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Two Unusual Polycyclic Polyprenylated Acylphloroglucinols, Including a Pair of Enantiomers from *Garcinia multiflora*

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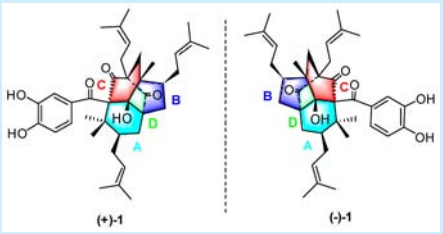
Supporting Information

ABSTRACT: Two polycyclic polyprenylated acylphloroglucinols, garcimulins A and B ((±)-**1** and **2**), including a pair of enantiomers with the unique caged tetracyclo[5.4.1.1^{1,5}.0^{9,13}]tridecane skeleton were isolated from *Garcinia multiflora*. Their structures and absolute configurations were determined by extensive analysis of spectroscopic data and electronic circular dichroism (ECD) calculations. Compounds **1** and **2** exhibited cytotoxic activities against five human cancer cell lines in vitro (IC_{50} 3.42−13.23 μM). The acidification of lysosomes in HeLa cell was obviously affected by compound **2**.



The famous *Garcinia* (Guttiferae), from which a huge number of polycyclic polyprenylated acylphloroglucinols (PPAPs), xanthones, and flavonoids with highly complicated ring systems and broad array of biological activities were reported,[1−4] has attracted continuous attention in natural products chemistry, synthetic chemistry, and pharmacology.[5−9] Particularly, gambogic acid from *Garcinia hanburyi* has been approved for the treatment of lung cancer in phase II clinical trials in China.[9] *Garcinia multiflora* Champ., a small evergreen tree, is widely distributed in the south of China. As a Chinese folkloric medicine, the bark is used as an external medicine to reduce inflammation.[10] As a part of our effort to search for antitumor active natural products,[11−13] two PPAPs garcimulins A and B ((±)-**1** and **2**), including a pair of enantiomers [(+)-garcimulin A (**1a**) and (−)-garcimulin A (**1b**)] with the unique caged tetracyclo[5.4.1.1^{1,5}.0^{9,13}]tridecane skeleton, were isolated from the leaves and twigs of *G. multiflora* (Figure 1). To the best of our knowledge, this is the first reported coexistence of enantiomers (**1a** and **1b**) and positional isomers (**1** and **2**) from the Guttiferae family. The intramolecular Diels−Alder reaction postulated during the biosynthesis appears to be pivotal for the production of new skeleton. Herein, we describe the structural elucidation, plausible biosynthetic pathway, and biological evaluation of the isolated compounds.

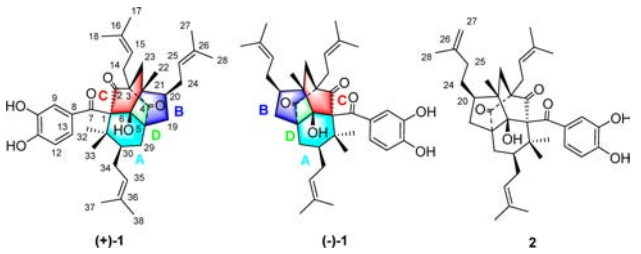


Figure 1. Structures of (+)-**1**, (−)-**1**, and **2**.

