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Psidials A−C, Three Unusual Meroterpenoids from the Leaves of *Psidium guajava* L

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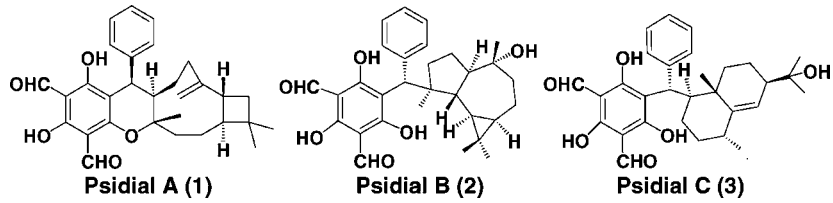
ABSTRACT



Three novel sesquiterpenoid-based meroterpenoids of psidials A−C (1−3) have been isolated from the leaves of *Psidium guajava* L. Their complete structures were elucidated by spectral and chemical methods, and that of 1 was confirmed by single-crystal X-ray diffraction analysis. Psidial B (2) and C (3) represented the new skeleton of the 3,5-diformylbenzyl phloroglucinol-coupled sesquiterpenoid. A possible biosynthetic pathway for 2−3 was postulated. 2−3 showed activity to enzyme PTP1B in 10 μM.

Psidium guajava L, a shrub or small tree, is grown throughout the tropics and subtropics and is known for its edible fruits. Its leaves are used in folk medicine as an anti-inflammatory and hemostatic agent, and for treating pulmonary diseases, coughs, vomiting, and diarrhea.[1] Previous investigations on the leaves of *Psidium guajava* L have led to the isolation of several triterpenoids,[2] flavonoids,[3] tannins,[4] and carotenoids.[5]

In our preliminary screening, the EtOAc part of the 70% EtOH extract of the leaves of *Psidium guajava* L showed significant anticancer activity.[6] One of our efforts to discover biologically significant anticancer properties from plant resources has led to the isolation of three novel compounds (psidials A−C) from the leaves of *Psidium guajava* L. Psidial A possessed the skeleton of 3,5-diformylbenzyl phloroglucinol-coupled caryophyllene.[7] Psidial B and C represented the new skeleton of the 3,5-diformylbenzyl phloroglucinol-coupled sesquiterpenoid. We report herein the isolation, structural elucidation, postulated biogenetic origin, and biological activity of **1**−**3**.

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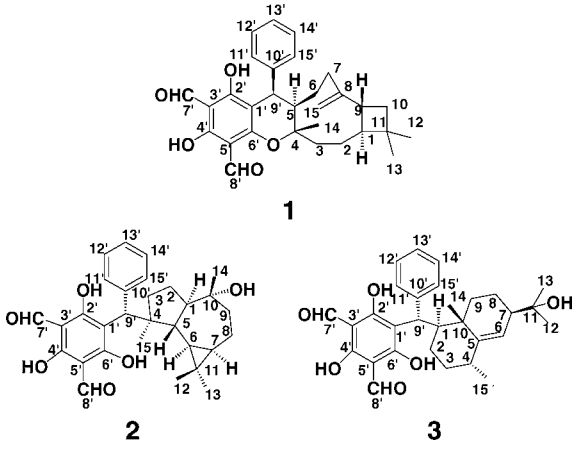
The powdered dried leaves of *Psidium guajava* L (8.0 kg) were extracted with 70% EtOH three times by heating, and the gum (2658 g) obtained by concentrating the 70% EtOH extract in vacuo was partitioned with EtOAc and *n*-BuOH successively. The EtOAc-soluble portion (386 g) was fractionated via silica gel column chromatography eluting with $CHCl_3$−MeOH (100:0 to 9:1, v/v) to give eight major fractions A_1−A_8. Fractions A_1 (30.4 g) and A_4 (6.4 g) showed anticancer activity and were separated via extensive column chromatography over silica gel, and were further purified by chromatography over sephadex LH-20 eluting with $CHCl_3$−MeOH (3:1) to afford psidial A (**1**) (80 mg), psidial B (**2**) (16 mg), and psidial C (**3**) (17 mg).



