MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. 2006; **44**: 727–730
Published online 20 April 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mrc.1830

MRC

Spectral Assignments and Reference Data

Complete assignments of ^{1}H and ^{13}C NMR data for seven arylnaphthalide lignans from *Justicia procumbens*

Meihua Yang,[1] Jun Wu,[2]* Fan Cheng[3] and Yuan Zhou[3]

[1] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100094, P.R. China
[2] Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, P.R. China
[3] Chemistry and Life Science College, China Three Gorges University, 8 University Road, Yichang 443002, P.R. China

Received 9 February 2006; revised 4 March 2006; accepted 7 March 2006

Three new arylnaphthalide lignans named 6′-hydroxy justicidin A (1), 6′-hydroxy justicidin B (2) and 6′-hydroxy justicidin C (3) have been isolated from the whole plant of *Justicia procumbens*, together with four known ones, neojusticin A (4), chinensinaphthol methyl ester (5), isodiphyllin (6) and taiwanin C (7). The complete assignments of ^{1}H and ^{13}C NMR chemical shifts for the new lignans and the ^{13}C NMR chemical shifts for the known lignans were obtained for the first time by means of 2D NMR techniques, including HSQC and HMBC.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; 2D NMR; arylnaphthalide lignan; *Justicia procumbens*; complete NMR assignments

INTRODUCTION

The whole plant of *Justicia procumbens* L. (Acanthaceae) is used as a herbal remedy for the treatment of fever, pain due to laryngopharyngeal swelling and cancer in China.[1] In Taiwan, the juice of this plant has also been used as a fish-killing material for many hundred years.[2] Previous phytochemical studies on this plant have afforded 10 diarylbutane lignans, 12 arylnaphthalide lignans and 7 arylnaphthalide lignan glycosides.[2–8] Recently, it was reported that justicidin A, isolated from the same plant, not only decreased the level of cytosolic Ku70 leading to apoptosis in human colorectal cancer cells[9] but also inhibited LPS-stimulated TNF-α release from RAW 264.7 macrophages in a concentration- and time-dependent manner.[10] In the continuing search for potential drug leads from acanthaceae plants, three new arylnaphthalide lignans, named 6′-hydroxy justicidin A (**1**), 6′-hydroxy justicidin B (**2**) and 6′-hydroxy justicidin C (**3**), have been isolated from the whole plant of *J. procumbens*, together with four known ones, neojusticin A (**4**),[2] chinensinaphthol methyl ester (**5**),[2,11] isodiphyllin (**6**)[12] and taiwanin C (**7**).[13] Though the ^{1}H NMR data of the above four known lignans **4–7** are well described in the literature, no completely assigned ^{13}C NMR data have been published. Here we give the exact and unambiguous ^{1}H and ^{13}C NMR assignments of these arylnaphthalide lignans for the first time, achieved with the aid of 2D NMR techniques, including HSQC and HMBC.

RESULTS AND DISCUSSION

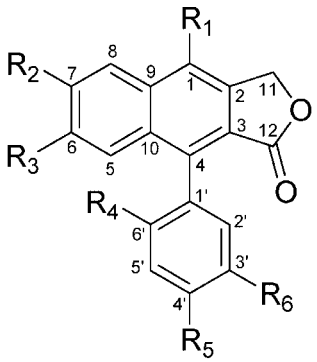
The ethanolic extract of the whole plant of *J. procumbens* was subjected to sequential extraction with petroleum ether and ethyl acetate as described in the Experimental section. The resulting ethyl acetate extract was chromatographed on silica gel, Sephadex LH-20 gel, and followed by preparative reverse-phase C_{18} HPLC to yield 6′-hydroxy justicidin A (**1**), 6′-hydroxy justicidin B (**2**), 6′-hydroxy justicidin C (**3**), neojusticin A (**4**), chinensinaphthol methyl ester (**5**), isodiphyllin (**6**) and taiwanin C (**7**) (Figs 1 and 2).

The structures of the known compounds **4–7** were determined by comparison of mass spectrometric (MS) and ^{1}H NMR data with those in the literature and reconfirmed by 2D NMR experiments. The ^{13}C NMR data of these compounds, which have not been reported so far, were first assigned unambiguously by HSQC and HMBC correlations, as shown in Tables 1–2.