Garcimulin A (**1**) was isolated as a light brown oil. A molecular formula of $C_{38}H_{50}O_6$ is implied by the HR-EI-MS (*m*/*z* 602.3610 [M]$^+$, calcd 602.3607) along with ^{1}H and ^{13}C NMR data with 14 degrees of unsaturation. The IR spectrum revealed the presence of hydroxyl (3343 cm^{-1}), carbonyl groups (1735, 1704, and 1635 cm^{-1}), and aromatic ring (1606 and 1450 cm^{-1}). In the ^{1}H NMR and HMQC spectra, a trisubstituted benzene ring [δ_H 7.81 (1H, s, H-9), 7.42 (1H, br s, H-13), 7.23 (1H, br s, H-12)], three olefinic protons [δ_H 5.54 (1H, t, *J* = 7.0 Hz, H-15), 5.08 (1H, t, *J* = 6.6 Hz, H-25), 4.97 (1H, d, *J* = 6.2 Hz, H-35)], and nine methyls were apparent (Table 1). The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) data (Table 1) revealed 38 carbon signals, including nine methyls, six methylenes, eight methines (six olefinic ones), and 15 quaternary carbons (six olefinic ones and three carbonyls). Further analysis of 1D NMR demonstrated the presence of a trisubstituted benzene ring, three carbonyls, and three prenylated groups, accounting for 10 degrees of unsaturation. The remaining four degrees of unsaturation suggested compound **1** to be tetracyclic. All the information mentioned above as well as the reported data of PPAPs-type compounds indicated **1** could be a PPAP derivative.[14,15]

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of 1 and 2 (δ in ppm, J in Hz) in Pyridine-d_5

	1		2	
no.	δ_H	δ_C	δ_H	δ_C
1		70.2		70.2
2		205.0		204.9
3		68.5		68.5
4		212.2		212.2
5		61.1		61.1
6	OH-6 6.02 (s)	84.9	OH-6 6.00 (s)	84.8
7		204.3		204.3
8		133.2		133.1
9	7.81 (s)	118.6	7.81 (d, 2.0)	118.6
10		146.8		146.8
11		152.2		152.1
12	7.23 (br s)	115.2	7.23 (d, 8.0)	115.2
13	7.42 (br s)	123.4	7.42 (dd, 8.0, 2.0)	123.4
14a	2.68 (dd, 15.0, 7.5)	26.8	2.68 (dd, 15.0, 7.0)	26.8
14b	2.81 (dd, 15.0, 7.5)		2.82 (dd, 15.0, 7.0)	
15	5.54 (t, 7.0)	120.0	5.54 (t, 7.0)	120.0
16		134.4		134.4
17	1.73 (s)	26.2	1.72 (s)	26.1
18	1.64 (s)	18.1	1.63 (s)	18.1
19α	2.14 (m)	38.8	2.16 (m)	38.8
19β	1.74 (m)		1.72 (m)	
20	1.63 (m)	50.6	1.57 (m)	50.2
21		45.9		46.0
22	1.22 (s)	20.2	1.20 (s)	20.2
23a	2.21 (m)	43.5	2.22 (m)	43.5
23b	1.79 (m)		1.82 (m)	
24a	2.26 (m)	33.4	1.51 (m)	32.9
24b			1.78 (m)	
25a	5.08 (t, 6.6)	124.3	1.78 (m)	36.7
25b			2.08 (m)	
26		131.8		146.2
27	1.71 (s)	25.9	4.79 (s); 4.81 (s)	110.3
28	1.65 (s)	18.2	1.72 (s)	22.6
29α	2.36 (dd, 12.9, 3.1)	32.1	2.39 (dd, 12.9, 3.1)	32.0
29β	1.95 (t, 12.9)		1.96 (t, 12.9)	
30		44.0	1.22 (m)	44.0
31		49.7		49.7
32	1.45 (s)	27.1	1.45 (s)	27.1
33	1.53 (s)	22.0	1.54 (s)	22.0
34a	1.78 (m)	29.2	1.79 (m)	29.2
34b	2.12 (m)		2.14 (m)	
35	4.97 (t, 6.2)	123.9	4.99 (t, 6.2)	123.9
36		132.9		132.9
37	1.58 (s)	25.8	1.59 (s)	25.8
38	1.54 (s)	17.9	1.56 (s)	17.9

