Novel and Anti-Inflammatory Constituents of *Garcinia subelliptica*

Jing-Ru Weng,[a] Chun-Nan Lin,*[a] Lo-Ti Tsao,[b] and Jih-Pyang Wang[b]

Abstract: Four novel phloroglucinol derivatives, garcinielliptones A (**1**), B (**2**), C (**3**), D (**4**), a novel triterpenoid, garcinielliptone E (**5**), and three known compounds were isolated from the seeds of *Garcinia subelliptic*a. The structures, including relative configurations, were elucidated by means of spectroscopic data. Known compounds garsubellin A (**6**) and garcinielliptin oxide (**7**) showed potent inhibitory effects on the release of β-glucuronidase, and β-glucuronidase and histamine, respectively, from peritoneal mast cells stimulated with compound 48/80 in a concentration-dependent manner with IC_{50} values of 15.6 ± 2.5, and 18.2 ± 3.6 and 20.0 ± 2.7 μM, respectively. Compound **7** showed potent inhibitory effects on the release of β-glucuronidase and lysozyme from neutrophils stimulated with formyl-Met-Leu-Phe(fMLP)/cytochalasin B (CB) in a concentration-dependent manner with IC_{50} values of 15.7 ± 3.0 and 23.9 ± 3.2 μM, respectively. Compound **7** also showed potent inhibitory effect on superoxide formation from neutrophils stimulated with fMLP/CB also in a concentration-dependent manner with an IC_{50} value of 17.9 ± 1.5 μM.

Keywords: anti-inflammatory activity · *Garcinia subelliptica* · NMR spectroscopy · phloroglucinol derivatives · terpenoids

Introduction

Recently, the isolation and characterization of several various constituents and antioxidant xanthones of the wood and root bark of *Garcinia subelliptica* Merr. (Guttiferae) have been reported.[1, 2] In the search for bioactive constituents in Formosan Guttiferae plants, we investigated bioactive constituents of the seeds of *G. subelliptica* and reported two novel triterpenoids named garcinielliptin oxide and garcinielliptone, respectively.[3, 4] In a continuing study of biologically active compounds in Formosan Guttiferae plants, we further investigated the constituents of the seeds of *G. subelliptica*; four novel phloroglucinols, garcinielliptones A–D (**1**–**4**), a novel triterpenoid, garcinielliptone E (**5**), and three known compounds, garsubellin A (**6**),[5] garcinielliptin oxide (**7**),[3] and garsubellin D (**8**),[6] were isolated from the seeds of this plant. In the present paper, the structure elucidations of the five novel compounds **1**–**5** and the anti-inflammatory effects of **1**, **2**, **6**, **7**, and **8** are reported.

[a] Prof. C.-N. Lin, J.-R. Weng
School of Pharmacy, Kaohsiung Medical University
Kaohsiung, Taiwan 807 (Republic of China)
Fax: (+886) 7-5562365
E-mail: lincna@cc.kmu.edu.tw

[b] L.-T. Tsao, Prof. J.-P. Wang
Department of Education and Research
Taichung Veterans General Hospital, Taichung
Taiwan 407 (Republic of China)

Results and Discussion

The molecular formula of garcinielliptone A (**1**) was determined to be $C_{30}H_{46}O_5$ by HR-FABMS (m/z 467.3122 $[M-1-H_2O]^-$) and DCIMS (m/z 485.2917 $[M-1]^-$), which was consistent with the ^{1}H and ^{13}C NMR data. The IR absorption of **1** implied the presence of OH (3424 cm^{-1}), CO (1724 cm^{-1}), conjugated ketone (1657 cm^{-1}), and C=C (1602 cm^{-1}) moieties. The ^{1}H NMR spectrum of **1** (see Experimental Section) resembles that of garsubellin A (**6**), measured in $CDCl_3$, except for the proton signals of H_2-7, H_2-17, H_2-18, Me-21, and H_2-22. The ^{13}C NMR spectrum of **1** (Table 1) also resembles that of **6**, measured in $CDCl_3$, except for the carbon signals of C-3, C-4, C-6, C-17, C-18, C-19, C-21, and C-22. This clearly indicates that **1** possesses the partial moiety represented as bold lines in **1** (Figure 1). The ^{1}H–^{1}H COSY correlations of H_2-22/H-23 and the HMBC correlations of H_2-22/C-1 and C-2 establish the connectivities between C-22 and C-23, and C-22 and C-2. The HMBC correlations of H_2-22/C-3, H_β-17/C-3, C-4, and C-5, C-19/Me-20 and Me-21, and Me-20/Me-21, the ^{1}H-^{1}H COSY correlation between H_2-17 and H_2-18, and the NOESY correlations of H_β-18/Me-20 and Me-21 establish the C-2 linked through C-3 and C-4 to C-5, and the 3-hydroxy-3-methylbutyl group located at C-4. The HMBC correlations of H_β-7/C-4 and C-5 establish the connectivities between C-4 and C-5, and C-4 and C-7. The HMBC correlation of C-6/Me-10 and Me-11 and NOESY correlation of Me-10/Me-29 confirm that the 1-oxopropyl group is linked to C-6 and the hydroxy group is linked to C-3. Based on the above results and the fact

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Table 1. ^{13}C NMR data for compounds **1**–**5** in $CDCl_3$.[a]

