Biflavonoids of *Calophyllum venulosum*

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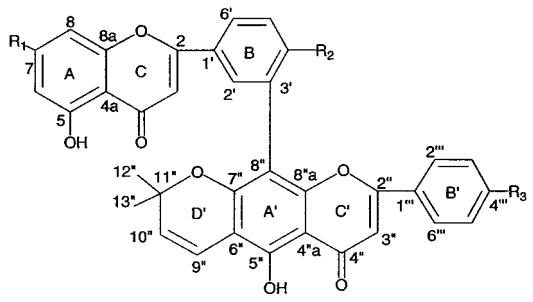
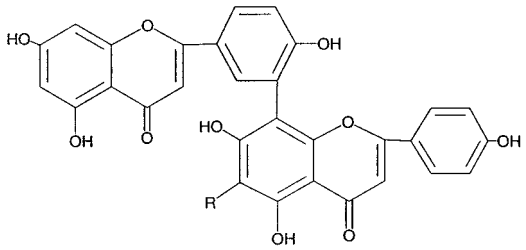
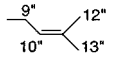
Four new biflavonoids [pyranoamentoflavone 7,4‴-dimethyl ether (**1**), pyranoamentoflavone 7,4′-dimethyl ether (**2**), 6″-(3-methyl-2-butenyl)amentoflavone (**3**), and 6″-(2-hydroxy-3-methyl-3-butenyl)amentoflavone (**4**)], as well as three known biflavonoids, [pyranoamentoflavone (**5a**), amentoflavone (**6**), and 2,3-dihydroamentoflavone (**7**)] have been isolated from the leaves of *Calophyllum venulosum*. The structures of the new compounds were established by spectroscopic data and chemical modification.

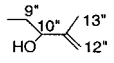
Since the classification of 20 structural types of biflavonoids by Geiger,[1] a steady stream of new biflavonoids has been reported continuously.[2−14] Lower plants have previously dominated as sources of biflavonoids, but it is noted that several higher plants have also afforded these compounds. Although the better-studied genus *Garcinia*[1,15−19] has provided biflavonoids, certain species from the genus *Calophyllum* (both in the same family Guttiferae) are now known also to furnish these compounds.[3,20,21] Amentoflavone, the main biflavonoid obtained from the genus *Calophyllum*, has been shown to inhibit HIV-reverse transcriptase, prevent histamine release from mast cells, and cause mice to enhance their production of interferon.[1] Three species of the genus *Calophyllum* have been studied for their biflavonoids.[3,20,21] *Calophyllum venulosum* Zoll., which is native to Malaysia, has not been chemically investigated before, and in this paper we report the isolation of four novel biflavonoids (**1**−**4**) together with three known ones (**5a**, **6**, and **7**) from the leaves of the plant.



1 R_1 = OMe, R_2 = OH, R_3 = OMe
2 R_1 = OMe, R_2 = OMe, R_3 = OH
5a R_1 = OH, R_2 = OH, R_3 = OH
5b R_1 = OMe, R_2 = OMe, R_3 = OMe



3 R = 

4 R = 

6 R = H

Results and Discussion

Compound **1** was isolated as a yellow powder. The FABMS (3-nitrobenzyl alcohol, Ar) of **1** gave a pseudomolecular ion at *m*/*z* 631 $[M - H]^-$ corresponding to a molecular formula of $C_{37}H_{28}O_{10}$, which was confirmed by HREIMS (found *m*/*z* 632.1697, calcd 632.1683). The UV spectrum of **1** in MeOH exhibited a maximum at 338 nm, which, on addition of NaOMe, underwent a bathochromic shift (to 400 nm), indicating the presence of a free C-4 or C-4‴ hydroxyl group. The addition of $AlCl_3$, $AlCl_3$−HCl, and NaOAc−H_3BO_3 caused no changes in the UV spectral data and indicated the absence of any 3′,4′- or 3‴,4‴-*ortho*-dihydroxyl system.[22]

