

Leonurun, a Novel Labdane Diterpenoid from *Leonotis leonurus*

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ABSTRACT

A novel labdane diterpenoid, leonurun, has been isolated from the leaves of *Leonotis leonurus*. This structure was determined from spectroscopic data and the relative stereochemistry from single-crystal X-ray diffraction analysis.

KEYWORDS

Labdane diterpenoid, *Leonotis leonurus*, NMR data, X-ray analysis.

Leonotis leonurus is a shrub found in many parts of South Africa and is colloquially known as wild dagga.¹ It has been used for many years as a traditional medicine to treat a number of ailments both orally (bronchitis, coughs, colds and others) and topically (piles, eczema, skin rashes, and boils).² The leaves of the plant have also been smoked for the relief of epilepsy, while a study carried out on the water extract of the plant does appear to indicate that some constituent of the leaves exhibits anti-convulsant activity.³ Our current interest in this plant lies in its potential positive inotropic activity.⁴

Other labdane diterpenoids have previously been extracted from *Leonotis leonurus*, most notably premarrubiin (2),⁵ the structure of which is closely related to that of leonurun (1) presented here. Reports of marrubiin (3) having been isolated from *Leonotis leonurus* have also been published,⁶ though it is thought that this may rather be an artifact of extraction with the marrubiin being formed from the premarrubiin present in the plant.⁵ Additional labdane diterpenoids extracted from *Leonotis leonurus* are a dilactone known as compound X^{7,8} and an α,β unsaturated ketone known as compound Y.⁷ It is worth noting that Laonigro *et al.*⁵ commented that in their hands no traces of compound X were found in *Leonotis leonurus* and attributed this to the fact that their specimens had been grown in Naples, Italy, while those from which compound X had been extracted were collected in the Eastern Cape, South Africa.^{7,8} Since we have isolated leonurun (1) in a significant quantity and no other researchers have previously identified this compound in *Leonotis leonurus* (though compounds from this plant have been characterized since the 1960's), we too have come to speculate that this is caused by a regional difference as our plant material was collected in the Western Cape, South Africa.

Experimental

Dried, powdered leaves of *Leonotis leonurus* were extracted with acetone and the material obtained was subjected to silica gel column chromatography with hexane/EtOAc, 7:3 used as eluent. After removal of the solvent *in vacuo* the concentrate fractions with an R_f of 0.43 were re-dissolved in ethanol and allowed to crystallize to afford leonurun (1) (m.p 105–106°C, $[\alpha]_D^{22.4} -27.9$ (c. 0.65, CHCl₃)). The yield from 1140 g of the dry

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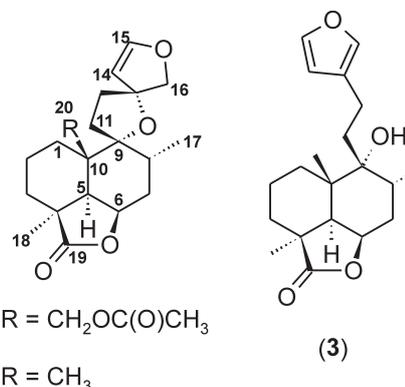


Figure 1 Structures of leonurun (1), premarrubiin (2) and marrubiin (3).

leaves was 547 mg. NMR spectra were collected at 25°C on a 600 Varian UnityInova spectrometer operating at 600 MHz for ¹H and 150 MHz for ¹³C. The sample was dissolved in CDCl₃ and the ¹H spectrum referenced to the residual solvent peak at 7.26 ppm and the ¹³C spectrum to the centre line of the solvent peak at 77.0 ppm. 2D NOESY and selective 1D NOE experiments were collected using a 0.8 sec mixing time.

