Bioactive Constituents of *Cedrelopsis microfoliata*

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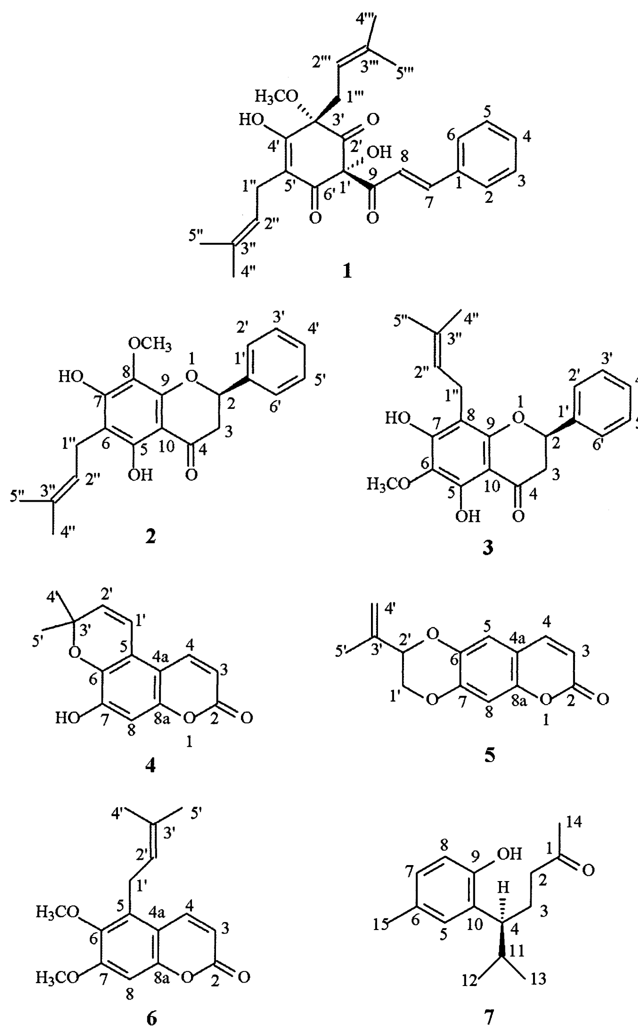
The stem bark of *Cedrelopsis microfoliata* has yielded the new compounds microfolian (**1**), microfolione (**2**), and microfolicoumarin (**6**) along with the known compounds agrandol (**3**), cedrecoumarin A (**4**), obliquin (**5**), and sesquichamaenol (**7**). Compounds **2**–**4** were found to show agonistic activity on both alpha- and beta-estrogenic receptors, and compounds **1**, **3**, and **4** were shown to inhibit the chemiluminescence of reactive oxygen metabolites and showed superoxide scavenging activity.

The genus *Cedrelopsis* has, at various times, been placed in the Sapindaceae, Rutaceae, and the Meliaceae families, but is now placed in the family Ptaeroxylaceae along with the genus *Ptaeroxylon* because of similarities in morphology and structure to the secondary xylem and pollen of *P. obliquum*.[1] The grouping of the Madagascan genus *Cedrelopsis* and the monospecific South African genus *Ptaeroxylon* into the Ptaeroxylaceae is supported by chemical evidence, since both were shown to contain a range of coumarins and chromones.[2–6] Recently, the stem bark of *C. grevei* has been found to contain the unusual limonoid derivatives cedmiline and cedmilinol.[7] In this investigation the stem bark of a second Madagascan *Cedrelopsis* species, *C. microfoliata* J.-F. Leroy, was examined.

