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Five biflavonoids from *Calycopteris floribunda* (Combretaceae)

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Abstract

The structures of five biflavonoids, 6″-demethoxyneocalycopterone (**1**), calyflorenone C (**2**), 6″-*epi*-calyflorenone B (**3**), 6″-*epi*-calyflorenone C (**4**) and calyflorenone D (**5**) from the green parts of *Calycopteris floribunda* were established by NMR and MS. Their NMR and chiroptical properties (CD, $[\alpha]_D^{20}$) were compared with those of the known *C. f.* biflavonoids **6**–**11**. Compound **1** represents a calycopterone derivative, **2**–**5** have a calyflorenone skeleton. With regard to one chiral center (C-6″), **4** and **3** are the respective epimers of **2** and **11**.

Keywords: *Calycopteris floribunda*; Combretaceae; Biflavonoids; Calycopterones; Calyflorenones; 6″-*Epi*-calyflorenones; Flavan-4-ol; Benzodihydrofuran; CD; Cytotoxicity

1. Introduction

The traditional Asian medicinal plant *Calycopteris floribunda* Lamk. (Combretaceae) (= *C. f.*) contains monoflavon-3-ols as well as two different structure types of biflavonoids, the calycopterones and calyflorenones. In both skeletons, two flavan-4-ol subunits are linked through a central dihydrofuran moiety with different attachment points (Mayer, 1999).

Recently, five minor constituents were separated from an EtOAc extract of the green parts of *C. f.* by sephadex and silica gel cc. From their MS and NMR spectra, **1** appeared to be a calycopterone derivative, whereas **2**–**5** had a calyflorenone skeleton. Additionally, for spectral comparison, $CDCl_3$ and C_6D_6 NMR spectra of calycopterone (**6**, Wall et al., 1994, published data: DMSO-d_6) were recorded.

2. Results and discussion

2.1. Structure elucidation

Calycopterone- (**1**, **6**, **7**, **8**) and calyflorenone-type (**2**, **3**, **4**, **5**, **9**, **10**, **11**) biflavonoids may be distinguished through their double bond equivalents, since the latter skeleton has one double bond less than the former one (Mayer, 1999). Until now, the parent compound calycopterone (**6**) (Wall et al., 1994) is the only phenolic biflavonoid from the leaf material investigated here. **6** crystallised fairly well, whereas all non-phenolic compounds with aromatic methoxy groups (**1**–**5**, **7**–**11**) were obtained either from eluent mixtures as viscous films or from petrol/Et_2O mixtures as amorphous powders.

When spectra of the known calycopterone derivatives **6**–**8** were consulted, **1** appeared as a 6″-demethoxy analogue of neocalycopterone (**7**) (Mayer, 1999). As against **7**, **1** has an additional olefinic proton ($\delta = 5.72$ ppm, sharp singlet) which is attached to an α,β-unsaturated ketone in α-position (C-6″, $\delta = 105$ ppm). This 6″-H shows HMBC correlations towards the β-carbon (C-7″, $\delta = 171$ ppm) and towards C-8″ and C-10″. **1** has a lower optical rotatory power (ORP, $[\Phi]_D^{20} = -1163$) than **6**–**8** [**6**: −1683 (Wall et al., 1994)]; [**7**: −1557, **8**: −1410 (Mayer, 1999)].

With regard to the calycopterones, the calyflorenone biflavonoids **2**–**4** and **9**–**11** have two chiral centers in addition. Their configuration is restricted at C-7″ and C-8″ because of the junction between the central dihydrofuran moiety and ring A′, whereas the α-keto-carbon C-6″ may have *R*- or *S*-configuration; an inversion is feasible here through a C-5″/C-6″ keto-enol-tautomerism.

The spectral data of calyflorenone C (**2**) closely resembled the known calyflorenones A (**9**) and B (**11**)

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(Mayer, 1999); since two hydroxyl groups were located at C-4 and C-4″, **2** was recognized as the C-4″-*O*-demethyl analogue of **11**.

From MS and NMR evaluations, also the biflavonoid **4** appeared as a C-4/C-4″- calyflorenone diol and **3** as its 4″-methyl ether. Another respective pair of calyflorenones (**2** and **11**) displays close spectral relationship with **4** and **3**; however, both pairs have opposite configurations at C-6″. In **3** and **4**, the stereochemistry in ring A′ was elucidated through the NOEs of 6″-H (see Fig. 1) which gave a characteristic sharp ^{1}H NMR singlet in all calyflorenone spectra. Thus, recorded in $CDCl_3$, the 6″-H when it is in α-position appears at ∼4 ppm (calyflorenones: **2**, $\delta = 3.96$; **9**, $\delta = 3.99$; **11**, $\delta = 4.00$), whereas in β-position, 6″-H is shifted downfield (**11** → **3**: $\Delta\delta \sim +0.1$ ppm; **2** → **4**: $\Delta\delta \sim +0.25$ ppm). This deshielding of the β-6″-H in **3** and **4** may be explained by a reinforced steric interaction with the 7″-β-methoxy group. With C_6D_6, α- and β-6″-H were not distinguished (see Table 1). As a concomitant, in $CDCl_3$ as

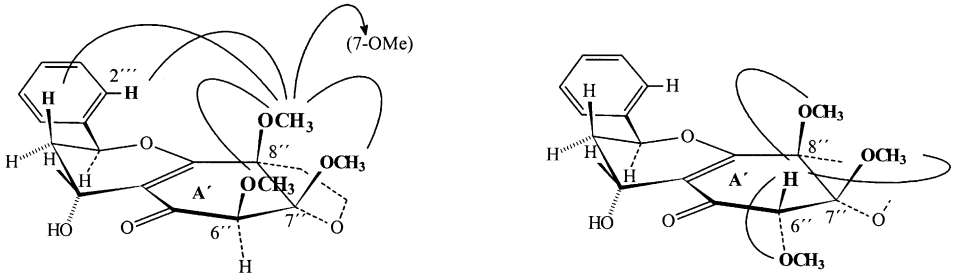


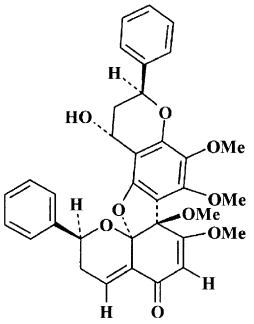
Fig. 1. Important NOEs of calyflorenone and 6″-*epi*-calyflorenone derivatives. *Left*: calyflorenone C (**2**), NOEs of the 8″-OMe group. *Right*: 6″-*epi*-calyflorenone C (**4**), NOEs of 6″-H.

Table 1
^{1}H NMR data of **1**–**6** (δ values [ppm] refer to $CDCl_3$ at 7.24 ppm or C_6D_6 at 7.16 ppm. OH signals were located by D_2O exchange.)

