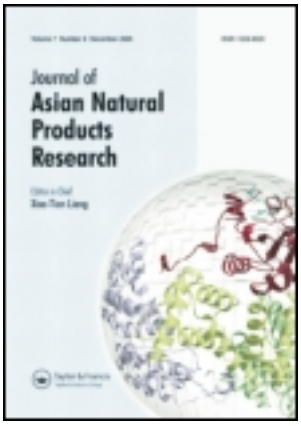

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Jian-Xiong Ma [a b c], Ming-Sheng Lan [c], Shi-Jin Qu [a], Jun-Jie Tan [a], Hong-Feng Luo [a], Chang-Heng Tan [a] & Da-Yuan Zhu [a]

[a] Department of Natural Medicinal Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, 201203, China

[b] Guangxi Medical University, Nanning, 530021, China

[c] Guangxi Institute of Medicinal Plant, Nanning, 530023, China

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Arylnaphthalene lignan glycosides and other constituents from *Phyllanthus reticulatus*

Jian-Xiong Ma[abc1], Ming-Sheng Lan[c1], Shi-Jin Qu[a], Jun-Jie Tan[a], Hong-Feng Luo[a], Chang-Heng Tan[a]* and Da-Yuan Zhu[a]

[a]*Department of Natural Medicinal Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China;* [b]*Guangxi Medical University, Nanning 530021, China;* [c]*Guangxi Institute of Medicinal Plant, Nanning 530023, China*

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Two new arylnaphthalene lignan glycosides, named reticulatusides A (**1**) and B (**2**), together with eight known compounds were isolated from the 95% EtOH extract of the whole plant of *Phyllanthus reticulatus*. The structures of the new compounds were elucidated by spectroscopic methods.

Keywords: reticulatuside A; reticulatuside B; arylnaphthalene lignan glycoside; *Phyllanthus reticulatus*

1. Introduction

Phyllanthus reticulatus Poir. (Euphorbiaceae) is a climbing shrub distributed in tropic area. In the Guangxi Zhuang national area of China, it is used as a folk medicine for the treatment of inflammation and rheumatism [1]. Pharmacological studies on the extracts of this plant showed antiplasmodial [2], antidiabetic [3], antimicrobial [4], cytotoxic [4], and hepatoprotective [5] bioactivities. Several phytochemical investigations on this plant revealed various constituents such as triterpenoids, phytosterols, coumarin [4], flavonoids, and other phenols [6]. As a continuous interest in Zhuang national medicines [7], we investigated the 95% EtOH extract of the title plant, which led to the isolation of two arylnaphthalene lignan glycosides, reticulatusides A (**1**) and B (**2**) (Figure 1), together with eight known compounds, spruceanol [8], cleistanthol [8], syringaresinol [9], phyllanthusmins B and C [10], (+)-lyoniresinol [11], (3*S*,5*R*,6*S*,9*R*)-megastigman-3,9-diol 3-*O*-α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranoside, and 7-megastigmen-3-ol-9-one 3-*O*-α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranoside [12]. The above-mentioned compounds were reported from this plant for the first time. In this paper, we reported the isolation and structure elucidation of **1** and **2**.

2. Results and discussion

Compound **1** showed the quasi-molecular ion peaks at *m/z* 697 $[M + Na]^+$, 1371 $[2M + Na]^+$, and 719 $[M + HCOO]^-$ in the positive and negative ESI-MS, respectively, suggesting the molecular formula $C_{32}H_{34}O_{16}$, which was further confirmed by the HR-ESI-MS. Two ESI-MS fragment peaks at *m/z* 543 $[M + H - 132]^+$ and 381 $[M + H - 132 - 162]^+$ indicated the existence of one pentose and one hexose in compound **1**. The sugar moiety of **1** was

*Corresponding author. Email: chtan@mail.shcnc.ac.cn

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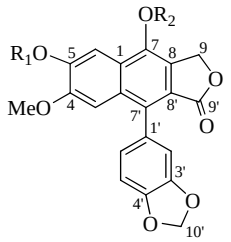

1 R_1 = Me, R_2 = Glu (6→1) Ara(f)
2 R_1 = Ara (p), R_2 = Glu

Figure 1. Structures of compounds **1** and **2**.

established to be α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranosyl based on the acid hydrolysis experiment and the following NMR data: (i) ^{1}H signals at δ 4.75 (1H, d, J = 7.7 Hz) and 4.86 (1H, overlapped by H_2O peak, HSQC cross-peak indicated as a br s); (ii) ^{13}C signals at δ 107.1 (d), 78.4 (d), 77.2 (d), 75.7 (d), 72.3 (d), and 69.1 (t), as well as 110.3 (d), 85.9 (d), 83.7 (d), 78.9 (d), and 63.0 (t); (iii) HMBC correlations between H-1‴ (δ_H 4.86) and C-6″ (δ_C 69.1). The ^{1}H NMR spectrum (Table 1) of the

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** (400 and 100 MHz, in CD_3OD).

