Journal of Natural Products
Vol. 58, No. 2, pp. 244-249, February 1995

PHYLLAMYRICINS A–C, THREE NOVEL LIGNANS FROM *PHYLLANTHUS MYRTIFOLIUS*

MEI-TSU LIN, SHOEI-SHENG LEE, and KARIN C.S. CHEN LIU*

School of Pharmacy, College of Medicine, National Taiwan University, 1 Jen-Ai Rd. Sec. 1, Taipei, Taiwan 100, Republic of China

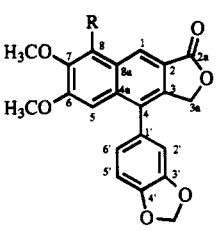
ABSTRACT.—Six lignans [**1–6**] of the aryl naphthalene type were isolated during the investigation of chemical constituents of *Phyllanthus myrtifolius*. Among these, phyllamyricins A [**1**], B [**2**], and C [**3**] are novel natural products. Retrojusticidin B [**4**] has been synthesized earlier, but was obtained here as the first natural occurrence of this compound. Compounds **5** and **6** were identified as justicidins A and B, respectively. Structural elucidation of the novel compounds was based mainly on nmr spectral analysis, which also led to the complete assignment of the ^{1}H- and ^{13}C-nmr data of compounds **1–4** and **6**.

Many plants of the genus *Phyllanthus* (Euphorbiaceae) are prominent in traditional medicine (1,2). In the search for biologically active compounds, plants of this genus have yielded a number of lignans (3–6) possessing cytotoxic activity. *Phyllanthus myrtifolius* Moon (Euphorbiaceae) is a small shrub indigenous to India and Ceylon (7) and has been introduced to other countries as a garden plant. To our knowledge, there is no prior report concerning the pharmacology and chemical constituents of this plant, nor about its use in folk medicine. Our interest in this plant was stimulated by its inhibitory activity on the DNA polymerase of Epstein-Barr virus (EBV). The MeOH extract of the aerial part of this plant was fractionated into hexane-, $CHCl_3$-, EtOAc-, *n*-BuOH-, and H_2O-soluble fractions. The $CHCl_3$-soluble fraction was repeatedly chromatographed on Si gel to yield six lignans.

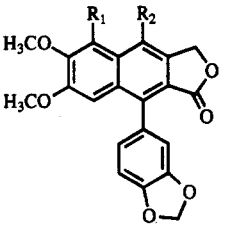
These six compounds each showed a uv absorption maximum around 260 nm and other absorptions of low intensity around 355, 310, 290, and 225 nm, characteristic of aryl naphthalene-type compounds such as justicidins E and F (8). The ir spectra of **1** and **3–6** all showed a lactone absorption (ca. 1760 cm^{-1}) which, in the case of **2**, was replaced by a conjugated carbonyl absorption (1678 cm^{-1}). The ^{1}H- and ^{13}C-nmr spectra of these compounds showed signals in common for a lactone methylene group (δ_H 5.15–5.40, δ_C ca. 70 ppm). In addition, the ^{13}C-nmr spectra of **1** and **3–6** showed a lactone carbon resonance around δ 170 ppm while that of **2** exhibited an aldehydic carbon signal (d, δ 194.3). These data, when taken together, revealed that compounds **1** and **3–6** are aryl naphthalides, while **2** is an aryl naphthalene with a formyl functionality. In addition, all of these compounds possess in common a methylenedioxy function (ir, ca. 930 cm^{-1}; ^{1}H nmr, AB quartet in the region δ 6.00–6.10; ^{13}C nmr, ca. 101.0 ppm).

Compound **1** showed a molecular ion at *m/z* 394.1055 in its hreims, corresponding to the formula $C_{22}H_{18}O_7$. Its ^{1}H-nmr spectrum exhibited five aromatic proton signals, two singlets (δ 8.68, 6.86), an ABX splitting pattern between δ 6.78 and 6.95, and three methoxy singlets at δ 3.80, 3.95, and 4.06, in addition to the signals for a methylenedioxy (δ 6.03 and 6.07, J_{gem}=0.9 Hz), and a methylene proton singlet (δ 5.16) (Table 1). These nmr data are characteristic for a five substituted 4-aryl-2,3-naphthalide derivative (8).