From the HREIMS of (1) an m/z value of 390.21021 was found which agreed with the calculated m/z for C₂₂H₃₀O₆ of 390.20424. The ¹H NMR spectrum of the compound (Fig. 2) correlated with this information as it integrated for 30 protons with the ¹³C NMR spectrum clearly displaying 22 signals. Besides the ¹H and ¹³C spectra, DEPT, ¹H-¹H COSY, HSQC and HMBC NMR spectra were also collected to assist in the elucidation of the structure. From the DEPT spectrum it was possible to conclude that there are three methyl groups, eight CH₂s, five CHs and six quaternary carbons in the compound. In the ¹H NMR spectrum a methyl doublet at 0.86 ppm (Me-17) could be clearly observed and two tertiary methyl groups at 1.25 (Me-18) and 2.02 ppm (Me-22), the latter chemical shift indicating an acetyl methyl. The two coupled downfield doublets at 5.14 (H-14) and 6.46 ppm (H-15) are characteristic of the vinyl protons of a cyclic enol ether. The methylene protons in this same ring (H-16) also appear at distinct chemical shifts of 4.07 and 4.42 ppm. The H-20 methylene protons being adjacent to the ester moiety appear at

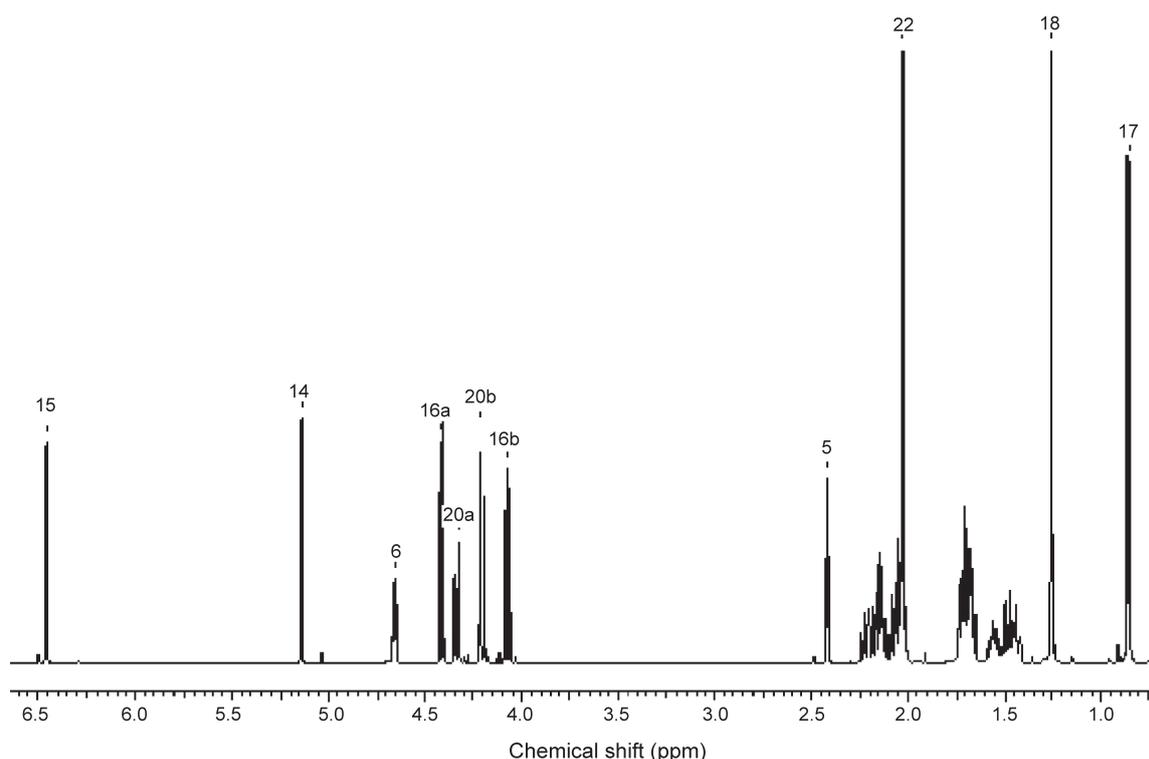


Figure 2 ^1H NMR spectrum of leonurun (**1**) in CDCl_3 with selected assignments shown.

4.20 and 4.34 ppm with the latter signal showing W-bond coupling of 1.7 Hz to one of the H-1 protons as indicated by the ^1H - ^1H COSY. This has also been observed in a closely related labdane diterpenoid known as leonitin⁹ (also isolated from plants in the *Leonotis* genus)^{9,10} with the same acetyl functionality.

