Chromanones and Dihydrocoumarins from *Calophyllum blancoi*

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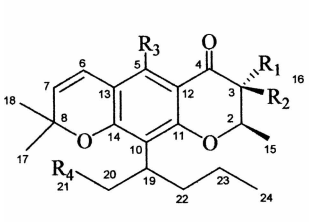
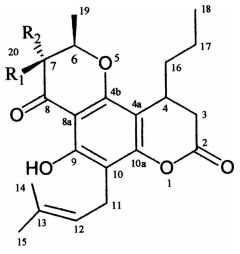
Received October 23, 2003; accepted December 1, 2003

Chromatographic fractionation of the acetone extract of the seeds of *Calophyllum blancoi* yielded six pyranochromanone derivatives; apetalic acid (1), isoapetalic acid (2), apetalic acid methyl ester (3), apetalic acid 5-*O*-acetate (4), isoapetalic methyl ester (5), and isoapetalic acid 5-*O*-acetate (6). In addition, one new dihydrocoumarin derivative, isorecedensolide (7), was also isolated together with recedensolide (8). The structures of the isolated compounds were established through analysis of NMR spectral data including 2D techniques as well as other physical and spectroscopic methods. Compounds 3, 4, 5, 7, and 8 showed mild activity against KB (human oral epidermoid carcinoma) and Hela (human cervical epitheloid carcinoma) tumor cell lines.

Key words *Calophyllum blancoi*; Guttiferae; pyranochromanone; apetalic acid; dihydrocoumarin; isorecedensolide

Numerous pyranocoumarins derivatives have been isolated from different tropical species of *Calophyllum* (Guttiferae).[1—4] Some of these compounds, as calanolides and inophyllums, were shown to inhibit HIV-1 replication and cytopathicity through their interaction with HIV-1 reverse transcriptase.[5—8] *Calophyllum blancoi* N.K.B. ROBSON (Guttiferae) is an evergreen, coastal tree in Taiwan, sometimes grown for ornamental purposes.[9] Pursuing our search for bioactive compounds from Taiwanese medicinal plants, it was deemed of interest to investigate the secondary metabolites of *C. blancoi* with potential medicinal activities. Chromatographic fractionation of the acetone extract of the seeds of *C. blancoi* yielded six pyranochromanone derivatives; apetalic acid (**1**),[10] isoapetalic acid (**2**),[11,12] apetalic acid methyl ester (**3**),[13] apetalic acid 5-*O*-acetate (**4**), isoapetalic methyl ester (**5**),[10,11] and isoapetalic acid 5-*O*-acetate (**6**). In addition, one new dihydrocoumarin derivative, isorecedensolide (**7**), was isolated together with recedesolide (**8**).[14] The structures of the isolated compounds were established through analysis of NMR spectral data including 2D techniques as well as other physical and spectroscopic methods. Apetalic (**1**) and isoapetalic acids (**2**) were previously isolated from some species of *Calophyllum*,[10—13] while recedensolide **8** was only reported in *C. recedens*.[14] Herein we report the high field ^{1}H- and ^{13}C-NMR data of some of these compounds which have not been published before. The cytotoxicity of the isolated compounds against KB, Hela and Med cell lines was investigated.





	R_1	R_2	R_3	R_4
1	H	CH_3	OH	COOH
2	CH_3	H	OH	COOH
3	H	CH_3	OH	COOMe
4	H	CH_3	OAc	COOH
5	CH_3	H	OH	COOMe
6	CH_3	H	OAc	COOH