Position	1	2	3	4	5
1	194.6	191.3	193.1	194.6	209.2
2	115.9	114.6	117.1	115.9	97.1
3	166.0	169.9	173.6	166.0	207.1
4	64.5	56.7	59.6	64.6	44.0
5	206.8	207.2	204.6	207.0	107.8
6	75.8	83.6	82.2	80.5	40.8
7	40.5	39.4	38.4	40.7	33.6
8	43.3	43.6	42.4	43.3	54.9
9	45.3	46.5	46.4	45.3	70.6
10	16.4	15.6	16.0	25.4	217.3
11	23.9	23.0	22.7	27.9	38.7
12	27.4	26.5	26.5	27.3	16.9
13	122.5	122.7	122.2	122.5	19.3
14	133.1	133.1	133.5	133.1	17.7
15	17.7	17.8	25.8	17.7	23.0
16	25.8	25.9	17.9	25.8	19.8
17	16.8	16.4	30.1	29.3	36.7
18	32.2	119.5	90.4	119.9	70.2
19	80.5	133.4	70.6	133.8	28.8
20	25.5	18.2	26.9	18.0	29.4
21	27.8	25.7	24.1	25.9	27.7
22	29.3	29.3	18.0	16.8	122.0
23	119.9	31.8	41.3	32.1	133.3
24	133.8	79.0	71.1	75.7	25.8
25	18.0	24.7	29.9	16.4	18.0
26	25.9	27.8	28.7	24.0	31.3
27	209.3	209.3	208.8	208.8	119.3
28	40.2	42.4	42.0	47.0	133.3
29	21.7	20.5	20.5	18.0	25.8
30	21.2	21.5	21.4	27.4	17.7
31				11.5	

[a] The number of protons directly attached to each C atom was verified by DEPT experiments. Signals obtained by $^1H-^1H$ COSY, HMQC, HMBC, and NOESY techniques and comparison with corresponding reported data.[3, 5]

that C-5 and C-6 are quarternary carbon atoms, C-5 and C-6 must be connected. Thus garcinielliptone A was characterized as **1** with a bicyclo[3.3.1]nonane moiety. The presence of characteristic peaks at m/z 468 $[M-H_2O]^-$, 467 $[M-H_2O-1]^-$, 399 $[468-a]^-$ (fragment a is defined in Figure 1) in its DCIMS also support the characterization of **1**.

Selected cross peaks were observed in the NOESY spectrum of **1**, as indicated in Figure 2. The relative configurations at C-4, C-6, and C-8 were deduced from the NOESY cross peaks of H_β-12/H_β-8, H_β-8/H_β-7, H_β-7/H_β-17, H_β-17/H_β-18, H_α-12/Me-10, Me-10/Me-29, and Me-10/Me-16, while 3-hydroxy-3-methylbutyl group at C-4, 1-oxopropyl group at C-6, and prenyl group at C-8 are on the β, α, and α sides of **1**, respectively.[5] From the above results, compound **1** was characterized as having a *trans*-bicyclo[3.3.1]nonane moiety (Figure 1). However, further experiments are required to elucidate the absolute stereochemistry of **1**.

Abstract in Chinese:

由福木種子分離得到四個新穎的 phloroglucinol 衍生物，garcinielliptones A (**1**) ，B (**2**) ，C (**3**) ，D (**4**) ，一個新穎的三帖類，garcinielliptone E (**5**)及三個已知化合物。化學結構及相對立體係由光譜資料證明。已知化合物 garsubellin A (**6**)及 garcinielliptin oxide (**7**)分別對老鼠腹腔巨噬細胞以 compound 48/80 引發之 β–glucuronidase，及β–glucuronidase 與組織胺釋放具有很強的抑制活性，且與濃度有關，IC_{50} 值分別為 15.6 ± 2.5 μM，及 18.2 ± 3.6 與 20.0 ± 2.7 μM。化合物 **7** 對老鼠嗜中性白血球以 formyl-Met-Leu-Phe (fMLP)/cytochalasin B (CB)引發之 β–glucuronidase 及溶小體酶釋放，其抑制活性呈現與濃度有關的現象，IC_{50} 值分別為 15.7 ± 3.0 及 23.9 ± 3.2 μ*M*。化合物 7 亦對老鼠嗜中性白血球以 fMLP/CB 引發過氧化物的形成，也呈現抑制活性與濃度有關的現象，且 IC_{50} 值為 17.9 ± 1.5 μM。

Based on the information from ^1H, COSY, and NOESY spectra, a computer-assisted 3D structure (Figure 2) was obtained by using the molecular modeling program CS CHEM 3D V3.5.1, with MM2 force-field calculations for energy minimization. The calculated distances between H_β-18 and H_β-17 (2.52 Å), H_β-17 and H_β-7 (2.48 Å), H_β-7 and H_β-8 (2.30 Å), H_β-8 and H_β-12 (2.41 Å), H_α-12 and Me-10 (3.76 Å), Me-10 and Me-16 (3.07 Å), and Me-10 and Me-29 (2.28 Å), are all less than 4.00 Å; these values are consistent with the well-defined NOESY experiments observed between each of these proton pairs. Thus garcinielliptone A (**1**) was characterized as 3-hydroxy-4-(3-hydroxy-3-methylbutyl-6-(1-oxopropyl)-2,8-diprenyl-8β-H-*trans*-bicyclo[3.3.1]non-2-en-1,5-dione (**1**).

The molecular formula of garcinielliptone B (**2**) was determined to be $C_{30}H_{44}O_4$ by HR-EIMS (m/z 468.3242 $[M]^+$), which was consistent with the ^1H and ^{13}C NMR data. The IR absorption of **2** implied the presence of CO (1724 cm^{-1}), conjugated ketone (1639 cm^{-1}), and C=C (1594 cm^{-1}) moieties. The ^1H NMR data of **2** were very similar to those of **1**, except for the absence of signals due to 3-hydroxy-3-methylbutyl group and the appearance of signals due to a 2,2-dimethylpyran moiety. The HMBC correlations of H_β-7/C-3, C-4, and C-5 and H_α-7/C-3 established the connectivities between C-4 and C-5, C-3 and C-4, and C-4 and C-7, and the HMBC correlations of H_β-17/C-4 and C-18, H_α-17/C-4, C-5, C-18, and C-19, H-18/Me-20, and Me-21/Me-20 confirmed that a prenyl group was linked at C-4. For the 2,2-dimethylpyran moiety, the connectivity between C-22 and C-23 was clearly revealed by the COSY data. The HMBC correlations of H_2-22/C-2, C-3, C-24 established that the 2,2-dimethylpyran moiety is fused on C-2–C-3 bond. In the ^{13}C NMR spectrum of **2** (Table 1), the chemical shift values of **2** were almost identical to corresponding data of **1** except for C-1, C-2, C-3, C-4, C-6, and C-17–C-26. The presence of significant peaks at m/z 453 $[M-Me]^+$, 399 $[453-a+H]^+$, and 357 $[M-2a-H]^+$ (fragment a is defined in Figure 1) in its EIMS also supported the characterization of **2**. Thus garcinielliptone B (**2**) was characterized as having a new 3,4-dihydro-2*H*-pyrano[2,6-*b*]bicyclo[3.3.1]-nonane skeleton.