The ^{1}H-NMR spectrum of **1** ($CDCl_3$) showed the presence of an AMX coupling system, with signals at δ 7.91 (H-6′, dd, J = 8.8, 2.4 Hz), 7.89 (H-2′, d, J = 2.4 Hz), and 7.25 (H-5′, d, J = 8.8 Hz), indicating that C-3′ was the position where two flavonoid units were linked together.[9] Two meta-coupled proton signals of H-6 and H-8 appeared at δ 6.50 and 6.60 (both d, J = 2.2 Hz); an AA′XX′ coupling system was established from the signals at δ 7.42 (H-2‴, H-6‴, d, J = 8.8 Hz) and 6.72 (H-3‴, 5‴, d, J = 8.8 Hz), and two proton signals appearing at δ 6.35 (H-3, s) and δ 6.34 (H-3″, s) excluded the possibility of any linkage between the two flavone moieties at C-3″, C-2‴, C-3‴, C-5‴ or C-6‴ with C-3′. Two protons of the chelated hydroxyl groups had resonances at δ 13.1 (OH-5″, s) and δ 12.8 (OH-5′, s). The proton signals appearing at δ 1.51 (3H, s) and δ 1.43 (3H, s) were assigned to H-13″ and H-12″ which, together with the two olefinic protons at δ 6.80 (H-9″, d, J = 10.0 Hz) and δ 5.69 (H-10″, d, J = 10.0 Hz), constituted a 2,2-dimethylpyran ring that could be fused to C-6″ and C-7″, as in the case of the known pyranoamentoflavone (**5a**).[3] This was confirmed by NOE difference experiments. Irradiation of the hydroxyl resonance (δ 13.1, OH-5″, s) caused an NOE enhancement of the proton at δ 6.80 ppm (H-9″). The results suggested that another connecting position for the two flavones was at C-8″. By comparison of its ^{1}H-NMR spectrum (Me_2CO-d_6, see Experimental Section) with that of pyranoamentoflavone (**5a**) (Me_2CO-d_6, see Table 1), it was observed that, while compound **1** showed

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Table 1. ^{1}H-NMR Assignments and Coupling Constants for Compounds **1**−**5b**[a]

proton(s)	**1**	**2**	**3**	**4**	**5a**	**5b**
3	6.35 (s)	6.62 (s)	6.73 (s)	6.70 (s)	6.70 (s)	6.63 (s)
6	6.50 (d 2.2)	6.37 (d 2.2)	6.24 (d 2.0)	6.24 (d 2.0)	6.24 (d 2.1)	6.36 (d 2.2)
8	6.60 (d 2.2)	6.42 (d 2.2)	6.50 (d 2.0)	6.50 (d 2.0)	6.53 (d 2.1)	6.41 (d 2.2)
2′	7.89 (d 2.4)	7.90 (d 2.4)	8.14 (d 2.3)	8.09 (d 2.3)	8.08 (d 2.5)	7.90 (d 2.4)
5′	7.25 (d 8.8)	7.15 (d 8.8)	7.24 (d 8.7)	7.23 (d 8.7)	7.27 (d 8.6)	7.15 (d 8.8)
6′	7.91 (dd 8.8,2.4)	7.97 (dd 8.8,2.4)	8.04 (dd 8.7,2.3)	8.03 (dd 8.7,2.3)	8.03 (dd 8.6,2.5)	7.97 (dd 8.8,2.4)
3″	6.34 (s)	6.57 (s)	6.65 (s)	6.65 (s)	6.70 (s)	6.59 (s)
9″	6.80 (d 10.0)	6.81 (d 10.0)	3.46 (d 7.1)	3.17 (m); 3.00 (m)	6.76 (d 10.0)	6.81 (d 10.0)
10″	5.69 (d 10.0)	5.63 (d 10.0)	5.35 (m)	4.47 (br t)	5.81 (d 10.0)	5.63 (d 10.0)
2‴,6‴	7.42 (d 8.8)	7.43 (d 8.8)	7.63 (d 8.9)	7.65 (d 8.7)	7.67 (d 8.6)	7.47 (d 8.8)
3‴,5‴	6.72 (d 8.8)	6.80 (d 8.8)	6.82 (d 8.9)	6.82 (d 8.7)	6.86 (d 8.6)	6.83 (d 8.8)
OH-5	12.8 (s)	12.8 (s)	13.01 (s)	12.98 (s)	12.99 (s)	12.8 (s)
OH-5″	13.1 (s)	13.2 (s)	13.46 (s)	13.66 (s)	13.5 (s)	13.2 (s)
MeO-7	3.82 (s)	3.84 (s)				3.84 (s)
MeO-4′		3.81 (s)				3.81 (s)
MeO-4‴	3.74 (s)				3.79 (s)	
Me-12″	1.51 (s)	1.44 (s)	1.80 (s)	5.05 (br s); 4.84 (br s)	1.50 (s)	1.44 (s)
Me-13″	1.43 (s)	1.39 (s)	1.67 (s)	1.87 (s)	1.43 (s)	1.39 (s)

[a] Multiplicity and coupling constants (Hz) are given in parentheses; compounds **1**, **2**, and **5b** were measured in $CDCl_3$, and compounds **3**, **4**, and **5a** were measured in Me_2CO-d_6.

