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Limonoid Derivatives from *Cedrelopsis grevei*

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Abstract: The stem bark of *Cedrelopsis grevei* has yielded β-amyrin and two novel limonoid derivatives, the pentanortriterpenoid, cedmilinol, and the hexanortriterpenoid, cedmiline. Structures were elucidated using NMR analysis and the "Logic for Structure Determination" program.

INTRODUCTION

Cedrelopsis grevei Bart., is a Madagascan medicinal plant, locally known as "Katrafay". The bark of this species is used to relieve muscle fatigue when placed in bath water. Pennington and Styles[1] grouped *Cedrelopsis* and *Ptaeroxylon* into the Ptaeroxylaceae family due to their similar morphology and the structure of the secondary xylem. The pollen of *Cedrelopsis* and *Ptaeroxylon* are very similar, unlike that of any Meliaceae pollen grain but similar to that of some Rutaceae. *Ptaeroxylon* had previously been placed in the Sapindaceae, the Rutaceae and, most popularly, in the Meliaceae.[1] The grouping together of *Cedrelopsis* and *Ptaeroxylon* was supported by chemical evidence, both had been found to contain a wide variety of coumarins and chromones, but no limonoids.[2,3,4,5,6] In the present work, the stem bark of *C. grevei* collected from the north-west of Madagascar was investigated and it yielded β-amyrin and two novel limonoid-derived compounds, cedmilinol **1** and cedmiline **2**. These results differed from those of a parallel study of the stem bark of the same species collected in the drier south of Madagascar. The major constituent of the stem bark from the southern specimen was again β-amyrin and a range of chromones and coumarins were also isolated as described earlier, [2,3,4,5,6] but no limonoids were found. The limonoid-derived compounds isolated in the present investigation are similar to those reported from the Cneoraceae, for example, cneorin K, 3.[7]

RESULTS AND DISCUSSION

HRMS of cedmilinol **1** showed a $[M]^+$ peak at *m/z* 440.1855 indicating a molecular formula $C_{25}H_{28}O_7$ (calcd: 440.1835). Peaks at *m/z* 422 $[M-18]^+$ and 404 $[M-36]^+$ showed the loss of one and two water molecules respectively respectively. The IR spectrum showed absorptions at 3450 cm^{-1} (OH stretch) and 1700 and 1750 cm^{-1} (C=O stretch). Resonances at δ6.39 (H-22), 7.42 (H-23) and 7.45 (H-21) indicated the presence of a limonoid β-substituted furanyl ring. However, the NMR spectra of cedmilinol **1** appeared rather different from those of known limonoids and the LSD (Logic for Structure Determination) Program[8] was used to confirm the structure. This program produces planar structures according to 2D NMR correlation data and sub-structural information provided by the user. The program indicated eighteen possible structures. The only one which accounted for the upfield quaternary carbon at δ29.3 (C-14) was structure **1** which enabled the incorporation of this carbon into a three-membered ring. None of the seventeen

