Constituents of the Pericarp of *Garcinia subelliptica*

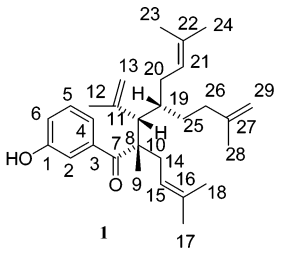
Chien-Chang Wu,[†] Jing-Ru Weng,[†] Shen-Jeu Won,[‡] and Chun-Nan Lin*,[†]

School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China, and Department of Microbiology and Immunology, National Cheng Kung University, Tainan, Taiwan 701, Republic of China

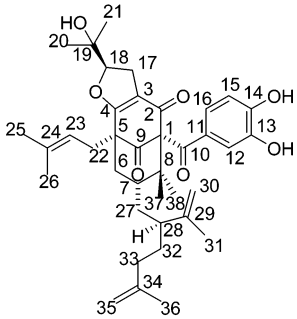
Received March 10, 2005

A new benzophenone, garcinielliptone FA (**1**), and a new benzoylphloroglucinol, garcinielliptone FB (**2**), along with six known compounds, were isolated from the pericarp of *Garcinia subelliptica*. The structures and relative configurations of **1** and **2** were elucidated by spectroscopic methods and supported by computer-generated molecular modeling. Compound **2** exhibited cytotoxic activity against several human cancer cell lines.

The isolation and characterization have been reported of various xanthone and phloroglucinol constituents of the wood and root bark of *Garcinia subelliptica* Merr. (Clusiaceae), with some of these compounds possessing inhibitory activity against DNA topoisomerases I and II.[1−3] In previous papers, we have isolated several new terpenoids and phloroglucinols from the seeds of *G. subelliptica*, and the anti-inflammatory activity of several terpenoids and phloroglucinols was investigated.[4−8] In the present study, a crude $CHCl_3$ extract of the pericarp of *G. subelliptica* was found to exhibit cytotoxic activity against the MCF-7, Hep 3B, and HT-29 human cancer cell lines, with IC_{50} values of 7.1, 5.5, and 8.2 μg/mL, respectively. Fractionation of this extract led to the isolation of a new benzophenone, garcinielliptone FA (**1**), a new benzoylphloroglucinol, garcinielliptone FB (**2**), and six known compounds, 1,7-dihydroxyxanthone,[9] cycloart-25-ene-3β,24-diol,[10] canophyllol,[11] canophyllic acid,[11] 5-hydroxymethylfurfural,[12] and I7,II4′-dimethylamentoflavone.[13] In the present paper, the structure elucidation and cytotoxicity of **1** and **2** are reported.



1



2

The molecular formula of **1** $[\alpha]_D^{25}$ 210° (*c* 0.04, acetone)} was determined to be $C_{29}H_{42}O_2$ by HREIMS [*m/z* 422.3138, M^+], which was consistent with its ^1H and ^{13}C NMR data. The IR absorption of **1** implied the presence of OH (3383 cm^{-1}), conjugated CO (1695 cm^{-1}), and aromatic (1583 cm^{-1}) moieties. The UV spectrum indicated the presence of a conjugated aromatic moiety λ_{max} [289 (4.18) and 252 (4.19) nm]. The ^1H NMR spectrum of **1** (Table 1) revealed the presence of proton signals for two γ,γ-dimethylallyl groups, three tertiary methyl groups, two methylenes, two methines, two olefinic groups, four aromatic protons, and a phenolic OH. The ^{13}C NMR spectrum of **1** showed the presence of a disubstituted benzene ring, seven methyls, four methylenes, two methines, eight olefinic carbons, and six quaternary carbons, including one oxygenated aromatic carbon, and a carbonyl carbon. The $^1H-^1H$ COSY correlations of H-4/H-5, H-5/H-6, H-10/H-19, H-20/H-21, H-19/H_2-25, and H_2-25/H_2-26 established the partial structures represented with bold lines in **1** (Figure S1, Supporting Information). The HMBC correlations of H_2-20/C-10 and C-19 established the connectivity between C-19 and C-20, and the HMBC correlations of Me-23/C-21 and C-22, and Me-24/C-21 and C-22, indicated that a prenyl group was linked to C-19. In turn, the HMBC correlations of H_2-26/C-25 and C-27, Me-28/C-26 and C-27, and H_2-29/C-27 suggested that an isopropenyl group was linked at C-26. The HMBC correlations of Me-9/C-8 and C-10, and H_2-14/C-7, were supportive of the linkage of C-7, Me-9, C-10, and C-14 at C-8. The HMBC correlations of Me-17/C-15 and C-16, H_2-14/C-16, and Me-12/C-11 and C-13, with C-10 present as a tertiary carbon, established that the remaining prenyl and isopropenyl groups were linked at C-8 and C-10, respectively. COSY correlations of H-4/H-5 and H-5/H-6 and the coupling constants of H-2, H-4, H-5, and H-6, with C-7 and C-3 present as quaternary carbons, confirmed the connectivity between C-3 and C-7. Thus, garcinielliptone FA was characterized as **1** as shown. The EIMS of **1** indicated significant peaks at *m/z* 407 $[M - Me]^+$, 121 $[a]^+$, and 93 $[b]^+$ (Figure S1, Supporting Information), which also supported the structure proposed for **1**. A NOESY experiment on **1** showed cross-peaks between Me-9/H_β-14, H-10, and H-19, H-10/H-19, and H-4/Me-28. The above result suggested that the methyl group at C-8 and the hydrogen group at C-10 and C-19, and the 3-hydroxybenzoyl group at C-8, the isopropenyl group at C-10, and the 2-isopropenyl ethyl group at C-19 are on the β- and α-side of **1**, respectively. From the ^1H NMR, COSY, and NOESY spectra, a computer-generated 3D structure of **1** was obtained by using the molecular modeling program, namely, CS CHEM 3D V3.5.1, with MM2 force-field calculations

