Benzophenone Derivatives from the Fruits of *Garcinia multiflora* and Their Anti-inflammatory Activity

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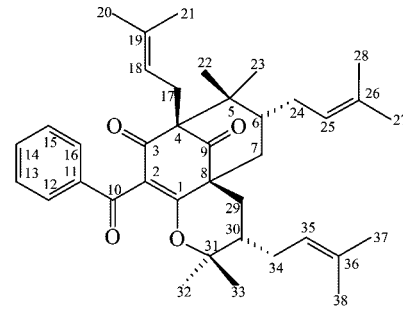
Five new benzophenone derivatives, 13,14-didehydoxyisogarcinol (**1**), garcimultiflorone A (**2**), garcimultiflorone B (**3**), 13-hydroxygarcimultiflorone B (**4**), and garcimultiflorone C (**5**), have been isolated from the fruits of *Garcinia multiflora*, together with seven known compounds (**6**−**12**). The structures of these new compounds were determined through spectroscopic and MS analyses. 13,14-Didehydoxyisogarcinol (**1**), garcimultiflorone A (**2**), garcimultiflorone B (**3**), and 13-hydroxygarcimultiflorone B (**4**) exhibited inhibition with an IC_{50} range of 0.11−5.58 μM on superoxide anion generation and elastase release by human neutrophils in response to fMet-Leu-Phe/cytochalasin B (fMLP/CB).

Garcinia multiflora (Guttiferae) Champ. is a small evergreen tree, distributed in South China, Hong Kong, and Taiwan.[1] Xanthones,[2−5] biflavonoids,[6] benzophenones,[7,8] acylphloroglucinols,[9−11] and their derivatives are widely distributed in plants of the genus *Garcinia*. Many of these compounds exhibit cytotoxic,[2,3,7] anti-inflammatory,[4,10,11] antitubercular,[5] anti-HIV,[6] and antioxidant[7] activities. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for *in vitro* anti-inflammatory activity, and *G. multiflora* has been found to be one of the active species. Five new benzophenone derivatives, 13,14-didehydoxyisogarcinol (**1**), garcimultiflorone A (**2**), garcimultiflorone B (**3**), 13-hydroxygarcimultiflorone B (**4**), and garcimultiflorone C (**5**), and seven known compounds (**6**−**12**) have been isolated and identified from the fruits of *G. multiflora.* This paper describes the structural elucidation of **1**−**5** and the anti-inflammatory activities of the isolates.

Results and Discussion

Chromatographic purification of the EtOAc-soluble fraction of a MeOH extract of fruits of *G. multiflora* on a silica gel column and preparative TLC afforded five new (**1**−**5**) and seven known compounds (**6**−**12**).

13,14-Didehydoxyisogarcinol (**1**) was isolated as colorless, amorphous powder, $[\alpha]^{25}_{D}$ −185. Its molecular formula, $C_{38}H_{50}O_4$, was determined on the basis of the positive HRESIMS ion at *m*/*z* 593.3610 $[M + Na]^+$ (calcd 593.3607) and supported by the ^1H, ^{13}C, and DEPT NMR data. The presence of carbonyl groups was revealed by the bands at 1724, 1679, and 1639 cm^{-1} in the IR spectrum and was confirmed by signals at δ 193.6, 194.0, and 206.4 in the ^{13}C NMR spectrum. The ^1H NMR data of **1** were similar to those of isogarcinol,[12] except that the C-2 benzoyl group [δ 7.38 (2H, t, J = 8.0 Hz, H-13 and H-15), 7.50 (1H, t, J = 8.0 Hz, H-14), and 7.73 (2H, d, J = 8.0 Hz, H-12 and H-16)] of **1** replaced the 3,4-dihydroxybenzoyl group of isogarcinol.[12] This was supported by HMBC correlations observed between H-12 (δ 7.73) and C-10 (δ 194.0), C-14 (δ 133.0), and C-16 (δ 128.9). The absolute configuration of **1** was evidenced by CD Cotton effects at 346 nm ([θ] +2510), 320 nm ([θ] −1217), 298 nm ([θ] +3843), 263 nm ([θ] −23 153), and 220 nm ([θ] +20 752) in analogy with those of isogarcinol.[12] According to the evidence above, the structure of



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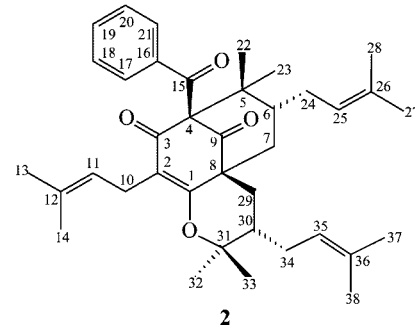

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1 was elucidated as 13,14-didehydoxyisogarcinol. This was further confirmed by ^1H−^1H COSY and NOESY (Figure 1) experiments. The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Figure 1) techniques.

Garcimultiflorone A (**2**) was obtained as an amorphous powder, and the sodium adduct ion $[M + Na]^+$ (*m*/*z* 593.3605) in the HRESIMS was consistent with the formula $C_{38}H_{50}O_4Na$. The presence of carbonyl groups was revealed by the bands at 1721, 1695, and 1641 cm^{-1} in the IR spectrum and was confirmed by the resonances at δ 193.2, 193.7, and 209.0 in the ^{13}C NMR spectrum. The ^1H NMR data (Table 2) of **2** were similar to those of **1**, except that the 2-isoprenyl [δ 1.61 (3H, s, H-14), 1.63 (3H, s, H-13), 3.03 (1H, dd, J = 13.6, 7.6 Hz, H-10), 3.08 (1H, dd, J = 13.6, 7.6 Hz, H-10), and 5.00 (1H, t, J = 7.6 Hz, H-11)] and 4-benzoyl groups [δ 7.22 (2H, t, J = 8.0 Hz, H-18 and H-20), 7.38 (1H, t, J = 8.0 Hz, H-19), 7.53 (2H, d, J = 8.0 Hz, H-17 and H-21)] of **2** replaced the 2-benzoyl and 4-isoprenyl groups of **1**. This was supported by the following NOESY and HMBC correlations: (a) HMBC correlations were observed between H-10 (δ 3.03,