	R_1	R_2
7	CH_3	H
8	H	CH_3

Results and Discussion

The NMR and FAB-MS data of **7** indicated a molecular formula of $C_{22}H_{28}O_5$ and nine degrees of unsaturation. The IR spectrum revealed absorption bands (cm^{-1}) at 1665 (conjugated carbonyl stretching) and 1771 (C=O stretching of lactone). The ^{13}C-NMR data of **7** (Table 2) displayed a carbonyl at δ 201.6, six quaternary aromatics at δ 103.4, 105.6, 110.3, 155.8, 156.2 and 160.2 along with one C*H* at δ 44.7, C*H*–O at δ 76.4, and two C*H*$_3$ at δ 19.5, 10.2 attributed to dimethyl chromanone moiety. The two methyls at δ_H 1.40 (3H, d, J=6.5 Hz) and 1.17 (3H, d, J=7.2 Hz) were assigned to H-19 and H-20 that are attached to the C-6 and C-7 of the chromanone moiety. The latter methyls were β-oriented as proved by the small coupling constant $J_{6,7}$ (3.4 Hz) and chemical shift values of C-6, C-7, C-19 and C-20 that matched their counterparts in **1**, **3** and **4**. This *cis*-substitution was supported by a strong nuclear Overhauser effect spectroscopy (NOESY) correlation between signals at δ 1.40 (H-19) and δ 1.17 (H-20). An *n*-propyl group was evidenced from the fragment ion at $[M-C_3H_7]^+$ two C*H*$_2$ signals at δ 34.5 (C-16) and 20.0 (C-17), and a C*H*$_3$ at δ 13.8 (C-18), and COSY correlations between H-17/H-16 and H-18. On the other hand, a dimethylallyl moiety was predicted by two C*H*$_3$ at δ 17.8 (C-15) and 25.7 (C-14), olefinic C*H* at δ 121.4 (C-12) and C*H*$_2$ at δ 21.3 (C-11) which were correlated to δ 1.66 (3H, s, H-15), 1.76 (3H, s, H-14), 5.20 (1H, t, J=7.1 Hz, H-12), and 3.30 (2H, d, J=7.1 Hz, H-11) respectively. The olefinic triplet of H-12 displayed heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 1) with signals of C-10, C-14 and C-15 while the doublet of H-11 exhibited cor-

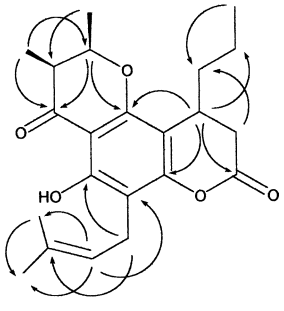


Fig. 1. Selected HMBC Correlations of **7**

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Table 1. ^{1}H-NMR Data (300 MHz, $CDCl_3$) of **1**—**6**[a,b]

Position	1	2	3	4	5	6
2	4.42 dq (6.5, 3.3)	4.09 dq (10.9, 6.5)	4.51 dq (6.5, 3.2)	4.60 dq (6.5, 3.2)	4.14 dq (11, 6.5)	4.16 dq (11, 6.5)
3	2.51 dq (7.2, 3.3)	2.51 dq (10.9, 7.2)	2.54 dq (7.2, 3.2)	2.50 dq (7.2, 3.2)	2.53 dq (11, 7.2)	2.44 dq (11, 7.2)
6	6.57 d (9.9)	6.59 d (9.9)	6.61 d (9.9)	6.34 d (9.9)	6.61 d (9.9)	6.33 d (9.9)
7	5.43 d (9.9)	5.45 d (9.9)	5.47 d (9.9)	5.59 d (9.9)	5.47 d (9.9)	5.59 d (9.9)
15	1.33 d (6.5)	1.47 d (6.5)	1.39 d (6.5)	1.36 d (6.5)	1.50 d (6.5)	1.49 d (6.5)
16	1.13 d (7.2)	1.18 d (7.2)	1.15 d (7.2)	1.08 d (7.2)	1.18 d (7.2)	1.13 d (7.2)
17	1.42 s	1.43 s	1.45 s	1.43 s	1.44 s	1.45 s
18	1.45 s	1.43 s	1.44 s	1.43 s	1.44 s	1.43 s
19	3.68 m	3.68 m	3.60 m	3.87 m	3.62 m	3.82 m
20	2.62 m	2.72 m	2.74 m	2.76 m	2.73 m	2.76 m
22	1.81 m	1.90 m	1.80 m	1.87 m	1.81 m	1.84 m
	1.52 m	1.48 m	1.47 m	1.57 m	1.54 m	1.57 m
23	1.17 m	1.12 m	1.19 m	1.19 m	1.15 m	1.16 m
24	0.84 t (7.3)	0.85 t (7.3)	0.86 t (7.2)	0.87 t (7.3)	0.86 t (7.3)	0.87 t (7.3)
Ph–O<u>H</u>	12.37 s	12.46 s	12.39 s	—	12.47 s	—
–COOC<u>H</u>$_3$	—	—	3.58 s	—	3.58 s	—
–CO–C<u>H</u>$_3$	—	—	—	2.39 s	—	2.40 s