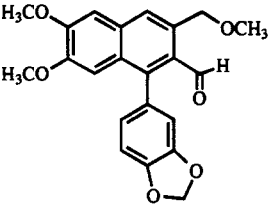
The arrangement of the five oxygenated substituents and the ^{1}H-nmr assignments of **1** were determined by a NOESY experiment, which exhibited nOes of 6-OMe (δ 3.80) to 7-OMe (δ 3.95) and H-5 (δ 6.86); 8-OMe (δ 4.06) to 7- OMe (δ 3.95) and H-1 (δ 8.68); the methylene protons at δ 5.16 to H-2′ (δ 6.80) and H-



1 R=OCH_3
4 R=H



3 R_1=OCH_3, R_2=H
5 R_1=H, R_2=OCH_3
6 R_1=H, R_2=H



2

6′ (δ 6.78); and H-5 to H-2′ and H-6′. This technique hence aided in the structure elucidation of **1** as 3-hydroxymethyl-6,7,8-trimethoxy-4-(3,4-methylenedioxyphenyl)-2-naphthoic acid γ-lactone.

The complete ^{13}C-nmr assignment of **1** (Table 2) was achieved through detailed analysis of two hetero COSY spectra (J_{CH}=140 Hz and 8 Hz). The data are summarized in Table 2. Among these, the signals of the nonoxygenated quaternary carbons, C-2, C-3, C-4, C-4a, C-8a, and C-1′, were distinguished by their long-range coupling (generally via three bonds) to H-1 (C-3, C-4a), H-3a (C-2, C-3, C-4, C-4a), H-5 (C-4, C-8a), and H-5′ (C-1′); while the signals of the oxygenated quaternary carbons, C-6, C-7, C-8, C-3′, and C-4′, were distinguished via three-bond coupling to H-1 (C-8), H-5 (C-7), 6-OMe (C-6), 7-OMe (C-7), 8-OMe (C-8), H-5′ (C-3′), H-2′ (C-4′), and H-6′ (C-4′). In addition, the hetero long-range COSY technique revealed three-bond coupling between the lactone carbon (C-2a, δ 171.6) and H-1 (δ 8.68), and between C-4 (δ 131.8) and H-3a (δ 5.16), to further support the structure designated for **1**. To our knowledge, **1** is a novel natural product and has been accorded the trivial name phyllamyricin A.

Compound **2** has a molecular formula of $C_{22}H_{20}O_6$, as deduced from its

TABLE 1. ^{1}H-Nmr (400.13 MHz) Data of **1–3** (δ in ppm, *J* in Hz).[a]

Proton	Compound			HMBC data of **3**	
	1	**2**	**3**	δ_H	δ_C
H-1	8.68 s	8.26 s	8.10 s	8.10	130.3 (C-3), 68.3 (2a), 147.34 (C-8)
H-2a		5.22 s	5.39 s	5.39	114.0 (C-1), 138.6 (C-2), 130.3 (C-3), 169.9 (C-3a)
H-3a	5.16 s	10.21 s			
H-5	6.86 s	7.10 s	6.91 s	6.91	139.7 (C-4), 153.4 (C-6), 143.0 (C-7), 128.2 (C-8a)
MeO-6	3.80 s	3.62 s	3.77 s	3.77	53.4 (C-6)
MeO-7	3.95 s	3.93 s	4.00 s	4.00	143.0 (C-7)
H-8		7.40 s			
MeO-8	4.06 s		4.07 s	4.07	147.3 (C-8)
H-2′	6.80 d (1.5)	7.07 d (1.5)	6.82 d (1.4)	6.82	139.7 (C-4), 147.5 (C-4′), 123.4 (C-6′)
H-5′	6.95 dd (1.5, 7.7)	7.06 dd (1.5, 7.8)	6.95 dd (1.4, 7.8)	6.95	128.3 (C-1′), 147.5 (C-3′)
H-6′	6.78 d (7.7)	6.92 d (7.8)	6.80 d (7.8)	6.80	139.7 (C-4), 147.5 (C-4′), 110.5 (C-2′)
-OCH_2O- ...	6.03 d (0.9)	6.07 d (0.9)	6.03 d (1.2)		
	6.07 d (0.9)	6.10 d (0.9)	6.08 d (1.2)		
MeO-2a		3.56 s			

[a]Data were recorded in $CDCl_3$, except for **2** (measured in C_5D_5N).