	Calycopterone derivatives						Calyflorenones C and D						6″-*Epi*-calyflorenones B and C					
	1			**6**			**2**			**5**			**3**			**4**		
Protons	$CDCl_3$	m*)	C_6D_6	$CDCl_3$	m	C_6D_6	$CDCl_3$	m	C_6D_6	$CDCl_3$	m	C_6D_6	$CDCl_3$	m	C_6D_6	$CDCl_3$	m	C_6D_6
2	5.17	*dd*	5.15	5.26	*dd*	5.30	5.21	*dd*	5.21	5.22	*dd*	5.27	5.23	*dd*	5.28[t)]	5.25	*dd*	5.30
3$_{ax}$	1.96	*ddd*	1.49	1.87	*ddd*	1.39	1.99	*ddd*	1.47	1.98[n)]	*ddbr*	1.52	1.99	*ddd*	1.46	2.02[u)]	*ddd*	1.44
3$_{eq}$	2.21[c)]	*dt*[#]	2.01	2.23	*dt*	2.02[g)]	2.229[k)]	*dt*	1.90	2.22[b)]	*dbr*	1.90	2.21	*dt*	1.86	2.24[s)v)]	*dt*[#]	1.86
4	4.87	*dd*	4.92	4.37	*t*	4.46	4.90	*dd*	4.62	4.92	*dd*	4.60	4.95	*dd*	4.57	5.01[w)]	*dd*	4.64
2′/6′	7.43	*dbr*	7.29	7.42	*dbr*	7.26	7.457	*dt*	7.33	7.46	*dd*	7.33	7.45	*dd*	7.29	7.47	*dbr*	7.31
3′/5′	7.37	*tbr*	7.10	7.32–	*tbr*[#]	7.12	7.38[l)]	*tbr*	7.12–	7.38[c)]	*tbr*[#]	7.15	7.37	*td*	7.13	7.38–	*tbr*	ovl.[a)]
4′	7.27	*tt*	7.02	{7.36[e)]; ∼7.29[f)]}	*tt*	7.06[h)]	7.323	*tt*	{7.17[m)]; 7.07}	7.32	*tt*	7.07	7.29	*tt*	7.07[p)]	{7.42[q)]; 7.34[r)]}	*tt*	7.05[b)]
2″	5.25	*dd*	5.30	5.23	*dd*	5.40	5.11	*dd*	5.27	5.12	*dd*	5.24	5.24	*dd*	5.36	5.13	*dd*	5.22
3″$_{ax}$	2.52	*ddd*	2.14 (**A**)	2.51	*ddd*	1.93–	2.017	*ddd*	1.66	1.98[n)]	*ddbr*	1.68	1.76[o)]	*ddd*[#]	1.50	1.96[x)]	*ddd*	1.66
3″$_{eq}$	2.68	*ddd*	2.09 (**B**)	2.70	*ddd*	{2.01[g)]; 6.98[i)]}	2.225[k)]	*dt*	2.04	2.22[b)]	*ddbr*	2.06	2.28	*dt*	2.01	2.24[s)y)]	*dt*[#]	2.06
4″	7.21	*dd*	7.20[a)]	7.22	*dd*		4.67	*dd*	4.70	4.75	*dd*	4.74	4.23	*t*	4.27	4.65[z)]	*dd*	4.60
6″	5.72	*s*	5.74	–	–	–	3.96	*s*	4.26	3.24α	*d*	3.52α	4.12	*s*	4.19	4.22[*)] [b)]	*s*	4.19
										2.91β	*d*	2.82β						
2‴/6″	7.38	*dbr*	7.31	7.37	*dbr*	7.28	7.435	*dd*	7.28	7.43	*dd*	7.27	7.42	*td*	7.32	7.43	*dbr*	7.27
3‴/5″	7.33	*tt*	7.17	{7.32–7.36[e)]}	*ovl.*	7.07[h)]	7.38[l)]	*tbr*	{7.09–7.14[m)]}	7.38[n)]	*td*[#]	7.10	7.39	*tbr*	7.16	{7.38–7.42[q)]}	*tbr*	ovl.[a)]
4‴	7.29	*tt*	7.11	∼7.29[f)]	*tt*	6.98[i)]	7.33	*tt*	7.04	7.33	*tt*	7.04	7.32	*tt*	7.06[p)]	7.35[r)]	*tt*	7.05[b)]
4-OH	2.20[c)]	*br*	?	–	–	–	2.18	*sbr*	2.27	2.30	*sbr*	2.57	1.77[o)]	*s*	–	∼3.0	*brs*	–
4″-OH	–	–	–	–	–	–	2.91	*br*	3.22	2.95	*sbr*	3.37	–	–	–	–	–	–
4-OMe	–	–	–	3.33	*s*	3.34	–	–	–	–	–	–	–	–	–	–	–	–
7-R[†]	4.00	*s*	4.02	6.47	*s* (OH)	6.82	3.53	*s*	3.70	3.50	*s*	3.65	3.52	*s*	3.69	3.51⊙	*s*	3.65
8-OMe	3.77[d)]	*s*	3.77	3.73	*s*	3.77	3.66	*s*	3.52	3.67	*s*	3.50	3.67	*s*	3.52	3.68	*s*	3.52
4″-OMe	–	–	–	–	–	–	–	–	–	–	–	–	3.39	*s*	3.36	–	–	–
6″-OMe	–	–	–	3.79	*s*	3.50	3.54	*s*	3.43	–	–	–	3.81	*s*	3.75	3.81◈	*s*	3.74
7″-OMe	3.53	*s*	2.96	4.11	*s*	3.62	3.55	*s*	3.45	3.49	*s*	3.29	3.53	*s*	3.61	3.54◘	*s*	3.61
8″-OMe	3.77[d)]	*s*	3.57	3.47	*s*	3.14	3.61	*s*	3.78	3.58	*s*	3.59	3.66	*s*	3.64	3.70⁂	*s*	3.63

