Neoflavonoid and Biflavonoid Constituents of Calophyllum inophylloide

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NEOFLAVONOID AND BIFLAVONOID CONSTITUENTS OF *CALOPHYLLUM INOPHYLLOIDE*

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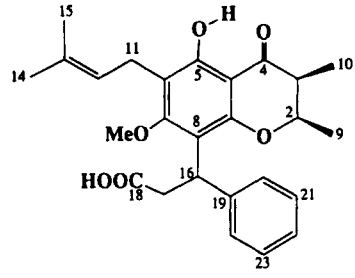
ABSTRACT.—A new neoflavonoid, (+)-(2*R*,3*S*)-2,3-dimethyl-5-hydroxy-6-(3-methylbut-2-enyl)-7-methoxy-8-(2-carboxyl-1-phenylethyl)-2,3-dihydrobenzopyran [**1**], and a new biflavonoid, pyranoamentoflavone [**2**], have been isolated and characterized from the heartwood extractives of *Calophyllum inophylloide* (Guttiferae). Other compounds present were amentoflavone [**3**], friedelin, and canophyllol.

The family Guttiferae contains about 40 genera and nearly 1000 species. In peninsular Malaysia, four genera (*Calophyllum, Garcinia, Mesua,* and *Mammea*) and 121 species are found in all kinds of habitats. Of these, 45 species have been recorded for the genus *Calophyllum* and 49 species for the genus *Garcinia* (1). Extracts and isolates of the family Guttiferae have been subjected to pharmacological and antimicrobial screening by several workers (2–6). Reported significant biological properties of isolates of some *Calophyllum* and *Garcinia* species (7) prompted us to study the chemistry of some Malaysian species, and in this paper we report the isolation of a new neoflavonoid, two biflavonoids of which one is new, and two friedelane-group triterpenes from the heartwood extract of *Calophyllum inophylloide* King, which is endemic to Malaysia.

Tlc examination of the leaf and heartwood extracts of *C. inophylloide* revealed that both extracts contained similar constituents. For isolation of the natural products, a sample of the dried and powdered heartwood of *C. inophylloide* was exhaustively extracted with hot MeOH in a Soxhlet apparatus. A partial separation of the extract was achieved by partitioning between EtOAc and H_2O. The organic layer was concentrated in vacuo and subjected to flash chromatography on Si gel. The nonpolar fractions yielded friedelin and canophyllol after recrystallization from petroleum ether. These two triterpenes were identified by their spectra and comparison with literature data (8,9).

The polar fractions were further subjected to Si gel cc and recrystallization from EtOAc/hexane or EtOAc to yield a neoflavonoid, compound **1**, and two biflavonoid compounds, **2** and **3**. Compound **1** was assigned the molecular formula $C_{26}H_{30}O_6$ from hrms measurement on the molecular ion, which was also the base peak at *m/z* 438. The ready elimination of a C_4H_7 chain giving rise to a peak at *m/z* 383 (80%) indicated that the compound has an isopentenyl side chain. From the ir spectrum, the presence of the following groups was inferred: monosubstituted phenyl (ν max 702 and 755 cm^{-1}), conjugated carbonyl (1639 cm^{-1}), and a carboxylic acid (1700 cm^{-1} and 2500–3400 cm^{-1}).

The ^{1}H-nmr spectrum of **1** in $CDCl_3$ showed poor resolution of the aromatic and methylene signals due to overlapping peaks. However, in pyridine the presence of the following signals could be inferred: 3-methylbut-2-enyl chain (δ 5.46, m, olefinic proton; δ 3.49, m, allylic protons; δ 1.78 and 1.63, two methyls), a monosubstituted phenyl (δ 7.64, d, J=7.5 Hz, H-20 and H-24; δ 7.37, dd, J_1 and J_2=7.5 Hz, H-21 and H-23; δ 7.22, d, J=7.5 Hz, H-22). An MeO signal was observed at δ 3.83, and a low



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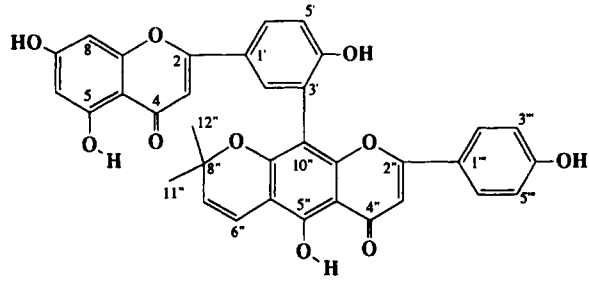