6′-Hydroxy justicidin A (**1**) was isolated as a pale yellow amorphous powder. Its molecular formula was determined as $C_{22}H_{18}O_8$ by the HR-TOF-MS spectrum (m/z 410.1000, calcd for $[M]^+$ 410.1002). The UV maxima at 203, 256, 307 nm and the IR (KBr) absorption bands at 3290, 2936, 1727, 1508, 1485, 1262, 1220, 1177, 1162 and 928 cm^{-1} suggested that it was an arylnaphthalide lignan with a γ-lactone. The ^{1}H NMR data (Table 1) of **1** contained the signals of four singlet aromatic protons (δ 6.59, 6.67, 6.91, 7.49 ppm), a methylenedioxy group (δ 5.981 ppm, broad s; 5.987 ppm, broad s), a γ-lactone methylene group (δ 5.67 ppm, 2H, broad s) and three methoxy groups (δ 3.65 ppm, s; 3.92 ppm, s; 4.11 ppm, s). The ^{1}H NMR data of **1** were similar to those of Justicidin A[1] except for the absence of an aromatic proton that led to the disappearance of an ABX system seen in the ^{1}H NMR spectrum of Justicidin A. The above observation suggested that an oxygenated group might be substituted at the 6′-C of the 1-aryl group of **1**. Considering the elemental composition of **1**, this group was characterized as a hydroxyl. On the basis of these results, the structure of **1** was identified as 6′-hydroxy justicidin A and its ^{13}C NMR data were assigned unambiguously by the HSQC and HMBC (Fig. 3) correlations, as shown in Table 2.

6′-Hydroxy justicidin B (**2**), a pale yellow amorphous powder, had a molecular formula of $C_{21}H_{16}O_7$ established by the HR-TOF-MS spectrum (m/z 380.0899, calcd for $[M]^+$ 380.0896). The ^{1}H and ^{13}C NMR data of **2** were almost the same as those of **1**, except for the absence of a methoxy group (δ_H 4.11 ppm, s; δ_C 59.6 ppm, q; 1-OCH_3 in **1**) and the presence of one more aromatic proton (δ_H 7.86 ppm, s). It was indicated that 1-methoxy group in **1** was replaced by an aromatic proton. This result was further confirmed by the HMBC correlation from this proton to C-2 (δ_C 139.9 ppm) and C-9 (δ_C 128.1 ppm) (Fig. 3). Therefore, the structure of **2** was identified as 1-demethoxy 6′-hydroxy justicidin A, namely 6′-hydroxy justicidin B. Its ^{13}C NMR data were assigned unambiguously by the HSQC and HMBC (Fig. 3) correlations, as shown in Table 2.

6′-Hydroxy justicidin C (**3**) was isolated as a pale yellow amorphous powder. Its molecular formula was determined as the



1: $R_1 = R_2 = R_3 = OCH_3$ $R_4 = OH$ R_5-$R_6 = -OCH_2O-$

2: $R_1 = H$ $R_2 = R_3 = OCH_3$ $R_4 = OH$ R_5-$R_6 = -OCH_2O-$

5: $R_1 = OCH_3$ R_2-$R_3 = -OCH_2O-$ $R_4 = H$ R_5=$R_6 = OCH_3$

6: $R_1 = OH$ R_2-$R_3 = -OCH_2O-$ $R_4 = H$ $R_5 = R_6 = OCH_3$

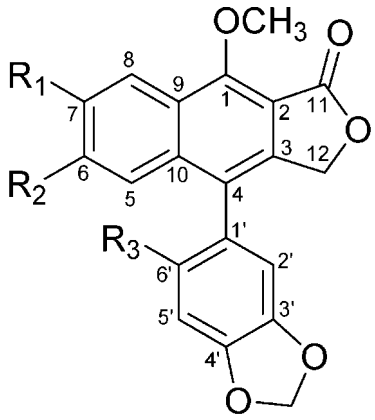
7: $R_1 = OCH_3$ R_2-$R_3 = -OCH_2O-$ $R_4 = H$ R_5-$R_6 = -OCH_2O-$

Figure 1. Structures of compounds **1–2** and **5–7**.

