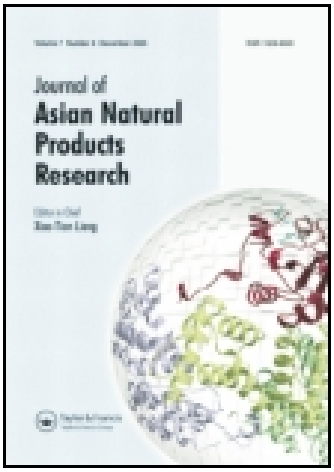

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Glycosides from the bark of *Machilus robusta*

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Six new glycosidic constituents (**1**–**6**), together with 10 known analogs, have been isolated from the bark of *Machilus robusta*. Structures of the new compounds, including the absolute configurations, were determined by spectroscopic and chemical methods as (−)-nectandrin B-β-D-glucopyranoside (**1**), (−)-(7*R*,7′*R*,8*S*,8′*R*)-4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan-4-*O*-β-D-glucopyranoside (**2**), (−)-(7*R*,7′*R*,8*S*,8′*R*)-4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan-4′-*O*-β-D-glucopyranoside (**3**), (−)-(8*S*,8′*R*)-4,4′-dihydroxy-3,3′,5′-trimethoxylignan-4′-*O*-β-D-glucopyranoside (**4**), (−)-(7*R*,8*R*)-syringylglycerol-8-*O*-β-D-glucopyranoside (**5**), and (−)-3-hydroxy-2-methyl-4-pyrone-3-*O*-β-D-xylopyranosyl-(1 → 6)-*O*-β-D-glucopyranoside (**6**), respectively.

Keywords: *Machilus robusta*; Lauraceae; 7,7′-epoxylignan glucosides; ligningluco-side; syringylglycerol glucoside; 2-methyl-4-pyrone diglycoside

1. Introduction

Species of the genus *Machilus* (Lauraceae) are sources of secondary metabolites with interesting chemical structures (lignans, butanolides, sesquiterpenes, alkaloids, and flavonoids) and significant bioactivities [1–3]. Several plants of this genus have long been used for the treatment of various diseases including edema, abdominal distension, pain, and inflammation in China [4]. As part of a program to assess the chemical and biological diversity of several traditional Chinese medicines, we have investigated three species of the genus, including *M. yaoshansis* [5–9], *M. wangchiana* [10,11], and *M. robusta* [12] widely distributed in the south of China. In our previous study, 28 metabolites were characterized from the EtOAc soluble portion of the ethanolic extract of the bark of *M. robusta*, and some of these compounds showed activity against HIV-1 replication and PC12 cell damage [12]. Continuing examination of the water soluble potion of the extract has resulted in the characterization of six new (**1**–**6**) (Figure 1) and 10 known glycosidic metabolites. Based on IUPAC recommendations for the nomenclature of lignans and neolignans [13], compounds **1**–**3** are categorized as 7,7′-epoxylignan β-D-glucosides, and **4** is a lignan β-D-glucoside. Compound **5** is a syringylglycerol 8-*O*-β-D-glucopyranoside with the 7*R*,8*R*-configuration (a pair of the structures with the same relative configuration was reported from *Alangium premnifolium* [14]), and **6** is an uncommon 2-methyl-4-pyrone diglycoside. In this paper, we describe the isolation and structure elucidation of these compounds.

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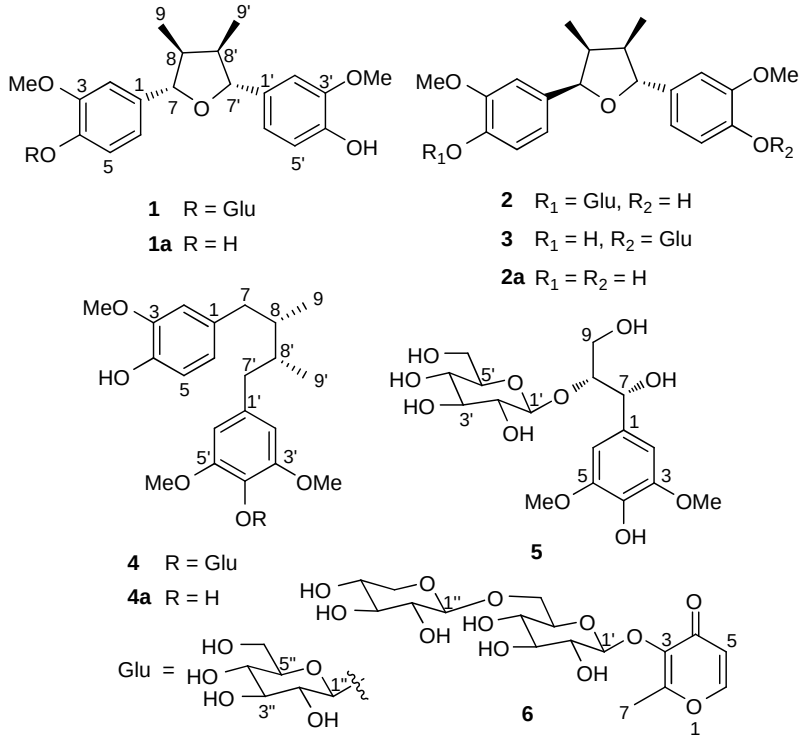

Figure 1. The structures of compounds **1**–**6**.

