Calycopterones and Calyflorenones, Novel Biflavonoids from *Calycopteris floribunda*

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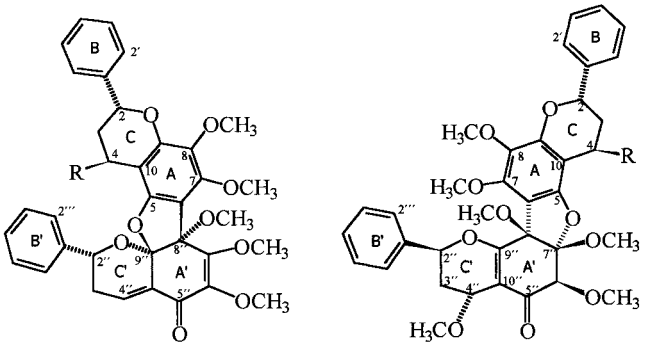
Neocalycopterone (**1**) and its methyl ether (**2**), along with two new biflavonoids, calyflorenones A (**3**) and B (**4**), were isolated from dried leaves of *Calycopteris floribunda* (Combretaceae). Structures **1**−**4** were investigated by spectroscopic methods. The relative stereochemistry of **3** and **4** was deduced through NOE and ROESY experiments, comparative CD and optical rotation evaluations. Cytotoxicity test results of **1** and **2** are reported. The monoflavonoid penduletin was isolated as a minor component from *C. floribunda* for the first time.

Calycopteris floribunda Lamk. (Combretaceae) is used in Asian traditional medicine systems.[1] The main flavonoid, calycopterin,[2] has anthelmintic[3] and antiviral[4] properties. Antimalarial activity of *C. floribunda* is mentioned,[1a] yet the constituents responsible for this activity are unknown. Three phenolic biflavonoids, named calycopterones, have been isolated from *C. floribunda* flowers collected in Thailand, and their cytotoxic activities against various human cancer cell lines were reported.[5] In the present study, *C. floribunda* leaves collected in India were investigated. Two nonphenolic calycopterone derivatives (**1** and **2**) were isolated along with two new biflavonoids (**3** and **4**). The calyflorenones A (**3**) and B (**4**) differ from the calycopterones by their fusion of the flavanol subunits and their saturation grade and thus represent a new biflavonoid skeleton.



1, R = -OH Neocalycopterone

1a, R = -OAc 4-Acetylneocalycopterone

2, R = -OCH_3 Neocalycopterone-4-methyl ether

3, R = -OCH_3 Calyflorenone A

4, R = -OH Calyflorenone B

Results and Discussion

An ethanolic extract of *C. floribunda* leaves was repeatedly extracted with diethyl ether. Combined ether extracts were partitioned between aqueous acetone and $CHCl_3$. The $CHCl_3$ extract was then submitted to gel permeation chromatography (GPC). The biflavonoids **1**−**4** were eluted prior to a monoflavonoid fraction containing small amounts of penduletin[6] and mainly calycopterin.[2] Compounds **1**−**4** were separated by silica LPLC or CC using lipophilic solvents to yield pure **2** and penduletin; **1** and **4** were obtained pure from RP-18 CC, and the final purification of **3** was achieved by DIAION filtration. Compounds **1**−**4** failed to give a phenolic reaction in TLC visualization tests. Molecular formulae of $C_{35}H_{34}O_{10}$ and $C_{36}H_{36}O_{10}$ were evident for **1** and **2** from their molecular peaks at *m/z* 614 and 628, respectively, with both having 19 double bond equivalents (dbe). While a $[M - 18]^{\bullet+}$ peak indicated an alcoholic group in **1**, in the MS of **2**, close to the mole peak, only cleavage of a methoxy radical is noticed.

The NMR spectra of **1** and **2** ($CDCl_3$) resemble those of calycopterone;[5] both show two ABMX proton systems, for one flavan-4-ol moiety (ring C) and one 2.5.6*H*-pyrane ring (ring C′). Furthermore, the DEPT spectra of **1** and **2** show two phenyl substituents, a ketal carbon (δ_C about 104 ppm) and an $\alpha\beta,\alpha'\beta'$-unsaturated carbonyl group (δ_C about 181 ppm). Due to the absence of phenolic groups in **1** and **2**, their A-rings show shift values different from those of calycopterone. Five and six methoxy groups are present in **1** and **2**, respectively. NMR differences of **1** and **2** are focused around C-3, thus indicating **2** to be the 4-methyl ether of **1**. With 4-O-methylation, C-4 is shifted downfield by 7.7 ppm, whereas the 4-H is moved upfield by 0.48 ppm ($CDCl_3$) or by 0.33 ppm (C_6D_6). Accordingly, **1** gave a monoacetate (**1a**) upon acetylation. The coupling constants observed around C-3 are almost identical in **1**, **1a**, and **2** and correspond to calycopterone.[5] While the axial and equatorial methylene protons at C-3″ display markedly different shifts in $CDCl_3$, the signals collapse into an AA′ high-order system when recorded in C_6D_6. Thus, the equatorial protons suffer an upfield shift (3″-H_{eq}: **3**, $\Delta\delta_H$ = 0.54 ppm; **4**, $\Delta\delta_H$ = 0.58 ppm) more pronounced than the axial protons (3″-H_{ax}: **3**, $\Delta\delta_H$ = 0.37; **4**, $\Delta\delta_H$ = 0.39). These AA′ pseudoquartets (3″-H_{ax}/3″-H_{eq}) obtained with both compounds in C_6D_6, do not reveal the real vicinal coupling constants with 2″-H; however, the splitting pattern of the 2″-protons (dd) reveal that the vicinal couplings $J_{2'',3''ax}$ and $J_{2'',3''eq}$ in C_6D_6 are converging, when compared with the $CDCl_3$ measurements. Consequently, in **1** and **2**, the C′-ring assumes different conformations in $CDCl_3$ and C_6D_6. NOE experiments with **1** and **2** were performed in both $CDCl_3$ and C_6D_6. Using aromatic solvent induced shifting effects, NOE experiments in both solvents allowed the assignment of all methoxy groups.