* To whom correspondence shound be addressed. Tel: +886 7 3121101, ext. 2163. Fax: +886 7 5562365. E-mail: lincna@cc.kmu.edu.tw.
[†] Kaohsiung Medical University.
[‡] Cheng Kung University.

10.1021/np050084v CCC: $30.25
Published on Web 07/07/2005

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of **1** (in $(CD_3)_2CO$) and **2** (in $CDCl_3$)

	1			**2**		
	δH	δC	HMBC	δH	δC	HMBC
1		157.9	7.02 (H-2), 7.22 (H-5), 7.05 (H-6)		78.9	1.34 (Me-37), 1.12 (Me-38)
2	7.02 (t, 2.0)	115.9	7.05 (H-6)		189.2	
3		139.8	7.22 (H-5)		117.5	2.95 (H_α-17), 3.00 (H_β-17)
4	6.92 (m)	121.0	7.05 (H-6)		177.1	1.86 (H_α-6), 1.92 (H_β-6), 2.95 (H_α-17), 3.00 (H_β-17), 1.69 (H_α-22), 2.23 (H_β-22)
5	7.22 (t, 7.7)	129.6	7.05 (H-6)		55.1	1.86 (H_α-6), 1.92 (H_β-6), 1.69 (H_α-22), 2.23 (H_β-22)
6	7.05 (m)	120.0	7.02 (H-2), 7.05 (H-4)	α 1.86 (m) β 1.92 (m)	35.1	
7		209.3	2.55 (H_α-14), 2.71 (H_β-14)	2.50 (m)	43.1	1.34 (Me-37), 1.12 (Me-38)
8		50.1	1.00 (Me-9)		48.2	1.86 (H_α-6), 1.92 (H_β-6), 1.34 (Me-37), 1.12 (Me-38)
9	1.00 (s)	27.1	2.71 (H_β-14)		205.6	1.86 (H_α-6), 1.92 (H_β-6), 1.69 (H_α-22), 2.23 (H_β-22)
10	1.53 (m)	47.2	1.00 (Me-9), 1.96 (H_2-20)		192.0	7.16 (H-12), 6.89 (H-16)
11		148.7	1.59 (Me-12)		129.5	6.55 (H-15)
12	1.59 (s)	25.9		7.16 (d, 2.0)	115.5	6.89 (H-16)
13	4.53 (brs)	113.2	1.59 (Me-12)		143.1	7.16 (H-12), 6.55 (H-15)
14	2.55 (m) 2.71 (m)	26.7			148.3	7.16 (H-12), 6.55 (H-15), 6.89 (H-16)
15	5.05 (m)	121.0	1.64 (Me-17)	6.55 (d, 8.4)	114.1	
16		135.2	2.71 (H_β-14), 1.64 (Me-17)	6.89(dd, 8.4, 2.0)	122.6	7.16 (H-12)
17	1.64 (s)	22.7		α 2.92 (dd, 15.2, 8.0) β 3.00 (dd, 15.2, 10.4)	27.3	
18	1.59 (s)	17.7		4.85 (t, 8.4)	92.5	2.95 (H_α-17), 3.00 (H_β-17), 1.29 (Me-20), 1.24 (Me-21)
19	2.65 (m)	44.1	1.96 (H_2-20)		71.9	2.95 (H_α-17), 3.00 (H_β-17), 1.29 (Me-20), 1.24 (Me-21)
20	1.96 (2H,m)	37.3		1.29 (s)	23.8	1.24 (Me-21)
21	4.89 (m)	125.2	1.96 (H_2-20), 1.60 (Me-23), 1.50 (Me-24)	1.24 (s)	23.7	1.29 (Me-20)
22		133.0	1.96 (H_2-20), 1.60 (Me-23), 1.50 (Me-24)	α 1.69 (dd, 14.4, 4.0) β 2.23 (dd, 14.4, 8.4)	34.3	
23	1.60 (s)	18.0	1.50 (Me-24)	4.95 (t, 8.0)	122.4	1.55 (Me-25), 1.67 (Me-26)
24	1.50 (s)	18.3	1.60 (Me-23)		133.9	1.55 (Me-25), 1.67 (Me-26)
25	1.53 (2H, m)	32.4	1.86 (H_2-26)	1.55 (s)	17.7	1.67 (Me-26)
26	1.86 (2H, m)	36.4	1.70 (Me-28)	1.67 (s)	25.0	
27		146.6	1.86 (H_2-26), 1.70 (Me-28), 4.65 (H_2-29)	α 1.45 (dd, 14.4, 7.6) β 2.14 (dd, 14.4, 4.4)	26.7	
28	1.70 (s)	26.2		2.50 (m)	43.7	4.72 (H_2-30)
29	4.65 (2H, s)	110.2			147.3	4.72 (H_2-30)
30				4.72 (2H, d, 5.2)	113.0	1.60 (Me-31)
31				1.60 (s)	17.7	
32				α 1.70 (m) β 2.13 (m)	31.7	
33				α 1.60 (m) β 1.92 (m)	40.8	4.67 (H_α-35), 4.72 (H_β-35)
34					146.1	1.71 (Me-36)
35				α 4.67 (d, 5.2) β 4.72 (d, 5.2)	109.3	1.71 (Me-36)
36				1.71 (s)	25.8	
37				1.34 (s)	22.9	1.12 (Me-38)
38				1.12 (s)	15.9	1.34 (Me-37)
OH	8.75 (s)					