The dried stem bark of *Cedrelopsis microfoliata* was extracted successively with hexane, dichloromethane, and methanol. The hexane extract yielded two novel compounds, the prenylated chalcone, microfolian (**1**), and a prenylated flavanone, microfolione (**2**), along with a known prenylated flavanone, agrandol (**3**). The dichloromethane extract yielded a new coumarin, microfolicoumarin (**6**), together with the known coumarins cedrecoumarin A (**4**) and obliquin (**5**) as well as the known sesquiterpenoid sesquichamaenol (**7**). Compounds **3**–**5** and **7** were identified by NMR and MS techniques and by comparison with published data.[4,8–10]

The HRMS of compound **1** was used to assign the molecular formula $C_{26}H_{30}O_5$. The IR spectrum showed the presence of hydroxy (3462 cm^{-1}) and ketone (1620, 1677, and 1714 cm^{-1}) groups. Its UV spectrum showed absorption maxima at 380, 238, and 223 nm due to the cinnamoyl chromophore and the conjugation of the whole molecule. A monosubstituted benzene ring was indicated by the presence of coupled ^{2}H NMR resonances at δ 7.62 (m, H-2, 6) and 7.40 (m, H-3, 4, 5). Resonances attributed to H-7 and H-8 of the chalcone structure occurred at δ 7.88 (d, 15.8 Hz) and 8.20 (d, 15.8 Hz), and corresponding ^{13}C NMR resonances occurred at δ 144.2 and 122.5. The C-9 resonance occurred at δ 184.6. These assignments were confirmed using HMBC and NOESY spectra. The structure of ring A was rather unusual, having keto groups at C-2′ and C-6′. The C-2′ and C-6′ resonances occurred at δ 194.8



and 191.6, respectively, in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum indicated the presence of two prenyl groups which were placed at the 3′ and 5′ positions. The H_2-1‴ resonance of the one prenyl group and the proton resonance of the methoxy group showed HMBC correlations to C-3′, which occurred at δ 84.4. Thus, a methoxy group was also placed at C-3′. The H_2-1‴ of the first prenyl group and the H_2-1″ resonance of the other prenyl group both showed HMBC correlations to the resonance at δ 168.3. Thus, this resonance was assigned to C-4′. The H_2-1″ resonance

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Table 1. ^{1}H, ^{13}C, HMBC, and NOESY Data for Compound **1**

position	δ_H[a]	δ_H[b]	δ_C	HMBC	NOESY
1			135.3		
2/6	7.62, m	7.63, m	128.4	C-3/5, C-4, C-7	H-7,8, H-3/5
3/5	7.40, m	7.38, m	128.9	C-1, C-2/6	H-2/6
4			130.6		
7	7.88, d (15.75)	7.90, d (15.75)	144.3	C-1, C-2/6, C-8, C-9	H-2/6
8	8.20, d (15.75)	8.22, d (15.75)	122.5	C-1, C-9	H-2/6, 3H-5‴
9			184.6		
1′			108.2		
2′			194.8		
3′			84.4		
4′			168.3		
5′			114.6		
6′			191.6		
1″	3.10[c]	3.15, t (7.10)	50.89	C-4′, C-5′, C-6′, C-2″, C-3″	3H-5″, H-2″
2″	5.13, t (7.14)	5.15, t (7.10)	121.8	C-4″, C-5″	2H-1″, 3H-4″
3″			131.3		
4″	1.66, s	1.70, s	25.0	C-2″, C-3″, C-5″	H-2″
5″	1.74, s	1.75, s	16.9	C-2″, C-3″, C-4″	2H-1″
1‴	2.57, t (7.51)	2.59, t (7.55)	39.2	C-2′, C-3′, C-4′, C-2‴, C-3‴	3H-5‴
2‴	4.86[d]	4.96, t (7.55)	115.5	C-4‴, C-5‴	3H-4‴
3‴			137.0		
4‴	1.54, s	1.63, s	24.9	C-2‴, C-3‴, C-5‴	H-2‴
5‴	1.47, s	1.50, s	16.8	C-2‴, C-3‴, C-4‴	2H-1‴, H-8
3′-OCH_3	3.10, s	3.21, s	52.9	C-3′	

[a] Spectrum run in CD_3OD (400 MHz). [b] Spectrum run in $CDCl_3$ (300 MHz). [c] Signal overlapped with methoxy signal. [d] Signal obscured by solvent peak.

