-Hu Wang & Xiao-Jiang Zhou (2015) Lignans from the bark of Zanthoxylum simulans, Journal of Asian Natural Products Research, 17:3, 232-238, DOI: 10.1080/10286020.2014.978303

To link to this article: http://dx.doi.org/10.1080/10286020.2014.978303

Journal of Asian Natural Products Research, 2015
Vol. 17, No. 3, 232–238, http://dx.doi.org/10.1080/10286020.2014.978303

Lignans from the bark of *Zanthoxylum simulans*

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(*Received 7 June 2014; final version received 14 October 2014*)

Investigation on the EtOAc extract of the bark of *Zanthoxylum simulans* led to the isolation of four new lignans including zanthoxylumin A (**1**), zanthoxylumin B (**2**), (−)-magnolin (**3**), and (−)-pinoresinol-di-3,3-dimethylallyl ether (**4**). Their structures were established by comprehensive analysis of the spectral data, especially 1D and 2D NMR spectra.

Keywords: *Zanthoxylum simulans*; Rutaceae; lignans

1. Introduction

The bark of *Zanthoxylum simulans* (Rutaceae) has been widely used as a folk medicine in China mainly to treat rheumatoid arthritis and swelling [1]. Alkaloids, coumarins, and lignans are major constituents in this genus [2,3]. However, up to now, the specific components against rheumatoid arthritis in this plant species were not well clarified [4–7]. As a part of our study on medicinal plants, we investigated the bark of this plant, from which four new lignans including zanthoxylumin A (**1**), zanthoxylumin B (**2**), (−)-magnolin (**3**), and (−)-pinoresinol-di-3,3-dimethylallyl ether (**4**) were isolated (Figure 1). Herein, we describe the isolation and structural elucidation of these compounds.

2. Results and discussion

Compound **1** was obtained as a colorless oil, and had a molecular formula $C_{18}H_{22}O_5$ from the HR-EI-MS at *m/z* 318.1459 $[M]^+$. Its IR spectrum revealed the existence of a five-membered lactone ring (1770 cm^{-1}) and benzene ring (1514 and 1464 cm^{-1}). ^1H NMR data (Table 1) implied an ABX system at δ_H 6.82 (dd, $J = 8.2$, 1.5 Hz), 6.85 (d, $J = 8.2$ Hz), and 6.88 (d, $J = 1.5$ Hz), indicative of the presence of a 1,3,4-trisubstituted benzene ring. In addition, ^{13}C NMR (Table 1) and DEPT spectra displayed 18 signals including one carbonyl carbon (δ_C 178.2), one benzylic oxygenated methine carbon (δ_C 86.0), two methine carbons (δ_C 48.1 and 46.0, respectively), two oxygenated methylene carbons (δ_C 70.0 and 69.8, respectively), three aromatic unsaturated quaternary carbons, and three aromatic unsaturated methine carbons, suggesting that **1** might be a lignan derivative with a five-membered lactone ring. Detailed analysis of the 1D and 2D NMR spectral data revealed that **1** was very similar to forsythenin [8], except for a methoxy moiety at the benzene ring being replaced by an oxygenated isopentenyl moiety. This oxygenated isopentenyl moiety can be confirmed by the ^1H NMR spectral data at δ_H 4.58 (2H, d, $J = 6.6$ Hz), 5.50 (1H, t,

