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Chalcones from the seed of *Cedrelopsis grevei* (Ptaeroxylaceae)

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Abstract

The seed of *Cedrelopsis grevei* (Ptaeroxylaceae) has yielded the known compounds uvangoletin, 5,7-dimethylpinocembrin, cardamonin, flavokawin B, 2′-methoxyhelikrausichalcone, and the novel prenylated chalcones, cedreprenone and cedrediprenone. Cedridiprenone has been shown to exhibit superoxide scavenging properties.

Keywords: *Cedrelopsis grevei*; Ptaeroxylaceae; Chalcone; Uvangoletin; 5,7-Dimethylpinocembrin; Cardamonin; Flavokawin B; 2′-Methoxyhelikrausichalcone; Cedreprenone; Cedrediprenone; Superoxide scavenger

1. Introduction

Cedrelopsis grevei is one of five Madagascan members of *Cedrelopsis*, which is grouped with the monospecific South African *Ptaeroxylon* genus into the Ptaeroxylaceae family. The Ptaeroxylaceae family has been grouped, at various times, with the Meliaceae, Rutaceae and Sapindaceae (Styles and Pennington, 1975). Previous investigations of the bark and wood of *C. grevei* and *Ptaeroxylon obliquum* have yielded a range of coumarins and chromones, both simple and prenylated (Schulte et al., 1973; Eshiett and Taylor, 1968; Dean and Robinson, 1971; McCabe et al., 1967; Kotsos, 1997), and two unusual limonoid derivatives, cedmiline and cedmilinol, have been isolated from *C. grevei* (Mulholland et al., 1999). The presence of these limonoids suggested a close relationship with the Cneoraceae family (Mulholland and Mahomed, 2000). The bark of this species is used in Madagascar as an additive to bath water in order to relieve muscle fatigue (Mullholand et al., 1999).

In this investigation, the fruit of *C. grevei* was investigated. No limonoid derivatives were detected, but a flavanone, five chalcones and a dihydrochalcone were isolated. Chalcones and flavanones have not been isolated previously from the Ptaeroxylaceae.

2. Results and discussion

The dichloromethane extract of the fruit and seeds yielded, after repeated column chromatography, seven compounds. The first four were identified as the known compounds, uvangoletin (2′,4′-dihydroxy-6′-methoxydihydrochalcone), **1**, cardamonin, **2**, flavokawin B (2′-hydroxy-4′,6′-dimethoxychalcone), **3** and 5,7-dimethylpinocembrin, **4**.

Although the *Dictionary of Natural Products* (1999) lists compound **5,** 2′-methoxyhelikrausichalcone, as being isolated previously from *Helichrysum aphelexoides* (Randriaminahy et al., 1992) the paper does not report this compound, but similar ring closed prenylated chalcones. The structures of compounds **1**–**4** were determined using NMR and HRMS techniques, and confirmed by comparison of physical and spectroscopic properties against literature data (Hufford and Oguntimein, 1980; Itokawa et al., 1981; Bick et al., 1972). Two further novel prenylated chalcones, cedreprenone, **6**, and cedrediprenone, **7**, were isolated. The compounds isolated make up an interesting biosynthetic series as shown in Fig. 1, all have unsubstituted B rings and 1,3,5-trioxygentaed ring A structures.