Psidial A (**1**) was obtained as a colorless flake crystal, mp 250 °C. $[\alpha]^{20}_D$ +104 (*c* 0.06, $CHCl_3$). The IR spectrum of **1** suggested the presence of a terminal double bond (3082 and 3036 cm^{-1}), conjugated carbonyl (1632 cm^{-1}), and a double bond (1604 cm^{-1}). Its maximum UV absorption at 278 nm (logε 4.31) was due to an aromatic ring. The negative ESIMS of **1** gave a quasi-molecular ion peak at *m*/*z* 473.3 $[M - H]^-$, while the positive ESIMS exhibited $[M + Na]^+$ at *m*/*z* 497.3. The HREIMS displayed the molecular ion at *m*/*z* 474.2408 (calcd 474.2406), which was consistent with a molecular formula of $C_{30}H_{34}O_5$ with 14 degrees of unsaturation. The 1D NMR data (Table 1) and HSQC spectrum of psidial A further revealed the presence of a 3,5-diformyl phloroglucinol (see Table 1), a benzyl [δ_H 7.15 (2H, d, J = 7.5 Hz), 7.26 (2H, t, J = 7.0 Hz), 7.22 (1H, t, J = 7.0 Hz), and 4.18 (1H, t, J = 5.5 Hz); δ_C 129.9 × 2, 128.0 × 2, 126.9, 138.6 and 35.0], three methyls [δ_H 0.96 (3H, s), 0.99 (3H, s), 1.17 (3H, s); δ_C 29.7, 21.7, and 22.2], and one terminal double bond [δ_H 5.01 and 5.04 (each 1H), s; δ_C 111.2 and 150.8] group. The aforementioned data implied that **1** possessed the feature of a 3,5-diformylbenzyl phloroglucinol-coupled sesquiterpenoid, the 3,5-diformylbenzyl phloroglucinol, and one double bond accounted for 11 out of 14 double bond equivalents.

Table 1. ^1H and ^{13}C NMR Spectroscopic Data of **1** in $CDCl_3$

no.	δ_H (mult, J in Hz)	δ_C	no.	δ_H (mult, J in Hz)	δ_C
1	1.48 (m)	59.3	15a	5.01 (s)	111.2
2a	1.61 (m)	23.9	15b	5.04 (s)	
2b	0.97 (m)		1′		107.5
3a	1.52 (m)	47.8	2′	13.44 (s)	168.5
3b	2.11 (dd, 14.0, 9.5)		3′		104.2
4		88.0	4′	13.09 (s)	167.5
5	2.06 (t, 8.0)	35.0	5′		104.7
6a	1.61 (m)	36.1	6′		164.1
6b	1.80 (t, 5.5)		7′	10.11 (s)	191.6
7a	2.26 (br d, 14.5)	35.5	8′	10.14 (s)	192.0
7b	2.58 (dt, 3.0, 13.5)		9′	4.18 (d, 5.5)	35.0
8		150.8	10′		138.6
9	2.43 (m)	42.2	11′	7.15 (d, 7.5)	129.9
10	1.59 (m)	24.4	12′	7.26 (t, 7.0)	128.0
11		34.7	13′	7.22 (t, 7.0)	126.9
12	0.96 (s)	29.7	14′	7.26 (t, 7.0)	128.0
13	0.99 (s)	21.7	15′	7.15 (d, 7.5)	129.9
14	1.17 (s)	22.2			