for energy minimization (Figure S2, Supporting Information). The calculated distances between Me-9/H_β-14 (2.459 Å), Me-9/H-10 (2.569 Å), Me-9/H-19 (3.202 Å), H-10/H-19 (3.008 Å), and H-4/Me-28 (2.992 Å) were all less than 4.00 Å. This is consistent with the well-defined NOESY interactions observed for each of these proton pairs. Thus, garcinielliptone FA (**1**) was characterized as 1-(3′-hydroxyphenyl)-3-isopropenyl-2β,7-dimethyl-2,4-di(3-methylbut-2-enyl)oct-7-en-1-one (**1**).

The molecular formula $C_{38}H_{50}O_7$ of **2** was established by HREIMS (*m/z* 618.3566, $[M]^+$). The IR spectrum exhibited OH (3416 cm^{-1}), CO (1730, 1694, and 1677 cm^{-1}), and aromatic (1613 cm^{-1}) moieties. The UV spectrum indicated the presence of an aromatic moiety [232 (4.07) and 282 (4.11) nm]. The ^{1}H NMR spectrum of **2** (Table 1) was very similar to that of hyperibone B,[11] except for the absence of signals due to a prenyl group and a phenyl group and the appearance of signals for a 2-isopropenyl hex-5-enyl group and an aromatic ABX system. The ^{1}H−^{1}H COSY correlations of H-27/H-28, H-28/H-32, and H-32/H-33 were used to determine the partial moieties represented with bold lines in **2** (Figure S1, Supporting Information), and the HMBC correlations of H_2-30/C-28, C-29, and C-31, H_2-35/C-33, and Me-36/C-34 and the NOESY correlation of H_α-6/H-28 established the 2-isopropenylhex-5-enyl moiety and confirmed that this group was linked to C-7.

In the ^{13}C NMR spectrum of **2** (Table 1), the chemical shift values were almost identical to the corresponding data

of hyperibone B,[11] except for the signals at C-6, C-11 to C-16, and C-27 to C-36. In addition to the above evidence, the aromatic ABX proton signals of ^{1}H NMR revealed a trisubstituted benzene ring, and the HMBC correlations of H-12/C-10 and H-16/C-10, with C-1 present as a quaternary carbon, confirmed that the 3,4-dihydroxybenzoyl group is located at C-1. The presence of fragment peaks at *m/z* 482 $[M - a + H]^+$, 413 $[482 - b]^+$, and 359 $[413 - c + 1]^+$ in the EIMS also supported the characterization of **2**.