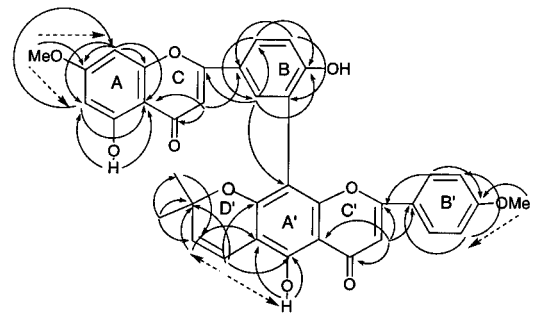
Table 2. ^{13}C-NMR Data of Compounds **1**−**5b**

position	**1**	**2**	**3**	**4**	**5a**	**5b**[a]
C-2	165.6	165.3	165.1	165.4	165.2	164.1
C-3	104.5 d[b]	104.0 d	104.4 d	104.4 d	103.8 d	104.4
C-4	183.5	183.5	183.1	183.2	183.1	182.4
C-4a	106.3	106.2	105.4	105.6	105.3	105.5
C-5	163.3	163.4	163.4	163.5	163.4	162.2
C-6	99.0 d	99.0 d	99.8 d	100.0 d	98.1 d	99.8 d
C-7	166.8	166.9	164.8	165.2	165.0	165.5
C-8	93.5 d	93.6 d	94.8 d	95.0 d	94.8 d	92.6 d
C-8a	159.0	159.0	158.9	159.1	158.9	157.7
C-1′	123.4	123.4	123.52	123.4	123.1	123.2
C-2′	132.8 d	132.3 d	132.97 d	132.8 d	132.6 d	131.2 d
C-3′	120.8	122.7	120.5	121.9	120.6	121.9
C-4′	160.5	162.1	161.1	160.5	160.2	160.7
C-5′	117.7 d	112.6 d	118.0 d	117.7 d	117.4 d	111.0 d
C-6′	129.1 d	129.4 d	129.3 d	128.8 d	128.9 d	127.8 d
C-2″	165.1	165.3	165.0	165.1	165.1	163.4
C-3″	104.6 d	104.0 d	103.8 d	103.8 d	104.2 d	103.8 d
C-4″	183.9	183.8	183.5	183.7	183.6	182.8
C-4″a	106.0	105.8	103.9	105.3	105.5	105.3
C-5″	157.3	157.1	164.9	160.4	157.0	156.2
C-6″	106.5	106.3	112.7	110.9	106.2	105.6
C-7″	158.2	157.9	159.8	162.6	157.8	156.8
C-8″	104.7	105.1	104.35	105.3	105.8	104.3
C-8″a	155.6	155.2	154.1	154.8	155.3	154.1
C-9″	116.2 d	116.2 d	22.5 t	30.0 t	116.1 d	115.7 d
C-10″	129.6 d	129.5 d	123.3 d	77.1 d	129.3 d	128.0 d
C-11″	79.4	79.4	131.8	148.2	79.2	78.2
C-12″	28.7 q	28.7 q	25.96 q	110.8 t	28.5 q	28.3 q
C-13″	28.9 q	28.9 q	18.0 q	19.0 q	28.8 q	28.3 q
C-1‴	124.5	124.2	123.5	123.5	123.2	123.4
C-2‴,6‴	129.2 d	129.3 d	129.1 d	129.3 d	129.2 d	127.7 d
C-3‴,5‴	115.6 d	117.1 d	116.7 d	116.9 d	116.8 d	114.5 d
C-4‴	164.0	162.2	161.7	162.0	162.0	162.5
MeO-7	56.6 q	56.7 q				55.8 q
MeO-4′		56.6 q				55.8 q
MeO-4‴	56.2 q					55.5 q

[a] Compound **5b** was run in $CDCl_3$; compounds **1**, **2**, **3**, **4**, and **5a** were measured in Me_2CO-d_6. [b] Multiplicity was determined by DEPT; all other carbon signals not labeled are singlets.

closely similar features to those corresponding to compound **5a**, only H-8 and H-3‴, 5‴ in **1** showed downfield shifts of $\Delta\delta$ 0.10 and 0.08 ppm, respectively, so that it may be concluded that compound **1** was a biflavonoid having an C-3′−C-8″ interflavonoid linkage as in pyranoamentoflavone,[3] corresponding to the amentoflavone series[20] with *O*-methylated sites at C-7 and C-4‴.