2. Results and discussion

Compound **1** was obtained as a white solid with $[\alpha]_D^{20}$ −22.5 ($c = 0.10$, MeOH). The IR spectrum of **1** showed absorption band for aromatic ring (1601 and 1515 cm^{-1}) functional groups. The (+)-ESI mass spectrum of **1** exhibited a quasi-molecular ion peak at *m/z* 529 $[M + Na]^+$ and (+)-HR-ESI-MS at *m/z* 529.2049 $[M + Na]^+$ indicated the molecular formula $C_{26}H_{34}O_{10}$. The ^1H NMR spectrum of **1** in MeOH-d_4 showed resonances attributable to two 1,3,4-trisubstitued phenyl rings at δ 7.03 (br s, H-2), 7.11 (d, $J = 8.5$ Hz, H-5), and 6.93 (br d, $J = 8.5$ Hz, H-6), and 6.95 (br s, H-2′), 6.74 (d, $J = 8.0$ Hz, H-5′), and 6.83 (br d, $J = 8.0$ Hz, H-6′) and two aromatic methoxy groups at δ 3.79 and 3.80. In addition, it displayed resonances assignable to two oxygen-bearing methines at δ 4.45 (d, $J = 5.5$ Hz, H-7) and 4.43 (d, $J = 6.0$ Hz, H-7′), two aliphatic methines at δ 2.27 (2H, m, H-8 and H-8′), and two aliphatic methyl groups at δ 0.96 (H_3-9) and 0.99 (H_3-9′), as well as characteristic signals due to a β-glucopyranosyl unit (Table 1) with an anomeric proton at δ 4.84 (d, $J = 7.5$ Hz). The ^{13}C NMR and DEPT spectra of **1** showed carbon signals corresponding to the above units (Table 1). The presence of four oxygen-bearing aromatic carbons ($\delta_C > 145$ ppm) in the ^{13}C NMR spectrum, in combination with the molecular composition, suggested that **1** was a 7,7′-epoxylignan β-glucopyranoside with two phenolic OH and two aromatic methoxy groups. This was confirmed by 2D NMR data analysis of **1**. Extensive analysis of gHSQC spectroscopic data of **1** provided unambiguous assignments of proton and corresponding carbon signals in the NMR spectra. $^1H-^1H$ gCOSY correlations of H-5/H-6, H-7/H-8/H_3-9,

Table 1. ^{1}H and ^{13}C NMR spectral data (δ) for compounds **1–4** in MeOH-d_4.

	1		**2**		**3**		**4**	
No.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		138.6		134.7		133.4		134.7
2	7.03 br s	112.2	6.95 br s	111.7	6.87 br s	111.2	6.65 d (1.5)	114.0
3		151.1		150.6		149.0		149.1
4		147.8		147.0		146.8		145.9
5	7.11 d (8.5)	118.2	7.09 d (8.5)	117.7	6.71 s	116.2	6.64 d (8.0)	116.2
6	6.93 br d (8.5)	120.4	6.82 br d (8.5)	119.9	6.71 s	120.1	6.56 dd (8.0, 1.5)	122.9
7a	4.45 d (5.5)	89.4	5.43 d (3.5)	86.1	5.41 br s	86.8	2.65 dd (14.0, 6.0)	40.2
7b							2.31 dd (14.0, 9.0)	
8	2.27 m	46.3	2.44 m	44.7	2.41 m	45.0	1.74 m	40.5
9	0.96 d (6.0)	13.3	0.55 d (6.0)	9.9	0.57 d (5.0)	10.1	0.81 d (7.0)	16.9
1′		134.8		136.9		139.4		140.6
2′	6.95 br s	111.7	6.91 br s	111.1	7.01 br s	112.0	6.37 s	108.2
3′		149.4		149.6		151.2		154.2
4′		147.7		148.5		147.8		134.8
5′	6.74 d (8.0)	116.4	6.70 d (8.0)	116.4	7.10 d (8.5)	118.1		154.2
6′	6.83 br d (8.0)	120.7	6.76 br d (8.0)	120.7	6.88 br d (8.5)	120.5	6.37 s	108.2
7′a	4.43 d (6.0)	88.9	4.57 d (9.5)	87.7	4.61 d (9.0)	87.3	2.72 dd (13.5, 4.5)	40.2
7′b							2.21 dd (13.5, 9.5)	
8′	2.27 m	45.8	2.44 m	48.6	2.41 m	48.8	1.72 m	40.1
9′	0.99 d (5.5)	13.5	0.92 d (5.5)	12.1	0.94 d (4.5)	12.3	0.79 d (7.0)	17.1
1″	4.84 d (7.5)	103.1	4.82 d (7.5)	103.1	4.83 d (7.5)	103.2	4.72 d (8.0)	106.0
2″	3.45 dd (7.5, 8.5)	75.2	3.43 dd (7.5, 9.0)	75.1	3.43 dd (7.5, 9.0)	75.2	3.40 dd (8.0, 9.0)	76.1
3″	3.42 dd (8.5, 9.0)	78.1	3.40 dd (8.5, 9.0)	78.0	3.39 dd (8.5, 9.0)	78.1	3.35 dd (8.5, 9.0)	78.1
4″	3.37 dd (8.5, 9.0)	71.6	3.37 dd (8.5, 9.0)	71.5	3.36 dd (8.5, 9.0)	71.6	3.36 dd (8.5, 9.0)	71.6
5″	3.36 m	78.5	3.35 m	78.3	3.35 m	78.5	3.15 m	78.6
6″a	3.81 dd (12.0, 4.5)	62.8	3.81 dd (12.0, 4.5)	62.6	3.79 dd (12.0, 4.5)	62.8	3.72 dd (12.5, 2.5)	62.9
6″b	3.63 dd (12.0, 2.5)		3.64 dd (12.0, 3.0)		3.63 br d (12.0)		3.61 dd (12.5, 5.5)	
O*Me*	3.80 s	57.0	3.80 s	56.8	3.83 s	57.1	3.75 s	2 × 57.3
O*Me*	3.79 s	56.7	3.78 s	56.5	3.78 s	56.7	3.74 s	56.7