*Correspondence to: Jun Wu, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, P.R. China.
E-mail: wwujun2003@yahoo.com

MRC

Spectral Assignments and Reference Data



3: $R_1 = R_2 = OCH_3$ $R_3 = OH$

4: $R_1\text{-}R_2 = -OCH_2O-$ $R_3 = H$

Figure 2. Structures of compounds **3–4**.

same as that of **1** by the HR-TOF-MS spectrum. The ^{1}H NMR data of **3** was very similar to those of **1**, except for the upfield shift of γ-lactone methylene protons (δ_H 5.07 ppm, double, J = 14.8 Hz; 5.23 ppm, double, J = 14.8 Hz) and the downfield shift of 1-OCH_3 (δ_H 4.20 ppm, s). According to the ^{1}H NMR spectral characteristics of the arylnaphthalide lignans (the methylene protons of the γ-lactone ring of the arylnaphthalide lignans appear between δ 5.08 ppm and 5.23 ppm when it is vicinal to the 1-aryl group, whereas they appear between δ 5.32 ppm and 5.52 ppm when it is opposite to the 1-aryl group) pointed out by Horii *et al.*,[14] the above observation disclosed that the methylene group of the γ-lactone ring in **3** was vicinal to the 1-aryl group as it was in justicidin C. Comparison of ^{1}H and ^{13}C NMR data of **3** with those of justicidin C[1] revealed that the 6′-H of justicidin C was substituted by a hydroxyl. On the basis of the above results, the structure of **3** was identified as 6′-hydroxy justicidin C. Its ^{13}C NMR data were assigned unambiguously by the HSQC and HMBC (Fig. 3) correlations, as shown in Table 2.

EXPERIMENTAL

Plant material

The whole plant of *J. procumbens* was collected from Jiangxi province and identified by Prof. Hubiao Chen, School of Pharmaceutical Sciences, Peking University. A voucher sample is kept in the Herbarium of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Compounds

The dried plant (5 kg) of *J. procumbens* was extracted with hot 95% ethanol three times successively. After removal of the solvent by evaporation, the residue was suspended in water and defatted with petroleum ether. The aqueous layer was further extracted with ethyl acetate. The resulting ethyl acetate extract (68 g) was subjected to silica gel CC using chloroform–methanol system (100 : 0 ~ 2 : 1) to yield 70 fractions. Fractions 23–26 (3 g) were combined and subjected to CC on silica gel, Sephadex LH-20 gel, followed by preparative reverse-phase C_{18} HPLC using an acetonitrile–water system (30 : 70 to 40 : 60) to yield compounds **1** (30 mg), **2** (20 mg), **3** (8 mg), **4** (5 mg), **5** (5 mg), **6** (5 mg) and **7** (3 mg).

6′-Hydroxy justicidin A (**1**). Pale yellow amorphous powder, HR-TOF-MS m/z: 410.1000, (calcd for $C_{22}H_{18}O8$: 410.1002). ^{1}H and ^{13}C NMR (DMSO-d_6): Tables 1 and 2.

6′-Hydroxy justicidin B (**2**). Pale yellow amorphous powder, HR-TOF-MS m/z: 380.0899, (calcd for $C_{21}H_{16}O_7$: 3807 : 380.0896). ^{1}H and ^{13}C NMR (DMSO-d_6):Tables 1 and 2.

6′-Hydroxy justicidin C (**3**). Pale yellow amorphous powder, HR-TOF-MS m/z: 410.0999,(calcd for $C_{22}H_{18}O_8$: 4108 : 410.1002). ^{1}H and ^{13}C NMR (DMSO-d_6): Tables 1 and 2.

Spectra

All NMR experiments were recorded on a Bruker AV-500 spectrometer operating at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, and equipped with an inverse-detection 5 mm probe (TBI probe,^{1}H 90° pulse width = 6.1 μs, ^{13}C 90° pulse width = 12.3 μs) and operating at room temperature with tetramethylsilane as internal standard. About 3–10 mg samples were dissolved in DMSO-d_6 or $CDCl_3$ (0.5 ml) to record the NMR spectra.

1D spectra were acquired using 64 K data points and spectral widths of 10 000 Hz and 27 500 Hz for ^{1}H and ^{13}C, respectively. 32 K data points were used for the processing with an exponential function for all 1D spectra.

