

Guajadial: An Unusual Meroterpenoid from Guava Leaves *Psidium guajava*

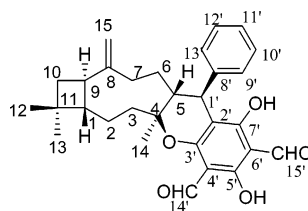
Xiao-Long Yang,^{†,§} Kun-Lung Hsieh,[‡] and Ji-Kai Liu^{*,†}

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China, HsiehS Biotech. Co., Ltd., Va. 11a, No.15 Road, Tan Thuan Export Processing Zone, District 7, Ho Chi Minh City, Vietnam, and Graduate School of Chinese Academy of Sciences, Beijing 100039, P. R. China

jkliu@mail.kib.ac.cn

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ABSTRACT



Guajadial (1)

Guajadial (1), a novel caryophyllene-based meroterpenoid, was isolated from the Leaves of *Psidium guajava* (guava). The structure and relative stereochemistry of guajadial (1) were elucidated by extensive spectroscopic analysis. A possible biosynthetic pathway for 1 was proposed.

Psidium guajava L., commonly known as guava, of the family Myrtaceae, is an indigenous medicinal plant used to control diabetes, hypertension, etc. in African and Asian folk medicine.^{1–6} Pharmacological investigations indicated that its bark, fruit, and leaves possess antimicrobial,¹ antidiabetic,^{3–5} hypoglycemic, and hypotensive,⁶ anticancer,⁷ anti-inflammatory,⁸ antiplaque,⁹ antiallergic,¹⁰ cardioprotective,¹¹ anti-

oxidant,¹² and antimutagenic activities.¹³ Previous investigations on the leaves of *P. guajava* have led to the isolation of several triterpenoids,^{2,14–19} flavonoids,^{1,20,21} tannins,²² and

* Corresponding author. Phone: 0086-871-5216327. Fax: 0086-871-5150227.

[†] Kunming Institute of Botany.

[‡] HsiehS Biotech. Co., Ltd.

[§] Graduate School of Chinese Academy of Sciences.

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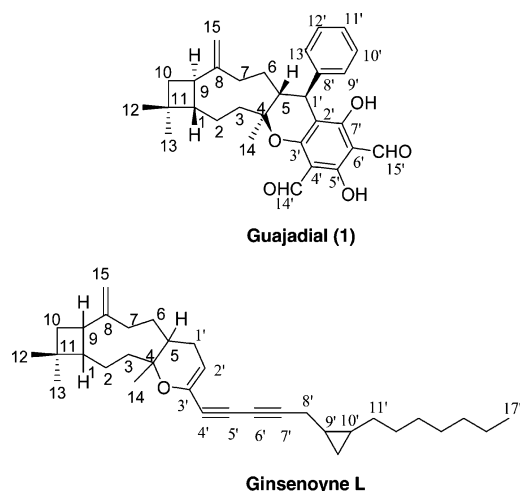
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carotenoids.²³ To find potentially bioactive secondary metabolites from *P. guajava* to appraise the ethnomedical properties, the present study was undertaken to investigate the chemical constituents of the leaves of *P. guajava* collected from south of Vietnam. A novel compound named guajadial (**1**) together with four known triterpenoids (ursolic acid,²⁴ 2 α -hydroxyursolic acid,²⁵ neriucoumaric acid,²⁶ 23-hydroxyursolic acid²⁷) and a sesquiterpene, clovandiol,²⁸ was obtained.

The air-dried powdered leaves (5 kg) of *P. guajava* were extracted with 95% EtOH (4 L \times 3) for 24 h. After removal of the solvent by evaporation, the residue (1200.0 g) was suspended in H₂O and then extracted successively with petroleum ether (1 L \times 3), ethyl acetate (1 L \times 4) and *n*-butanol (1 L \times 4). Each solvent was evaporated off under reduced pressure to yield extracts of petroleum ether (86.0 g), ethyl acetate (90.0 g), and *n*-butanol (40.0 g). The ethyl acetate (86.0 g) part was subjected to column chromatography (silica gel), eluted with petroleum ether/acetone (100:0, 98:2, 95:5, 90:10, 80:20, 50:50 (v/v)) to afford six fractions (A–F). Guajadial (**1**, 20.0 mg) was obtained from fraction B eluted with petroleum ether/acetone (100:1).