a) Chemical shifts (δ) in ppm. *b*) Coupling constants (J) in parentheses in Hz.

Table 2. ^{13}C-NMR (75 MHz, $CDCl_3$) Assignments of Compounds **2** and **4**—**8**[a,b]

C	2	4	5	6	7	8
2	79.0 d	76.2 d	78.9 d	78.7 d	167.5 s	167.5 s
3	45.8 d	45.4 d	45.8 d	47.0 d	37.0 t	36.8 t
4	199.4 s	194.1 s	199.4 s	193.1 s	28.4 d	28.5 d
4a					105.6 s	105.6 s
4b					156.2 s	156.1 s
5	157.1 s	144.7 s	157.1 s	144.5 s		
6	115.7 d	115.5 d	115.8 d	115.6 d	76.4 d	79.1 d
7	125.7 d	129.2 d	125.6 d	129.2 d	44.5 d	46.5 d
8	78.2 s	77.9 s	78.1 s	77.8 s	201.6 s	199.7 s
8a					103.4 s	104.0 s
9					160.2 s	159.8 s
10	109.0 s	116.0 s	109.3 s	116.1s	110.3 s	110.3 s
10a					155.8 s	155.9 s
11	160.0 s	158.0 s	157.1 s	158.1 s	21.3 t	21.3 t
12	101.9 s	106.1 s	101.9 s	106.9 s	121.4 d	121.4 d
13	102.6 s	109.0 s	102.5 s	109.1 s	132.4 s	132.4 s
14	157.0 s	160.0 s	157.1 s	159.7 s	25.7 q	25.7 q
15	19.5 q	15.9 q	19.6 q	19.6 q	17.8 q	17.8 q
16	10.5 q	9.0 q	10.5 q	10.4 q	34.5 t	34.3 t
17	28.4 q	28.3 q	28.4 q	28.3 q	20.0 t	19.9 t
18	28.2 q	28.3 q	28.3 q	28.3 q	13.8 q	13.8 q
19	30.4 d	30.9 t	30.7 d	30.9 d	16.3 q	19.5 q
20	38.8 t	38.3 t	38.8 t	38.3 t	9.4 q	10.2 q
21	179.7 s	175.6 s	173.7 s	175.9 s		
22	35.6 t	35.2 t	35.6 t	35.3 t		
23	20.9 t	20.8 t	21.0 t	20.9 t		
24	14.1 q	14.1 q	14.0 q	14.1 q		
COO<u>C</u>H$_3$			51.3 q			
O<u>C</u>OCH$_3$		169.2 s		169.2 s		
OCO<u>C</u>H$_3$		21.0 q		21.0 q		

a) Multiplicities were obtained from DEPT. *b*) Assignment obtained from HMQC and COSY spectra.

relations with the quaternary olefinic carbons at δ 132.4 (C-13) and δ 160.2 (C-9). This confirmed the presence of the dimethylallyl moiety and determined its attachment at C-10. The carbonyl at δ 167.5 was assigned to a dihydrocoumarin moiety and it showed HMBC correlation with H-4 (δ 3.30) which in turn was correlated to <u>C</u>H$_2$ at δ 20.0 (C-17). In addition, a COSY connectivity was observed between H-4/H-3 supporting the placement of the *n*-propyl group at C-4 of the dihydrocoumarin moiety. Thus the structure of **7** was established as isorecedensolide. It is worthy to mention that **7** is one of the rare examples of the isolation of a dihydrocoumarin, and not a coumarin, from the genus *Calophyllum*.[8]