Notes: ^{1}H NMR data (δ) were measured in MeOH-d_4 for **1–4** at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H–^{1}H COSY, HSQC, and HMBC experiments.

H-5′/H-6′, and H-7′/H-8′/H_3-9′ verified the structure units with vicinal coupling protons in **1**. In the HMBC spectrum of **1**, long-range correlations from H-7 to C-1, C-2, C-6, C-8, C-9, C-7′, and C-8′ and from H-7′ to C-1′, C-2′, C-6′, C-8′, C-9′, C-7, and C-8, in combination with their chemical shifts, confirmed the 7,7′-epoxylignan skeleton. Meanwhile, HMBC correlations of C-3 with H-2, H-5 and 3-OCH_3 and correlations of C-3′ with H-2′ and H-5′ and 3′-OCH_3 demonstrated that the two OCH_3 groups were located at C-3 and C-3′ in **1**, respectively. In addition, an HMBC correlation from H-1″ to C-4 revealed that the β-glucopyranosyl unit was attached to C-4. Thus, the planar structure of **1** was elucidated as 4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan-4-*O*-β-glucopyranoside.

In the NOESY spectrum of **1**, correlations of H-7/H_3-9 and H-7′/H_3-9′ indicated the 7,8-*trans*-7′,8′-*trans* configuration. Enzymatic hydrolysis of **1** produced a sugar and **1a**. The sugar was identified as D-glucose by TLC comparison with an authentic sample and the positive specific rotation, $[\alpha]_D^{20} + 45.1$ ($c = 0.13$, H_2O) [7]. The ^{1}H NMR and specific rotation $\{[\alpha]_D^{20} \approx 0$ ($c = 0.12$, MeOH)$\}$ data of **1a** were identical to those of nectandrin B [15–18]. Therefore, the structure of compound **1** was determined as (−)-nectandrin B-β-D-glucopyranoside.

Compound **2** was obtained as a white solid. The spectroscopic data of **2** indicated that it was an isomer of **1**. This was confirmed by 2D NMR data analysis, particularly, HMBC correlations from H-2, H-5, H-6, and H-1″ to C-4 confirmed that the β-glucopyranosyl moiety was located at C-4 in **2**. Comparison of the NMR data of **2** with those of **1** (Table 1) indicated that the chemical shifts of H-7 and H_3-9 and H-7′ and H_3-9′ were significantly different from those of **1**. This, combined with the coupling constant values of $J_{7,8}$ (3.5 Hz) and $J_{7',8'}$ (9.5 Hz), demonstrated that **2** had the 7,8-*cis*-7′,8′-*trans* configuration [17,19–23]. The elucidation was further confirmed by NOE enhancements of H_3-9 and H_3-9′ upon irritation of H-7′ in the NOE difference experiment of **2**. Enzymatic hydrolysis of **2** produced glucose $\{[\alpha]_D^{20} + 42.1$ ($c = 0.08$, H_2O)$\}$ and **2a** $\{[\alpha]_D^{20} -35.6$ ($c = 0.16$, MeOH)$\}$. The NMR data of **2a** were consistent with those of machilin-I [17] and 3′,3″-dimethoxylarreatricin [23], which have the same relative configuration, but both the absolute configurations have not been determined. The CD spectra displayed negative Cotton effects corresponding to the ^1L_a and ^1L_b transitions of the aromatic units at 231 ($\Delta\varepsilon$ −9.74) and 278 ($\Delta\varepsilon$ −2.29) nm for **2** and at 231 ($\Delta\varepsilon$ −0.32) and 272 ($\Delta\varepsilon$ −0.11) nm for **2a**, which are opposite to those of chicanine [20]. Since the absolute configuration of chicanine was determined by chemical transformation to (−)-galcitin [20] and the chirality at C-7 and C-7′ was reported to be expected to have a major influence on the electronic transition of the aromatic chromaphore [24], the configuration of **2** was assigned as 7*R*,7′*R*,8*S*,8′*R*. Therefore, compound **2** was determined to be (−)-(7*R*,7′*R*,8*S*,8′*R*)-4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan-4-*O*-β-D-glucopyranoside.