J [Hz] **1**: 2,3$_{ax}$: 12.7; 2,3$_{eq}$: 1.7; 3$_{ax}$,3$_{eq}$: 14.8; 3$_{ax}$,4: 3.8; 3$_{eq}$,4: 2.3; 2″,3″$_{ax}$: 11.8; 2″,3″$_{eq}$: 4.0; 3″$_{ax}$,3″$_{eq}$: 19.5; 3″$_{ax}$,4″: 2.3; 3″$_{eq}$,4″: 5.7. **2**: 2,3$_{ax}$: 12.3; 2,3$_{eq}$: 1.5; 3$_{ax}$,3$_{eq}$: 15.0; 3$_{ax}$,4: 3.6; 3$_{eq}$,4: 2.2; 2″,3″$_{ax}$: 12.3; 2″,3″$_{eq}$: 2.3; 3″$_{ax}$,3″$_{eq}$: 15.0; 3″$_{ax}$,4″: 3.8; 3″$_{eq}$,4″: 2.1. **3**: 2,3$_{ax}$: 12.2; 2,3$_{eq}$: 1.6; 3$_{ax}$,3$_{eq}$: 14.8; 3$_{ax}$,4: 3.6; 3$_{eq}$,4: 1.8; 2″,3″$_{ax}$: 12.8; 2″,3″$_{eq}$: 2.5 3″$_{ax}$,3″$_{eq}$: 14.8; 3″$_{ax}$,4″:2.2; 3″$_{eq}$,4″:∼2.5. **4**: 2,3$_{ax}$: 12.3; 2,3$_{eq}$: 1.6; 3$_{ax}$,3$_{eq}$: 14.7; 3$_{ax}$,4: 3.7; 3$_{eq}$,4: 2.2; 2″,3″$_{ax}$: 12.5; 2″,3″$_{eq}$: 2.6; 3″$_{ax}$,3″$_{eq}$: 14.7; 3″$_{ax}$,4″: 3.8; 3″$_{eq}$,4″: 2.2. **5**: 2,3$_{ax}$: 12.2; 2,3$_{eq}$: 1.8; 3$_{ax}$,3$_{eq}$: 14.8; 3$_{ax}$,4: 3.7; 3$_{eq}$,4: 1.8; 2″,3″$_{ax}$: 12.7; 2″,3″$_{eq}$: 2.2; 3″$_{ax}$,3″$_{eq}$: 14.5; 3″$_{ax}$,4″: 3.8; 3″$_{eq}$,4″: 2.0. **6**: 2,3$_{ax}$: 12.2; 2,3$_{eq}$: 1.9; 3$_{ax}$,3$_{eq}$: 14.0; 3$_{ax}$,4: 2.7; 3$_{eq}$,4: 2.4; 2″,3″$_{ax}$: 11.0; 2″,3″$_{eq}$: 3.5; 3″$_{ax}$,3″$_{eq}$: 19.4; 3″$_{ax}$,4″: 2.3; 3″$_{eq}$,4″: 5.0. [†]R = −OH or −OMe. *) multiplicities from $CDCl_3$ spectra unless noted[#] (C_6D_6). [a)] overlapped by benzene. [b)-s)] overlapping, location through HH-COSY, HMBC or HMQC. [t)] + D_2O: 5.31 ppm. [u)] + D_2O: 1.94 ppm. [v)] + D_2O: 2.22 ppm. [w)] + D_2O: 4.93 ppm. [x)] + D_2O: 1.93 ppm. [y)] + D_2O: 2.25 ppm. [z)] + D_2O: 4.64 ppm. [*)] + D_2O: 4.20 ppm. ⊙ + D_2O: 3.49 ppm. ◈ + D_2O: 3.82 ppm. ◘ + D_2O: 3.52 ppm. ⁂ + D_2O: 3.69 ppm.

Table 2
CD data of calyflorenones and calycopterones



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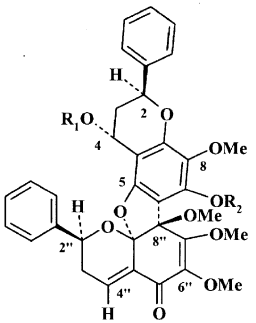
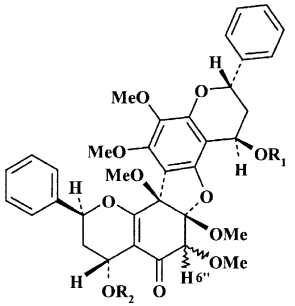
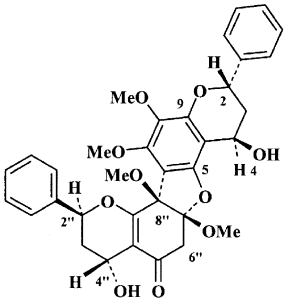


6 $R_1 = CH_3$ $R_2 = H$
7 $R_1 = H$ $R_2 = CH_3$
8 $R_1 = CH_3$ $R_2 = CH_3$



2 $R_1 = H$ $R_2 = H$ 6'': ß-OMe
3 $R_1 = H$ $R_2 = CH_3$ 6'': α-OMe
4 $R_1 = H$ $R_2 = H$ 6'': α-OMe
9 $R_1 = CH_3$ $R_2 = CH_3$ 6'': ß-OMe
10 $R_1 = CH_3$ $R_2 = CH_3$ 6'': α-OMe
11 $R_1 = H$ $R_2 = CH_3$ 6'': ß-OMe



5

Structure type	**2** Calyflorenone type	**11** Calyflorenone type	**9** Calyflorenone type	**10** 6″-*Epi*-calyflorenone type	**3** 6″-*Epi*-calyflorenone type	**4** 6″-*Epi*-calyflorenone type	**5** 6″-Demethoxy-calyflorenone type	**1** 6″-Demethoxy-calycopterone type	**6** Calycopterone
Functional groups	4-OH/4″-OH	4-OH/4″-OMe	4-OMe/4″-OMe	4-OMe/4″-OMe	4-OH/4″-OMe	4-OH/4″-OH	4-OH/4″-OH	4-OH	4-OMe, 7-OH
Δ ϵ* > 330 nm (λ)	$+49 \times 10^3$ (344^{p})	$+484 \times 10^3$ (334^{p})	–	–	–	–	–	–	–
Δ ϵ 300–320 nm (λ)	-72×10^3 (314^{t})	$+137 \times 10^3$ (311^{t})	-1640×10^3 (308^{t})	–	–	–	-165×10^3 (314^{t})	-614×10^3 (294^{t})	-672×10^3 (314^{t})
Δ ϵ ∼275 nm (λ)	$+533 \times 10^3$ (273^{p})	$+3096 \times 10^3$ (275^{p})	$+5815 \times 10^3$ (273^{p})	$+3390 \times 10^3$ (277^{p})	$+814 \times 10^3$ (274^{p})	$+1455 \times 10^3$ (274^{p})	$+909 \times 10^3$ (272^{p})	–	$+517 \times 10^3$ (264^{psh})
Δ ϵ ∼245 nm (λ)	-98×10^3 (248^{psh})	-53×10^3 (244^{psh})	-521×10^3 (243^{psh})	$+1491 \times 10^3$ (242^{p})	–	–	–	$+1255 \times 10^3$ (242^{p})	$+1490 \times 10^3$ (244^{p})
Δ ϵ 205–215 nm (λ)	-1690×10^3 (216^{t})	-7139×10^3 (216^{t})	-20127×10^3 (216^{t})	-6188×10^3 (215^{t})	-3788×10^3 (216^{t})	-6322×10^3 (215^{t})	-3029×10^3 (215^{t})	(∼230^{psh})	(∼222^{psh}, ∼213^{psh})
Δ ϵ ∼200 nm (λ)	$+241 \times 10^3$ (197^{p})	$+2502 \times 10^3$ (200^{p})	$+3095 \times 10^3$ (199^{p})	$+3472 \times 10^3$ (197^{psh})	$+620 \times 10^3$ (195^{p})	$+1022 \times 10^3$ (195^{p})	$+266 \times 10^3$ (196^{p})	-1614×10^3 (204^{t})	-2446×10^3 (202^{t})