	1		**2**	
Site	^{13}C	^{1}H	^{13}C	^{1}H
1	132.2		128.3	
2	129.1		130.2	
3	107.35/107.32[a]	7.05/7.04[a] s	108.0	7.13/7.12[a] s
4	152.0		151.9	
5	153.7		151.5	
6	103.03/103.02[a]	8.14/8.13[a] s	106.8	8.25 s
7	146.6		146.2	
8	132.80/132.71[a]		132.0	
9	69.6	5.81/5.80[a] d (15.0)	69.6	5.62/5.61[a] d (15.0)
		5.41/5.40[a] d (15.0)		5.464/5.459[a] d (15.0)
1′	130.39/130.35[a]		130.2	
2′	112.2/112.0[a]	6.83/6.77[a] br s	112.0	6.81/6.80[a] d (1.5)
3′	149.3		149.3	
4′	149.3		149.3	
5′	109.31/109.24[a]	6.92 d 7.7	109.3	6.97 d (7.9)
6′	125.1/124.9[a]	6.79/6.76[a] br d (7.7)	125.0	6.77 dd (7.9, 1.5)
7′	137.9		137.8	
8′	120.5		120.9	
9′	172.6		172.1	
10′	102.6	6.03/6.02[a] s	102.6	6.04/6.06 s
MeO-5	57.0	4.00 s		
MeO-4	56.3	3.70 s	56.5	3.75 s
Glu-1″	107.1	4.75 d (7.7)	107.3	4.77 d (7.6)
2″	75.7	3.75 dd (9.1, 7.7)	75.1	3.59 t-like (7.6)
3″	78.4	3.48 t (9.1)	78.3	3.69 dd (9.0, 7.6)
4″	72.34/72.38[a]	3.36 t-like (9.4)	71.7	3.40 t-like (9.0)
5″	77.24/77.21[a]	3.49 m	78.2	3.72 ddd (9.0, 3.4, 2.8)
6″	69.13/69.09[a]	3.61 dd (10.6, 2.9)	62.9	3.70 dd (12.0, 3.4)
		4.03 dd (10.6, 1.6)		3.97 dd (12.0, 2.8)
Ara-1‴	110.29/110.26[a]	4.86 br s (overlapped)	101.5	5.29 d (7.2)
2‴	83.78/83.74[a]	3.94 br s	73.0	3.98 dd (9.2, 7.2)
3‴	78.9	3.80 dd (6.3, 2.9)	74.5	3.64 dd (9.2, 3.5)
4‴	85.9	3.78 m	70.0	3.86 br s
5‴	63.0	3.57 dd (11.9, 4.2)	67.9	3.92 dd (12.5, 2.2)
		3.55 dd (11.9, 2.8)		3.51 br d (12.5)

[a] Splitted signals caused by atropisomerism.

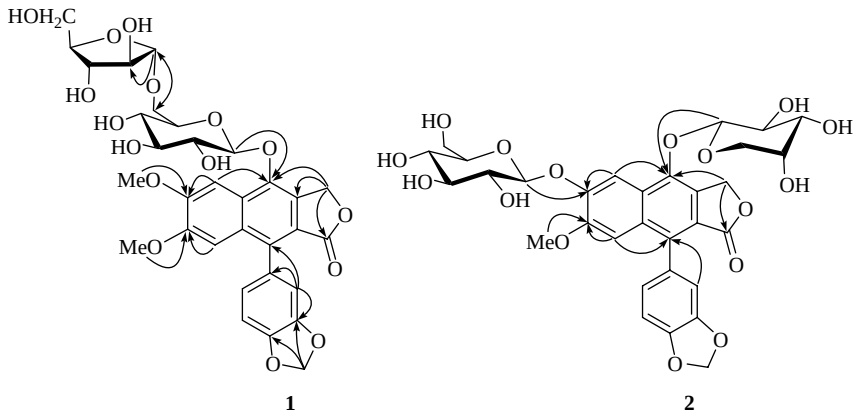

Figure 2. Selected HMBC correlations (H to C) of **1** with **2**.

aglycone of **1** showed signals for one 1,3,4-trisubstituted aromatic ring at δ 6.92 (1H, d, $J = 7.7$ Hz), 6.83 (1H, br s), and 6.79 (1H, br d, $J = 7.7$ Hz); two isolated olefinic protons at δ 8.14 and 7.05 (each 1H, s); one dioxymethylene at δ 6.03 (2H, s); two methoxyls at δ 4.00 and 3.70 (each 3H, s); and an isolated oxygenated methylene at δ_H 5.81 and 5.41 (each d, $J = 15.0$ Hz). The ^{13}C NMR spectrum (Table 1) of **1** displayed 21 carbons (12 sp^2 C, 5 sp^2 CH, 2 OMe, 1 OCH_2O, and 1 CH_2) for the aglycone moiety of **1**. The above evidences indicated **1** to be an arylnaphthalene glycoside [13].