The structure of **1** was subsequently established by a comprehensive analysis of 2D NMR spectroscopy. A series of HMBC (Figure 1) correlations from H_3-12 to C-1, C-10, and C-11, from H_3-13 to C-1, C-10, and C-11 allowed the connection of C-1, C-10, C-12, and C-13 to the quaternary carbon C-11. It can be concluded that C-12 and C-13 connected with the same carbon (C-11). From H_2-15 to C-7, C-8, and C-9, from H-9 to C-1, C-2, C-7, C-8, C-10, C-11, and C-15, from H-1 to C-2, C-3, C-8, C-9, C-10, C-11, C-12, and C-13, as well as a careful comparison of chemical shift values of the above protons and carbons with those of caryophyllene, suggested that **1** contained the basic structural unit of caryophyllene. The mutual HMBC correlations of H-9′/C-1′, C-2′, C-6′, C-10′, C-11′, C-15′, C-4, and C-5, H-7′/C-2′, C-3′, and C-4′, H-8′/C-4′, C-5′, and C-6′ confirmed the presence of 3,5-diformylbenzyl phloroglucinol, which was coupled with a caryophyllene moiety via the C-5−C-9′ bond as judged by the HMBC correlations of H-5/C-9′ and H-9′/C-5. The only leftover uncertainty for the planar structure of **1** was the remaining one degree of unsaturation, which required the presence of an additional ring. Observation of the ^{13}C NMR data of the reported macrocarpals and euglobal[8,9] indicated that the aromatic C-6′ bearing a hydroxyl group (as in the cases of macrocarpals) normally appeared at δ_C 170, while the etherified aromatic C-6′ generally appeared at 163. The relatively upfield shifted C-6′ resonated at δ_C 88.0, suggesting that an ether bridge existed between C-6′ and C-4 to form a ring, so the gross structure

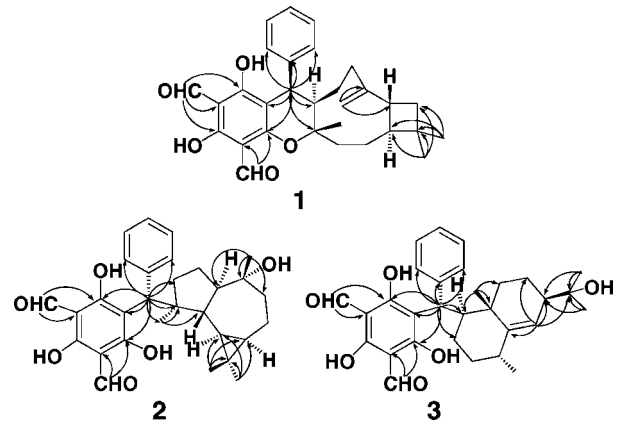


Figure 1. Key HMBC (H→C) correlations of **1**−**3**.

of **1** was established as depicted and this was finally confirmed by the performance of a single-crystal X-ray diffraction of **1** (Figure 2).

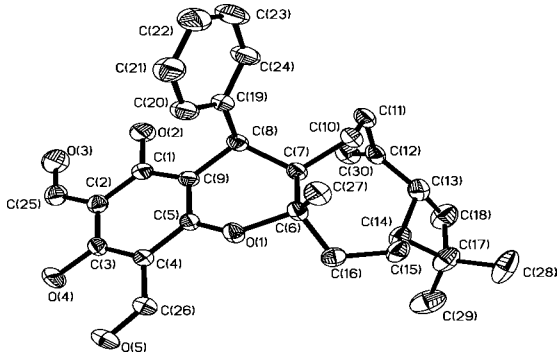


Figure 2. Single-crystal X-ray structure of **1**.

