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Guadial A and Psiguadials C and D, Three Unusual Meroterpenoids from *Psidium guajava*

Meng Shao,[†,‡] Ying Wang,[*,†,§] Yu-Qing Jian,[†,§] Xiao-Jun Huang,[†,§] Dong-Mei Zhang,[†,§] Qing-Fa Tang,[‡] Ren-Wang Jiang,[†,§] Xue-Gang Sun,[‡] Zhi-Ping Lv,[‡] Xiao-Qi Zhang,[*,†,§] and Wen-Cai Ye[*,†,§]

Institute of Traditional Chinese Medicine & Natural Products and JNU-HKUST Joint Laboratory for Neuroscience & Innovative Drug Research, Jinan University, Guangzhou 510632, P. R. China, and College of Traditional Chinese Medicine, Southern Medical University, Guangzhou 510515, P. R. China

chywc@yahoo.com.cn; wangying_cpu@163.com; xqzhang74@hotmail.com

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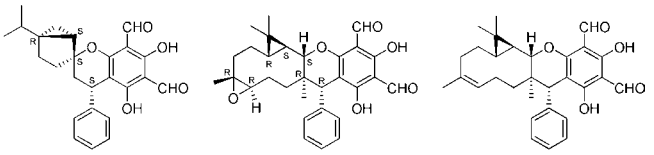
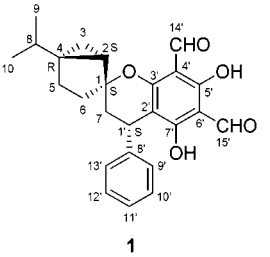
ABSTRACT



The first monoterpene-based meroterpenoid (1) and two novel sesquiterpene-based ones (2 and 3) with unprecedented skeletons were isolated from the leaves of *Psidium guajava*. Their structures with absolute configuration were elucidated by extensive spectroscopic studies. A plausible biosynthetic pathway for all meroterpenoids from the title plant is also proposed. Compounds 2 and 3 showed significant cytotoxicity toward HepG2 and HepG2/ADM cells.

The leaves of *Psidium guajava* L. (Myrtaceae) have long been used in China as folk medicine for the treatment of diarrhea and hyperglycemia. In recent years, several sesquiterpene-based meroterpenoids with unprecedented skeletons have been isolated from the leaves of *P. guajava* by several research groups.[1,2] Some of the *Psidium* meroterpenoids exihibited significant biological activities including inhibitory effects on protein tyrosine phosphatase 1B (PTP1B) and the growth of human hepatoma cell (HepG2).[1,2] The main structural difference of these sesquiterpene-based *Psidium* meroterpenoids could be attributed to the skeleton of the terpenoid moiety as well as the coupling pattern between benzyl phloroglucinol dialdehyde and terpenoid. Two plausible biosynthetic pathways and a short biomimetic synthesis relative to *Psidium* meroterpenoids had been also reported.[1,3] However, there is still confusion about their biogenetic pathway.

As a result of our continuing study on this plant, the first monoterpene-based meroterpenoid, guadial A (**1**), and two novel sesquiterpene-based meroterpenoids, psiguadials C and D (**2** and **3**), were isolated. In this paper, the



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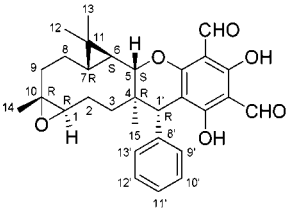

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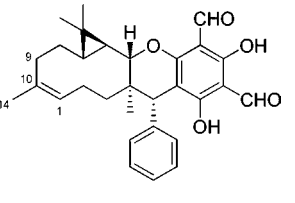

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[†] Institute of Traditional Chinese Medicine & Natural Products, Jinan University.

[‡] College of Traditional Chinese Medicine, Southern Medical University.

[§] JNU-HKUST Joint Laboratory for Neuroscience & Innovative Drug Research, Jinan Universiy.

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biogenetic route of all meroterpenoids from the title plant and cytotoxic effects of **1**–**3** were also described.

Guadial A (**1**) was isolated as a colorless oil. The molecular formula was established as $C_{25}H_{26}O_5$ by HR-ESI-MS at *m*/*z* 429.1671 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_5Na$ 429.1673). The UV spectrum of **1** showed the absorptions maxima at 284 and 302 nm. The IR spectrum exhibited the characteristic absorptions for hydroxyl (3500 cm^{-1}), aldehyde (2927 cm^{-1}), and benzene ring (1632, 1436, and 697 cm^{-1}). The ^1H and ^{13}C NMR spectra displayed the presence of two chelated phenolic hydroxyls, two aldehydes, a monosubstituted benzene ring, a hexasubstituted benzene ring, and two methyls. The above spectral data suggested that **1** was a benzylphloroglucinol dialdehyde–terpene derivative.[1,2] Detailed analysis of $^1H-^1H$ COSY, HSQC, and HMBC spectra resulted in unambiguous assignment of all ^1H and ^{13}C NMR signals as shown in Table 1.