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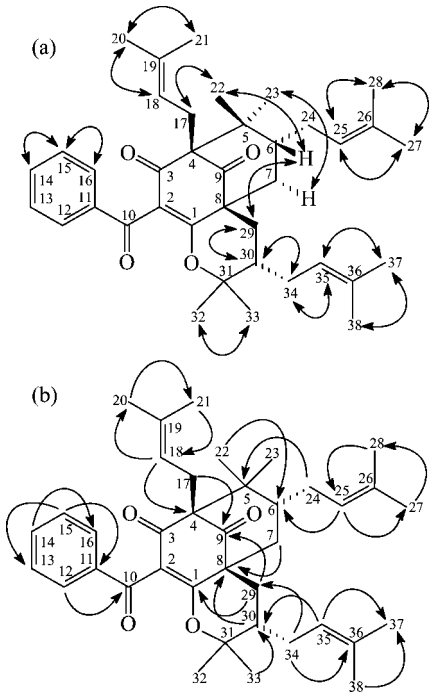


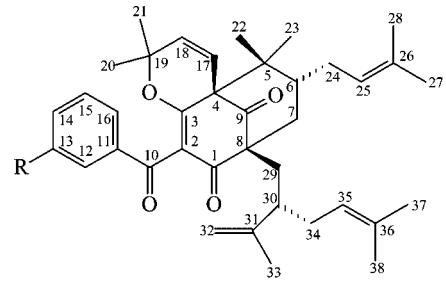
Figure 1. NOESY (a) and HMBC (b) correlations of **1**.

Table 1. ^{1}H NMR Data of **1** and **2**[a]

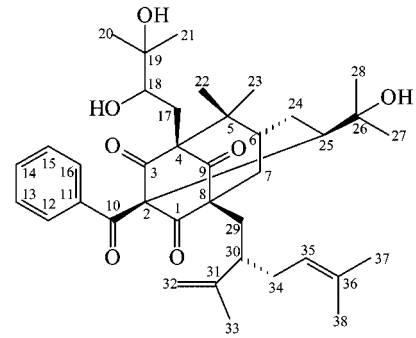
	1	**2**		
position	δ_H *J* (Hz)	δ_H *J* (Hz)	NOE	HMBC
6	1.42 m	1.46 m	7, 22, 24, 29	4, 5, 8, 22, 25
7	1.47 m	1.85 m	23, 24	1, 5, 8, 9, 24
	2.13 dd (13.6, 4.4)	2.52 d (14.0)	6	1, 5, 9
10		3.03 dd (13.6, 7.6)	11, 14	1, 3, 12
		3.08 dd (13.6, 7.6)	11, 14	1, 2, 3
11		5.00 br t (7.6)	10, 13	2, 13
12	7.73 br d (8.0)			
13	7.38 br t (8.0)	1.63 s	11, 14	11, 14
14	7.50 br t (8.0)	1.61 s	10, 13	11, 12, 13
15	7.38 br t (8.0)			
16	7.73 br d (8.0)			
17	2.43 dd (13.6, 5.0)	7.53 d (8.0)	18	15, 19
	2.69 dd (13.6, 8.0)			
18	4.87 dd (8.0, 5.0)	7.22 t (8.0)	17, 19	16, 20
19		7.38 t (8.0)	18, 20	17, 21
20	1.64 s	7.22 t (8.0)	19, 21	16, 18
21	1.60 s	7.53 d (8.0)	20, 22	15, 19
22	1.11 s	1.36 s	6, 21	4, 5, 6, 23
23	0.75 s	1.45 s	7, 24	4, 5, 6
24	1.73 m	2.08 m	6, 23, 25	5, 6, 7, 26
	2.19 m			5, 26
25	5.07 t (7.2)	4.88 br t (6.8)	24, 27	6, 26, 28
27	1.71 s	1.70 s	25, 28	25, 26, 28
28	1.58 s	1.56 s	27	25, 27
29	0.98 m	1.66 m	6, 7, 30	1, 8, 9, 31
	3.03 dd (14.0, 3.6)	1.91 m	7, 30	1, 7, 8, 9, 34
30	2.01 m	1.90 m	29, 33, 34	8, 32, 35
32	1.21 s	1.54 s	33, 34	30, 31, 33
33	0.89 s	1.20 s	32, 30	30, 32
34	1.79 m	1.80 m	30, 35	29, 31, 36
	2.02 m	2.15 m	30, 35	31, 36
35	5.18 t (6.8)	5.08 br t (6.8)	34, 37	30, 36, 38
37	1.77 s	1.73 s	35, 38	35, 38
38	1.60 s	1.61 s	34, 37	35, 37

[a] Recorded in $CDCl_3$ at 400 MHz. Values in ppm (δ). *J* (in Hz) in parentheses.

3.08) and C-1 (δ 167.7), C-3 (δ 193.2), and C-12 (δ 131.9), (b) HMBC correlations were observed between H-17 (δ 7.53) and C-15 (δ 193.7) and C-19 (δ 131.8), and (c) NOESY correlations were observed between H-21 (δ 7.53) and H-22 (δ 1.36). The relative configuration of **2** was elucidated on the basis of NOESY experiments. The NOESY cross-peaks between H-21/H-22, H-22/H-6, H-6/H-29, H_α-7/H-23, H_α-7/H-24, and H-23/H-24 suggested that H-6, the 4-benzoyl group, and the bond between C-8 and C-29 are β-oriented, and the isoprenyl group at C-6 is α-oriented. To further clarify the relalive configuration of **2**, a computer-assisted 3D structure was obtained by using the molecular modeling program CS CHEM 3D Ultra 10.0, with MM2 force-field calculations for energy minimization. The calculated distances between H-21/H-22 (3.096 Å), H-22/H-6 (2.617 Å), H-6/H-29 (3.855 Å), H-6/H_β-7 (2.354 Å), H_α-7/H-23 (2.271 Å), and H_α-7/H_α-24 (2.798 Å) are all less than 4 Å. This is consistent with the NOESY interactions between each of these proton pairs. The structure of garcimultiflorone A (**2**) was further confirmed by the ^{1}H−^{1}H COSY, NOESY DEPT, HSQC, and HMBC techniques (Table 1).