The ^{13}C-NMR spectrum of **1** showed two methoxyl signals at δ 56.2 and 56.6. The carbons of rings B, C, A′, and D′ (2 to 4 and 1′ to 13″)[23] showed carbon chemical shift values identical to those corresponding to pyranoamentoflavone (**5a**). The chemical shift differences of the carbons in A and B′ rings between these two compounds, mainly C-6, C-7, C-8, C-4‴, C-3‴ and C-5‴, gave further support for methoxyl substituents at positions C-7 and C-4‴ (Table 2). In NOE difference experiments, NOE enhancements between H-6/H-8 at δ 6.50/6.60 (d, J = 2.2 Hz) and 7-OMe at δ 3.82 (s) as well as between H-3‴/H-5‴ at δ 6.72 (d, J = 8.8 Hz) and 4‴-OMe at 3.74 (s) as shown in Figure 1 also supported the above suggestion. Therefore, compound



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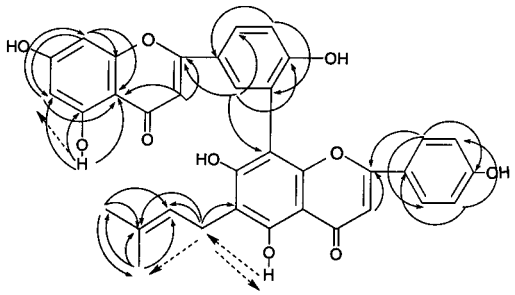

3

Figure 1. Major NOE difference (hashed arrow) and HMBC correlations of compounds **1** and **3**.

1 was identified as the new compound, pyranoamentoflavone 7,4‴-dimethyl ether.

Compound **2** was isolated as a light yellow powder and had a FABMS (3-nitrobenzyl alcohol, Ar) (*m*/*z* 631, $[M - H]^-$) similar to that of compound **1**, indicating that it was an isomer of **1**. The UV spectrum of compound **2** in MeOH showed a λ_{max} at 338 nm, which, on addition of NaOMe, showed a bathochromic shift to 372 nm, suggesting the presence of 4′-OH and/or 4‴-OH.[22] Like compound 7,4′,4‴-trimethylpyranoamentoflavone (**5b**), compound **2** exhibited the presence of an AMX coupling system with signals at δ 7.97 (H-6′, dd, J = 8.8, 2.4 Hz), δ 7.90 (H-2′, d, J = 2.4 Hz) and δ 7.15 (H-5′, d, J = 8.8 Hz), two singlets at δ 6.62 (H-3, s) and δ 6.57 (H-3″, s), a set of peaks at δ 6.81 (H-9″, d, J = 10.0 Hz), δ 5.63 (H-10″, d, J = 10.0 Hz), δ 1.44 (Me-12″, s) and δ 1.39 (CH_3-13″, s) belonging to a dimethylpyran ring, a *meta*-coupling system with peaks at δ 6.37 (H-6, d, J = 2.2 Hz) and δ 6.42 (H-8, d, J = 2.2 Hz), and two low-field singlets at δ 12.8 (OH-5) and δ 13.2 (OH-5″) due to the two chelated hydroxyl groups. Both compounds showed nearly identical chemical shifts and coupling constants except that the signals of H-2‴,6‴ and H-3‴,5‴ of compound **2** appeared at δ 7.43 (d, J = 8.8 Hz) and δ 6.80 (d, J = 8.8 Hz), while those of compound **5b** appeared at δ 7.47 (d, J = 8.8 Hz) and δ 6.83 (d, J = 8.8 Hz) instead, with only slight upfield shifts of $\Delta\delta$ 0.04 and 0.03 ppm, respectively, for H-2‴, 6‴ and H-3‴, 5‴. Based on these data, it may be concluded that compound **2** had the pyranoamentoflavone skeleton with two methoxyl groups at C-7 and C-4′ similar to compound **5b** but with a hydroxyl group at C-4‴. This was further confirmed by NOE difference experiments. Irradiation of the signal at δ 3.84 (OMe-7, s) caused peak enhancements at δ 6.37 (H-6, d, J = 2.2 Hz) and 6.42 (H-8, d, J = 2.2 Hz). A comparison of its ^{13}C-NMR spectrum with that of compound **5a** (Table 2) supported these assignments. In their ^{13}C-NMR spectra, it was observed that only the carbons in rings A and B showed different chemical shifts, while all other chemical shifts were closely comparable for compounds **2** and **5a**.[23] Furthermore, the carbons in rings A, B, and C exhibited chemical shifts similar to those of abiesin (a 3′,6″-biflavone).[24] Therefore, the only positions available as sites for methylation were C-7 and C-4′. Thus, compound **2** was determined structurally as pyranoamentoflavone 7,4′-dimethyl ether.