In the HMBC spectrum of **1** (Figure 2), obvious cross-peaks of MeO (δ_H 3.70)/C-4 (δ_C 152.0), MeO (δ_H 4.00)/C-5 (δ_C 153.7), and H-1″ (δ_H 4.75)/C-7 (δ_C 146.6) were observed, demonstrating that the two methoxyls and the sugar linkage were on C-4, C-5, and C-7 of the arylnathalene skeleton, respectively. Furthermore, HMBC correlations between H_2-10′/C-3′ and C-4′, and H_2-9/C-7, C-8, and C-9′, substantiated further the structure 3′,4′-methylenedioxy-4,5-dimethoxy-7-hydroxy-2,7′-cycloligna-7,7′-dien-9′,9-olide 7-*O*-α-L-arabinofuranosyl(1 → 6)-β-D-glucopyranoside for reticulatuside A (**1**). As indicated by Al-Abed *et al.* [13], some ^{1}H and ^{13}C signals of **1** exist in pairs due to the equilibrium between two conformational isomers resulting from the slow rotation of the sugar unit at room temperature (rt) around the glucosidic linkage with the naphthalide ring system.

Reticulatuside B (**2**) was obtained as yellowish amorphous powder. Its molecular formula was determined to be $C_{31}H_{32}O_{16}$ by the HR-ESI-MS. After acid hydrolysis, **2** gave D-glucose and L-arabinose as sugar moiety. The ^{1}H and ^{13}C NMR spectra (Table 1) of **2** displayed signals for an arylnaphthalene skeleton which was nearly superposed with that of **1**. Two sugar units were identified to be β-glucopyranosyl and α-arabinopyranosyl on the basis of NMR data at δ_H 4.77 (1H, d, $J = 7.6$ Hz) and δ_C 107.3 (d), 78.3 (d), 78.2 (d), 75.1 (d), 71.7 (d), and 62.9 (t), as well as at δ_H 5.29 (1H, d, $J = 7.2$ Hz) and δ_C 101.5 (d), 74.5 (d), 73.0 (d), 70.0 (d), and 67.9 (t) [14,15]. Comparing the ^{1}H and ^{13}C NMR spectral data of **2** with those of **1**, the most important differences were that **2** lacked signals for the second methoxyl, and **2** had a terminal glucosyl ($\delta_{C\text{-}6''}$: 62.9 of **2** vs. 69.1 of **1**) and a pyran-configuration arabinosyl.

The structure of reticulatuside B was finally elucidated to be 3′,4′-methylenedioxy-4-methoxy-5-α-L-arabinofuranosyloxy-7-β-D-glucopyranosyloxy-2,7′-cycloligna-7,7′-dien-9′,9-olide (**2**) by the HMBC correlations (Figure 2) between H-1‴/C-7, H-1‴/C-5, and MeO/C-4.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a PerkinElmer 341 polarimeter (Perkin Elmer, Inc., Wellesley, MA, USA). UV spectra were obtained on a Shimadzu UV-2550 spectrophotometer (Shimadzu Corp., Kyoto, Japan). IR spectra were recorded on a Thermo Nicolet Magna-IR 750 FTIR spectrometer (Nicolet Instrument, Inc., Madison, WI, USA) with KBr tablet. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were acquired on a Bruker AM-400 spectrometer with tetramethylsilane (TMS) as an internal standard. ESI-MS and HR-ESI-MS data were measured on a Bruker Esquire 3000plus and a Finnigan LCQ DECA mass spectrometers, respectively. Silica gel (200–300 meshes, Qingdao Haiyang Chemical Group Co., Qingdao, China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), and ODS-A gel (Mitsubishi Chemical Industries Co., Ltd., Tokyo, Japan) were used for column chromatography (CC), and precoated plates of silica gel (HSGF254; Qingdao Haiyang Chemical Group Co., Qingdao, China) were used for TLC.

3.2 Plant material

The fresh whole plants of *P. reticulatus* were collected in Yongning County, Guangxi Province, China. The plant was identified by Prof. D.-Y. Zhu of Shanghai Institute of Materia Medica. A voucher specimen (No. 09-108) is deposited in the Herbarium of Shanghai Institute of Materia Medica.

