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Isolation and biomimetic synthesis of (±)-calliviminones A and B, two novel Diels–Alder adducts, from *Callistemon viminalis*

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ABSTRACT

(±)-Calliviminones A (**1**) and B (**2**), two Diels–Alder adducts of polymethylated phloroglucinol and myrcene with unprecedented spiro-[5.5] undecene skeleton, were isolated from the fruits of *Callistemon viminalis*. Structural elucidation was accomplished by NMR spectra studies, and the biomimetic synthesis of compounds **1** and **2** confirmed the pivotal role of Diels–Alder reaction in the plausible biosynthetic pathway. Compounds **1** and **2** were also the first example of Carbon Diels–Alder adducts between phloroglucinol and terpene. Bioactivity scan indicated that **1** and **2** showed moderate inhibition on NO production on lipopolysaccharide-induced RAW264.7 macrophages.

Naturally occurring adducts of acylphloroglucinol and terpene constitute a large family of natural products that display diverse molecular architectures and a wide range of biological profiles, such as antibacterial, insecticidal and antiproliferative properties.[1–3] *Callistemon viminalis* (Myrtaceae) is a shrub native to Australia, which has also been cultivated in the south of China and used for treatment of cold and arthralgia in Chinese folk medicine.[4] Previous researches indicated that polymethylated phloroglucinols are prominent secondary metabolites of the genus *Callistemon*.[5,6]

As a part of our research program to discover natural products with a unique molecular architecture in Myrtaceae family, two novel adducts of polymethylated phloroglucinol and acyclic monoterpene (myrcene) possessed an unprecedented spiro-[5.5] undecene skeleton, named (±)-calliviminones A and B, were isolated from the fruits of *C. viminalis* collected in Guangdong province of China. The unique spiro skeleton was likely formed via a [4+2]-cycloaddition between $\Delta^{6(7)}$ of isobutyl syncarpic acid moiety and $\Delta^{1'(2')}$, $^{3'(4')}$ of myrcene unit in **1**, and those were between $\Delta^{6(7)}$ and $\Delta^{4'(3')}$, $^{2'(1')}$ in **2**, which were the first example of Diels–Alder adducts between phloroglucinol and terpene units in Carbon Diels–Alder manner. Both **1** and **2** were synthesized successfully based on the hypothesis of biosynthetic pathway, which confirmed the pivotal role of Diels–Alder reaction in the biosynthetic pathway. Bioactivity screen showed that compounds **1** and **2** had inhibition on nitric oxide (NO) production on lipopolysaccharide-induced RAW264.7 macrophages with IC_{50} of 29.9 and 28.8 μM, respectively. Herein, we reported the isolation, structural elucidation, biomimetic synthesis, and inhibitory activities on NO production.

Compound **1** was isolated as colorless gum, and its molecular formula was determined to be $C_{24}H_{36}O_3$ by its $[M+H]^+$ quasi-molecular ion at *m/z* 373.2739 (calcd for $C_{24}H_{37}O_3$, 373.2737) in the HRESIMS. The absorption bands at 1699 and 1645 cm^{-1} in IR spectrum indicated the presence of carbonyl and doubled bond groups. Consideration of the ^1H and ^{13}C NMR spectra in conjunction with information from HSQC spectrum, the presence of an isobutyl syncarpic acid unit could be approved by the following characteristic resonance: δ_H 1.41, 1.39, 1.38, 1.34, 1.68, 2.18, 0.88, 0.83; δ_C 213.0, 208.8, 208.7, 67.1, 56.9, 56.6, 41.4, 30.2, 26.4, 26.1, 25.2, 24.9, 24.2, 19.2.[6,7] This conclusion was also confirmed by the key HMBC correlations (Fig. 2) of H-8 (δ_H 1.68) with C-9 (δ_C 24.2) and C-7 (δ_C 41.4); H-7 (δ_H 2.18) with C-6 (δ_C 67.1); Me-13 (δ_H 1.39) with C-1 (δ_C 208.7) and C-6 (δ_C 67.1) and Me-12 (δ_H 1.38) with C-5 (δ_C 208.8) and C-6 (δ_C 67.1). The remaining 10 carbon atoms were classified to be two methyl groups, four methylenes, and four olefinic carbons based on the HSQC correlations, which may formed a monoterpene moiety. Considering the 24

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carbon atoms and 7° of unsaturation in the structure of **1**, together with the number of methyls, it could be speculated that compound **1** was an adduct of polymethylated phloroglucinol with monoterpene.

