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Lunamarin C, a new terpenoid coumarin from *Clausena heptaphylla*

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The petroleum ether extract of the leaves of *Clausena heptaphylla* afforded a new coumarin, lunamarin C (**1**). Its structure was determined by extensive analysis of spectral data, including 2D NMR and by comparison with structurally related compounds, lunamarins A (**2**) and B (**3**).

1. Introduction

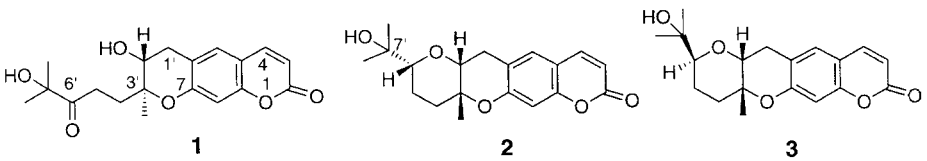
Clausena heptaphylla Wight & Arn., (Bengali name – Karanphul) is a shrub or small tree found in south and southeast Asia. Although, plants belonging to this genus have been extensively studied before very little attention has been given to *C. heptaphylla.* Previous investigations of *C. heptaphylla* have revealed a number of carbazole alkaloids [1–4], coumarins [5], monoterpenes [6–8], 2-methylanthraquinone [9], and a limonoid [10]. As part of our continuing investigations of rutaceous species of Bangladesh, we studied a methanolic extract *C. heptaphylla* and reported the isolation of coumarins, lunamarins A (**2**), B (**3**) [11] and clausmarin A [12]. This paper describes the isolation and structure determination of another new terpenoid coumarin, lunamarin C (**1**) from a petroleum ether extract of the same species.

2. Investigations, results and discussion

The air dried and powdered leaves of *C. heptaphylla* were extracted with petroleum ether (b.p. 40–60 °C) in a Soxhlet apparatus. Repeated preparative TLC of the concentrated crude extract over Si gel PF_{254} yielded a new coumarin, lunamarin C (**1**). The identity of **1** was established by extensive spectroscopic studies, including 2D NMR techniques. Lunamarin C (**1**) showed pseudomolecular ion peaks at m/z 347 $[M + H]^+$ (CI-MS) and 329 $[M–H_2O + H]^+$ (FAB-MS), which suggested a molecular formula $C_{19}H_{22}O_6$ with 16 atomic mass units more than the isomeric coumarins, lunamarins A and B (**3**) [11]. The UV spectrum of **1** was indicative of a 7-oxygenated coumarin [13] and the IR spectrum displayed bands corresponding to hydroxyl, carbonyl and aromatic functionalities. The ^1H and ^{13}C NMR spectral data of lunamarin C (Table) were in close correspondence to those of lunamarin A (**2**) and B (**3**) [11] suggesting a close structural similarity. However, the signals due to H-6′, and C-6′ observed in the NMR spectra of compounds **2** and **3** were absent from those recorded for **1**. In addition the C-4′ and C-5′ methylene protons, especially the latter ones were more deshielded than the corresponding protons in lunamarins A (**2**) and B (**3**) [11]. The lack of couplings from the C-5′ methylene protons to any protons other than to H_2-4′ in the COSY and TOCSY spectra suggested the absence of a protonated carbon next to C-5′. HMBC correlations from the C-7′ methyl protons at δ 1.17 and 1.19 to δ_C 211.4 confirmed the presence of a carbonyl group at C-6′, which was also supported by HMBC correlations from H_2-4′ to δ_C 211.4. Therefore, lunamarin C (**1**) was a tricyclic coumarin as compared to the tetracycllic nature of lunamarins A (**2**) and B (**3**). The relative stereochemistries at C-2′ and C-3′ in lunamarin C (**1**) were determined by selective 1D NOESY experiments. Irradiation at the resonance frequency of H-2′ caused significant enhancement of H_β-1′ (δ 2.64), while irradiation of the C-3′ methyl signal produced strong enhancement of H_α-1′ (δ 2.93), indicating that unlike lunamarins A (**2**) and B (**3**), the C-3′ methyl group and H-2′ in lunamarin C (**1**) are on the opposite side of the plane. On the basis of the above spectral data structure **1** was assigned to lunamarin C. Although monoterpenoid coumarins have previously been isolated from *C. heptaphylla* [11, 12], *C. pentaphylla* [14] and *C. excavata* [15], this is the first report of occurrence of any coumarin containing a carbonyl group at C-6′.

