

Isolation and Biomimetic Synthesis of (\pm)-Guajadial B, a Novel Meroterpenoid from *Psidium guajava*

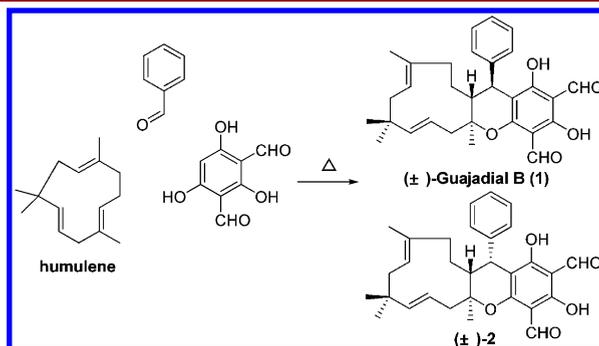
Yuan Gao,^{†,‡,§} Gang-Qiang Wang,[†] Kun Wei,[†] Ping Hai,[‡] Fei Wang,^{†,‡} and Ji-Kai Liu^{*,†}

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China, BioBioPha Co. Ltd., Kunming 650201, People's Republic of China, and Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

jkliu@mail.kib.ac.cn

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ABSTRACT



(\pm)-Guajadial B (**1**), an unusual humulene-based meroterpenoid, was isolated as a racemate from the leaves of *Psidium guajava*, collected from Vietnam. The structure of this novel secondary metabolite was established on the basis of extensive analysis of NMR spectra and confirmed by biomimetic synthesis in a domino three-component coupling reaction.

Psidium guajava L. (Myrtaceae) is an evergreen shrub grown in tropical and subtropical regions as a food and is also an indigenous medicinal plant used for the treatment of inflammatory, diabetes, hypertension, wounds, pain, fever, vomiting, and diarrhea.¹ Our previous studies on the leaves of *P. guajava* led to the isolation of a rare caryophyllene-based meroterpenoid guajadial.² Subsequently, several structurally novel merosessquiterpenoids were

isolated from the same plant.^{3,4} Recent studies by Thompson et al. have verified the proposed biomimetic approach of guajadial via a hetero-Diels–Alder reaction between caryophyllene and an *o*-quinone methide conducted in water as a universal biological solvent.⁵ Our efforts in this area have been continuing, and we would now like to report the isolation, structure elucidation, and biomimetic synthesis of another unusual humulene-based meroterpenoid (\pm)-guajadial B (**1**) from *P. guajava*.

Leaves of *P. guajava* (15 kg) were collected from south of Vietnam and extracted with MeOH at rt. After filtration, the methanolic extract was evaporated under reduced pressure to obtain a residue (ca. 1400 g), which was fractionized by silica gel column chromatography using petroleum ether containing an increasing amount of acetone. Successive purification of 2.5 g of the fraction eluted with petroleum ether/acetone (100:1) by Sephadex

[†] Kunming Institute of Botany.

[‡] BioBioPha Co. Ltd.

[§] Graduate University of Chinese Academy of Sciences.

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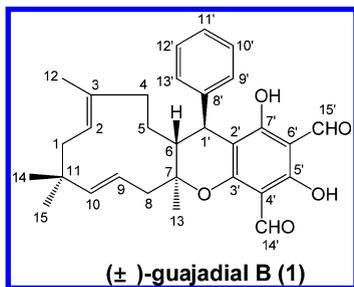
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LH-20 (CHCl₃/MeOH, 1:1) and preparative HPLC on a ZORBAX SB-C18 column (90%→95% CH₃CN in H₂O and 0.1% formic acid over 5 min followed by 95% CH₃CN in H₂O and 0.1% formic acid to 17 min, 20 mL/min, 280 nm) afforded 6 mg of guajadial B (**1**, *t_R* = 9.2 min).



Compound (**±**)-**1** was obtained as an amorphous powder. The ion at *m/z* 474.2417 (calcd 474.2406) in its high-resolution EI mass spectrum gave a molecular formula of C₃₀H₃₄O₅, which was in accordance with the ¹H and ¹³C NMR data (Table 1). The IR spectrum suggested the presence of hydroxyl (3431 cm⁻¹) and conjugated carbonyl (1634 cm⁻¹) functionalities. The UV spectrum of (**±**)-**1** showed the absorption maxima at 276 and 335 (sh) nm. Analysis of the ¹H NMR data revealed signals due to two H-bonded phenolic hydroxyls at δ_H 13.48 and 13.11 (each 1H, s), two aldehydes at δ_H 10.10 and 10.07 (each 1H, s), a monosubstituted benzene ring at δ_H 7.16–7.28 (5H, m), and four methyls at δ_H 1.05, 1.05, 1.23, 1.44 (each 3H, s). The ¹³C NMR (DEPT) spectrum showed a total of 30 carbon signals, including two aldehyde resonances at δ_C 192.2 (d) and 191.6 (d); signals for a monosubstituted phenyl at δ_C 128.3 (4 × d) and 126.5 (d); a set of carbons due to a hexasubstituted aromatic ring at δ_C 105.7 (s), 163.2 (s), 104.2 (s), 168.3 (s), 104.2 (s), and 169.3 (s); four methyls at δ_C 16.7 (q), 19.9 (q), 23.9 (q), and 29.8 (q); signals ascribed to two double bonds at δ_C 119.3 (d), 123.0 (d), 136.3 (s), and 143.1 (d); and eight remaining aliphatic carbons. Detailed analysis of its 2D NMR spectra and comparison of the 1D NMR data with those of (**±**)-**2** resulted in unambiguous assignment of all ¹H and ¹³C NMR signals as shown in Table 1.