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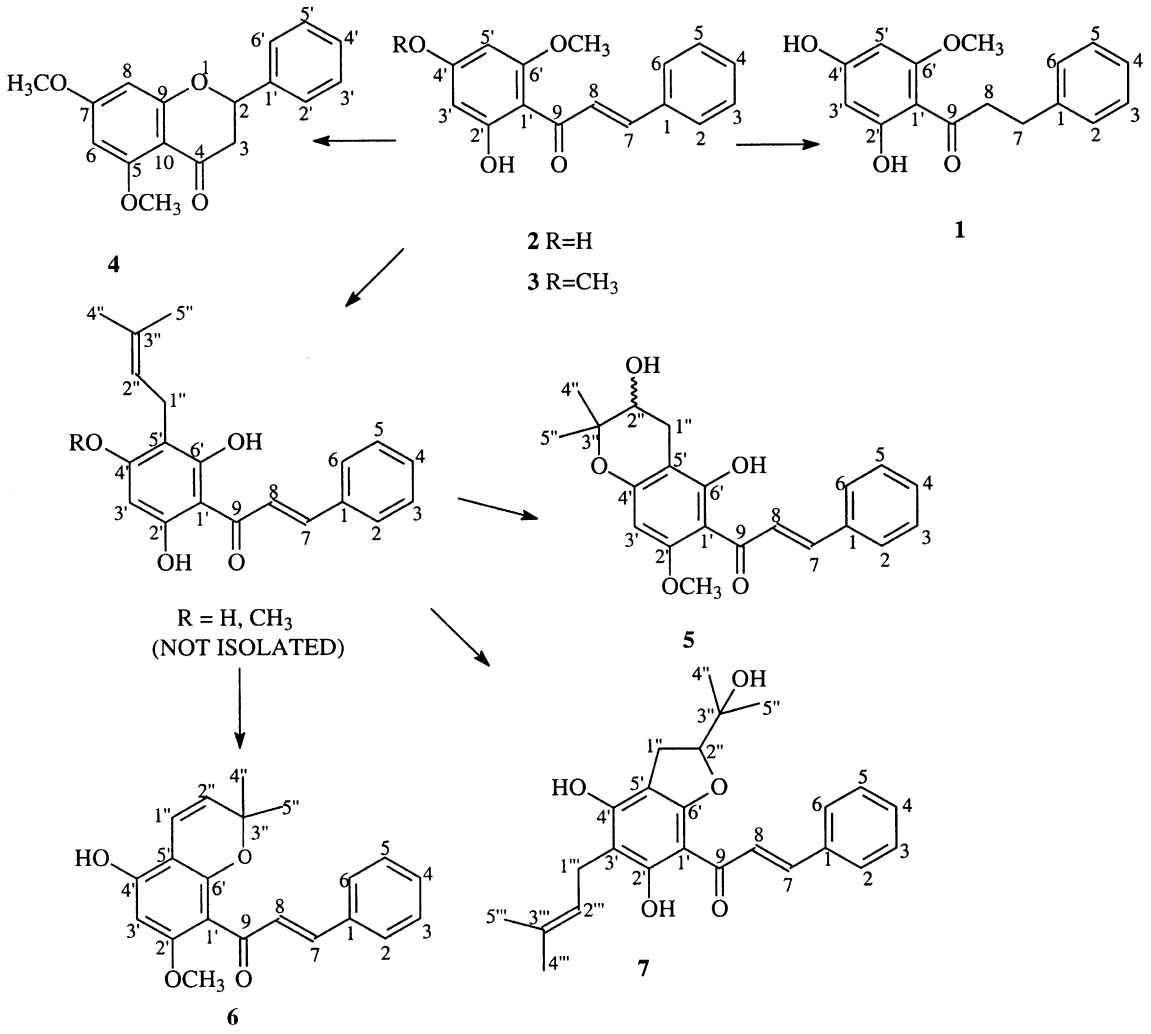

Fig. 1. Biosynthetic series of compounds isolated from the fruit and seeds of *Cedrelopsis grevei*.

Compound **5**, 2′-methoxyhelikrausichalcone, $C_{21}H_{22}O_5$, was isolated as yellow crystals. The ^{1}H NMR spectrum indicated that the compound was similar to the chalcones **2** and **3**, with an unsubstituted ring B (δ 7.61, 2H, m, H-2,6 and δ 7.40, 3H, m, H-3,4,5), and resonances due to the *trans*-α,β-unsturated ketone protons at δ 7.65 and 8.04 (2H, J = 15.8 Hz, H-7,8). The ketone carbonyl carbon occurred at δ 193.0 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum showed the presence of a single aromatic proton in ring A. This was placed at C-3′ based on a ^{3}J correlation with C-1′. A three-proton singlet at δ 3.84 indicated the presence of a methoxy group. The NOESY spectrum showed a correlation with the H-3′ proton resonance indicating it was present at either C-2′ or C-4′. Two singlets at δ 1.36 and δ 1.41 ascribed to methyl group protons (3H-4″ and 3H-5″) and an AMX system with resonances at δ 3.77 (dd, J = 6.8, 5.5, H-2″), δ 2.80 (dd, J = 17.0, 5.5, H-1″) and δ 2.47 (dd, J = 17.0, 6.8, H-1″), with corresponding ^{13}C NMR resonances at δ 68.1 (CH, C-2″) and δ 25.6 (CH_2, C-1″) and a further resonance at δ 78.6 (C-3″) indicated that a hydroxylated prenyl group was present. The two H-1″ protons showed HMBC correlations with C-4′ and C-6′, so the group was placed at C-5′. The molecular formula indicated the presence of an extra ring, which was supported by the non-equivalent methyl groups. There were two options: forming an ether linkage between C-3″ and either C-4′ or C-6′. No NOESY correlation was seen between hydrogens on the hydroxylated prenyl group and either H-8 or H-6 (as seen in compounds **6** and **7**), so the ether linkage had to occur between C-3″ and C-4′. This meant the methoxy group occurred at C-2′. This was confirmed by an HMBC correlation between C-2′ and the methoxy group proton resonance. All hydrogen and carbon resonances could be assigned from the HSQC and HMBC spectra and are given in Table 1.