*[$\Delta A \times cm^2 \times mol^{-1}$]; calc. $\lambda_{max}(\pi \rightarrow \pi^*)$ for the calycopterone chromophore: 292 nm; calc. $\lambda_{max}(\pi \rightarrow \pi^*)$ for the calyflorenone chromophore: 267 nm; ^{p} peak; ^{psh} peak as shoulder; ^{t} trough.

well as in C_6D_6, distinct ^{13}C deshieldings were observed in **3** and **4** for the C-6″ (by ~5 ppm) and for the 6″-methoxy group (by ~2.5 ppm) which receives less steric interaction in 6″-α-orientation (in the 6″-*epi*-calyflorenones) than in **2** and **11**, where both the 6″- and 7″-methoxy groups are in *β*-position (see Fig. 1). The same observations had been made with 6″-*epi*-calyflorenone A (**10**) (Mayer, 2000). Accordingly, the steric crowding of the 7″- methoxy group in **2** and **11** was expressed by ^{13}C shifts slightly lower than in **3** and **4**. In summary, the diastereomeric divergence at C-6″ (calyflorenones, C-6″ = *S*, vs. 6″-*epi*-calyflorenones, C-6″ = *R*) was reflected in pronounced NMR shift differences which range up to the γ-carbon (C-9″). However, the same item was scarcely reflected in their CD spectra (see Table 2) and neither in their ORP.

A mole peak of 602.2147 Da gave the mf $C_{34}H_{34}O_{10}$ for **5**, and including NMR results, a calyflorenone skeleton was recognised. Instead of a one-proton singlet (6″-H) as in the representatives **2**, **3** and **4**, the ^1H NMR spectrum of **5** featured an AB system of a methylene group at C-6″ without vicinal couplings. The respective β-6″-H was identified through its NOEs with the neighbouring 7″- and 8″-methoxy groups, whereas the geminal α-6″-H showed only weak NOEs with 4-H and 3-H_{eq} (see Fig. 2). Thereby, **5** (calyflorenone D) was identified as a 6″-demethoxy analogue of **2** or **4**, respectively.

SIMS (solvent induced magnetic shifting) was a useful tool for the data correlations and was observed in the ^1H NMR spectra of all *C. f.* biflavonoids. For instance, the shift values of methoxy groups were located only through two HMBC experiments in different solvents. Different SIMS results were observed for calycopterones and calyflorenones (see Table 1). Thus, a change from $CDCl_3$ to C_6D_6 exerted upfield shifts for almost all hydrogens, but most pronounced for 3-H_{ax} (~ +0.5) and for 3″-H_{ax} (~ +0.3) in both skeletons. With regard to $CDCl_3$, the 7″-methoxy groups showed upfield shifts by 0.6 ppm in C_6D_6 in the calycopterones, while less

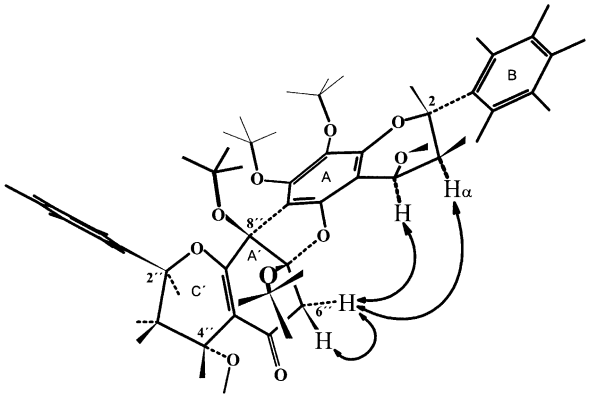


Fig. 2. HYPERCHEM simulation of calyflorenone D (**5**), the 6″-demethoxy analogue of calyflorenone C (**2**) and 6″-*epi*-calyflorenone C (**4**). Hydrogens are omitted except for the NOEs of α-6″-H.

distinct (0.1–0.2) in the calyflorenones and vice versa (~0.1 deshielding) in the 6″-*epi*-calyflorenones. In the calyflorenones **2** and **9**, α-6″-H suffered about 0.3 ppm upfield shift in C_6D_6 but remained almost unaffected in the 6″-*epi*-calyflorenones where it is in *β*-position.

D_2O exchanges were performed in $CDCl_3$, and along with expected simplifications of multiplicities, certain signals were shifted upfield (4-H in the 6″-*epi*-calyflorenones **3** and **4**). Only in the most polar biflavonoid **4**, 3-H_{ax} (2.02–1.94 ppm) was drifted into the 3″-H_{ax} signal, thus D_2O addition was disadvantageous in this case, whereas simultaneously 3-H_{eq} (2.24–2.22 ppm) and 3″-H_{eq} (2.24–2.25 ppm) were resolved. No such D_2O shift effects have been observed in the calycopterone derivatives so far.

2.2. Circular dichroism

CD spectra (Figs. 4 and 5) allow a swift discrimination of the two biflavonoid skeletons (Mayer, 1999). Without perceptible explanations, only **2** and **11** displayed troughs of very low amplitudes between 335 and 345 nm. Between 300 and 315 nm, small troughs were also apparent in the calyflorenones **2**, **9**, **11** and in the 6″-demethoxycalyflorenone derivative **5**; they should be due to n–π* transients. In contrast, the 6″-*epi*-calyflorenones (**3** and **4**) did not show any CE >300 nm. In all *C. f.* biflavonoids, π–π* transients of the α,β-unsaturated ketones were expected between 265 and 295 nm; they are obviously superposed by strong ^1L_b CD bands of the benzoid rings A and A′ next to the chiral carbons 2 and 2″. As compared to (2*S*, 4*R*)-*trans*-flavan-4-ol (Snatzke et al., 1973), the CEs there observed were not emerging in the CD spectra of C.f. biflavonoids. In ring A′ which is attached to a dihydrobenzofuran moiety, the same stereochemistry as in the (−)-*cis*-pterocarpans (with 6a*R*,11a*R* there) is mimicked by C-7″ and C-8″, and the CD spectra of the calyflorenones resembled those (Antus et al., 2001; Szarvas et al., 2000) but didn′t show agreement with the characteristic CD shape observed in the *trans*-pterocarpans (Kiss et al., 2003).