The chemical shifts of the quaternary carbons at 89.11 (C-9) and 91.64 ppm (C-13) in the ^{13}C spectrum are quite distinct and provide evidence for the presence of the two-spiro fused five-membered rings. The presence of the lactone and the acetyl moieties are further indicated by the carbonyl peaks at 182.60 (C-19) and 170.10 ppm (C-21), respectively. The HMBC spectrum allowed the labdane skeleton in the ^1H and ^{13}C NMR spectra to be assigned. This 2D spectrum also allowed connection of the functional groups identified in the 1D spectra above to the labdane backbone. Correlations from the H-20 methylene signal to those carbons C-21, C-1, C-10 and C-9 confirmed the position of the acetyl group. Correlations from the spiro carbon C-13 to H-11, H-12, H-14, H-15 and H-16 and spiro carbon C-9 to Me-17, H-5, H-11, H-7, H-8 and H-20 confirmed the position of the two spiro-fused ring moiety at C-9. Table 1 shows the full assignment of the ^1H and ^{13}C spectra.

The NOESY spectrum of leonurun (**1**) showed correlations between Me-18, H-5 and H-6. A correlation from H-6 to one of the protons of H-7, and a correlation from this same proton to Me-17 indicated that Me-18, H-5, H-6 and Me-17 are all on the same face of the molecule. The spectrum also indicates that there is a NOE correlation between Me-17 and one of the H-16 protons giving an indication as to the orientation of the ring containing the enol ether. These mentioned are all clear NOE correlations which give some indication as to the relative stereochemistry of the molecule; however, this still leaves doubt as to the stereochemistry at C-10, C-9 and C-13. Other NOE correlations are present in the spectrum such as that between one of the H-20 protons and either H-8 or H-12. However, as the signals of these two protons overlap it is difficult to assess whether the correlation is between H-20 and H-8 or H-20 and H-12. The other

correlations present in the spectrum are similarly difficult to interpret due to overlapping signals in the ^1H spectrum. As the characterization of leonurun by NMR spectroscopy had led to the elucidation of a novel structure and the compound was crystalline, it was a natural step to obtain an X-ray crystal structure (Fig. 3, showing both of the molecules in the asymmetric unit) which was able to provide the relative stereochemistry

Table 1 NMR spectroscopic data of compound **1**.

Position	δ_{C}	δ_{H} (J Hz)
1	22.66	1.70 m (eq), 1.47 m (ax)
2	17.97	1.70 m (eq), 1.56 m (ax)
3	28.36	2.14 m (eq), 1.47 m (ax)
4	43.58	
5	46.82	2.41 d (4.6)
6	75.77	4.66 dd (1.7, 12.5)
7	31.86	2.14 m (eq), 1.70 m (ax)
8	32.73	2.04 m
9	89.11	
10	42.46	
11	31.66	2.23 m, 1.70 m
12	37.43	2.04 m
13	91.64	
14	107.36	5.14 d (2.6)
15	148.23	6.46 d (2.6)
16	80.16	4.42 d (10.6), 4.07 d (10.6)
17	17.67	0.86 d (6.5)
18	23.41	1.25 s
19	182.60	
20	65.94	4.34 d (12.5), 4.20 dd (1.7, 12.5)
21	170.10	
22	21.03	2.02 s

