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A NEW DITERPENE OF *LEONOTIS NEPETAEFOLIA*

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Abstract - A new labdane diterpenoid, leonotinic acid (**1**), possessing an α,β-butenolide unit, was isolated from the aerial parts of *Leonotis nepetaefolia*. It was identified on the basis of 1D and 2D NMR including ^{1}H-^{1}H COSY, HSQC, HMBC and T-ROESY spectroscopic techniques.

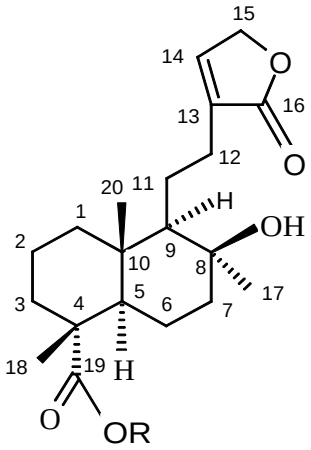
Leonotis nepetaefolia Aiton f. is a member of the Lamiaceae family and there are approximately fifteen known tropical species of this genus. The plant is native to tropical Africa and rather common; in Barbados it is considered a weed, where it is especially abundant on abandoned agricultural land.[1] The plant is commonly referred to as ball bush/head, lion head/tail, button weed, rabbit food, man piaba, honeysuckle and Lord Lavington, to name a few.[1,2] A tea made from its leaves is used to treat coughs, fever, stomach ache and skin ailments. It is also used in ethnomedicine to treat kidney disease, rheumatism, dysmenorrhea and worms. In India the ash of the inflorescence is used to treat burns and antibacterial activity of the MeOH and EtOAc extracts of the plant against *Pseudomonas aeruginosa* was reported.[3,4] A number of diterpenes have been reported from *L. nepetaefolia*, in addition to iridoid and phenylethanoid glycosides.[5-14] In continuation of our investigation of medicinal plants of Barbados, the aerial parts of *L. nepetaefolia* was investigated. This led to the isolation of a new labdane diterpene, designated leonotinic acid (**1**).

Compound (**1**) was obtained as crystalline needles and had the molecular formula $C_{20}H_{30}O_5$, as determined by HREIMS. The IR spectrum had bands at 3395 and 1744 cm^{-1} indicative of hydroxy and α,β-unsaturated (γ-lactone moieties respectively. Another IR band at 2517 cm^{-1} indicated the presence of a carboxylic acid functionality, which was confirmed by the ready conversion of **1** to the methyl ester (**2**).

The ^{1}H NMR spectrum of compound (**1**) (Table 1), had resonances due to three tertiary methyl groups at δ 1.03, 1.20 and 1.46, and these had direct connectivity to carbons at δ 22.1, 28.9, and 31.3, on the basis of an HSQC experiment. The oxymethylene group of the (γ-lactone had a broad singlet at δ 4.80, and this had HSQC connectivity to the oxygenated carbon at δ 70.6. Also, the olefinic hydrogen at δ 7.23 (1H, br s, H-14) was

directly attached to the carbon at δ 144.8, and was part of the α-substituted γ-lactone unit. The ^{1}H-^{1}H COSY spectrum showed cross-peaks between the olefinic proton at δ 7.23 and the oxymethylene protons at δ 4.80 (H_2-15) and those at δ 2.29 (H_2-12).

The HMBC spectrum (Table 1), displayed long range correlations between the C-17 methyl group and C-7, C-8, and C-9, while the C-18 methyl group had correlations to the C-19 carboxyl group, in addition to C-3, C-4, and C-5. In the T-ROESY spectrum, cross peaks were observed between the C-18 and C-20 methyl



	R
1	H
2	Me

groups, and this indicated that they were on the same side of the molecule and that they were β-oriented. On the other hand, T-ROESY cross peaks were observed between the C-17 methyl group and the methine protons at δ 1.26 (dd, *J* = 11.8, 4.1 Hz, H-5) and δ 1.09 (m, H-9), which indicated that they were all α-oriented. This information led to the assignment of compound (**1**) as 15,16-epoxy-8β-hydroxy-16-ketolabd-13-en-19-oic acid, for which the trivial name leonotinic acid is suggested.

EXPERIMENTAL

Melting points were determined using a Fisher/Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter with Na 589 line in MeOH. IR spectra were obtained on a Nexus 870 FT-IR spectrophotometer. A Hewlett-Packard 8452A Diode Array spectrophotometer was used to obtain UV spectra in methanol solutions. MS data were recorded on a micromass 70-250S spectrometer at an ionising voltage of 70 eV. NMR spectra were acquired on a Bruker Avance DRX 400 MHz spectrometer in $CDCl_3$ or $CDCl_3$ and two drops of CD_3OD as solvent, using TMS

Table 1. ^{13}C, ^{1}H NMR and HMBC Spectral Data for Leonotinic acid (**1**).

