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Coumarins from *Cedrelopsis grevei* (Ptaeroxylaceae)

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Abstract

The stem bark of *Cedrelopsis grevei* Baill. has yielded the first reported examples of 5-prenylated coumarins, cedrecoumarin A and B as well as the known coumarins, cedrelopsin, scoparone, *O*-methylcedrelopsin and norbraylin, and the known chromones ptaeroglycol and ptaeroxylinol.

Keywords: *Cedrelopsis grevei*; *Ptaeroxylon obliquum*; Ptaeroxylaceae; β-Amyrin; Scoparone; *O*-methylcedrelopsin; Norbraylin; Cedrecoumarin A; Cedrecoumarin B; Ptaeroglycol; Ptaeroxylinol; α and β estrogen receptor agonist; Superoxide anion scavenger

1. Introduction

The monotypic genus *Ptaeroxylon* has given difficulty to botanists, who have placed this genus in the Sapindaceae, Rutaceae and most popularly, in the Meliaceae. In their generic monograph on the Meliaceae, Styles and Penington (1975) state that the genus *Cedrelopsis* is closely related to *Ptaeroxylon* and very similar to it in morphology and in the structure of the secondary xylem. The pollen of *Cedrelopsis* and *Ptaeroxylon* have been found to be very similar, unlike that of any known Meliaceous pollen grain but similar to that of some Rutaceae. They concluded that *Cedrelopsis* and *Ptaeroxylon* do not belong to the Meliaceae and that there is insufficient evidence to place them in either the Sapindaceae or Rutaceae. Thus, these genera are now considered to form a separate family, the Ptaeroxylaceae, which has been shown to be chemically distinct from both the Rutaceae and Meliaceae and to contain a range of chromones and some unusual coumarins (Dean et al., 1967a, b; Dean and Taylor, 1966; Eshiett and Taylor, 1968; Dean and Robinson, 1971).

Cedrelopsis grevei Baill. is one of seven species of the genus *Cedrelopsis* which is confined to Madagascar. This species is commonly referred to as "Katrafay" by the local people, and it is believed to relieve muscular fatigue when the bark is soaked in bath water. Two specimens have been investigated, one specimen from the wetter north-western part of Madagascar which resulted in the isolation of the pentacyclic triterpenoid, β-amyrin, as well as two novel limonoid-derived compounds, a pentanortriterpenoid, cedmilinol **1**, and a hexanortriterpenoid, cedmiline **2** (Mulholland et al., 1999; Fig. 1). The isolation of these limonoid derivatives led to the investigation of the second specimen collected in the drier south of Madagascar.

Compounds which have been isolated previously from *Cedrelopsis grevei* include the 6,7-oxygenated chromones ptaeroxylin (Eshiett and Taylor, 1968), alloptaeroxylin, alloptaeroxylin methyl ether, peucinin, heteropeucinin, greveiglycol and ptaeroglycol (Dean and Robinson, 1971) as well as the coumarin cedrelopsin (Eshiett and Taylor, 1968). Ptaeroxylin, alloptaeroxylin (Dean and Taylor, 1966) and ptaeroglycol (Dean et al., 1967b) have also been isolated from the South African *Ptaeroxylon obliquum* (Ptaeroxylaceae).

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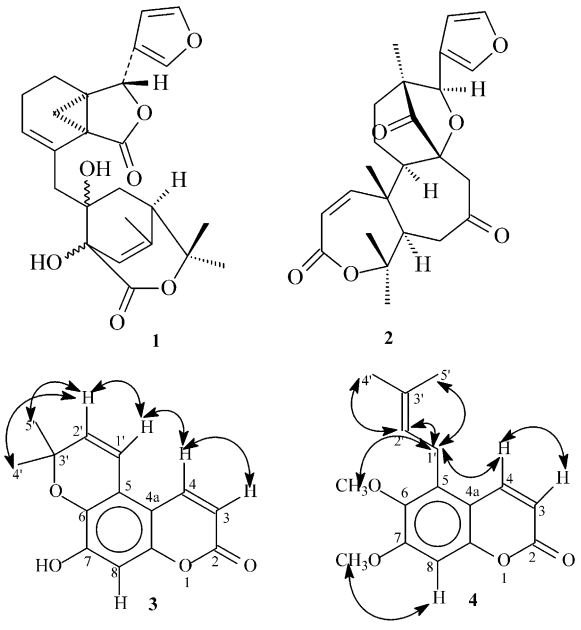

