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Psiguadials A and B, Two Novel Meroterpenoids with Unusual Skeletons from the Leaves of *Psidium guajava*

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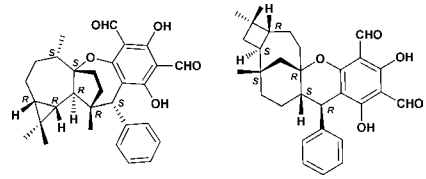
ABSTRACT



Psiguadial A 1 Psiguadial B 2

Psiguadials A (1) and B (2), two novel sesquiterpenoid-diphenylmethane meroterpenoids with unusual skeletons, along with a pair of known epimers, psidial A (3) and guajadial (4), were isolated from the leaves of *Psidium guajava*. Their structures with absolute configurations were elucidated by means of NMR, X-ray diffraction, and quantum chemical CD calculation. Compounds 1, 2, and 4 exhibited potent inhibitory effects on the growth of human hepatoma cells.

Psidium guajava L. (Myrtaceae) is an evergreen shrub distributed widely in the tropical areas of Africa, south America, and southeast Asia. The leaves of this plant had been used for the treatment of diarrhea and hyperglycemia as a folk and traditional Chinese medicine for centuries. Its safety and efficacy were evaluated by pharmacological and clinical investigations in recent years.[1−4] Previous phytochemical investigations of this plant had led to the isolation of more than forty compounds including triterpenoids,[5−7] sesquiterpenoids,[8] and flavonoids,[9,10] etc. Recently, several novel sesquiterpenoid-based meroterpenoids with relative configurations had been reported from this plant.[11−13] The

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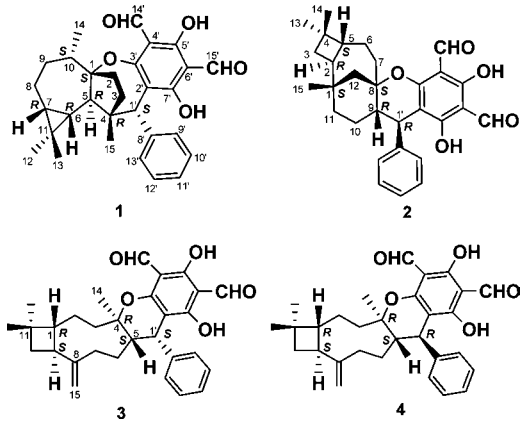
skeletons of these compounds were characterized with a unique connection pattern between sesquiterpenoid and diphenylmethane moiety.

As a part of our continuing search for structural unique and biologically interesting constituents from traditional Chinese medicines,[14,15] two novel meroterpenoids with unusual skeletons, psiguadials A (**1**) and B (**2**), together with a pair of known epimers, psidial A (**3**) and guajadial (**4**), were isolated from the EtOH extract of the leaves of *P. guajava*. On the basis of the different sesquiterpenoid moieties, **1** and **2** could be regarded as a globulol-based and a caryolane-based meroterpenoids, respectively. Herein, the isolation and structural elucidation of **1** and **2** are described. The absolute configurations of **1**−**4** were determined by single crystal X-ray diffraction in combination with CD experiments.



Psiguadial A (**1**) was isolated as colorless needles. The molecular formula of **1** was established as $C_{30}H_{34}O_5$ by HR-EI-MS (*m/z* 474.2403 $[M]^+$, calcd 474.2406). The UV spectrum of **1** in $CHCl_3$ showed absorptions maxima at 229, 296, and 347 nm (ϵ 2370, 7157, and 2133). The IR absorptions at ν_{max} 2964 and 1632 cm^{-1} suggested the presence of aldehyde and benzene functionality in **1**. Analysis of the NMR spectra of **1** revealed signals due to two phenolic hydroxyls [δ_H 13.76 and 13.53 (each 1H, br s)], four methyls [δ_H 1.13, 0.91, 0.24 (each 3H, s) and 1.09 (3H, d, J = 6.8 Hz); δ_C 28.4, 24.9, 17.4 and 14.3], two aldehydes [δ_H 10.38 and 10.12 (each 1H, s); δ_C 193.7 and 191.8], a monosubstituted benzene ring [δ_H 7.11−7.22 (5H, m); δ_C 140.9, 130.3 (× 2), 127.6 (× 2) and 126.4], and a hexasubstituted benzene ring (δ_C 170.5, 168.2, 166.7, 114.2, 108.2 and 105.0). With the aid of $^1H-^1H$ COSY, HSQC and HMBC experiments, all the ^1H and ^{13}C NMR signals of **1** were assigned as shown in Table 1.