Psidial B (**2**) was obtained as an amorphous powder, mp >300 °C, $[\alpha]^{20}_{D}$ −48.9 (*c* 0.0075, MeOH). The IR spectrum of **2** exhibited a hydroxyl group (3273 cm^{-1}, br s), and a conjugated carbonyl (1622 cm^{-1}) and methyl (1448 cm^{-1}) group, respectively. The aromatic ring and conjugated carbonyl functional groups were also indicated by the UV absorption bands at λ_{max}^{MeOH}(log ε) 204 (4.61), 274 (4.47), and 390 (3.97) nm. The negative ESIMS of **2** gave a quasi-molecular ion peak at *m*/*z* 491.4 $[M - H]^-$, while the positive ESIMS exhibited $[M + Na]^+$ at *m*/*z* 515.3. The HRESIMS $[M + Na]^+$ displayed the molecular ion at *m*/*z* 515.24367 (calcd 515.24096), which was consistent with a molecular formula of $C_{30}H_{36}O_6$ with 13 degrees of unsaturation. A careful comparison of chemical shift values of **2** with those of **1** revealed that **2** contained the structural unit of a 3,5-diformyl phloroglucinol (see Table 2). The data for **2** implied that **2** possessed the feature of a 3,5-diformylbenzyl phloroglucinol-coupled sesquiterpenoid. The 3,5-diformylbenzyl phloroglucinol accounted for 10 out of 13 double bond equivalents.

The structure of **2** was subsequently established by a comprehensive analysis of 2D NMR spectroscopy. A series of HMBC (Figure 1) correlations from H_3-14 to C-1, C-9, and C-10 allowed the connection of C-1, C-9, and C-14 to the quaternary carbon C-10, from H_3-15 to C-3, C-4, and C-5 allowed the connection of C-3, C-5, and C-15 to the quaternary carbon C-4, from H_3-12 to C- 6, C-7, and C-11, from H_3-13 to C-6, C-7, and C-11 allowed the connection of C-6, C-7, C-12, and C-13 to the quaternary carbon C-11. It can be concluded that C-12 and C-13 connected with the same carbon (C-11). From H-6 to C-1, C-4, C-5, C-7, C-8, and C-11, from H-1 to C-2, C-3, C-4, C-5, C-6, and C-10, as well as a careful comparison of chemical shift values of the above protons and carbons with those of viridiflorol,[10] demonstrated that **2** contained the basic structural unit of viridiflorol. The mutual HMBC correlations of H-9′/C-3, C-4, C-5, C-15, C-1′, C-2′, C-6′, C-10′, C-11′, and C-15′ confirmed that 3,5-diformylbenzyl phloroglucinol was coupled with a viridiflorol moiety via the C-4−C-9′ bond as judged by the HMBC correlations of H-9′/C-4 and C-4 was the quaternary carbon. The aromadendran accounted for the leftover 3 out of 13 double bond equivalents. In light of the evidence mentioned above, the planar structure of **2** was finally established as depicted.

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data of **2**−**3**[a]

	2			**3**	
no.	δ_H (mult, *J* in Hz)	δ_C	no.	δ_H (mult, *J* in Hz)	δ_C
1	1.88 (m)	59.1	1	2.66 (m)	45.3
2	1.47 (m)	25.4	2a	1.23 (m)	23.6
3a	1.09 (m)	38.4	2b	1.25 (m)	
3b	1.91 (m)		3a	1.35 (m)	33.0
4		48.0	3b	1.37 (m)	
5	1.28 (m)	45.8	4	2.39 (m)	39.2
6	0.44 (m)	30.8	5		149.1
7	0.39 (m)	25.5	6	5.42 (d, 3.0)	122.7
8a	0.76 (m)	20.0	7	1.83 (m)	45.1
8b	1.62 (m)		8a	1.37 (m)	19.6
9a	1.37 (t, 12.5)	44.5	8b	1.37 (m)	
9b	1.59 (m)		9a	1.15 (m)	35.2
10		72.9	9b	1.19 (m)	
11		19.2	10		39.0
12	0.29 (s)	15.3	11		71.4
13	0.76 (s)	28.5	12	0.99 (s)	27.3
14	1.09 (s)	20.2	13	0.95 (s)	27.2
15	1.29 (s)	23.0	14	1.15 (s)	22.6
			15	1.03 (d, 7.5)	22.2
1′		108.9	1′		111.6
2′		169.6	2′		168.6
3′		105.3	3′		105.2
4′		169.6	4′		168.6
5′		105.3	5′		105.2
6′		169.6	6′		168.6
7′	9.91 (s)	191.5	7′	9.97 (s)	192.1
8′	9.94 (s)	191.8	8′	9.97 (s)	192.1
9′	4.38 (s)	48.0	9′	4.36 (d, 9.0)	40.3
10′		142.9	10′		144.4
11′	7.48 (d, 7.0)	130.9	11′	7.38 (d, 8.0)	128.9
12′	7.17 (t, 6.5)	127.3	12′	7.18 (t, 7.0)	127.8
13′	7.09 (t, 7.0)	125.6	13′	7.07 (t, 7.0)	125.4
14′	7.17 (t, 6.5)	127.3	14′	7.18 (t, 7.0)	127.8
15′	7.48 (d, 7.0)	130.9	15′	7.38 (d, 8.0)	128.9