Compound **3** showed spectroscopic data similar to those of **2**, except that the chemical shifts of the phenyl moieties were changed in the NMR spectra of **3** (Table 1). This suggested that the β-glucopyranosyl moiety was located at C-4′ in **3** instead of at C-4 in **2**. The suggestion was confirmed by HMBC correlations from H-7′ to C-2′, C-6′, C-8′, and C-9′; from H-2′ and H-6′ to C-7′ and C-4′; and from H-1″ to C-4′, as well as by NOE enhancements of H_3-9 and H_3-9′ upon irradiation of H-7′ ($J_{7',8'} = 9.0$ Hz). The CD spectrum of **3** displayed Cotton effects at 230 ($\Delta\varepsilon$ −13.9) and 281 ($\Delta\varepsilon$ −2.6) nm, which is similar to those of **2**. This indicated that **3** had the absolute configuration identical to that of **2**,

which was proved by enzymatic hydrolysis of **3** produced D-glucose {$[\alpha]_D^{20}$ + 42.8 (c = 0.14, H_2O)} and **2a**. Therefore, the structure of compound **3** was determined as (−)-(7*R*,7′*R*,8*S*,8′*R*)-4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan-4′-*O*-β-D-glucopyranoside.

Compound **4**, an amorphous powder with $[\alpha]_D^{20}$ −18.8 (c = 0.12, MeOH), exhibited a quasi-molecular ion peak at *m/z* 545 $[M + Na]^+$ in the positive mode ESI-MS. The molecular formula of **4** ($C_{27}H_{38}O_{10}$) was determined by HR-ESI-MS at *m/z* 545.2363 $[M + Na]^+$, which was supported by the NMR data (Table 1). The NMR spectra of **4** in MeOH-d_4 showed resonances attributed to a 1,3,4-trisubstituted phenyl, a symmetrically 1′,3′,4′,5′-tetrasubstituted phenyl, three aromatic methoxy, two methines, two methylenes, and two methyls, together with characteristic signals due to a β-glucopyranosyl unit. These data suggested that **4** was a lignan glucoside with the typical nucleus, which was confirmed by 2D NMR data analysis. In particular, HMBC correlations from H-2′/H-6′ and H-1″ to C-4′ and from OC*H*$_3$-3′/OC*H*$_3$-5′ to C-3′/C-5′ demonstrated unequivocally that the β-glycopyranosyl moiety and two methoxy groups were symmetrically substituted on the same phenyl unit. Enzymatic hydrolysis of **4** produced D-glucose {$[\alpha]_D^{20}$ + 40.3 (c = 0.08, H_2O)} and **4a**. The ^{1}H NMR data and specific rotation {$[\alpha]_D^{20}$ + 2.5 (c = 0.56, $CHCl_3$)} of **4a** were in good agreement with those of (+)-(8*S*,8′*R*)-4,4′-dihydroxy-3,3′,5′-trimethoxylignan isolated from the same plant [12]. Thus, the structure of compound **4** was characterized as (−)-(8*S*,8′*R*)-4,4′-dihydroxy-3,3′,5′-trimethoxylignan-4′-*O*-β-D-glucopyranoside.