One positive CE at ca. 275 nm appeared in all calyflorenones and 6″-*epi*-calyflorenones, and analogously in the calycopterones (**1**, **6**, **7**, **8**), however, here only as a shoulder at lower (~5–10 nm) *λ* values. Secondly, between 242 and 248 nm, the calycopterones (**1**, **6**, **7**, **8**) showed pronounced positive CEs (see Fig. 4 and Table 2), whereas in the same *λ* region, the calyflorenones **2**, **7**, **9**, **11** (with C-6″ having *S*-configuration) were recognised by small peak shoulders underneath the Δ*A* zero line, and the 6″-*epi*-calyflorenones **3** and **4** (with C-6″ *R*-) as well as **5** (no chirality at C-6″) did not have any CEs (see Fig. 5 and Table 2). Therefore, this range (about 240–250 nm) is discriminating best between the two biflavonoid skeletons. Furthermore, intense ^1L_a-band troughs at ca. 215 nm (calyflorenones)

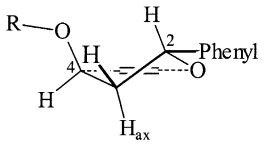


Fig. 3. Ring C confirmation of **1**–**11**.

or 200 nm (calycopterones) were observed; the calyflorenones gave peaks at 190–200 nm due to the ^1B band, whereas in the calycopterones, peaks corresponding to ^1B may be assumed <190 nm; thus, both bands appear to be red-shifted in the calyflorenones.

In summary, the contributions of the benzylic chiral carbons C-2/C-2″ may not be estimated; and for the unsaturated ring C' of the calycopterones, no CD predictions are available. Obviously, the chiral carbons C-7″ and C-8″ in the calyflorenones or C-8″ and C-9″ in the calycopterones, making up the interflavanol linkage, are responsible for the different CD shapes of the two skeletons.

An epimerisation of the chiral calyflorenone-C-6″ seems not to be significant for the CD and may not be recognised here at first sight; and neither the ORP reflects different C-6″ configurations in the diastereomer pairs **2** and **4** ($[\Phi]_D^{20} = -108$ and -138) or **3** and **11** ($[\Phi]_D^{20} = -194$ and -198).

2.3. Stereochemistry

Except for the ring C′ in the calycopterone skeleton, the aliphatic rings C and C′ of the *C. f.* biflavonoids represent flavan-4-ol moieties. Their aliphatic four-spin systems have similar coupling constants (see Table 1) which are in accordance with the half-chair conformation of a 2.5-*trans*-disubstituted cyclohexene; the aromatic substituent is in a pseudo-equorial conformation (Pouget et al., 2000), and there is a P-helicity of the heterocyclic ring (Antus et al., 2001; see Fig. 3). Only combined ROESY results of recordings in C_6D_6 and $CDCl_3$ gave satisfactory informations about the calyflorenone stereochemistry within the rings C/A′/C' and about NOE interactions between the rings A′ and C as well as NOEs between the 7- and the 8-methoxy groups (see Figs. 1 and 2).

From their biosynthetic origin, both biflavonoid skeletons should have the (2*S*/4*R*)-configuration of natural flavan-4-ols which implies levorotatory properties (Rákosi et al., 1970). Since the relative stereochemistry

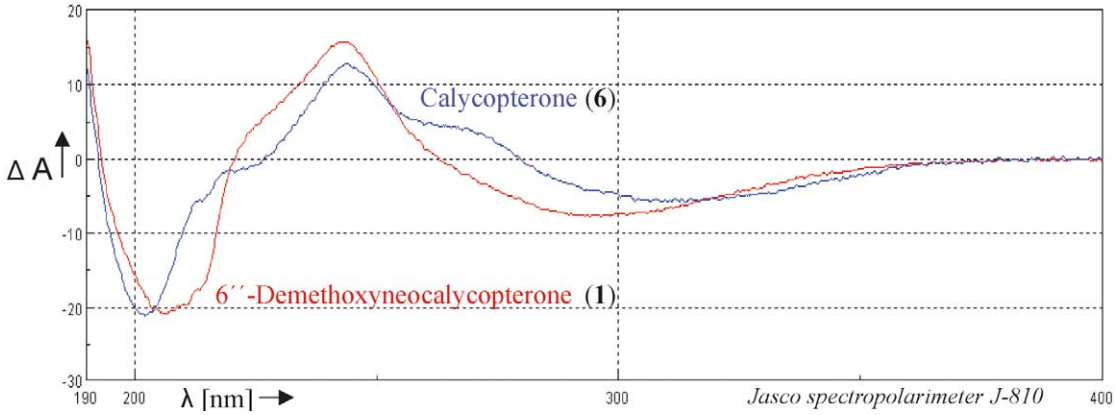


Fig. 4. CD spectra of the calycopterone derivatives **1** and **6**.

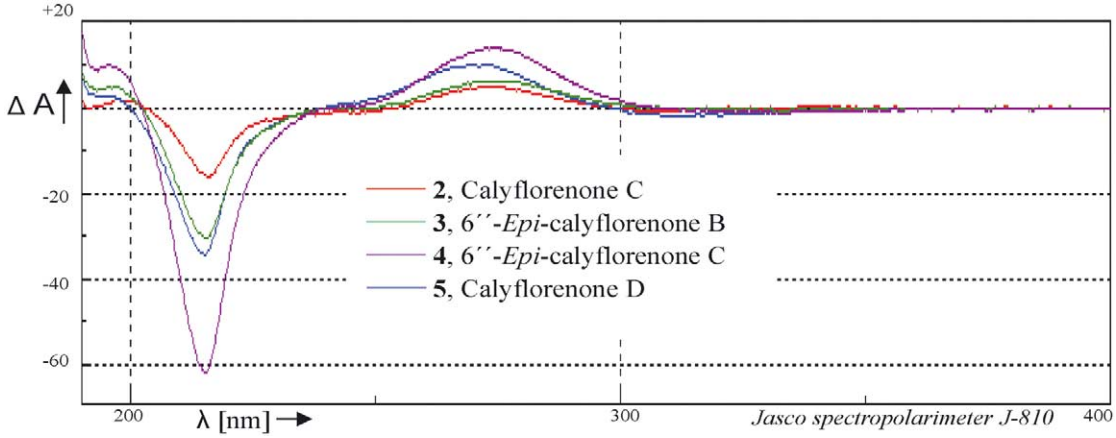


Fig. 5. CD spectra of the calyflorenone derivatives **2**, **3**, **4** and **5**.

of calycopterone (**6**) was confirmed through a single crystal X-ray structure (Wall et al., 1994), the absolute configuration would then be as depicted (2*S*, 4*R*, 2″*S*, 8″*S*, 9″*S*), and therefore, for the calyflorenones a (2*S*, 4*R*, 2″*S*, 4″*R*, 6″*S*, 7″*R*, 8″*R*)-configuration may be assumed.