The $^1H-^1H$ COSY spectrum showed the presence of five spin coupling systems in bold as shown in Figure 1. In the HMBC spectrum, the correlations between H_3-9/H_3-10 and C-4, between H-2/H_2-7 and C-6, between H_2-3 and C-5, and between H_2-3/H_2-5 and C-8 proved the presence of a monoterpenoid moiety with a 3/5 bicyclic ring system and an isopropyl group at C-4 (**1a**). Furthermore, the HMBC correlations between H-5 and C-1, and between H_2-7 and C-2/C-6 established an oxa-spiro[5.5] ring. Based on the molecular formula information, the remaining oxygen atom was assigned to bridge C-1 and C-3′. Comparison of the NMR data of **1** with those of euglobal-Ib[4] suggested that their structrues were very similar except that the isopropyl group at C-1′ in euglobal-Ib was replaced by a monosubstituted benzene ring in **1**.

The relative configuration of **1** could be deduced by its ROESY spectrum. In the ROESY spectrum, the correlations between H-3a and H-6a, between H-3b and H-8, between H-2 and H-1′, between H-7a and H-6b, as well as between H-7b and H-2 indicated that these protons were cofacial, respectively. Thus, the relative configuration of **1** was established as shown in Figure 2.

The absolute configuration of **1** was elucidated by quantum chemial CD calculation.[2,5] The overall predicted CD spectra of (1*S*,2*S*,4*R*,1′*S*)-**1** and (1*R*,2*R*,4*S*,1′*R*)-**1** were compared with the experimental one (Figure 3). The result showed that the spectrum calculated for (1*S*,2*S*,4*R*,1′*S*)-**1** was consistent with the experimental one. Therefore, the absolute configuration of **1** was determined as 1*S*,2*S*,4*R*,1′*S*.

Psiguadial C (**2**) was obtained as colorless needles. The molecular formula was assigned as $C_{30}H_{34}O_6$ by its HR-EI-MS (*m*/*z* 490.2352 $[M]^+$, calcd for $C_{30}H_{34}O_6$ 490.2350). The UV and IR spectra of **2** showed the characteristic absorptions for *Psidium* meroterpenoids.[1,2] The NMR spectra of **2** also displayed the signals for benzylphloroglucinol dialdehyde and sesquiterpenoid moieties. With the aid of $^1H-^1H$ COSY, HSQC, and HMBC experiments, all of the ^1H and ^{13}C NMR signals of **2** were assigned as shown in Table 2.

The presence of two spin systems of H-1–H-3 and H-5–H-9 in the $^1H-^1H$ COSY spectrum, as well as the HMBC correlations between H-5 and C-15, between H-15 and C-3, and between H-14 and C-1/C-9 suggested the existence of a 10-membered carbon ring in **2** (Figure 1). The HMBC correlations between H-12/H-13 and C-6/C-7 indicated the presence of a cyclopropane ring between

Table 1. NMR Data of **1** (in $CDCl_3$, *J* in Hz)[a]

no.	δ_H	δ_C	no.	δ_H	δ_C
1		88.9	1′	4.21 (dd, 7.3, 9.5)	35.1
2	1.35 (dd, 3.6, 7.8)	28.3	2′		103.7
3	a 0.82 (dd, 3.6, 5.2)	12.2	3′		165.9
	b 0.45 (dd, 5.2, 7.8)		4′		104.6
4		34.6	5′		168.6
5	1.67 (m)	24.5	6′		104.3
6	a 1.70 (m)	33.6	7′		169.9
	b 1.64 (m)		8′		144.7
7	a 2.01(dd, 9.5, 14.3)	42.3	9′	7.15 (br d, 7.0)	126.8
	b 2.34 (dd, 7.3, 14.3)		10′	7.29 (br d, 7.0)	128.7
8	1.37 (m)	32.6	11′	7.21 (t, 7.0)	126.5
9	0.97 (d, 6.8)	19.8	12′	7.29 (br d, 7.0)	128.7
10	0.91 (d, 6.8)	19.7	13′	7.15 (br d, 7.0)	126.8
5′-OH	13.53 (s)		14′	10.10 (s)	192.5
7′-OH	13.17 (s)		15′	10.13 (s)	191.8

[a] Overlapped signals were reported without designating multiplicity.