The $^1H-^1H$ COSY spectrum of **1** revealed the presence of three spin systems (C-2 to C-3, C-5 to C-14, and C-9′ to C-13′, Figure 1). The HMBC spectrum showed the correlations between H_3-12/H_3-13 and C-6/C-7, between H_3-14 and C-1, as well as between H-5/H_3-15 and C-3. The above data indicated the presence of a sesquiterpenoid moiety with 3/7/5 carbon rings system (**1a**). Comparison of the NMR data assigned to **1a** with those of (+)-globulol[16] suggested that they were very similar, except that the C-1 methine was

Table 1. NMR Data of **1** and **2** (J in Hz)[a]

	1		**2**	
no.	δ_H	δ_C	δ_H	δ_C
1	−	104.1	−	33.4
2	a 2.01 b 1.88	36.6	2.16	37.0
3	a 1.90 b 1.63	37.9	α 1.52 β 1.37	35.5
4	−	47.8	−	35.1
5	2.36 (d, 11.9)	48.7	1.82 (m)	44.1
6	0.29 (dd, 8.8, 11.9)	26.3	α 1.65 (m) β 1.41 (m)	20.1
7	0.58	23.9	α 1.93 β 1.58	29.4
8	α 1.26 (m) β 1.83 (m)	20.3	−	84.1
9	α 1.74 (m) β 1.93 (m)	30.3	1.68	50.0
10	2.49 (m)	39.9	1.49 (m)	23.9
11	−	19.2	α 1.41 β 1.10	37.6
12	0.91 (s)	28.4	a 2.08 b 1.29	47.5
13	0.24 (s)	14.3	1.02 (s)	30.6
14	1.09 (d, 6.8)	17.4	1.01 (s)	20.7
15	1.13 (s)	24.9	0.86 (s)	26.1
1′	4.49 (s)	53.2	3.49 (d,11.5)	40.4
2′	−	114.2	−	105.7
3′	−	166.7	−	163.5
4′	−	108.2	−	104.6
5′	−	168.2	−	168.5
6′	−	105.0	−	104.2
7′	−	170.5	−	169.6
8′	−	140.9	−	143.4
9′	7.13	130.3	7.18	128.2
10′	7.22	127.6	7.23	126.2
11′	7.11	126.4	7.18	128.2
12′	7.22	127.6	7.23	126.2
13′	7.13	130.3	7.18	128.2
14′	10.38 (s)	193.7	10.08 (s)	191.4
15′	10.12 (s)	191.8	10.08 (s)	192.3
5′- OH	13.76 (s)		13.51 (s)	
7′- OH	13.53 (s)		13.04 (s)	

[a] Overlapped signals were reported without designating multiplicity.

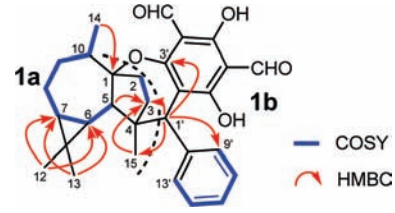


Figure 1. Key $^1H-^1H$ COSY and HMBC correlations of **1**.

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replaced by an oxygenated quaternary carbon and the absence of hydroxyl group at C-10 in **1a**. Moreover, the ^{1}H and ^{13}C NMR signals assigned to **1b** were very similar to those of psidial A (**3**), indicating that **1** had the same diphenylmethane moiety as **3**. The HMBC correlations between H-1′ and C-3/C-15 indicated that **1a** and **1b** were connected via C-4−C-1′ bond. Furthermore, the obvious downfield shift of C-1 (δ_C 104.1) and upfield shift of C-3′ (δ_C 166.7) suggested that **1a** and **1b** were connected via an oxygen atom to form a seven-member ring (Figure 1).