Guajadial (**1**) was obtained as white powder, $[\alpha]_D^{26} = -23.1$ (*c* 0.5, acetone). The molecular formula of **1** was determined to be C₃₀H₃₄O₅ on the basis of HR-FAB-MS⁺ (calcd for [M + H]⁺ *m/z* 475.2484; found, 475.2469) with 14 degrees of unsaturation and its ¹³C NMR (DEPT) spectrum including signals for two carbonyl carbons, two quaternary carbons, eight aromatic and olefinic quaternary carbons, five aromatic methine carbons, one olefinic methylene carbon, four methine carbons, five methylene carbons and three methyl carbons. The IR spectrum of **1** exhibited CHO (2726 cm⁻¹), double band (1633 cm⁻¹), methyl (1440 cm⁻¹), and hydroxyl group (3440 cm⁻¹, brs), respectively.



The connectivity of the protons and C-atoms was established by the ¹H, ¹³C HSQC spectrum. The cross-peaks

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between H-1 and H-2/H-9, H-2 and H-1/H-3, H-5 and H-6/H-1', H-6 and H-5/H-7, H-9 and H-1/H-10 were observed in the ¹H-¹H COSY spectrum (Figure 1). It allowed

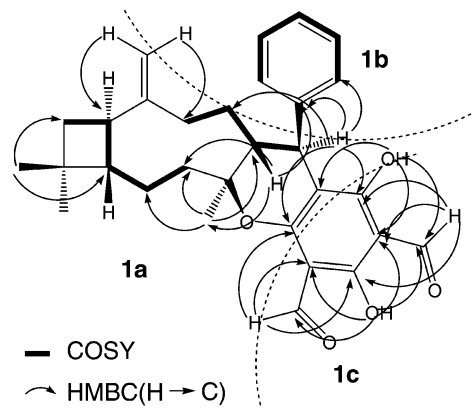


Figure 1. Fragments and key COSY and HMBC correlations of guajadial (**1**).

establishment of two H-atom systems, one at C-3 through C-2, C-1, C-9 to C-10, and the other at C-1' through C-5, C-6 to C-7.

Table 1. ¹³C NMR (100 MHz) Data for Guajadial (**1**) in CDCl₃

| no. | δ C | no. | δ C | no. | δ C | no. | δ C |
|-----|------------|-----|------------|-----|------------|-----|------------|
| 1 | 53.2 (d) | 7 | 35.4 (t) | 15 | 110.2 (t) | 8' | 143.7 (s) |
| 2 | 22.2 (t) | 8 | 150.7 (s) | 1' | 43.4 (d) | 9' | 128.7 (d) |
| 3 | 37.1 (t) | 9 | 41.3 (d) | 2' | 105.5 (s) | 10' | 127.9 (d) |
| 4 | 84.3 (s) | 10 | 36.7 (t) | 3' | 163.4 (s) | 11' | 126.2 (d) |
| 5 | 43.4 (d) | 11 | 33.8 (s) | 4' | 104.1 (s) | 12' | 127.9 (d) |
| 6 | 30.4 (t) | 12 | 30.3 (q) | 5' | 168.3 (s) | 13' | 128.7 (d) |
| | | 13 | 22.0 (q) | 6' | 104.3 (s) | 14' | 192.1 (d) |
| | | 14 | 21.2 (q) | 7' | 169.5 (s) | 15' | 191.5 (d) |

Comparison of the ¹H and ¹³C NMR data (Tables 1 and 2) of **1** with ginsenoyne L from the roots of *Panax ginseng* showed chemical shifts values from C-1 to C-15 and from C-1' to C-3' similar to those of ginsenoyne L,²⁹ which strongly suggested a similar partial structure from C-1 to C-15 and from C-1' to C-3' of both compounds. The distinct differences between them at C-1' and C-2' are the following: the chemical shifts of **1** [δ C 43.9 (d, C-1'); 106.3 (s,

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Table 2. ^1H NMR (400 MHz) Data for Guajadial (**1**) in CDCl_3