Compound **3** was isolated as a yellow powder. The HREIMS determined the molecular ion as $C_{35}H_{26}O_{10}$ (found *m*/*z* 606.1536, calcd 606.1526), corresponding to a prenylated biflavonoid. The analysis of its NMR data including DEPT, NOE, HMQC, and HMBC spectra, allowed for an unambiguous assignment of all proton and carbon signals (Tables 1 and 2). In the HMBC spectrum of compound **3** (Figure 1), a chelated hydroxyl proton exhibited long-range correlations to three carbons, one of which must be oxygenated. It was observed that the less deshielded chelated hydroxyl signal (δ_H 13.01) correlated with a protonated carbon (δ 99.8, C-6) and two substituted carbons (δ 163.4 and 105.4). The oxygenated carbon signal (δ 163.4) was readily assigned to C-5, while the signal at δ 105.4, which correlated to H-3 as well as OH-5, was assigned to C-4a. ^2J-correlations of H-3 to the oxygenated carbon at δ 165.1, C-2 and a ^3J-coupling to a substituted carbon at δ 123.5 extended the structure to ring B. A carbon with a chemical shift δ 123.5, which was coupled to H-5′ of the ABX system, was assigned to C−1′ of the trisubstituted benzene ring. The C-4′ carbon, which was identified by ^3J-correlations from H-2′ and H-6′, was shown to be oxygenated from its chemical shift (δ 161.1). The substituted carbon at δ 120.5 (s) must be C-3′ as it was coupled to H-5′. The HMBC spectrum depicted that H-2′ was also coupled to a substituted carbon C-8″ (δ 104.35), which did not correlate to the other protons of ring B. This carbon must belong to the second monomer, and it was linked to C-3′. Because C-3′ was not coupled to other protons, apart from those in ring B, C-8″ must be flanked by two substituted carbons. This pointed to a C-3′−C-8″ linkage between the two monomers. The ^{1}H-NMR spectrum of **3** showed two singlets (3H each) at δ 1.80 and 1.67, a broad triplet (1H) at δ 5.35 (J = 7.1 Hz), and a doublet at δ 3.46 (2H, J = 7.1 Hz) indicating the presence of a prenyl (3,3-dimethylallyl) group in the molecule. In the ^{13}C-NMR spectrum, it was observed that, except for the extra prenyl group signals in compound **3** all the chemical shift values were similar for compounds **3** and amentoflavone (**6**), with only C-6″ in compound **3** displaying a 12.8 ppm downfield shift. Irradiation of the more deshielded chelated hydroxyl (δ 13.46, OH-5″, s) caused NOE enhancements of the protons at δ 3.46 (H-9″, d, J = 7.1 Hz), which confirmed the position of the prenyl group to be at C-6″ and showed that the chemical shift of C-6″ (δ 112.7) was closely comparable to analogous values of similar known compounds.[25] Therefore, **3** was established structurally as 6″-(3-methyl-2-butenyl)amentoflavone.

The ^{1}H-NMR spectrum of compound **4** had features similar to that of compound **3**. The analysis of the ^{1}H-NMR spectrum revealed AMX (H-2′, H5′, and H-6′), AA′XX′ systems (H-2‴,6‴ and H-3‴,5‴), two hydrogen-bonded 5-hydroxyls, and two *meta*-coupled doublets (H-6

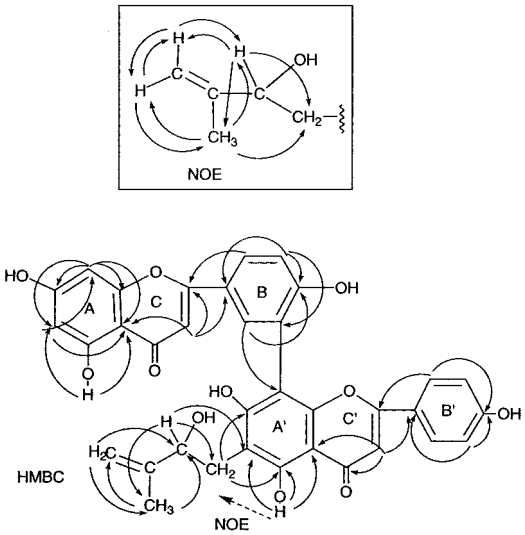

Figure 2. Major NOE and HMBC correlations of compound **4**.

and H-8). Other signals could be assigned to single protons (H-3 of C-ring/H-3″ of C′-ring) and a 2-hydroxy-3-methyl-3-butenyl system. To our knowledge, only a few flavonoids carrying a 2-hydroxy-3-methyl-3-butenyl substituent have been isolated from plants.[3,26−29] In one ^{1}H-NMR spectrum run in Me_2CO-d_6, Ha-9″ and Hb-9″ of **4** were observed as multiplets overlapping with hydroxyl proton signals, while in DMSO-d_6 both appeared as two doublets, and H-10″ was a broad unresolved doublet. The spectral data indicated that compound **4** had the same biflavonoid skeleton as compound **3** but with a different prenyl substituent, namely, 2-hydroxy-3-methyl-3-butenyl and 3-methyl-2-butenyl, respectively. As with compounds **1** and **3**, an HMBC experiment (Figure 2) revealed ^3J coupling linking 2′-H to C″-8. Therefore, the biflavonoid must be linked through C-3′ of one monomer to C-8″ of the second monomer, with C-6″ being occupied by the 2-hydroxy-3-methyl-3-butenyl group. In the HMBC spectrum, H-9″ was correlated to C-5″, C-6″ and C−7″, which further confirmed the substituted position. Completely unambiguous assignments of all ^{13}C-NMR resonances (Table 2) were obtained through HMBC and HMQC experiments. Compound **4** showed a molecular ion at m/z 622 ($C_{35}H_{26}O_{11}$), and the ready loss of H_2O from the allylic alcohol group provided an ion at m/z 604.1356 (calcd for $C_{35}H_{24}O_{10}$, 604.1369) in the HREIMS, which confirmed the molecular formula. The biflavonoid **4** was therefore determined as 6″-(2-hydroxy-3-methyl-3-butenyl)amentoflavone.