Compound **5** was isolated as an amorphous powder. The spectroscopic features of **5** were similar to those of 7,8-*threo*-syringylglycerol 8-*O*-β-D-glucopyranosides [14,25]. 2D NMR data analysis confirmed that **5** had the same planar structure as the reported compounds. The 7,8-*threo*-configuration of **5** was indicated by the $J_{7,8}$ and $\Delta\delta_{C8-C7}$ values [25]. However, comparison of the NMR data of **5** and the co-occurring (7*S*,8*S*)-syringylglycerol 8-*O*-β-D-glucopyranoside indicated that H-8 and C-8 in **5** were shielded by $\Delta\delta_H$ −0.03 ppm and $\Delta\delta_C$ −0.7 ppm, respectively, whereas H-1′ was deshielded by $\Delta\delta_H$ + 0.15 ppm. This suggested that **5** had the (7*R*,8*R*)-configuration. Enzymatic hydrolysis of **5** yielded β-D-glucose {$[\alpha]_D^{20}$ + 41.7 (c = 0.16, H_2O)} and **5a**. The ^{1}H NMR data of **5a** were identical to those of (7*S*,8*S*)-syringylglycerol, however, the specific rotation of **5a**, $[\alpha]_D^{20}$ −25.4 (c = 0.25, MeOH), was opposite to that of (7*S*,8*S*)-syringylglycerol [25]. This confirmed that **5** had the 7*R*,8*R*-configuration. Therefore, the structure of compound **5** was assigned as (−)-(7*R*,8*R*)-syringylglycerol 8-*O*-β-D-glucopyranoside. Although a pair of 7,8-*threo*-syringylglycerol 8-*O*-β-D-glucopyranosides was reported from *A. premnifolium* [14], the absolute configuration was not determined.

Compound **6** was obtained as a yellowish solid. Its molecular formula $C_{17}H_{24}O_{12}$ was determined by positive mode HR-ESI-MS at *m/z* 443.1168 $[M + Na]^+$ combined with the NMR data (Table 2). The NMR data of **6** were similar to those of the co-occurring spatholosineside A, 3-hydroxy-2-methyl-4-pyrone 3-*O*-α-L-rhamnopyranosyl-(1 → 6)-*O*-β-D-glucopyranoside [26], except that the L-rhamnopyranosyl unit in spatholosineside A was replaced by a β-glycosyl unit. Acid hydrolysis of **6** produced β-D-xylopyranose and β-D-glucopyranose, as identified by GC analysis of trimethylsiyl L-cysteine of the derivative of hydrolysate and the authentic sugars [27]. This suggested that **6** was a derivative of spatholosineside A with the β-D-xylopyranosyl unit substituting the L-rhamnopyranosyl unit. The elucidation was confirmed by 2D NMR data analysis

Table 2. ^{1}H and ^{13}C NMR spectral data (δ) for compounds **5** and **6** in MeOH-d_4.

	5		**6**[a]	
No.	δ_H	δ_C	δ_H	δ_C
1		132.6		
2	6.65 s	105.9		165.0
3		149.4		143.8
4		136.6		177.5
5		149.4	6.39 d (5.5)	117.7
6	6.65 s	105.9	7.94 d (5.5)	157.5
7	4.60 d (7.5)	75.3	2.40 s	16.2
8	3.79 m	86.9		
9a	3.45 dd (12.0, 3.0)	62.6		
9b	3.25 dd (12.0, 6.0)			
1′	4.46 d (7.0)	104.5	4.73 d (7.0)	105.5
2′	3.26 dd (7.0, 9.0)	75.6	3.31 dd (7.0, 9.0)	75.7
3′	3.34 dd (9.0, 9.0)	78.1	3.33 dd (9.0, 8.5)	78.2
4′	3.26 dd (9.0, 8.5)	71.8	3.34 dd (8.5, 9.0)	71.5
5′	3.28 m	78.5	3.34 m	77.8
6′a	3.83 dd (12.0, 1.0)	62.9	3.99 brd (11.5)	70.2
6′b	3.63 dd (12.0, 5.0)		3.64 dd (11.5, 6.0)	
O*Me*-3/-5	3.79	57.1		

Notes: ^{1}H NMR data (δ) were measured in MeOH-d_4 for **5** and **6** at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H–^{1}H COSY, HSQC, and HMBC experiments.

[a] Data for the β-D-xylopyranosyl moiety of **6**: δ_H 4.18 (1H, d, J = 7.5 Hz, H-1″), 3.10 (1H, dd, J = 7.5 and 9.0 Hz, H-2″), 3.22 (1H, dd, J = 9.0 and 8.5 Hz, H-3″), 3.41 (1H, m, H-4″), 3.77 (1H, dd, J = 11.5 and 5.0 Hz, H-5″a), and 3.11 (1H, dd, J = 11.5 and 7.0 Hz, H-5″b); δ_C 105.8 (C-1″), 75.1 (C-2″), 78.0 (C-3″), 71.5 (C-4″), and 67.2 (C-5″).

of **6**. In particular, in the HMBC spectrum of **6**, correlations of H-5/C-3 and C-6; H-6/C-2, C-4, and C-5; and H_3-7/C-2 and C-3 verified the 2-methyl-4-pyrone moiety. In addition, an HMBC correlation of H-1′/C-3 confirmed that the β-D-glucopyranosyloxy unit was located at C-3 of the 2-methyl-4-pyrone moiety, while a correlation of H-1″/C-6′ proved the location of the β-D-xylopyranosyloxy unit at C-6′ of the β-D-glucopyranosyloxy unit in **6**. Thus, the structure of compound **6** was determined as 3-hydroxy-2-methyl-4-pyrone 3-*O*-β-D-xylopyranosyl-(1 → 6)-*O*-β-D-glucopyranoside.