3 R = H
4 R = OH



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Garcimultiflorone B (**3**) was obtained as a colorless, amorphous powder. HRESIMS gave an $[M + Na]^+$ ion at *m/z* 591.3446 (calcd for $C_{38}H_{48}O_4Na$, 591.3450), consistent with a molecular formula of $C_{38}H_{48}O_4Na$. Carbonyl groups were indicated by the bands at 1723, 1699, and 1646 cm^{-1} in the IR spectrum and were confirmed by resonances at δ 192.2, 193.4, and 206.9 in the ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR data of **3** were similar to those of **1**, except that the 2,2-dimethyl-3,4-dehydropyran moiety at C-3,4 and the lavandulyl group at C-8 of **3** replaced the isoprenyl group at C-4 and 2,2-dimethyl-3-(3-methylbut-2-enyl)pyrano moiety at C-1,8 of **1**. This was supported by the following NOESY and HMBC correlations: (a) NOESY correlations were observed between H-18 (δ 5.24) and H-17 (δ 6.45), H-20 (δ 0.54), and H-21 (δ 1.38), (b) HMBC correlations were observed between H-17 (δ 6.45) and C-3 (δ 167.3), C-4 (δ 70.9), C-5 (δ 50.0), C-9 (δ 192.2), and C-19 (δ 83.2), (c) NOESY correlations were observed between H-30 (δ 2.59) and H-29 (δ 1.78), H-32 (δ 4.59), H-34 (δ 2.06), and H-35 (δ 5.02), and (d) HMBC correlations were observed between H-29 (δ 1.78, 2.17) and C-1 (δ 206.9), C-7 (δ 43.3), C-8 (δ 62.5), C-9 (δ 192.2), C-31 (δ 148.4), and C-34 (δ 33.0). The NOESY experiment of **3** showed selected cross-peaks as shown in the 3D drawing (Figure 2). The relative configuration of **3** was deduced from the NOESY cross-peaks of H-17/H-22, H-22/H-6, H-6/H-29, H_α-7/H-23, and H_α-7/H_α-24. Consequently, H-6, the lavandulyl group at C-8, and the bond between C-4 and C-17 are β-oriented, and the isoprenyl group at C-6 is α-oriented. Based on

Table 2. ^{1}H NMR Data of **3**−**5**[a]

	3	**4**			**5**		
position	δ_H *J* (Hz)	δ_H *J* (Hz)	NOE	HMBC	δ_H *J* (Hz)	NOE	HMBC
6	1.45 m	1.46 m	7, 22, 24	4, 5, 7, 22, 25	2.07 m	7, 22, 24	4, 5, 8, 22, 23, 25
7	2.13 m	2.13 m	6, 25	1, 5, 8, 9, 29	1.80 d (13.4)	24	1, 8, 9, 24
					2.68 dd (13.4, 6.8)	6	1, 8, 9, 24
12	7.71 br d (8.0)	7.15 t (2.0)		10, 13, 16	7.31 br d (7.6)	13, 27, 33	10, 11, 14
13	7.29 br t (8.0)				7.29 br t (7.6)	12, 14	11, 15
14	7.44 br t (8.0)	6.94 br dd (8.0, 2.0)	15	12, 13, 16	7.42 br t (7.6)	13, 15	12, 16
15	7.29 br t (8.0)	7.15 t (8.0)	14, 16	11, 13	7.29 br t (7.6)	14, 16	11, 13
16	7.71 br d (8.0)	7.24 br d (8.0)	15	10, 11, 14	7.31 br d (7.6)	15, 20	10, 11, 14
17	6.45 d (10.4)	6.46 d (10.4)	18, 22	3, 4, 5, 9, 19	1.64 dd (14.8, 3.2)	18, 20, 22	3, 9
					3.44 dd (14.8, 11.6)	21	3, 4, 5, 9, 19
18	5.24 d (10.4)	5.27 d (10.4)	17, 21	4, 19, 20, 21	4.93 dd (11.6, 3.2)	17, 20	4, 20, 21
20	0.54 s	0.65 s	21	18, 21	1.19 s	16, 18	18, 21
21	1.38 s	1.40 s	18, 20	18, 19, 20	1.18 s	17, 37	18, 19, 20
22	1.39 s	1.38 s	6, 17, 23	4, 5, 6	1.34 s	6, 17	4, 5, 6
23	1.49 s	1.48 s	7α, 22, 24	4, 5, 6, 22	1.48 s	7, 24	4, 6, 22
24	2.09 m	2.09 m	6, 25	5, 7, 26	1.49 m	25	5, 7, 26
	2.29 br d (15.2)	2.28 br d (14.2)	6, 25	5, 7, 26	2.34 m	25	5, 7
25	4.86 br t (6.8)	4.85 br t (6.8)	24, 27	6, 27, 28	2.72 t	24, 27	1, 2, 3, 6, 10, 28
27	1.68 s	1.66 s	25, 28	25, 26, 28	1.31 s	25, 28	25, 28
28	1.53 s	1.53 s	27	25, 27	1.17 s	27	25, 27
29	1.78 dd (14.2, 4.6)	1.89 dd (14.0, 5.5)	30	1, 7, 8, 9, 31, 34	1.84 dd (14.4, 4.0)	30	1, 7, 8, 9, 31
	2.17 dd (14.2, 9.4)	2.09 dd (14.0, 8.6)	30	1, 7, 8, 9	2.27 dd (14.4, 9.6)	7	1, 8, 9
30	2.59 m	2.59 m	29, 32, 34	8, 31, 32, 35	2.59 m	33, 34	8, 32, 35
32	4.59 br s	4.61 br s	30	30, 31, 33	4.61 br s	29, 34	30, 31, 33
	4.68 br s	4.69 br s	30, 33	30, 31	4.69 br s	33, 33	30, 31, 33
33	1.60 s	1.61 s	32	30, 31, 32	1.56 s	12, 32	30, 31, 32
34	2.06 m	2.02 br t (7.0)	30, 35	29, 31, 36	2.08 m	30, 32	29, 31, 36
35	5.02 br t (6.8)	5.02 br t (7.0)	34, 37	30, 37, 38	5.03 br t (6.8)	30, 37	30, 36, 37, 38
37	1.68 s	1.68 s	35, 38	35, 36, 38	1.68 s	21, 35, 38	35, 38
38	1.60 s	1.59 s	37	35, 37	1.61 s	34, 37	35, 37
OH-13		5.18 br s					

[a] Recorded in $CDCl_3$ at 400 MHz. Values in ppm (δ). *J* (in Hz) in parentheses.