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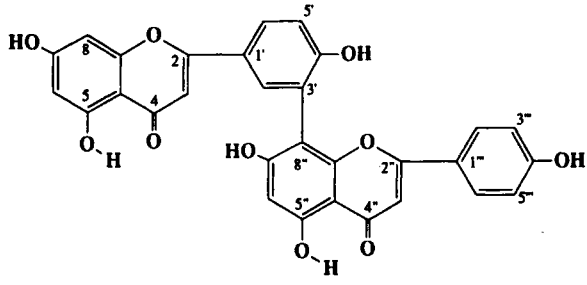

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field signal at δ 12.81 was assignable to a chelated OH group. A single proton, low field signal at δ 5.55 (dd, J_1 and J_2=7.8 Hz, benzylic proton) was coupled to non-equivalent methylene proton signals at δ 3.73 and 3.59. Each of the methylene protons was a doublet of doublets with coupling constants J_1=15.8 and J_2=7.8 Hz. An Me doublet at δ 1.06 (J=6.6 Hz) was coupled to a proton signal at δ 4.60 (dq, J_1=3.5 Hz, J_2=6.6 Hz) which was coupled to an adjacent proton signal at δ 2.68 (dq, J_1=3.5 Hz, J_2=7.3 Hz). The latter was coupled to an Me doublet at δ 0.98 (J=7.3 Hz). The coupling constant of 3.5 Hz between the two adjacent protons indicated a cis-equatorial-axial orientation for protons H-2 and H-3, as in isochapelieric acid (10).

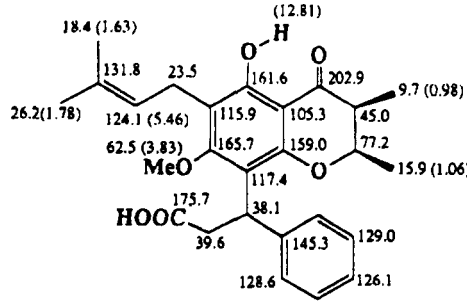
The ^{13}C-nmr chemical shift assignments of compound **1** were obtained from proton-decoupled, off-resonance decoupling, and J-modulated experiments. The presence of five Me carbons, two methylene carbons, nine methine carbons, and ten quaternary carbons, totalling 26 carbon atoms, was revealed. The connectivities between protons and carbons were observed by HMQC and HMBC nmr experiments (Table 1). Important observations from these nmr experiments were as follows. (a) The benzylic proton at δ 5.55,

TABLE 1. ^{13}C-nmr Spectral Data for Compounds **2** and **3**.

Carbon	Compound		
	2[a] (C,D,N)	**3** (DMSO-d_6)	**3** (C,D,N)
C-2	165.1	164.1	166.2
C-3	104.2	103.2	104.7
C-4	182.2	181.9	183.2
C-4a	105.5	104.0	105.8
C-5	163.7	161.6	164.2
C-6	100.6	98.8	100.4
C-7	166.4	163.9	165.1
C-8	95.3	94.2	95.4
C-8a	159.1	157.6	159.0
C-1′	122.8	120.3	122.2
C-2′	132.9	127.9[b]	133.1
C-3′	121.2	121.7[c]	121.4
C-4′	161.6	159.6	161.8
C-5′	117.7	116.4	117.8
C-6′	128.9	131.6[b]	128.8
C-2″	165.3	164.3	165.3
C-3″	104.5	102.8	104.1
C-4″	182.7	182.2	183.6
C-4″a	106.2	104.0	105.8
C-5″	155.4	160.8	163.0
C-5″a	106.4	—	—
C-6″	116.5	99.1	100.5
C-7″	129.1	161.9	163.6
C-8″	79.1	104.1	105.5
C-8″a	—	154.7	156.4
C-9″a	157.8	—	—
C-10″	106.4	—	—
C-10″a	157.1	—	—
C-11″	28.6	—	—
C-12″	29.0	—	—
C-1‴	122.8	121.4[c]	122.4
C-2‴	129.4	128.3	129.3
C-3‴	117.4	116.0	117.4
C-4‴	163.4	161.0	163.2
C-5‴	117.4	116.0	117.4
C-6‴	129.4	128.3	129.3