The known compounds were identified by comparing the spectroscopic data with those reported in the corresponding literature as (+)-isolariciresinol-9′-*O*-β-xylopyranoside [28,29], (+)-5′-methoxy-isolariciresinol-9′-*O*-β-xylopyranoside [30,31], (+)-lyoniresinol-9′-*O*-β-D-xylopyranoside [32], (+)-(8*S*,8′*S*)-4,4′- dihydroxy-3,3′,5,5′-tetramethoxylignan-9,9′-diol 9-*O*-β-D-xylopyranoside [33], 4-hydroxy-3-methoxyphenol-β-D-[6-*O*-(4-hydroxy-3,5-dimethoxylbenzoate)]-glucopyranoside [34], (−)-2,6-dimethoxy-*p*-hydroquinone 1-*O*-β-D-glucopyranoside [35], (−)-3,5-dimethoxy-4-hydroxyphenol 1-*O*-β-D-glucopyranoside [36], (−)-4-hydroxy-3-methoxyphenyl 1-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside [37], (7*S*,8*S*)-syringylglycerol 8-*O*-β-D-glucopyranoside [14,25], and spatholosineside A [26].

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on P-2000 polarimeter (JASCO, Tokyo, Japan). UV spectra were measured on a JASCOP-650 spectrometer (JASCO). CD spectra

were recorded on a JASCO J-815 CD spectrometer (JASCO). IR spectra were recorded on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission; Thermo Electron Corporation, Madison, WI, USA). NMR spectra were obtained at 500 MHz for ^{1}H, and 125 MHz for ^{13}C, respectively, on an Inova 500 spectrometer (Varian Associates, Inc., Palo Alto, CA, USA) in MeOH-d_4 with solvent peaks used as references. ESI-MS and HR-ESI-MS data were measured using an AccuToFCS JMS-T100CS spectrometer (Agilent Technologies, Ltd, Santa Clara, CA, USA). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector (Waters Corporation, Milford, MA, USA), with an Prevail (250 × 10 mm i.d.) column packed with C_{18} (5 μm) (Alltech Associates, Inc., Deerfield, IL, USA). TLC was carried out with glass precoated silica gel GF_{254} plates (Qingdao Marine Chemical, Inc.). Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating. Unless otherwise noted, all chemicals were obtained from commercially available sources and were used without further purification.

3.2 Plant material

The bark of *M. robusta* was collected at Dayao Mountain, Guangxi Province, China in August 2006. The plant was identified by Mr Guang-Ri Long (Guangxi Forest Administration, Guangxi, China). A voucher specimen (No. 041) was deposited at the Herbarium of the Department of Natural Product Chemistry, Institute of Materia Medica.

3.3 Extraction and isolation

The air-dried bark of *M. robusta* (5.3 kg) was powdered and extracted with 45 liters of aq. 95% EtOH at room temperature for 3 × 48 h. The EtOH extract was evaporated under reduced pressure to yield a dark brown residue (535 g). The residue was suspended in H_2O (2000 ml) and partitioned with EtOAc (8 × 2000 ml). The aq. phase (196.3 g) was applied to a HDP100 macroporous adsorbent resin (1000 g, dried weight) column. A successive elution of the column with H_2O, 30%, 50%, and 95% EtOH (5000 ml each) yielded four corresponding fractions after removing of solvents. The fraction (22.3 g) eluted by 50% EtOH was separated by medium pressure liquid chromatography over reversed-phase silica gel eluting with a gradient of increasing MeOH (30–100%) in H_2O to give fractions A–D on the basis of TLC analysis. Subsequent separation of fraction B (5.52 g) on normal silica gel CC, eluting with a gradient of increasing MeOH (0–100%) in $CHCl_3$, afforded fractions B_1–B_5. Fraction B_3 (1.98 g) was chromatographed over Sephadex LH-20, using MeOH:H_2O (70:30) as the eluting solvent, to give three subfractions $B_{3\text{-}1}$–$B_{3\text{-}3}$. $B_{3\text{-}2}$ (0.30 g) and $B_{3\text{-}3}$ (0.22 g) were separately isolated by reversed-phase semipreparative HPLC, using mobile phase of MeOH:H_2O (20:80) (flow rate: 2 ml/min; detection wavelength: 230 nm) to afford **1** (15.0 mg, R_t = 34.2), **2** (10.6 mg, R_t = 31.4), and **3** (11.2 mg, R_t = 32.7) from $B_{3\text{-}2}$; and **5** (16.9 mg, R_t = 22.7) and **6** (5.9 mg, R_t = 26.3) from $B_{3\text{-}3}$. Fraction C (2.82 g) was separated on normal silica gel CC, eluting with a gradient of increasing MeOH (10–50%) in $CHCl_3$, to afford subfractions C_1–C_3. C_2 (0.85 g) was further chromatographed over Sephadex LH-20, using MeOH as the elution solvent, and then isolated by reversed-phase semipreparative HPLC by using MeOH:H_2O (30:70) as mobile phase

(flow rate: 2 ml/min; detection wavelength: 254 nm) to yield **4** (20.8 mg, $R_t = 23.9$).