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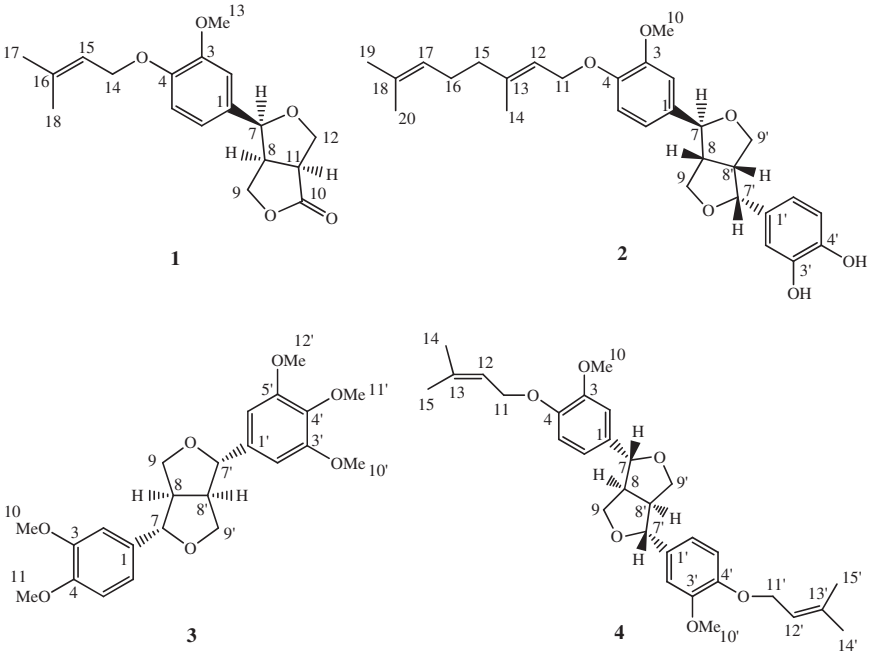

Figure 1. Chemical structures of compounds **1**–**4**.

$J = 6.6$ Hz), 1.77 (3H, s), 1.73 (3H, s). The key HMBC correlation between H_2-14 and C-4 (δ_C 148.4) indicated that the isopentyl moiety was connected to C-4 of the 1,3,4-trisubstituted benzene ring via an ether bond (Figure 2). The ROESY spectrum showed the correlations between H-8 and H-7/H-11/H-9a, H-11 and H-7/H-8/H-9a/H-12a, indicating these protons to be on the same side. Thus, the relative configuration of compound **1** was assigned as shown in Figure 2 and named as zanthoxylumin A.

Compound **2** was isolated as a white solid, and had the molecular formula $C_{29}H_{36}O_6$ as determined by HR-EI-MS at *m/z* 480.2530 $[M]^+$. IR and NMR spectra established that **2** possessed tetrahydrofuran lignan skeleton [9,10]. The ^{1}H NMR data (Table 1) exhibited the presence of two ABX systems at δ_H 6.91 (1H, d, $J = 8.0$ Hz), 6.89 (1H, dd, $J = 8.0$, 1.2 Hz), 6.98 (1H, d, $J = 1.2$ Hz) and δ_H 6.72 (1H, d, $J = 8.0$ Hz), 6.65 (1H, dd, $J = 8.0$, 1.4 Hz), 6.80 (1H, d, $J = 1.4$ Hz), suggesting two 1,3,4-trisubstituted benzene rings. Meanwhile, signals for one geranyloxy group were observed in the NMR spectra of **2** (Table 1), which was further supported by COSY correlations of H_2-11/H-12, H_2-15/H_2-16/H-17 and by HMBC correlations of H_2-11 to C-12 and C-13, and H_2-15 to C-12, C-13, C-14, C-16, and C-17, and H-17 to C-19 and C-20. The key HMBC correlations between H_2-11 and C-4 indicated that the geranyloxy group was directly connected to C-4 of the 1,3,4-trisubstituted benzene ring (Figure 2). The general features of its NMR spectroscopic data (Table 1) were markedly similar to those of planispine B [11]. Detailed comparison of the NMR and MS data of these two compounds indicated that a methoxy group (δ_C 55.8, δ_H 3.84) in planispine B was replaced by a hydroxyl group in **2**. Previous studies have suggested that the essential elements of the furofurano lignan skeleton have regular stereo-

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1**–**4**.