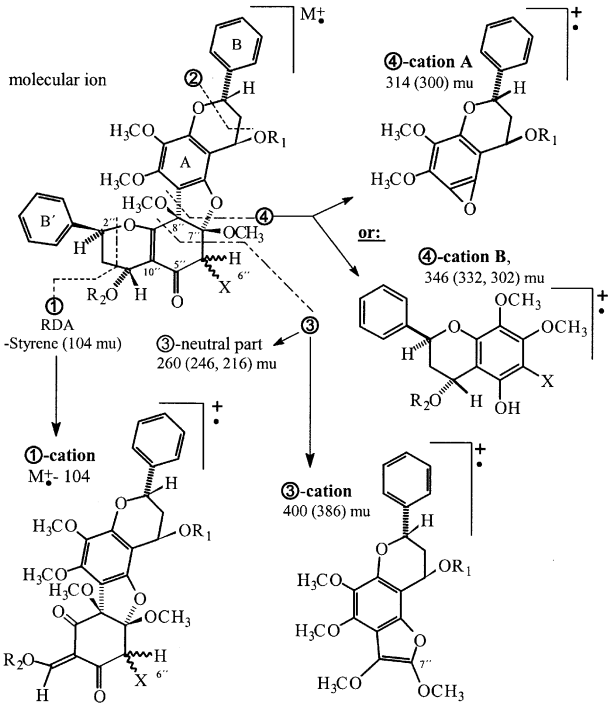
2.4. Keto–enol-tautomerism

A common precursor (**X**) for both the calycopterone and the calyflorenone biflavonoids with a single C–C linkage is likely to be formed through single electron oxidation of a phenolic flavan-4-ol and subsequent radical coupling thereof. Rotamers of the semiquinoid × could give the intermediates **Cpt** or **Cfl**, (see Scheme 2) by Michael-type additions. The calycopterone skeleton could thus arise by an attachment at C-9″, whereas a nucleophilic attack towards C-7″ would give either a calyflorenone or a 6″-*epi*-calyflorenone derivative when the C-5″/C-6″-enol is transformed to the ketone.

As to invert the configuration at C-6″ chemically and thus prove such a possible keto-enol tautomerism in the calyflorenones, the isolated epimers **3** and **11** were treated with acidic or alkaline methanol. Hereby, only degradation was observed. Therefore, both calyflorenones and C-6″-*epi*-calyflorenones are rather native constituents than products of a keto–enol tautomerism, which could not be excluded during extraction procedures.

2.5. MS fragmentation

In the complex mass spectra of **1**–**11**, [$M_{\bullet}^{+}$] ions gave prominent peaks, and cascades of simple eliminations predominated. A common loss of H_2O occured in the 4- and 4″-hydroxy derivatives, and displacement of MeOH from the [$M_{\bullet}^{+}$]ion was observed only where the respective 4- or 4″-methoxy groups were present. Although in the 4-/4″-dihydroxy derivatives **2**, **4**, **5**, a different splitting of MeOH was feasible by a 1.2-elimination at C-6″/C-7″, the expected fragments ([$M_{\bullet}^{+}$]−32) were not found here. As was proved by single-peak-HRMS, one fragmentation was omnipresent which involved the segregation of styrene. This RDA could occur twice, subsequently in ring B or B′, but the non-aromatic site (rings B'/C') should be preferred. When proceeding from the [$M_{\bullet}^{+}$] ion, obviously only one styrene molecule is cut off ([$M_{\bullet}^{+}$]-104, calyflorenones: Scheme 1, ① or ② in each *C. f.* biflavonoid, since [$M_{\bullet}^{+}$]-104-104 peaks are missing. Two more bisection reactions were observed in the calyflorenones: Most likely, the [$M_{\bullet}^{+}$] ions undergo a rearrangement ③ by cleaving the bonds C6″–C7″ along with C8″–C9″; the resulting ③$^{+}_{\bullet}$ ion was present in all MS (e.g. **2**: $C_{21}H_{22}O_7$, *m*/*z* 386.1373, by HR). Furthermore, a cleavage ④ of the central furan moiety seems to be favoured to give an arene oxide (④ $\mathbf{A}_{\bullet}^{+}$ ion or ④ **A**-neutral part) and leave the rings A′B′C″ as an aromatic system (④ $\mathbf{B}_{\bullet}^{+}$ ion or ④ **B**-neutral part, a flavan-4-ol). The respective cations, either ④ $\mathbf{A}_{\bullet}^{+}$ or ④ $\mathbf{B}_{\bullet}^{+}$, have been found in all calyflorenone derivatives.



Scheme 1. Radical cations in the MS of calyflorenone derivatives.

2.6. Cytotoxicity

When the anticancer activity of **7** and **8** was examined by the NCI, general cytotoxicity in mice (hollow fiber assay) was the limiting item. The respective mechanism for the calycopterones (e.g. **6**) is unknown (Wall et al., 1994). In a brine shrimp lethality experiment, both types of biflavonoids acted as cytotoxic agents. Receptor binding studies are needed to elucidate possible cellular targets for these biflavan-4-ol derivatives. Recently, interest has been adressed to flavan-4-ol monomers: Some synthetic representatives were chosen to be tested for antiproliferative and antiaromatase properties (Pouget et al., 2000).