Table 2. ^{1}H NMR Data for Compounds **2**–**6** (400 MHz, CD_3OD)

proton	**2**	**3**	**4**	**5**	**6**
2	5.48, dd (12.8, 3.1)	5.40, dd (12.6, 3.1)			
3	(a) 3.09, dd (17.2, 12.8) (b) 2.78, dd (17.2, 3.1)	(a) 3.05, dd (17.2, 12.6) (b) 2.78, dd (17.2, 3.1)	6.19, d (9.7)	6.26, d (9.5)	6.25, d (9.8)
4			8.08, d (9.7)	7.55, d (9.5)	7.75, d (9.8)
5				6.98, s	
8			6.63, s	6.85, s	6.72, s
1′			6.79, d (10.0)	(a) 4.01, dd (11.4, 8.1) (b) 4.35, dd (11.4, 2.4)	3.55, d (6.6)
2′	7.51, d (7.1)	7.48, d (7.1)	5.90, d (10.0)	4.52, dd (8.1, 2.4)	5.02, t (6.6)
3′	7.40, d (7.5)	7.35, d (7.1)			
4′	7.36, m	7.37, m	1.43, s	(a) 5.11, d (0.7) (b) 5.17, d (0.7)	1.80, s
5′	7.40, d (7.5)	7.35, d (7.1)	1.43, s	1.84, s	1.67, s
6′	7.51, d (7.1)	7.48, d (7.1)			
1″	3.22, d (7.1)	3.21, d (7.1)			
2″	5.18, t (7.1)	5.14, t (7.1)			
4″	1.63, s	1.56, s			
5″	1.74, s	1.60, s			
OCH_3	3.73, s	3.78, s			3.77, s[a]
OCH_3					3.90, s[b]

[a] Position C-6. [b] Position C-7.

showed a HMBC correlation with C-6′. The C-1′ resonance occurred in an unusual downfield position of δ 108.2, and the remaining hydroxyl group (indicated by the molecular formula) was placed at this position. A NOESY interaction between H-8 and H_3-5‴ indicated that the prenyl group at C-3′ and the chalcone chain were *cis*. These assignments were supported by NOESY and HMBC correlations as shown in Table 1. Thus, **1** (microfolian) was assigned the structure 1′,4′-dihydroxy-2′,6′,9-triketo-3′-methoxy-3′,5′-diprenylchalcone.

The ^{1}H NMR spectra (Table 2) for the flavanones agrandol (**3**) and microfolione (**2**) were very similar, and **2** was found to be a structural isomer of agrandol. Compound **3** has been isolated previously from the root bark of *Dioclea grandiflora* (Leguminosae).[8] HRMS of both compounds indicated the molecular formula $C_{21}H_{22}O_5$. The IR spectrum of **2** showed the presence of hydroxy (3382 cm^{-1}) and ketone (1637 cm^{-1}) groups. The UV spectrum of **2** showed absorption maxima at 345, 297, and 208 nm due to the presence of a conjugated aromatic system. The presence of a flavanone structure was indicated by an AMX system with resonances at δ 5.48 (dd, J = 12.8, 3.1 Hz, H-2), δ 3.09 (dd, J = 17.2, 12.8 Hz, H-3a), and δ 2.78 (dd, J = 17.2, 3.1 Hz, H-3b) and the resonance occurring at δ 196.2 ascribable to C-4 for **2**. In **3**, prenylation occurs at C-8 and a methoxy group is present at C-6. These groups are interchanged in microfolione (**2**). This was determined using the NOESY spectra for these compounds. In agrandol (**3**), a positive NOESY correlation was seen to occur between the H_2-1″ and H-4″ resonances of the prenyl group and H-2′,6′, but in microfolione (2), a NOESY interaction was observed between the methoxy group proton resonance and H-2′. The H_2-1″ proton resonance showed HMBC correlations with C-5, C-6, and C-7, and the methoxy group proton resonance showed a HMBC correlation with C-8, which confirmed the proposed structure for **2** (microfolione; 5,7-dihydroxy-8-methoxy-6-prenylflavanone).