In the HMBC spectrum (Figure 1), cross-peaks from H-1' to C-2', C-3', C-7', C-8', C-9', and C-13' implied the existence of a 3,5-diformylbenzyl phloroglucinol moiety (**1a**), which was confirmed by careful comparison of chemical shift values with those of guajadial,² a novel 3,5-diformylbenzyl phloroglucinol-coupled sesquiterpenoid mentioned earlier. The HSQC spectrum established the connectivity of the protons and the C-atoms, and the ¹H–¹H COSY correlations between H-1 and H-2, H-5 and H-4/H-6, and H-9 and H-8/H-10 revealed the presence of three spin systems (C-1/C-2, C-4/C-5/C-6, and C-8/C-9/C-10) (Figure 1). A series of HMBC cross-peaks from C(3)-Me to C-2, C-3, and C-4; from C(7)-Me to C-6, C-7, and C-8; and from C(11)-Me to C-10, C-11, and C-1 allowed the construction of a basic structural unit of humulene (**1b**). Significant HMBC correlations from H-1' to C-5, C-6, and C-7 and from H-6 to C-1' and C-8' confirmed that the

Table 1. ¹H and ¹³C NMR Spectroscopic Data for (**±**)-**1** in CDCl₃

no.	δ _H (J in Hz)	δ _C
1α	2.04 (dd, 12.9, 11.8)	41.3 (t)
1β	1.64 (dd, 12.9, 4.1)	
2	4.52 (dd, 11.8, 4.1)	123.0 (d)
3		136.3 (s)
4α	1.56 (dd, 13.1, 6.9)	37.4 (t)
4β	0.58 (dd, 13.1, 11.0)	
5α	1.36 (dd, 14.9, 11.0)	30.7 (t)
5β	1.42 (ddd, 14.9, 7.4, 6.9)	
6	2.15 (dd, 10.1, 7.4)	43.4 (d)
7		85.2 (s)
8α	2.36 (dd, 15.0, 9.3)	42.4 (t)
8β	2.66 (ddd, 15.0, 1.4, 1.0)	
9	5.18 (ddd, 16.0, 9.3, 1.4)	119.3 (d)
10	5.14 (dd, 16.0, 1.0)	143.1 (d)
11		38.6 (s)
12	1.44 (s)	16.7 (q)
13	1.23 (s)	19.9 (q)
14	1.05 (s)	23.9 (q)
15	1.05 (s)	29.8 (q)
1'	3.60 (d, 10.1)	44.7 (d)
2'		105.7 (s)
3'		163.2 (s)
4'		104.2 (s)
5'		168.3 (s)
6'		104.2 (s)
7'		169.3 (s)
8'		144.6 (s)
9'	7.19 (m)	128.3 (d)
10'	7.26 (m)	128.3 (d)
11'	7.19 (m)	126.5 (d)
12'	7.26 (m)	128.3 (d)
13'	7.19 (m)	128.3 (d)
14'	10.10 (s)	192.2 (d)
15'	10.07 (s)	191.6 (d)
5'-OH	13.48 (s)	
7'-OH	13.11 (s)	

3,5-diformylbenzyl phloroglucinol was coupled with a humulene moiety via a C-6–C-1' bond as evidenced by ¹H–¹H COSY cross-peaks between H-6 and H-5/H-1'. According to the molecular formula information, it is plausible to deduce that the oxygen atom leftover was to bridge C-7 at δ_C 85.2 and C-3' at δ_C 163.2, to form a six-member ring.

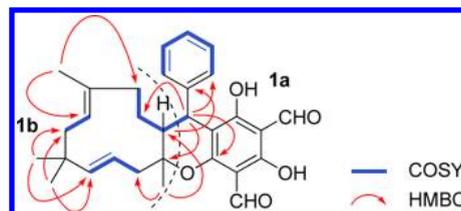


Figure 1. Key ¹H–¹H COSY and HMBC correlations of (**±**)-**1**.