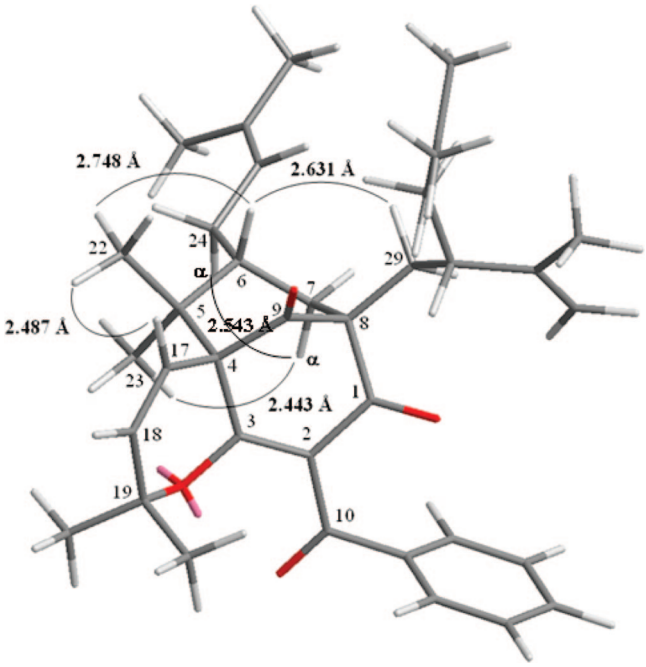

Figure 2. Selected NOESY correlations and relative configuration of **3**.

the information from the ^{1}H NMR, COSY, and NOESY spectra, a computer-generated 3D structure was obtained by using the above-mentioned molecular modeling program with MM2 force-field calculations for energy minimization. The calculated distances between H-17/H-22 (2.487 Å), H-22/H-6 (2.748 Å), H-6/H-29 (2.631 Å), H_α-7/H-23 (2.443 Å), and H_α-7/H_α-24 (2.543 Å) are all less than 4.00 Å; this is consistent with the well-defined NOESY observed for each of the proton pairs. The structure of garcimultiflorone B (**3**) was further confirmed by the ^{1}H−^{1}H COSY, NOESY, DEPT, HSQC, and HMBC experiments (Figure 3).

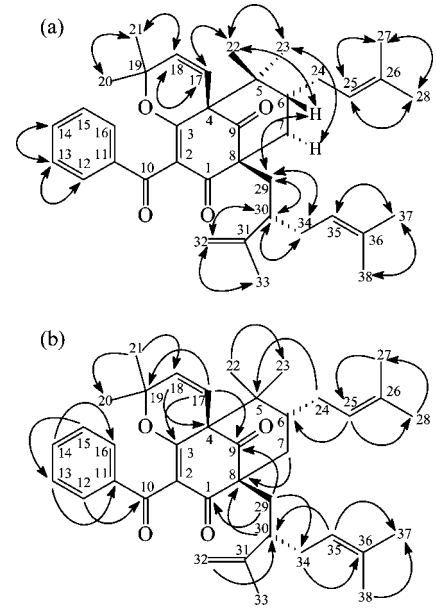

Figure 3. NOESY (a) and HMBC (b) correlations of **3**.

13-Hydroxygarcimultiflorone B (**4**) was isolated as an amorphous powder. The ESIMS of **4** afforded an $[M + Na]^+$ ion at *m*/*z* 607, implying a molecular formula of $C_{38}H_{48}O_5$, which was confirmed by HRESIMS. The IR spectrum showed the presence of OH (3365 cm^{-1}) and carbonyl (1720, 1700, and 1641 cm^{-1}) groups. Comparison of the ^{1}H NMR data (Table 2) of **4** with those of **3** suggested that their structures were closely related, except that OH-13 [δ 5.18 (1H, br s, D_2O exchangeable)] of **4** replaced H-13 [δ 7.29 (1H, t, J = 8.0 Hz)] of **3**. This was supported by HMBC correlations

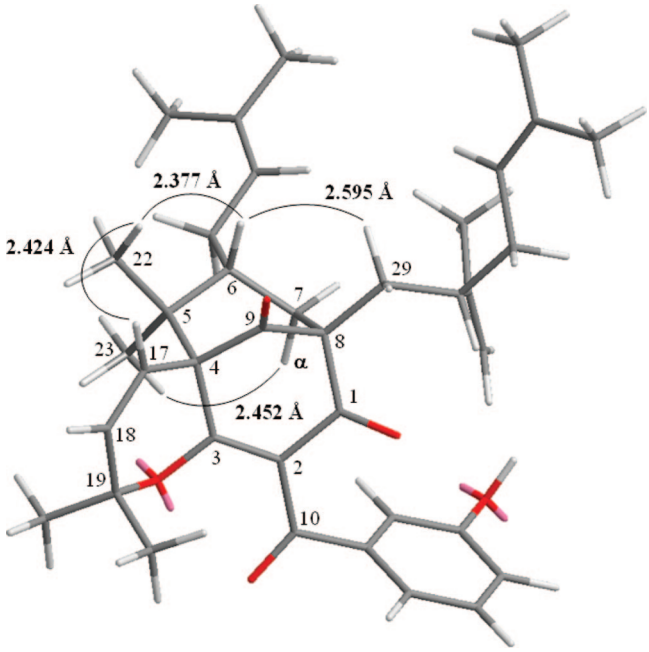

Figure 4. Selected NOESY correlations and relative configuration of **4**.

between OH-13 (δ 5.18) and C-13 (δ 155.4) and C-12 (115.0). Compound **4** showed similar NOESY cross-peaks (Figure 4) between H-17/H-22, H-22/H-6, H-6/H-29, $H_α$-7/H-23, and H-23/H-24 when compared to **3**, and their relative configurations have to be identical. The structure of **4** was thus elucidated as 13-hydroxygarcimultiflorone B. This structure was supported by $^1H-^1H$ COSY and NOESY experiments (Table 2), and ^{13}C NMR assignments were confirmed by DEPT, HSQC, and HMBC techniques (Table 2).