Comprehensive analysis of the 1D and 2D NMR spectra (Figure 2) indicated that compound **1** featured a unique caged tetracyclo[5.4.1.1^{1,5}.0^{9,13}]tridecane skeleton. The HMBC correlations of a proton signal at δ_H = 6.02 ppm (OH-6) with C-1 (δ_C 70.2), C-5 (δ_C 61.1), and C-6 (δ_C 84.9) indicated a linkage of C-1, C-5, and OH-6 to C-6. Moreover, a six-membered ring (A-ring) was established by the HMBC correlations of Me-32 to C-1, C-30, and C-31, and H$_2$-29 to C-5, C-6, and C-31, along with ^{1}H−^{1}H COSY cross-peaks of H$_2$-29/H-30. The HMBC correlations of Me-22/C-6, C-20, and C-21, indicated that C-6, C-20, and C-22 were linked through C-21 as shown in Figure 2. In addition, ^{1}H−^{1}H COSY correlations of H$_2$-19/H-20 and

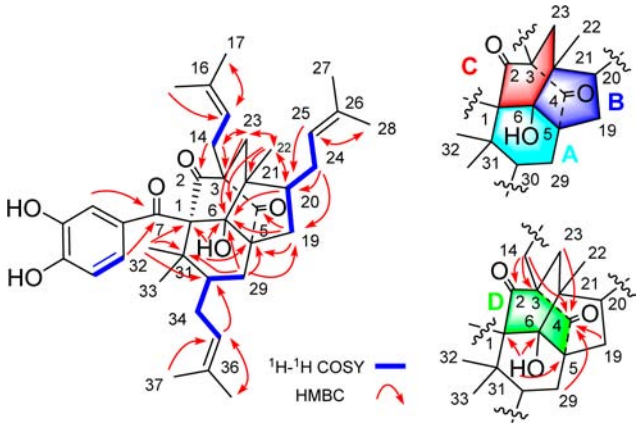

Figure 2. Key HMBC and ^{1}H−^{1}H COSY correlations of **1**.

HMBC correlations from H$_2$-19 to C-5 and C-6, and from H-20 to C-6 could assign the five-membered ring B with a methyl at C-21.

In addition, the HMBC correlations of Me-22 to C-6 (δ_C 84.9), C-21 (δ_C 45.9), and C-23 (δ_C 43.5) revealed the connection of C-23 and C-6 via C-21. In the HMBC spectrum, cross-peaks of H$_2$-14 to C-2 (δ_C 205.0), C-3 (δ_C 68.5), C-4 (δ_C 212.2), and C-23 implied that C-2, C-4, C-14, and C-23 were connected to each other through C-3. Similarly, the key correlations from both H$_2$-14 and H$_2$-23 to C-3 and C-4, and from both H$_2$-19 and H$_2$-29 to C-4 and C-5, implied the linkage of C-3 and C-5 through C-4. Two "loose ends" of carbonyl C-2 (δ_C 205.0) and benzophenone C-7 (δ_C 204.3) and the severely downfield-shifted signal at δ_C = 70.2 ppm (C-1) along with the remaining one degree of unsaturation implied that two carbonyls C-2 and C-7 were connected via C-1, which was in good agreement with the literature.[15] Thus, the bicyclo[2.2.2] was constructed, forming C and D rings. The 1D and 2D NMR could easily assign three prenylated groups at C-3, C-20, and C-30, respectively. Therefore, the above evidence established the planar structure of **1**.

The relative configuration of **1** was assigned by the ROESY experiment (Figure 3), in which cross-peaks from 6-OH to H-

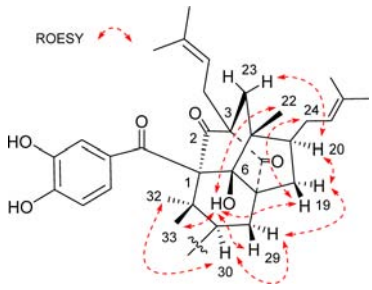

Figure 3. Key ROESY correlations of **1**.

19β, Me-22, H-29β, and Me-33, and from H-19β to H-24a, indicated that these groups were cofacial and randomly assigned as β-oriented. In contract, the evident ROESY correlations of H-30/H-29α, H-30/Me-32, H-29α/H-19α, H-19α/H-20, and H-20/H-23b implied α-orientation of these groups. Thus, two prenylated groups at C-20 and C-30 occupied the β-orientation. Because of the rigid caged tetracyclo[5.4.1.1^{1,5}.0^{9,13}]tridecane core, the prenylated group at C-3 was fixed as α-oriented.