	1		**2**		**3**		**4**	
No.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		131.0		135.7		133.5		133.6
2	6.88 d (1.5)	109.1	6.98 d (1.2)	111.3	6.89 d (1.8)	109.2	6.90 br s	109.4
3		149.8		150.3		149.3		149.7
4		148.4		149.3		148.8		148.0
5	6.85 d (8.2)	112.7	6.91 d (8.0)	115.3	6.82 d (8.2)	111.1	6.85 br s	112.8
6	6.82 dd (8.2, 1.5)	118.3	6.89 dd (8.0, 1.2)	120.0	6.86 dd (8.2, 1.8)	118.4	6.85 br s	118.3
7	4.62 d (6.9)	86.0	4.42 d (7.0)	89.4	4.72 d (4.5)	85.9	4.75 d (4.2)	86.0
8	3.10–3.16 m	48.1	2.89–2.92 m	55.9	3.06–3.12 m	54.6	3.10–3.14 m	54.3
9a, b	4.50 dd (9.8, 6.8), 4.33 dd (9.8, 2.0)	69.8	4.08 d (9.3), 3.81–3.83 m	72.0	3.88–3.90 m, 4.24–4.28 m	72.1	3.88 dd (8.7, 3.6), 4.26 dd (8.7, 6.8)	71.9
10		178.2	3.84 s	56.6	3.88 s	56.1	3.88 s	56.1
11	3.45 ddd (9.1, 9.1, 3.8)	46.0	4.58 d (6.4)	67.1	3.86 s	56.1	4.57 d (6.7)	65.9
12	4.37 dd (9.1, 9.1), 4.19 dd (9.1, 3.8)	70.0	5.44–5.46 m	121.4			5.51 td (6.7)	120.0
13	3.88 s	55.9		142.2				137.9
14	4.58 d (6.6)	65.7	1.72 s	16.8			1.76 s	26.1
15	5.50 t (6.6)	119.7	2.05–2.07 m	40.8			1.72 s	18.4
16		137.9	2.10–2.14 m	27.5				
17	1.77 s	25.8	5.08–5.10 m	125.1				
18	1.73 s	18.2		132.7				
19			1.65 s	26.0				
20			1.60 s	17.9				
1′				131.1		137.0		133.6
2′			6.80 d (1.4)	114.2	6.55 s	102.8	6.90 br s	109.4
3′				147.1		153.6		149.7
4′				146.2		137.5		148.0
5′			6.72 d (8.0)	116.2		153.6	6.85 br s	112.8
6′			6.65 dd (8.0, 1.4)	117.9	6.55 s	102.8	6.85 br s	118.3
7′			4.79 d (5.3)	83.7	4.74 d (4.5)	86.2	4.75 d (4.2)	86.0
8′			3.32–3.35 m	51.4	3.06–3.12 m	54.2	3.10–3.14 m	54.3

9′a, b	3.30–3.32 m, 3.79-3.81 m	70.9	3.88–3.90 m, 4.24–4.28 m	71.9	3.88 dd (8.7, 3.6), 4.26 dd (8.7, 6.8)	71.9
10′			3.85 s	56.3	3.88 s	56.1
11′			3.81 s	61.1	4.57 d (6.7)	65.9
12′			3.85 s	56.3	5.51 td (6.7)	120.0
13′						137.9
14′					1.76 s	26.1
15′					1.72 s	18.4

chemistry, namely, the chemical shifts of C-7 and C-7′ in their ^{13}C NMR spectra have diagnostic values in the different types of furofurano lignans [3,7,11]. More specifically, the chemical shifts of C-7 and C-7′ were 89.4 and 83.7 ppm, respectively, indicating that the relative configurations were determined as 8*S**, 7*R**, 8′*S**, and 7′*S**. The ROESY spectrum further showed the correlations between H-7 and H-2/H-9a/H-9′a, H-8 and H-8′/H-9′b, H-7′ and H-9b/H-8′. Therefore, the relative configuration of compound **2** was elucidated as shown in Figure 2 and named as zanthoxylumin B.

Compound **3** was obtained as a colorless oil. The HR-EI-MS (*m/z* 416.1838 $[M]^+$, calcd for 416.1835) suggested its molecular formula to be $C_{23}H_{28}O_7$. ^{1}H and ^{13}C NMR data (Table 1) showed the characteristic pattern of a lignan with tetrahydrofuran moieties. NMR data of **3** were nearly the same as those of the known lignan (+)-magnolin [12]. The only difference is in the optical rotation value, (+)-magnolin was positive $[\alpha]_D^{20}$ + 53.4 (c = 3.1, $CHCl_3$) [12], but **3** was negative $[\alpha]_D^{23.5}$ −15.3 (c = 0.1, $CHCl_3$). Thus, they are enantiomers, and compound **3** was determined as (−)-magnolin.