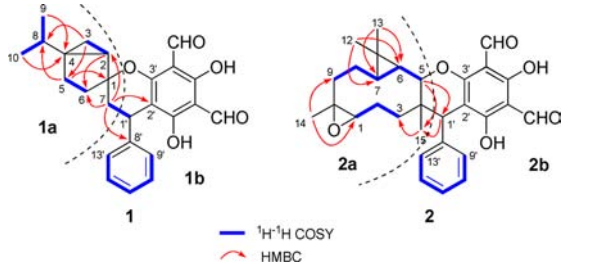

Figure 1. Key $^1H-^1H$ COSY and HMBC correlations of **1** and **2**.

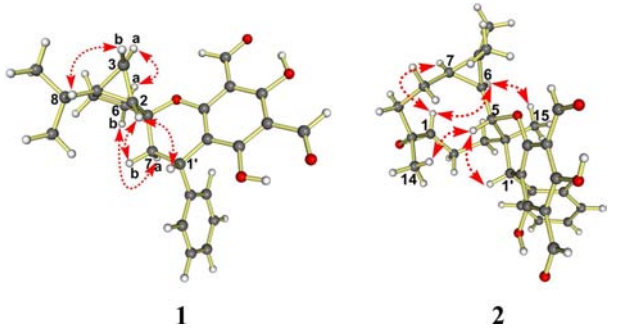

Figure 2. Key ROESY correlations of **1** and **2**.

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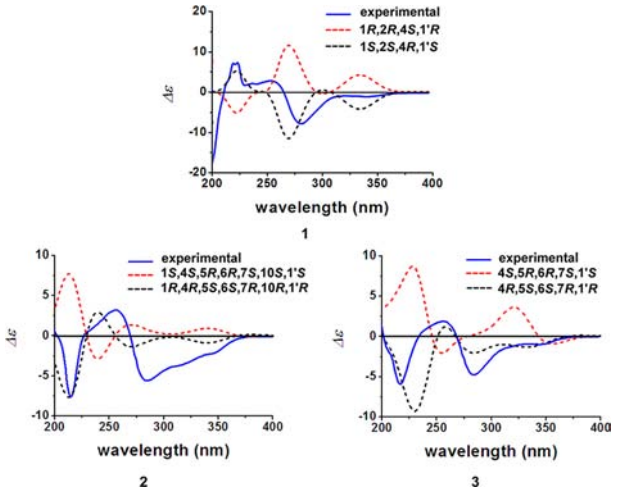


Figure 3. Calculated and experimental CD spectra of **1**–**3**.

C-6 and C-7. The above data indicated the presence of a sesquiterpenoid moiety with 3/10 consecutive carbocyclic system (**2a**). The HMBC correlations between H-1′ and C-15 and between H-5 and C-1′ (Figure 1) indicated that the sesquiterpenoid moiety (**2a**) and benzylphloroglucinol dialdehyde (**2b**) were connected via a C-4−C-1′ bond. Moreover, the obvious upfield chemical shifts of C-3′ (δ_C 165.8) and the oxygenated methine C-5 (δ_C 84.0) suggested the oxygen atom was bridged C-5 and C-3′ to form a dihydropyran ring.

The relative stereochemistry of **2** could be elucidated by a ROESY experiment. The NOE correlations between H-5 and H-14/H-1′, between H-1 and H-6/H-7, and between H-15 and H-6 established the relative configuration of 2 as shown in Figure 2.

The structure and stereochemistry of **2** were further confirmed by an X-ray experiment. In the X-ray structure of **2** (Figure 4), the 10-membered ring was *trans*-fused with the dihydropyran and epoxide rings but *cis*-fused with the cyclopropane ring. The final refinement on the CuKα data resulted in a Flack parameter of 0.01(13), allowing an unambiguous assignment of the absolute structure of **2** (1*R*,4*R*,5*S*,6*S*,7*R*,10*R*,1′*R*), which was consistent with the result obtained by the following quantum chemical CD calculation experiment (Figure 3).

