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Acylphloroglucinols from *Callistemon lanceolatus* DC.

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ABSTRACT

Five acylphloroglucinols, named callistenones A–E together with six known acylphloroglucinols, triterpenoids, and *C*-methylflavonoids were isolated from the leaves of *Callistemon lanceolatus*. Their structures were characterized by spectroscopic methods. Some of the compounds showed very strong antibacterial activity.

1. Introduction

Infectious diseases are a major problem worldwide because of the emergence of resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA). *S. aureus* can cause a wide range of diseases from skin infections, hospital-acquired bacteremia to life-threatening infections such as necrotizing fasciitis and pneumonia, septic arthritis, osteomyelitis, and endocarditis. MRSA now has emerged as a widespread cause of community infection. There is a concern because MRSA is becoming resistant to multiple antibiotics.[1] Therefore, there is a need to find new antimicrobial agents to combat this pathogen. Natural products isolated from plants are an important source for the discovery of new antimicrobial substances. We have screened extracts from some Myrtaceae plants collected in Thailand for antibacterial activity and found that a crude extract from the leaves of *Callistemon lanceolatus* DC. (Myrtaceae), which is known as crimson bottlebrush, was very effective at inhibiting the growth of *S. aureus*. This prompted us to identify the active constituents from the leaves of *C. lanceolatus*.

2. Result and discussion

The CH_2Cl_2 extract of the leaves of *C. lanceolatus* was tested for antibacterial and antifungal activities. The results showed that the extract was growth inhibitory against *S. aureus* ATTC25923, and MRSA SK1 with an MIC of 4 μg/mL for both strains, but showed no effect against *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, *Callistemon neoformans* ATCC90113, *Callistemon albicans* NCPF3153, and *Microsporum gypseum* at a concentration of 200 μg/mL. Investigation of the active compounds present in the CH_2Cl_2 extract has resulted in the isolation of 11 compounds. On the basis of ^1H, ^{13}C NMR, COSY, HMQC, and HMBC spectra as well as by comparison with previous reports, callistenones A–E (**1**–**5**) were identified as new acylphloroglucinol derivatives while the remaining six compounds were found to be the known compounds, flavesone (**6**),[2] leptospermone (**7**),[2] rhodomyrtosone D (**8**),[3] endoperoxide G3 (**9**),[4] betulinaldehyde (**10**),[5] and 5-hydroxy-7,4′-dimethoxy-6,8-dimethylflavone (**11**).[6] This is the first time that compounds **6**–**9** have been isolated from *C. lanceolatus* (Fig. 1).

Callistenone A (**1**) was obtained as a yellowish gum. A molecular ion peak $[M+H]^+$ at *m/z* 429.2270 in an HRFABMS represents $C_{25}H_{33}O_6$ corresponded to a molecular formula of $C_{25}H_{32}O_6$. The EIMS spectrum of **1** exhibited a base peak at *m/z* 385.0 $[M-CH(CH_3)_2]$, which suggested facile loss of an isopropyl group from the molecule. The IR spectrum exhibited stretching bands for

