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Short communication

# Acylphloroglucinols from the fruits of *Callistemon viminalis*

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Five new acylphloroglucinols, callistenones L-P (**1–5**), and three known compounds watsonianone A, callistenones F and H were isolated from the fruits of *Callistemon viminalis*. Callistenones L and M possessed a bisfuran fused-ring skeleton. Compounds **3–5** were acylphloroglucinol condensed with a $\beta$-triketone moiety via a pyrane. Their structures were established from analyses of NMR spectra, CD spectra and X-ray crystallography. Compound **8** showed antibacterial activity against *S. aureus* with MIC value of 20.3 μg/mL and *E. coli* with MIC value of 15.6 μg/mL.

## 1. Introduction

*Callistemon viminalis*, is a flowering plant belonging to the family Myrtaceae and widely distributed throughout southern China. Extracts of the leaves from this plant have been used as folk medicine for treatment of cold and arthralgia in China (State Administration of Traditional Chinese Medicine of the People&s Republic of China, 1999). Acylphloroglucinols derivatives are prominent secondary metabolites in the Myrtaceae family and show outstanding antibacterial and anti-inflammatory properties (Singh and Bharate, 2006; Nishiwaki et al., 2015; Zhang et al., 2016; Umehara et al., 1998; Cottiglia et al., 2012; Rattanaburi et al., 2013; Hirantat et al., 2012). There have also been reports that they have antioxidant properties (Muller et al., 2010).

Previous studies, a series of hybrids of $\beta$-triketone derivative with terpene were isolated from the title plant (Wu et al., 2015a,b, 2016, 2017). Continuing research for comprehensively characterizing the acylphloroglucinol composition of *C. viminalis* led to the isolation of five new acylphloroglucinols (**1–5**) and three known compounds, watsonianone A (Carroll et al. 2013), callistenones F and H (Liu et al., 2016). Callistenones N-P are characterized by a phloroglucinol ring connected via an isobutyl or isopentyl bridge to one syncarpic acid. Callistenones L and M possessed an intriguing bis-furan acylphloroglucinol core. All the isolated compounds were tested for their cytotoxic activity, but showed no significant activity ($IC_{50}$ > 50 μM), against tumor cell lines (HepG-2, U2-OS and MCF-7). In addition, only compound **8** showed antibacterial activity against *S. aureus* with MIC value of 20.3 μg/mL and *E. coli* with MIC value of 15.6 μg/mL. Herein, the isolation, structural elucidation and biological activity of these compounds are reported.

## 2. Results and discussion

Compound **1** was obtained as a white powder. The HRESIMS spectrum showed a pseudomolecular ion peak at *m/z* 469.2235 [M-H]$^-$ (calcd. 469.2232) corresponding to a molecular formula of $C_{27}H_{34}O_7$ with 11 ° of unsaturation. The $^{13}C$ NMR and HSQC spectra showed three carbonyls ($\delta_C$ 198.4; 211.3; 203.9), one methylene ($\delta_C$ 51.7), two methines ($\delta_C$ 26.1; 35.5) and nine methyls ($\delta_C$ 24.2; 23.1; 26.1; 24.4; 22.8; 22.9; 15.8; 15.8; 7.6). The NMR spectra suggested **1** was a hybrid of $\beta$-triketone derivative and acylphloroglucinol. The most deshielded signal at $\delta_C$ 203.9 (C-1′) belonged to the carbonyl of an isovaleryl group (C-1′ – C-5′) linked to a phloroglucinol skeleton. The resonances of four singlet methyl groups at $\delta_H$ 1.51 (Me-10), 1.41 (Me-11), 1.41 (Me-13) and 1.34 (Me-12) in the $^1H$ NMR spectrum (Table 1) and the HMBC correlations of Me-12 with C-3, Me-11 with C-3 and C-1 indicated the presence of $\beta$-triketone moiety. The phloroglucinol moiety was combined to a $\beta$-triketone moiety via a bisfuran fused-ring bearing the isopropyl group, which was confirmed by the HMBC correlations (Fig. 2) of H-9 to C-4a, C-4b, C-8, C-8a, C-9a and C-1 as well as of Me-3″ to C-1″ ($\delta_C$ 129.2). The structural features of **1** were similar to those of rhodomyrtosone A, except for the aromatic proton in rhodomyrtosone A replaced by a methyl group ($\delta_H$ 2.05, s, Me-7) (Hiranrat and Mahabusarakam, 2008). This was supported by the HMBC correlations of 7-Me to C-8, C-7 and C-6. Moreover, the correlation between H-9 and H-2″ in the ROESY experiment provided the assignment of a *cis* relative stereochemistry.