Selected cross peaks were observed in the NOESY experiment of **2**, as indicated in Figure 2. The relative configurations at C-4, C-6, and C-8 were deduced from NOESY cross peaks of H_β-7/H_β-17, H_β-8/Me-10, and Me-10/Me-30 while prenyl groups at C-4 and C-8, and 1-oxopropyl group at C-6 are on the β, α, and β sides of **2**, respectively.[5]

Based on the information from ^1H, COSY, and NOESY spectra, a computer-assisted 3D structure (Figure 2) was obtained by using the above-mentioned molecular modeling program, with MM2 force-field calculations for energy minimization. The calculated distances between H_β-7 and H_β-17 (2.57 Å), H_β-8/Me-10 (2.24 Å), Me-10/Me-30 (2.55 Å),

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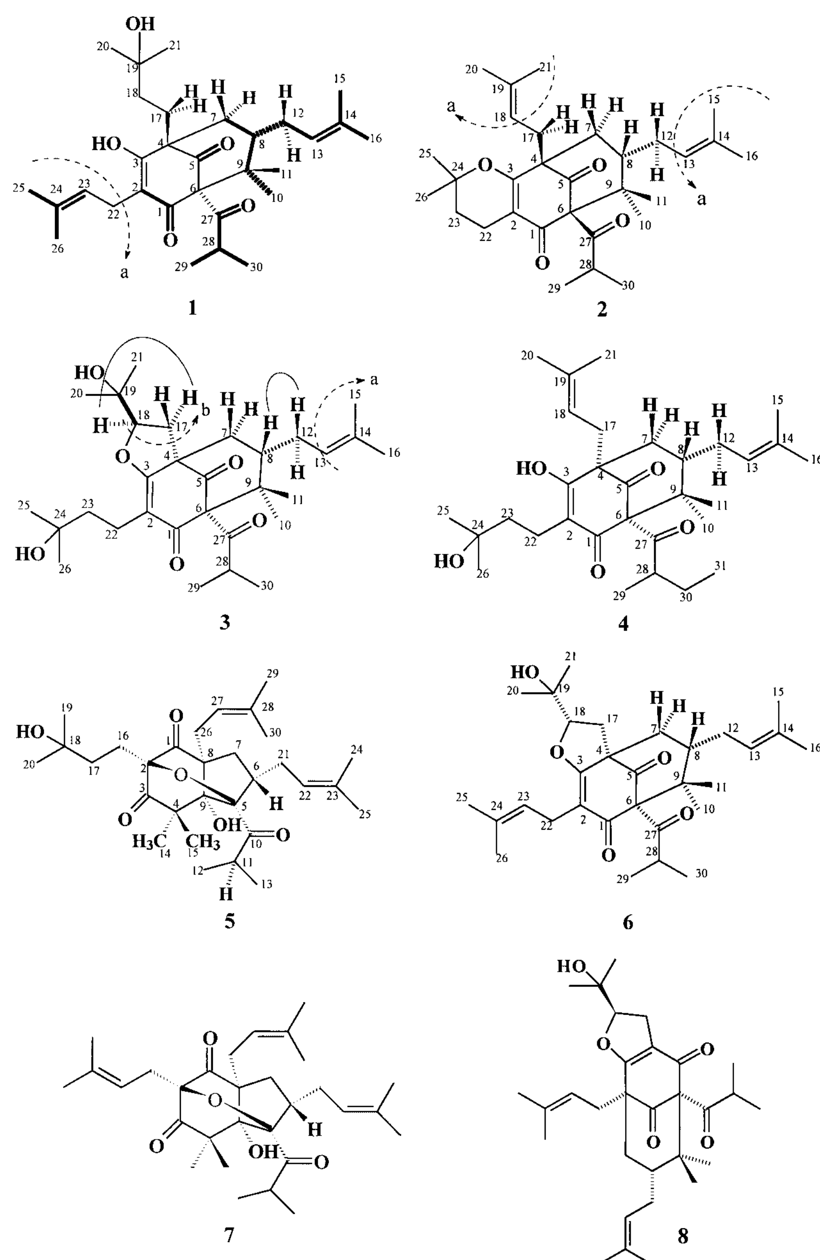

Figure 1. Structures of **1**–**8**, substructure (bold lines) of **1**, selected NOESY cross peaks of **3**, and MS fragmentation patterns of **1**–**3**.

are all less than 4.00 Å; these values are consistent with the well-defined NOESY experiments observed between each of these proton pairs. Therefore, garcinielliptone B (**2**) was characterized as 2,3-(2,2-dimethyl-3,4-dihydro-2H-pyran)-6-(1-oxopropyl)-4,8-diprenyl-8β-H-*cis*-bicyclo[3.3.1]non-1,5-dione.

The molecular formula of garcinielliptone C (**3**) was determined to be $C_{30}H_{46}O_6$ by HR-EIMS (*m*/*z* 502.3305 $[M]^+$), which was consistent with the ^{1}H and ^{13}C NMR data. The IR absorption of **3** implied the presence of OH (3416 cm^{-1}), CO (1723 cm^{-1}), conjugated ketone (1631 cm^{-1}), and C=C (1605 cm^{-1}) moieties. The ^{1}H NMR spectrum of **3** (see Experimental Section) was very similar to that of **6**, except for the absence of signals due to a prenyl group and the appearance of signals due to a 3-hydroxy-3-methylbutyl group. The HMBC correlations of H_2-22/C-1, C-2, and C-3, established the connectivities between C-1 and C-2, C-2 and C-3, and C-2 and C-22, and the HMBC correlations of H_2-22/C-23 and C-24, Me-25/C-23, C-24, and C-26, Me-26/C-23, C-24, and C-25 confirmed that the prenyl group linked at C-2. In the ^{13}C NMR spectrum of **3** (Table 1), the chemical shift values of **3** were almost identical to the corresponding data of **6** except for C-22–C-26. In the EIMS of **3**, the base peak at *m*/*z* 415 was attributed to the fragment $[M-b-H]^+$, and significant peak at *m*/*z* 446 ($[M-a-H]^+$) (fragments a and b are defined in Figure 1) also supported the characterization of **3**. Thus, garcinielliptone C (**3**) was characterized as a bicyclo[3.3.1]nonane skeleton.