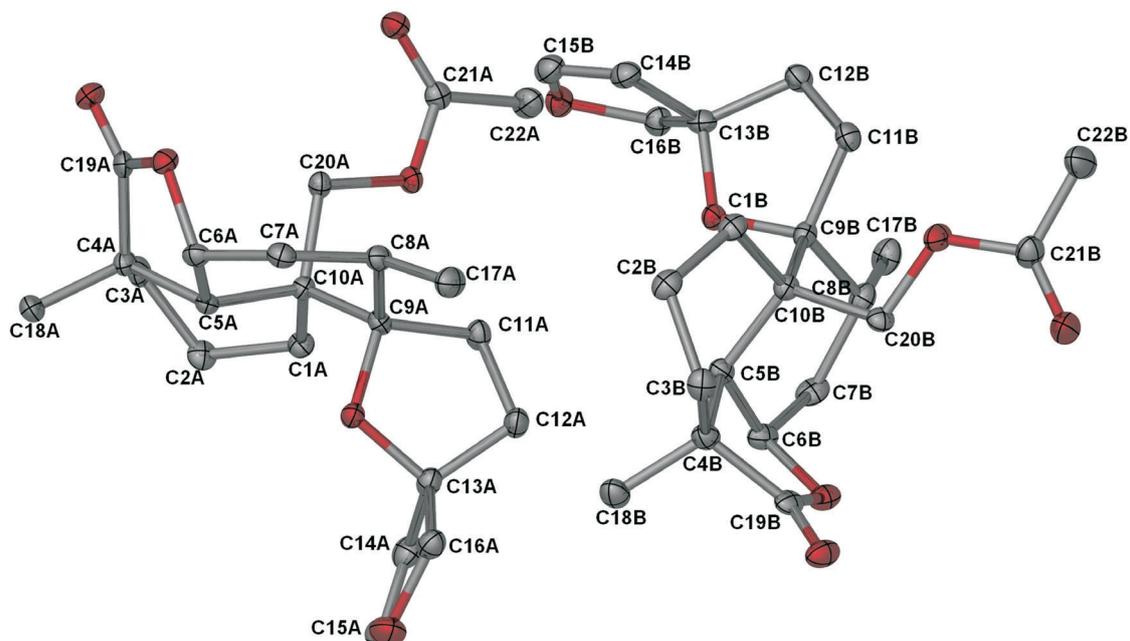


Figure 3 X-ray crystallographic structure of compound **1** showing both molecules in the asymmetric unit (A and B). Hydrogen atoms have been omitted for clarity.

of the molecule.¹¹ The X-ray crystal structure indicates that the stereochemistry at C-4 is pro-*S* while all the remaining centres are pro-*R*. The relative stereochemistry that was determined for four of the chiral centres from the NOESY experiment is thus in agreement with that obtained from the crystal structure.

In this study, a new previously unidentified labdane diterpenoid was isolated from leaves of *Leonotis leonurus* collected in the Western Cape, South Africa, and has been named leonurun. Initial investigation has indicated that the compound has positive inotropic activity and further studies are being pursued.

Acknowledgements

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References and Notes

- 1 M. Roberts, *Indigenous Healing Plants*, Southern Book Publishers, Cape Town, South Africa, 1990, 223 pp.
- 2 G. Scott, E.P. Springfield and N. Coldrey, *Pharm. Biol.* 2004, **42**, 186–213.
- 3 E. Biennu, G.J. Amabeoku, P.K. Eagles, G. Scott and E.P. Springfield, *Phytomedicine*, 2002, **9**, 217–223.
- 4 K. Obikeze, *Cardiovascular effects of Leonotis leonurus extracts in normotensive rats and in isolated perfused rat heart*, M.Pharm. thesis, University of the Western Cape, Cape Town, South Africa, 2004.
- 5 G. Laonigro, R. Lanzetta, M. Parrilli, M. Adinolfi and L. Mangoni, *Gazz. Chim. Ital.*, 1979, **109**, 145–150.
- 6 D.E.A. Rivett, *S. Afr. J. Chem. Soc.*, 1964, 1857–1858.
- 7 E.R. Kaplan and D.E.A. Rivett, *J. Chem. Soc. (C)*, 1968, 262–266.
- 8 G.J. Kruger and D.E.A. Rivett, *S. Afr. J. Chem.*, 1988, **41**, 124–125.
- 9 S. Habtemariam, A.I. Gray and P.G. Waterman, *J. Nat. Prod.*, 1994, **57**, 1570–1574.
- 10 G.A. Eagle, E.R. Kaplan, K. Naidu and D.E.A. Rivett, *J. Chem. Soc., Perkin Trans. I*, 1978, 994–997.
- 11 Crystallographic data (excluding structure factors) for the structure of leonurun (**1**) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 606716. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail deposit@ccdc.cam.ac.uk].