TABLE 2. ^{13}C-Nmr (100.61 MHz) Data of **1–4** and **6** (δ in ppm).[a]

Carbon	Compound					Hetero long-range COSY data of **1**	
	1	**2**	**3**	**4**	**6**	δ_C	δ_H
C-1	120.6 d	124.9 d	114.0 d	124.1 d	118.2 d		
C-2	120.8 s	134.6 s	138.6 s	121.3 s	139.46 s	120.8	5.16 (H-3a)
C-3	139.2 s	128.8 s	130.3 s	137.9 s	118.4 s	139.2	8.68 (H-1), 5.16 (H-3a)
C-4	131.8 s	145.8 s	139.7 s	131.8 s	133.1 s	131.8	5.16 (H-3a), 6.86 (H-5)
C-4a.....	132.9 s	127.5 s	119.9 s	131.6 s	128.8 s	132.9	8.68 (H-1)
C-5	100.1 d	106.2 d	102.1 d	103.9 d	105.8 d		
C-6	155.6 s	150.7 s	153.4 s	152.0 s	150.0 s	155.6	3.80 (MeO-6)
C-7	141.1 s	152.8 s	143.0 s	150.1 s	151.7 s	141.1	6.86 (H-5), 3.95 (MeO-7)
C-8	149.0 s	107.3 d	147.3 s	107.6 d	106.0 d	149.0	8.68 (H-1), 4.06 (MeO-8)
C-8a.....	125.5 s	132.5 s	128.2 s	129.8 s	139.5 s	125.5	6.86 (H-5)
C-1′.....	129.6 s	130.7 s	128.3 s	129.7 s	128.3 s	129.6	6.95 (H-5′)
C-2′.....	109.4 d	108.6 d	110.5 d	109.5 d	110.6 d		
C-3′.....	148.3 s	148.3 s	147.5 s	148.2 s	147.5 s	148.3	6.03 and 6.07 (3-OCH_2O-4′), 6.95 (H-5′)
C-4′.....	147.6 s	148.1 s	147.5 s	147.6 s	147.5 s	147.6	6.80 (H-2′), 6.78 (H-6′)
C-5′.....	109.0 d	111.6 d	108.2 d	109.0 d	108.2 d		
C-6′.....	122.6 d	124.8 d	123.4 d	122.7 d	123.4 d		
6-OMe.....	55.8 q	55.4 q	55.8 q	55.9 q	55.8 q		
7-OMe.....	61.1 q	55.9 q	61.2 q	56.0 q	56.0 q		
8-OMe.....	61.7 q		61.5 q				
2a-OMe		58.7 q					
C-2a.....	171.6 s	73.2 t	68.3 t	171.5 s	68.0 t	171.6	8.68 (H-1), 5.16 (H-3a)
C-3a.....	69.4 t	194.3 d	169.9 s	69.4 t	169.9 s		
-OCH_2O-...	101.4 t	102.1 t	101.2 t	101.4 t	101.2 t		

[a]Data were recorded in $CDCl_3$ except for **2** (measured in C_5D_5N).

hreims. The ^{1}H-nmr spectrum of **2** showed six aromatic proton signals, three singlets (one more than **1**), an ABX splitting system, three methoxy singlets, in addition to the aldehydic proton singlet (δ 10.21) the methylenedioxy (AB quartet), and the methylene proton singlet (δ 5.22) (Table 1). Analysis of the chemical shifts and coupling patterns of these six aromatic protons suggested that C-2, C-3, C-6, C-7, C-3′, and C-4′ were substituted by a methylenedioxy, two methoxyls, a formyl and a methoxymethyl group. From a biogenetic viewpoint, the formyl and methoxymethyl group would most likely be located at C-2 and C-3, respectively.

The exact location of the substituents was determined by a ROESY nmr experiment. The critical data found were that the singlet at δ 8.26 (H-1) correlated to the singlets at δ 7.40 (H-8), 3.56 (2a-OMe) and 5.22 (H-2a), and the signal of the aldehydic proton (δ 10.21, H-3a) correlated to those of H-2a, H-2′, and H-6′. These results established **2** as 2-methoxymethyl-3-formyl-6,7-dimethoxy-4-(3,4-methylenedioxyphenyl)-naphthalene.