The relative configurations at C-4 and C-6 are determined by comparing the relative stereochemistry of **6**.[5] The NOESY experiment of **3** showed cross peaks between H_β-8/H_β-12 and H_α-17/H-18 (Figure 1). Based on the above NOESY experiment and the values of coupling constants of H_2-17 and H-18 in the ^{1}H NMR spectrum, we can say that the 2-α-hydroxyisopropyl and prenyl groups are on β and α sides of **3**, respectively. Therefore, garcinielliptone C (**3**) was characterized as 2-(3-hydroxy-3-methylbutyl)-3,4-(2-α-hydroxyisopropyl-dihydrofurano)-6-(1-oxopropyl)-8-prenyl-8β-H-*cis*-bicyclo[3.3.1]-non-2-en-1,5-dione.

The molecular formula of garcinielliptone D (**4**) was determined to be $C_{31}H_{48}O_5$ by HR-EIMS (*m*/*z* 498.3340 $[M-2]^+$), which is consistent with the ^{1}H and ^{13}C NMR data. The IR absorption of **4** implied the presence of OH (3416 cm^{-1}), CO (1724 cm^{-1}), conjugated ketone (1646 cm^{-1}), and C=C (1602 cm^{-1}) moieties. The ^{1}H NMR spectrum of **4** were very similar to those of **1**, except for the presence of signals due to an 1-oxobutyl group and the absence of signals due to 1-oxopropyl group. In the ^{13}C NMR spectra of **4**, the chemical shift values of **4** were almost identical those of **1** except for C-10, C-11, and C-17–C-26, C-28, C-29, C-30, and C-31. Additionally, ^{1}H–^{1}H COSY and HMQC of **4** indicated the presence of a *sec*-butyl group, which was not present in the structure of **1**. These spectral data suggest that **4** is a phloroglucinol derivative, in which the

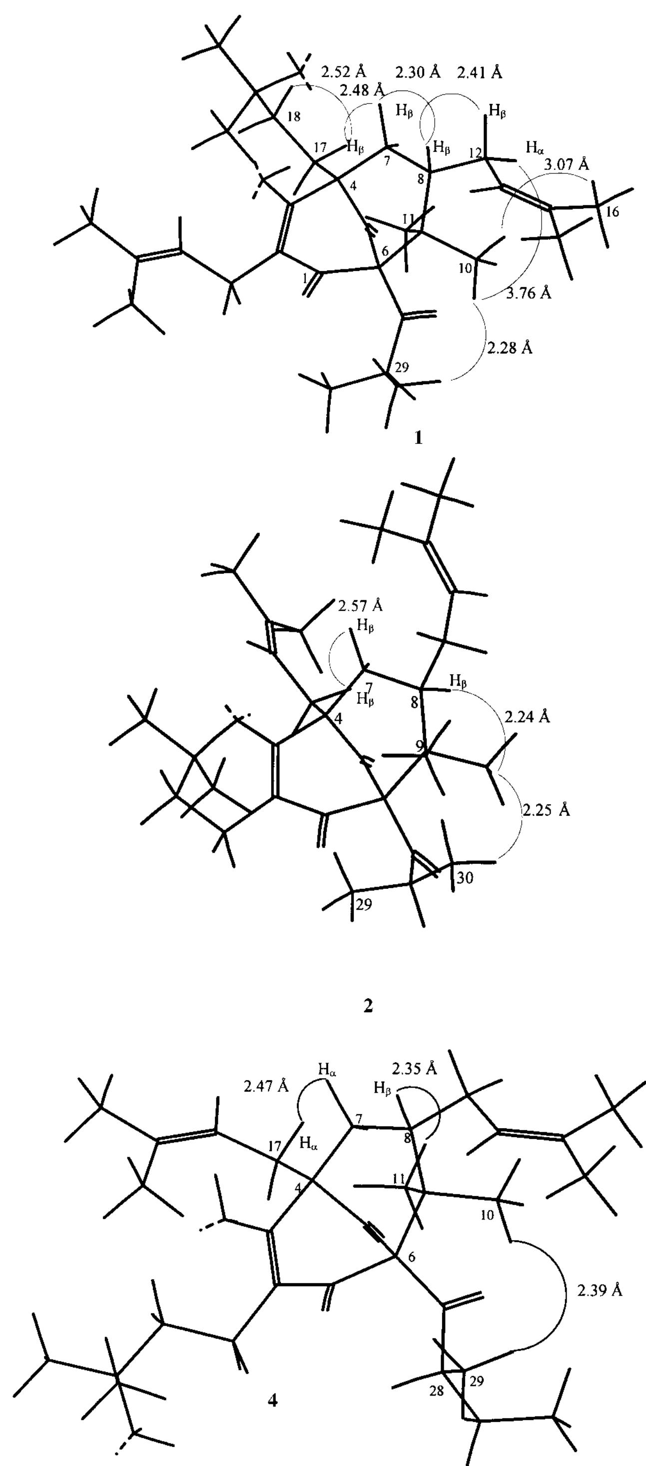

Figure 2. Selected NOESY correlations and relative stereochemistry for **1**, **2**, and **4**.

