New labdane-type diterpenoids from the leaves of *Leonotis leonurus* (Lamiaceae)

D. Naidoo[a*], V. Maharaj[a], N.R. Crouch[b], A. Ngwane[c]

[a] *Bioprospecting Research Group, Biosciences Division, CSIR, Pretoria, 0001, South Africa*
[b] *Ethnobotany Unit, South African National Biodiversity Institute, P.O. Box 52099, Berea Road, 4007 South Africa / School of Chemistry, University of KwaZulu-Natal, Durban, 4041, South Africa*
[c] *Division of Medical Microbiology, Department of Medicine, University of Cape Town, K-45 OMB GSH, Observatory 7925, South Africa*

* Corresponding author. Tel.: +27-12-841-4077; fax: +27-12-841-4790.
E-mail address: DNaidoo2@csir.co.za (D. Naidoo).

Abstract

Keywords: *Leonotis leonurus*; Lamiaceae; labdane-type diterpenoids; 9,13-Epoxy-6-hydroxy-16,15-labdanolide; 9,13-Epoxy-6-hydroxy-15,16-labdanolide

1. Subject and source

The genus *Leonotis* (Pers.) R.Br. comprises about 10 species, and has been placed within subfamily Lamioideae of the Lamiaceae. Genus members range in habit from tall herbs to small trees. All are native to tropical and/or southern Africa, with *Leonotis nepetifolia* (L.) R.Br. naturalized in the tropics (Harley et al., 2004).

Leonotis leonurus (L.) R.Br., commonly referred to as Wild dagga or Lion's ear, is a robust perennial shrub which grows usually to 2 m tall and is widespread throughout eastern South Africa, growing amongst rocks in grassland (Iwarsson, 1985). The plant has found a wide variety of medicinal applications: as a purgative, emmenagogue and for chronic skin ailments (Pappe, 1868), anthelmintic (tapeworms), snakebite, colds (Githens, 1949), corpulence, epilepsy, indigestion, haemorrhoids, muscular cramps, bronchitis, tuberculosis (Watt & Breyer-Brandwijk,

1962) coughs, asthma (Felhaber, 1997), feverish headaches (Byrant, 1970), dysentery and chest infections (Gerstner, 1941). Watt & Breyer-Brandwijk (1962) describe further medicinal applications both within and beyond its natural distribution range. Ethnoveterinary applications against fowl sickness and gall sickness in cattle have been documented (Hulme, 1954). The aqueous leaf extract of *L. leonurus* has also been reported to possess antinociceptive, anti-inflammatory and hypoglycemic properties as well as activity against type-2 diabetes mellitus (Ojewole, 2005). Based on its well-documented traditional usage profile for respiratory ailments, and its *in vitro* antibacterial activity (Kamatou et al., 2006), *L. leonurus* was identified as a potential source of novel anti-tuberculosis compounds. It was screened accordingly and profiled phytochemically.

Leaf material of *Leonotis leonurus* (L.) R.Br. was harvested from Clanwilliam, Western Cape Province, South Africa, and a voucher (*BP00444*, PRE) lodged at the National Herbarium for verification purposes.

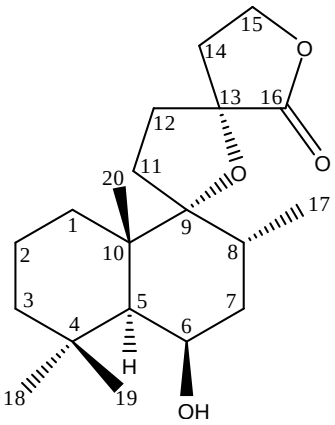
2. Previous work

Leonotis leonurus has previously yielded labdane-type diterpenoids: leonurun (McKenzie et al., 2006), marrubiin (Sagitdinova et al., 1996) and compound X (Kruger et al., 1988; Habtemariam. et al., 1994). The bioactivity of such medicinally-important labdane triterpenoids as forskolin has been reviewed by Lukhoba et al. (2006); the recorded pharmacology of compounds of this subclass accounts for the rational use of *L. leonurus* in treating obesity, digestive disorders and muscular cramps. This species has earlier also been reported to contain tannins, quinones, saponins, alkaloids and triterpenoids (Laonigro et al., 1979).