The amorphous appearance of **1** and **2** and failure of crystallization attempts raised doubt about the optical

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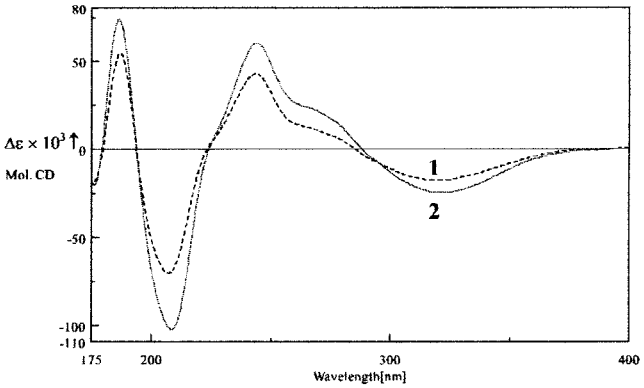


Figure 1. CD spectra of **1** and **2**.

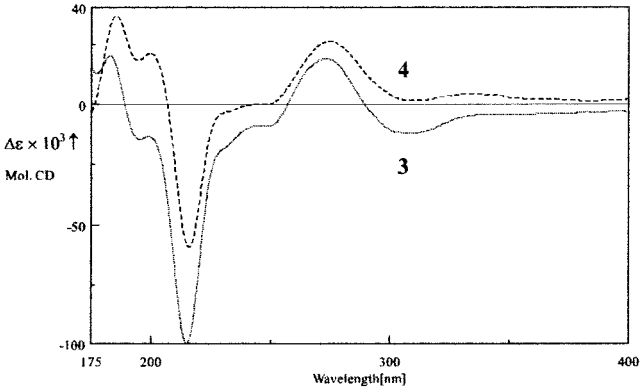
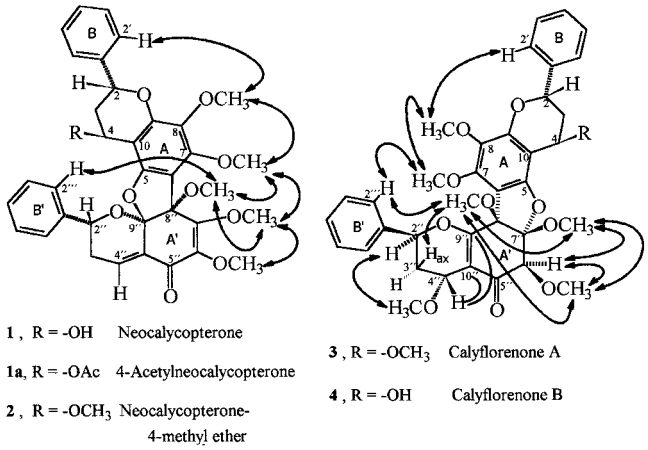


Figure 2. CD spectra of **3** and **4**.

purity of both compounds. Thus, the ^{1}H NMR behavior of **1** was checked in the presence of the chiral shift reagent *l*-Eu[hfc]$_3$[7] which could interfere with the flavanol carbons C-2 and C-4 to form diastereotopic complexes. At a molar ratio of *l*-Eu(hfc)$_3$/**1** of 1:100, the carbonyl group of **1** was the only complexing site, and a downfield shift of the 6″-methoxy group resulted first. Similar effects toward the protons of ring C were noticed only at the 10-fold *l*-Eu(hfc)$_3$ concentration, and particularly the C-3 methylene protons were shifted downfield. As no signal splitting or doubling of the benzylic or carbinol protons was observed, the optical purity of **1** was assumed. Compounds **1** and **2** show molar optical rotation values of less than −1400, similar to calycopterone.[5] Thus, from far-reaching conformity in their NMR and optical rotation data, the absolute configuration of **1** and **2** is obviously the same as in calycopterone, the latter proved by X-ray analysis. The CD spectra of **1** and **2** (Figure 1) demonstrate their identical stereochemistry, where the methylated derivative **2** shows greater $\Delta\epsilon^{25}$ values.