Table 1. ^{1}H (500 MHz) NMR spectral data for compounds **1–6** detected in DMSO-d_6, and 7 in $CDCl_3$ (J in Hz)

No.	1	2	3	4	5	6	7
1	–	7.86, s	– v	–	–	–	–
5	6.91, s	6.94, s	6.89, s	6.91, s	6.84, s	6.83, s	7.06, s
8	7.49, s	7.43, s	7.59, s	7.63, s	7.53, s	7.58, s	7.56, s
11	5.67. br s	5.39, br s	–	–	5.66, br s	5.33, br s	5.50, br s
12	–	–	5.07, d, 14.8	5.20, br s	–	–	–
–	–	–	5.23, d, 14.8	5.18, br s	–	–	–
2′	6.59, s	6.63, s	6.77, s	6.96, d, 1.6	6.81, d, 1.8	6.81, d, 1.6	6.77, d, 1.6
5′	6.57, s	6.59, s	6.65, s	7.07, d, 8.0	7.06, d, 8.0	7.05, d, 8.0	6.95, d, 7.8
6′	–	–	–	6.85, dd, 8.0, 1.6	6.77, dd, 8.0, 1.8	6.76, dd, 8.0, 1.6	6.75, dd, 7.8, 1.6
6-OCH_2O-7	–	–	–	6.17, br s	6.15, br s	6.12, br s	6.04, d, 1.4
–	–	–	–	–	–	–	6.07, d, 1.4
3′-OCH_2O–4′	5.987, br s	5.990, br s	5.98, br s	6.09, br s	–	–	6.07, br s
–	5.981, br s	5.997, br s	6.01, br s	–	–	–	–
1-OCH_3	4.11, s	–	4.20, s	4.15, s	4.07, s		4.08, s
6-OCH_3	3.92, s	3.91, s	3.92, s	–	–	–	–
7-OCH_3	3.65, s	3.65, s	3.70, s	–	–	–	–
3′-OCH_3	–	–	–	–	3.97, s	3.82, s	–
4′-OCH_3	–	–	–	–	3.86, s	3.69, s	–

Table 2. ^{13}C (125 MHz) NMR spectral data for compounds **1–6** detected in DMSO-d_6, and 7 in $CDCl_3$

No.	1	2	3	4	5	6	7
1	147.6, s	118.6, d	154.4, s	155.0, s	147.9, s	145.2, s	148.4, s
2	124.4, s	139.9, s	109.6, s	110.7, s	125.3, s	122.5, s	125.7, s
3	120.2, s	118.7, s	139.9, s	140.2, s	119.6, s	119.2, s	119.7, s
4	130.7, s	136.2, s	123.8, s	127.5, s	133.7, s	130.7, s	135.1, s
5	106.4, d	105.6, d	104.6, d	101.8, d	102.9, d	102.7, d	104.0, d
6	151.6, s	151.5, s	149.7, s	148.6, s	149.8, s	148.9, s	149.9, s
7	150.3, s	149.7, s	152.1, s	151.5, s	148.8, s	148.5, s	148.9, s
8	101.0, d	106.7, d	101.9, d	99.8, d	98.2, d	98.2, d	98.6, d
9	130.1, s	128.1, s	133.4, s	135.0, s	131.3, s	131.3, s	132.3, s
10	125.4, s	132.9, s	123.0, s	125.0, s	126.7, s	124.8, s	127.7, s
11	67.1, t	68.1, t	168.9, s	168.9, s	66.8, t	66.7, t	66.5, t
12	169.6, s	169.7, s	69.0, t	69.0, t	169.2, s	169.8, s	169.4, s
1′	113.6, s	113.2, s	113.1, s	129.3, s	127.3, s	127.7, s	128.4, s
2′	111.0, d	110.4, d	109.9, d	110.2, d	114.1, d	114.4, d	110.7, d
3′	140.1, s	139.8, s	140.4, s	147.6, s	148.5, s	148.4, s	147.5, s
4′	147.7, s	147.7, s	148.0, s	148.2, s	148.3, s	148.2, s	147.5, s
5′	98.1, d	97.8, d	98.4, d	109.3, d	111.4, d	111.4, d	108.2, d
6′	150.0, s	149.6, s	149.5, s	123.6, d	122.6, d	122.8, d	123.7, d
6-OCH_2O-7	–	–	–	102.8, t	102.4, t	102.1, t	101.2, t
3′-OCH_2O-4′	101.3, t	101.0, t	101.3, t	101.8, t	–	–	101.8, t
1-OCH_3	59.6, q	–	63.2, q	63.6, q	59.6, q	–	60.0, q
6-OCH_3	56.1, q	55.8, q	55.7, q	–	–	–	–
7-OCH_3	55.7, q	55.3, q	55.5, q	–	–	–	–
3′-OCH_3	–	–	–	–	55.7, q	55.6, q	–
4′-OCH_3	–	–	–	–	55.6, q	55.7, q	–