All the isolated compounds were tested for their cytotoxic activity against KB, Hela and Med. As shown in Table 4, compounds **3**—**5**, **7**, and **8** showed mild activity against KB and Hela tumor cell lines while **1**, **2**, and **6** were inactive.

Table 3. Cytotoxicity of Compounds **1**—**8** against Human Tumor Cells (ED_{50}, μg/ml)[a)]

Compound	KB[b)]	Hela[c)]	Med[d)]
1	13.64	17.73	(—)[e)]
2	11.29	12.77	(—)[e)]
3	7.61	8.94	9.91
4	6.18	6.95	10.83
5	6.37	7.79	8.69
6	13.15	16.79	13.37
7	9.34	9.89	11.79
8	6.81	6.27	12.49
Doxorubicin	0.15	0.14	0.19

a) The concentration that inhibits 50% of the growth of human tumor cell lines after 72 h exposure according to the method described in the experimental section. *b*) Oral epidermoid carcinoma. *c*) Human cervical epitheloid carcinoma. *d*) Human medulloblastoma. *e*) ED_{50} >20 μg/ml.

Experimental

Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. Low-resolution electron impact (EI)-MS and FAB-MS spectra were recorded on a VG Quattro 5022 mass spectrometer. The ^{1}H-, ^{13}C-NMR, COSY, ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), HMBC, and NOESY spectra were recorded on a Bruker FT-300 spectrometer. The chemical shifts are given in δ (ppm) and coupling constants in Hz. Silica gel 60 (Merck) was used for column chromatography (CC), and pre-coated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC.

Plant Material The plant, *Calophyllum blancoi*, was collected from Lan-Yu, Taiwan, in September 2001 and authenticated by one of the authors (Y. C. Shen). A voucher specimen was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan. The ripe seeds were collected and dried at room temperature.

Extraction and Isolation Ripe seeds of *C. blancoi* (830 g) were crushed and then extracted with acetone (1 l×3). The combined extract was concentrated *in vacuo* to give a crude extract, which was partitioned between EtOAc and water (1 : 1). The EtOAc layer was concentrated to give an oily residue (73 g), which was partitioned with *n*-hexane, MeOH and water (4 : 3 : 1). The MeOH/water layer was concentrated (12.5 g) and applied on top of a Si gel column (300 g) and gradiently eluted with an *n*-hexane/EtOAc mixture to afford ten fractions A1—A10. The fractions A8 and A9 yielded **1** (3.6 g), and fraction A6 afforded **2** (1.67 g). Part of fractions A2—A4 (300 mg) was CC on Si gel and eluted with *n*-hexane/EtOAc of increasing polarity (100 : 1—10 : 1) to give fractions B2-1 (43 mg) and B2-2 (75 mg). Fraction B2-1 was subjected to preparative TLC over Si gel and developed with *n*-hexane/CH_2Cl_2/MeOH (20 : 15 : 1) to yield **8** (18 mg) and **5** (8.6 mg). Fraction B2-2 was treated in the same way to yield **7** (40 mg) and **3** (10.3 mg). Part of fraction A5 (100 mg) was subjected to preparative TLC on Si gel and developed with *n*-hexane/EtOAc (3 : 1) to give **4** (19 mg) and **6** (5.5 mg).

Apetalic Acid (**1**): Yellow oil; $[\alpha]_D^{25}$ +23.8° (c=1.0, CH_2Cl_2); EI-MS m/z 388 $[M]^+$; IR (CH_2Cl_2) ν_{max} 3300 (bonded O–H st.), 1710 (C=O st.), 1665 (conj. C=O) cm^{-1}; UV (CH_3CN) λ_{max} nm (log ε): 268 (4.66), 281 (4.12), 340 (4.31); ^{1}H-NMR (300 MHz, $CDCl_3$): Table 1.