Three prenylated coumarins, cedrecoumarin A (**4**), obliquin (**5**), and microfolicoumarin (**6**), were also isolated. The first two have been reported to occur in the related

Table 3. ^{13}C NMR Data for Compounds **2**−**6** (100 MHz, CD_3OD)

carbon	2	3	4	5	6
2	79.6	79.2	162.5	161.4	161.4
3	43.2	43.2	111.4	114.4	113.1
4	196.2	197.1	140.3	143.2	141.2
4a			107.3	113.2	111.1
5	156.9[a]	152.9	117.6	114.7	126.1
6	108.4	128.9	137.8	140.9	143.9
7	157.7[a]	157.4[a]	150.0	146.8	156.4
8	128.3	107.8	102.6	105.1	98.7
8a			150.4	149.5	152.6
9	151.8	155.7[a]			
10	101.8	102.0			
1′	139.2	139.4	116.7	67.8	25.0
2′	126.0	126.0	133.5	75.9	122.3
3′	128.5	128.4	76.5	139.4	133.1
4′	128.4	128.3	26.3	115.0	18.4
5′	128.5	128.4	26.3	19.2	25.9
6′	126.0	126.0			
1″	21.1	21.7			
2″	122.4	122.4			
3″	130.7	130.6			
4″	24.9	24.9			
5″	16.8	16.8			
O*C*H_3	60.5	59.9			61.43[b]
O*C*H_3					56.31[c]

[a] Assignments may be interchanged. [b] Position C-6. [c] Position C-7.

species *Cedrelopsis grevei*[4] and *Ptaeroxylon obliquum*,[9] respectively. HRMS of microfolicoumarin (**6**) indicated the molecular formula $C_{16}H_{18}O_4$. The IR spectrum of **6** showed the presence of a ketone group at 1710 cm^{-1}. The UV spectrum showed absorption maxima at 299 and 205 nm due to the conjugated aromatic system. The ^{1}H NMR spectrum showed H-3 and H-4 occurring as a pair of doublets ($J = 9.8$ Hz) at δ 6.25 and 7.75, respectively. The C-2 carbonyl resonance occurred at δ 161.4 (Table 3). The NOESY spectrum showed a correlation between H-4 and H-1′and H-2′ of the prenyl group, indicating that this group was attached at C-5. H-1′ also showed a NOESY correlation with one of the two methoxy groups at δ 3.77, which assigned the methoxy group at C-6. The second methoxy group at δ 3.90 and a singlet proton at δ 6.72 were left to be placed at C-7 and C-8. The proton singlet at δ 6.72 showed HMBC correlations with C-4a and C-8a, and the second methoxy group at δ 3.90 was thus placed at C-7. This is in accordance with coumarins previously isolated from the Ptaeroxylaceae which all have oxygenated substituents at C-6 and C-7.[11] Accordingly, microfolicoumarin (**6**) was assigned as 6,7-dimethoxy-5-prenylcoumarin.

The final compound isolated was the known sesquiterpenoid (−)-sesquichamaenol, which has been isolated previously from *Juniperus formosana*[10] and *Chamaecyparis formosensis*.[12] As the published NMR data are incomplete, full assignments are given in the Experimental Section.

Compounds **2**−**4** were found to be active in assays for agonistic activity on both alpha- and beta-estrogen receptors in ranges from 10 to 100 μg/mL (20−50% stimulation).[13] However, activity was low compared to the standard genistein, which is active in the same assay at 3−30 ng/mL (10−100% stimulation).