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It could be presumed that garcimulin A (**1**) is a pair of enantiomers since the CD spectrum is a line as well as the small optical activity ($[\alpha]_D^{21}$ −4.76). Luckily, with the help of CHIRALPAK IC column, (+)-garcimulin A (**1a**) and (−)-garcimulin A (**1b**) with the opposite Cotton effects in CD spectra and opposite optical rotations were successfully obtained, which was further confirmed by the same 1D NMR data of **1a** and **1b**. The absolute configurations of compounds **1a** and **1b** were determined by comparing the experimental with the computational electronic circular dichroism (ECD) spectra (Figure 4).[16]

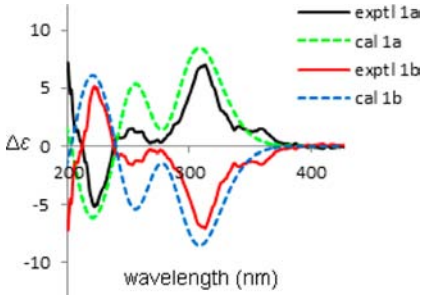

Figure 4. Calculated and experimental ECD spectra of **1a** and **1b**.

Garcimulin B (**2**) gave the molecular formula of $C_{38}H_{50}O_6$ on the basis of HR-EI-MS (m/z 602.3604 $[M]^+$, calcd 602.3607), the same as **1**. Comparison of the 1D NMR data with those of **1** implied that they possessed a similar structure except for the presence of a terminal double bond in **2** instead of a nonterminal double bond in the latter. The HMBC correlations from Me-28 and H_2-25 to C-26 (δ_C 146.2) and C-27 (δ_C 110.3) assigned the terminal double bond at C-26 and C-27. The prenylated group with the terminal double bond was connected to C-20 by the HMBC correlations of H_2-25 with C-20. The relative configuration of **2** was identical to that of **1** by analysis of the ROESY spectrum. In addition, the CD spectrum of **2** matched well with that of **1b**, establishing the absolute configuration of **2** (Supporting Information).

Structurally, (±)-**1** and **2** possessed the same skeleton of PPAP derivatives and unique caged tetracyclo[5.4.1.1^{1,5}.0^{9,13}]tridecane core with a slight difference at the side chain, but their optical characters were poles apart. A double-bond shift in the course of biosynthesis was presumed to be the key to explain the phenomenon. Biogenetically, both (±)-**1** and **2** were originated from the same precursor **i**, while the shift of a double bond differed their destiny. The key intermediate **iii** might undergo an intramolecular Diels−Alder reaction[15] in two different faces to form racemic mixture (**iv** and **vi**), which then converted to corresponding **1a** and **1b** after the migration of a double bond. However, the intermediate **ix** with a chiral center could also undergo an intramolecular Diels−Alder reaction in the left face to get **x**, which could get **2** through the migration of a double bond and a prenylated group.

Three compounds (**1a**, **1b**, and **2**) were evaluated for their cytotoxicities against five human tumor cell lines[13] (HL-60, SMMC-7721, A-549, MCF-7, and SW480) and their inhibitory activity against LPS-induced NO production in RAW264.7 cells (Table 2).[17] Interestingly, compounds **1a** and **2** were found to have moderate cytotoxicities against the cancer cell lines in vitro (IC_{50} 3.42−13.23 μM), while **1b** did not.[18,19] In an anti-inflammatory assay, compound **2** exhibited a moderate inhibitory