Isoapetalic Acid (**2**): Yellow oil; $[\alpha]_D^{25}$ −23.0° (c=1.0, CH_2Cl_2); FAB-MS m/z 389 $[M+H]^+$; EI-MS m/z 388 $[M]^+$, 329 $[M^+-CH_2-COOH]$; IR (CH_2Cl_2) ν_{max} 3300 (bonded O–H st.), 1708 (C=O st.), 1663 (conj. C=O) cm^{-1}; UV (CH_3CN) λ_{max} nm (log ε): 264 (4.53), 284 (4.38), 343 (4.18); ^{1}H-NMR (300 MHz, $CDCl_3$): Table 1; ^{13}C-NMR (75 MHz, $CDCl_3$): Table 2.

Apetalic Acid Methyl Ester (**3**): Yellow oil; $[\alpha]_D^{25}$ +20.0° (c=1.0, CH_2Cl_2); EI-MS m/z 402 $[M]^+$, IR (CH_2Cl_2) ν_{max} 3320 (bonded O–H st.), 1715 (C=O st.), 1667 (conj. C=O) cm^{-1}; UV (CH_3CN) λ_{max} nm (log ε): 290 (4.03), 341 (3.99); ^{1}H-NMR (300 MHz, $CDCl_3$): Table 1.

Apetalic Acid 5-*O*-Acetate (**4**): Yellow oil; $[\alpha]_D^{25}$ +77.1° (c=1.0, CH_2Cl_2); EI-MS m/z 430 $[M]^+$; IR (CH_2Cl_2) ν_{max} 3300 (bonded O–H st.), 1713 (C=O st.), 1668 (conj. C=O), 1250 (C–O st. of acetate) cm^{-1}; UV (CH_3CN) λ_{max} nm (log ε): 289 (4.11), 3.44 (5.43); ^{1}H-NMR (300 MHz, $CDCl_3$): Table 1; ^{13}C-NMR (75 MHz, $CDCl_3$): Table 2.

Isopetalic Acid Methyl Ester (**5**): Yellow oil; $[\alpha]_D^{25}$ −83.7° (c=1.0, CH_2Cl_2); EI-MS m/z 402 $[M]^+$; IR (CH_2Cl_2) ν_{max} 3310 (bonded O–H st.), 1712 (C=O st.), 1663 (conj. C=O) cm^{-1}; UV (CH_3CN) λ_{max} nm (log ε): 287 (3.95), 290 (3.94), 340 (3.80); ^{1}H-NMR (300 MHz, $CDCl_3$): Table 1; ^{13}C-NMR (75 MHz, $CDCl_3$): Table 2.

Isopetalic Acid 5-*O*-Acetate (**6**): Yellow oil; $[\alpha]_D^{25}$ −37.6° (c=1.0, CH_2Cl_2); EI-MS m/z 430 $[M]^+$; IR (CH_2Cl_2) ν_{max} 1711 (C=O st.), 1663 (conj. C=O), 1250 (C–O st. of acetate) cm^{-1}; UV (CH_3CN) λ_{max} nm (log ε): 285 (4.69), 342 (4.25); ^{1}H-NMR (300 MHz, $CDCl_3$): Table 1; ^{13}C-NMR (75 MHz, $CDCl_3$): Table 2.