High resolution mass spectrometry of compound **6**, cedreprenone, a yellow crystalline material, indicated a molecular formula of $C_{21}H_{20}O_4$. The ^{1}H NMR spectrum was very similar to that of compound **5** with resonances ascribable to an unsubstituted aromatic ring B

Table 1
: ^{1}H and ^{13}C NMR Data for compounds **5**–**7** (400 MHz, CD_3OD)

Proton	5	6	7	Carbon	5	6	7
				1	135.7	135.6	135.6
2	7.61, *m*	7.62, *m*	7.65, *m*	2	128.0	128.0	128.1
3	7.40, *m*	7.41, *m*	7.37, *m*	3	128.8	128.8	128.7
4	7.40, *m*	7.41, *m*	7.37, *m*	4	129.9	130.1	130.0
5	7.40, *m*	7.41, *m*	7.37, *m*	5	128.8	128.8	128.7
6	7.61, *m*	7.62, *m*	7.65, *m*	6	128.0	128.0	128.1
7	7.65, *d* (15.8)	7.71, *d* (15.8)	7.74, *d* (15.8)	7	141.2	142.0	142.2
8	8.04, *d* (15.8)	8.09, *d* (15.8)	8.20, *d* (15.8)	8	128.0	127.4	126.5
				9	193.0	192.9	191.1
				1′	106.1	106.0	101.6
2′				2′	165.9	161.4	162.9
3′	6.10, *s*	6.10, *s*		3′	91.8	92.3	108.2
4′				4′	164.2	166.8	158.7
5′				5′	100.3	103.3	103.9
6′				6′	155.0	155.5	160.8
1″	2.80, *dd* (17.0, 5.5) 2.47, *dd* (17.0, 6.8)	6.56, *d* (9.9)	3.04, *m*	1″	25.6	116.3	27.0
2″	3.77, *dd* (6.8, 5.5)	5.52, *d* (9.9)	4.78, *t* (8.6)	2″	68.1	124.8	91.0
				3″	78.6	78.1	71.0
4″	1.36, *s*	1.52, *s*	1.38, *s*	4″	20.5	27.0	24.7
5″	1.41, *s*	1.52, *s*	1.28, *s*	5″	24.7	27.0	24.7
1‴			3.25, *d* (7.6)	1‴			21.2
2‴			5.16, *bt* (6.8)	2‴			123.1
				3‴			130.1
4‴			1.74, *s*	4‴			16.8
5‴			1.63, *s*	5‴			24.8
OCH_3	3.84, *s*	3.86, *s*		$O\underline{C}H_3$	55.2	55.3	

(δ 7.62, 2H, m, H-2, 6 and δ_H 7.41, 3H, m, H-3, 4, 5), *trans*-orientated H-7 and H-8 protons, (δ 7.71, 8.09, 2H, $J = 15.8$ Hz, H-7, 8), a proton at C-3′ (δ 6.10) and a methoxy group proton resonance at δ 3.86. The resonance ascribed to C-9 occurred at δ 192.9. The AMX system of compound **5** was absent, but a second set of doublets occurred at δ 6.56 and δ 5.52 ($J = 9.9$ Hz,), which were attributed to alkene protons, H-1″ and H-2″ of the prenyl group. A six proton methyl group proton singlet was present at δ 1.52 (s, 6H, 3H-4″, 3H-5″), the chemical shift indicating that they were vinylic, and a resonance at δ 78.1 indicated that C-3″ was again oxygenated. The pattern of substitution on ring A was again confirmed using the NOESY and HMBC spectra.

