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Chemical constituents from the branches of *Alangium barbatum* and their anti-inflammatory activities

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ABSTRACT

Two new sesquiterpenes (**1** and **2**), one new alkaloid (**4**), one new neolignan glycoside (**14**), together with 19 known compounds were isolated from the branches of *Alangium barbatum*. Their structures were established by spectroscopic analysis. The absolute configuration of **21** was determined for the first time by single-crystal X-ray diffraction analysis with Cu-Kα irradiation. Some of isolates were evaluated for their inhibitory effects on NO production in LPS-induced RAW264.7 macrophages. Compounds **1**, **3** and **9** at 10 μM concentrations exhibited mild inhibitory effects on NO production in the range of 25.0 to 32.4. Moreover, these compounds at 25 μM concentration were also found to mildly suppress NF-κB activation in TNF-α induced expression of NF-κB-Luc in Hela cells with inhibition percent in the range of 15.0 to 25.0%.

1. Introduction

Alangium barbatum, belonging to the genus *Alangium*, is a deciduous shrub, and widely distributed in south of China (Zhang et al., 2009). The root of the genus *Alangium* has been traditionally used for rheumatism, leprosy, arthritis, helmintiasis, skin diseases, dysentery, inflammations, and hypertension (Hung et al., 2009; Anjum et al., 2002; Zhang et al., 2017). Previous work on this genus has shown the presence of the alkaloids, phenolic glycosides, terpenoids and lignans (Itoh et al., 2001; Tamaki et al., 2000; Pailee et al., 2015; Otsuka et al.,1996). In this study, we have investigated the constituent of *A. barbatum*, and this has resulted in the isolation of two new sesquiterpenes (**1** and **2**), one new alkaloid (**4**) and one new neolignane glycoside (**14**), and 19 known compounds (Figure S36). Herein, we describe the isolation, structure elucidation of these compounds, along with their inhibitory effects on NO production in LPS-induced RAW264.7 macrophages, and against NF-κB activation in TNF-α induced expression of NF-κB-Luc in Hela cells (Figs. 1 and 2).

2. Results and discussion

A molecular formula of $C_{15}H_{22}O_2$ and 5 degrees of unsaturation were determined for **1** on the basis of the HRESIMS 233.1574 $[M-H]^-$ (calcd for 233.1547). The ^{13}C NMR spectrum of **1** showed fifteen carbon signals, including six aromatic carbons, three methylene carbons, three methine carbons and three methyls. $^1H-^1H$ COSY and HSQC analyses revealed three isolated spin systems $C(11)H_3-C(1)H-C(2)H_2$, $C(2)H_2-C(3)H_2$-C(4)H, C(4)H-C(13)H-$C(14)H_3$/$C(15)H_3$. The ^1H and ^{13}C-NMR spectra (Table 1) implied that **1** was a cadinane architecture, closely related to a previous structure for 6-hydroxycalamenene except for the presence of the hydroxyl at C-12 (Salmoun et al., 2007). The location of the hydroxyl group was determined to be at C-12 from the HMBC correlations between H_2-12 with C-6 (δ 153.4), C-7 (δ 122.0), and C-8 (δ 127.4). The relative configuration of **1** was determined by NOESY experiment. The NOESY correlation between H-1 and H-4 suggested the two protons were on the same side (Fig. 3). The absolute configuration at C-4 of **1** was 4*S* on the basis of the positive rotatory and chemical shift at C-3 (Salmoun et al., 2007). Thus, the structure of **1** was determined and named as (1*S*, 4*S*)-6, 12-dihydroxycalamenene.

Compound **2** had the molecular formula $C_{22}H_{24}O_3$, by HRESIMS at *m/z* 337.1781 $[M+H]^+$. The ^1H NMR spectrum of **2** (Table 1) revealed signals of a set of ortho-disubstituted aromatic nucleus system [δ_H 6.83 (td, J = 7.5, 1.5 Hz, 1 H), 6.56 (dd, J = 7.5, 1.5 Hz, 1 H), 6.40 (td, J = 7.5, 1.5 Hz, 1 H), 6.29 (dd, J = 7.5, 1.5 Hz, 1 H)], two aromatic protons and one olefinic proton [7.35 (s, 1 H), 6.94 (s, 1 H), 5.88 (s,

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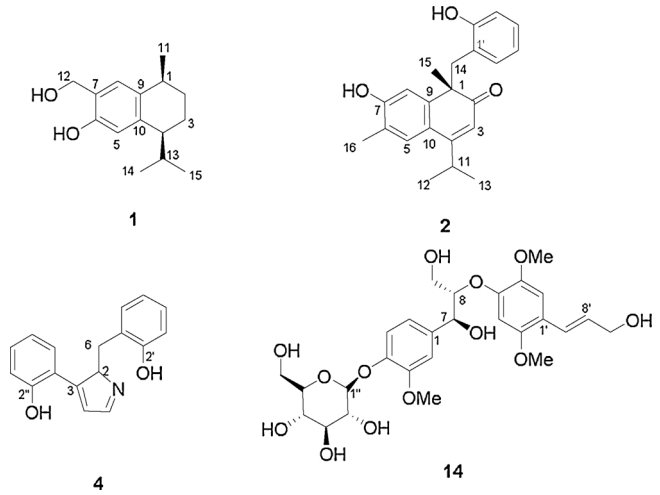

Fig. 1. Chemical structures of compounds **1**,**2**,**4**,**14**.