The relative stereochemistry for (**±**)-**1** was established by analysis of its ROESY data (Figure 2) and proton coupling

constants. Key correlations between H-1' and H₃-13 and between H-6 and the aromatic protons revealed the α -axial orientation for H-1' and H₃-13 and β -axial orientation for H-6. These observations are in good agreement with the coupling constants ($J_{1'-6} = 10.1$ Hz) which indicated a *trans* diaxial relationship for H-1' and H-6, as well as with the chemical shift value of H-4 β at δ_{H} 0.58 (the noticeable upfield shift being due to the shielding by the aromatic ring current of 1'-phenyl). The ROESY correlations of H₃-12 \leftrightarrow H-1 α and H-2 \leftrightarrow H-4 β supported the *E*-geometry of the C-2/C-3 olefin. The *E*-geometry of C-9/C-10 olefin was consistent with the large coupling constants observed ($J_{9-10} = 16.0$ Hz) as well as with ROESY correlations (H-9 \leftrightarrow H₃-14/H₃-15 and H-10 \leftrightarrow H-8 α). In Figure 2, the 3D-presentation of (\pm)-**1** originated from the conformation with minimized energy by MM2 calculation using ChemBio3D software. Conformationally, key ROESY correlations of H-6 \leftrightarrow H-9/H-2 and H-10 \leftrightarrow H₃-12 demonstrated that H-6, H-9, and H-2 were on the same side, while H-10 and H₃-12 were on the opposite side as presented in Figure 2. This secondary metabolite occurs as a racemate, as evidenced by the lack of optical activity. The racemic nature was also supported by subsequent HPLC analysis of (\pm)-**1** on a chiral phase column, in which two distinct chromatographic peaks appeared with a ratio of 1:1.

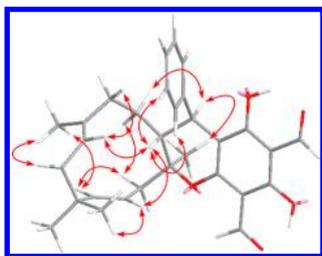


Figure 2. Key ROESY correlations of (\pm)-**1**.

To confirm our proposed structure of (\pm)-guajadial B (**1**),⁶ the biomimetic synthesis of (\pm)-**1** was designed based on a hetero-Diels–Alder reaction strategy. Our retrosynthetic analysis of (\pm)-**1** was shown in Scheme 1; a [4 + 2] cycloaddition between natural α -humulene **6** and the *o*-quinone methide **5** was enlisted to deliver (\pm)-guajadial B (**1**) in a biomimetic manner. In turn, the *o*-quinone methide **5** was envisioned to arise from a Knoevenagel condensation⁷ between diformylphloroglucinol **3** and benzaldehyde **4**. For synthetic efficiency and brevity, the preparation of the *o*-quinone methide **5** and subsequent biomimetic [4 + 2] cycloaddition could be performed in one pot.

Indeed, a similar strategy had been applied in the biomimetic synthesis of guajadial by Thompson et al.⁵

(6) (\pm)-Guajadial B (**1**): white powder; $[\alpha]_{\text{D}}^{25} 0$ (*c* 0.20, CHCl₃); UV (MeOH) λ_{max} : 276, 335 (sh) nm; IR (KBr) ν_{max} 3441, 2958, 2927, 2858, 1634, 1442, 1383, 1303, 1191, 1157, 1047, 843, 756, 699, 608 cm⁻¹; EI-MS: *m/z* 474 [M]⁺ (100), 405 (45), 391 (62), 323 (15), 309 (25), 279 (20), 271 (58), 233 (21), 167 (25), 149 (52); HR-EI-MS: *m/z* 474.2417 (calcd for C₃₀H₃₄O₅, 474.2406).

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Scheme 1. Retrosynthetic Approach to (\pm)-Guajadial B (**1**)

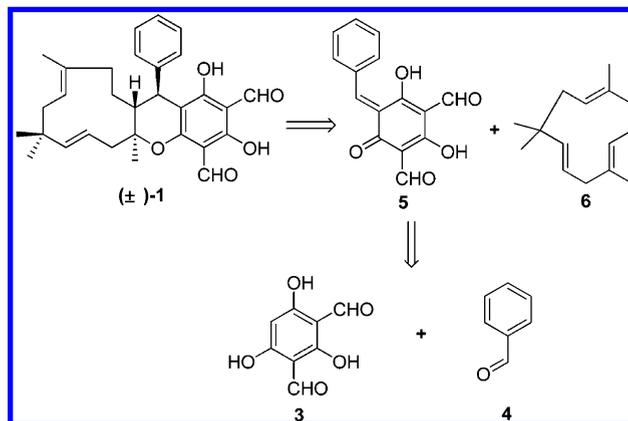
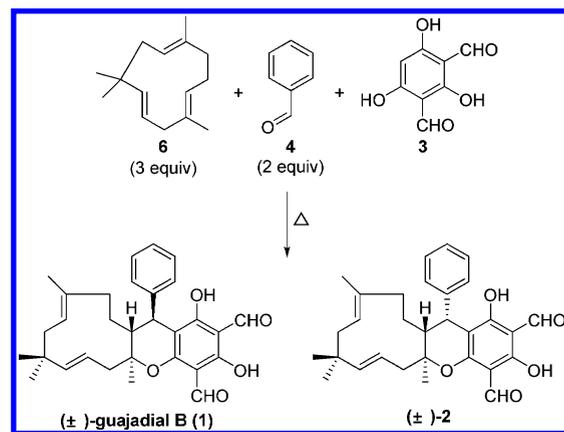