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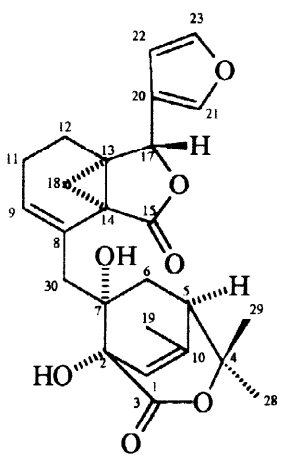
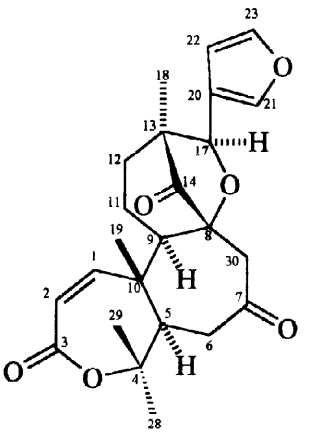
structures excluded contained a cyclopropane ring, making **1** the only acceptable solution to the problem. Using COSY, HMQC, and HMBC spectra all ^{1}H and ^{13}C NMR resonances could be assigned (Table 1). The stereochemistry at H-17 could not be determined from the ROESY spectrum. In their study on the tricoccins, Epi and Mondon[7] reported that the chemical shift of H-22 differs significantly depending on whether H-17 is α or β. H-22 occurs at δ6.91 in tricoccin R_9 which has H-17α and at δ6.45 in tricoccin R_{12} which has H-17β. Only marginal differences were found between H-17 in these two structures. In cedmilinol, H-22 occurred at δ6.39 indicating that H-17 was β. A NOE difference experiment was performed in order to confirm this. Irradiation of H-17 led to the enhancement of one of the H-12 protons but not the cyclopropane ring protons. If H-17 had been in the α–orientation, a positive NOE would have been obtained for one of the C-18 cyclopropane ring protons. H-5α occurs at δ2.18. A model showed that the hydroxy group at C-2 must therefore also be in the α-orientation. The ROESY spectrum indicated that the hydroxy groups at C-2 and C-7 were on the same side of the molecule however the stereochemistry of these hydroxy groups with respect to the chiral centres in the "northern" half of the molecule remains undetermined. Attempts at acetylation of the tertiary hydroxy groups (Ac_2O/py/DMAP) and attempts to form the acetonide using 2,2-dimethoxypropane were unsuccessful, possibly due to steric hindrance. The biosynthesis of cedmilinol **1** is thought to initially follow a path similar to that reported for the cneorins and tricoccins[7]. Contraction of ring D, the formation of the 13,14,18-cyclopropane ring, cleavage of the C-9, C-10 bond and incorporation of the C-30 methyl group into ring B has previously been described[7]. A suggested biosynthesis for cedmilinol starting with a typical limonoid, **4**, is given in scheme 1. Either ring D contraction or ring B enlargement could occur first.



1



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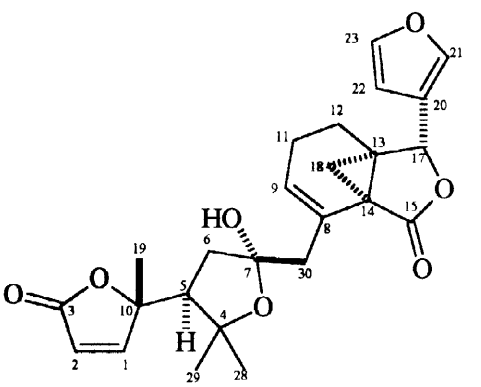
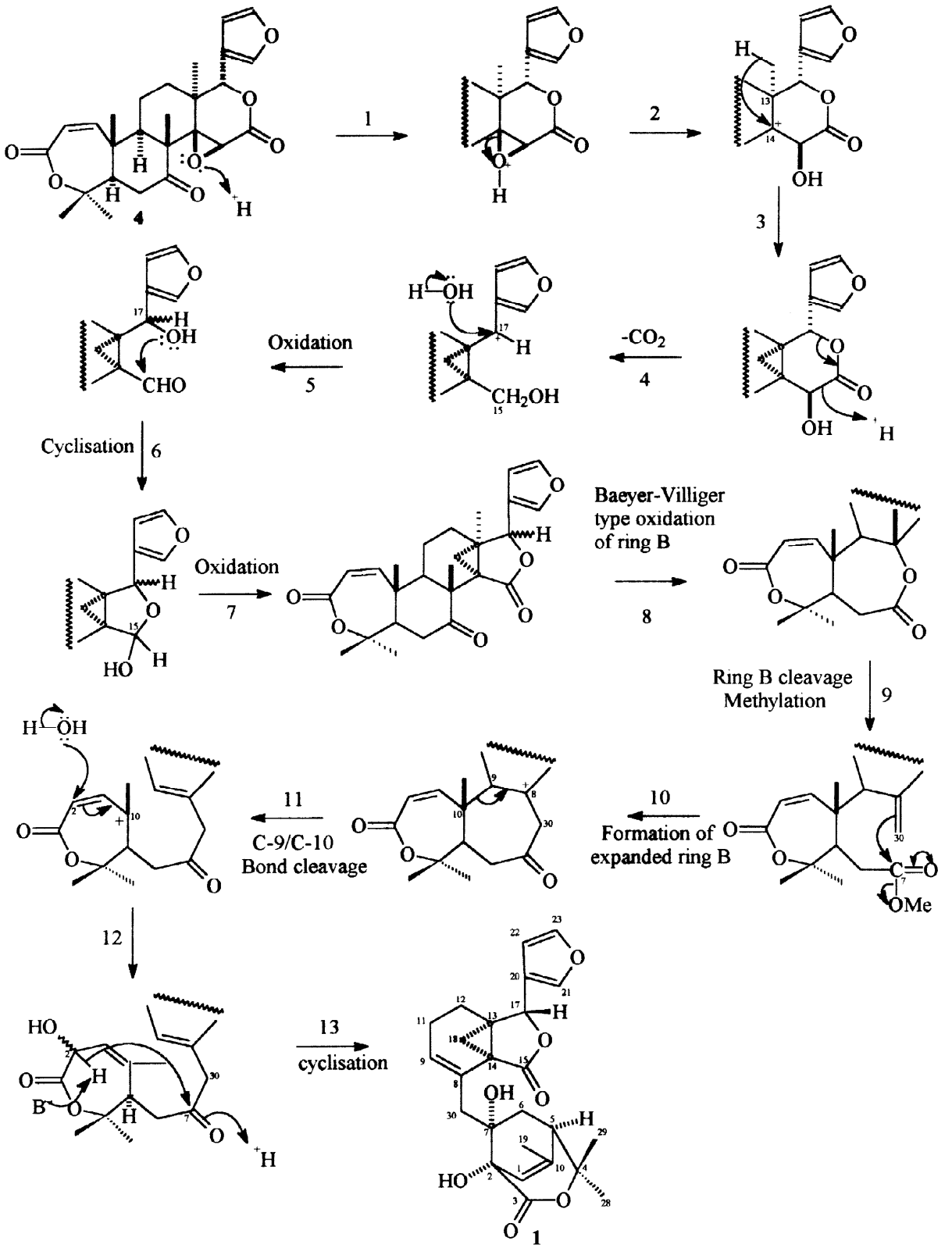