Fig. 1. Cedmilinol (**1**), cedmiline (**2**), cedrecoumarin A (**3**) and cedrecoumarin B (**4**) (arrows indicate NOE correlations).

2. Results and discussion

In the present study, the stem bark of *C. grevei* Baill. obtained from the dry southern part of Madagascar was investigated. The species furnished β-amyrin, the 6,7-dioxygenated coumarins cedrelopsin, scoparone, *O*-methylcedrelopsin, norbraylin and two novel coumarins, cedrecoumarin A **3** and cedrecoumarin B **4** (Fig. 1), as well as the known chromones ptaeroglycol and ptaeroxylinol. The cedrecoumarins are of particular interest as they are the first examples of 5-prenylated coumarins to be reported.

Cedrecoumarin A **3** was found to be an angular pyranocoumarin and the structural isomer of norbraylin. The high resolution mass spectrum of this compound exhibited a molecular ion at m/z 244.0731, indicating the molecular formula $C_{14}H_{12}O_4$. The nine double bond equivalents present suggested the presence of a three-ring coumarin. A fragment peak at m/z 229 ($[M\text{-}15]^+$) is typical of pyranocoumarins and results from the loss of one of the geminal methyl groups, to form the stable benzopyrylium ion (Murray et al., 1982). The IR spectrum showed peaks at 3370 (O–H stretching), and 1710 cm^{-1} (C=O stretching), the latter frequency being typical for the α-pyrone carbonyl stretching (Murray et al., 1982). The ^1H NMR spectrum of 3 showed two one proton doublets at δ_H 8.08 and 6.19 which were assigned to H-4 and H-3 of the coumarin nucleus based on HMBC correlations with C-2. Resonances at δ_H 1.43 (2×3H), 5.90 (1H) and 6.79 (1H) were assigned to 3H-4′ and 3H-5′, H-2′ and H-1′ of the pyran ring. The carbon atom, C-1′ of the pyran ring was bonded to C-5 based on results of HMBC and NOESY experiments. Firstly, a NOESY correlation was seen between H-1′ and H-4 and this could only occur if C-1′ was attached to C-5. Secondly, the HMBC spectrum showed correlations between H-1′ and C-4a, C-5 and C-6, confirming attachment of the isoprenyl group at C-5. The one proton singlet at δ_H 6.63 in the ^1H NMR spectrum of **3** was allocated to H-8 as a correlation was observed between this proton and C-4a and C-6 in the HMBC spectrum. No correlations with H-8 were seen in the NOESY spectrum, supporting this assignment. The remaining hydroxy group was placed at C-7. The remaining quaternary carbons were assigned using the HMBC spectrum (Table 1.).