Me C-30 group of **1** is replaced by an ethyl group. The HMBC correlations of H_2-17/C-3, C-4, C-5, C-7, C-18, and C-19, H_2-22/C-2, C-3, C-23, and C-24, Me-29/C-27, C-28, and C-30, H_2–30/C-28, Me-31/C-28 and C-30 established that the prenyl group, 3-hydroxy-3-methylbutyl, and *sec*-butyl group were linked to C-8, C-2, and C-27, respectively. The HMBC correlations of C-6/Me-10 and Me-11, and Me-10/Me-11, and NOESY correlations of Me-10/Me-29 and H_β-8/Me-11 established Me-10 and Me-11, and C-27, were linked to C-9 and C-6, respectively.

Selected cross peaks were observed in the the NOESY spectrum of **4** as shown in Figure 2. The relative configurations at C-4, C-6, and C-8 are deduced from NOESY cross peaks of H_α-7/H_α-17, H_β-8/Me-11, and Me-10/Me-29 while the prenyl groups at C-4 and C-8, and 1-oxobutyl group at C-6 are all on the α side of **4**.[5]

Based on the information from ^{1}H, COSY, and NOESY spectra, a computer-assisted 3D structure (Figure 2) was obtained by using the above-mentioned molecular modeling program, with MM2 force-field calculations for energy minimization. The calculated distances between H_α-7 and H_α-17 (2.47 Å), H_β-8/Me-11 (2.35 Å), Me-10/Me-29 (2.39 Å), are all less than 4.00 Å; these values are consistent with the well-defined NOESY experiments observed between each of these proton pairs. Therefore, garcinielliptone D (**4**) was characterized as 2-(3-hydroxy-3-methylbutyl)-3-hydroxy-6-(1-oxobutyl)-4,8-diprenyl-8β-H-*cis*-bicyclo[3.3.1]non-2-en-1,5-dione.

The molecular formula of garcinielliptone E (**5**) was determined to be $C_{30}H_{46}O_6$ by HR-EIMS (*m*/*z* 502.3288 $[M]^+$), which was consistent with the ^{1}H and ^{13}C NMR data. The IR absorption of **5** implied the presence of OH (3313 cm^{-1}) and three CO (1769, 1739, 1675 cm^{-1}) moieties. The ^{1}H NMR spectrum of **5** resembled that of garcinielliptone oxide (**7**),[3] except for the absence of signals due to a prenyl group and the appearance of signals due to a 3-hydroxy-3-methylbutyl group. The ^{13}C NMR spectrum of **5** (Table 1) also resembled to those of **7** except for C-16–C-20, indicating that **5** was a triterpenoid. From the above information in conjunction with the molecular formula, we suggest that compound **5** possesses a *cis*-bicyclo[4.3.0]nonane moiety with a hydroxy group at C-9.[3]

The HMBC correlations of H_2–17/C-19 and C-20, Me-19/C-18, Me-20/C-18 and NOESY correlations of Me-19/Me-14, Me-20/Me-14 confirmed that the 3-hydroxy-3-methylbutyl group was linked to C-2. The presence of significant peaks at *m*/*z* 484 $[M-H_2O]^+$, 388 $[M-H_2O-Me_2CCHCH_2CO+H]^+$, 345 $[M-C_3H_7-H_2O-Me_2CCHCH_2CO+H]^+$, 319 $[M-C_3H_7-H_2O-Me_2CCHCH_2CO-CO+3H]^+$ in its EIMS also supported the characterization of **5**. Therefore, garcinielliptone E (**5**) was characterized as 2-(3-hydroxy-3-methylbutyl)-9-hydroxy-5-(1-oxopropyl)-6,8-diprenyl-6β-H-*cis*-bicyclo[4.3.0]non-2,5-oxo-1,3-dione. Garcinielliptone E (**5**) is the second example of triterpenoid with a novel skeleton.

The anti-inflammatory activity of **1**, **2**, and **6**–**8** were studied in vitro by measuring the inhibitory effects on the chemical mediator released from mast cells and neutrophils. Compounds **6** and **7** showed potent inhibitory effects on the release of β-glucuronidase, and β-glucuronidase and histamine, respectively, from peritoneal mast cells stimulated with compound 48/80 (10 $\mu g\,mL^{-1}$) in a concentration-dependent manner with IC_{50} values of 15.6 ± 2.5, and 18.2 ± 3.6 and 20.0 ± 2.7 μM, respectively, while **1**, **2**, and **8** had no significant inhibitory effects (Table 2). These results indicate the cleavage of dihydrofurano ring from chemical bond between O-C-3 and C-18 in **6** did not enhance the inhibitory effects. As shown

Table 2. The inhibitory effects of **1**, **2**, and **6**–**8** on the release of β-glucuronidase and histamine from rat peritoneal mast cells stimulated with compound 48/80 (10 μg mL^{-1}).

Compound	IC$_{50}$ [μM][a]	
	β-Glucuronidase	Histamine
1	> 30 (38.6 ± 2.4)	> 30 (36.1 ± 1.3)
2	> 30 (31.7 ± 1.2)	> 30 (34.9 ± 3.9)
6	15.6 ± 2.5	> 30 (46.7 ± 3.7)
7	18.2 ± 3.6	20.2 ± 2.7
8	> 30 (47.7 ± 2.8)	> 30 (37.6 ± 3.6)
mepacrine	20.6 ± 1.2	50.1 ± 4.1

[a] When 50% inhibition could not be reached at the highest concentration, the % of inhibition is given in parentheses. Data are presented as the mean ± s.e.m. ($n = 3$–5). Mepacrine was used as a positive control.

in Table 2, there is a clear indication that compounds which possess a *cis*-bicyclo[4.3.0]nonane moiety (i.e., **7**) have potent inhibitory effects on mast cell degranulation stimulated with compound 48/80, and the inhibitory effects of **6** and **7** are stronger than that of mepacrine. Mepacrine was used in this experiment as a positive control.