Compound **2** had the same molecular formula $C_{27}H_{34}O_7$ as compound **1**, as established from HRESIMS. The NMR data revealed that compound **2** had similar planar structure as **1** with the difference being the positions of the isovaleryl group and the methyl group. Compound **2**

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**Table 1**
$^{1}$H (500 MHz) and $^{13}$C (125 MHz) NMR Spectroscopic Data for **1** and **2** in $CDCl_3$.

| No. | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ |
| 1 | | 198.4 | | 198.7 |
| 2 | | 55.2 | | 55.1 |
| 3 | | 211.3 | | 211.4 |
| 4 | | 45.7 | | 45.8 |
| 4a | | 179.8 | | 179.8 |
| 4b | | 157.5 | | 161.5 |
| 5 | | 101.4 | | 100.3 |
| 6 | | 164.1 | | 166.2 |
| 7 | | 107.4 | | 106.9 |
| 8 | | 157.6 | | 154.2 |
| 8a | | 103.6 | | 102.9 |
| 9a | | 113.4 | | 113.4 |
| 9 | 4.50, s | 45.4 | 4.49, s | 45.5 |
| 10 | 1.34, s | 23.1 | 1.35, s | 23.6 |
| 11 | 1.41, s | 26.1 | 1.41, s | 25.8 |
| 12 | 1.41, s | 24.4 | 1.43, s | 24.4 |
| 13 | 1.51, s | 24.2 | 1.50, s | 24.4 |
| 1′ | | 203.9 | | 206.6 |
| 2′ | 2.98, dd (14.0, 6.5) | 51.7 | 3.09, dd (15.0, 7.0) | 53.1 |
| | 2.76, dd (14.0, 6.5) | | 2.97, dd (15.0, 7.0) | |
| 3′ | 2.17, m | 26.1 | 2.25, m | 25.4 |
| 4′ | 1.00, d (7.0) | 22.8 | 0.99, d (7.0) | 22.9 |
| 5′ | 0.98, d (7.0) | 22.9 | 0.97, d (7.0) | 23.0 |
| 1” | | 129.2 | | 129.0 |
| 2” | 2.38, m | 35.5 | 2.40, m | 35.4 |
| 3” | 1.09, d (7.0) | 15.8 | 1.06, d (7.0) | 15.7 |
| 4” | 1.08, d (7.0) | 15.8 | 1.03, d (7.0) | 15.7 |
| 6-OH | 13.58, s | | 14.45, s | |
| 8-OH | 9.82, s | | 9.98, s | |
| 7-Me | 2.05, s | 7.6 | | |
| 5-Me | | | 2.04, s | 7.9 |

was therefore proposed to be a regioisomer of **1** (Gervais et al., 2015). The HMBC correlations (Fig. 2) from 5-Me ($\delta_H$ 2.04, s) to C-5 ($\delta_C$ 100.3), C-4b ($\delta_C$ 161.5), C-6 ($\delta_C$ 166.2) and that of H-9 ($\delta_H$ 4.49, s) to C-4b ($\delta_C$ 161.5), C-8a ($\delta_C$ 102.9), C-8 ($\delta_C$ 154.2) were consistent with the methyl group being bonded to C-5 and the isovaleryl group attached at C-7. The NOE enhancements between H-9 ($\delta_H$ 4.49, s) and H-2″ ($\delta_H$ 2.40, m) indicated that H-9 and the isopropyl protons were oriented on the same side. Thus, the structure of compound **2** was determined as shown in Fig. 1. Furthermore, compounds **1** and **2** were isolated as

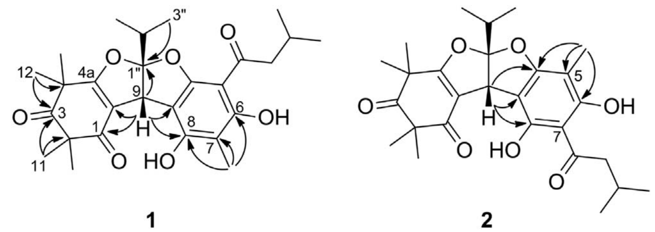


**Fig. 2.** Key HMBC correlations of compounds **1** and **2**.

racemates respectively, which was verified by a Chiral Lux 5 u Cellulose-2 column (250 × 4.6 mm) (Fig. S3).