Amentoflavone (**6**) and 2,3-dihydroamentoflavone (**7**), two very common biflavonoids, were identified by comparing their spectroscopic data with those reported.[30] Pyranoamentoflavone (**5a**), a known compound first isolated from *C. inophylloide*,[3] was methylated to give compound **5b** (see Experimental Section), and its structure was confirmed by the application of spectroscopic data including 2D NMR spectra. This represents a second report of a pyranoamentoflavone from a plant source. Biflavonoids with prenyl groups generally are rare in nature.[2,3,31] So far, biflavones (with C-3−C-8″ linkage) carrying a prenyl group have been isolated only from the genus *Calophyllum*; thus, the occurrence of such compounds in *C. venulosum* may be of chemotaxonomic significance. Except for this feature, the biflavonoids elaborated by *Calophyllum* and *Garcinia* from the family Guttiferae show similarities in being of the flavone−flavone, flavanone−flavone, flavanone−flavanone, and flavanone−flavanol types. From this and other studies[3,20,21] it is seen that the potential diversity of biflavonoids from the genus *Calophyllum* may in time rival those of the better studied *Garcinia* species. It would be interest to conduct cytotoxicity and anti-HIV studies on the new biflavonoids obtained in this investigation inasmuch as such bioactivities have been found for their simpler analogues.[1,32]

Experimental Section

General Experimental Procedures. A Bausch and Lamb hot-stage instrument was used to measure melting points (uncorrected). IR spectra were recorded on a Bio-Rad FT-IR spectrometer, and UV spectra were recorded on a Hewlett Packard 8425A diode array spectrometer. NMR spectra were recorded using Bruker ACF 300 [300 MHz (^{1}H) and 75 MHz (^{13}C)] and AMX 500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] instruments in $CDCl_3$, Me_2CO-d_6, and DMSO-d_6 solutions with TMS as an internal standard, unless otherwise stated. EIMS were run on a Micromass VG 7035 mass spectrometer at 70 eV. Liquid chromatography was performed on Si gel (Kieselgel 60, particle size 0.04-0.063 mm) and Sephadex LH20. TLC was run on precoated Si gel glass plates (Merck silica gel 60F_{254}).

Plant Material. The leaves of *Calophyllum venulosum* Zoll. (Guttiferae) were collected in June 1995, from Danum Valley, Sabah, Malaysia. The voucher specimen (JTP195) has been deposited at the Herbarium of the Forest Research Centre, Sepilok, Sabah, Malaysia.

Extraction and Isolation. Dried and powdered leaves of *C. venulosum* (2 kg) were successively and exhaustively extracted with hot EtOH (10 L × 5). Evaporation *in vacuo* reduced the extract to a residue of 210 g. After re-extraction with *n*-hexane, the residue was extracted with EtOAc (500 ml × 10), and concentration *in vacuo* gave a residue (50 g). This residue was then subjected to Si gel column chromatography and eluted with solvent mixture of increasing polarity (*n*-hexane−Me_2CO) beginning with 10:1. Further separation of the fraction was achieved by Si gel flash chromatography and column chromatography on Sephadex LH20 with $CHCl_3$−MeOH (1:1). Compounds **5a** and **6** were isolated in relatively large amounts (75 mg and 500 mg, respectively). Compounds **1**, **2**, and **3** were purified on preparative TLC with $CHCl_3$−EtOAc (8:1) and $CHCl_3$−MeOH (10:1), respectively, with yields of 10 mg, 5 mg, and 20 mg respectively. Compound **7** was obtained in a small amount (3 mg), while the yield of compound **4** was somewhat higher (20 mg).