3

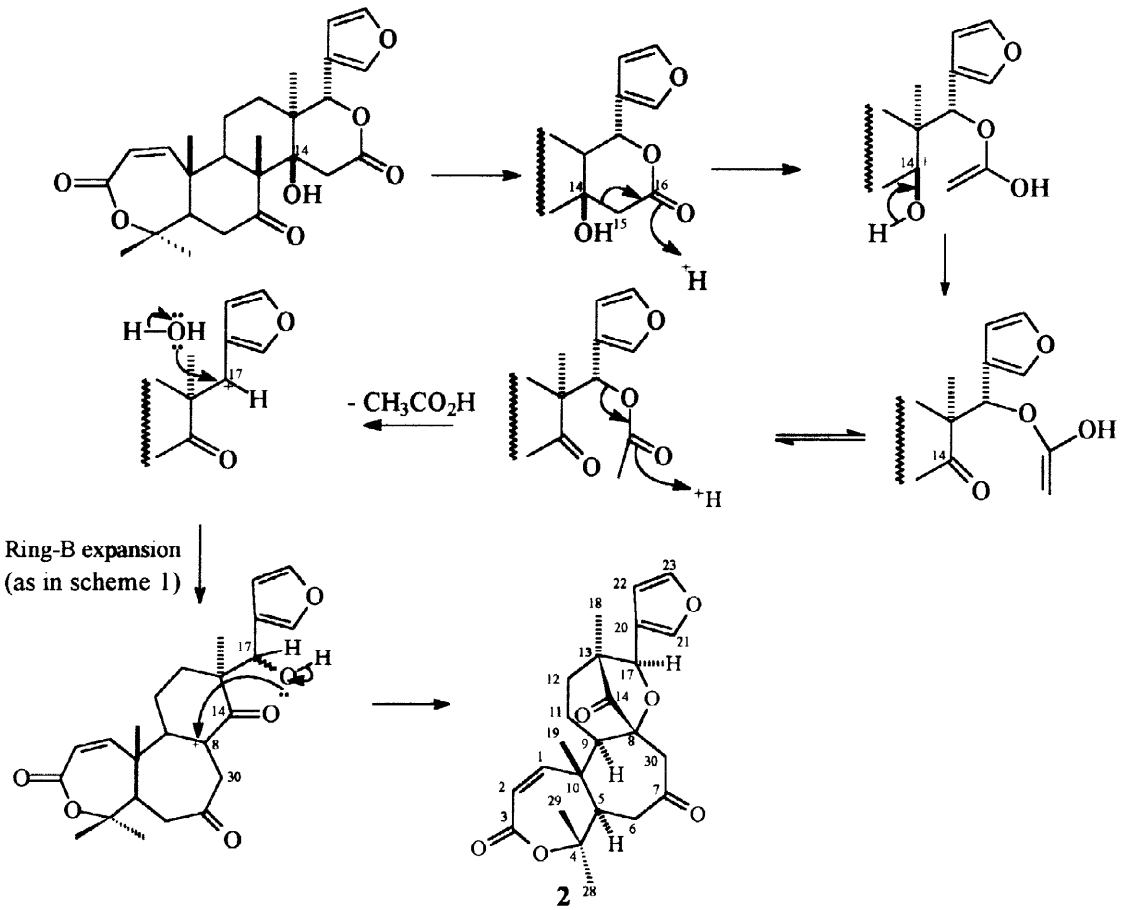
HRMS of cedmiline **2** gave a $[M]^+$ peak at *m/z* 412.1890 corresponding to a molecular formula $C_{24}H_{28}O_6$ (calcd: 412.1886). The IR spectrum showed absorptions at 1640 cm^{-1} (C=C stretch) and 1690 and 1740 cm^{-1} (C=O stretch). The ^{1}H NMR spectrum showed the typical furanyl ring protons at δ7.34 (H-23), 7.37 (H-21) and 6.04 (H-22). Using the LSD programme, only one possible structure was obtained giving structure **2** for cedmiline. Using COSY, HMQC, and HMBC spectra, all ^{1}H and ^{13}C NMR resonances could be assigned for **2** (Table 1). The large coupling constants for H-5 and H-6 were in agreement with an α-axial position for H-5. In the ROESY spectrum, the intense through-space interaction observed between H-5, H-9 and CH_3-28 suggested a α-position for H-9 and the methyl group. The intense ROE correlations between CH_3-28, and the H-6 resonance at δ2.64 implied an α-orientation for this proton. The last intense ROE interactions between CH_3-19, CH_3-29 and the second H-6 at δ2.46 were in