Calyflorenones A (**3**) and B (**4**) were detected (TLC) by observing spots under UV$_{254\ nm}$ and by their brownish color with spray reagents (anisaldehyde/H_2SO_4 or $Ce^{IV}(SO_4)_2$. The HREIMS displayed molecular formulas of $C_{37}H_{40}O_{11}$ and $C_{36}H_{38}O_{11}$ for **3** and **4**, respectively, suggesting a biflavonoid skeleton with one dbe less than the calycopterone type. Mass fragments due to the cleavage of methoxy radicals, MeOH and styrene are seen in both compounds, whereas loss of water occurs only in **4**. The UV spectra of **3** and **4** reveal three maxima at about 215, 255, and 290 nm. A n → π^* transition, >300 nm, due to an $\alpha\beta$-unsaturated ketone, is hidden. However, this is reflected by the CD curve (Figure 2). Addition of trifluoroacetic acid to the UV solutions leads to irreversible degradation of compounds **3** and **4**, and simultaneously, the UV maximum



1, R = -OH Neocalycopterone
1a, R = -OAc 4-Acetylneocalycopterone
2, R = -OCH$_3$ Neocalycopterone-4-methyl ether
3, R = -OCH$_3$ Calyflorenone A
4, R = -OH Calyflorenone B

Figure 3. Important NOEs of **1**−**4** (Arrows) and HMBC Correlations (Bows) of 4″-H in **3**.

at about 220 nm is increased. The ^{1}H NMR spectra of both **3** and **4** display two ABMX spin systems which closely resemble those of ring C in **1** and **2**. Thus, two flavanol subunits are substantiated for **3** and **4**, with a staggered conformation of the protons of rings C and C′. NOEs are observed between the benzylic protons 2/2″ and the 4/4″ methoxyl groups nearby. Since the axial protons of the 3- and 3″-methylene groups are overlapping as well as their respective equatorial protons, and both methylene groups display similar coupling constants, assignments were made only through a H−H COSY experiment. From long-range correlations between the benzylic proton 2-H and the neighboring 2′/6′-aromatic protons as well as between 2″-H and 2‴/6‴-H, and also through the C−H long-range correlations 4-H↔C-10/C-9 and 4″-H↔C-9″/C-10″, the almost isochronic shifts of the 3 and 3″ methylene protons of rings C and C′ are discerned. The 4″ proton signal shows a shift similar to the benzylic 4-H, this is explained by the allylic position of 4″-H next to the olefinic bond C-9″−C-10″. Their ^{13}C NMR shifts indicate a conjugated double bond next to a carbonyl group in ring A′ (C-5″), with an oxygen function in the β-position (C-9″). In the ^{1}H NMR spectra of **3** and **4**, seven or six methoxyls appear, respectively, and both compounds show a characteristic one-proton sharp singlet at δ around 4 ppm. The corresponding hydrogen is obviously attached to a methoxylated carbon in ring A′ and does not have coupling partners in an α- or β-position. This singlet shows HMBC and ACCORD[8] correlations toward five carbon atoms: the geminal methoxy group (6″-OMe), the carbonyl group C-5″, the ketal carbon C-7″, the quarternary carbon C-8″, and the olefinic carbon C-10″. It is devoid of an HMBC cross signal with the olefinic carbon C-9″ and must be, consequently, fixed to the carbon in the opposite ring position (C-6″). Thus, it indicates the arrangement of ring A′.

In addition to the two unsubstituted phenyl rings B and B′ in both **3** and **4**, a third aromatic ring (A) is evident from a subset of six carbons. By their ^{13}C NMR shift values, four of them are recognized as oxygenated and two as quarternary, and moreover, a 1.2.3.5-arrangement of the oxidized carbons on ring A is evident for compounds **3** and **4** (C-5/-7/-8/-9). The entire ^{1}H and ^{13}C NMR data imply a close conformity of the flavanol subunits (rings A, B, C) of compounds **1**−**4**. However, **3** and **4** have an interflavanol junction different from **1** and **2**, which is evident by analysis of their NOEs. In **3** and **4**, four sets of protons create a sequence of NOEs: 2‴/6‴-H ↔ 7-OMe ↔ 8-OMe ↔ 2″/6″-H. Herewith, one flank of the calyflorenone skeleton (rings A, B, and B′) is characterized. The interflavanol junction