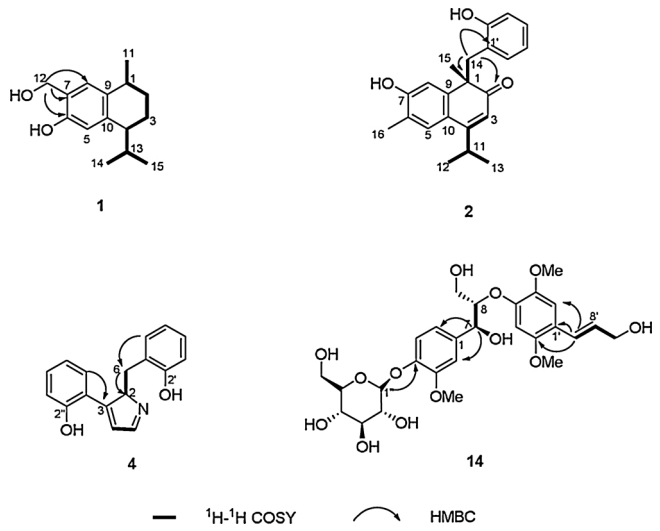

Fig. 2. The Key ^{1}H–^{1}H COSY and HMBC correlations for compounds**1**, **2**, **4**, and **14**.

1 H)], one methylene proton [3.01 (d, J = 13.8 Hz, 2 H)], one methine proton [3.16 (m, 1 H)] and two methyl singlets and two methyl doublets [2.20 (s, 3 H), 1.49 (s, 3 H), 1.19 (d, J = 6.7 Hz, 3 H), 1.02 (d, J = 6.8 Hz, 3 H)]. The NMR data resembled those of the known compound lacinilene C, in which the hydroxyl group at C-1 was replaced by 2′-hydro-phenylmethyl (Zhang et al., 2013). The only chiral carbon (C-1) of **2** was determined to be an *S* absolute configuration based on a positive Cotton effect at 318 nm (Fig. 4) in the CD spectrum (Zhang et al., 2013). The key HMBC correlations between H_2-14 to C-1, C-2, C-1′, C-2′, and C-9 indicated that the 2′-hydroxy- phenylmethyl was attached to C-1. Thus, **2** was determined and named as lacinilene E.

Compound **4** was isolated as brown oil. Its molecular formula was deduced as $C_{17}H_{15}NO_2$ by HR-ESIMS at *m/z* 265.1103 $[M+H]^+$. The ^{1}H NMR spectra indicated the presence of two sets of ortho-disubstituted aromatic nucleus system [δ_H 6.79 (dd, J = 8.0, 1.0 Hz, H-3′), 7.05 (td, J = 7.8, 1.9 Hz, H-4′), 6.66 (td, J = 7.4, 1.1 Hz, H-5′), 6.94 (dd, J = 7.4, 1.6 Hz, H-6′); 8.03 (d, J = 8.5 Hz, H-3″), 7.75 (ddd, J = 8.4, 6.9, 1.3 Hz, H-4″), 7.59 (ddd, J = 8.3, 6.8, 1.2 Hz, H-5″), 8.42 (d, J = 8.0 Hz, H-6″)], two olefinic protons [δ_H 7.71 (d, J = 4.6 Hz, H-4), 8.80 (d, J = 4.6 Hz, H-5)], one methylene [δ_H 5.82 (dd, J = 8.6, 4.0 Hz, H-2)] and one methine proton [δ_H 3.27 (t, J = 3.5 Hz, H-6a), 2.90 (dd, J = 13.7, 8.6 Hz, H-6b)]. The ^{1}H–^{1}H COSY and HSQC analyses on **4** indicated the presence of four isolated spin systems C(4)

Table 1
^{1}H (500 MHz) and^{13}C NMR (125 MHz) spectroscopic data for compounds **1** and **2**.