The relative configurations at C-1, C-5, C-7, and C-18 in **2** were determined by comparing with the relative stereochemistry of hyperibone B.[11] The NOESY experiment of **2** showed a cross-peak between H_α-6/H-28 and H_β-6/H-7 and suggested that the 2-isopropenylhex-5-enyl group is on the α-side of **2**. From the ^{1}H NMR, COSY, and NOESY spectra, a computer-generated 3D structure of **2** was obtained as described in **1**. The calculated distances between H_α-6/H-28 (2.829 Å) and H_β-6/H-7 (2.447 Å) were all less than 4.00 Å. This is consistent with the well-defined NOESY interactions observed for each of these proton pairs. Therefore, garcinielliptone FB (**2**) was characterized as 8,8-dimethyl-1-(3,4-dihydroxybenzoyl)-3,4-[2*β*-(2-hydroxyisopropyl)-2,3-dihydrofurano]-5-(*γ*,*γ*-dimethylallyl)-7-(2-isopropenylhex-5-enyl)-7*β*-*H*-*cis*-bicyclo[3,3,1]nona-3-en-2,9-dione (**2**).

An in vitro microassay for cytotoxicity of **2** was performed using MTT.[14,15] The cytotoxicity of **1** and **2** was studied against a number of human cancer cell lines.[14] Compound **1** exhibited no cytotoxic activity, while compound **2** showed marginal cytotoxic activity against the MCF-7, Hep 3B, and HT-29 cell lines in a concentration-dependent manner with IC_{50} values of 6.8, 6.3, and 11.2 *μ*g/mL. The positive control, 5-fluorouracil (5-Fu), showed potent cytotoxic activity against the MCF-7, Hep 3B, and HT-29 cell lines with IC_{50} values of 0.12, 7.2×10^{-2}, and 7.4×10^{-2} *μ*g/mL, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a JASCO model DIP-370 digital polarimeter. UV spectra were obtained on a JASCO model UV−vis spectrophotometer. IR spectra were recorded on a Hitachi model 260-30 spectrophotometer. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian Unity-400 spectrometer. EIMS were obtained on a JMS-HX100 mass spectrometer.

Plant Material. The fresh pericarps of *G. subelliptica* (15.3 kg) were collected at Kaohsiung, Taiwan, in July 2001. A voucher specimen (2001-3) has been deposited at the Department of Medicinal Chemistry, School of Pharmacy, Kaohsiung Medical University.

Extraction and Isolation. The fresh pericarps of *G. subelliptica* (15.3 kg) were extracted with $CHCl_3$ at room temperature. The $CHCl_3$ extract was concentrated under reduced pressure to afford a brown residue (196 g). The residue (196 g) was fractionated by chromatography over silica gel, using *n*-C_6H_{14}−EtOAc (19:1), *n*-C_6H_{14}−EtOAc (9:1), *n*-C_6H_{14}−EtOAc (4:1), and *n*-C_6H_{14}−EtOAc (2:1), to afford fractions A, B, C, and D. Fraction A contained mixtures of aliphatic compounds, fraction D contained intractable mixtures of phloroglucinols, and only fractions B and C were examined in detail. Fraction B was rechromatographed over silica gel, and elution with *n*-C_6H_{14}−acetone (4:1) yielded **1** (3 mg), while elution with *n*-C_6H_{12}−acetone (5:1) yielded cycloart-25-ene-3*β*,24-diol (9.1 mg), canophyllol (6.1 mg), and canophyllic acid (6.3 mg), and elution with $CHCl_3$ yielded 1,7-dihydroxyxanthone (2.8 mg). Fraction C was chromatographed over silica gel, and elution with *n*-C_6H_{14}−acetone (5:1) yielded **2** (3 mg), elution with *c*-C_6H_{12}−EtOAc (4:1) yielded I7,II 4′-dimethylamentoflavone (1 mg), and elution with CH_2Cl_2 yielded 5-hydroxymethylfurfural (18 mg). 1,7-Dihydroxyxanthone, cycloart-25-ene-3*β*,24-diol, canophyllol, canophyllic acid, I7,II4′-dimethylamentoflavone, and 5-hydroxymethylfurfural were identified by spectroscopic methods and compared with spectroscopic data obtained from the literature.[9−13]

Garcinielliptone FA (1): yellow oil; $[\alpha]_D^{25}$ 210° (*c* 0.04, acetone); UV (MeOH) λ_{max} (log ϵ) 289 (4.48), 252 (4.49) nm; IR (KBr) ν_{max} 3383, 1708, 1583 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz), see Table 1; EIMS *m/z* 422 $[M]^+$ (1), 407 (1), 121 (75), 93 (22), 69 (100); HREIMS *m/z* $[M]^+$ 422.3138 (calcd for $C_{29}H_{42}O_2$, 422.3184).