Further examination of a hetero long-range-COSY spectrum (J=8 Hz) also supported the assigned structure for **2**. Three critical supportive observations were that C-1 (δ 124.9), assigned from its one-bond coupling to H-1 (δ 8.26) in the hetero COSY spectrum, was three-bond coupled to H-8 (δ 7.40) and H-2a (δ 5.22); C-2a was coupled to 2a-OMe (δ 3.56), and C-2 (δ 134.6) was coupled to H-2a and the aldehydic proton (δ 10.21, H-3a). The locations of the other two methoxyls at C-6 and C-7 were designated by the observation of three-bond coupling of C-6 (δ 150.7) to H-8 and a methoxy group (δ 3.62), and of C-7 (δ 152.8) to H-5 (δ 7.10) and 7-OMe (δ 3.93). The placement of the methylenedioxy group at C-3′ and C-4′ was conducted by a process of elimination. Based on the analysis of the hetero-COSY (^1J

and long range) and ROESY spectra, the ^{1}H- and ^{13}C-nmr data were unambiguously assigned (Table 1 and 2). Compound **2** appears to be a novel natural product and has been named phyllamyricin B. However, the related 2-formyl-3-methoxymethyl isomer has been synthesized (9).

Compound **3** was found to have the same molecular formula ($C_{22}H_{18}O_7$) as **1**, as deduced from the hreims. The ^{1}H-nmr spectrum of **3** showed a close resemblance to that of **1** (Table 1). The major differences apparent were the signals of H-1 (δ 8.68) and the methylene protons (H-2a, δ 5.16) in **1** were observed at δ 8.10 and 5.39, respectively, in **3**. The chemical shift of the methylene protons in the lactone ring occurring at δ 5.39 suggested **3** to be a 4-aryl-3,2-naphthalide (8). Observed nOes of H-1 (δ 8.10) to 8-OMe (δ 4.07) and H-2a (δ 5.39) and of H-5 (δ 6.91) to 6-OMe (δ 3.77), H-2′ (d, δ 6.82), and H-6′ (dd, δ 6.80) in the NOESY spectrum, confirmed **3** as 2-hydroxymethyl-6,7,8-trimethoxy-4-(3,4-methylenedioxy-phenyl)-3-naphthoic acid γ-lactone. The novel substance **3** has been named phyllamyricin C. The ^{13}C-nmr assignments of **3** (Table 2) were made from the HMBC spectrum, which also confirmed its structure by the observation of the three-bond coupling of H-1 (δ 8.10) to the methylene carbon (C-2a, δ 68.3).

Compound **4** has a molecular formula of $C_{21}H_{16}O_6$, assigned from the eims $[M]^+$ at *m/z* 364, being 30 mass units fewer than **1**. The ^{1}H-nmr spectrum was very similar to that of **1** except in the lack of a MeO singlet and the presence of an aromatic proton singlet consistent with the eims data. In particular, the signals of the ABX system for H-2′, H-5′, and H-6′, the AB system for the methylenedioxy protons, and the methylene singlet of the lactone ring were almost superimposable in the ^{1}H-nmr spectrum of **1** and **4**. The signal of H-1 in **4** shifted from δ 8.68 in **1** to δ 8.24, suggesting that the adjacent position at C-8 is unsubstituted. This proposal was supported by nOe observations which upon irradiation of H-1 (δ 8.24) or 7-OMe (δ 4.01), enhanced H-8 (δ 7.25). Compound **4** is therefore 3-hydroxymethyl-6,7-dimethoxy-4-(3,4-methylenedioxyphenyl)-2-naphthoic acid γ-lactone, which has been synthesized earlier and was named retrojusticidin B (10). This is the first natural occurrence of this compound.

The spectral data (^{1}H-nmr, eims, ir, and uv) of **5** and **6** were identical to those described for justicidins A (11) and B (3), respectively. The nOe results also confirmed their structures and facilitated the ^{1}H-nmr assignments. The ^{13}C-nmr assignments (Table 2) for compounds **4** and **6** have not been published before and were made in this study by the use of hetero COSY and hetero long-range COSY nmr techniques.