3.3 Extraction and isolation

The air-dried and powdered whole plants of *P. reticulates* (5 kg) were extracted three times with 20 liters of 95% EtOH at rt. The concentrated extract was partitioned between H_2O and petroleum ether, EtOAc and *n*-BuOH, respectively. The EtOAc fraction (15 g) was subjected to silica gel CC with gradient $CHCl_3$–MeOH (100:1 → 5:1, v/v) as eluant to gain subfractions E1–E6. Fr. E2 (1.0 g) was purified by silica gel CC ($CHCl_3$–MeOH, 30:1), following Sephadex LH-20 CC ($CHCl_3$–CH_3OH, 1:1) to achieve spruceanol (20 mg) and syringaresinol (9 mg). By the similar procedure, cleistanthol (18 mg) and phyllanthusmin B (15 mg) were obtained from Fr. E4. Fr. E5 was separated into Frs E5.1–5.4 by silica gel CC ($CHCl_3$–MeOH, 10:1). Phyllanthusmin C (8 mg) and (+)-lyoniresinol (17 mg) were isolated from Frs E5.2 and E5.3, respectively, after the purification of Sephadex LH-20 CC (MeOH). The *n*-BuOH fraction (100 g) was subjected to silica gel CC ($CHCl_3$–MeOH, 20:1, 10:1, 6:1, 3:1, finally MeOH) to afford Frs B1 − B5. Fr. B3 (10.0 g) was further isolated by silica gel CC ($CHCl_3$–MeOH–H_2O, 5:1:0.1) to get Frs B3.1 − 3.5. Fr. B3.3 furnished **1** (9 mg) and 7-megastigmen-3-ol-9-one 3-*O*-α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranoside (9 mg) after purification by ODS-A gel CC (MeOH–H_2O, 30–70%) and Sephadex LH-20 CC (MeOH). By the same procedure, Fr. B3.4 afforded **2** (12 mg) and (3*S*,5*R*,6*S*,9*R*)-megastigman-3,9-diol 3-*O*-α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranoside (6 mg).

*3.3.1 Reticulatuside A (**1**)*

Yellowish amorphous powder; $[\alpha]_D^{25}$ − 82 (c = 0.065, MeOH). UV λ_{max} (MeOH) nm (log ε): 263 (4.69). IR (KBr) ν_{max}: 3423, 2923, 1734, 1623, 1508, 1434, 1265, 1230, 1170, 1074, 1039, and 771 cm^{-1}. For ^{1}H and ^{13}C NMR spectral data, see Table 1. ESI-MS *m/z*: (positive ion model) 697 $[M + Na]^+$, 1371 $[2M + Na]^+$, 543 $[M + H - 132]^+$, and 381 $[M + H - 132 - 162]^+$; (negative ion model) 719 $[M + HCOO]^-$ and 1347 $[2M - H]^-$. HR-ESI-MS *m/z*: 697.1748

$[M + Na]^+$ (calcd for $C_{32}H_{34}O_{16}Na$, 697.1745).

3.3.2 Reticulatuside B (2)

Yellowish amorphous powder, $[\alpha]_D^{25} - 22$ ($c = 0.045$, MeOH). UV λ_{max} (MeOH) nm ($\log \varepsilon$): 259 (4.69). IR (KBr) ν_{max}: 3415, 2921, 1749, 1618, 1508, 1384, 1257, 1074, 1037, and 775 cm^{-1}. For ^1H and ^{13}C NMR spectral data, see Table 1. ESI-MS *m/z*: (positive ion model) 661 $[M + H]^+$, 683 $[M + Na]^+$, 1343 $[2M + Na]^+$; (negative ion model) 705 $[M + HCOO]^-$, 1319 $[2M - H]^-$. HR-ESI-MS *m/z*: 683.1586 $[M + Na]^+$ (calcd for $C_{31}H_{32}O_{16}Na$, 683.1588).

3.4 Analysis of the sugar moiety

Compounds **1** and **2** (1 mg each) were refluxed in 2 N HCl–dioxane (1:1 v/v, 2 ml) for 2 h. On cooling, the mixture was neutralized with $NaHCO_3$. After extraction with AcOEt, the aqueous layer was concentrated by blowing with N_2. The residue was purified by CC of Sephadex LH-20 ($MeOH–H_2O = 1:1$, v/v) to give the sugar moiety. The purified sugar and standard D-glucose and L-arabinose (Sigma, St. Louis, MO, USA) were treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (1 ml) at 60°C for 1 h. Then, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.02 ml) at 60°C for 1 h. The supernatant was applied to gas chromatography (GC) analysis (Supelco; 230°C, N_2). D-Glucose and L-arabinose were detected from **1** and **2** by comparing its retention time with those of the authentic samples ($t_R = 16.8$ and 10.2 min for D-glucose and L-arabinose, respectively).

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Note

1. These authors contributed equally to this work.

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