in **3** and **4** must then be, next to a C-6↔C-8″ bond, realized through a ketal formation on ring A′, which is supported by ^{13}C NMR shifts at about 110 ppm (C-7″). Since a bridge between ring A and ring A′ (toward C-8″ or C-6″) would not match the observed NOEs, a dihydrofuran ring with a C-5↔O↔C-7″ attachment is assumed, in contrast to the calycopterone-type biflavonoids, where a C-5↔O↔C-9″ linkage occurs. In both **3** and **4**, three sp^2 carbons imply some rigidity in ring A′, so that a transoid attachment of the dihydrofuran moiety on ring A′ is unlikely. All eight possible variants of a fusion between rings A and A′, including transoid attachments, were built with Dreiding models and their optimum geometry was visualized by PC calculations (HYPERCHEM). Equatorial arrangements of the aromatic rings B and B′ and axial methoxyl groups on C-4/-4″ as well as axial benzylic protons 2-H/2″-H were taken into account, as had been demonstrated by NOEs and couplings. The resulting 3D models and molecular energies do not rule out trans fused variants, these are excluded only by a NOE between the 7″- and 8″-methoxy groups in **3** and **4**, and thus, a cis fusion of ring A′ is established. Considering the NOE between the 8″-methoxy group and the 2‴/6‴-protons, together with the absence of a steric interaction between 8″-OMe and the 2″-proton which are situated still closer, the latter two must be attached on opposite sides of the plane which is put up by the C9″−C10″ double bond. This relation is supported by another NOE of 8″-OMe with 3″-H_{ax}, which is fixed in a transoid position with regard to 2″-H. Furthermore, a moderate NOE between the 6″- and 8″-methoxyls proves both groups to have a steric 1−3 interaction on ring A′. H-6″ shows NOEs with 6″-OMe and 7″-OMe, and thus, it should be equatorial. In summary, the mutual dependences of the chiral centers C-4″↔C-2″, along with C-3″↔C-8″ and C-2″↔C-8″↔C-7″↔C-6″, are implied by respective NOEs. From these steric limitations, the configuration depicted as **3** and **4** (or the enantiomer) is concluded. Therein, ring A′ may form an envelope conformation with C-6″ down, thus permitting an almost planar alignment of the enone chromophor. Since, so far, the configuration of flavan-4-ols from higher plants is regarded as 2(*S*),4(*R*), along with a negative optical rotation,[9] the configuration 2*S*,4*R*,2″*S*,4″*R*,6″*R*,7″*S*,8″*S* is tentatively proposed for calyflorenone A (**3**). Calyflorenone B (**4**) is the 4-nor-compound of **3**. The different ketone chromophors of calycopterones and calyflorenones are characterized by their carbonyl ^{13}C shifts and also through their CD spectra. Here, n→π* transitions of unsaturated ketones are reflected by negative CEs at about 320 nm, albeit respective absorptions are not detectable in the UV spectra. In contrast to **3** and **4**, there is a cross conjugation of the 5″-carbonyl group in **1** and **2** which may explain the appearance of a pair of overlapping CD peaks in the region of the π→π* transition (230−300 nm). It is difficult to draw further conclusions from these CD spectra, regarding the stereochemistry, since the impacts of the chiral centers C-6″/-7″/-8″ cannot be calculated[10] and application of general rules may be misleading when applied to an almost planar enone.[11] Both biflavonoid skeletons show different molar rotation values: The calycopterone derivatives, known so far, range from $\Phi = -1400$ to -1900, whereas calyflorenones A (**3**) and B (**4**) show $\Phi = -265$ and -198, respectively.

Penduletin[6] was isolated from mother liquors of calycopterin[2] by CC. The mp, UV, MS, and ^{1}H NMR shifts[6b,12] are in agreement with published data, and δ_C values (acetone-d_6) closely resemble those of penduletin-4′-methyl ether.[13] Noteworthy, next to calycopterin and 3′-methoxycalycopterin, penduletin is the third incidence of a flavonol aglycon that *C. floribunda* has in common with *Digitalis thapsi*.[2a,14]

Compounds **1** and **2** were submitted to the National Cancer Institute (Bathesda, MD) for anticancer screening. Both compounds showed broad, rather unspecific, cytotoxicity toward all human cancer cell lines of the NCI panel.[15] Compound **1** exhibited LC_{50} values of less than 10^{-5} M in 50% of 48 tested cell lines, and **2** matched this with 65% of 50 tested cell lines. Thus, **1** and **2** show growth inhibition of human cancer cell lines roughly in the same order of magnitude as the phenolic calycopterone derivatives.[5] Due to problems in further supply of **2**, only **1** is presently under investigation in a secondary in vivo testing by the NCI.

Experimental Section

General Experimental Procedures. Melting temperatures are uncorrected. Optical rotation; Perkin-Elmer 241, [α] in deg × g^{-1} × cm^2 × 10^{-1}, λ = 589 nm. CD, Jasco J720 spectropolarimeter, λ = 400−175 nm, sensitivity 20 mdeg, resolution 0.2 nm, $\Delta\epsilon$ [ΔA × cm^2 × mol^{-1}],[10] recorded in trifluoroethanol. IR; Perkin-Elmer 298, cited as $\tilde{\nu}$ [cm^{-1}]. NMR (C_6D_6, $CDCl_3$, used as references, δ [ppm], J [Hz]); Varian XL300. Bruker AMX 500: HMBC, delay 60 corresponding to ∼8 Hz; HMQC, delay 3.5 corresponding to 140 Hz; ACCORD, delay 125−60 corresponding to 4−8 Hz, 512 exp., each 128 scans; ROESY, mixing time 450 1024 exp., each 64 scans. NOEs (those of interest) were obtained by single experiments or by ROESY evaluation, and are cited as one-directional only, e.g. 1(−H) ↔ 2(−H), 3(−H). EIMS; Kratos 50, 70 eV, HR around MP only. UV; Perkin-Elmer Lambda 2; LPLC, Lobar A & B (Merck); silica gel MN 60, 230−400 mesh (Macherey & Nagel); DIAION HP-20 (Mitsubishi, Düsseldorf, FRG); Sephadex LH-20 (Pharmacia, Uppsala, Sweden).