Pyranoamentoflavone 7,4‴-dimethyl ether (1): yellow powder ($CHCl_3$); mp 240-242 °C; UV (MeOH) λ_{max} (log ϵ) 270 (4.55), 310 (4.38), 338 (4.53) nm; NaOMe 268, 300, 348, 400 nm; $AlCl_3$ 280, 300, 328, 364 (sh) nm; $AlCl_3$−HCl 280, 300, 328, 364 (sh) nm; NaOAc 270, 312, 340; NaOAc−H_3BO_3 270, 310, 338 nm; IR (KBr) ν_{max} 3448, 2983, 2936, 1657, 1611, 1511, 1501, 1437, 1372, 1337, 1293, 1266, 1208, 1183, 1165, 1131, 1046, 841 cm^{-1}; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Me_2CO-d_6) data, see Tables 1 and 2; ^{1}H-NMR (Me_2CO-d_6, 300 MHz) δ 1.43 ppm (3H, s, Me-13″), 1.50 (3H, s, Me-12″), 3.82 (3H,

s, MeO-4‴), 3.89 (3H, s, MeO-7), 5.80 (1H, d, J = 10.0 Hz, H-10″), 6.32 (1H, d, J = 2.2 Hz, H-6), 6.63 (1H, d, J = 2.2 Hz, H-8), 6.74 (1H, s, H-3″), 6.75 (1H, s, H-3), 6.76 (1H, d, J = 10 Hz, H-9″), 6.94 (2H, d, J = 8.8 Hz, H-3‴,5‴), 7.28 (1H, d, J = 8.8 Hz, H-5′), 7.74 (2H, d, J = 8.8 Hz, H-2‴, 6‴), 8.07 (1H, dd, J = 8.8, 2.4 Hz, H-6′), 8.10 (1H, d, J = 2.4 Hz, H-2′), 12.98 (1H, s, OH-5), and 13.56 (1H, s, OH-5″); EIMS m/z 632 $[M]^{\cdot+}$ (54), 617 (100), 603 (6), 559 (2), 309 (10); HREIMS m/z 632.1697 (calcd for $C_{37}H_{28}O_{10}$, 632.1683).

Pyranoamentoflavone 7,4′-dimethyl ether (2): yellow powder ($CHCl_3$); mp 290-292 °C; UV (MeOH) λ_{max} (log ϵ) 270 (4.53), 312 (4.40), 338 (4.54) nm; NaOMe 272, 372 nm; $AlCl_3$ 282, 302, 330, 368 (sh) nm; $AlCl_3$−HCl 282, 300, 328, 368 (sh) nm; NaOAc 272, 312, 334 nm; NaOAc−H_3BO_3 296, 312, 332 nm; IR (KBr) ν_{max} 3521, 2929, 2861, 1665, 1603, 1457, 748, 701 cm^{-1}; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Me_2CO-d_6) data, see Tables 1 and 2; ^{1}H-NMR (Me_2CO-d_6, 300 MHz) δ 1.41(3H, s, Me-13″), 1.46 (3H, s, Me-12″), 3.86 (3H, s, MeO-4′), 3.88 (3H, s, MeO-7), 5.78 (1H, d, J = 10.0 Hz, H-10″), 6.32 (1H, d, J = 1.9 Hz, H-6), 6.64 (1H, d, J = 1.9 Hz, H-8), 6.68 (1H, s, H-3″), 6.75 (1H, d, J = 10.0 Hz, H-9″), 6.78 (1H, s, H-3), 6.83 (2H, d, J = 8.8 Hz, H-3‴,5‴), 7.38 (1H, d, J = 8.8 Hz, H-5′), 7.59 (2H, d, J = 8.8 Hz, H-2‴,6‴), 8.14 (1H, d, J = 2.4 Hz, H-2′), 8.19 (1H, dd, J = 8.8, 2.4 Hz, H-6′), 12.94 (1H, s, OH-5), and 13.57 (1H, s, OH-5″); EIMS m/z 632 $[M]^{\cdot+}$ (32), 617 (100), 603 (4), 559 (16); HREIMS m/z 632.1700 (calcd for $C_{37}H_{28}O_{10}$, 632.1683).

6″-(3-methyl-2-butenyl)amentoflavone (**3**): isolated as a yellow powder (MeOH): mp > 300 °C; UV (MeOH) λ_{max} (log ϵ) 276 (4.53), 336 (4.54) nm; NaOMe 279, 372 nm; $AlCl_3$ 276, 354, 367 (sh) nm; $AlCl_3$−HCl 278, 356, 368 (sh) nm; NaOAc 290, 336 nm; NaOAc−H_3BO_3 290, 338 nm; IR (KBr) ν_{max} 3461, 2936, 2855, 1653, 1561, 1545, 1456, 1383, 1104, 671 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR (Me_2CO-d_6) data, see Tables 1 and 2; FABMS m/z 605 $[M - H]^-$ (16), 587 (24), 543 (36), 423 (22), 281 (18); HREIMS m/z 606.1536 (calcd for $C_{35}H_{26}O_{10}$, 606.1526).