[a]Assignments were by COSY, NOESY, HMQC, and HMBC 2D nmr methods.
[b]Values from Markham *et al.* (11) reassigned.
[c]Interchangeable.

directly bonded to the carbon atom at δ 38.1 (C-16), showed ^2J correlations with carbon atoms at δ 39.6 (C-17), 117.4 (C-8), 145.3 (C-19), and ^3J correlations with carbon atoms at 128.6 (C-20 and 24), 159.0 (C-8a), 165.7 (C-7), 175.7 (C-18). Thus, the placement of the 2-carboxyl-1-phenylethyl group must be at C-8. (b) The protons of the methylene group of the 3-methylbut-2-enyl substituent (H-11) showed ^2J couplings with carbon atoms at δ 115.9 (C-6) and 124.1 (C-12), and ^3J interactions with the carbon signals at δ 124.1 (C-13), 161.6 (C-5), and 165.7 (C-7). The substituent must as a consequence be placed at C-6. The complete ^{13}C-nmr chemical shift assignments of **1** are given in Figure 1. Compound **1** is, therefore, (+)-(2*R*,3*S*)-2,3-dimethyl-5-hydroxy-6-(3-methylbut-2-enyl)-7-methoxy-8-(2-carboxyl-1-phenylethyl)-2,3-dihydrobenzopyran.



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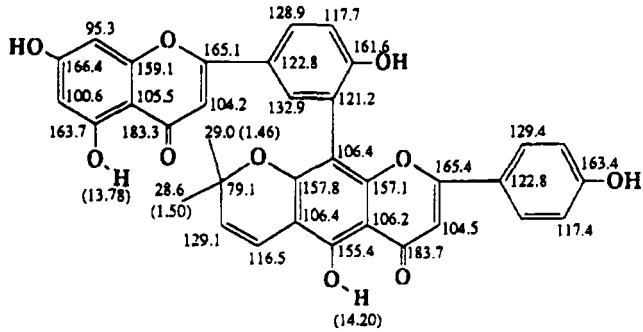

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FIGURE 1. ^{1}H- and ^{13}C-nmr data for compounds **1** and **2**.

Methylation of compound **1** with Me_2SO_4 gave a methyl ester, but the chelated OH group was not methylated. Based on the ms and ^{1}H-nmr data, this derivative was assigned as (+)-(2*R*,3*S*)-2,3-dimethyl-5-hydroxy-6-(3-methylbut-2-enyl)-7-methoxy-8-(2-methoxycarbony-1-phenylethyl)-2,3-dihydrobenzopyran.

The fabms of compound **2** showed an $[M+1]^{+}$ of 605, which agreed with the formula $C_{35}H_{24}O_{10}$. The characteristic uv maxima of 346, 303, and 235 are indicative of a flavonoid structure. The ir spectrum showed two carbonyl peaks at 1658 and 1605 cm^{-1} and a broad OH peak between 3100 and 3400 cm^{-1}. The ^{1}H-nmr spectrum (250 MHz, C_5H_5N) of **2** showed the presence of two chelated OH groups (δ 14.20 and 13.78), a 2,2-dimethyl-2*H*-pyran ring (δ 1.46 and 1.50, gem-dimethyl; 7.01, d, J=10 Hz, methine; 5.68, d, J=10 Hz, methine), and a para-substituted benzene ring (δ 7.93, d, J=8.8 Hz, H-2″, -6″; 7.17, d, J=8.8 Hz, H-3″, -5″). The spectra also showed the presence of other aromatic protons at δ 8.40 (1H, d, J=2.4 Hz), 7.94 (1H, dd, J_1=8.6 Hz, J_2=2.4 Hz), 7.43 (1H, d, J=8.6 Hz), 6.84 (1H, d, J=2.1 Hz) and 6.77 (1H, d, J=2.1 Hz). Two olefinic proton singlets were observed at δ 7.04 and 6.98, and a broad peak centered near 5.20 ppm, integrating for three protons, was due to three OH groups.