Garcinielliptone FB (2): yellow oil; $[\alpha]_D^{25}$ −66° (*c* 0.175, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 232 (4.07), 282 (4.11) nm; IR (KBr) ν_{max} 3416, 1730, 1694, 1677, 1613 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m/z* 619 $[M + 1]^+$ (2), 482 (4), 413 (2), 359 (45), 69 (100); HREIMS *m/z* $[M]^+$ 618.3566 (calcd for $C_{38}H_{50}O_7$, 618.3556).

Cytotoxicity Bioassays. Assays for cytotoxicities against human hepatomacellular carcinoma (Hep 3B), human breast adenocarcinoma (MCF-7), and human colorectal adenocarcinoma (HT-29) cell lines were performed by a method described previously.[15]

Acknowledgment. This work was supported by a grant from the National Science Council of the Republic of China (NSC 92-2320-B037-017).

Supporting Information Available: Figures showing substructures (bold lines) and MS fragmention patterns for **1** and **2** and selective NOESY correlations and relative configuration of **1** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Iinuma, M.; Tosa, H.; Tanaka, T.; Shimano, R.; Asai, F.; Yonemori, S. *Phytochemistry* **1994**, *35*, 1355−1360.
(2) Minami, H.; Kinoshita, M.; Fukuyama, Y.; Kodama, M.; Yoshizawa, T.; Sugiura, M.; Nakagaw, K.; Tago, H. *Phytochemistry* **1994**, *36*, 501−506.
(3) Tosa, H.; Iinuma, M..; Tanaka, T.; Nozaki, H.; Ikeda, S.; Tsutsui, Ken.; Tsutsui, Kimiko; Yamada, M.; Fujimori, S. *Chem. Pharm. Bull.* **1997**, *45*, 418−420.
(4) Lin, C. N.; Kiang, C. W.; Lu, C. M.; Wu, R. R.; Lee, K. H. *J. Chem. Soc., Chem. Commun.* **1996**, 1315−1316.
(5) Chung, M. I.; Su, H. J.; Lin, C. N. *J. Nat. Prod.* **1998**, *61*, 1015−1016.
(6) Weng, J. R.; Tsao, L. T.; Wang, J. P.; Lin, C. N. *Chem. Eur. J.* **2003**, *9*, 1958−1963.
(7) Weng, J. R.; Lin, C. N.; Tsao, L. T.; Wang, J. P. *Chem. Eur. J.* **2003**, *9*, 5520−5527.
(8) Weng, J. R.; Tsao, L. T.; Wang, J. P.; Wu, R. R.; Lin, C. N. *J. Nat. Prod.* **2004**, *67*, 1796−1799.
(9) Iinuma, M.; Tosa, H.; Tanaka, T.; Riswan, S. *Phytochemistry* **1996**, *42*, 245−247.
(10) Greca, M. D.; Fiorentino, A.; Monaco, P.; Previtera, L. *Phytochemistry* **1994**, *35*, 1017−1022.
(11) Ali, M. S.; Mahmud, S.; Perveen, S.; Ahmad, V. U.; Rizwani, G. H. *Phytochemistry* **1999**, *50*, 1385−1389.
(12) Jabbar, A.; Taleb, M.; Rashid, M. A.; Hasan, C. M. *Fitoterapia* **1995**, *66*, 377.
(13) Harborne, J. B.; Mabry, T. J.; Mabry, H. *The Flavonoids*; Academic Press: New York, 1975; p 49.
(14) Cell lines, human hepatomacellular carcinoma (Hep 3B), human breast adenocarcinoma (MCF-7), and human colorectal adenocarcinoma (HT-29), were obtained from the American Type Culture Collection (ATCC; Rockville, MD).
(15) Wang, L. W.; Su, H. J.; Yang, S. Z.; Won, S. J.; Lin, C. N. *J. Nat. Prod.* **2004**, *67*, 1182−1185.

NP050084V