The relative configuration of **1** could be deduced by a ROESY experiment. In the ROESY spectrum, correlations between H-6 and H_3-15/H_3-13/H-10/H-3a, as well as between H_3-15 and H-1′ suggested that these protons had the same orientation (Figure 2). Fortunately, crystals suitable for a

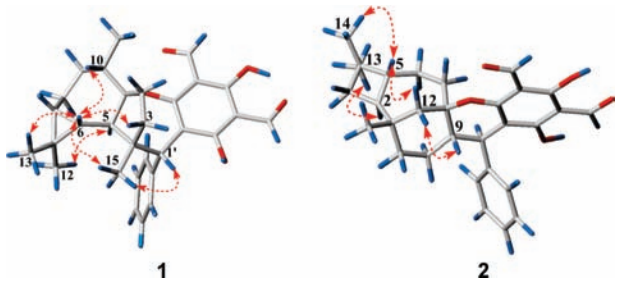

Figure 2. Key ROESY correlations of **1** and **2**.

single-crystal X-ray diffraction experiment were obtained. Thus, the structure and stereochemistry of **1** were further confirmed by this experiment (Figure 3). In the globulol

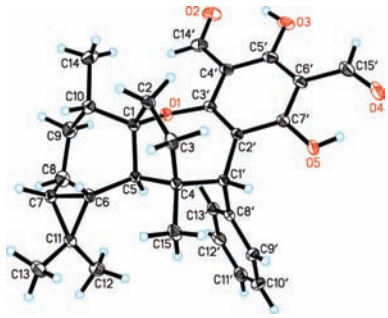

Figure 3. X-ray structure of **1**.

moiety (**1a**), the three-member ring was *cis*-fused to the seven-member ring which was *cis*-fused to the five-member ring. In the diphenylmethane moiety (**1b**), the two phenyl rings made a dihedral angle 76.7°. Bearing five oxygen atoms on the molecule, the final refinement on the CuKα data resulted in a Flack parameter of 0.1 (2), allowing an unambiguous assignment of the absolute structure.

Psiguadial B (**2**) was obtained as colorless needles. The molecular formula of **2** was assigned as $C_{30}H_{34}O_5$ by HR-EI-MS (*m/z* 474.2403 $[M]^+$, calcd 474.2406). The NMR spectra of **2** also revealed the presence of two phenolic hydroxyls [δ_H 13.51 and 13.04 (each 1H, br s)], three methyls [δ_H 1.02, 1.01, and 0.86 (each 3H, s); δ_C 30.6, 26.1 and 20.7], two aldehydes [δ_H 10.08 (2H, s); δ_C 192.3 and 191.5], a monosubstituted benzene ring and a hexasubstituted benzene ring. A comparison of the NMR data of **2** with those of **1** (Table 1) indicated that **2** had the same diphenylmethane moiety as **1** (**2b** in Figure 4).

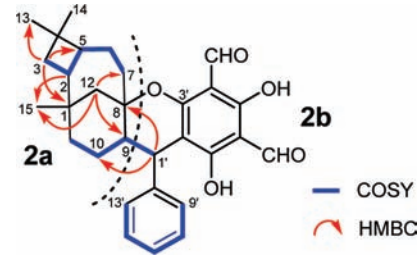

Figure 4. Key $^{1}H-^{1}H$ COSY and HMBC correlations of **2**.

The $^{1}H-^{1}H$ COSY spectrum of **2** suggested the presence of three spin systems in bold as shown in Figure 4. The HMBC correlations between H_2-12 and C-7/C-9/C-15, as well as between H_2-3 and C-1/C-5/C-13 allowed the establishment of the planar structure of **2a**, which was a sesquiterpenoid moiety with 4/7/6 rings system (Figure 4). Comparison of the 1D and 2D NMR data of **2a** with those of the known compound (+)-caryolane-1,9-β-diol[17] showed that they were analogous. Different from (+)-caryolane-1,9-β-diol, the four-member ring in **2a** was fused to the seven-member ring via C-2 and C-5 instead of C-6 and C-7. This assignment was supported by the HMBC correlations between H_2-3 and C-1 as well as between H-2 and C-15.

The connection of **2a** and **2b** could be deduced by an HMBC experiment and molecular formula information. Thus, in the HMBC spectrum, correlations between H-1′ and C-8/C-10 suggested that **2a** and **2b** were connected through the C-1′−C-9 bond. According to the molecular formula information, the remaining oxygen atom was to bridged C-8 and C-3′ to form a six-member ring (Figure 4).