Isorecedensolide (**7**): Yellowish oil; $[\alpha]_D^{25}$ +47.0° (c=1.0, CH_2Cl_2); FAB-AM m/z 373 $[M+H]^+$; EI-MS m/z 372 $[M]^+$, 329 $[M-C_3H_7]^+$; IR (CH_2Cl_2) ν_{max} 3300 (bonded O–H st.),1771 (C=O st. of lactone), 1665 (conj. C=O) cm^{-1}; UV (CH_3CN) λ_{max} nm (log ε): 283 (4.20), 291 (3.99), 342 (4.01); ^{1}H-NMR (300 MHz, $CDCl_3$): δ: 1.45 (2H, m, H-3), 3.30 (1H, m, H-4), 4.57 (1H, dq, J=6.5, 3.4 Hz, H-6), 2.60 (1H, dq, J=7.3, 3.4 Hz, H-7), 3.30 (2H, d, J=7.1 Hz, H-11), 5.20 (1H, t, J=7.1 Hz, H-12), 1.76 (3H, s, H-14), 1.66 (3H, s, H-15), 2.76 (1H, m, H-16a), 2.68 (1H, m, H-16b), 1.31 (1H, m, H-17a), 1.24 (1H, m, H-17b), 0.91 (3H, t, J=7.3, H-18), 1.40 (3H, d, J=6.5 Hz, H-19), 1.17 (3H, d, J=7.2 Hz, H-20), 12.17 (–OH); ^{13}C-NMR (75 MHz, $CDCl_3$): Table 2.

Recedensolide (**8**): Yellowish oil; $[\alpha]_D^{25}$ −76.1° (c=1.0, CH_2Cl_2); FAB-AM m/z 373 $[M+H]^+$; EI-MS m/z 372 $[M]^+$, 329 $[M-C_3H_7]^+$; IR (CH_2Cl_2) ν_{max} 3300 (bonded O–H st.), 1771 (C=O st. of lactone), 1665 (conj. C=O) cm^{-1}; UV (CH_3CN) λ_{max} nm (log ε): 285 (4.22), 340 (3.97); ^{1}H-NMR (300 MHz, $CDCl_3$): δ: 1.45 (2H, m, H-3), 3.27 (1H, m, H-4), 4.18 (1H, dq, J=10.8, 6.5 Hz, H-6), 2.60 (1H, dq, J=10.8, 7.3 Hz, H-7), 3.31 (2H, d, J=7.1 Hz, H-11), 5.19 (1H, t, J=7.1 Hz, H-12), 1.79 (3H, s, H-14), 1.66 (3H, s, H-15), 2.78 (1H, m, H-16a), 2.63 (1H, m, H-16b), 1.40 (2H, m, H-17), 0.90 (3H, t, J=7.3, H-18), 1.51 (3H, d, J=6.5 Hz, H-19), 1.23 (3H, d, J=7.2 Hz, H-20), 12.27 (–OH); ^{13}C-NMR (75 MHz, $CDCl_3$): Table 2.

Cytotoxicity Assay The cells for assay were cultured in RPMI-1640 medium supplemented with a 5% CO_2 incubator at 37 °C. The cytotoxicity assay depends on the binding of methylene blue to fixed monolayers of cells at pH 8.5, washing the monolayer, and releasing the dye by lowering the pH value. Samples and control standard drugs were prepared at a concentration of 1, 10, 40, and 100 μg/ml. After seeding 2880 cells/well in a 96-well microplate for 3 h, 20 μl of sample or standard agent was placed in each well and incubated at 37 °C for 3 d. After removing the medium from the microplates, the cells were fixed with 10% formaldehyde in 0.9% saline for 30 min, then dyed with 1% (w/v) methylene blue in 0.01 M borate-buffer (100 μl/well) for 30 min. The 96-well plate was dipped into a 0.01 M borate-buffer solution four times in order to remove the dye. Then, 100 μl/well of EtOH–0.1 M HCl (1 : 1) was added as a dye eluting solvent, and the absorbance was measured on a microtiter plate reader (Dynatech, MR 7000) at a wavelength of 650 nm. The ED_{50} value was defined by a comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance.

Acknowledgments The authors are grateful to the National Science Council, Republic of China for financial support (grant # NSC 92-2811-B-110-003). We also acknowledge Ms. Shin-Ching Yu of NSC Southern MS for mass measurements.

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