Plant Material. Fresh leaves of wild *C. floribunda* shrubs, not flowering (10 kg), were collected in Margao, Goa province, India, in September 1991 and dried for 1 week in-house (Goa University, Chemistry Department). A voucher specimen of a flowering plant is deposited at the Pharmazeutisches Institut, Universität Bonn (Kreuzbergweg 26, D-53115 Bonn, Germany).

Extraction and Separation. Dried leaves (4 kg) were hand-crushed and macerated once with EtOH for 1 week. Evaporation yielded 332 g of an oily extract (**A**). Extract **A** (50 g) was suspended 10 times in ether (500 mL each), and insoluble parts separated off by centrifugation. Combined ether solubles made up 19 g (**B**). Total extract **B** was shaken with 400 mL of acetone. Under stirring, 800 mL of H_2O was added. The suspension was shaken three times with 600 mL of $CHCl_3$. Combined $CHCl_3$ layers, containing solids, were evaporated with EtOH repeatedly to remove H_2O and gave 14.8 g of $CHCl_3$ soluble fraction **C**. Four times, fraction **C** (2.5 g) was dissolved in 10 mL of a 1:1 mixture of $CHCl_3$ and EtOH and was then placed on a sephadex column (250 g of LH-20) and eluted with the same mixture. Biflavonoid fractions were recognized by their brown color on spraying TLC plates with anisaldehyde/H_2SO_4 reagent and were eluted prior to a mixture containing penduletin and calycopterin. Combination gave 1.36 g crude biflavonoid fraction **D**. Fraction **D** was repeatedly chromatographed on silica gel, using petroleum ether−butanone−acetone−ethyl acetate (55:15:15:15, system 1) as eluent. The subfractions were further separated with system 1 or petroleum ether−ethyl acetate (1:1, system 2) on Lobar A or B columns to yield pure **2** and impure samples of **1**, **3**, and **4**.

Neocalycopterone (1): obtained impure from CC (system 1, R_f = 0.30). Purification by Lobar-RP18 CC (70% MeOH), 580 mg, amorphous, pale yellow, mp 135−138 °C (ether/petroleum ether); $[\alpha]^{15}_D$ −254 ($CHCl_3$, c = 0.211), corresponding to $[\phi]^{15}_D$ −1557. UV, EtOH, λ_{max} (ϵ): 288 nm (10 300), 209 nm (60 500). CD, $c = 7.33 \times 10^{-5}$, $\Delta\epsilon^{25}$ (nm): 0 (397.5), -2.48×10^6 (320.0), 0 (284.9), $+1.37 \times 10^6$ (270, sh), $+5.79 \times 10^6$ (243.6), 0 (223.5), -9.66×10^6 (207.4), 0 (194), $+7.41 \times 10^6$

Table 1. ^{13}C NMR Shifts [δ] of **1**−**4** ($CDCl_3$)

C atom	**1**	**2**	**3**	**4**
2	74.1	73.7	74.0	74.0
3	37.7	35.5	34.6[c]	37.7
4	59.0	66.7	67.5	59.0
5	152.1[a]	151.1[a]	151.9	151.4
6	109.4	108.8	109.4	109.9
7	152.6	152.4	152.3	152.2
8	140.0	139.9	136.7	136.9
9	151.2[a]	152.0[a]	151.9	151.7
10	105.1	102.1	102.6	104.6
1′	140.8	140.8[b]	140.9	140.6
2′/6′	126.2	125.7	126.1	126.2
3′/5′	128.6	128.3	128.6	128.6[d]
4′	128.0	128.5	128.3	128.4[e]
2″	71.0	70.7	76.3	76.4
3″	33.4	33.4	34.5[c]	34.5
4″	138.2	137.4	66.7	66.7
5″	181.7	181.4	191.2	190.9
6″	136.8	136.4	79.2	79.3
7″	159.0	158.9	109.2	109.6
8″	88.4	88.3	86.2	85.9
9″	104.4	103.5	168.2	167.9
10″	132.0	131.9	111.4	111.7
1‴	140.8	140.9[b]	139.8	139.7
2‴/6‴	125.9	126.1	126.3	126.1
3‴/5‴	128.6	128.4	128.7	128.7[d]
4‴	128.1	128.5	128.1	128.4[e]
4-OMe		56.2	56.7	
7-OMe	61.4	61.5	61.4	61.4
8-OMe	61.0	60.8	61.0	61.0
4″-OMe			56.9	56.9
6″-OMe	61.0	60.8	59.7	59.8
7″-OMe	61.6	61.3	51.9	52.2
8″-OMe	54.3	54.2	55.7	55.6

[a−e] Shift values with superscripts: interchangeable or overlapping.