agreement with a β-postion all these protons. The stereochemistry of H-17 was determined using NOE experiments. Irradiation of H-17 led to a positive enhancement of H-9α and one of the H-12 protons. A model showed that this was



Scheme 1: The Proposed Biosynthesis of Cedmilinol **1**

only possible when H-17 was in the α-orientation. The suggested biosynthetic pathway for cedmiline **2** is given in Scheme 2. In this proposed scheme, acetic acid is lost from ring D. The 14-hydroxy, 16-lactone limonoids suggested as a precursor for **2** are well known.[9,10]



Scheme 2 : The Proposed Biosynthesis of Cedmiline **2**

MATERIALS AND METHODS

General procedures: Melting points were determined on a Kofler micro-hot stage apparatus and are uncorrected. Optical rotations were measured at room temperature using an Optical Activity AA-5 Polarimeter together with a series A2 stainless steel (200 mm) unjacketed flow tube. IR spectra were recorded with a Nicolet Impact 400 D spectrometer which was calibrated against an air background. ^{1}H, ^{13}C NMR, ^{1}H-^{1}H COSY, HMBC, HMQC and ROESY spectra were recorded with a Bruker DRX-500 spectrometer at Reims. NOE experiments were performed on a Varian Gemini-300 NMR spectrometer in Durban. Chemical shifts (δ) are expressed in ppm with reference to TMS. HRMS and EIMS were recorded at the Cape Technikon on Finnigan 1020 GCMS and Kratos 9/50 HRMS instuments.

Plant material: The stem bark of *C. grevei* Bart. was collected and identified by Dr M Randrianarivelojosia. The voucher specimen (001-Mj/M.Dul) was deposited in the herbarium of the Laboratory of Pharmocology, EES Sciences, at the University of Antananarivo, Madagascar.

Table 1 ^{1}H, ^{13}C and HMBC NMR data for cedmilinol **1** and ^{1}H, ^{13}C, HMBC and ROESY NMR data for cedmiline **2** ($CDCl_3$, 500 MHz, δ values given in ppm, *J* given in Hz in parenthesis)