The introduction of formyl-Met-Leu-Phe (fMLP) (1 μM)/cytochalasin B (CB) (5 μg mL^{-1}) stimulated the release of β-glucuronidase and lysozyme from rat neutrophils. Compound **7** had potent and concentration-dependent inhibitory effects on neutrophil degranulation, while **1**, **2**, **6**, and **8** did not show significant inhibitory effects (Table 3). These results indicated that triterpenoids with bicyclo[3.3.1]nonane moiety did not show significant inhibitory effects. Trifluoperazine was used in this experiment as a positive control.

Table 3. The inhibitory effects of **1**, **2**, and **6**–**8** on the release of β-glucuronidase and lysozyme from rat neutrophils stimulated with fMLP (1 μM)/CB (5 μg mL^{-1}).

Compound	IC$_{50}$ [μM][a]	
	β-Glucuronidase	Lysozyme
1	> 30 (−3.0 ± 3.4)	> 30 (13.5 ± 4.8)
2	> 30 (16.4 ± 2.8)	> 30 (10.0 ± 1.5)
6	> 30 (17.5 ± 1.1)	> 30 (34.6 ± 3.1)
7	15.7 ± 3.0	23.9 ± 3.2
8	> 30 (25.4 ± 3.4)	> 30 (−5.2 ± 4.5)
trifluoperazine	12.2 ± 0.3	13.2 ± 0.7

[a] When 50% inhibition could not be reached at the highest concentration, the % of inhibition is given in parentheses. Data are presented as the mean ± s. e. m. ($n = 3$–5). Trifluoperazine was used as a positive control.

The introduction of fMLP (0.3 μM)/CB (5 μg mL^{-1}) or phorobol myristate acetate (PMA) (3 nM) stimulated superoxide anion generation in rat neutrophils. Compound **7** had potent inhibitory effect on fMLP/CB-induced superoxide anion generation, while **1**, **2**, **6**, and **8** had no significant inhibitory effects (Table 4). These results also indicated that triterpenoids which possess a *cis*-bicyclo[4.3.0]nonane moiety have potent inhibitory effects on fMLP/CB-induced superoxide anion generation. Both fMLP and PMA activate NADPH oxidase to produce superoxide anion, but through different cellular signaling mechanisms.[7] The observations that **7** had no appreciable effect on PMA-induced response suggests the involvement of PMA-independent signaling pathway. The inhibitory effect of compouds **3**–**5** on mast cell and neutrophil degranulation are currently being undertaking.

Table 4. The inhibitory effects of **1**, **2**, and **6**–**8** on superoxide anoion generation in rat neutrophils stimulated with fMLP (0.3 μM)/CB (5 μg mL^{-1}) or PMA (3 nM).

Compound	IC$_{50}$ [μM][a]	
	fMLP/CB	PMA
1	> 30 (−12.5 ± 7.3)	> 30 (15.7 ± 5.2)
2	> 30 (23.4 ± 1.8)	> 30 (9.9 ± 4.2)
6	> 30 (17.5 ± 1.1)	> 30 (34.6 ± 3.1)
7	17.9 ± 1.5	> 30 (43.6 ± 3.1)
8	> 30 (30.0 ± 1.6)	> 30 (41.8 ± 3.9)
trifluoperazine	6.2 ± 0.3	7.6 ± 0.3

[a] When 50% inhibition could not be reached at the highest concentration, the % of inhibition is given in parentheses. Data are presented as the mean ± s.e.m. ($n = 3$–5). Trifluoperazine was used as a positive control.

Compound **6** also induced choline acetyltransferase (ChAT) activity in P10 rat septal neuron cultures.[5] The present results suggest that compounds **6** and **7** may have anti-inflammatory effects, because they inhibited the chemical mediators released from mast cells and neutrophils.

Experimental Section

General: Optical rotations: JASCO model DIP-370 digital polarimeter. IR spectra: Hitachi 260–30 spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H and ^{13}C NMR spectra: Varian Unity-400 spectrometer; 400 and 100 MHz, respectively; δ in ppm, J in Hz. MS: JMS-HX100 mass spectrometer; m/z (rel. %)

Plant material: The fruits of *G. subelliptica* were collected at Kaohsiung, Taiwan, in July 2001. A voucher specimen (2003) has been deposited at the Department of Medicinal Chemistry, School of Pharmacy, Kaohsiung Medical University.

Extraction and isolation: The fresh seeds (7.5 kg) obtained from the fresh fruits (22.8 kg) of *G. subelliptica*, were extracted with chloroform at room temperature. The $CHCl_3$ extract was concentrated under reduced pressure to afford a brown residue (130 g). This residue was subjected to column chromatography (silica gel). Elution with *n*-hexane/acetone (3:1) yielded **1** (20 mg), **2** (18 mg), and **6** (31 mg). Elution with $CHCl_3$/acetone (9:1) yielded **3** (15 mg), **4** (14 mg), and **5** (8 mg). Elution with *n*-hexane/ethyl acetate (10:1) yielded **7** (11 mg), and **8** (12 mg). The known compounds **6**, **7**, and **8** were identified by spectral methods and compared with spectral data reported in literature.[3, 5, 6]

Garcinielliptone A (1): Colorless oil; $[\alpha]_D = -33$ ($c = 0.62$ in $CHCl_3$); IR (film on NaCl): $\tilde{\nu} = 3424$ (OH), 1724 (C=O), 1657, 1602, 1443 cm^{-1}; ^{1}H NMR ([D$_1$]$CHCl_3$): $\delta = 1.00$ (s, H$_3$-11), 1.05 (d, $J = 6.4$ Hz, H$_3$-29), 1.13 (d, $J = 6.4$ Hz, H$_3$-30), 1.25 (s, H$_3$-10), 1.29 (dd, $J = 12.0$, 7.6 Hz, H$_\alpha$-7), 1.33 (s, H$_3$−20), 1.35 (m, H$_\beta$-8), 1.39 (s, H-21), 1.50 (s, H$_3$-15), 1.56 (dd, $J = 13.2$, 5.2 Hz, H$_\alpha$-12), 1.63 (s, H$_3$-16), 1.63 (s, H$_3$-25), 1.65 (s, H$_3$-26), 1.69 (dd, $J =$ 9.2, 6.4 Hz, H$_\alpha$-18), 1.77 (m, H$_\beta$-18), 1.77 (m, H$_\beta$-7), 2.05 (dd, $J = 13.5$, 5.2 Hz, H$_\beta$-12), 2.37 (m, H-28), 2.43 (m, H$_2$−17), 2.43 (m, H$_2$-22), 4.90 (t, $J = 7.2$ Hz, H-13), 5.00 ppm (t, $J = 7.2$ Hz, H-23); ^{13}C NMR: see Table 1; DCIMS: m/z (%): 485.2917 $[M-1]^-$ (3), 468.2695 (100), 406.1470 (7), 390.1648 (37); HR-FABMS: calcd for $C_{30}H_{43}O_4^-$: 467.3161; found: 467.3122 $[M-1-H_2O]^-$.