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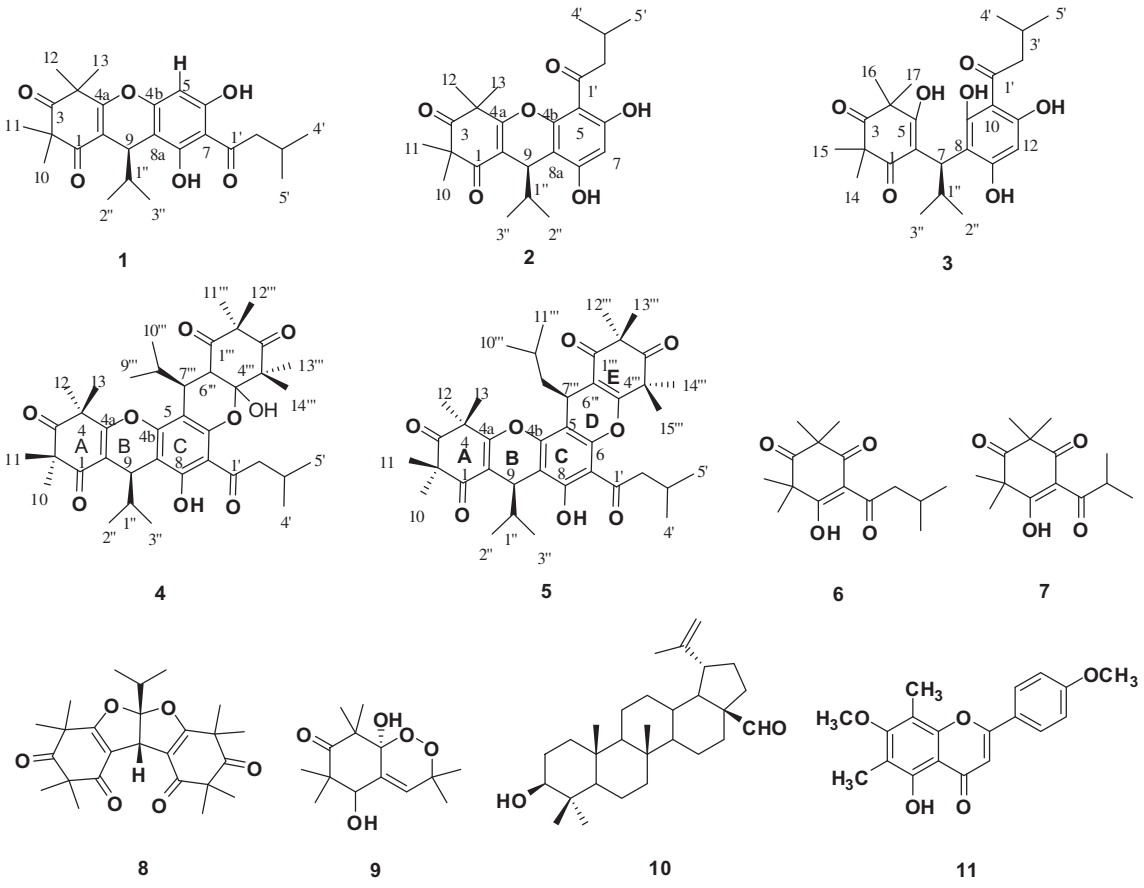

Fig. 1. Structures of compounds **1**–**11** from *C. lanceolatus*.