No	δ_C	**1** δ_H (J in Hz)	δ_C	**2** δ_H (J in Hz)
1	22.2	2.71 (m, 1 H)	51.6	
2	30.6	1.91 (dd, J = 7.4, 3.4 Hz, 2 H)	206.1	
		1.32 (m, 1 H)		
3	21.5	1.79 (m, 1 H)	116.8	5.88 (s, 1 H)
		1.54 (m, 1 H)		
4	43.1	2.60 (m, 1 H)	164.2	
5	114.6	6.75 (s, 1 H)	127.3	7.35 (s, 1 H)
6	153.4		122.5	
7	122.0		157.2	
8	127.4	6.87 (s, 1 H)	113.4	6.94 (s, 1 H)
9	144.7		146.0	
10	131.7		121.7	
11	22.3	1.22 (d, J = 6.9 Hz, 3 H)	28.5	3.16 (m, 1 H)
12	61.2	4.81 (d, J = 2.2 Hz, 2 H)	20.9	1.19 (d, J = 6.7 Hz, 3 H)
13	31.9	2.15 (m, 1 H)	21.3	1.02 (d, J = 6.8 Hz, 3 H)
14	17.2	0.68 (d, J = 6.8 Hz, 3 H)	42.5	3.01 (d, J = 13.8 Hz, 1 H)
15	21.2	0.96 (d, J = 6.8 Hz, 3 H)	25.1	1.49 (s, 3 H)
16			14.6	2.20 (s, 3 H)
1′			123.3	
2′			155.2	
3′			114.2	6.56 (dd, J = 8.0,1.0 Hz, 1 H)
4′			126.9	6.83 (dd, J = 8.0,1.0 Hz, 1 H)
5′			118.1	6.40 (td, J = 7.5, 1.1 Hz, 1 H)
6′			130.3	6.29 (dd, J = 7.5, 1.1 Hz, 1 H)

H − C(5)H, C(6)H_2 − C(2)H, C(3′)H-C(4′)H-C(5′)H-C(6′)H, C(3″)H-C(4″)H-C(5″)H-C(6″)H (Fig. 2). The key HMBC correlations from H-6″ to C-3 (δ 147.2), H-6′ to C-6 (δ 40.5), and H-6 to C-2 (δ 68.9) and C-5 (δ 149.5) suggested the location of the hydroxy-phenylmethyl at C-2, the 2-hydroxy-phenyl at C-3, respectively. The NMR data of **4** showed great similarity to those of the known compound 2,5-dihydro-3-phenyl-2-(phenylmethyl)-1*H*-Pyrrole except for the absent of the double bond at N-1 to C-5 and the hydroxy group at C-2″ (Guthrie et al., 1955). Thus, **4** was identified as a new natural product for which the name 2-(2′-hydroxy-phenylmethyl)-3-(2″-hydroxy-phenyl)-2H- pyrrole was proposed.

Compound **14** was isolated as brown oil, which molecular formula ($C_{27}H_{36}O_{13}$) was assigned by HRESIMS at *m/z* 591.2041 $[M+Na]^+$. The 1D in combination with 2D NMR data for **14** were summarized in experimental section, which were found to be similar with the reported data for citrusin B (Li et al., 2013), except for the configuration at C-7 and C-8. The relative orientation at C-7 and C-8 were elucidated to be *trans* based on the coupling constant (J = 6.5 Hz) and the NOESY correlations between H-7 and H-9; H-8/H-2 and H-6. The CD spectra of **14** displayed negative Cotton effects at 220–260 nm (Fig. 5) indicating that **14** possessed an 8*S* configuration. Thus, the absolute configuration at 7-position in **14** was determined to be 7*S*. Consequently, the structure of **14** was determined to be (7*S*, 8*S*)-threo-3,2′,5′-trimethoxy-7,9-dihydroxy-8-O-4′-neolignan-4-O-β-D–glucopyranoside.

The structures of the other known compounds were elucidated by lacinilene C (**3**) (Zhang et al., 2007), cyclo-(L-Pro-L-Leu) (**5**) (Sawadsitang et al., 2015), 3-hydroxy-benzenemethanol (**6**) (Bao et al., 2007), 3-hydroxy-4-methoxy- benzaldehyde (**7**) (Bata et al., 2016), benzoic acid, 2, 4-dihydroxy- 3, 6-dimethyl- methyl ester (**8**) (Li et al., 2015a,b), 1, 2-benzenedicarboxylic acid, 1, 2-bis-(2- methylpropyl) ester (**9**) (Eid and Metwally, 2017), 2-hydroxy-benzenemethanol (**10**) (Li et al., 2015a,b), 1,1'-oxybis-2, 5- bis-(1,1-dimethylethyl) -benzene (**11**) (Anjum et al., 2002b), (7*S*,8*R*)-glehlinoside H (**12**) (Ren et al., 2018), (7*S*,8*R*)-glehlinoside H (**13**) (Ren et al., 2018), 2-(β-D-glucopyranosyloxy)-6-hydroxy-ethyl ester (**15**) (Zhang et al., 2013), (2-