C	δ_C	δ_H (*J* in Hz)	**HMBC**
1	36.6	1.68 m	H-3, H-5
		1.18 m	H-3, H-5
2	19.3	<1.99>[a] m	
3	38.2	2.13 br d (14.5)	H-1, H-5, H-18
		1.00 dd (14.5, 4.4)	
4	43.7		H-3, H-5, H-18
5	48.3	1.26 dd (11.8, 4.1)	H-3, H-18, H-20
6	22.0	1.90 m	H-7
		1.82 m	H-7
7	37.1	1.57 m	H-6, H-17
		1.48 m	H-6, H-17
8	73.2		H-6, H-7, H-17
9	59.9	1.09 m	H-7, H-11, H-17, H-20
10	39.1		H-5, H-6, H-11, H-20
11	24.8	2.02 dt (11.5, 3.0)	
		1.61 m	
12	28.5	<2.29>[a] t (7.4)	H-11, H-14
13	134.7		H-11, H-12, H-14, H-15
14	144.8	7.23 br s	H-12, H-15
15	70.6	<4.80>[a] s	H-14
16	175.5		H-12, H-14, H-15
17	31.3	1.46 s	H-7, H-9
18	28.9	1.20 s	H-3
19	180.5		H-3, H-5, H-18
20	22.1	1.03 s	H-5

[a] Average value for an incompletely resolved CH_2 group.

as internal standard. Column chromatography was performed using Merck silica gel (grade 9385,230-400 mesh, 60 Å). Analytical TLC was done using Aldrich aluminium backed plates coated with silica gel with 254 nm fluorescent indicator.

Plant Materials -- *Leonotis nepetaefolia* was collected from Codrington Hill, St. Michael, Barbados in October 2001. The plant material was identified by Prof. Sean Carrington, Department of Biological and Chemical Sciences, University of the West Indies, Cave Hill Campus. A voucher specimen has been deposited in the National Herbarium (BAR) located on this Campus.

Extraction and Isolation – The dried, aerial parts of *Leonotis nepetaefolia* (1.87 kg) was macerated in MeOH (14.5 L) and left to stand for two days. This procedure was repeated once. The combined methanolic extracts were evaporated *in vacuo* and then resuspended in a 2:1 MeOH-H_2O and extracted with CH_2Cl_2 (2×250 mL). The crude CH_2Cl_2 extract (15.3 g) was subjected to flash column chromatography, using gradient elution with hexane-acetone systems (5-100%). Twelve major fractions were obtained on the basis of TLC monitoring of the individual fractions. Further column chromatography of fraction #6 (4.4 g) yielded compound (**1**) (12 mg), as colourless needles after crystallization from MeOH.

Leonotinic acid (**1**). Colourless needles (MeOH): mp 222-223 ºC; $[\alpha]^{20}_D$ +6.9º (*c* 0.16, MeOH); IR (KBr) ν_{max} 3395, 3030, 2517, 1744, 1712, 1651, 1079 cm^{-1}; UV (MeOH) λ_{max} 214 nm (log ε 2.9); ^1H NMR and ^{13}C NMR spectral data, see Table 1; EIMS *m/z* (rel. int.) 350 $[M]^+$ (5), 332 (26), 287 (8), 271 (15), 234 (75), 219 (12), 179 (16), 109 (100), 81 (25); HREIMS 350.2106 $[M]^+$ (calcd for $C_{20}H_{30}O_5$, 350.2094).

Leonotinic acid methyl ester (**2**). Colourless prisms (MeOH): mp 103-104 ºC; $[\alpha]^{20}_D$ +9.4º (*c* 0.32, MeOH); IR (film) ν_{max} 3442, 1746, 1724, 1647, 1093 cm^{-1}; UV (MeOH) λ_{max} 208 nm (log ε 2.3); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.17 (1H, br s, H-14), 4.77 (2H, br s, H-15a,b), 3.64 (3H, s, 19-OMe), 2.31 (2H, t, *J* = 7.5 Hz, H-12a,b), 2.15 (1H, br d, *J* = 14.4 Hz, H-3a), 2.00 (1H, m, H-11a), 1.96 (1H, m, H-2a), 1.82 (2H, m, H-6a,b), 1.70 (1H, m, H-1a), 1.65 (1H, m, H-11b), 1.57 (1H, m, H-7a), 1.50 (1H, m, H-7b), 1.49 (1H, m, H-2b), 1.46 (3H, s, H-17), 1.27 (1H, m, H-5), 1.19 (1H, m, H-1b), 1.17 (3H, s, H-18), 1.09 (1H, m, H-9), 1.02 (1H, ddd, 14.4, 14.4, 4.0, H-3b), 0.91 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz): δ177.6 (C-19), 174.6 (C-16), 144.0 (C-14), 134.8 (C-13), 73.3 (C-8), 70.2 (C-15), 59.6 (C-9), 51.3 (19-OMe), 48.4 (C-5), 43.9 (C-4), 38.9 (C-10), 38.1 (C-3), 37.5 (C-7), 36.3 (C-1), 31.7 (C-17), 28.7 (C-18), 28.6 (C-12), 24.7 (C-11), 22.1 (C-6), 21.9 (C-20), 19.2 (C-2); EIMS *m/z* (rel. int.) 364 $[M]^+$ (4), 333 (1), 219 (28), 81 (100); HREIMS 364.2239 $[M]^+$ (calcd for $C_{21}H_{32}O_5$, 364.2250).

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