Garcimultiflorone C (**5**) had a molecular formula of $C_{38}H_{52}O_7$ as determined by positive-ion HRESIMS. The presence of OH and carbonyl groups was revealed by the bands at 3417, 1731, and 1694 cm^{-1}, respectively, in the IR spectrum. Comparison of the ^1H NMR data (Table 2) of **5** with those of **3** suggested that their structures are related except that the 2,3-dihydroxy-3-methylbutyl group at C-4, the 3-hydroxy-3-methylbutyl group at C-6, and the linkage between C-2 and C-25 of **5** replaced the 2,2-dimethyl-3,4-dehydroxypyran moiety at C-3,4 and the isoprenyl group at C-6 of **3**. This was supported by the following NOESY and HMBC correlations: (a) HMBC correlations were observed between H-17 (δ 3.44) and C-3 (δ 208.7), C-5 (δ 50.7), C-9 (δ 205.7), and C-19 (δ 84.1), (b) NOESY correlations were observed between H-18 (δ 4.93) and H-17 (δ 1.64 and 3.44), H-20 (δ 1.19), and H-21 (δ 1.18), (c) HMBC correlations were observed between H-25 (δ 2.72) and C-1 (δ 203.9), C-2 (δ 65.7), C-3 (δ 208.7), C-6 (δ 44.6), and C-10 (δ 192.4), and (d) NOESY correlations were observed between H-25 (δ 2.72) and H-24 (δ 1.49 and 2.34), H-27 (δ 1.31), and H-28 (δ 1.17). The NOESY experiment of **5** showed selected cross-peaks as indicated in the 3D drawing (Figure 5). The relative configuration of **5** was deduced from the NOESY cross-peaks of H-12/H-33, H-16/H-20, H-21/H-37, and H-12/H-27, showing that the benzoyl group at C-2, the 2,3-dihydroxy-3-methylbutyl group at C-4, the lavandulyl group at C-8, and the 2-hydroxypropan-2-yl group at C-25 are β-oriented. Based on the information from the ^1H NMR, COSY, and NOESY spectra, a computer-generated 3D structure was obtained by using the above-mentioned molecular modeling program with MM2 force-field calculations for energy minimization. The calculated distances between H-12/H-33 (3.536 Å), H-16/H-20 (2.891 Å), H-21/H-37 (3.446 Å), and H-12/H-27 (2.820 Å) are all less than 4.00 Å; this is consistent with the well-defined NOESY observed for each of the proton pairs. The structure of garcimultiflorone C (**5**) was further confirmed by the $^1H-^1H$ COSY, NOESY, DEPT, HSQC, and HMBC techniques (Table 2).

The known isolates were readily identified by comparison of physical and spectroscopic data (UV, IR, ^1H NMR, $[α]_D$, and MS) with corresponding authentic samples or literature values, and this included two coumarins, aesculetin dimethyl ether (**6**)[13] and 6,7,8-trimethoxycoumarin (**7**),[14] a triterpene, squalene (**8**),[15] a benzopyran, δ-tocotrienol (**9**),[16] and three steroids, β-sitostenone (**10**)[17] and a mixture of β-sitosterol (**11**)[18] and stigmasterol (**12**).[18]

Neutrophils accumulate at sites of inflammation and immunological reaction in response to locally existing chemotactic mediators. The bacterial *N*-formyl peptides, such as formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), are some of the first identified and most potent chemoattractants for neutrophils.[19] When fMLP was used as a stimulant, cytochalasin B (CB), a priming agent, was incubated for 3 min before activation by peptide (fMLP/CB). In this study, the effects on neutrophil pro-inflammatory responses of compounds isolated from the fruits of *G. multiflora* were evaluated by suppressing fMLP/CB-induced superoxide anion ($O_2^{\bullet-}$) generation and elastase release by human neutrophils. The inhibitory activity data on neutrophil pro-inflammatory responses are shown in Table 3. Diphenyleneiodonium and phenylmethylsulfonyl fluoride were used as positive controls for superoxide anion generation and elastase release, respectively. From the results of our biological tests, the following conclusions can be drawn: (a) Compounds **1**−**5** exhibited inhibitory activities ($IC_{50} \leq 7.21$ μM) on human neutrophil $O_2^{\bullet-}$ generation; (b) compounds **1**−**4** inhibited fMLP/CB-induced elastase release with IC_{50} values ≤ 4.65 μM; (c) among the benzophenone analogues (**1**−**5**), compounds **3** and **4**, with a 2,2-dimethyl-3,4-dehydropyran moiety at C-3,4, exhibited more effective inhibition than analogues **1**, **2**, and **5** against fMLP/CB-induced $O_2^{\bullet-}$ generation and elastase release; (d) compound **3**, with a benzoyl group at C-2, showed stronger inhibition than the analogous benzophenone **4**, with a 3-hydroxybenzoyl group at C-2, against fMLP/CB-induced $O_2^{\bullet-}$ generation and elastase release; (e) garcimultiflorone B (**3**) was the most effective among the isolated compounds, with IC_{50} values of 0.11 ± 0.04 and 0.14 ± 0.02 μM, respectively, against fMLP/CB-induced superoxide anion generation and elastase release.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Jasco DIP-370 polarimeter in $CHCl_3$. CD spectra were recorded on a Jasco J-810 spectropolarimeter. UV spectra were obtained on a Jasco UV-240 spectrophotometer. IR spectra (KBr or neat) were recorded on a Perkin-Elmer system 2000 FT-IR spectrometer. NMR spectra, including COSY, NOESY, HMBC, and HSQC experiments, were recorded on a Varian Unity 400 spectrometer operating at 400 MHz (^1H) and 100 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ) using TMS as an internal standard. EI, ESI, and HRESI mass spectra were recorded on a Bruker APEX II mass spectrometer. HREI mass spectra were recorded on a JEOL JMX-HX 110 mass spectrometer. Silica gel (70−230, 230−400 mesh) (Merck) was used for CC. Silica gel 60 F-254 (Merck) was used for TLC and PTLC.