The high resolution mass spectrum of cedrecoumarin B **4** showed a molecular formula $C_{16}H_{18}O_4$ (m/z 274.1191). The IR spectrum showed C–H aromatic stretching at 3010 cm^{-1}, a carbonyl stretching band at 1730 cm^{-1}, and a C=C stretching band at 1605 cm^{-1}. This carbonyl stretch frequency is typical of the α-pyrone carbonyl group stretch of a coumarin (Murray et al., 1982). The ^1H NMR spectrum of this compound indicated it was a prenylated coumarin with two methoxy groups. Two one-proton doublets at δ_H 8.00 ($J=9.5$ Hz) and 6.31 ($J=9.5$ Hz) were allocated to H-4 and H-3 respectively as in cedrecoumarin A **3** and the corresponding ^{13}C NMR resonances occurred at δ_C 143.3 and 113.1. The isoprenyl group was again placed at C-5 based on NOE experiments. Irradiation of H-4 gave a positive NOE for the two-proton doublet at δ_H 3.65 which was assigned to 2H-1′. Irradiation of the 2H-1′ (δ_H 3.65) resonance showed a NOE enhancement of H-4, H-2′, the methoxy group proton resonance at δ_H 3.81 and the vinyl methyl group proton resonance at δ_H 1.87. Thus a methoxy group was placed at C-6 and the methyl proton resonance was assigned to 3H-5′. Irradiation of the H-2′ (δ_H 5.10) resonance gave a NOE enhancement of the 2H-1′ resonance and the methyl proton resonance at δ_H 1.73 which could be assigned to 3H-4′. The resonances at δ_C 25.4, 123.6, 25.9 and 18.2 in the ^{13}C NMR spectrum were assigned to C-1′, C-2′, C-4′ and C-5′, respectively. Irradiation of the resonance at δ_H 6.94 attributed to H-8 showed NOE enhancement of only the methoxy group proton resonance at δ_H 3.98. The fact that enhancement of only one methoxy group proton resonance occurred on irradiation of the signal at δ_H 6.94 confirms the assignment of this resonance to H-8. Remaining ^{13}C NMR assignments are given in Table 1. Cedrecoumarin B decomposed on standing.

Cedrecoumarin A was found to be active at 10 μg/ml in assays for agonistic activity on ER (estrogen receptor) α and ER β using human embryonal kidney cells transfected with either ER α or ER β, and both with the luciferase reporter gene (Kuiper et al., 1998). The activity of cedrecoumarin A on ER β was more pronounced (60% of the maximum stimulation by 10^{-11} M 17-β-estradiol) in comparison with ER α (40%). However,

Table 1
^{1}H and ^{13}C NMR data of cedrecoumarin A **3** and cedrecoumarin B **4**

Pos.	**3**, ^{1}H[a]	**3**, ^{13}C[b]	**3**, HMBC correlations	**3**, NOESY interactions	**4**, ^{1}H[c]	**4**, ^{13}C[d]	**4**, NOE interactions
2		162.50	H-3,4			163.5	
3	6.19, *d* (9.7)	111.40		H-4	6.31, *d* (9.5)	113.1	H-4
4	8.08, *d* (9.7)	140.28		H-3,1′	8.00, *d* (9.5)	143.3	H-3, 2H-1′
4a		107.31	H-1′ 3,4,8			112.2	
5		117.64	H-4,1′,2′			133.9	
6		137.84	H-1′,2′,8			154.0	
7		149.99	H-8			158.1	H-8
8	6.63, *s*	102.63	H-4		6.94, *s*	99.7	7-OCH_3
8a		150.43	H-4,8			145.3	
6-OCH_3		–			3.81, *s*	61.4	2H-1′
7-OCH_3		–			3.98, *s*	56.7	
1′	6.79, *d* (10.0)	116.72	H-4,2′	H-4,2′	3.65, *d* (6.5)	25.4	H-4,2′,3H-5′, 6-OCH_3
2′	5.90, *d* (10.0)	133.49	H-1′,3H-4′,3H-5′	H-1′, 3H-4′, 3H-5′	5.10, *m*	123.6	2H-1′, 3H-4′
3′		76.49				133.7	
4′	1.43, *s*	26.25	H-1′,2′	H-1′,2′	1.73, *s*	25.9	H-2′
5′	1.43, *s*	26.25	H-1′,2′	H-1′,2′	1.87, *s*	18.2	2H-1′