Compound **4** was isolated as a white solid. Its molecular formula was established as $C_{30}H_{38}O_6$ (*m/z* 494.2673 $[M]^+$, calcd for 494.2668) on the basis of its HR-EI-MS analyses, but in the ^{13}C NMR spectrum, only 15 carbon signals were observed, which indicated the existence of a symmetrical structure in **4**. ^{1}H and ^{13}C NMR data (Table 1) also implied **4** was a lignan with tetrahydrofuran moieties as compound **3**. All NMR spectral data of **4** were extremely close to those of the known lignan (+)-pinoresinol-di-3,3-dimethylallyl ether [13]. The only difference is in the optical rotation value, (+)-pinoresinol-di-3,3-dimethylallyl ether was positive $[\alpha]_D^{25}$ + 41.6 (c = 0.13, $CHCl_3$) [13], but **4** was negative $[\alpha]_D^{26.8}$ −32.1

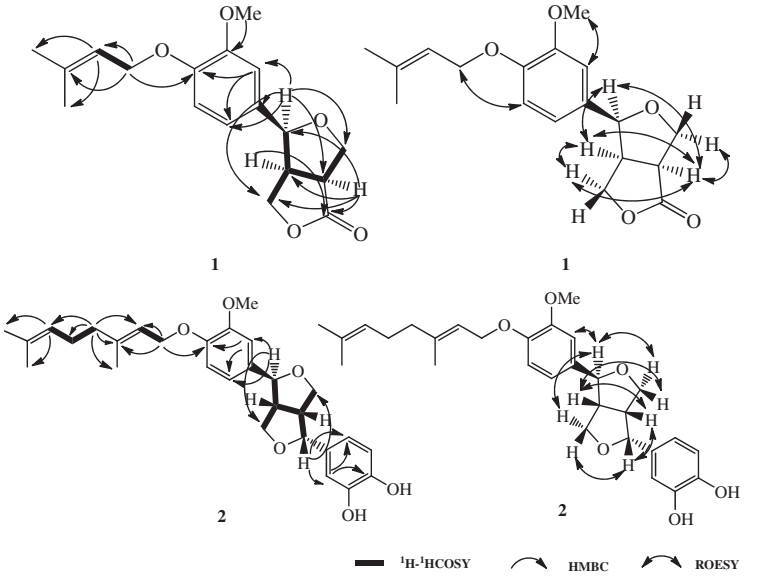

Figure 2. Key HMBC, ^{1}H–^{1}H COSY, and ROESY correlations of compounds **1** and **2**.

($c = 0.3$, $CHCl_3$). Therefore, they are also enantiomers, and compound **4** was identified as (−)-pinoresinol-di-3,3-dimethylallyl ether.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). UV spectra were measured on a Shimadzu UV-2401PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR spectra were obtained on a Tensor 27 (Bruker Optics Gmbh, Ettlingen, Germany) with KBr pellets. NMR spectra were recorded on a Bruker AV-400 or a DRX 500 or a Bruker Avance III-600 MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). EI-MS was obtained on a Finnigan-4510 spectrometer. ESI-MS and HR-EI-MS were determined with an API QSTAR Pulsar 1 spectrometer (MDS Sciex, Concord, ON, Canada). Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), RP-18 gel (40–63 μm, Daiso Co., Osaka, Japan), and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography.

3.2 Plant material

The bark of *Z. simulans* was purchased from Hunan Corporation of Materia Medica, Hunan Province, China, in February 2011, and authenticated by the corresponding author (X.J. Zhou). A voucher specimen (No. ZHXJ-0012) has been deposited at our laboratory in Hunan University of Chinese Medicine.