It has been reported that 4-aryl-3,2-naphthalides such as justicidin A [**5**] possess cytotoxic activity while 4-aryl-2,3-naphthalides do not have this effect (11). Whether or not the anti-EBV effect of these lignans follows such structural requirements is under investigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were measured on a Fisher-Johns melting point apparatus and are uncorrected. Ir spectra were recorded on a Jasco A-100 infrared spectrometer. Uv spectra were measured on a Hitachi 150-20 spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-300 or AMX-400 FT spectrometer in $CDCl_3$ (99.5%) or C_5D_5N (99.5%) using residual solvent peaks as reference standards; 2D nmr spectra were recorded using the Bruker standard pulse program: in the hetero COSY, hetero long-range COSY, and HMBC experiments, values used were $\Delta=1$ sec and $J=140$ Hz, 8 Hz, and 8 Hz, respectively. The correlation maps consisted of 96×1 K data points per spectrum, each composed of 320–480 transients for hetero COSY and hetero long-range COSY; for the HMBC experiment, the correlation maps consisted of 256×1 K data points per spectrum, each composed of 16 transients. In phase-sensitive NOESY and ROESY experiments, the mixing times were 0.6 sec and 50 msec, respectively, and $\Delta=3$ sec, with the correlation maps consisting of 128×1 K

data points per spectrum, each composed of 32–64 transients. Ms spectra were recorded on a TSQ-46c GC-MS-MS-DS spectrometer (eims) and a JEOL JMX-HX 110 spectrometer (hreims).

EXTRACTION AND ISOLATION.—The garden plant *P. myrtifolius* was cultivated at the College of Medicine, National Taiwan University. A voucher herbarium specimen is deposited at the School of Pharmacy, National Taiwan University. The dried and powdered aerial parts (9.7 kg), harvested in August 1992, were extracted with 95% EtOH (10 liters×5). The EtOH extract (690 g) suspended in H_2O was successively partitioned with $CHCl_3$, EtOAc, and BuOH, to give four fractions weighing 170 g ($CHCl_3$), 29 g (EtOAc), 250 g (BuOH), and 238 g (H_2O), respectively. The $CHCl_3$ fraction was further triturated with hexane to give hexane-soluble (108 g) and $CHCl_3$-soluble (59 g) fractions.

The $CHCl_3$-soluble fraction (50.0 g) was chromatographed over Si gel (finer than 230 mesh, 1.5 kg) eluted with MeOH (0% to 20%) in $CHCl_3$ and 120 ml fractions. The eluents were combined to give fractions A to K, following Si gel tlc analysis [MeOH-$CHCl_3$ (1:19), uv 254 nm]. Fraction C (14.5 g) was recrystallized from MeOH/$CHCl_3$ to afford compound **1** (5.79 g). The residue (6.90 g), combining fraction D (1.68 g) and the mother liquor of fraction C, was subjected to cc on Si gel (230–400 mesh, 300 g) eluted with Me_2CO (1–5%) in toluene, with 15-ml fractions being collected. Following tlc analysis [toluene-Me_2CO (98:2) and uv 254 nm], eluates of similar tlc profile were combined to give nine fractions. Recrystallization of fractions III (427.4 mg), II (28.2 mg), VI (1.1 g), VII (8.3 mg), and VIII (261.0 mg) from MeOH/$CHCl_3$ yielded **1** (216.0 mg), **2** (4.3 mg), **4** (360.0 mg), **5** (1.5 mg), and **6** (189.2 mg), respectively. Compound **3** (2.2 mg) was obtained via two successive Si gel cc (230–400 mesh, 5 g) steps on fraction IV (100.4 mg), with toluene as eluent.