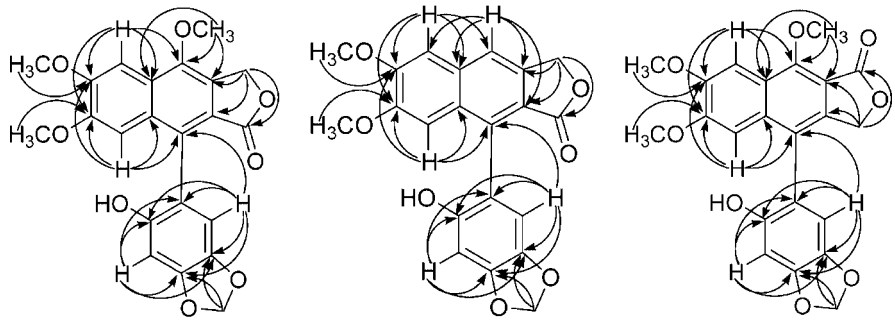


Figure 3. Selected HMBC correlations for compound **1–3**.

Standard pulse sequences were used for 2D spectra. Spectral widths of 5000 Hz and 25 000 Hz were used for ^1H and ^{13}C, respectively. Relaxation delays of 2.0 s were used for all 2D NMR experiments. The 2D spectra used 2048 × 512 (HSQC), and 4096 × 512 (HMBC) data point matrices, which were zero filled to 1024 × 1024, 2048 × 1024 and 4096 × 1024, respectively. Non-shifted qsine-bell window functions were used along the F_1 and F_2 axes for all 2D spectra. The HMBC experiments used an 80 ms delay time to obtain ^1H and ^{13}C long-range correlations. Z-PFGs were used to obtain HSQC, HMBC spectra. Data processing was carried out with Bruker XWINNMR 3.50 programs.

Acknowledgement

This research was financially supported by a grant (code: 04100729) from the Natural Science Foundation of Guangdong Province.

REFERENCES

1. Fukamiya N, Lee KH. *J. Nat. Prod.* 1986; **49**: 348.
2. Chen CC, Hsin WC, Ko FN, Huang YL, Ou JC, Teng CM. *J. Nat. Prod.* 1996; **59**: 1149.
3. Ohta K, Munakata K. *Tetrahedron Lett.* 1970; **11**: 923.
4. Okigawa M, Maeda T, Kawano N. *Chem. Pharm. Bull.* 1970; **18**: 862.
5. Asano J, Chiba K, Tada M, Yoshii T. *Phytochemistry* 1996; **42**: 713.
6. Chen CC, Hsin WC, Huang YL. *J. Nat. Prod.* 1998; **61**: 227.
7. Day SH, Lin YC, Tsai ML, Tsao LT, Ko HH, Chung MI, Lee JC, Wang JP, Won SJ, Lin CN. *J. Nat. Prod.* 2002; **65**: 379.
8. Weng JR, Ko HH, Yeh TL, Lin HC, Lin CN. *Arch. Pharm. Pharm. Med. Chem.* 2004; **337**: 207.

Spectral Assignments and Reference Data

9. Lee JC, Lee CH, Su CL, Huang CW, Liu HS, Lin CN, Won SJ. *Carcinogenesis* 2005; **26**: 1716.
10. Tsao LT, Lin CN, Wang JP. *Mol. Pharmacol.* 2004; **65**: 1063.
11. Teng CM, Yu SM, Chen CC, Huang YL, Huang TF. *Thromb. Res.* 1990; **59**: 121.
12. González AG, Pérez JP, Trujillo JM. *Tetrahedron* 1978; **34**: 1011.
13. Lin YT, Lo TB, Wang KT, Weinstein B. *Tetrahedron Lett.* 1967; **8**: 853.
14. Horii Z, Tsujiuchi M, Momose T. *Tetrahedron Lett.* 1969; **10**: 1079.