

Triterpene constituents from the leaves of *Melicope indica*

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Phytochemical investigation of a petroleum ether extract of *Melicope indica* afforded two unusual pentacyclic triterpenes viz. neohop-13(18)-en-3 α -ol (**1**) and fern-8(9)-en-3 β -ol (**2**) and the ubiquitous steroids, stigmasterol and sitosterol. The structures of **1** and **2** were independently elucidated on the basis of 2D NMR data and confirmed by comparison with those of related compounds. While compound **1** is a new natural product, this is the first report of occurrence of fern-8(9)-en-3 β -ol (**2**) from the genus *Melicope*.

1. Introduction

Melicope indica (Forst. f.) Wight is a shrubby Rutaceous plant with slender branches, which is endemic of south Indian hills, Nilgiri Mountains and woods near the Avalanches [1]. This is the only species belonging to the genus *Melicope* that is known to peninsular India and has apparently not been used in traditional medicine. Three alkaloids and three flavonoids [2], two of which exhibiting antiviral activity [3] have been reported from this plant. In search for the biologically active constituents of *M. indica* we have isolated and characterized two biogenetically interesting triterpenoids (**1**, **2**) along with mixtures of sitosterol, stigmasterol and fatty materials. Although compound **1** has been previously obtained in laboratory by two or three-step transformations of naturally occurring triterpenes [4, 5], this has not been obtained from a natural source and its high-resolution ^1H - and ^{13}C NMR spectral data are presented here for the first time.

2. Investigations, results and discussion

This paper deals with the isolation and structure elucidation of two pentacyclic triterpenes characterized as neohop-13(18)-en-3 α -ol (**1**) and fern-8(9)-en-3 β -ol (**2**) from the leaves of *M. indica*. The FABMS of both **1** and **2** displayed the $[\text{M} + \text{H}]^+$ ions at m/z 427 and ^{13}C NMR spectra showed 30 carbon resonances, which suggested the molecular formula of $\text{C}_{30}\text{H}_{50}\text{O}$. In the FABMS of **1**, a fragment at m/z 409 $[\text{M} + \text{H} - \text{H}_2\text{O}]^+$ corresponding to the loss of water was suggestive of the presence of a hydroxyl group. Two intense peaks, one at m/z 206 $[\text{M} + \text{H} - 221]^+$ and another at m/z 190 $[\text{M} + \text{H} - 237]^+$, which are common in most of the pentacyclic triterpenoids, indicated that compound **1** belonged to the hopane or lupane group [6]. The ^1H NMR spectrum of **1** (Table) showed a complex pattern of six tertiary methyls at δ 0.81, 0.84, 0.86, 0.88, 0.96 and 1.12 (3H each, s) and two secondary methyls at δ 0.95 and 0.91 (3H, each, d, $J = 6.5$ Hz) and a 1H multiplet at δ 3.41 ($W_{1/2} = 6$ Hz) characteristic of an oxymethine proton at C-3, whereas no vinylic proton was observed. The ^1H and ^{13}C NMR spectral data of **1** were

almost identical to those of neohop-13(18)-en-3 β -ol (**3**) [7] (Table) suggesting a very close structural similarity between these two compounds. However, the small half width of the oxymethine proton signal required it to have an equatorial (β) configuration and thus revealed the hydroxyl function in **1** to be axial (α) [7–9]. The small differences observed in the ^{13}C signals of C-1 to C-5 and the methyl groups attached to C-4, between compound **1** and neohop-13(18)-en-3 β -ol (**3**) and a close resemblance of these ^{13}C NMR resonances between the former one and arborinol (**4**) [7] were in support of the proposed structure. Therefore, compound **1** was an epimer of neohop-13(18)-en-3 β -ol (**3**). The 2D NMR spectral data of **1** obtained by COSY-45, HSQC and HMBC experiments were in agreement with the structure. Thus, compound **1** was identified as neohop-13(18)-en-3 α -ol. Although **1** has previously been obtained by strong acidic isomerization and subsequent hydrolysis of hop-17(21)-en-3 α -yl acetate [5] and also by acetylation, dehydration and subsequent hydrolysis of isolangidiol [4], this is the first report of its isolation as a natural product.

The structure of fern-8(9)-en-3 β -ol (isomotioli) (**2**) was independently solved by 2D NMR studies and confirmed by comparison with previously reported values [10]. Compound **2** represents the second report of its isolation from the family Rutaceae as it was previously isolated from *Evodia hortensis* (Rutaceae) [11] and also found twice

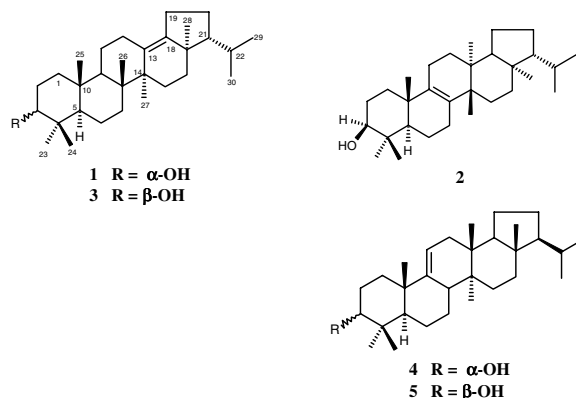


Table: NMR assignments for neohop-13(18)-en-3 α -ol (1) in CDCl₃ (500 MHz for ¹H-NMR and 125 MHz for ¹³C-NMR), its C-3 epimer (3) and allied arborinol (4) [7]