Compound **3** was obtained as a colorless gum. Its deprotonated molecule peak at *m/z* 441.2284 [M-H]$^-$ in the HRESIMS spectrum corresponded to a molecular formula of $C_{26}H_{34}O_6$ with 10° of unsaturation. The $^{1}$H NMR spectrum of **3** showed signals for a chelated hydroxyl group ($\delta_H$ 13.69, 6-OH), an aromatic proton ($\delta_H$ 6.31, H-7), a methoxy group ($\delta_H$ 3.86, 8-OMe), a disubstituted isobutyl group ($\delta_H$ 4.30, d, $J$ = 4.0, H-9; 1.85, m, H-1″; 0.76, d, $J$ = 7.0, Me-2″ and 0.74, d, $J$ = 7.0, Me-3″), a 2-methylbutyry group ($\delta_H$ 3.78, m, H-2′; 1.97, m, H-3′; 1.50, m, H-3′; 0.98, t, $J$ = 7.0, Me-4′ and 1.22, d, $J$ = 7.0, Me-5′) and four singlet methyl groups of a *β*-triketone moiety ($\delta_H$ 1.43, s, Me-12; 1.37, s, Me-13; 1.44, s, Me-11; 1.61, s, Me-10). HMBC correlations from H-9 to C-8b and C-8a indicated that **3** was a hybrid of acylphloroglucinol and *β*-triketone, and they were connected via an isobutyl bridge, which was similar with that of callistenone B (Rattanaburi et al., 2013). The differences between **1** and callistenone B were that the isovaleryl group and 8-OH in callistenone B were replaced by 2-methylbutyry group and 8-OMe, respectively. This difference was confirmed by the HMBC correlations of Me-5′ ($\delta_H$ 1.22, d, $J$ = 7.0) with C-2′ ($\delta_C$ 46.9) and C-1′ ($\delta_C$ 208.8) and the correlations of 8-OMe ($\delta_H$ 3.86, s) with C-8 ($\delta_C$ 162.4) and C-7 ($\delta_C$ 96.8). The absolute configurations of **3** was determined by compared the CD spectrum with that of myrtucommulone B (Hans et al., 2015). The CD spectrum of **3** (Supporting information, Fig. S1.6) displayed a positive Cotton-effect at 313 nm ($\Delta\varepsilon$ +6.7), confirming the (9*R*) absolute configuration for **3**. Thus, compound **3** was assigned as 6-hydroxy-8-methoxy-9*R*-isopropyl-2,2,4,4-tetramethyl-5-(2-methylbutyryl)-4,9-dihydroxanthene-1,3-dione.

The HRESIMS of compound **4** indicated a molecular formula of $C_{25}H_{32}O_6$ (*m/z* 427.2124 [M-H]$^-$, calcd for 427.2126). Detailed

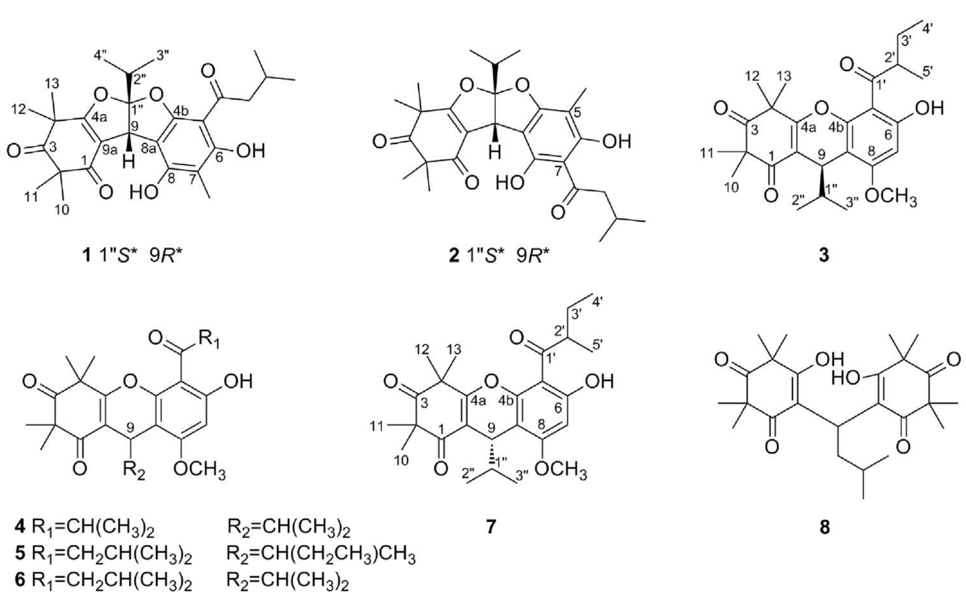


**Fig. 1.** Structures of compounds **1–8**.