Plant Material. The fruit of *G. multiflora* was collected from Mudan, Pingtung County, Taiwan, in September 2007 and identified by Dr. I. S. Chen. A voucher specimen (Chen 6061) was deposited in the Faculty of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Separation. The dried fruit of *G. multiflora* (3 kg) was pulverized and extracted with MeOH (3 × 10 L) for 3 days. The MeOH extracts were concentrated under reduced pressure at 35 °C, and the residue (330 g) was partitioned between EtOAc and H_2O (1:1). The EtOAc layer was concentrated to give a residue (fraction A, 167 g). The water layer was further extracted with *n*-BuOH, and the *n*-BuOH-soluble part (fraction B, 55 g) and the water-solubles (fraction C, 95 g) were separated. Fraction A (120 g) was chromatographed on silica gel (70−230 mesh, 4.8 kg), eluting with CH_2Cl_2, gradually increasing the polarity with MeOH to give 14 fractions: A1 (8.5 L, CH_2Cl_2), A2 (6 L, CH_2Cl_2/MeOH, 98:1), A3 (5 L, CH_2Cl_2/MeOH, 95:

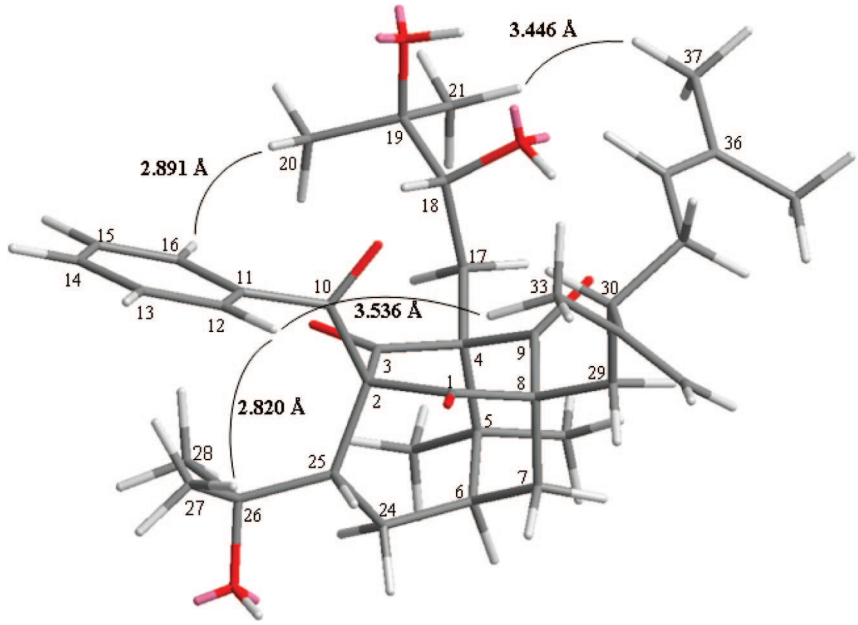

Figure 5. Selected NOESY correlations and relative configuration of **5**.

Table 3. Inhibitory Effects of **1**−**12** on Superoxide Radical Anion Generation and Elastase Release by Human Neutrophils in Response to fMet-Leu-Phe/Cytochalasin B[a]

compound	IC_{50} (μM)[b]	
	superoxide anion generation	elastase release
13,14-didehydoxyisogarcinol (**1**)	0.88 ± 0.14	1.16 ± 0.40
garcimultiflorone A (**2**)	5.58 ± 1.19	4.65 ± 0.25
garcimultiflorone B (**3**)	0.11 ± 0.04	0.14 ± 0.02
13-hydroxygarcimultiflorone B (**4**)	0.40 ± 0.10	0.86 ± 0.12
garcimultiflorone C (**5**)	7.21 ± 1.61	12.10 ± 1.63
aesculetin dimethyl ether (**6**)	>30	>30
6,7,8-trimethoxycoumarin (**7**)	>30	>30
squalene (**8**)	>30	>30
δ-tocotrienol (**9**)	14.8 ± 2.66	15.12 ± 5.93
β-sitostenone (**10**)	>30	>30
mixture of β-sitosterol (**11**) and stigmasterol (**12**)	>30	>30
diphenyleneiodonium	1.70 ± 0.77	
phenylmethylsulfonyl fluoride		203.5 ± 32.8

[a] Diphenyleneiodonium and phenylmethylsulfonyl fluoride were used as positive controls. Results are presented as average ± SEM (n = 4). [b] Concentration necessary for 50% inhibition (IC_{50}).