The presence of an isopentene moiety (C-6′ to C-10′) was determined by the cross peaks between H-7′ (δ_H 5.05) and C-9′ (δ_C 25.8) and C-10′ (δ_C 17.8); H-6′ (δ_H 2.07) and C-5′ (δ_C 37.2) and C-7′ (δ_C 124.3) in the HMBC spectrum. The fragments of C-3′ to C-6′ and C-1′ to C-4′ were established by the ^{1}H–^{1}H COSY and the HMBC spectra (Fig. 2), which indicated that the monoterpene moiety of **1** possessed myrcene skeleton. The key HMBC correlations between these two parts, H-1′α (δ_H 2.48) to C-6 (δ_C 67.1) and C-1 (δ_C 208.7), H-7 (δ_H 2.18) to C-4′ (δ_C 28.0) and C-3′ (δ_C 138.4), indicated that the isobutyl syncarpic acid and the myrcene moieties were connected through C-1′ to C-6 and C-4′ to C-7 bonds, which was also confirmed by the correlations of H-7 with H-4′ and of H-1′ with H-2′ in the ^{1}H–^{1}H COSY spectrum (Fig. 2). Thus, the planar structure of **1** was demonstrated as shown in Figure 1, which possessed spiro-[5.5] undecene skeleton constructed of an isobutyl syncarpic acid and a myrcene moieties.

Due to the optical rotation measured to be zero, **1** was a racemic mixture of enantiomers with the stereogenic center at C-7 and the relative configuration of compound **1** was established through ROESY spectrum (Fig. 2). Cross peaks from Me-10 to Me-12 and Me-9 to Me-13 indicated that the above protons were on the same side and H-7 was located at opposite side and adopted as β-configuration. The ROESY correlation from H-1′α (δ_H 2.48) to H-4′α (δ_H 2.23) and Me-14 indicated that H-1′α (δ_H 2.48) was present on the underside of the cyclohexene ring and adopted as α-configuration. That was also confirmed by the obviously downfield shift of H-1′α affected by the negative shielding effect of the olefinic bond and two carbonyls at C-1 and C-5. Aforementioned information indicated that the cyclohexene ring adopted a half chair conformation.

Compound **2**, colorless gum, has the same molecular formula as that of **1** established by HRESIMS. Similar NMR data (Table 1),

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** and **2** in $CDCl_3$

No.	1		2	
	δ_H (mult; *J*, Hz)	δ_C	δ_H (mult; *J*, Hz)	δ_C
1		208.7		208.9
2		56.6		56.4
3		213		213.2
4		56.9		56.3
5		208.8		208.9
6		67.1		68.8
7	2.18, m[a]	41.4	2.07, m[a]	40.5
8	1.68, m[a]	30.2	1.58, m[a]	29.7
9	0.88, d (7.0)	24.2	0.88, d (7.0)	24.3
10	0.83, d (7.0)	19.2	0.79, d (7.0)	19
11	1.41, s	26.4	1.44, s	26.3
12	1.38, s	24.9	1.38, s	24.4
13	1.39, s	25.2	1.40, s	26.1
14	1.34, s	26.1	1.33, s	26.6
1′α	2.48, dd (17.5, 3.0)	31.5	2.21, d (17.5)	24.1
1′β	2.14, m[a]		1.98, m[a]	
2′	5.26, br s	115.4	5.35, br s	119.3
3′		138.4		133.7
4′α	2.23,m[a]	28	2.49, dd (17.5, 1.5)	31.3
4′β	2.00, m[a]		2.06, m[a]	
5′	1.97, m[a]	37.2	1.98, m[a]	37.6
6′	2.07, m[a]	26.4	2.10, m[a]	26.2
7′	5.05, t (7.0)	124.3	5.07, tt (7.0, 1.5)	124.3
8′		131.6		131.7
9′	1.67, s	25.8	1.66, s	25.8
10′	1.59, s	17.8	1.59, s	17.8

[a] Singal pattern unclear due to overlapping.