Compounds **1**, **3**, and **4** were found to be active inhibitors of the (luminol-induced) chemiluminescence of reactive oxygen metabolites generated by human polymorphonuclear leucocytes activated with opsonized zymosan (IC_{50} values 4.0, 3.2, and 13 μg/mL, respectively) and to scavenge superoxide anions in a cell-free system (IC_{50} value 3.0, 3.0, and 0.2 μg/mL), suggesting potential antiinflammatory activity for these compounds.[14,15]

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler micro-hot stage melting apparatus and are uncorrected. Optical rotations were measured at room temperature in methanol or chloroform using an Optical Activity AA-5 polarimeter together with a series A2 stainless steel (4 × 200 mm) unjacketed flow tube. Concentrations are quoted as g/100 mL. UV spectra were recorded on a Varian DMS 300 UV−visible spectrophotometer, using methanol as solvent. IR spectra were recorded on a Nicolet Impact 400D spectrometer on sodium chloride plates and calibrated against an air background. NMR spectra were recorded using a Varian Inova 400 MHz spectrometer. HRMS were recorded using a Kratos high-resolution MS 9/50 spectrometer, and EIMS on an Agilent GC 6890 and an Agilent MS 5973 GC−MS instrument. Si gel (Merck, type 60, 230−400 mesh) was used for column chromatography.

Plant Material. Stem bark of *Cedrelopsis microfoliata* J.-F. Leroy (581 g) was collected at Ankarafantsika in the northwest of Madagascar in November 1999, and a voucher specimen has been retained at the University of Antananarivo (07-99/nJ/nDul).

Extraction and Isolation. A portion of the dried plant material (108 g) was extracted successively with hexane, dichloromethane, and methanol for 24 h each. NMR spectroscopy of the methanol extract showed only the presence of sugars, so it was not investigated further. The hexane (3.26 g) and dichloromethane (2.48 g) extracts were separated using column chromatography over Si gel (Merck 9385) with varying ratios of hexane/dichloromethane for the hexane extract and dichloromethane/ethyl acetate for the dichloromethane extract. The hexane extract yielded compounds **1**−**3**, and the dichloromethane extract yielded compounds **4**−**7**. Agrandol (**3**), cedrecoumarin A (**4**), obliquin (**5**), and sesquichamaenol (**7**) have been reported previously, and the structures of these compounds were confirmed by comparison against literature data.[4,8−10,12] However, as literature NMR assignments for these compounds are incomplete, NMR data for these compounds are also provided. NMR data for compounds **3**−**5** are included in Tables 2 and 3.

Microfolian (1): yellow oil (32 mg); $[\alpha]_D^{22}$ +11.62° (*c* 0.086, CH_2Cl_2); UV λ_{max} (CH_2Cl_2) (log ϵ) 380 (4.23), 238 (4.08), 223 (3.99) nm; IR (NaCl) ν_{max} 3462, 2924, 2850, 1620, 1583, 1522, 1441, 1386 cm^{-1}; EIMS *m*/*z* [M$^+$] 438 (42), 370 (100), 299 (44), 239 (63), 221 (33), 195 (79), 131 (78), 103 (31); HRMS *m*/*z* 438.20428 [M$^+$], $C_{26}H_{30}O_6$ requires 438.20424.

Microfolione (2): white crystals (35 mg); mp 172−173 °C; $[\alpha]_D^{22}$ +37.5° (*c* 1.0, CH_2Cl_2); UV λ_{max} (MeOH) (log ϵ) 345 (4.53), 297 (5.13), 208 (5.44) nm; IR (NaCl) ν_{max} 3382, 2971, 2923, 2849, 1637, 1466, 1380, 1337 cm^{-1}; EIMS *m*/*z* [M$^+$] 354 (100), 339 (14), 299 (43), 235 (32), 195 (39), 194 (57); HRMS *m*/*z* 354.14543 [M$^+$], $C_{21}H_{22}O_5$ requires 354.14672.

Microfolicoumarin (6): yellow oil (5 mg); UV λ_{max} (MeOH) (log ϵ) 299 (4.54), 205 (5.08) nm; IR (NaCl) ν_{max} 3403, 2965, 2929, 2873, 1710, 1519, 1457, 1426, 1365 cm^{-1}; EIMS *m*/*z* [M$^+$] 274 (100), 259 (11), 243 (11), 219 (10), 217 (45); HRMS *m*/*z* 274.12097 [M$^+$], $C_{16}H_{18}O_4$ requires 274.12050.