1), A4 (6 L, CH_2Cl_2/MeOH, 90:1), A5 (7 L, CH_2Cl_2/MeOH, 85:1), A6 (5 L, CH_2Cl_2/MeOH, 80:1), A7 (8 L, CH_2Cl_2/MeOH, 70:1), A8 (6 L, CH_2Cl_2/MeOH, 60:1), A9 (5 L, CH_2Cl_2/MeOH, 50:1), A10 (7 L, CH_2Cl_2/MeOH, 30:1), A11 (5 L, CH_2Cl_2/MeOH, 30:1), A12 (5 L, CH_2Cl_2/MeOH, 5:1), A13 (5 L, CH_2Cl_2/MeOH, 1:1), A14 (5 L, MeOH). Fraction A2 (7.8 g) was chromatographed further on silica gel (70−230 mesh, 310 g) eluting with *n*-hexane/acetone (10:1) to give 11 fractions (each 1.2 L, A2-1−A2-11). Fraction A2-6 (230 mg) was purified further by preparative TLC (silica gel, *n*-hexane/acetone, 10:1) to obtain **2** (4.8 mg) (R_f = 0.46) and **3** (22.6 mg) (R_f = 0.71). Fraction A2-8 (225 mg) was purified further by preparative TLC (silica gel, *n*-hexane/acetone, 5:1) to afford **5** (38 mg) (R_f = 0.29). Fraction A2-9 (192 mg) was purified further by preparative TLC (silica gel, *n*-hexane/acetone, 70:1) to obtain **10** (10.5 mg) (R_f = 0.30). Fraction A3 (7.5 g) was chromatographed further on silica gel (70−230 mesh, 290 g) eluting with *n*-hexane/acetone (8:1) to give 12 fractions (each 550 mL, A3-1−A3-12). Fraction A3-6 (205 mg) was purified further by preparative TLC (silica gel, $CHCl_3$/acetone, 70:1) to yield **6** (4.6 mg) (R_f = 0.47) and **7** (2.8 mg) (R_f = 0.46). Fraction A3-9 (318 mg) was purified by MPLC (11 g silica gel, 230−400 mesh, *n*-hexane/EtOAc, 20:1, 200 mL fractions) to obtain nine subfractions: A3-9-1−A3-9-9. Fraction A3-9-2 (22 mg) was purified further by preparative TLC (silica gel, *n*-hexane/acetone, 3:1) to obtain **8** (12.4 mg) (R_f = 0.85). Fraction A4 (5.1 g) was chromatographed further on silica gel (70−230 mesh, 240 g) eluting with $CHCl_3$/EtOAc (20:1) to give 10 fractions (each 1.3 L, A4-1−A4-10). Fraction A4-6 (440 mg) was purified by MPLC (19 g silica gel, 230−400 mesh, $CHCl_3$/EtOAc, 10:1, 250 mL fractions) to obtain nine subfractions: A4-6-1−A4-6-9. Fraction A4-6-3 (85 mg) was purified further by preparative TLC (silica gel, $CHCl_3$/EtOAc, 5:1) to obtain **9** (28.0 mg) (R_f = 0.41). Fraction A5 (8.3 g) was chromatographed further on silica gel (70−230 mesh, 280 g) eluting with $CHCl_3$/acetone (30:1) to give 13 fractions (each 1.2 L, A5-1−A5-13). Fraction A5-3 (167 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 10:1) to provide **1** (3.5 mg) (R_f = 0.26). Fraction A5-7 (220 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/EtOAc, 30:1) to yield a mixture of **11** and **12** (12 mg) (R_f = 0.78). Fraction A7 (5.6 g) was chromatographed further on silica gel (70−230 mesh, 240 g) eluting with $CHCl_3$/acetone (20:1) to give 10 fractions (each 1.5 L, A7-1−A7-10). Fraction A7-3 (215 mg) was purified by MPLC (9 g silica gel, 230−400 mesh, $CHCl_3$/EtOAc, 30:1, 135 mL fractions) to obtain eight subfractions: A7-3-1−A7-3-8. Fraction A7-3-3 (35 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/EtOAc, 30:1) to obtain **4** (6.3 mg) (R_f = 0.71).

Biological Assay. The effect of the isolated compounds on neutrophil pro-inflammatory response was evaluated by monitoring the inhibition of superoxide anion generation and the release of elastase in fMLP/CB-activated human neutrophils in a concentration-dependent manner.

Preparation of Human Neutrophils. Human neutrophils from venous blood of healthy, adult volunteers (20−28 years old) were isolated using a standard method of dextran sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes.[20] Purified neutrophils containing >98% viable cells, as determined by the trypan blue exclusion method,[21] were resuspended in a Ca^{2+}-free HBSS buffer at pH 7.4 and were maintained at 4 °C prior to use.

Measurement of Superoxide Anion Generation. Measurement of superoxide anion generation was based on the SOD-inhibitable reduction of ferricytochrome *c*.[22,23] In brief, after supplementation with 0.5 mg/mL ferricytochrome *c* and 1 mM Ca^{2+}, neutrophils (6×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with different concentrations (10−0.01 μg/mL) of compounds or DMSO (as control) for 5 min. Cells were incubated with cytochalasin B (1 μg/mL) for 3 min prior to the activation with 100 nM formyl-L-methionyl-L-leucyl-L-phenylalanine for 10 min. Changes in absorbance with the reduction of ferricytochrome *c* at 550 nm were continuously monitored in a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan). Calculations were based on differences in the reactions with and without SOD (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome *c* (ϵ = 21.1/mM/10 mm).

Measurement of Elastase Release. Degranulation of azurophilic granules was determined by measuring elastase release as described previously.[23] Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. Briefly, after supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (6×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with compounds for 5 min. Cells were stimulated with fMLP (100 nM)/CB (0.5 μg/mL), and changes in absorbance at 405 nm were monitored continuously in order to assay elastase release. The results were expressed as the percent of elastase release in the fMLP/CB-activated, drug-free control system.

13,14-Didehydroxyisogarcinol (1): colorless, amorphous powder; $[\alpha]^{25}_D$ −185 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 237 (3.96), 287 (sh, 3.70) nm; CD (MeOH) $[\theta]_{346}$ +2510, $[\theta]_{327}$ 0, $[\theta]_{320}$ −1217, $[\theta]_{309}$ 0, $[\theta]_{298}$ +3843, $[\theta]_{289}$ 0, $[\theta]_{263}$ −23 153, $[\theta]_{237}$ 0, $[\theta]_{220}$ +20 752; IR (KBr) ν_{max} 1724 (C═O), 1679 (C═O), 1639 (C═O) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.2 (C-23), 18.0 (C-28), 18.0 (C-38), 18.1 (C-21), 21.3 (C-32), 22.1 (C-22), 24.7 (C-17), 25.8 (C-27), 25.8 (C-37), 26.1 (C-20), 27.7 (C-24), 27.7 (C-29), 28.5 (C-33), 29.6 (C-34), 40.7 (C-30), 42.4 (C-7), 42.9 (C-6), 46.1 (C-5), 52.8 (C-8), 70.4 (C-4), 86.8 (C-31), 113.9 (C-2), 120.1 (C-18), 121.4 (C-35), 122.5 (C-25), 128.4 (C-13), 128.4 (C-15), 128.9 (C-12), 128.9 (C-16), 133.0 (C-14), 133.2 (C-26), 133.7 (C-36), 134.3 (C-19), 137.5 (C-11), 171.0 (C-1), 193.6 (C-3), 194.0 (C-10), 206.4 (C-9); ESIMS *m/z* 593 $[M + Na]^+$; HRESIMS *m/z* 593.3610 $[M + Na]^+$ (calcd for $C_{38}H_{50}O_4Na$, 593.3607).

