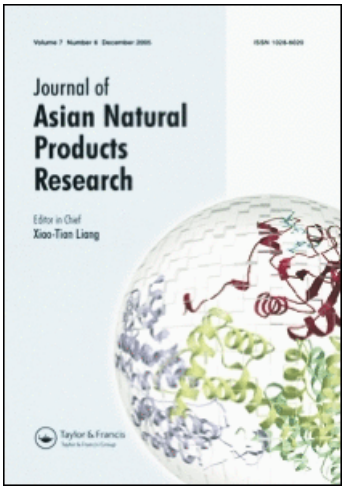

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Two new constituents from mangrove *Bruguiera gymnorrhiza*

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A lignan and two aromatic compounds were isolated from the branches of the mangrove plant, *Bruguiera gymnorrhiza*. They were brugunin A (**1**), bruguierol D (**2**) and 2,3-dimethoxy-5-propylphenol (**3**). Among them, **1** and **2** were new compounds; **3** was isolated from a natural source for the first time. The structures of these compounds were determined by NMR spectroscopic studies as well as chemical evidence.

Keywords: Mangrove; *Bruguiera gymnorrhiza*; Brugunin A; Bruguierol D

1. Introduction

Bruguiera gymnorrhiza (L.) (Rhizophoraceae) is an evergreen mangrove tree, widely distributed in tropical and subtropical coastlines. Previous investigations on this plant have shown the presence of diterpenes, triterpenes, flavonoids, steroids, hydrocarbons in its leaves and the outer layer of the root bark [1–5]. In a continuation of our work on constituents of *Bruguiera gymnorrhiza*, we obtained one lignan and two aromatic compounds, named brugunin A (**1**), bruguierol D (**2**) and 2,3-dimethoxy-5-propylphenol (**3**). In the present paper, we report the isolation and structure elucidation of them.

2. Results and discussion

Compound **1** was obtained as a white powder. The molecular formula of **1** was assigned as $C_{25}H_{32}O_8$ by HRESI-MS at *m/z* 459.2022 $[M - H]^-$. The IR spectrum indicated the presence of hydroxyls (3399 cm^{-1}) and benzene ring (1611, 1499, 1456 cm^{-1}). ^1H NMR data (see table 1) showed three aromatic proton signals, one at δ 6.38 (1H, s) and the other two with the same chemical shift at δ 6.29 (2H, s); four methoxy signals at δ 3.85, 3.80 (two methoxyls) and 3.09 and two methyl signals at δ 1.29 and 1.33. The ^{13}C NMR spectrum (see table 1) and a DEPT experiment on **1** indicated a total of 25 carbons. The carbon types were

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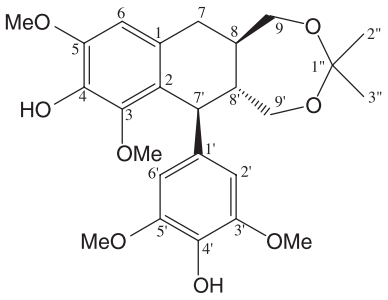
Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) data of **1** in $CDCl_3$ (δ in ppm).

No.	δ_C	δ_H	*No.*	δ_C	δ_H
1	128.1 s		1′	138.7 s	
2	125.8 s		2′	105.6 d	6.29 1H, s
3	146.2 s		3′	146.7 s	
4	137.1 s		4′	132.9 s	
5	145.7 s		5′	146.7 s	
6	105.8 d	6.38 1H, s	6′	105.6 d	6.29 1H, s
7	33.2 t	2.52 2H, brd, $J = 7.1$	7′	45.1 d	3.51 1H, d, $J = 10.0$
8	41.8 d	1.64 1H, m	8′	51.6 d	1.60 1H, m
9	65.8 t	3.57 1H, m; 3.68 1H, m	9′	63.9 t	3.65 2H, m
3-OMe	58.8 q	3.09 3H, s	1″	101.1 s	
5-OMe	56.0 q	3.85 3H, s	2″	24.8 q	1.29 3H, s
3′-OMe	56.5 q	3.80 3H, s	3″	24.9 q	1.33 3H, s
5′-OMe	56.5 q	3.80 3H, s			

determined from the DEPT and HMQC experiments as six methyls (four methoxyls at δ 56.0, 56.5, 56.5 and 58.8), six methines (three aromatic methines at δ 105.6, 105.6 and 105.8), three methylenes (two oxymethylenes at δ 63.9 and 65.8) and 10 quaternary carbons. The NMR data of **1** were very similar with those of the known compound (+)-lyoniresinol [6], which was also obtained from the same plant, except for three more carbon signals including one quaternary carbon at δ 101.1 and two methyl signals at δ 24.8 and 24.9 in **1**. The structure of **1** was established by detailed COSY, HMQC and HMBC (see figure 1) spectral analysis. The stereochemistry of **1** was proposed as 7′*S*,8′*R*,8*R* on the basis of its hydrolysate **1a** which showed the same R_f and optical rotation values and ^{1}H NMR data with those of (+)-lyoniresinol [7,8]. Therefore, the structure of compound **1** was elucidated as 7′*S*,8′*R*,8*R*-4,4′-dihydroxy-3,3′,5,5′-tetramethoxy-2,7′-cyclolignin-9,9′-acetonide and named brugunin A. Brugunin A (**1**) is probably an artefact that could have been formed when acetone was used for washing out the residue in the distillation flask during chromatographic fractionation. Indeed, it was observed that this compound was formed when lyoniresinol was dissolved in acetone solution set aside at room temperature for two days.