Sesquichamaenol (7): white crystals (7 mg); mp 108−109 °C; $[\alpha]_D^{22}$ −5.95° (*c* 0.042, CH_2Cl_2); UV λ_{max} (MeOH) (log ϵ) 284 (4.22), 204 (5.00) nm; IR (NaCl) ν_{max} 3439, 2984, 2935, 2867, 1740, 1648, 1611, 1457, 1390, 1346, 1303 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.20 (2H, m, H_2-2), 2.08 (1H, m, H-3a), 1.73 (1H, m, H-3b), 2.56 (1H, m, H-4), 6.82 (1H, d, $J = 1.7$ Hz, H-5), 6.84 (1H, dd, $J = 8.1$, 1.7 Hz, H-7), 6.65 (1H, d, $J = 8.1$ Hz, H-8), 1.83 (1H, m, H-11), 0.71 (3H, d, $J = 6.6$ Hz, H_3-12), 0.99 (3H, d, $J = 6.6$ Hz, H_3-13), 2.03 (3H, s, H_3-14), 2.23 (3H, s, H_3-15), 4.95 (1H, s); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 208.7 (C-1), 42.0 (C-2), 27.0 (C-3), 44.0 (C-4), 128.7 (C-5), 130.0 (C-6), 127.5 (C-7), 115.9 (C-8), 152.0 (C-9), 130.2 (C-10), 33.4 (C-11), 21.6 (C-12), 21.2 (C-13), 30.4 (C-14), 21.1 (C-15); EIMS *m*/*z* [M$^+$] 234 (19), 191 (15), 176 (18), 164 (30), 163 (25), 133 (75), 121 (54), 43 (100).

Estrogen Receptor (ER) α and β Assays. Human 293 embryonal kidney cells were stably transfected with either ER α or ER β combined with a luciferase response element (reporter gene).[13] In this system 17β-estradiol can be measured at concentrations as low as 10^{-14} M; maximum responses are detected at concentrations of 10^{-11} M. The maximum response is taken as 100%, and estrogenic activities of test samples are expressed as percentage of this maximum response at a certain concentration.

Chemiluminescence Assay. Polymorphonuclear leukocytes (PMNs) were prepared from buffycoat residues from healthy volunteers, after centrifugation in Ficoll-Hypaque, according to manufacturer's instructions (Amersham Pharmacia, Uppsala, Sweden). Cells were diluted to 1×10^{-7} PMNs per mL HBSS (Hank's Buffered Salt Solution) and dispensed in white 96-well flat-bottom microtiter plates in 50 μL amounts. Subsequently, 50 μL of an appropiate dilution range of a test sample and 50 μL of luminol (0.1 mM) were added to each well. The cells were activated with 50 μL (human) serum-treated (opsonized) zymosan (0.8 mg/mL), after which luminescence of each well was monitored every 2 min during a 30 min period, in a Titertek Luminoskan luminometer (TechGen International, Zellik, Belgium). Maximum peak levels were used to calculate the inhibitory activity of the test samples compared with a control. Controls consisted of cells with luminol and buffer.[14,15]

Superoxide Anion Scavenging Assay. In 96-well, white flat-bottom microtiter plates test compounds were serially diluted in PBS (phosphate buffer saline; pH 7.4) to a final volume of 50 μL. Hypoxanthine (50 μL; final concentration 1 mM), lucigenin (50 μL; 0.1 mM), and either buffer (PBS) or superoxide dismutase (SOD; 25 μL; 10 U/mL) were added. Superoxide anion production was initiated by the addition of 25 μL of xanthine oxidase (10 mU/mL), and chemiluminescence was monitored every min for 0.5 s during 15 min using a Fluoroskan Ascent FL luminometer (Labsystems, Breda, Netherlands). Activity of test compounds was calculated from the SOD-inhibitable part of the chemiluminescence signal. To exclude direct effects of test compounds on xanthine oxidase activity, uric acid formation was determined spectrophotometrically at 290 nm.[15]

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