6″-(2-Hydroxy-3-methyl-3-butenyl)amentoflavone (**4**): obtained as a yellow powder (MeOH); mp 230-232 °C; UV (MeOH) λ_{max} (log ϵ) 268 (4.50), 286 (4.55), 336 (4.50) nm; NaOMe 276, 392 nm; $AlCl_3$ 280, 350, 386 (sh) nm; $AlCl_3$−HCl 280, 346, 388 nm; NaOAc 276, 344 nm; NaOAc−H_3BO_3 268, 286, 338 nm; IR (KBr) ν_{max} 3407, 1651, 1613 cm^{-1}; ^{1}H- and ^{13}C-NMR (Me_2CO-d_6) data, see Tables 1 and 2; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 1.77 (3H, s, Me-13″), 2.82 (1H, dd, J = 13.6, 7.9 Hz, Ha-9″), 2.96 (1H, dd, J = 13.6, 3.8 Hz, Hb-9″), 3.40 (1H, br s, H-10″), 4.70 (1H, br s, Ha-12″), 4.86 (1H, br s, Hb-12″), 6.16 (1H, d, J = 2.0 Hz, H-6), 6.35 (1H, d, J = 2.0 Hz, H-8), 6.59 (2H, d, J = 8.6 Hz, H-3‴,5‴), 6.71 (1H, s, H-3″), 6.81 (1H, s, H-3), 7.01 (1H, d, J = 8.6 Hz, H-5′), 7.63 (2H, d, J = 8.6 Hz, H-2‴,6‴), 7.94 (1H, dd, J = 8.6, 2.4 Hz, H-6′), 8.20 (1H, d, J = 2.4 Hz, H-2′), 12.01 (1H, s, OH-5), and 13.58 (1H, s, OH-5″); EIMS m/z 622 $[M]^{\cdot+}$ (1), 604 (15), 589 (32); FABMS m/z $[M + H]^+$ 623 (2).

Pyranoamentoflavone (5a): obtained as a yellow powder (MeOH); mp and EIMS identical to literature data;[3] ^{1}H-NMR and ^{13}C-NMR (Me_2CO-d_6) data, see Tables 1 and 2 (compound **5a** was recorded in pyridine-d_5);[3] UV (MeOH) λ_{max} (log ϵ) 270 (4.59), 286 (4.55), 312 (4.60), 342 (4.65) nm; NaOMe 274, 396 nm; $AlCl_3$ 262, 298, 328, 366 nm; $AlCl_3$−HCl 262, 298, 328, 366 nm; NaOAc 292, 338 nm; NaOAc−H_3BO_3 290, 338 nm; IR (KBr) ν_{max} 2867-3390, 1646, 1619, 1576, 1464, 1437, 1352, 1285, 1165, 1123, 1032, 837 cm^{-1}.

Pyranoamentoflavone 7,4′,4‴-Trimethyl ether (5b). Pyranoamentoflavone (**5a**) (20 mg) was treated with an excess of ethereal CH_2N_2 at room temperature. Evaporation of the solvent afforded pyranoamentoflavone trimethyl ether (**5b**) as a yellow solid ($CHCl_3$) (16 mg, 80%). The TLC-purified yellow powder had mp 180-182 °C; UV (MeOH) λ_{max} (log ϵ) 270 (4.40), 310 (4.55), 336 (4.45) nm; NaOMe 282 nm; $AlCl_3$ 260, 278, 298, 328, 376 (sh) nm; $AlCl_3$−HCl 262, 278, 298, 328, 370 (sh) nm; NaOAc 272, 290, 334 nm; NaOAc−H_3BO_3 270, 290, 332 nm; IR (KBr) ν_{max} 3440, 1650, 1605 cm^{-1}; ^{1}H and ^{13}C-NMR ($CDCl_3$) data, see Tables 1 and 2; EIMS m/z 646 $[M]^{\cdot+}$ (15), 631 (15), 573 (2), 58 (30), 43 (100).

Amentoflavone (6): isolated as a yellow powder (MeOH); mp, EIMS, ^{1}H-NMR and ^{13}C-NMR data identical to the literature values;[25] UV (MeOH) λ_{max} (log ϵ) 270 (4.80), 336 (4.75) nm; NaOMe 276, 382 nm; $AlCl_3$ 276, 302, 350, 382 (sh) nm; $AlCl_3$−HCl 278, 300, 348, 380 (sh) nm; NaOAc 290, 338 nm; NaOAc−H_3BO_3 290, 338 nm; IR (KBr) ν_{max} 3380, 3172, 2923, 1657, 1611, 1570, 1428, 1360, 1287, 1167, 1030, 947 cm^{-1}.

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