hydroxyl, saturated carbonyl, and unsaturated carbonyl groups at 3265, 1716, and 1624 cm^{-1}, respectively. The presence of carbonyl groups was also deduced from the carbon resonances at δ_C 207.6 (C-1′), 197.9 (C-1), and 212.6 (C-3) in the ^{13}C NMR spectrum (Table 1). In addition the ^{13}C NMR spectrum contained signals for 8 sp^2 hybridized carbons between δ_C 94.2 and 168.5 and 14 aliphatic carbons upfield of δ_C 56.6. The ^1H NMR spectrum (Table 1) showed resonances of four tertiary methyl groups at δ_H 1.37 (H_3-10), 1.31 (H_3-11), 1.57 (H_3-12), and 1.43 (H_3-13). In the HMBC spectrum, H_3-10 and H_3-11 showed HMBC correlations to carbonyl carbons C-1 (δ_C 197.9) and C-3 (δ_C 212.6) whereas H_3-12 and H_3-13 showed correlations to the carbonyl carbon C-3 (δ_C 212.6) and an oxygenated vinylic carbon C-4a (δ_C 168.5). These data indicated the presence of a β-triketone moiety.[3] The presence of a di-*C*-substituted phloroglucinol moiety was deduced from HMBC correlations observed from an aromatic proton (δ_H 6.34, s, H-5) to two oxygenated aromatic carbons at δ_C 158.0 (C-4b) and δ_C 164.0 (C-6) and two upfield non-protonated sp^2 carbons at δ_C 108.5 (C-7) and δ_C 104.8 (C-8a), and from a phenolic hydroxyl proton (δ_H 14.22, 8-OH) also to the two upfield non-protonated sp^2 carbons C-7 and C-8a in addition to an oxygenated aromatic carbon at δ_C 160.9 (C-8). COSY correlations between two equivalent secondary methyl protons H_3-4′ and H_3-5′ (δ_H 0.96, d, J=6.6 Hz) and a methine proton H-3′ (δ_H 2.22–2.30, m) and further correlations from H-3′ to methylene protons H_2-2′ (δ_H 3.99 dd, J=13.6, 6.6 Hz and δ_H 3.07, dd, J=13.6, 6.6 Hz) in addition to HMBC correlations from H_2-2′ and H-3′ to the carbonyl carbon at δ_C 207.6 indicated that an isovaleryl group was present in the molecule. Since the phenolic proton (8-OH) resonated significantly downfield it must be strongly intramolecular hydrogen bonded suggesting that the isovaleryl group was attached to the carbon *ortho* to it.[7] COSY correlations between the resonances at δ_H 4.26 (d, J=3.6 Hz, H-9), 1.92–2.01 (m, H-1″), 0.77 (d, J=6.6 Hz, H_3-2″), and 0.75 (d, J=6.6 Hz, H_3-3″) were in agreement with the presence of an isobutyl group. This group was attached to both the β-triketone and phloroglucinol moieties since HMBC correlations were observed from H-9 to C-1 (δ_C 197.9), C-8 (δ_C 160.9), C-8a (δ_C 104.8), C-9a (δ_C 112.6), C-4a (δ_C 168.5), and C-4b (δ_C 158.0). Finally, the molecular formula for **1** dictated that an ether linkage was present between C-4a and C-4b. Thus callistenone A was assigned as 6,8-dihydroxy-9-isopropyl-2,2,4,4-tetramethyl-7-(3-methylbutyryl)-4,9-dihydroxanthene-1,3-dione. The absolute configuration at C-9 remains unassigned in **1**.

Callistenone B (**2**) was a yellowish gum. Compound **2** had molecular formula $C_{25}H_{32}O_6$, as derived from the ion peak at m/z 429.2285 $[M+H]^+$ by HRFABMS and other spectroscopic data (UV, IR, MS, ^1H NMR, and ^{13}C NMR) were very similar to those of **1** with the only significant difference being the non-equivalent methylene signals of the isovaleryl group (δ_H 3.27, H-2′a and δ_H 3.01, H-2′b for **2**; δ_H 3.07, H-2′a and δ_H 2.99, H-2′b for **1**). Callistenone B was therefore proposed to be a regioisomer of **1**. HMBC correlations (Table 1) from H-7 to C-5 (δ_C 104.7), C-6 (δ_C 164.2), C-8 (δ_C 161.0), C-8a (δ_C 103.9), and that of 6-OH to C-5 (δ_C 104.7), C-6 (δ_C 164.2), C-8a (δ_C 103.9) were consistent with the aromatic proton being bonded to C-7 and the isovaleryl group attached at C-5. Therefore 5-(3-methylbutanoyl)-6,8-dihydroxy-9-isopropyl-2,2,4,4-tetramethyl-2*H*-xanthene-1,3(4*H*,9*H*)-dione was assigned for callistenone B (**2**).

Callistenone C (**3**) was a yellowish gum. Its molecular ion $[M]^+$ in the HREIMS at m/z 446.2305 corresponded to the molecular formula of $C_{25}H_{34}O_7$. The IR spectrum showed absorption bands for a conjugated carbonyl group at 1652 cm^{-1}, a non-conjugated carbonyl group at 1715 cm^{-1}, and a hydroxyl group at 3163 cm^{-1}. Its ^1H and ^{13}C NMR spectra in $CDCl_3$ (Table 2) showed doubling of all