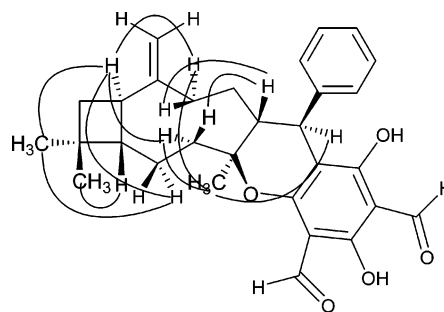
| no. | δH (J in Hz) | no. | δH (J in Hz) | no. | δH (J in Hz) |
|------------|----------------------------|-----|----------------------------|-------|----------------------------|
| 1 | 1.91 (m) | 10 | 1.55 (m) | 7' | |
| 2 α | 1.30 (m) | 11 | | 8' | |
| 2 β | 1.91 (m) | 12 | 1.03 (s) | 9' | 7.20 (m) |
| 3 α | 2.15 (m) | 13 | 1.00 (s) | 10' | 7.20 (m) |
| 3 β | 1.91 (m) | 14 | 1.29 (s) | 11' | 7.20 (m) |
| 4 | | 15 | 4.06, 4.56 (s) | 12' | 7.20 (m) |
| 5 | 2.25 (m) | 1' | 3.40 (d, 10.4) | 13' | 7.20 (m) |
| 6 | 1.55 (m) | 2' | | 14' | 10.11 (s) |
| 7 α | 1.91 (m) | 3' | | 15' | 10.09 (s) |
| 7 β | 1.65 (m) | 4' | | 3'-OH | 13.46 (s) |
| 8 | | 5' | | 5'-OH | 13.08 (s) |
| 9 | 2.35 (m) | 6' | | | |

C-2') is absent in ginsenoside L [δ_{C} 29.0 (t, C-1'); 111.2 (d, C-2')].

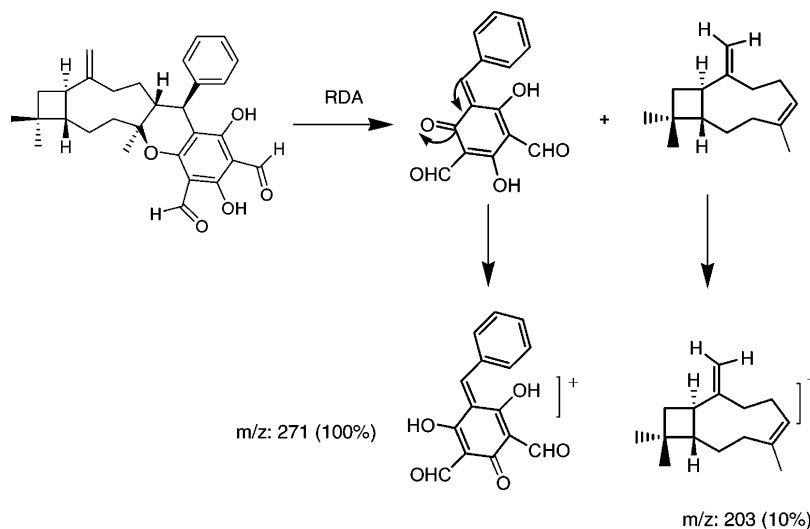
The HMBC spectra of **1** (Figure 1) demonstrated the following correlations from H-12 to C-1, C-10, C-11, and C-13, from H-13 to C-1, C-10, C-11, and C-12, from H-14 to C-2, C-3, C-4, and C-5, and from H-15 to C-1, C-6, C-7, C-8, and C-9. Finally, the partial structure of **1a** was established (Figure 1). The second partial structure of a single-substituted aromatic ring **1b** (Figure 1) was established by analysis of the ^{13}C and ^1H NMR spectra [δ_{C} 128.5 (d, $\times 2$), 127.9 (d, $\times 2$), 126.2 (d), 145.0 (s); δ_{H} 7.26 (m, 5H)]. Similarly, fragment **1c** was established by analysis of the detected correlations in HMBC experiments as following correlations: H-14' with C-3', C-4', and C-5'; H-15' with C-5', C-6', and C-7'; 5'-OH with C-4', C-5', C-6', C-14', and C-15'; 7'-OH with C-2', C-3', C-6', C-7', and C-15'. The linkages between fragments **1a**, **1b**, and **1c** were clearly detected from C-1' to C-8', from C-2' to C-7', and from C-3' to C-4' by the HMBC correlations: H-1' with C-4, C-5, C-6, C-2', C-3', C-8', C-9', and C-7'; 7'-OH with C-2' and C-3'; H-14' with C-3'. The EI-MS

spectrum of **1** showed the major fragments ion peaks at m/z 271 (100%) and 203 (10%), which may be formed through retro-Diels–Alder and rearrangement reactions of **1** (Scheme 1). The major fragment ionic formula of **1** at m/z 271 (100%) was determined to be $\text{C}_{15}\text{H}_{11}\text{O}_5^+$ on the basis of HR-FAB-MS $^+$ (calcd for $[\text{M}]^+$ m/z 271.0606; found, 271.0619), which further confirmed the presence of fragments **1b** and **1c**. In light of the evidence mentioned above, the planar structure of **1** was finally established (Figure 1).