(187.0),0 (179.9), −2.70 × 10^6 (175.4). IR (KBr), 3470 br, 3100 w, 3070 w, 3040 w, 2940, 2840, 1670 s, 1630 vs, 1600 vs, 1450, 1330, 1025 vs, 925, 760, 700. NMR, see Tables 1 and 2. NOEs (C_6D_6): 2 ↔ 2′6′,3_{ax},3_{eq}; 2′6′ ↔ 8-OMe; 2″ ↔ $3''_{AB}$,4″; 4″ ↔ 2″6″; 7-OMe ↔ 8-,6″-OMe, 8″-OMe; 8-OMe ↔ 2′6′; 6″-OMe ↔ 7″-OMe; 7″-OMe ↔ 7-OMe, 8″-OMe; 8″-OMe ↔ 2′6′. EIMS (*m*/*z*, rel. int.): 614.2142 (18, $M^{\bullet+}$, calcd for $C_{35}H_{34}O_{10}$: 614.2152), 596 (100), 565 (45), 533 (15), 427 (20), 411 (22), 381 (20), 368 (30), 353 (48), 314 (80), 299 (50), 267 (35), 115 (43), 104 (60).

4-Acetylneocalycopterone (1a): 1 (10 mg) was stirred 24 h with 1 mL acetic anhydride and 4 mL pyridine at room temp. Work up gave **1a** (6 mg), mp 121−123 °C (EtOAc). ^{1}H NMR ($CDCl_3$): 5.11 dd (12.5/2.2, 2-H); 2.27 dt (15.3/∼2/∼2, 3-H_{eq}); 2.12 ddd (15.3/12.5/∼4, 3-H_{ax}); 5.96 dd (3.8/2.2, 4-H); 5.20 dd (11.3/4.8, 2″-H); 2.68 ddd (20/5.5/4.0, 3″-H_{eq}); 2.47 ddd (20/11.8/∼2.5, 3″-H_{ax}), 7.23 ddbr (5.5/2.3, 4″-H); 2.04 s (additional methyl). IR (KBr), 3450 br, 1665, 1630 s, 1595 s, 1025 vs.

Neocalycopterone-4-methyl ether (2): 60 mg obtained from CC (system 1, R_f = 0.46, then system 2, R_f = 0.28), amorphous, pale yellow, mp 115−116 °C (ether/petroleum ether). $[\alpha]^{19}_D$ −225 ($CHCl_3$, *c* = 0.120), corresponding to $[\phi]^{19}_D$ −1410. UV, EtOH, λ_{max} (ϵ): 288 nm (8600), 209 (60 000); +NaOMe, 294 nm (unchanged), 210 nm (2 × 10^5). CD, *c* = 7.96 × 10^{-5}, $\Delta\epsilon^{25}$(nm): −3.15 × 10^6 (321.4), 0 (287.9), +2.45 × 10^6 (272, sh), +7.52 × 10^6 (244.2), 0 (224.3), −12.93 × 10^6 (208.6), 0 (193.9), +9.22 × 10^6 (186.6), −2.58 × 10^6 (176). IR (KBr), 3450 br, 3060 w, 3040 w, 2940, 2830, 1670, 1630 s, 1595 s, 1450, 1325 s, 1095 s, 1025 vs, 920, 700. NMR, see Tables 1 and 2. NOEs ($CDCl_3$): 4-OMe ↔ 4, 8-OMe; 7-OMe ↔ 8-OMe, 7″-OMe, 8″-OMe; 8-OMe ↔ 8″-OMe; 6″-OMe ↔ 7″-OMe, 8″-OMe; 7″-OMe ↔ 8″-OMe. EIMS (*m*/*z*, rel. int.): 628.2312 (100, $M^{\bullet+}$, calcd for $C_{36}H_{36}O_{10}$: 628.2309), 597 (22), 555 (8), 524 (10), 443 (14), 339 (3), 215 (4), 121 (18), 105 (23).