[a] Recorded at 500 and 125 MHz for ^{1}H and ^{13}C, respectively. ^{1}H and ^{13}C NMR spectroscopic data of **2**−**3** in DMSO-d_6.

The relative stereochemistry of **2** was elucidated by analysis of ^{1}H−^{1}H coupling constants, NOESY experiment, and chemical shifts with macrocarpal-A.[11] The same relative stereochemistry of C-1 and C-5 in **2** as in macrocarpal-A was deduced from the similar carbon and proton chemical shifts, protons coupling constants, and NOESY correlations found in **2**. To observe the NOE interactions among H-1, H-6, H-7, H-13, and H-15 would indicate the H-6, H-7, and

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methyl group at C-13 and C-15 should be α-oriented. However, no NOE correlation peaks were observed between H-1 and H-14, while NOE interactions were observed among H-5, H-12, H-14, and H-9′, indicating the H-9′ and methyl group at C-12 and C-14 should be *β*-oriented (Figure 1).

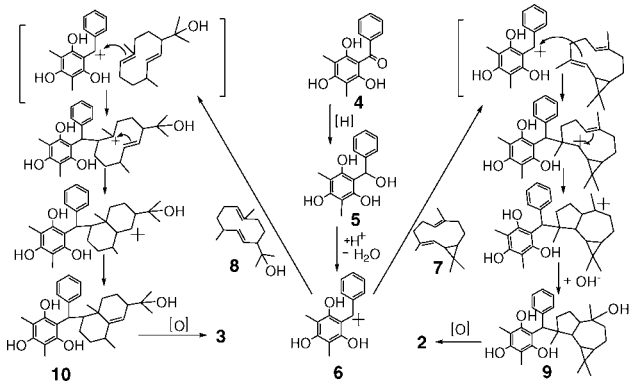
Psidial C (**3**) was obtained as an amorphous powder, mp 292−293 °C, $[\alpha]^{20}_{D}$ +90.4 (*c* 0.0075, MeOH). The IR spectrum of **3** exhibited a hydroxyl group (3390 cm^{-1}, br s), conjugated carbonyl (1612 cm^{-1}), and methyl (1443 cm^{-1}), respectively. The aromatic ring and conjugated carbonyl functional groups were also indicated by the UV absorption bands at λ_{max}^{MeOH}(log *ε*) 202 (4.31), 274 (4.20), and 388 (3.76) nm. The negative ESIMS of **3** gave a quasi-molecuar ion peak at *m*/*z* 491.4 $[M - H]^-$, while the positive ESIMS exhibited $[M + Na]^+$ at *m*/*z* 515.3. The HRFABMS $[M + Na]^+$ displayed the molecular ion peak at *m*/*z* 515.2437 (calcd 515.2410), which was consistent with a molecular formula of $C_{30}H_{36}O_6$ with 13 degrees of unsaturation. A careful comparison of chemical shift values of **3** with those of **1** also revealed that **3** contained the structural unit of a 3,5-diformyl phloroglucinol (see Table 2). The data for **3** implied that **3** possessed the feature of a 3,5-diformylbenzyl phloroglucinol-coupled sesquiterpenoid. The 3,5-diformylbenzyl phloroglucinol accounted for 10 out of 13 double bond equivalents.