3.3 Extraction and isolation

The dried powdered bark of *Z. simulans* (30 kg) was extracted with ethanol (2×180 l) to give an extract (3370 g), which was suspended in water and partitioned by petroleum ether and EtOAc (each 4×8 l), respectively. The EtOAc extract (470 g) was fractionated by a silica gel column eluted with $CHCl_3$ with increasing amounts of methanol to afford seven fractions (Frs 1–7). Fr. 2 (89 g) was divided into five parts (Frs 2-1–2-5) by an MCI gel CHP 20P column eluting with

gradient aqueous MeOH. Fr. 2-5 (12 g) was first subjected to silica gel column ($CHCl_3/Me_2CO$, 50:1), followed by Sephadex LH-20 ($CHCl_3$/MeOH, 6:4), and further purified by semi-preparative HPLC (SHIMADZU LC-10A HPLC system, Ultimate XB-C-18, 5 μm, 10 × 250 mm) eluting with 50% aqueous MeOH (flow rate, 2 ml/min) to produce compound **1** (24 mg; t_R 17.02 min) and compound **3** (16 mg; t_R 23.37 min). Fr. 2-1 (7.5 g) was chromatographed by silica gel column ($CHCl_3/Me_2CO$, 60:1), Sephadex LH-20 ($CHCl_3$/MeOH, 6:4) and finally preparative thin layer chromatography ($CHCl_3/Me_2CO$, 20:1) to give compound **2** (9 mg; R_f 0.45) and compound **4** (45 mg; R_f 0.69).

3.3.1 *Zanthoxylumin A (**1**)*

Colorless oil; $[\alpha]_D^{21}$ −42.8 ($c = 0.16$, $CHCl_3$); UV ($CHCl_3$): λ_{max} (log ε) 280.6 (2.67), 240.0 (2.85) nm; IR (KBr): ν_{max} 1770, 1630, 1514, 1464, 1423, 1383, 1263, 1038 cm^{-1}; for ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data, see Table 1; EI-MS: *m/z* 318 $[M]^+$; HR-EI-MS: *m/z* 318.1459 $[M]^+$ (calcd for $C_{18}H_{22}O_5$, 318.1467).

3.3.2 *Zanthoxylumin B (**2**)*

White solid; $[\alpha]_D^{18.6}$ −43.7 ($c = 0.11$, MeOH); UV (MeOH): λ_{max} (log ε) 280.5 (2.95), 227.5 (3.43) nm; IR (KBr): ν_{max} 1630, 1610, 1514, 1463, 1419, 1384, 1267, 1138 cm^{-1}; for ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data, see Table 1; ESI-MS (positive): *m/z* 503 $[M + Na]^+$; HR-EI-MS: *m/z* 480.2530 $[M]^+$ (calcd for $C_{29}H_{36}O_6$, 480.2512).

3.3.3 *(−)-Magnolin (**3**)*

Colorless oil; $[\alpha]_D^{23.5}$ −15.3 ($c = 0.1$, $CHCl_3$); UV (C_6H_{14}): λ_{max} (log ε) 276.0 (2.53), 203.6 (3.71) nm; IR (KBr): ν_{max} 1514, 1463, 1419, 1383, 1126 cm^{-1}; for ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data, see Table 1; EI-MS: *m/z* 416 $[M]^+$; HR-EI-MS: *m/z* 416.1838 $[M]^+$ (calcd for $C_{23}H_{28}O_7$, 416.1835).

3.3.4 *(−)-Pinoresinol-di-3,3-dimethylallyl ether (**4**)*

White solid, $[\alpha]_D^{26.8}$ −32.1 ($c = 0.3$, $CHCl_3$); UV ($CHCl_3$): λ_{max} (log ε) 281.6 (3.13), 241.2 (3.37) nm; IR (KBr): ν_{max} 1585, 1516, 1466, 1417, 1264, 1236, 1139, 1069, 1028 cm^{-1}; for ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data, see Table 1; ESI-MS (positive): *m/z* 517 $[M + Na]^+$; HR-EI-MS: *m/z* 494.2673 $[M]^+$ (calcd for $C_{30}H_{38}O_6$, 494.2668).

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 31170336), a Project from the Traditional Chinese Medicine Scientific Research Fund of Hunan Province, China (No. 201122), and a Project from Science and Technology Innovative Research Team in Higher Educational Institutions of Hunan Province, China (No. 15).

Note

1. These authors contributed equally to this paper.

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