Phyllamyricin A [**1**].—Mp 231–232°; uv (MeOH) λ max (log ε) 354 (sh, 3.63), 326 (3.94), 295 (3.79), 259 (4.69), 228 (4.36) nm; ir (KBr) ν max 3004, 3000, 2950, 1765 (C=O, lactone), 1615, 1498, 1482, 1400, 1348, 1270, 1237, 1200, 1156, 1120, 1108, 1088, 1030, 1000, 938, 924 cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; NOESY data ($CDCl_3$) H-1<−>MeO-8<−>MeO-7<−>MeO-6<−>H-5<−>H-2′, H-5<−>H-6′, H-2′<−>H-3a<−>H-6′<−>H-5′; eims (70 eV) *m/z* $[M]^+$ 394 (100), 395 (20), 379 (10), 365 (5), 349 (13), 335 (4), 321 (6), 307 (7); hreims *m/z* $[M]^+$ 394.1055 (calcd for $C_{22}H_{18}O_7$, 394.1052).

Phyllamyricin B [**2**].—Mp 140–141°; uv (MeOH) λ max (log ε) 355 (sh, 3.58), 315 (4.34), 299 (4.32), 266 (4.83), 228 (4.47) nm; ir (KBr) ν max 3000, 2940, 1678 (C=O, aldehyde), 1620, 1503, 1488, 1470, 1428, 1265, 1235, 1160, 1117, 1040, 1008, 930, 920 cm^{-1}; ^{1}H-nmr data see Table 1; ^{13}C-nmr data, see Table 2; ROESY data (C_5D_5N) MeO-7<−>H-8<−>H-1<−>H-2a, H-1<−>MeO-2, H-2a<−>H-3a<−>H-2′, H-3a<−>H-6′<−>H-5′, H-5<−>MeO-6; hetero long-range COSY data (in C_5D_5N) C-1 (δ 124.9) to H-2a (δ 5.22), C-2 (δ 134.6) to H-2a and H-3a (δ 10.21), C-2a (δ 73.2) to MeO-3a (δ 3.56), C-3 (δ 128.8) to H-1 (δ 8.26), C-4 (δ 145.8) to H-5 (δ 7.10), C-4a (δ 127.5) to H-8 (δ 7.40), and H-1, C-6 (δ 150.7) to MeO-6 (δ 3.62) and H-8, C-7 (δ 152.8) to MeO-7 (δ 3.93), and H-5, C-8 (δ 107.3) to H-1, C-8a (δ 132.5) to H-5, C-1′ (δ 130.7) to H-5′ (δ 7.06), C-3′ (δ 148.3) to H-5′, C-4′ (δ 148.1) to H-2′ (δ 7.07), C-6′ (δ 124.8) to H-2′; eims *m/z* $[M]^+$ 380 (100), 381 (20), 365 (80), 348 (27), 335 (21), 318 (10), 305 (8), 289 (18); hreims *m/z* $[M]^+$ 380.1252 (calcd for $C_{22}H_{20}O_6$, 380.1260).

Phyllamyricin C [**3**].—Mp 190–191°; uv (MeOH) λ max (log ε) 361 (3.62), 316 (sh, 3.83), 290 (3.93), 259 (4.64), 230 (4.37) nm; ir (KBr) ν max 3010, 2930, 1759 (C=O, lactone), 1620, 1595, 1505, 1482, 1460, 1338, 1355, 1230, 1216, 1170, 1038, 1005, 930, 860 cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; NOESY data, MeO-8<−>H-1<−>H-2a, MeO-7<−>MeO-6<−>H-5<−>H-2′, H-5′<−>H-6′<−>H-5; eims *m/z* $[M]^+$ 394 (100), 395 (23), 379 (5), 349 (29), 321 (15); hreims *m/z* $[M]^+$ 394.1054 (calcd for $C_{22}H_{18}O_7$, 394.1052).