The structure of **3** was subsequently established by a comprehensive analysis of 2D NMR spectroscopy. A series of HMBC (Figure 1) correlations from H_3-14 to C-1, C-9, and C-10 allowed the connection of C-1, C-9, and C-14 to the quaternary carbon C-10, from H_3-12 to C-7, C-11, and C-13, from H_3-13 to C-7, C-11, and C-12 allowed the connection of C-7, C-12, and C-13 to the quaternary carbon C-11. It can be concluded that C-12 and C-13 connected with the same carbon (C-11). From H-1 to C-2, C-3, C-5, C-10, C-14, from H-6 to C-4, C-5, C-7, C-8, C-10, and C-11, as well as a careful comparison of chemical shift values of above protons and carbons with those of rosifoliol,[12] demonstrated that **3** contained the basic structural unit of rosifoliol. The mutual HMBC correlations of H-9′/C-1, C-2, C-10, C-1′, C-2′, C-6′, C-10′, C-11′, and C-15′ confirmed 3,5-diformylbenzyl phloroglucinol was coupled with rosifoliol moiety via the C-1−C-9′ bond as judged by the HMBC correlations of H-9′/C-1 and H-1/C-9′. Eudesmane accounted for the leftover 3 out of 13 double bond equivalents. In light of the evidence mentioned above, the planar structure of **3** was finally established as depicted.

The relative stereochemistry of **3** was elucidated by analysis of $^1H-^1H$ coupling constants, NOESY experiment, and chemical shifts with **2**. The same relative stereochemistry of C-9′ in **3** as in **2** was deduced from similar carbon and proton chemical shifts, and proton coupling constants, and NOESY correlations found in **3**. That the configuration H-9′ was also supported by the coupling constant value ($J_{1,9'}$ = 9.0 Hz). Observing NOE interactions between H-9′ and H-14 would indicate that a methyl group at C-14 should be *β*-oriented. However, no NOE correlation peaks were observed between H-1 and H-9′, while NOE interactions were observed among H-1, H-7, and H-15, which indicated the H-1, H-7, and methyl group at C-15 should be α-oriented (Figure 1).

Bicyclogermacrane-type intermediate **7** and allogermacrane-type intermediate **8** have been isolated from the leaves of *Psidium guajava* L. The biogenetic precursor of key 3,5-dimethyl-2,4,6-trihydroxybenzophenon **4**,[13] which has been isolated from the leaves of *Psidium guajava* L, can obtain the intermediate **5** by reduction. Intermediate **5** can generate **6**[14] under acidic condition, then **6** attacks intermediate **7**[15] in anti-Markovnikov fashion or **6** attacks intermediate **8**[16] in Markovnikov fashion, and **2** and **3** can be generated from **9** and **10** by oxidation, respectively. Therefore a plausible biosynthetic pathway for compounds **2**−**3** could be proposed through this route (Scheme 1).

Scheme 1. Hypothetical Biogenetic Route of **2**−**3**



Psidials A−C showed activity to protein tyrosine phosphatase 1B (PTP1B), inhibition rates of enzyme PTP1B were 1.7%, 61.7%, and 38.8% in 10 *μ*M respectively, but Psidials A−C were inactive to several human cancer cell lines (IC_{50} > 10 *μ*M) including human ovarian cancer cell line (A 2780), colon cancer cell line (HCT-8), hepatoma cell line (Bel-7402), lung cancer cell line (A549), and human gastric adenocarcinoma cell line (BGC-823).

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Supporting Information Available: IR, UV, MS, crystallographic data, 1D and 2D NMR spectra, as well as proposed biogenesis of psidials A−C. This material is available free of charge via the Internet at http://pubs.acs.org.

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