Garcinielliptone B (2): Colorless oil; $[\alpha]_D = -23$ ($c = 0.11$ in $CHCl_3$); IR (film on NaCl): $\tilde{\nu} = 1724$ (C=O), 1639, 1594 cm^{-1}; ^{1}H NMR ([D$_1$]$CHCl_3$): $\delta = 0.99$ (s, H$_3$-10), 1.01 (d, $J = 6.4$ Hz, H$_3$-30), 1.11 (d, $J = 6.4$ Hz, H$_3$-29), 1.18 (m, H$_\alpha$-12), 1.21 (s, H$_3$-11), 1.21 (m, H$_2$-23), 1.22 (s, H$_3$-25), 1.30 (m, H$_\alpha$-7), 1.35 (s, H$_3$-26), 1.54 (s, H$_3$-15), 1.65 (s, H$_3$-16), 1.65 (s, H$_3$-21), 1.66 (s, H$_3$-20), 1.77 (m, H$_2$-23), 1.79 (dd, $J = 12.4$, 3.2 Hz, H$_\beta$-7), 2.06 (m, H$_\beta$-8), 2.06 (m, H-28), 2.13 (m, H$_\beta$-12), 2.35 (m, H$_\alpha$-17), 2.39 (m, H$_2$-22), 2.45 (m, H$_\beta$-17), 4.91 (t, $J = 6.8$ Hz, H-13), 4.98 ppm (t, $J = 6.8$ Hz, H-18); ^{13}C NMR: see

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Table 1; EIMS: m/z (%): 468 $[M]^+$ (33), 399 (34), 357 (100), 331 (62), 277 (67); HR-EIMS: calcd for $C_{30}H_{44}O_4^+$: 468.3239; found: 468.3242 $[M]^+$.

Garcinielliptone C (3): Colorless oil; $[\alpha]_D = -40$ ($c = 0.16$ in $CHCl_3$); IR (film on NaCl): $\tilde{\nu} = 3416$ (OH), 1723 (C=O), 1631, 1605 cm^{-1}; ^1H NMR ($[D_1]CHCl_3$): $\delta = 0.97$ (d, $J = 6.8$ Hz, H_3-30), 1.02 (s, H_3-10), 1.06 (d, $J = 6.8$ Hz, H_3-29), 1.19 (s, H_3-21), 1.20 (s, H_3-26), 1.24 (s, H_3-25), 1.24 (s, H_3-11), 1.37 (s, H_3-20), 1.46 (m, H_α-7), 1.48 (m, H_2-23), 1.50 (m, H_β-8), 1.53 (s, H_3-16), 1.67 (s, H_3-15), 1.75 (dd, $J = 12.8$, 5.8 Hz, H_α-17), 1.75 (dd, $J = 12.8$, 5.8 Hz, H_α-12), 1.93 (m, H-28), 1.99 (m, H-7), 2.14 (m, H_β-12), 2.50 (m, H_2-22), 2.64 (dd, $J = 12.8$, 11.2 Hz, H_β-17), 4.54 (dd, $J = 11.2$, 5.8 Hz, H_α-18), 4.92 ppm (t, $J = 7.2$ Hz, H-13); ^{13}C NMR: see Table 1; EIMS: m/z (%): 502 $[M]^+$ (14), 468 (18), 415 (11), 347 (39), 305 (24), 287 (28), 234 (48); HR-EIMS: calcd for $C_{30}H_{46}O_6^+$: 502.3294; found: 502.3305 $[M]^+$.

Garcinielliptone D (4): Colorless oil; $[\alpha]_D = -22$ ($c = 0.12$ in $CHCl_3$); IR (film on NaCl): $\tilde{\nu} = 3416$ (OH), 1724 (C=O), 1646, 1602 cm^{-1}; ^1H NMR ($[D_1]CHCl_3$): $\delta = 0.81$ (t, $J = 7.6$ Hz, H_3-31), 1.00 (s, H_3-25), 1.04 (d, $J = 6.8$ Hz, H_3-29), 1.25 (s, H_3-26), 1.32 (s, H_3-10), 1.33 (m, H_α-12), 1.36 (dd, $J = 13.6$, 7.2 Hz, H_α-7), 1.41 (s, H_3-11), 1.49 (s, H_3-15), 1.58 (m, H_β-8), 1.58 (m, H_α-30), 1.62 (s, H_3-16), 1.63 (s, H_3-21), 1.64 (s, H_3-20), 1.77 (m, H_2-23), 1.81 (m, H_β-7), 1.95 (m, H_β-12), 2.04 (m, H_β-30), 2.09 (m, H-28), 2.39 (m, H_2-17), 2.47 (m, H_2-2), 4.89 (t, $J = 7.2$ Hz, H-13), 4.95 ppm (t, $J = 7.2$ Hz, H-18); ^{13}C NMR: see Table 1; EIMS: m/z (%): 498 $[M-2]^+$ (1), 482 (35), 425 (14), 357 (90), 291 (97); HR-EIMS: calcd for $C_{31}H_{46}O_5^+$: 498.3345; found: 498.3340 $[M-2]^+$.