[a] 300 MHz, CD_3OD.
[b] 100 MHz, CD_3OD.
[c] 300MHz, CD_3OD, Sample decomposed—no HMBC/NOESY spectra obtained.
[d] 75MHz, CD_3OD.

activity was low compared to the standard genistein which is active in the same ER α and β assays at 30 ng/ml (80 and 160%, respectively). Cedrecoumarin A was found to inhibit luminol-enhanced chemiluminescence of reactive oxygen metabolites generated by human polymorphonuclear leukocytes activated with opsonized zymosan (IC_{50} 3.2 μg/ml) and to scavenge superoxide anions in a cell free system (IC_{50} 3.0 μg/ml) suggesting anti-inflammatory activity for this compound (Smit et al., 2000; Van den Worm, 2001).

3. Experimental

3.1. General

IR spectra were recorded with a Nicolet Impact 400 D spectrometer on sodium chloride plates and calibrated against an air background. EIMS were obtained using a Finnigan 1020 spectrophotometer operating at 70 eV. HRMS were obtained using a Kratos High Resolution MS 9/50 spectrometer. UV spectra were recorded with a Varian DMS 300 UV–visible spectrophotometer using dichloromethane as solvent. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer in CD_3OD. HMBC and NOESY spectra for **3** were recorded on a Varian Unity Inova 400 MHz spectrometer.

3.2. Extraction and isolation

The stem bark of *C. grevei* was collected in Madagascar by Dr. M. Randrianarivelojosia and a voucher specimen (No.: 002-Mj/M.Dul) was retained at the University of Antananarivo in Madagascar.

A sample of the dried powdered stem-bark (1051.7 g) was extracted with hexane using a Soxhlet apparatus, to yield 35.8 g of extract. A 7.5 g sample of the hexane extract was examined and after repeated column chromatography over silica gel (Merck 9385), using dichoromethane/ethyl acetate in varying proportions, yielded β-amyrin (105 mg) (Razdan et al., 1987), the coumarins cedrelopsin (5 mg) (Eshiett and Taylor, 1968), scoparone (13 mg) (Razdan et al., 1987), *O*-methylcedrelopsin (10 mg) (Kokwaro et al.,1983), norbraylin (3 mg) (Deshmukh et al., 1976) and two oxepin ring chromones, ptaeroglycol (26 mg) (Dean et al., 1967b; Dean and Robinson, 1971) and ptaeroxylinol (31 mg) (Dean et al., 1967b) which were identified by comparison of their physical and spectroscopic data against literature values as referenced above. The novel coumarins cedrecoumarin A **3** and cedrecoumarin B **4** were also isolated.

3.2.1. Cedrecoumarin A **3**

9-Hydroxy-7,7-dimethyl-2H,7H-benzo[1,2-b:3,4-b]dipyran-2-one (13 mg), yellow gum, HRMS *m*/*z* 244.0731 ($C_{14}H_{12}O_4$ requires 244.0735) EIMS *m*/*z* (rel. int.) 244 (26), 229 (100), 201 (10), 100 (1); IR ν_{max} (cm^{-1}): 3370, 1710; UV λ_{max} nm (log ε): 316 (2.6), 275 (1.8), 232 (4.1); ^{1}H and ^{13}C NMR data are given in Table 1.

3.2.2. Cedrecoumarin B **4**

6,7-Dimethoxy-5-(3-methyl-2-butenyl)-2H-1-benzopyran-2-one, 6,7-dimethoxy-5-prenylcoumarin (12 mg), yellow gum, HRMS *m*/*z* 274.1191 ($C_{16}H_{18}O_4$ requires 274.1205) EIMS *m*/*z* (rel. int.) 274 (90), 244 (8), 233

(17), 205 (12), 167 (145), 149 (100); IR ν_{max} (cm^{-1}) : 3010, 1730, 1605; UV λ_{max} nm (log ε): 335 (1.9), 296 (1.9), 259, 231 (4.1); ^{1}H and ^{13}C NMR data are given in Table 1.

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