The aromatic proton H-3′ showed HMBC correlations with C1′, 2′, 4′ and 5′ and a NOESY correlation with the 2′-OCH_3 group. The H-1″ olefinic proton showed an HMBC correlation to C-6′ and a NOESY correlation to H-2″, while H-2″ showed an HMBC correlation to C-5′ and a NOESY correlation to H-1″, 3H-4″ and 3H-5″. The 3H-4″ and 3H5″ proton resonances showed HMBC correlations to C-2″ and C-3″. The molecular formula indicated an extra ring and again an ether linkage between C-3″ and either C-4′ or C-6′ was possible. However, NOESY interactions between the 3H-4″ and 3H-5″ proton resonances and H-6 and H-8 were present and this could only be possible if the ether linkage occurred between C-3″ and C-6′, giving structure **6**. The fact that the two methyl groups are equivalent is due to the planarity of the molecule. All hydrogen and carbon resonances could be assigned from the HSQC and HMBC spectra and are given in Table 1.

Compound **7**, cedrediprenone, was isolated as a yellow crystalline material of molecular formula $C_{25}H_{28}O_5$. The ^{1}H NMR spectrum was again similar to those of compounds **5** and **6** with resonances ascribable to an unsubstituted aromatic ring B (δ 7.65, 2H, m, H-2,6 and δ 7.37, 3H, m, H-3,4,5) and *trans*-orientated H-7 and H-8 protons, (δ 7.74, 8.20, ea 1H, $J = 15.8$ Hz, H-7,8). The resonance ascribed to C-9 occurred at δ 191.1. The extra five carbons in the molecular formula along with the presence of four methyl group singlets, two AMX coupled systems and the absence of the H-3′ proton resonance indicated that compound **7** was di-prenylated. These two groups were tentatively placed at C-3′ and C-5′ in line with the 2,4,6-oxygenation pattern of ring A. The first of these groups had vinylic methyl group proton resonances at δ 1.63 and δ 1.74 (3H-4‴, 3H-5‴), which showed HMBC correlations to an alkene proton triplet at δ 5.16, (H-2‴). This resonance showed coupling in the COSY spectrum with a doublet ascribed

to two equivalent protons of a methylene group at δ 3.25 (2H-1‴). This prenyl group was placed at C-3′ because the H-1‴ protons showed a ^3J HMBC correlation with C-2′ and C-4′. The second group was characterised by the presence of two non-equivalent methyl group proton resonances at δ 1.28 and 1.38 (3H-4″, 3H-5″), a coupled system consisting of two superimposed methylene group proton resonances at δ 3.04 (2H-1″) and δ 4.78 (H-2″). The corresponding C-2″ occurred at δ 91.0 and the fully substituted C-3″ resonance occurred at δ 71.0 indicating both these carbons were attached to oxygen. The molecular formula required an extra ring and this was supported by the presence of two non-equivalent methyl group proton resonances. However, when compared to compound **5**, the resonances for the 2″ position with H-2″ at δ_H 4.78 and C-2″ at δ_C 91.0 are too far downfield for a hydroxypyran group, in which H-2″ occurs at δ_H 3.77 and C-2″ at δ_C 68.1 in compound **5**. Flavanones with the prenyl group cyclised to the furan ring were reported by Roussis et al. (1987) and had similar chemical shifts for the proton and carbon resonances at the 2″ position. This suggested the prenyl group had cyclisation to a furan ring in compound **7**. The C-3″ carbon therefore had a hydroxy group attached to it and the C-2″ carbon was involved in the cyclisation. Again, two possible ether linkages were possible- between C-2″ and either the oxygen atoms at C-4′ or C-6′. The latter was shown to occur by the presence of correlations in the NOESY spectrum between H-6 and H-8 and the 3H-4″ and 3H-5″ proton resonances. All assignments and the positioning of the alkyl groups were confirmed using HSQC, HMBC and NOESY spectra. ^{1}H and ^{13}C NMR assignments for compound **7**, cedrediprenone are given in Table 1.

Cedrediprenone (**7**) was found to be active at inhibiting the luminol—enhanced chemiluminescence of reactive oxygen metabolites generated by human polymorphonuclear leucocytes activated with opsonized zymosan (IC_{50} 8.1 μg/ml) and to scavenge superoxide anions in a cell free system (IC_{50} 0.2 μg/ml) suggesting anti-inflammatory activity for cedrediprenone (Smit et al., 2000; Van den Worm, 2001). 2′-methoxyhelikrausichalcone (**5**) and cedreprenone (**6**) were found to be inactive in the same bioassays.