C/H	1			3		4	
	¹³ C	¹ H mult J (Hz)	HMBC*	¹³ C	¹ H mult J (Hz)	¹³ C	¹ H mult J (Hz)
1	33.9 (t)	1.29, 1.46		39.0		30.4	
2	25.7 (t)	1.93, 1.54		27.4	1.56, 1.64	25.7	
3	76.5 (d)	3.41, <i>m</i>	C-1, C-5	78.9		76.3	3.43, dd, 2.8, 2.8
4	37.8 (s)	—		38.9		37.8	
5	49.6 (d)	1.25	C-4, C-7, C-9, C-23, C-24	55.7	0.73	46.6	
6	18.7 (t)	1.43		18.5		21.4	1.43, 1.58
7	34.6 (t)	1.46, 1.60		34.5		26.6	1.24, 1.82
8	41.7 (s)**	—		41.4**		41.0	
9	52.3 (d)	1.48	C-8, C-10, C-11	52.2	1.34	148.8	
10	37.8 (s)	—		37.5		39.6	
11	21.8 (t)	1.22, 1.51		21.7		114.1	5.26, ddd, 6.1, 1.8, 1.8
12	26.9 (t)	2.29, 1.90	C-13, C-18	26.7	2.31, 1.89	36.1	
13	131.8 (s)	—		131.5		36.8	
14	42.6 (s)**	—		42.1**		38.3	
15	29.5 (t)	1.85, 1.27	C-13	29.4		29.6	
16	38.2 (t)	1.27, 1.78	C-18	37.9		35.9	
17	42.9 (s)	—		42.7		42.9	
18	141.4 (s)	—		141.8		52.1	
19	26.7 (t)	2.29, 2.23	C-13, C-18	26.5	2.27, 2.20	20.2	1.23, 1.37
20	27.8 (t)	1.83, 1.34	C-18	27.6	1.84, 1.35	28.2	1.23, 1.85
21	59.4 (d)	1.05	C-17, C-20, C-28, C-29, C-30	59.2		59.6	
22	30.0 (d)	1.56		29.8		30.8	
23	28.4 (q)	0.96	C-3, C-4, C-5, C-24	27.9	0.98	28.3	0.96
24	22.4 (q)	0.84	C-3, C-4, C-5, C-23	15.4	0.76	22.5	0.88
25	16.8 (q)	0.86	C-1, C-5, C-9, C-10	16.8	0.83	21.9	1.05
26	18.9 (q)	0.88	C-7, C-8, C-9	18.6	0.86	17.1	0.82
27	27.0 (q)	1.12	C-13, C-14, C-15	26.7	1.10	15.3	0.77
28	18.1 (q)	0.81	C-16, C-17, C-18, C-21	17.9	0.80	14.0	0.76
29	23.1 (q)	0.95, d, 6.5	C-21, C-22, C-30	23.0	0.94, d, 6.7	22.1	0.89, d, 6.4
30	23.3 (q)	0.91, d, 6.5	C-21, C-22, C-29	23.1	0.90, d, 6.7	23.0	0.83, d, 6.4

* Key correlations

** Assignments in a vertical column may be interchanged

from *Strychnos potatorum* (Loganiaceae) [12], and *Euphorbia supina* (Euphorbiaceae) [13]. Two triterpenoids of arborinane-type, arborinol (4) and isoarborinol (5) have also been reported from another Rutaceous species, *Glycosmis arborea* [14–16]. Thus, the limited distribution of hopane or fernane triterpenes in this family demonstrates a chemotaxonomic relationship among the genera *Melicope*, *Evodia* and *Glycosmis*.

3. Experimental

3.1. General

The ¹H NMR spectra were obtained in CDCl₃ on a Varian VXR-500S instrument operating at 500 MHz, while the ¹³C NMR spectra were obtained on the same instrument at 125 MHz. Inverse-detected heteronuclear correlations were measured using the HSQC (optimised for ¹J_{CH} = 140 Hz) and HMBC (optimised for ¹J_{CH} = 8.3 Hz) pulse sequences with a pulsed-field gradient. The chemical shifts (δ) and coupling constants (J) are expressed in parts per million and hertz, respectively. The MS were recorded on a JEOL SX 102 mass spectrometer (resolving power = 10,000) using *m*-nitrobenzyl alcohol (NBA) or polyethylene glycol as matrix.

3.2. Plant material

Leaves of *M. indica* were collected from Baldha Garden, Dhaka in June 2000. The plant was identified by Professor M. Salar Khan, Senior consultant, Bangladesh National Herbarium (BNH), where a voucher specimen has been deposited under the accession number DACB-12653.

3.3. Extraction and isolation

The air-dried plant material was ground to a coarse powder and 115.0 g was extracted in a Soxhlet apparatus using 2.5 L of petroleum ether (60–80 °C). The extract was filtered and then evaporated under reduced pres-

sure at 40 °C using a Büchi rotary evaporator to have 3.0 g of the gummy concentrate. A portion of the petroleum ether extract (2.0 g) was chromatographed over Kieselgel 60 (70–230 mesh), and the column was eluted with petroleum ether-EtOAc, EtOAc, and EtOAc-MeOH mixtures of increasing polarity, with a total of 105 fractions collected (each 30 ml). Evaporation of the solvents from column-fractions 21 to 27, followed by repeated washings of the crystalline deposits with *n*-hexane and finally with petroleum ether-CHCl₃ mixtures gave 11.8 mg of 1, while similar treatments of fractions 29 to 33 yielded 14.3 mg of 2.

Neohop-13(18)-en-3 α -ol (1): Colourless needles; FABMS: *m/z* 427 [M + H]⁺ (appropriate for C₃₀H₅₀O + H⁺); ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) see Table.

Fern-8(9)-en-3 β -ol (2): Colourless needles; FABMS: *m/z* 427 [M + H]⁺ (appropriate for C₃₀H₅₀O + H⁺); ¹H NMR and ¹³C NMR data were identical to literature values [8].

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