3. Experimental

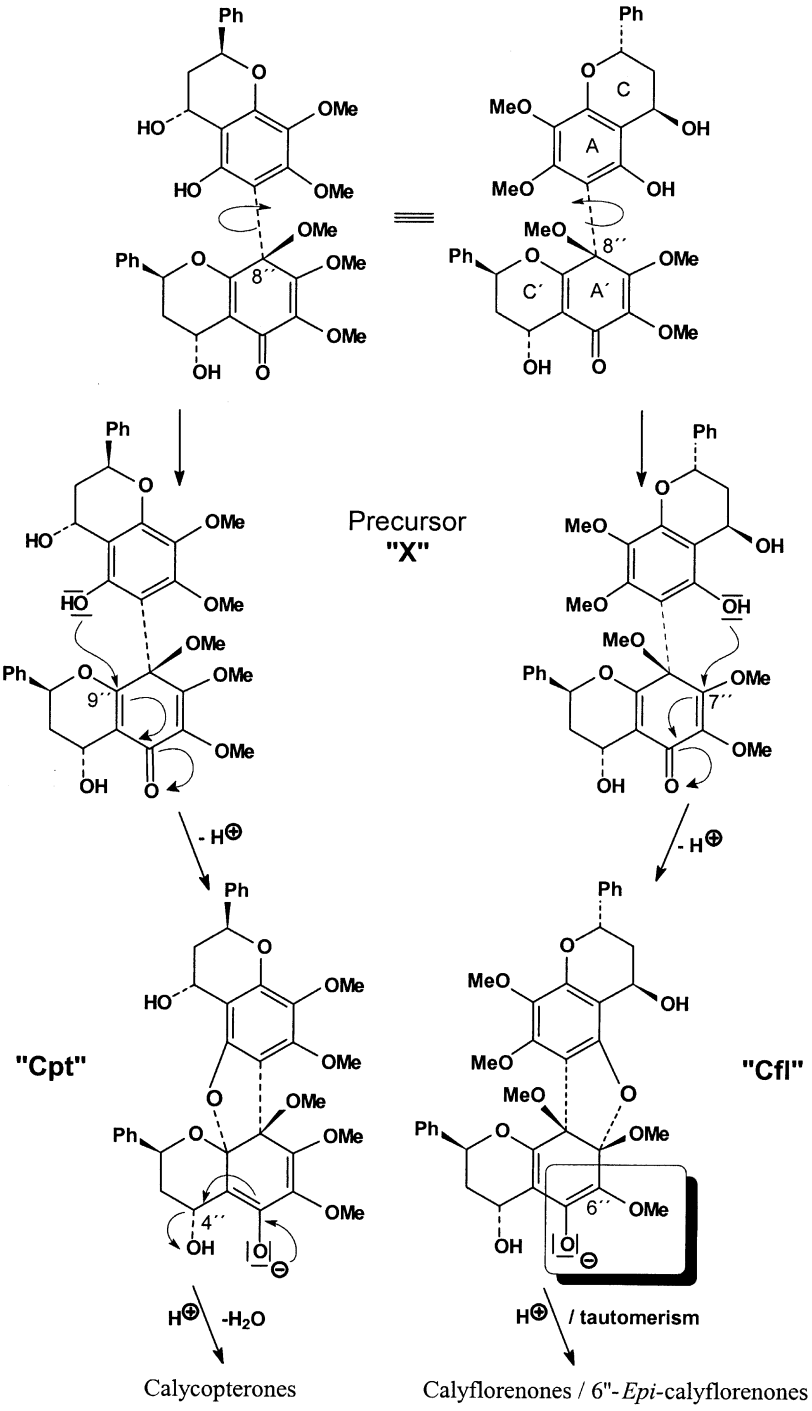
3.1. General

Mps are uncorr. UV: Perkin-Elmer Lambda 2, EtOH solns. IR: Perkin-Elmer FT-1600, ν [cm^{-1}]. Optical rotation: Perkin-Elmer 241, $CHCl_3$ solns., [α] = [deg. × g^{-1} × cm^2 × 10^{-1}], λ = 589 nm. CD: Jasco spectropolarimeter

J810, range 400–195 nm, sensitivity 20 mdeg, resoln. 0.2 nm, $\Delta\epsilon$ [$\Delta A \times cm^2 \times mol^{-1}$], trifluoroethanol, (Table 2). NMR (C_6D_6, $CDCl_3$, TMS as reference, σ [ppm], J [Hz], Tables 1 and 3): Bruker AMX 500; data correlation by 2D NMR (^{13}C: HMQC, HSQC, HMBC; ^{1}H: HH-COSY, ROESY, Bruker standard procedures). EIMS: Thermoquest Finnigan MAT 95 XL, 70 eV, single-HR at [$M^{+}_{\bullet}$] and for some ions of interest, rel. intensities in brackets. LPLC: Lobar A & B (Merck). Cc: silica gel MN, 60 mesh (Macherey & Nagel). Cer(IV)sulphate reagent: 0.3 g $Ce(SO_4)_2$/0.1 l conc. nitric acid (65%).

3.2. Plant material

C. f. leaves were collected at Margao, Goa province, India, in September 1991, air-dried and hand-crushed; see lit. (Mayer, 1999). A voucher specimen of a flowering plant is deposited at the authors address (Goa 91–C.f.).



Scheme 2. Feasible biosynthetic steps towards the calyflorenones and 6″-*epi*-calyflorenones.

Table 3
^{13}C NMR data of **1–6** (δ [ppm] ($CDCl_3$))

Carbon	1	2	3	4	5	6
2	73.9	73.9	74.0	74.1	73.9	73.7
3	37.6	37.6	37.6	37.6	37.6	35.5
4	58.9	59.0	58.6	58.3	59.0	66.9
5	152.0	151.2	152.3	152.2[b)]	152.1[d)]	151.7
6	109.1	109.6	108.9	108.6	108.6	104.1
7	152.7	152.2	151.0	151.0	152.2[d)]	148.2
8	136.7	136.8	136.7	136.7	136.5	139.5
9	151.1	151.7	151.7	151.9[b)]	151.6	151.0
10	105.1	104.3	104.1	104.2	104.2	99.4
1′	140.8	140.5	140.6	140.7	140.5	141.1
2′6′	126.1	126.1	126.1	126.2	126.1	126.2
3′5′	128.5	128.6	128.5	128.4[c)]	128.6[e)]	128.5
4′	128.0	128.0[a)]	128.0	128.0	128.0	128.0
2″	70.9	76.6	76.4	76.6	76.5	70.8
3″	33.3	36.8	35.1	36.6	36.6	33.1
4″	137.3	59.2	66.2	58.9	58.6	137.5
5″	184.4	192.9	191.5	193.7	193.5	181.6
6″	105.0	78.6	84.1	84.2	43.2	131.3
7″	171.2	108.9	107.8	108.0	109.1	157.9
8″	86.9	86.1	87.3	87.7	85.2	87.6
9″	105.6	166.7	166.1	164.8	166.1	103.3
10″	132.0	113.7	111.6	113.9	115.9	132.1
1‴	140.7	139.4	139.6	139.4	139.4	140.5
2‴6‴	125.8	126.0	125.8	126.0	126.2	125.8
3‴5‴	128.5	128.6	128.6	128.5[c)]	128.5[e)]	128.6
4‴	128.0	128.3[a)]	128.3	128.4	128.4	128.0
4-OMe	–	–	–	–	–	56.1
7-OMe	61.4	61.4	61.2	61.3	61.2	–
8-OMe	60.9	60.9	60.9	60.9	60.9	61.0
4″-OMe	–	–	57.0	–	–	–
6″-OMe	–	59.4	62.1	62.1	–	61.0
7″-OMe	56.7	51.7	53.4	53.5	51.4	61.7
8″-OMe	54.5	55.7	54.7	54.8	54.6	53.6

[a–e]Interchangeable/overlapping.

3.3. Extraction and isolation

One kg crushed *C. f.* leaves were macerated 3× with EtOAc (total 15 l). Evapn. and removal of H_2O by azeotropic distillation with EtOH yielded an oily extract of about 18 g. Successive exclusion cc thereof (Sephadex LH 20®, EtOH 96%) gave 1.25 g crude biflavonoid fraction **BF** with **6** as the main component (~90%). **BF** was separated by silica gel (1280 g) cc with CH_2Cl_2 – MeCOEt–Me_2CO–EtOAc (70 + 10 + 10 + 10, *system 3*) into two subfractions (R_f): **I** (0.8–0.35), and **II** (0.35–0). **I** and **II** were separated further with *system 3* or petrol (60–80 °C)–MeCOEt–Me_2CO–EtOAc (25 + 25 + 25 + 25, *system 4*) on Lobar A or B columns to give pure **1–6** as colourless gums. From Et_2O solns., **1–6** were obtained as pale amorphous powders after addn. of petrol and subsequent evapn. TLC monitoring was performed with cc eluents, detn.: (1) UV (254 nm), (2) spraying and subsequent heating (~300 °C) the glass plates [either cer(IV)sulphate reagent (calyflorenones orange, calycopterones olive-brownish) or anisaldehyde/H_2SO_4 reagent (all *C. f.* biflavonoids brown)].