Table: NMR spectral assignments for lunamarin C (1) in C_6D_6

C/H	^{13}C	^1H mult *J* (Hz)	HMBC
2	160.2		
3	112.9	5.88 d (8.5)	C-2, C-10
4	142.6	6.62 d (8.5)	C-2, C-5, C-9
5	123.2	6.37 s	C-4, C-7, C-9
6	124.0		
7	163.2		
8	98.0	6.53 s	C-6, C-7, C-9, C-10
9	156.4		
10	112.7		
1′	29.2	H_α 2.93 dd (16.5, 7.0)	C-6, C-7, C-3′, C-4′
	29.2	H_β 2.64 dd (16.5, 10.0)	C-6, C-2′, C-3′
2′	87.6	4.35 dd (10.0, 7.0)	C-4′, 3′-Me
3′	74.4		
3′-Me	20.4	0.85 s	C-2′, C-3′, C-4′
4′	29.9	1.84 m	C-2′, C-5′, C-6′
		1.52 m	C-2′, C-3′, C-5′, C-6′
5′	32.0	2.16 m	
		2.08 m	C-3′, C-4′, C-6′
6′	211.4		
7′	80.0		
7′-Me	27.4	1.19 s	C-6′, C-7′, 7′-Me
7′-Me	27.4	1.17 s	C-6′, C-7′, 7′-Me



1 **2** **3**

3. Experimental

3.1. General

The ^{1}H- (500 MHz) and ^{13}C-(125 MHz)NMR spectra were recorded in C_6D_6 on a Varian VXR 500S spectrometer and the chemical shifts are reported in ppm relative to the residual non deuterated solvent signals. Inverse detected heteronuclear correlations were measured using HSQC (optimized for $^1J_{CH} = 140$ Hz) and HMBC (optimized for $^nJ_{CH} = 8.3$ Hz) pulse sequences. TOCSY and COSY-45 spectra were used to determine the proton-proton connectivities. UV and IR spectra were obtained on a Beckman DU-640 and Perkin-Elmer 1600 FTIR spectrometer, respectively. Optical rotation was measured with a Perkin-Elmer 241 polarimeter. MS and accurate mass measurements were performed on a JEOL SX102 mass spectrometer.

3.2. Plant material

The leaves of *C. heptaphylla* were collected from a forest in Cox's Bazar, Bangladesh in August 1997 and were identified by Mr. Md. Yusuf, Senior Scientific Officer, Bangladesh Council of Scientific & Industrial Research (BCSIR), Chittagong. A voucher specimen for this collection is maintained at the Herbarium of the Department of Botany, University of Dhaka under the accession number, Rutaceae-07.

3.3. Extraction and isolation

The powdered leaves (910 g) were extracted with petroleum ether (b.p. 40–60 °C) in a Soxhlet apparatus. An aliquot of the the concentrated crude extract was subjected to preparative TLC over Si gel PF_{254} using toluene-EtOAc-AcOH (70 : 30 : 1) to provide an impure sample of lunamarin C (**1**). Repeated purification of the slightly impure coumarin using toluene-EtOAc-AcOH (90 : 10 : 1) yielded **1** (2.0 mg).
Lunamarin C (**1**): Colorless gum; $[\alpha]_D$ −2.0° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 335, 297, 259, 249, 222 nm; IR (film) υ_{max} 3444, 1731, 1715, 1627, 1568, 1396, 1266, 1123, 1016, 960, 823, 720 cm^{-1}; CIMS m/z 347 $[M + H]^+$, 329 $[M + H]^+$; HR-FABMS *m/z* 329.1391 (calcd for $C_{19}H_{23}O_6$ $[M + H]^+$, 329.1389); ^{1}H- and ^{13}C NMR data (Table).

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