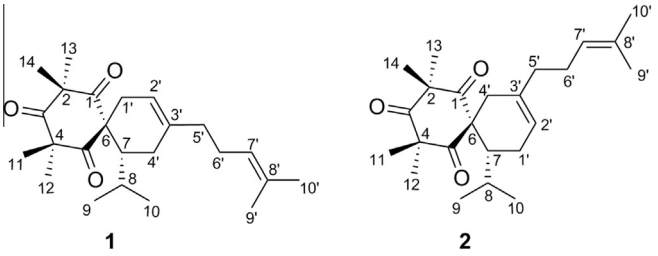


Figure 1. Structures of compounds **1** and **2**.

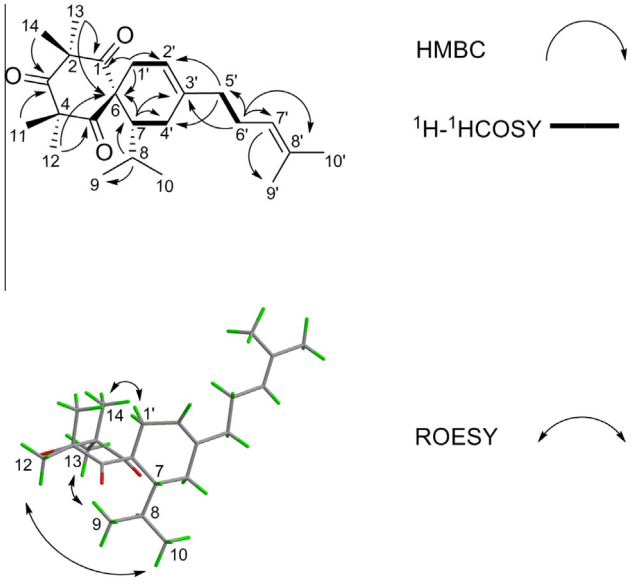
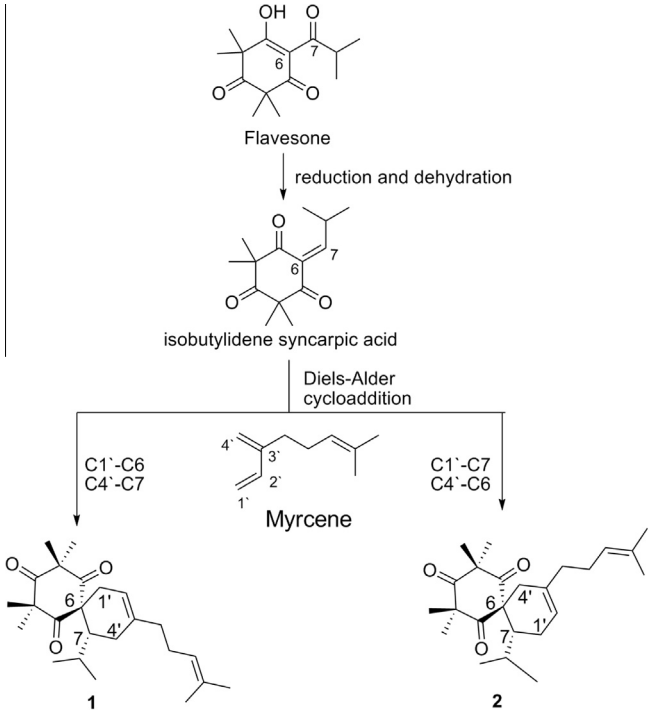


Figure 2. Key HMBC (→), ^{1}H–^{1}H COSY (—) and ROESY (↔) correlations of **1**.



Scheme 1. The plausible biogenetic pathway for **1** and **2**.