Calyflorenone A (3): impure by Lobar CC, system 1, R_f = 0.40. Purification by DIAION filtration (EtOH 100%); 135 mg, amorphous, pale yellow, mp 93−94 °C (ether/petroleum ether). $[\alpha]^{15}_D$ −40.2, $CHCl_3$, *c* = 1.000), corresponding to $[\phi]^{15}_D$ −265. UV, EtOH, λ_{max} (ϵ): 292 nm (sh, 5800), 258 nm (11 200), 213 nm (50 300). CD, *c* = 4.54 × 10^{-5} (nm): −1.64 × 10^6 (307.6), 0 (295.7), +5.81 × 10^6 (272.6), 0 (253.7), t −7.12 × 10^5 (248.6), −5.20 × 10^5 (244.6, r), −2.01 × 10^7 (215.6), 0 (204.3), +3.09 × 10^6 (199.2), +2.38 × 10^6 (195.0, t), 1.02 × 10^7 (186.2), 0 (179.9), −2.33 × 10^6 (176.8). IR (KBr), 3440 br, 2930, 2840, 1665, 1630−1600 br, 1450, 1250, 1195, 1170, 1135, 1085 vs, 1030,

Table 2. ^{1}HNMR Data [δ] of **1**−**4** ($CDCl_3$ or C_6D_6)[l]

	1			**2**			**3**			**4**	
proton	$CDCl_3$	m[a]	C_6D_6	$CDCl_3$	m	C_6D_6	$CDCl_3$	m	C_6D_6	$CDCl_3$	m
2	5.20	dd	5.22	5.29	dd	5.55	5.28	dd	5.51	5.24	dd
3_{ax}	1.98	ddd	1.54	1.90	ddd	1.52	1.88	ddd	1.59	1.97	ddd
3_{eq}	2.23	dt	2.08	2.24	dt	2.14	2.30[b]	dt	2.11	2.23	m
4	4.89	dd	4.94	4.41	t	4.61	4.42	t	4.26	4.91	m
2′/6′	7.47	m	7.38	[7.23−7.44[d]]	m[e]	7.37 [f]	7.46 [i]	dm	7.42	7.47	m
3′/5′	7.36	m	7.14	[7.23−7.44[d]]	m	7.11−7.26 [g]	7.39	m	7.22	7.39	m
4′	7.28	m	7.07	[7.23−7.44[d]]	m	7.06	7.35[h]	m	7.18	7.34	m
2″	5.23	dd	5.30	5.20	dd	5.35	5.21	dd	5.44	5.21	dd
$3''_{ax}$	2.52	ddd	2.15[c]	2.50	ddd	2.11[b]	1.85	ddd	1.54	1.86	ddd
$3''_{eq}$	2.69	ddd	2.15[c]	2.69	ddd	2.11[b]	2.30[b]	dt	2.06	2.30	dt
4″	7.28	m	7.21	∼7.3	m	7.15	4.31	t	4.48	4.32	m
6″	−		−	−		−	3.99	s	4.29	4.01	s
2‴/6‴	7.39	m	7.36	[7.23−7.44[d]]	m[e]	7.37 [f]	7.46 [i]	m	7.40	7.47	m
3‴/5‴	7.36	m	7.25	[7.23−7.44[d]]	m	7.11−7.26 [g]	7.41	m	7.25	7.41	m
4‴	7.30	m	7.14	[7.23−7.44[d]]	m	7.15	7.35[h]	m	7.18	7.36	m
4-OH	2.13	m	2.14	−		−	−		−	2.36	m
4-OMe	−	−	3.34	s	3.44	3.52	s	3.43[k]	−		
7-OMe	4.04	s	4.11	4.09	s	3.92	3.58	s	3.80	3.58	s
8-OMe	3.79	s	3.74	3.72	s		3.67	s	3.59	3.69	s
4″-OMe	−		−	−		−	3.42	s	3.43[k]	3.42	s
6″-OMe	3.78	s	3.70	3.78	s	3.72	3.57	s	3.54	3.58	s
7″-OMe	4.10	s	3.93	4.04	s	4.12	3.59	s	3.62	3.60	s
8″-OMe	3.55	s	3.65	3.55	s	3.63	3.60	s	3.86	3.60	s

[a] Multiplicities from $CDCl_3$ or C_6D_6 either. [b] Together as a dquartet. [c] Together as a pseudoquintet. [d−k] Interchangeable, overlapping or doubled. [l] *J* [Hz]: **1**, 2,3_{ax}, 12.2; 2,3_{eq}, 1.8; 3_{ax},3_{eq}, 14.3; 3_{ax},4, 3.8; 3_{eq},4, 2.0; 2″,$3''_{ax}$, 11.8; 2″,$3''_{eq}$, 4.0; $3''_{ax}$,$3''_{eq}$, 19.8; $3''_{ax}$,4″, 2.3; $3''_{eq}$,4″, 5.5. **2**: 2,3_{ax}, 12.7; 2,3_{eq}, 2.3; 3_{ax},3_{eq}, 14.4; 3_{ax},4, 3.0; 3_{eq},4, 2.3; 2″,$3''_{ax}$, 11.3; 2″,$3''_{eq}$, 4.0; $3''_{ax}$,$3''_{eq}$, 19.8; $3''_{ax}$,4″, 2.3; $3''_{eq}$,4″, 5.7. **3**, 2,3_{ax}, 12.8; 2,3_{eq}, 2.3; 3_{ax},3_{eq}, 14.5; 3_{ax},4, 2.8; 3_{eq},4, 2.5; 2″,$3''_{ax}$, 13.0; 2″,$3''_{eq}$, 2.8; $3''_{ax}$,$3''_{eq}$, 14.5; $3''_{ax}$,4″, 2.8; $3''_{eq}$,4″, 2.6. **4**, 2,3_{ax}, 12.4; 2,3_{eq}, 1.8; 3_{ax},3_{eq}, 14.6; 3_{ax},4, 3.7; 3_{eq},4, 2.2; 2″,$3''_{ax}$, 13.0; 2″,$3''_{eq}$, 2.7; $3''_{ax}$,$3''_{eq}$, 14.5; $3''_{ax}$,4″, 2.8; $3''_{eq}$,4″, 2.5.