3.3.1 (−)-Nectandrin B-β-D-glucopyranoside (**1**)

Yellowish solid; $[\alpha]_D^{20}$ −22.5 ($c = 0.10$, MeOH); UV (MeOH) λ_{max} (log ε) 229 (0.18), 279 (0.07) nm; CD (MeOH) $\Delta\varepsilon_{210\,nm}$ +4.20, $\Delta\varepsilon_{233\,nm}$ −3.14; IR (film) ν_{max} 3448, 3389, 2958, 1601, 1515, 1461, 1388, 1270, 1226, 1157, 1126, 1076, and 1049 cm^{-1}; for ^1H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) spectroscopic data, see Table 1; positive-mode ESI-MS: *m/z* 529 $[M + Na]^+$; negative-mode ESI-MS: *m/z* 505 $[M-H]^-$; HR-ESI-MS: *m/z* 529.2049 $[M + Na]^+$ (calcd for $C_{26}H_{34}O_{10}Na$, 529.2044).

3.3.2 (−)-(7R,7′R,8S,8′R)-4,4′-Dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan-4-O-β-D-glucopyranoside (**2**)

Yellowish solid; $[\alpha]_D^{20}$ −25.3 ($c = 0.29$, MeOH); UV (MeOH) λ_{max} (log ε) 230 (0.38), 278 (0.16) nm; CD (MeOH) $\Delta\varepsilon_{231\,nm}$ −9.74, $\Delta\varepsilon_{278\,nm}$ −2.29; IR (film) ν_{max} 3386, 2965, 1597, 1514, 1456, 1417, 1384, 1272, 1223, 1073, and 1036 cm^{-1}; for ^1H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) spectroscopic data, see Table 1; positive-mode ESI-MS: *m/z* 529 $[M + Na]^+$; negative-mode ESI-MS: *m/z* 505 $[M - H]^-$; HR-ESI-MS: *m/z* 529.2055 $[M + Na]^+$ (calcd for $C_{26}H_{34}O_{10}Na$, 529.2044).

3.3.3 (−)-(7R,7′R,8S,8′R)-4,4′-Dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan-4′-O-β-D-glucopyranoside (**3**)

Yellowish solid; $[\alpha]_D^{20}$ −54.5 ($c = 0.46$, MeOH); UV (MeOH) λ_{max} (log ε) 231 (0.33), 281 (0.12) nm; CD (MeOH) $\Delta\varepsilon_{231\,nm}$ −13.92, $\Delta\varepsilon_{281\,nm}$ −2.61; IR (film) ν_{max} 3398, 2964, 1600, 1514, 1460, 1424, 1384, 1268, 1226, 1158, 1123, 1073, and 1039 cm^{-1}; for ^1H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) spectroscopic data, see Table 1; positive-mode ESI-MS: *m/z* 529 $[M + Na]^+$; negative-mode ESI-MS: *m/z* 505 $[M-H]^-$; HR-ESI-MS: *m/z* 529.2056 $[M + Na]^+$ (calcd for $C_{26}H_{34}O_{10}Na$, 529.2044).

3.3.4 (−)-(8S,8′R)-4,4′-Dihydroxy-3,3′,5′-trimethoxylignan-4′-O-β-D-glucopyranoside (**4**)

Amorphous powder; $[\alpha]_D^{20}$ −18.8 ($c = 0.12$, MeOH); UV (MeOH) λ_{max} (log ε) 230 (0.32), 278 (0.10) nm; CD (MeOH) $\Delta\varepsilon_{217\,nm}$ −2.19, $\Delta\varepsilon_{234\,nm}$ +0.65, $\Delta\varepsilon_{278\,nm}$ +0.53; IR (film) ν_{max} 3411, 2958, 1594, 1515, 1462, 1423, 1272, 1243, 1131, 1071, 894, and 816 cm^{-1}; for ^1H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) spectroscopic data, see Table 1; positive-mode ESI-MS: *m/z* 545 $[M + Na]^+$; negative-mode ESI-MS: *m/z* 521 $[M-H]^-$; HR-ESI-MS: *m/z* 545.2363 $[M + Na]^+$ (calcd for $C_{27}H_{38}O_{10}Na$, 545.2357).