Compound **2** was isolated as colourless oil. The molecular formula $C_{12}H_{18}O_4$ of **2** was determined by HRESI-MS at *m/z* 225.1133 $[M - H]^-$. UV and IR spectra indicated the presence of hydroxyl and benzene ring. The ^{13}C NMR (see table 2) and DEPT experiments of **2** showed 12 carbons, of which six carbons formed a petasubstituted benzene ring and the others represented three methoxy groups, one methyl and two methylene groups. ^{1}H NMR (see table 2) data showed one aromatic proton at δ 6.48 (1H, s); three methoxy signals at δ 3.76, 3.90 ($2 \times OCH_3$). Furthermore, two methylenes and one methyl formed a propyl fragment, which was also confirmed by COSY data. HMBC experiment (see figure 1) determined the substituted position of hydroxy, methoxy and propyl groups. Thus, **2** was elucidated as 2,3,4-trimethoxy-5-propylphenol, named bruguierol D.

2,3-Dimethoxy-5-propylphenol (**3**), which had been produced by chemical synthesis according to the literature [9], was also isolated from this plant, but the detailed NMR data and assignment had not been reported. We assigned the NMR data of **3** by HMBC, HMQC and COSY experiments (see table 2).



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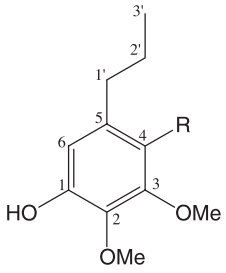
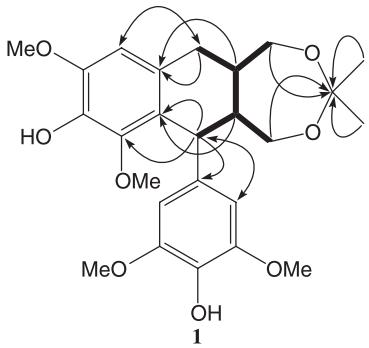


2 R=OMe
3 R=H



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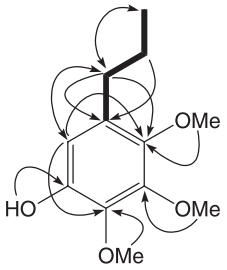

2

Figure 1. HMBC and COSY correlations of **1** and **2**.

3. Experimental

3.1 General experimental procedures

NMR spectra were measured on Bruker DPX-300 and Bruker DPX-500 spectrometer; IR spectra were recorded on Bruker IFS55 spectrometer; UV spectra were determined on Varian

Table 2. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) data of **2** and **3** in $CDCl_3$ (δ in ppm).

C	***2***		***3***	
	δ_C	δ_H	δ_C	δ_H
1	144.7 s	5.43 OH, brs	148.9 s	
2	138.0 s		133.5 s	
3	145.8 s		152.1 s	
4	144.8 s		104.4 d	6.26 1H, d, $J = 1.8$
5	131.5 s		139.0 s	
6	109.9 d	6.48 1H, s	107.7 d	6.41 1H, d, $J = 1.8$
1′	31.6 t	2.48 2H, t, $J = 8.0$	38.2 t	2.46 2H, t, $J = 8.1$
2′	23.8 t	1.57 2H, m	24.4 t	1.56 2H, m
3′	14.1 q	0.94 3H, t, $J = 7.3$	13.8 q	0.90 3H, t, $J = 7.3$
2-OMe	60.6 q	3.90 3H, s	60.9 q	3.85 3H, s
3-OMe	61.2 q	3.76 3H, s	55.8 q	3.83 3H, s
4-OMe	61.1 q	3.90 3H, s		

UV–Visible Cary spectrophotometer; ESI-MS were taken by triple quadrupole mass spectrometer (Quattro VG Biotech and Finnigan MAT 311A) and HRESI-MS were obtained on Bruker Daltonics, APEX II spectrometer; Optical rotation were obtained on Propol Digital Automatic Polarimeter; Silica gel 60 M (Macherey-Nagel, 230–400 mesh) was used for column chromatography, TLC, silica gel plates (Macherey-Nagel, Sil G/UV_{254}, 0.20 mm). Spots were detected under a UV lamp and after staining with anisaldehyde/H_2SO_4; Sephadex LH-20 (Pharmacia) was used for column chromatography.

3.2 Plant material

Samples of the branch of *Bruguiera gymnorrhiza* were collected in Xiamen, China, in June 2002 and authenticated by Professor Peng Lin, Xia Men University, China. A voucher sample of the plant is deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing, China (MXM004). The branch was air-dried and milled.