Garcimultiflorone A (2): colorless, amorphous powder; $[\alpha]^{25}_D$ −173 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 247 (4.32), 282 (4.22) nm; CD (MeOH) $[\theta]_{317}$ +7884, $[\theta]_{274}$ +13 903, $[\theta]_{257}$ 0, $[\theta]_{248}$ − 6559, $[\theta]_{234}$ 0, $[\theta]_{227}$ +1884; IR (KBr) ν_{max} 1721 (C═O), 1695 (C═O), 1641 (C═O) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.9 (C-14), 17.9 (C-28), 18.0 (C-38), 21.3 (C-33), 22.0 (C-10), 22.1 (C-23), 25.8 (C-13), 25.9 (C-27), 25.9 (C-37), 26.8 (C-22), 28.3 (C-32), 29.1 (C-24), 29.4 (C-29), 30.0 (C-34), 37.9 (C-7), 39.9 (C-30), 47.8 (C-6), 48.7 (C-5), 49.4 (C-8), 77.9 (C-4), 84.2 (C-31), 120.7 (C-11), 121.3 (C-35), 123.9 (C-2), 124.9 (C-25), 127.8 (C-18), 127.8 (C-20), 128.3 (C-17), 128.3 (C-21), 131.8 (C-19), 131.9 (C-12), 132.4 (C-26), 134.2 (C-36), 136.9 (C-16), 167.7 (C-1), 193.2 (C-3), 193.7 (C-15), 209.0 (C-9); ESIMS *m/z* 593 $[M + Na]^+$; HRESIMS *m/z* 593.3605 $[M + Na]^+$ (calcd for $C_{38}H_{50}O_4Na$, 593.3607).

Garcimultiflorone B (3): colorless, amorphous powder; $[\alpha]^{25}_D$ −132 (*c* 0.96, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 247 (4.56), 322 (3.95) nm; CD (MeOH) $[\theta]_{343}$ + 6518, $[\theta]_{327}$ 0, $[\theta]_{308}$ −5802, $[\theta]_{257}$ −6884, $[\theta]_{244}$ 0, $[\theta]_{222}$ +13 938; IR (KBr) ν_{max} 1723 (C═O), 1699 (C═O), 1646 (C═O) cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.9 (C-28), 17.9 (C-33), 17.9 (C-38), 23.4 (C-23), 25.7 (C-37), 25.8 (C-27), 27.3 (C-22), 28.2 (C-20), 29.3 (C-24), 30.4 (C-21), 33.0 (C-34), 35.2 (C-29), 43.3 (C-7), 43.8 (C-30), 48.7 (C-6), 50.0 (C-5), 62.5 (C-8), 70.9 (C-4), 83.2 (C-19), 112.2 (C-32), 112.7 (C-2), 114.5 (C-17), 123.0 (C-35), 124.1 (C-18), 124.8 (C-25), 127.9 (C-13), 127.9 (C-15), 128.6 (C-12), 128.6 (C-16), 131.5 (C-36), 132.1 (C-14), 132.6 (C-26), 136.9 (C-11), 148.4 (C-31), 167.3 (C-3), 192.2 (C-9), 193.4 (C-10), 206.9 (C-1); ESIMS *m/z* 591 $[M + Na]^+$; HRESIMS *m/z* 591.3446 $[M + Na]^+$ (calcd for $C_{38}H_{48}O_4Na$, 591.3450).

13-Hydroxygarcimultiflorone B (4): colorless, amorphous powder; $[\alpha]^{25}_D$ −115 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 252 (4.34), 314 (3.94) nm; CD (MeOH) $[\theta]_{342}$ +2509, $[\theta]_{327}$ 0, $[\theta]_{302}$ −4837, $[\theta]_{254}$ −4363, $[\theta]_{242}$ 0, $[\theta]_{221}$ +8110; IR (KBr) ν_{max} 3365 (OH), 1720 (C═O), 1700 (C═O), 1641 (C═O) cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.9 (C-28), 18.0 (C-33), 18.0 (C-38), 23.5 (C-23), 25.7 (C-27), 25.8 (C-37), 27.3 (C-22), 28.4 (C-20), 29.4 (C-24), 30.5 (C-21), 32.8 (C-34), 35.4 (C-29), 43.3 (C-7), 43.8 (C-30), 48.7 (C-6), 50.2 (C-5), 62.5 (C-8), 71.2 (C-4), 83.4 (C-19), 112.3 (C-32), 112.6 (C-2), 114.6 (C-17), 115.0 (C-12), 119.4 (C-14), 121.3 (C-16), 123.2 (C-35), 124.3 (C-18), 124.9 (C-25), 129.2 (C-15), 132.4 (C-36), 132.7 (C-26), 138.5 (C-11), 148.4 (C-31), 155.4 (C-13), 167.4 (C-3), 192.3 (C-9), 192.9 (C-10), 207.1 (C-1); ESIMS *m/z* 607 $[M + Na]^+$; HRESIMS *m/z* 607.3395 $[M + Na]^+$ (calcd for $C_{38}H_{48}O_5Na$, 607.3399).

Garcimultiflorone C (5): colorless, amorphous powder; $[\alpha]^{25}_D$ −25.3 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (3.90), 244 (3.89), 278 (sh, 3.05), 305 (2.51) nm; CD (MeOH) $[\theta]_{308}$ −1489, $[\theta]_{269}$ 0, $[\theta]_{265}$ +185, $[\theta]_{259}$ 0, $[\theta]_{243}$ −1663, $[\theta]_{233}$ 0, $[\theta]_{217}$ +2790; IR (KBr) ν_{max} 3417 (OH), 1731 (C═O), 1694 (C═O) cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.0 (C-28), 18.0 (C-33), 18.0 (C-38), 20.8 (C-20), 21.6 (C-21), 22.6 (C-22), 25.2 (C-23), 25.7 (C-37), 28.7 (C-27), 31.3 (C-17), 31.5 (C-24), 33.4 (C-34), 34.6 (C-29), 42.1 (C-25), 43.4 (C-30), 44.6 (C-6), 44.9 (C-7), 50.7 (C-5), 65.7 (C-2), 67.4 (C-8), 82.1 (C-4), 84.1 (C-19), 85.7 (C-18), 88.5 (C-26), 112.5 (C-32), 122.5 (C-35), 127.9 (C-13), 127.9 (C-15), 128.9 (C-12), 128.9 (C-16), 132.2 (C-14), 132.2 (C-36), 135.2 (C-11), 148.8 (C-31), 192.4 (C-10), 203.9 (C-1), 205.7 (C-9), 208.7 (C-3); ESIMS *m/z* 643 $[M + Na]^+$; HRESIMS *m/z* 643.3615 $[M + Na]^+$ (calcd for $C_{38}H_{52}O_7Na$, 643.3611).

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