3.3.5 (−)-(7R,8R)-Syringylglycerol 8-O-β-D-glucopyranoside (**5**)

Amorphous powder; $[\alpha]_D^{20}$ −48.0 ($c = 0.53$, MeOH); UV (MeOH) λ_{max} (log ε) 238 (0.04), 281 (0.01) nm; CD (MeOH) $\Delta\varepsilon_{23\,nm}$ +0.05, $\Delta\varepsilon_{280\,nm}$ +0.04; IR (film) ν_{max} 3392, 2940, 1617, 1520, 1466, 1424, 1322, 1222, 1125, 1082, 894, and 837 cm^{-1}; for ^1H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) spectroscopic data, see Table 2; positive-mode ESI-MS: *m/z* 429 $[M + Na]^+$; negative-mode ESI-MS: *m/z* 405 $[M-H]^-$; HR-ESI-MS: *m/z* 429.1371 $[M + Na]^+$ (calcd for $C_{17}H_{26}O_{11}Na$, 429.1367).

*3.3.6 (−)-3-Hydoxy-2-methyl-4-pyrone 3-O-β-D-xylopyranosyl-(1 → 6)-O-β-D-glucopyranoside (**6**)*

Yellowish solid; $[\alpha]_D^{20}$ −74.0 ($c = 0.15$, MeOH); UV (MeOH) λ_{max} (log ε) 257 (0.44), 205 (0.70) nm; IR (film) ν_{max} 3397, 2920, 1651, 1441, 1375, 1287, 1253, 1191, 1108, 1072, 992, 972, and 834 cm^{-1}; for ^1H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) spectroscopic data, see Table 2; positive-mode ESI-MS: *m/z* 443 $[M + Na]^+$; negative-mode ESI-MS: *m/z* 419 $[M-H]^-$; HR-ESI-MS: *m/z* 443.1168 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_{12}Na$, 443.1160).

*3.3.7 Enzymatic hydrolysis of **1**–**5***

A solution of each compound (**1**–**5**, 5–8 mg) in H_2O (3 ml) was hydrolyzed with snailase (30 mg, LJ0427B2011Z, Shanghai Sangon Biotech Co. Ltd, Shanghai, China) at 37°C for 12 h. The reaction mixture was extracted with EtOAc (3 × 3 ml). The H_2O phases of the hydrolysates of **1**–**5** were separately concentrated to dryness and then eluted with CH_3CN:H_2O (8:1) on a silica gel column to yield glucose with $[\alpha]_D^{20}$ values that ranged from +40.3 to +45.1 ($c = 0.08$–0.16, H_2O). The solvent system $CHCl_3$:MeOH:H_2O (8:5:1) was used for TLC identification of glucose (R_f, 0.30). EtOAc extracts of the hydrolysates were separately concentrated to dryness, and then eluted on a silica gel column with 40–70% EtOAc in petroleum ether to yield nectandrin B {**1a**, 1.2 mg, $[\alpha]_D^{20} \approx 0$ ($c = 0.12$, MeOH)} [14,16,17] from **1**, (−)-(7*R*,7′*R*,8*S*,8′*R*)-4,4′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan {**2a**, $[\alpha]_D^{20}$ −35.6 ($c = 0.16$, MeOH)} [16,22] 2.0 mg from **2** and 1.6 mg from **3**, (+)-(8*S*,8′*R*)-4,4′-dihydroxy-3,3′,5′-trimethoxylignan {**4a**, 5.6 mg, $[\alpha]_D^{20}$ + 2.5 ($c = 0.56$, $CHCl_3$)} [12] from **4**, and (−)-(7*R*,8*R*)-syringylglycerol {**5a**, 2.5 mg, $[\alpha]_D^{20}$ −25.4 ($c = 0.25$, MeOH)} [24] from **5**, respectively.

*3.3.8 Acid hydrolysis of **6***

Compound **6** (3.5 mg) was refluxed in 2 N HCl (5.0 ml) at 80°C for 6 h. The reaction mixture was extracted with EtOAc (3 × 5 ml). The H_2O layer was evaporated under reduced pressure. After addition of H_2O (5 ml), the acidic solution was evaporated again, and this procedure was repeated until a neutral solution was obtained. The neutral solution was evaporated and dried *in vacuo* to furnish a monosaccharide residue. The residue was dissolved in pyridine (0.5 ml) and 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was maintained at 60°C for 2 h, evaporated under a stream of N_2, and dried *in vacuo*. Next, 0.2 ml of *N*-trimethylsilylimidazole was added, and the resultant reaction mixture was maintained at 60°C for 1 h. The mixture was partitioned between *n*-hexane and H_2O (2 ml each), and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, DB-5 (30 m × 0.25 mm × 0.25 μm); detector, FID; detector temperature, 280°C; injection temperature, 250°C; initial temperature, 100°C for 2 min and subsequent increase to 280°C at the rate of 10°C/min; final temperature, 280°C for 5 min; carrier, N_2 gas. The absolute configurations of the sugars were determined by comparing the retention times of their trimethylsilyl-L-cysteine derivatives with those of authentic sugars prepared by a similar procedure. The retention times of the trimethylsilyl-L-cysteine derivatives of the sugars were as follows: D-glucose, 19.57 min, and D-xylopyranose, 17.66 min.

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