3.4. 6″-Demethoxyneocalycopterone (1) $C_{34}H_{32}O_9$

Pale amorphous solid, mp 157–159 °C (Et_2O/petrol). R_f values: 0.44 (*system 3*); 0.48 (*system 4*). $[\alpha]_D^{20} = -199.14$ ($c = 0.350$), $[\Phi]_D^{20} = -1163.4$. UV, λ_{max} [nm] (ϵ): 295 sh, 257 nm (18,350), 212 nm (68,770). IR: 3394 br, 2930, 1669 s, 1630 vs, 1602 vs, 1456 s, 1234 vs, 1170, 1068, 1045 vs, 913, 765, 700. MS: 584.2027 Da ([$M_{\bullet}^{+}$], 63, calc. for $C_{34}H_{32}O_9$: 584.2046); 566 (100); 551 (6); 537 (5); 536 (10); 535 (25); 519 (5); 504 (8); 503 (12); 480 (22, ①$_{\bullet}^{+}$); 449 (6, (1)$^{+}$−31 mu); 448 (8, ①$_{\bullet}^{+}$ −32 mu); 417 (17); 351 (22); 345 (11); 265 (8); 115 (7); 105 (4); 104 (3).

3.5. Calyflorenone C (2) $C_{35}H_{36}O_{11}$

Pale amorphous solid, mp 185 C (Et_2O/petrol). R_f values: 0.36 (*system 3*); 0.51 (*system 4*). $[\alpha]_D^{20} = -17.089$ ($c = 0.158$), $[\Phi]_D^{20} = -108.02$. UV, λ_{max} [nm], (ϵ): 294 (6350); 258 (12830); 213 (52230). IR: 3424 br, 2933, 2835, 1662, 1635, 1602 s, 1457, 1260, 1194, 1163, 1126, 1090 s, 1020 vs, 801, 699. MS: 632,2279 Da ([$M_{\bullet}^{+}$], 100, calc. for $C_{35}H_{36}O_{11}$: 632,2258); 614 (32); 569 (10); 566 (8); 552 (5); 528.1649 (17, ①$_{\bullet}^{+}$, calc. for $C_{27}H_{28}O_{11}$: 528.1632); 465 (12); 447 (7); 399 (7); 392 (10); 386.1373 (11, ③$_{\bullet}^{+}$, calc. for $C_{21}H_{22}O_7$: 386.1366); 368 (6), 364 (7); 361 (9); 353 (13); 314 (8, ④A$_{\bullet}^{+}$); 304 (7); 295 (14); 283 (7); 267 (23); 104 (23); 75(13).

3.6. 6″-Epi-calyflorenone B (3) $C_{36}H_{38}O_{11}$

Pale amorphous solid, mp 110–112 °C (Et_2O/petrol). R_f values: 0.31 (*system 3*); 0.54 (*system 4*). $[\alpha]_D^{20} = -30.05$ ($c = 0.183$), $[\Phi]_D^{20} = -194.23$. UV, λ_{max} [nm], (ϵ): 294 sh (7820); 257 (17,090); 212 (63,650). IR: 2928, 2829, 1675, 1619,s, 1609s, 1457, 1330, 1289, 1248, 1195s, 1161s, 1092s, 1027vs, 761, 700. MS: 646.2411 Da ([$M_{\bullet}^{+}$], 100, calc. for $C_{36}H_{38}O_{11}$: 646.2414); 630 (6); 629 (18); 628 (41); 615 (6); 597 (6); 566 (5); 542 (8, ①$_{\bullet}^{+}$); 511 (10); 479 (5); 430 (10); 412 (23); 381 (5); 353 (5); 318 (8); 314 (15, ④A$_{\bullet}^{+}$); 295 (14); 267 (13); 183 (5); 151 (19); 149 (15); 147 (25); 109 (30); 104 (14).

3.7. 6″-Epi-calyflorenone C (4) $C_{35}H_{36}O_{11}$

Pale amorphous solid, mp 117–119 °C (Et_2O/petrol). R_f values: 0.17 (*system 3*); 0.39 (*system 4*). $[\alpha]_D^{20} = -21.86$ ($c = 0.183$), $[\Phi]_D^{20} = -138.19$. UV, λ_{max} [nm] (ϵ): 295 nm (11,430), 257 nm (23,370), 215 nm (75,760). IR: 2930, 2852, 1675, 1616 s, 1457, 1252, 1193, 1161, 1094 s, 1041 s, 1061 s, 1020 vs, 870, 760, 700. MS: 632.2254 Da ([$M_{\bullet}^{+}$], 100, calc. for $C_{35}H_{36}O_{11}$: 632,2258); 616 (5); 615 (15); 614 (31); 601 (3); 596 (8); 566 (7); 534 (6); 528 (13,

(1)$^{+}_{\bullet}$); 497 (7); 399 (6); 392 (8); 381 (7); 372 (6); 353 (13); 314 (27, ④A$^{+}_{\bullet}$); 295 (13); 267 (23); 115 (10); 104 (35).

3.8. Calyflorenone D (5) $C_{34}H_{34}O_{10}$

Pale amorphous solid, mp 108–114 °C (Et_2O/petrol). R_f values: 0.30 (*system 3*); 0.49 (*system 4*). $[\alpha]_D^{20} = -27.47$ ($c = 0.142$), $[\Phi]_D^{20} = -165.37$. UV, λ_{max} [nm], (ϵ): 295 (5920); 257 (12,750); 211 (47,740). IR: 3372, 2925, 2851, 1653 s, 1457, 1327, 1288, 1194, 1162, 1089, 1023 s, 869, 760, 700. MS: 602.2147 Da ([M$^{+}_{\bullet}$], 100, calc. for $C_{34}H_{34}O_{10}$: 602,2152), 585 (15), 584 (38), 566 (28), 535 (10), 498 (15, ①$^{+}_{\bullet}$), 480 (14, ①$^{+}_{\bullet}$-18), 449 (12), 448 (10, ①$^{+}_{\bullet}$-18-32), 417 (29), 351 (15), 284 (8, ④B$^{+}_{\bullet}$-H_2O); 267 (16), 265 (16); 104 (23).

3.9. Calycopterone (6)

Colourless prisms, mp 117 °C (lit: 222–223 °C), MS and IR are identical with lit. (Wall et al. 1994); additional NMR data (^1H, $CDCl_3/C_6D_6$, Table 1; ^{13}C, $CDCl_3$, Table 3) resemble those recorded in DMSO-d_6 (ibidem).

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