	Cedmilinol 1				**Cedmiline 2**			
Pos.	δ_H	δ_C	HMBC correlations	Pos.	δ_H	δc	HMBC correlations	ROESY
1	5.58 d (1.8)	127.5	C-19, 5	1	6.17 d (13.2)	152.2	C-2, 3, 5, 9, 10, 19	
2		81.7		2	5.92 d (13.2)	119.5	C-1, 3, 9, 10	
3		171.5		3		166.1		
4		86.8		4		84.0		
5	2.18 d (5.6)	48.2	C-1, 6, 7, 10, 19	5α	2.97 dd (14.5, 4.2)	52.3	C-4, 6, 7, 9, 10, 29	H-9α, 28, 30α
6	1.81 m 2.02 m	32.2	C-2, 4, 5, 7, 10, 30	6α 6β	2.64 dd (4.2, 16.9) 2.46 dd (4.2, 16.9)	46.4	C- 4, 5, 7, 10, 30	H-28, 30α H-19, 28, 29
7		79.2		7		206.7		
8		130.4		8		81.5		
9	5.29 d (6.9)	124.9	C-11, 14, 30	9α	2.04 m	63.8	C-1, 10, 14, 19	
10		141.1		10		47.4		
11	1.97 m 2.25 m	21.0	C-8, 9	11α 11β	1.86 m 2.15 m	21.7	C-8, 9, 12, 13	H-19
12	1.72 ud (12.2, 6.1) 2.25 m	20.6	C-11, 13, 14, 17, 18	12	1.64 m 2.04 m	40.5	C-9, 11, 13, 14, 17, 18	H-18 H-18
13		36.0		13		51.4		
14		29.3		14		213.6		
15		176.6		17	5.09 s	78.8	C- 8, 12, 13, 14, 20, 21, 22	
17	5.34 s	78.5	C-12, 13, 15, 18, 20, 21, 22	18	0.87 s	14.8	C-11, 12, 13, 14, 17	H-12
18	1.46 m (2H)	22.8	C-8, 12, 13, 14, 15, 17	19	1.23 s	19.8	C-1, 5, 9, 10	H-6β, 11β, 29
19	1.86 d (1.9)	23.3	C-1, 5, 10	20		124.9		
20		121.5		21	7.37 brs	140.4	C-20, 22, 23	
21	7.45 brs	139.9	C-20, 22, 23	22	6.04 brs	108.3	C-20, 21, 23	
22	6.39 brs	108.5	C-20, 21, 23	23	7.34 brs	144.1	C-20, 21, 22	
23	7.42 brs	143.9	C-20, 21	28	1.43 s	32.3	C-3, 4, 5, 29	H-5α, 6α, 6β
28	1.41 s*	28.5*	C-4, 5, 29	29	1.51 s	20.7	C-4, 5, 28	H-6β, 19
29	1.49 s*	27.7*	C-4, 5, 28	30α 30β	3.43 d (12.6) 2.37 d (12.6)	45.9	C-6, 7, 8, 9, 14	H-5α, 6α
30	1.81 d (14.3) 3.64 d (14.3)	42.1	C-6, 7, 8, 9, 14					
C_2-OH	4.55 s		C-1, 2, 3, 7					
C_7-OH	4.79 s		C-6, 7, 30					

*Assigments may be interchanged

Extraction and isolation: Powdered and dried stem bark of *C.grevei* (176 g) was extracted using a Soxhlet apparatus with refluxing hexane. The hexane extract was concentrated and after repeated column chromatography over silica gel (Merck 9385) with hexane/dichloromethane/ethyl acetate as solvent furnished three compounds, β-amyrin (613 mg) whose structure was confirmed by comparison against literature data,[11] and the novel compounds cedmilinol **1** (736 mg) and cedmiline **2** (490 mg).

Cedmilinol (1): yellow oil; $[\alpha]_D$ +3.3 ($CHCl_3$; *c* 0.23); IR ν_{max} (NaCl) cm^{-1} 3450 (br), 1750, 1700; EIMS ***m/z*** 440 (M^+, 24), 422 (14), 404 (2), 273 (30), 43 (100); HRMS 440.1855 $[M]^+$; $C_{25}H_{28}O_7$ requires 440.1835. ^1H and ^{13}C NMR data are given in Table 1.

Cedmiline (2): recrystalised from ethyl acetate as colourless plates; $[\alpha]_D$ +17.1 ($CHCl_3$; *c* 0.146); mp 297-298°C; IR ν_{max} (NaCl) cm^{-1} 1740, 1690, 1640; EIMS ***m/z*** 412 (M^+, 5), 274 (11), 256 (32), 205 (40), 83 (100), 43 (89); HRMS 412.1890 $[M]^+$; $C_{24}H_{28}O_6$ requires 412.1886. ^1H and ^{13}C NMR data are given in Table 1.

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