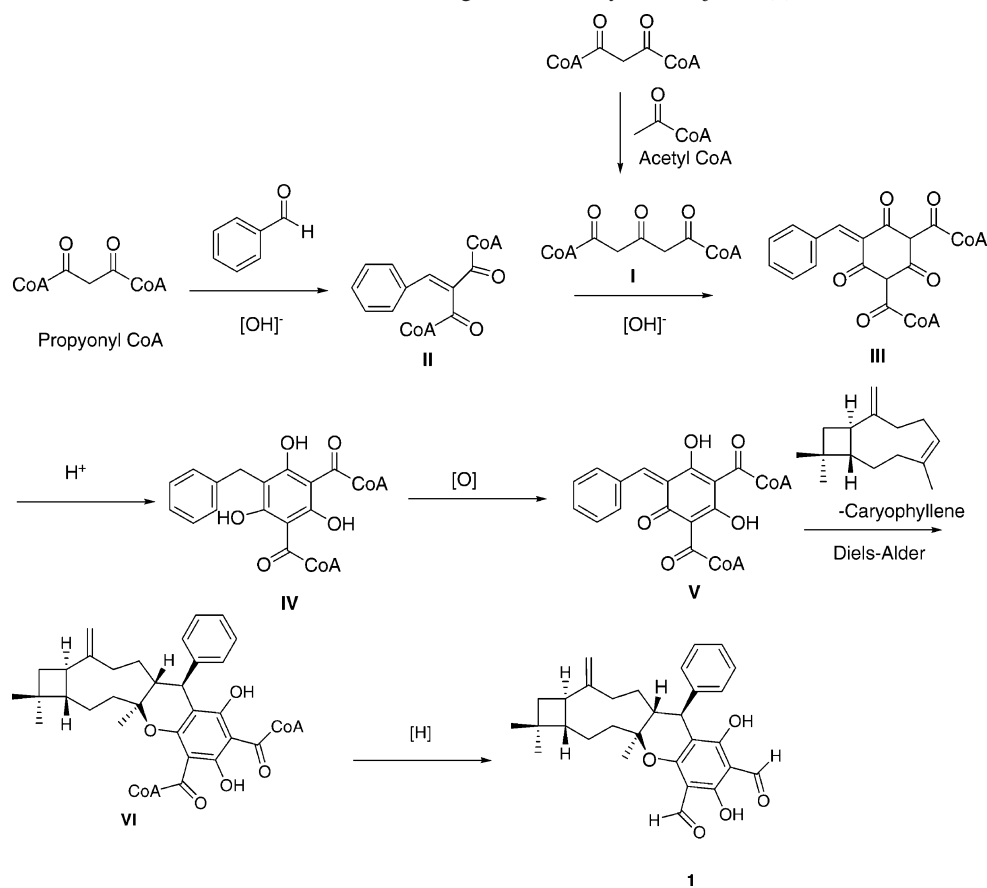
The relative configurations of all of the chiral centers of guajadial (**1**) were elucidated by analysis of the ROESY data, proton coupling constants, and chemical shifts with β -caryophyllene.³⁰ The same relative stereochemistry of C-1 and C-9 in **1** as in β -caryophyllene were deduced from the similar carbon and proton chemical shifts, protons coupling constants, and ROESY correlations found in **1**. To observe the NOE interactions (Figure 2) between H-9, H-2 α and H-3 α

**Figure 2.** Selected ROESY correlations exhibited by guajadial (**1**).

and H-7 α ; between H-14, H-3 α , H-1' and H-7 α indicated the H-1' and methyl group at C-4 should be α -oriented. However, no NOE correlation peaks were observed between

Scheme 1. Possible Formation for Major Fragments of Guajadial (**1**) in EI-MS Spectrum

Scheme 2. Possible Biogenetic Pathway for Guajadial (**1**)



H-14 and H-5, while observed NOE interactions between H-5, H-3 β , and H-7 β indicated that the H-5 is β -oriented. The configuration H-1' was also supported by the coupling constant value ($J_{5,1'} = 10.4$ Hz) (Figure 2). We also propose a biogenetic pathway to account for the plausible formation of **1** (Scheme 2).

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Supporting Information Available: Detailed description of the experimental procedures, copies of the NMR (1D and 2D), and MS spectra of guajadial (**1**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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