The molecular formula of psiguadial D (**3**) was established to be $C_{30}H_{34}O_5$ by its HR-EI-MS at *m*/*z* 474.2395 $[M]^+$ (calcd for $C_{30}H_{34}O_5$ 474.2406). Comparison of the ^1H and ^{13}C NMR data of **3** with those of **2** revealed that they were very similar except for the presence of an olefinic group (δ_H 5.31; δ_C 127.5 and 130.7) in **3** instead of the epoxide unit in **2**. In the HMBC spectrum, correlations between H-1 and C-9/C-14 indicated that the double bond was located at C-1 and C-10. Finally, the absolute configuration of **3** was established to be 4*R*,5*S*,6*S*,7*R*,1′*R* by quantum chemial CD calculation experiment (Figure 3).

To date, nine meroterpenoids had been isolated from *P. guajava*.[1,2] Two plausible biosynthetic pathways relative to these *Psidium* meroterpenoids have also been reported.[1]

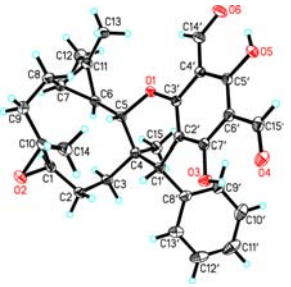


Figure 4. X-ray structure of **2**.

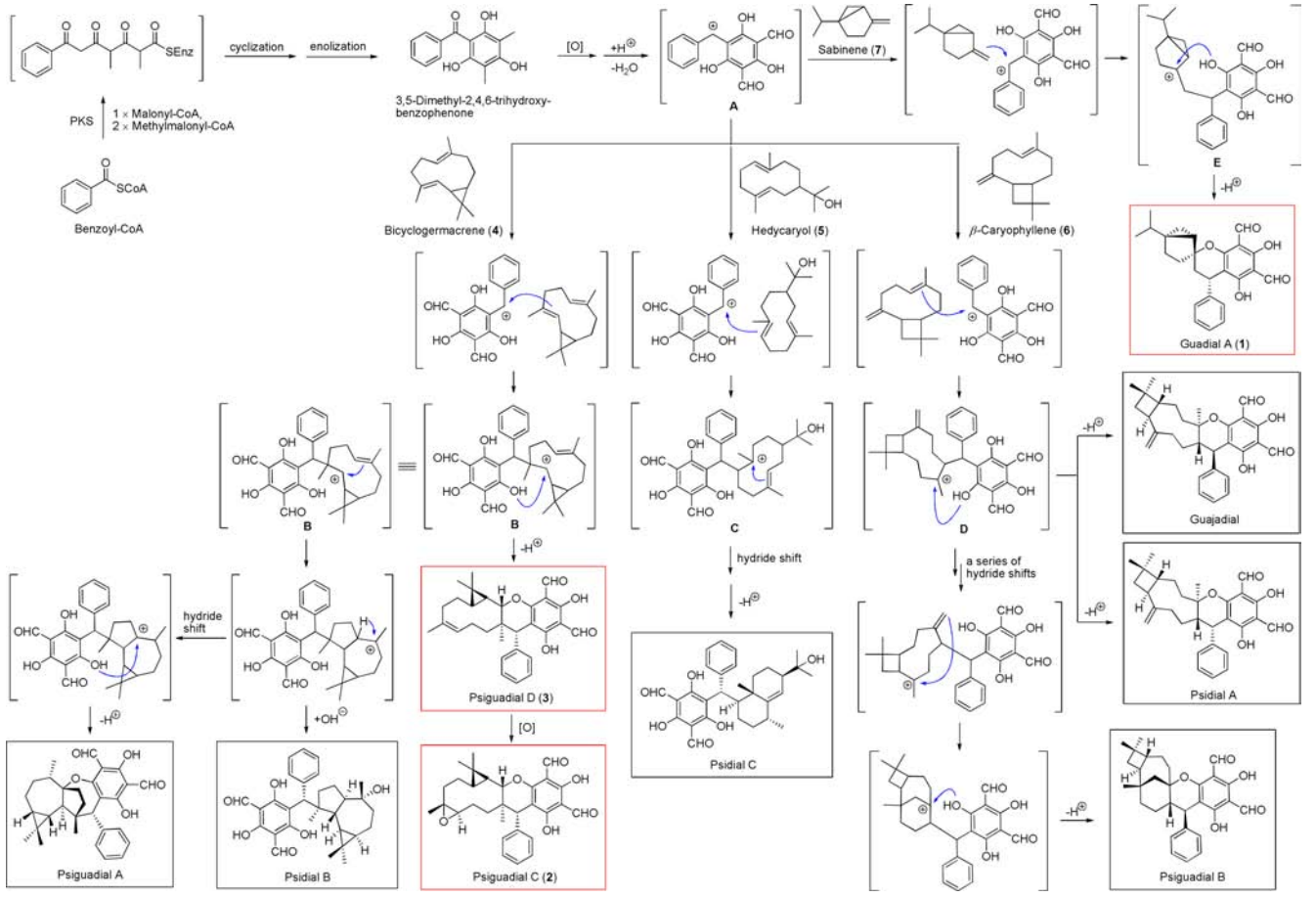
Table 2. NMR Data of **2** and **3** (in $CDCl_3$, *J* in Hz)[a]