Scheme 1. Plausible Biosynthetic Pathways of (±)-1 and 2

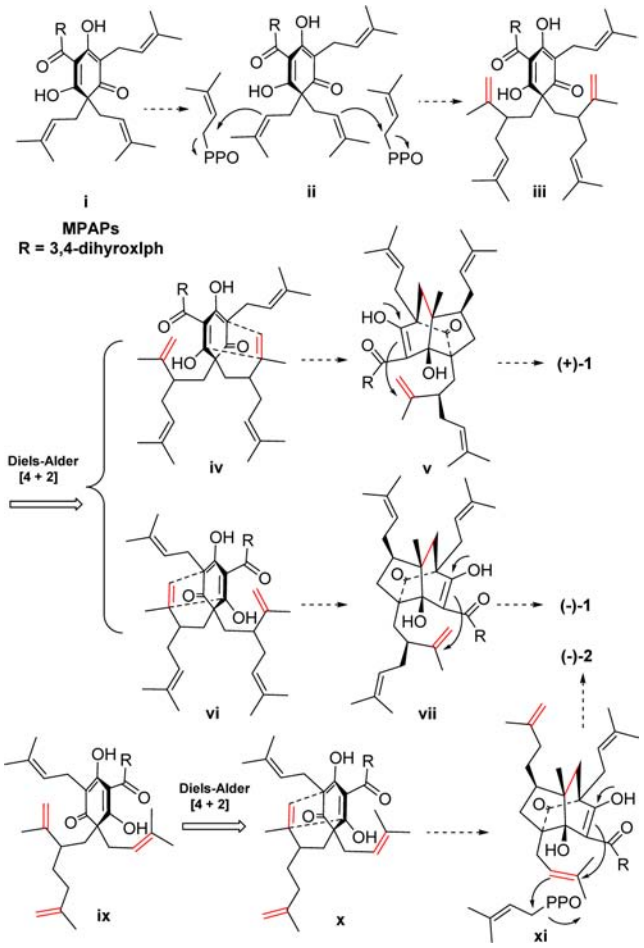

Table 2. Cytotoxicities and Anti-Inflammations of Compounds 1a, 1b, and 2 (IC_{50} μM)

no.	HL-60	SMMC-7721	A-549	MCF-7	SW480	NO
1a	3.42	4.19	4.51	4.18	7.22	>20
1b	>20	>20	>20	>20	>20	>20
2	12.85	7.57	7.10	12.24	13.23	15.10
P[a]	1.01	5.29	6.13	13.62	14.03	0.16

[a]Positive controls for cytotoxicities and anti-inflammations were DDP and MG-132, respectively.

effect on LPS-stimulated NO production in RAW 264.7 cells with the IC_{50} value of 15.1 μM.

Since cancer cells use autophagy as a survival strategy to provide essential biomolecules required for cell viability under metabolic stress, the autophagy is important to the development of cancer.[20,21] Autophagy takes place in lysosome, and acidification of lysosomes is critical to the development of cancer.[20,21] Thus, the effect on the acidification of lysosomes could be regarded as an indicator of anticancer ability. Four compounds [(±)-**1**, **1a**, **1b**, and **2**] were tested for the effect of lysosomal acidification in HeLa cell, and **2** exhibited strong suppression of the lysosomal acidification (Figure 5). To the best of our knowledge, this is the first reported PPAP effecting lysosomal acidification.

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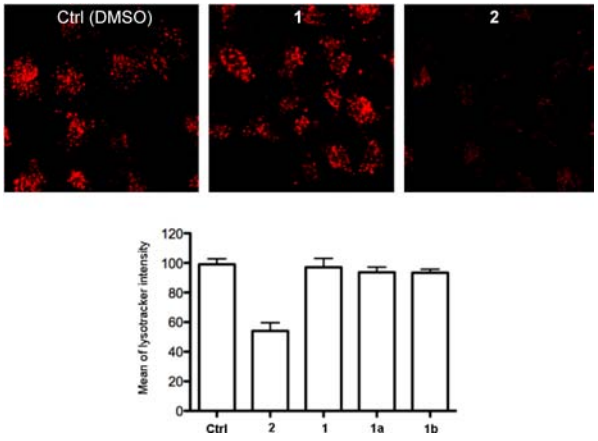

Figure 5. Effect of compounds (±)-**1**, **1a**, **1b**, and **2** (20 μM) on lysosomal acidification in HeLa cell.

■ ASSOCIATED CONTENT

Supporting Information

Computational details of **1a** and **1b**, experimental procedures, and spectroscopic data for (±)-**1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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