Garcinielliptone E (5): Colorless oil; $[\alpha]_D = -51$ ($c = 0.18$ in $CHCl_3$); IR (film on NaCl): $\tilde{\nu} = 3313$ (OH), 1769, 1739, 1675 cm^{-1} (C=O); ^1H NMR ($[D_1]CHCl_3$): $\delta = 0.87$ (m, H_β-6), 0.87 (d, $J = 6.8$ Hz, H_3-13), 0.93 (s, H_3-14), 1.09 (d, $J = 6.8$ Hz, H_3-12), 1.09 (m, H_α-17), 1.18 (m, H_β-17), 1.18 (m, H_α-21), 1.19 (s, H_3-19), 1.22 (s, H_3-20), 1.22 (m, H_α-7), 1.25 (s, H_3-15), 1.54 (s, H_3-25), 1.55 (s, H_3-30), 1.67 (s, H_3-24), 1.68 (s, H_3-29), 1.71 (td, $J = 13.4$, 4.4 Hz, H_α-16), 1.87 (dd, $J = 14.0$, 3.6 Hz, H_β-7), 2.07 (br d, $J = 13.2$ Hz, H_β-21), 2.32 (td, $J = 13.4$, 4.4 Hz, H_β-16), 2.40 (d, $J = 8.4$ Hz, H_2-26), 3.22 (m, H-11), 4.91 (t, $J = 7.6$ Hz, H-22), 5.33 ppm (t, $J = 7.2$ Hz, H-27); ^{13}C NMR: see Table 1; EIMS: m/z (%): 484 (12), 416 (11), 388 (26), 345 (54), 319 (100); HR-EIMS: calcd for $C_{30}H_{46}O_6^+$: 502.3294; found: 502.3288 $[M]^+$.

Mast cell degranulation: Heparinized Tyrode's solution was injected into the peritoneal cavity of exsanguinated rat (Sprague–Dawley, 250–300 g). After abdominal massage, the cells in the peritoneal fluid were harvested and then separated in 38 % bovine serum albumin in glucose-free Tyrode's solution. The cell pellets were washed and suspended in Tyrode's solution. Cell suspension was pre-incubated at 37 °C with DMSO or drugs (3, 10, 30, 50, or 100 µM) for 3 min. Fifteen minutes after the addition of compound 48/80 (10 $\mu g\,mL^{-1}$), β-glucuronidase (phenolphthalein-β-D-glucuronide as substrate, 550 nm) and histamine (o-phthadialdehyde condensation, 350/450 nm) in the supernatant were determined. The total content was measured after treatment of the cell suspension with Triton X-100. The percentage released was determined.[8] To eliminate the effect of the solvent on the mast cell degranulation, the final concentration of DMSO was fixed at 0.5 %.

Neutrophil degranulation: Blood was withdrawn from a rat and mixed with EDTA. After dextran sedimentation, Ficoll–Hypaque separation, and hypotonic lysis of the residual erythrocytes, neutrophils were washed and suspended in Hanks' balanced salt solution (HBSS) to 1×10^7 cells mL^{-1}.[9] The cell suspension was pre-incubated at 37 °C with DMSO or drugs for 10 min, and then stimulated with fMLP (1 µM)/CB (5 $\mu g\,mL^{-1}$). After 45 min, the lysozyme and β-glucuronidase in the supernatant were determined.[10, 11] The total content was measured after treatment of the cell suspension with Triton X-100 and the percentage released was calculated. The final volume of DMSO was ≤ 0.5 %.

Superoxide anion formation: Superoxide anion formation was measured in term of superoxide dismutase inhibitable cytochrome c reduction.[12] Neutrophil suspension was preincubated with 0.5 % DMSO or drugs for 3 min, and then superoxide dismutase or HBSS was added into the blank and test wells, respectively. After addition of cytochrome c, reaction was initiated by stimulating with fMLP (0.3 µM)/CB (5 µg/ml) or PMA (3 nM). Thirty minutes later, the reaction was terminated by centrifugation, and the absorbance changes of supernatant were monitored at 550 nm in a microplate reader. The final concentration of drugs in DMSO was fixed at 0.5 %.

Statistical analysis: Data are presented as the mean ± s.e.m. Statistical analyses were performed using the least significant difference test method after analysis of variance. P values < 0.05 were considered to be significant. Analysis of the regression line was used to calculate IC_{50} values.

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[1] M. Iinuma, H. Tosa, T. Tanaka, R. Shimano, F. Asai, S. Yonemori, *Phytochemistry* **1994**, *35*, 1355–1360.

[2] H. Minami, M. Kinoshita, Y. Fukuyama, M. Kodama, T. Yoshizawa, M. Sugiura, K. Nakagaw, H. Tago, *Phytochemistry* **1994**, *36*, 501–506.

[3] C. N. Lin, C. W. Kiang, C. M. Lu, R. R. Wu, K. H. Lee, *Chem. Commun.* **1996**, 1315–1316.

[4] M. I. Chung, H. J. Su, C. N. Lin, *J. Nat. Prod.* **1998**, *61*, 1015–1016.

[5] Y. Fukuyama, A. Kuwayama, H. Minami, *Chem. Pharm. Bull.* **1997**, *45*, 947–949.

[6] Y. Fukuyama, H. Minami, A. Kuwayama, *Phytochemistry* **1998**, *49*, 853–857.

[7] A. W. Segal, A. Abo, *Trends Biochem. Sci.* **1993**, *18*, 43–47.

[8] J. P. Wang, S. L. Raung, C. N. Lin, C. M. Teng, *Eur. J. Pharmacol.* **1994**, *251*, 35–42.

[9] J. P. Wang, S. L. Raung, Y. H. Kuo, C. M. Teng, *Eur. J. Pharmacol.* **1995**, *288*, 341–348.

[10] D. R. Absolom, *Methods Enzymol.* **1986**, *132*, 92–179.

[11] A. J. Barrett, *A Laboratory Handbook*, Elsevier, Amsterdam, **1972**, pp. 118–120.

[12] S. B. Corradin, J. Mauel, S. D. Donini, E. Quattrocchi, P. Ricciardi-Casragnoli, *Glia* **1993**, *7*, 255–262.

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