	2		**3**	
no.	δ_H	δ_C	δ_H	δ_C
1	2.93 (dd, 7.2, 1.2)	64.0	5.31 (dd, 10.4, 3.3)	127.5
2	a 2.11 (dd, 7.2, 14.9)	22.5	a 2.76 (m)	23.4
	b 1.84 (m)		b 2.10 (m)	
3	1.68	31.6	1.57	35.4
4		40.6		41.4
5	3.85 (d, 6.7)	84.0	3.71 (d, 7.1)	85.3
6	0.98 (m)	26.6	0.95	27.0
7	0.67 (m)	31.7	0.66 (td, 1.4, 10.4)	31.6
8	a 2.03 (dd, 5.5, 13.9)	21.3	a 1.92	22.4
	b 1.16		b 0.96	
9	a 2.21 (dd, 5.4, 12.4)	39.3	2.09	38.1
	b 1.17			
10		60.4		130.7
11		21.6		19.8
12	1.20 (s)	19.2	1.20 (s)	30.3
13	1.20 (s)	30.3	1.13 (s)	19.3
14	1.34 (s)	17.2	1.72 (s)	17.5
15	0.77 (s)	18.5	0.73 (s)	18.6
1′	4.23 (s)	43.9	4.39 (s)	43.9
2′		104.6		105.2
3′		165.8		166.4
4′		104.3		104.5
5′		168.3		168.3
6′		104.8		104.2
7′		170.6		170.6
8′		139.7		140.4
9′	6.79 (d, 7.7)	127.6	6.78 (d, 7.8)	127.5
10′	7.15	127.4	7.15	127.5
11′	7.23	126.5	7.23	126.3
12′	7.19	130.1	7.33	130.2
13′	7.31	127.7	7.33	127.8
14′	10.13 (s)	192.1	10.12 (s)	192.2
15′	10.13 (s)	191.7	10.10 (s)	191.6
5′-OH	13.60 (s)		13.60 (s)	
7′-OH	13.15 (s)		13.14 (s)	

[a] Overlapped signals are reported without designating multiplicity.

Because of the findings of key biogenetic intermediate and terpene precursors in this plant, the plausible biogenetic route of *Psidium* meroterpenoids could be proposed as shown in Scheme 1. First, the benzoyl-CoA was successively condensed with one molecule of malonyl-CoA and

Scheme 1. Plausible Biosynthetic Pathway for *Psidium* Meroterpenoids from *P. guajava*



two molecules of methylmalonyl-CoA. Then, cyclization and enolization should lead to the formation of intermediate 3,5-dimethyl-2,4,6-trihydroxybenzophenon, which had been previously isolated from the same plant.[6] The intermediate could be oxidized and then generated a carbocation **A**. As a cationic initiator, the carbocation **A** could respectively couple with different terpene precursors including bicyclogermacrene (**4**), hedycaryol (**5**), β-caryophyllene (**6**), and sabinene (**7**), which had been detected in the plant (see the Supporting Information), to generate carbocations **B**−**E**. Further rearrangement of carbocations **B**−**E** could construct different meroterpenoid skeletons to afford guajadial,[1a] psidials A−C,[1b] psiguadials A and B,[2] as well as compounds **1**−**3**.

The cytotoxic effects of **1**−**3** were evaluated by MTT assay in doxorubicin-sensitive and -resistant human hepatoma cells (HepG2 and HepG2/ADM) as described previously.[2] Compounds **2** and **3** exhibited potent inhibitory effects on the growth of HepG2 cells with IC_{50} values of 104.5 ± 13.71 nM and 128.3 ± 18.2 nM, respectively. The cytotoxicity of **2** and **3** in HepG2/ADM, with IC_{50} values of 21.06 ± 1.25 μM and 23.65 ± 1.71 μM, differed significantly from that in HepG2 cells. As with other *Psidium* meroterpenoids,[2] **2** and **3** might also be substrates for the efflux transporter P-glycoprotein 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**−**3**, CIF files of **2**, quantum chemical CD calculations of **1**−**3**, and GC−MS analysis of **4**−**7**. These materials are available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.