The ^{13}C nmr chemical shifts of **2** (completely proton-decoupled, off-resonance decoupling, and J-modulated experiments) revealed the presence of 35 carbon atoms, of which two were Me carbons, thirteen were methine carbons and the remaining were quarternary carbons. Two-dimensional nmr (HMQC, HMBC, NOESY) techniques were also used to determine proton and carbon connectivities. The most important observation from the HMBC experiment was an observed ^{3}J correlation of the aromatic proton at δ 8.40 (H-2′) with the carbon at 106.4 ppm (C-10″). On this basis compound

2 must be a 3'-8" linked biflavonoid belonging to the amentoflavone series of biflavonoids. The assignments follow logically from those for the known amentoflavone [**3**], while only the signals relating to the A-ring carbons of flavonoid **2** require the application of established substituent effect rules for their assignment (10) apart from the results from the HMBC experiment. The above spectral information confirmed the structure of **2** as a pyranoamentoflavone. Characteristic ^{13}C-nmr chemical shift assignments of **2** are given in Figure 1.

The ir and uv spectra of compound **3** indicated that it had a flavonoid structure whereas the ms fragmentation indicated it to be a biflavonoid. Further elucidation of the structure by ^{1}H- and ^{13}C-nmr (completely proton-decoupled, off-resonance decoupling and *J*-modulated experiments) revealed compound **3** as amentoflavone. The physicochemical and spectral data of compound **3** are identical to those of amentoflavone as reported by Markham *et al.* (11).

EXPERIMENTAL

PLANT MATERIAL.—The leaves and heartwood of *C. inophylloide* were collected in January 1990 from Pasoh, Negri Sembilan in peninsular Malaysia. A voucher specimen has been deposited at the herbarium of the Forest Research Institute of Malaysia, Kepong.

EXTRACTION AND ISOLATION OF COMPOUNDS.—A small amount of the leaves and heartwood (5 g each) were soaked in MeOH separately, and the extracts were subjected to tlc screening. Tlc behaviors indicated that the extracts contained similar constituents. The heartwood (2.0 kg) was air-dried and ground to pass through a 40–60 mesh screen. The sample was soaked in *n*-hexane overnight to remove fatty and waxy materials. It was then extracted with MeOH in a Soxhlet apparatus for 20 h. The MeOH extract was concentrated in vacuo to give a dark brown gummy residue (108 g). The residue was partitioned between EtOAc and H_2O. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a dark brown residue (35 g). A portion of the solid (20 g) was subjected to flash chromatography on a Si gel (230–400 mesh) column.The column was eluted with solvent mixtures of increasing polarity (hexane, hexane/EtOAc, EtOAc, EtOAc/MeOH), and 15 fractions were collected.

Fractions 1–6 were combined, concentrated to small volume, and kept at room temperature. A crystallizate separated out, which on recrystallization from petroleum ether (60–80°) afforded canophyllol as white needles (765 mg, 0.038%), mp 283–284° [lit. (9) 282°]. The mother liquor of canophyllol was submitted to preparative tlc over Si gel in toluene-EtOAc (19:1), followed by recrystallization from petroleum ether (60–80°), to yield friedelin as white needles (65 mg, 0.003%), mp 261–263° [lit. (8) 263°]. The two compounds also had ^{1}H-nmr spectra identical to those reported (8,9). Fractions 7–12 were combined and separated by Si gel cc (230–400 mesh) with CH_2Cl_2/EtOAc gradients of increasing polarity, followed by recrystallization from EtOAc/hexane (7:3) to give compounds **1** (180 mg, 0.009%) and **2** (95 mg, 0.0048%). Fractions 13–15 were combined and chromatographed on a Si gel (230–400 mesh) column with EtOAc/MeOH gradients of increasing polarity followed by recrystallization from EtOAc to yield compound **3**. Compound **3** was identified as amenotoflavone by comparison of its spectral data with literature values (11).