3. Experimental

The fruit of *C. grevei* Baill. (432 g) was collected at Beza Mahafaly, (in the south of Madagascar) in May 1999, and a voucher specimen retained at the Labarotoire de Pharmacodynamie of the Faculty of Science at the University of Antananarivo (MJ/MDUL01-99). The dried fruit was extracted successively on a Labcon shaker with dichloromethane (2 l) and methanol (2 l) and the solvent removed under reduced pressure to yield extracts of 2.76 g and 4.83 g respectively. TLC of the crude dichloromethane and methanol extracts showed a similar chromatographic pattern, so the methanol extract was not investigated further. NMR spectra were recorded in CD_3OD on a Varian 400 MHz spectrometer. ^{1}H and ^{13}C NMR data of **1**–**7** are given in Table 1. HRMS and EIMS were recorded at the Cape Technikon on Kratos HRMS 9/50 and Finnigan 1020 GC MS instruments. IR spectra were recorded on a Nicolet Impact 400D instrument.

Isolation of compounds **1**–**7**. The dichloromethane extract (2.76 g) was separated using silica gel column chromatography (Merck 9385) and eluted with an ethyl acetate:hexane step gradient of 5, 10, 20, 40, 75 and 100% ethyl acetate in hexane, collecting 30×30 ml fractions for each step. Elution with 5% EtOAc in hexane afforded a yellow crystalline material, cedreprenone, **6** (18 mg) and a yellow crystalline compound, flavokawin B (2′-hydroxy-4′,6′-dimethoxychalcone, **3**, (8 mg; Itokawa et al., 1981). Elution with 20% EtOAc in hexane gave three yellow crystalline products, compounds **1**, uvangoletin (2′,4′-dihydroxy-6′-methoxydihydrochalcone), (12 mg) (Hufford and Oguntimein, 1980), **5**, 6′-methoxyhelikrausichalcone (18 mg) and **2**, cardamonin (15 mg) (Itokawa et al., 1981). Compounds **4**, 5,7-dimethylpinocembrin, (22 mg; Bick et al., 1972) and **7,** cedridiprenone (17 mg) were eluted with 40% EtOAc in hexane. Compounds **1**–**4** were identified by comparison of their physical and spectroscopic data against literature values as referenced above.

6′-Methoxyhelikrausichalcone, **5**, 18 mg, yellow crystalline, mp 127–128 °C, HRMS [M^+] at *m*/*z* 354.14690, $C_{21}H_{22}O_5$ requires 354.14672. EIMS: *m*/*z* (rel. int.): 354 (94.29), 283 (30.25), 277 (40.95), 205 (39.67), 179 (100). IR ν_{max}^{NaCl} cm^{-1}: 3425, 2937, 2850, 1639, 1450, 1348, 1231, 1157, 1114, 1052. UV λ_{max}^{MeOH} nm (log ε): 338 (4.36), 205 (4.19). $[\alpha]_D^{22}$−263° (MeOH, *c* = 0.19). ^{1}H and ^{13}C NMR data are given in Table 1.

Cedreprenone, **6**, 18 mg, yellow crystalline, mp 134 °C, HRMS [M^+] at *m*/*z* 336.13675, $C_{21}H_{20}O_4$ requires 336.13616. EIMS: *m*/*z* (rel. int.): [M^+] 336 (37.94), 321 (52.84), 283 (8.70), 279 (17.57), 251 (15.34), 231 (29.13), 217 (61.67), 167 (39.00), 149 (100). IR ν_{max}^{NaCl} cm^{-1}: 3750, 2925, 2859, 2377, 1649, 1571, 1406, 1348, 1168, 1131, 1041. UV λ_{max}^{MeOH} nm (log ε): 336 (5.40), 291 (5.40), 218 (5.05). ^{1}H and ^{13}C NMR data are given in Table 1.

Cedrediprenone, **7**, 17 mg, yellow crystalline, mp 153 °C, HRMS [M^+] at *m*/*z* 408.19489, $C_{25}H_{28}O_5$ requires 408.19367. EIMS: *m*/*z* (rel. int.): 408 (100), 393 (23.22), 375 (9.64), 365 (12.80), 353 (20.00), 276 (14.59), 231 (13.12), 189 (18.00), 177 (13.98), 131 (14.57), 103 (12.96). IR ν_{max}^{NaCl} cm^{-1}: 3377, 2934, 2862, 1629, 1569, 1432, 1366, 1258, 1180, 1085. UV λ_{max}^{MeOH} nm (log ε): 346 (4.74), 268 (4.84), 261 (4.83), 216 (4.11). $[\alpha]_D^{22}$−3.62° (CH_2Cl_2, *c* = 0.069). ^{1}H and ^{13}C NMR data are given in Table 1.

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