Retrojusticidin B [**4**].—Mp 223–224°; uv λ max (MeOH) (log ε) 352 (sh 3.46), 317 (4.08), 297 (sh 3.98), 256 (4.74), 230 nm (4.47); ir ν max (KBr) 2940, 1750, 1620, 1503, 1482, 1438, 1385, 1342, 1263, 1240, 1230, 1160, 1152, 1040, 1007, 930, 910 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 3.82 (3H, s, MeO-6), 4.01 (3H, s, MeO-7), 5.17 (2H, s, H-3a), 6.04 and 6.07 (2H, each d, *J*=1.0 Hz, -OC*H*$_2$O-), 6.80 (1H, dd, *J*=8.0 and 1.6 Hz, H-6′), 6.81 (1H, d, *J*=1.6 Hz, H-2′), 6.95 (1H, d, *J*=8.0 Hz, H-5′), 7.06 (1H, s, H-5), 7.25 (1H, s, H-8), 8.24 (1H, s, H-1); ^{13}C-nmr data, see Table 1; nOe data, 6-OMe to H-5 29%, 7-OMe to H-8 33%, H-1 to H-8 14%, H-3a to H-2′ and H-6′ 16%; hetero long-range COSY data, C-2a (δ 171.5) to H-3a, C-3 (δ 137.9) to H-3a and H-1, C-4 (δ 131.8) to H-5 and H-6′, C-4a (δ 131.6) to H-8, C-6 (δ 152.0) to 6-OMe and H-8, C-7 (δ 150.1) to 7-OMe and H-5, C-8 (δ 107.6) to H-1, C-8a (δ 129.8) to H-5, C-1′ (δ 129.7) to H-5′, C-3′ (δ 148.2) to H-2′ and H-5′, C-4′ (δ 147.6) to H-6′; eims (70 eV) *m/z* $[M]^+$ 364 (100) (assigned to $C_{21}H_{16}O_6$), 365 (18), 335 (24), 319 (3), 305 (4).

^{1}H-Nmr and hetero long-range COSY nmr data of justicidin B [**6**].—^{1}H nmr ($CDCl_3$) δ 3.79 (s, 6-

OMe), 4.02 (s, 7-OMe), 5.35 (s, H-2a), 6.01 and 6.06 (d, J_{gem}=1.3 Hz, -OCH_2O-), 6.80 (dd, J=8.0 and 1.5 Hz, H-6′), 6.83 (d, J=1.5 Hz, H-2′), 6.94 (d, J=8.0 Hz, H-5′), 7.08 (s, H-5), 7.16 (s, H-8), 7.68 (s, H-1); hetero long-range COSY data, C-1 (δ 118.2) to H-8, C-2 (δ 139.46) to H-2a, C-3 (δ 118.4) to H-1, C-4 (δ 133.1) to H-5, C-3a (δ 169.9) to H-2a, C-4a (δ 128.8) to H-1 and H-8, C-6 (δ 150.0) to 6-OMe and H-8, C-7 (δ 151.7) to 7-OMe and H-5, C-8a (δ 139.5) to H-5, C-1′ (δ 128.3) to H-5′, C-6′ (δ 123.4) to H-2′.

ACKNOWLEDGMENTS

The authors are grateful to Department of Health, Taiwan, Republic of China, for the financial support of this research under the grant DOH 83-HR-308.

LITERATURE CITED

1. D.W. Unander, G.L. Webster, and B.S. Blumberg, *J. Ethnopharmacol.*, **30**, 233 (1990).
2. D.W. Unander, G.L. Webster, and B.S. Blumberg, *J. Ethnopharmacol.*, **34**, 97 (1991).
3. G.R. Pettit, G.M. Cragg, M.I. Suffness, D. Gust, F.E. Boettner, M. Williams, J.A. Saenz-Renauld, P. Brown, J.M. Schmidt, and P.D. Ellis, *J. Org. Chem.*, **49**, 4258 (1984).
4. P. Satyanarayana, P. Subrahmanyam, and K.N. Viswanatham, *J. Nat. Prod.*, **51**, 44 (1988).
5. G.R. Pettit and D.E. Schaufelberger, *J. Nat. Prod.*, **51**, 1104 (1988).
6. B. Singh, P.K. Agrawal, and R.S. Thakur, *J. Nat. Prod.*, **52**, 48 (1989).
7. T.-I. Yang, "A List of Plants in Taiwan," Vol. 1, Natural Publishing Co., Taipei, 1982, p. 832.
8. Z.-I. Horii, M. Tsujiuchi, K.-I. Kanai, and T. Momose, *Chem. Pharm. Bull*, **25**, 1803 (1977).
9. R.S. Ward, P. Satyanarayana, and B.V Gopala Rao, *Tetrahedron Lett.*, **22**, 3021 (1981).
10. A. Pelter, R.S. Ward, P. Satyanarayana, and P. Collins, *Tetrahedron Lett.*, **23**, 571 (1982).
11. N. Fukamiya and K.-H. Lee, *J. Nat. Prod.*, **49**, 348 (1986).

Received 15 April 1994