(+)-(2R,3S)-2,3-Dimethyl-5-hydroxy-6-(3-methylbut-2-enyl)-7-methoxy-8-(2-carboxyl-1-phenylethyl)-2,3-dihydrobenzopyran [**1**].—Pale cream needles (EtOAc/hexane): mp 170–171°, $[\alpha]^{25}D$ +30° (*c*=1, $CHCl_3$); R_f 0.74 on Si gel (EtOAc); found $[M]^+$ 438.1953 ($C_{26}H_{30}O_6$ requires 438.1931); uv λ max EtOH 362, 288, 228 nm; ir ν max (KBr) 3400, 2500, 1700, 1639, 755, 702 cm^{-1}, ^{1}H nmr (250 MHz, C_5H_5N) δ 12.81 (1H, s, chelated 5-OH), 7.64 (2H, d, *J*=7.5 Hz, H-20, H-24), 7.37 (2H, dd, J_1 and J_2=7.5 Hz, H-21, H-23), 7.22 (1H, d, *J*=7.5 Hz, H-22), 5.55 (1H, dd, J_1 and J_2=7.8 Hz, H-16), 5.46 (2H, m, H-12), 4.60 1H, m, J_1=6.6 Hz, J_2=3.5 Hz, H-2), 3.83 (3H, s, OMe), 3.73 and 3.59 (1H, dd, J_1=15.8 Hz, J_2=7.8 Hz, H-17), 3.49 (2H, m, H-11), 2.68 (1H, m, J_1=7.3 Hz, J_2=3.5 Hz, H-3), 1.78 (3H, s, Me-14), 1.63 (3H, s, Me-15), 1.06 (3H, d, *J*=6.6 Hz, Me-9), 0.98 (3H, d, *J*=7.3 Hz, Me-10); ^{13}C-nmr (75 MHZ, $CDCl_3$) chemical shifts see Figure 1; eims (70 eV, 180° source temperature) *m/z* (rel. int. %) 438 (100), 423 (24), 383 (81), 379 (52), 323 (25), 311 (21), 289, (35), 91 (30).

Methylation of **1**.—Compound **1** (18 mg) in dry Me_2CO (5 ml) was treated with Me_2SO_4 (0.5 ml) and dry K_2CO_3 (20 mg). The product was a yellow semisolid Me ester derivative: found $[M]^+$ 452.2221 ($C_{27}H_{32}O_6$ requires 452.2199); ^{1}H nmr ($CDCl_3$) δ 12.27 (1H, s, chelated 5-OH), 7.32–7.15 (5H, m, monosubstituted phenyl protons H-21 to H-24), 5.22 (1H, dd, H-16), 5.02 (1H, m, H-12), 4.55 (1H, m, H-2), 3.62 (3H, s, OMe), 3.61 (3H, s, OMe), 3.26 (4H, m, H-11, H-17), 2.62 (1H, m, H-3), 1.76 (3H, s, Me-14), 1.69 (3H, s, Me-15), 1.25 (3H, d, *J*=6.6 Hz, Me-9), 1.10 (3H, d, *J*=7.3 Hz, Me-10).

Pyranoamentoflavone [**2**].—Yellow crystals (EtOAc): mp 228–230° (dec), R_f 0.69 on Si gel (EtOAc); fabms (nitrobenzyl alcohol, Xe) *m/z* (rel. int. %) $[M+H]^+$ 605 (60%), 589 (16), 306 (10), 292 (23), 274 (21), 260 (19), 242 (22), 228 (11), 216 (10), 170 (18), 155 (21), 139 (37), 121 (90), 107 (35), 65 (100) ($[M]^+$ is deduced to be $C_{35}H_{24}O_{10}$); uv λ max EtOH 346, 303, 235 nm; ir ν max (KBr) 2400–3400 (broad OH), 1658 (C=O), 1605 (C=O) cm^{-1}; ^1H nmr (250 MHz, C_5D_5N) δ 14.20 (1H, s, 5″-OH), 13.78 (1H, s, 5-OH), 8.40 (1H, d, J=2.4 Hz, H-2′), 7.94 (1H, dd, J_1=8.6 Hz, J_2=2.4 Hz, H-6′), 7.93 (2H, d, J=8.8 Hz, H-2‴, H-6‴), 7.43 (1H, d, J=8.6 Hz, H-5′), 7.17 (2H, d, J=8.8 Hz, H-3‴, H-5‴), 7.04 (1H, s, H-3″), 7.01 (1H, d, J=10 Hz, H-6″), 6.98 (1H, s, H-3), 6.84 (1H, d, J=2.1 Hz, H-8), 6.77 (1H, d, J=2.1 Hz, H-6), 5.68 (1H, d, J=10 Hz, H-7″), 1.50 (3H, s, Me-11″), 1.46 (3H, s, Me-12″); ^{13}C nmr (75 MHz, C_5D_5N) see Table 1.

Amentoflavone [**3**].—Compound **3** was isolated as yellow crystals, mp 247–249°, and the ^{13}C-nmr spectral data (75 MHz, C_5D_5N) are summarized in Table 1.

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