3.3 Extraction and isolation

The pulverised plant material (6.1 kg) was macerated with methanol (25 L) at room temperature three times for 2 weeks. The combined methanol extracts were concentrated and yielded 282.6 g of a crude extract. A suspension of this crude extract in distilled water was extracted with EtOAc and n-BuOH respectively to yield 23.6 g of a dried EtOAc extract, 39.6 g of a n-BuOH extract and an aqueous residue. The EtOAc extract was subjected to silica gel column chromatography (6 × 50 cm) eluting with $CHCl_3$/MeOH (50:1–1:1). The eluents were combined to 25 fractions (1–25) on the basis of TLC analysis. Fraction 14 was separated by column chromatography on silica gel (3 × 50 cm, cyclohexane/EtOAc = 3:1) and yield 10 fractions (A_1–A_{10}) on the basis of TLC analysis. Fr. A_8 (31.4 mg) was subjected to sephadex LH-20 column chromatography (2 × 80 cm, MeOH) and yielded **1** (12.8 mg). Fraction 5 was subjected to sephadex LH-20 column chromatography (3.5 × 120 cm, $CHCl_3$), then further purified by silica gel column chromatography (1.5 × 30 cm, hexane/EtOAc = 8:1) to give **2** (6.0 mg) and **3** (3.0 mg).

3.3.1 Brugunin A (1). White amorphous solid; $[\alpha]_D^{20}$ + 7.6 (*c* 0.19, $CHCl_3$); IR (film) ν_{max} 3399, 2936, 2838, 2360, 2341, 1708, 1611, 1499, 1456, 1425, 1363, 1301, 1216, 1110, 1096, 1070, 1025 cm^{-1}; UV (MeOH): λ_{max} (log ε) 279 (3.52) nm; + HCl 276 (3.55) nm; + NaOH 251 (3.74) nm; ^{1}H NMR and ^{13}C NMR, see table 1; ESI-MS *m/z* 483.2 $[M + Na]^+$, 943.3 $[2M + Na]^+$, 478.2 $[M + NH_4]^+$, 461.2 $[M + H]^+$; HRESI-MS *m/z* 459.2022 $[M - H]^-$ (calcd for $C_{25}H_{31}O_8$, 459.2019).

Hydrolysis of **1**. Brugunin A (**1**, 6 mg) in MeOH (5 ml), and 1 N HCl (1 ml) was heated to 60°C for 2 h. After cooling, the reaction mixture was neutralised with saturated aqueous sodium bicarbonate. The methanol was removed under reduced pressure using a rotary evaporator and the remaining aqueous mixture was extracted with EtOAc (3 times). The combined organic layers were evaporated to dryness under vacuum and the residue was applied to silica gel column purification eluting with cyclohexane-acetone (1:1) to afford **1a** (4.1 mg).

1a, White amorphous solid; $[\alpha]_D^{20}$ + 60.2 (*c* 0.20, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.43 (1H, s, H-6), 6.30 (2H, s, H-2′, 6′), 4.02 (1H, d, 7.5, H-7′), 3.86 (3H, s, CH_3O-5), 3.80

(1H, m, H-9a), 3.78 (6H, s, CH_3O-3′, 5′), 3.76 (1H, m, H-9b), 3.59 (2H, m, H-9′), 3.29 (3H, s, CH_3O-3), 2.65 (1H, dd, 14.3, 11.6, H-7a), 2.57 (1H, dd, 14.3, 4.6, H-7b), 1.90 (1H, m, H-8′), 1.75 (1H, m, H-8); ESI-MS *m/z* 443.2 $[M + Na]^+$, 863.4 $[2M + Na]^+$, 438.3 $[M + NH_4]^+$, 421.2 $[M + H]^+$, 419.3$[M - H]^-$.

3.3.2 2,3,4-Trimethoxy-5-propylphenol (2). Colourless oil; IR (film) ν_{max} 3395, 2919, 2850, 2360, 2341, 1592, 1508, 1487, 1465, 1420, 1371, 1198, 1110, 1097, 1008 cm^{-1}; UV (MeOH): λ_{max} (log ε) 282 (2.97) nm; + HCl 277 (2.87) nm; + NaOH 291 (3.06) nm; ^1H NMR and ^{13}C NMR, see table 2; ESI-MS *m/z* 249.2 $[M + Na]^+$, 227.2 $[M + H]^+$; HRESI-MS *m/z* 225.1133 $[M - H]^-$ (calcd for $C_{12}H_{17}O_4$, 225.1127).

3.3.3 2,3-Dimethoxy-5-propylphenol (3). Colourless oil; ^1H NMR and ^{13}C NMR, see table 2; ESI-MS *m/z* 219.2 $[M + Na]^+$, 197.2 $[M + H]^+$; HRESI-MS *m/z* 195.0505 $[M - H]^-$ (calcd for $C_{11}H_{15}O_3$, 195.1021).

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