700. NMR, see Tables 1 and 2. NOEs ($CDCl_3$ or C_6D_6): 2 ↔ 3_{ax}, 3_{eq}, 4, 4-OMe, 8-OMe; 3_{eq} ↔ 3_{ax}, 4, 4-OMe; 4 ↔ 3ax, 4-OMe, 8-OMe; 2″ ↔ $3''_{ax}$, $3''_{eq}$, 4″-OMe, 7-OMe; $3''_{ax}$ ↔ 8″-OMe; $3''_{eq}$ ↔ 2″,4″,4″-OMe; 4″ ↔ 2″, $3''_{ax}$, 4″-OMe; 6″ ↔ 6″-OMe, 7″-OMe; 7-OMe ↔ 8-OMe, 2‴/6‴, 3‴/5‴; 8-OMe ↔ 2′/6′; 4″-OMe ↔ 8″-OMe; 6″-OMe ↔ 7″-OMe, 8″-OMe; 7″-OMe ↔ 4″-OMe, 8″-OMe. EIMS (*m*/*z*, rel. int.): 660.2589 (100, $M^{\bullet+}$, calcd for $C_{37}H_{40}O_{11}$: 660.2571), 630 (6), 629 (8), 628 (4), 597 (14), 556 (7), 493 (5), 413 (5), 345 (5), 318 (4), 147 (18), 121 (17),105 (10), 75 (12).

Calyflorenone B (4): obtained impure by CC (system 1, $R_f = 0.31$, then system 2, $R_f = 0.13$). Purification by Lobar-A-RP18 CC (70% MeOH), 25 mg, amorphous, pale yellow, mp 113−115 °C (ether/petroleum ether). $[\alpha]^{15}_D$ −30.6 ($CHCl_3$, $c = 0.157$), corresponding to $[\phi]^{15}_D$ −198. UV, EtOH, $\lambda_{max}(\epsilon)$: 296 nm (7500), 259 nm (14 000), 212 nm (59 000), on NaOMe addition: 296 and 258 nm (both unchanged int.), 211 nm (1.56×10^5). CD, $c = 8.36 \times 10^{-5}$, $\Delta\epsilon^{25}$ (nm): $+4.84 \times 10^5$ (333.6), $+1.37 \times 10^5$ (311.0, t), $+3.09 \times 10^6$ (274.6), 0 (250.7), -5.28×10^4 (244.4, sh) -7.14×10^6 (216.0), 0 (206.9), $+2.50 \times 10^6$ (199.8), $+2.14 \times 10^6$ (194.6, t), $+4.40 \times 10^6$ (185.6), 0 (176.7). IR (KBr), 3430 br, 3075 w, 3040 w, 2940, 2840, 1665, 1635, 1600s, 1450, 1195, 1170, 1090 vs, br, 1020 s, 765, 700. NMR, see Tables 1 and 2. NOEs (by ROESY, $CDCl_3$): 2 ↔ 3_{ax}, 3_{eq}, 4, 8-OMe, 2′/6′; 3_{ax} ↔ 3_{eq}, 4, 2′/6′; 3_{eq} ↔ 4, 2′/6′, 3′/5′; 4 ↔ 4-OMe, 7-OMe, 6″-H, 7″-OMe; 2″ ↔ 2‴/6‴, $3''_{ax}$, $3''_{eq}$, 4″, 4″-OMe, 7-OMe; $3''_{ax}$ ↔ $3''_{eq}$, 4″, 8″-OMe; $3''_{eq}$ ↔ 4″, 4″-OMe; 4″ ↔ 4″-OMe, 6″-OMe; 6″-H ↔ 6″-OMe, 7″-OMe. EIMS (*m*/*z*, rel. int.): 646.2421 (100, $M^{\bullet+}$, calcd for $C_{36}H_{38}O_{11}$: 646.2414), 628 (16), 583 (12), 542 (10), 479 (7), 399 (6), 318 (12), 295 (14), 267 (10), 147 (33), 121 (10), 104 (5), 75 (23).

Penduletin. Mother liquors of calycopterin were chromatographed repeatedly by CC ($CHCl_3$−acetone−butanone−ethyl acetate, 88:4:4:4; penduletin, $R_f = 0.26$, calycopterin, $R_f = 0.33$). Pale yellow amorphous powder, mp 218−220 °C (lit.,[6a] 216−217 °C; lit.,[6b] 222 °C).

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