Table 2. Condition Screen for the Biomimetic Synthesis of (\pm)-Guajadial B (**1**)



entry	conditions	<i>t</i> (°C)	time	yield ^a (%)	
				1	2
1	satd. NaCl	100	24 h	24	8
2	5% PTS _(aq) ^b	100	24 h	34	11
3	5% PTS _(aq) excess 6 (10 equiv)	100	24 h	31	9
4	AcOH + NaOAc (0.1 equiv)	80	24 h	39	14
5	AcOH + NaOAc (0.1 equiv)	80	72 h	43	18
6	AcOH + NaOAc (0.1 equiv)	80	120 h	45	19

^a HPLC yields with MeOH/H₂O condition. ^b PTS: PEG-600/ α -Tocopherol-based diester of Sebacic acid.

However, the situation with the biomimetic approach is obscured by the issue that there are three endocyclic double bonds in the 11-membered sesquiterpene ring, each of which is potentially capable of reacting with *o*-quinone methide **5**. It is known, however, that the trisubstituted double bond situated furthest from the *gem*-dimethyl

group of α -humulene **6** is the more reactive alkene; due to the steric hindrance, we believe the syn-addition of *o*-quinone methide **5** must involve the trisubstituted olefin. To determine whether this strategy is chemically feasible for the synthesis of (\pm)-guajadial B (**1**), preparation of diformylphloroglucinol **3**, a precursor of *o*-quinone methide **5**, was required. The precursor **3** was readily synthesized in 82% yield by using Hintermann's conditions.⁸

With diformylphloroglucinol **3** in hand, various conditions were investigated to determine the feasibility of effecting its addition to (\pm)-**1**. Our initial attempt to synthesize (\pm)-**1** was carried out by using water as the reaction medium, and NaCl was added due to its dehydrating nature.⁹ Heating the three-compound mixture in saturated brine for 24 h afforded the desired (\pm)-guajadial B (**1**) in a 24% yield, together with another compound which was later assigned as (\pm)-**2**, an C-1' epimer of (\pm)-**1**, in 8% yield (Table 2, entry 1). Considering the unequal dispersal of the reactants in water, surfactant has been used to decrease the heterogeneity.¹⁰ In an aqueous solution of 5 wt % PEG-60/ α -Tocopherol-based diester of Sebacic acid (PTS) as a non-ionic surfactant, a better chemical yield of 45% with 11% (\pm)-**2** was achieved (Table 2, entry 2). The same procedure was applied except that 10 equiv of **6** were added, resulting in a total yield of 40% with 9% (\pm)-**2** (Table 2, entry 3). Higher efficiency in the synthesis of (\pm)-**1** was sought by using the conditions of Thompson et al.⁵ Treatment of the mixture in the presence of sodium acetate in acetic acid

under conventional heating at 80 °C for 24 h resulted in a yield of 53% with 14% (\pm)-**2** (Table 2, entry 4). Extending the heating period to 3 days gave a promising yield of 61% with 18% (\pm)-**2** (Table 2, entry 5). However, when we extended the period by an additional 2 days, no significant improvement in yield was observed at the expense of reaction time (Table 2, entry 6). Subsequent column chromatography was followed by HPLC purification of the cleanest fractions, resulting in an unoptimized 27% isolated yield of (\pm)-guajadial B (**1**), identical by NMR, TLC, and HPLC comparison with the natural product, and a 6% yield of (\pm)-**2**. Consistent with their nonenzymatic route, both products occur as racemates as evidenced by the lack of optical activity and by HPLC analyses on a chiral phase column.

In summary, we have isolated and characterized the humulene-based meroterpenoid (\pm)-guajadial B (**1**) from *P. guajava* L. We revealed that when using water as a universal biological solvent, the naturally occurring (\pm)-**1** is the favored monoadduct of α -humulene **6** with *o*-quinone methide **5** generated under nonenzymatic conditions at elevated temperatures. This offers positive evidence that a similar route may occur to provide (\pm)-**1** biosynthetically.

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Supporting Information Available. Synthetic procedures and analytic data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.

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