The ROESY correlations between H-12a (δ_H 2.08) and H-5/H_3-14 as well as between H-12b (δ_H 1.29) and H-9 suggested that these protons were cofacial and assigned as β-orientation (Figure 2). The *trans*-fusion between the four-member ring and the seven-member ring was also determined by the ROESY experiment (Figure 2). However, no NOE correlation was observed between H-1′ and H-9, suggesting that H-1′ was α-oriented. Indeed, the dihedral angel between H-1′ and H-9 was almost 180° in a energy minimized conformation of **2** calculated by SYBYL software (Figure 5), which was consistent with the observed coupling constant

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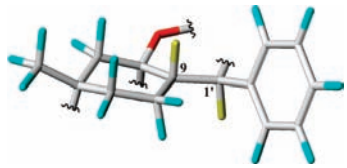

Figure 5. Partial structure with the most reasonable conformation of **2** calculated by Grid search of SYBYL 7.0 software.

($J_{9,1'} = 11.5$ Hz) between H-1′ and H-9. On the basis of the above results, the relative configuration of **2** was elucidated as shown in Figure 2.

Compounds **3** and **4** were identified by comparison of their physiochemical properties and spectral data with those reported in the literatures.[11,13] Their absolute configurations were not known previously but were determined by X-ray diffraction experiments. Similar to **1**, the final refinement on the CuKα data of **3** and **4** resulted in a suitable low Flack parameters 0.1 (2) and 0.0 (8), respectively, permitting assignment of their absolute configurations (see Supporting Information).

The quantum chemical CD calculation method was used to further establish the absolute configurations of **1**−**4**.[18] The preliminary conformational distribution search was performed by CHEM3D software using the MM2 force field overlaid with key correlations observed in the ROESY spectrum. The corresponding minimum geometries were further fully optimized by using DFT at the B3LYP/6-31G(d) level as implemented in the Gaussian 09 program package. The stable conformers obtained were submitted to CD calculation by TDDFT [B3LYP/6-31G(d)] method. The overall predicted CD spectra of **1**−**4** were subsequently compared with the experimental ones, which revealed a good agreement between the calculated and the measured CD curves (Figure 6). Therefore, the stereostructures of **1**−**4** were established.

The antitumor activities of **1**−**4** were detected by MTT assay in doxorubicin-sensitive and -resistant human hepatoma cells (HepG2 and HepG2/ADM). Compounds **1**, **2**, and **4** exhibited potent inhibitory effects on the gowth of HepG2 cells with IC_{50} values of 61.07 ± 1.77, 45.62 ± 1.41, and

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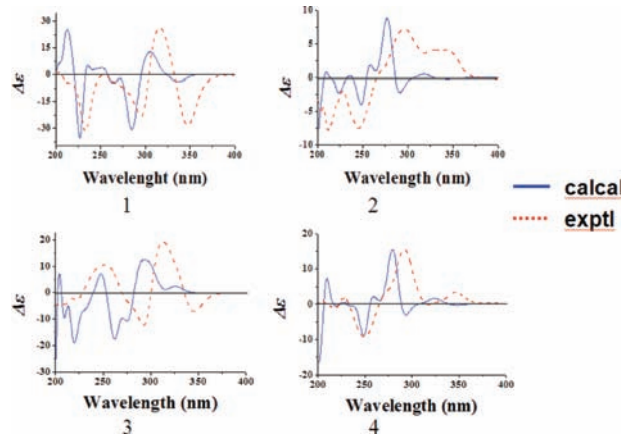

Figure 6. Calculated and experimental CD spectra of **1**−**4**.

157.90 ± 0.44 nM, respectively, whereas **3** had no signifcant effect even at a concentration of 10 μM. These results indicated that the configuration at the C-1′ position might be important for their cytotoxicities. Furthermore, it was interesting to find that compounds **1**, **2**, and **4** showed much weaker antiproliferative activities in HepG2/ADM cells than that in HepG2 cells, with IC_{50} values of 17.94 ± 0.21, 25.09 ± 0.21, and 56.37 ± 0.41 μM, respectively. The similar structure−activity relationship of meroterpenoids was observed in HepG2 and HepG2/ADM cells. Given that the HepG2/ADM cells overexpress the P-glycoprotein pump, it is likely that the meroterpenoids are substrates for the pump.

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Supporting Information Available: Detailed description of the experimental procedure, a listing of UV, IR, HR-EI-MS, and NMR spectra of compounds **1** and **2**, CIF files of **1**, **3**, and **4**, and quantum chemical CD calculations of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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