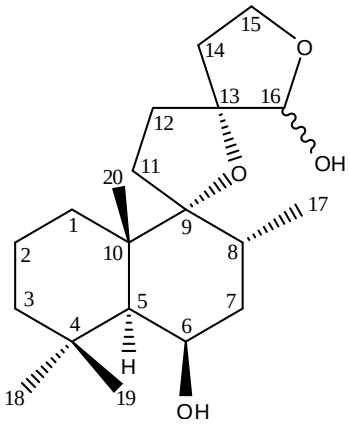
3. Present work

Leaves of *L. leonurus* were dried, ground and extracted separately using water and 1:1 methanol:dichloromethane resulting in aqueous and organic extracts. Both extracts were tested in an *in vitro* biological assay against *Mycobacterium tuberculosis* using the method of Lall and Meyer (1999). The organic extract showed greater than 99% growth inhibition when tested at 1000 μg/ml with rifampicin as the positive control (2 μg/ml), and was considered to have potent activity. The organic

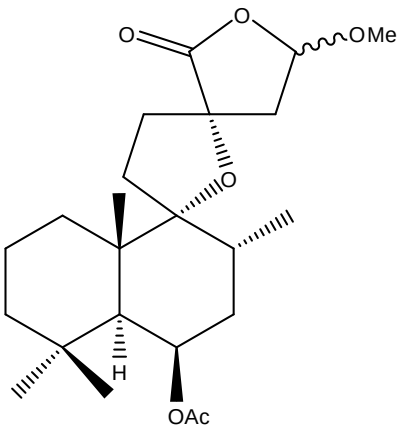
extract was subjected to fractionation by column chromatography over silica gel (Merck 5554) using varying proportions of ethyl acetate in dichloromethane leading to a mixture of 2 compounds which were further separated by preparative thin layer chromatography over pre-coated silica glass plates (Merck, SIL G-25 UV_{254}, 0.25 mm, 20 x 20 cm). New labdane-type diterpenoids were isolated: 9,13-Epoxy-6-hydroxy-labdan-16,15- olide **1** and 9,13:15,16-Diepoxy-6,16-labdanediol **2**. The structures of compounds **1** and **2** were identified by interpretation of the NMR spectral data (^{1}H, ^{13}C, DEPT, COSY, NOESY, HSQC and HMBC), as well as by comparison of the spectral data with those of closely related compounds previously reported from *Leonotis* and *Vitex* L., (Habtemariam et al., 1994; Tasdemir et al., 1997; Tasdemir et al., 1998; Ono et al., 2001; Ono et al., 2002). Table **1** shows the NMR spectral data of compounds **1** and **2** compared to the known 6-Acetoxy-9,13-epoxy-15-methoxy-labdan-16,15-olide **3** previously isolated from *Vitex rotundifolia* L.



1



2



3

Table 1
NMR spectral data of compounds **1**, **2** and **3** (δ in ppm, *J* in Hz)

Position	Compound **1**		Compound **2**		Compound **3**	
	δ_H	δ_c	δ_H	δ_c	δ_H	δ_c
1a b	1.58 (1H) 1.3 (1H)	34.0	1.63 (1H) 1.31 (1H)	34.3	1.81 1.32 (1H, m)	33.7
2a b	1.55 (1H) 1.5 (1H)	18.7	1.60 (1H) 1.49 (1H)	18.7	1.6 1.54	18.7
3a b	1.26 (1H) 1.18 (1H, *J* = 4.5)	44.0	1.29 (1H, *J* = 4.5) 1.17 (1H)	44.1	1.27 1.2 (1H, ddd, *J* = 4.0, 13.5, 13.5)	43.9
4		34.4		35.6		34.1
5	1.31 (1H, d, *J* = 2.5)	49.7	1.33 (1H, d, *J* = 2.5)	50.0	1.5 (1H, d, *J* = 3.0)	48.7
6	4.31 (1H, ddd, *J* = 2.5, 2.5, 2.5)	68.0	4.35 (1H, ddd, *J* = 2.5, 2.5, 2.5)	67.9	5.38 (1H, ddd *J*= 3.0, 3.0, 3.0)	70.6
7a b	1.7 (1H, ddd, *J* = 2.5, 3.0, 14.0) 1.4 (1H, ddd, *J* = 2.5, 3.0, 14.0)	40.7	1.75 (1H, ddd, *J* = 2.5, 3.0, 14.0) 1.44 (1H, ddd, *J* = 2.5, 3.0, 14.0)	40.7	1.63 (1H, ddd *J* = 3.0, 14.0, 14.0) 1.54	36.8
8	2.3 (1H, d, *J* = 3.0)	30.6	2.24 (1H, d, *J* = 3.0)	30.8	2.13	31.2
9		95.5		94.4		95.4
10		43.0		42.8		43.3
11a b	2.35 (1H) 1.8 (1H)	29.6	2.24 (1H) 1.80 (1H)	29.2	2.3 1.82	29.5
12a b	2.15 (1H) 2.0 (1H)	33.5	2.13 (1H) 1.94 (1H)	33.3	2.3 2.11	35.8
13		83.3		90.6		84.5
14a b	2.4 (1H, dd, *J* = 6.0, 13.0) 2.2 (1H, dd, *J* = 3.5, 13.0)	37.2	2.35 (1H, dd, *J* = 3.5, 13.0) 1.88 (1H, dd, *J* = 6.0, 13.0)	36.4	2.59 (1H, dd *J* = 5.5, 13.5) 2.15 (1H, dd *J* = 3.5, 13.5)	43.9
15a b	4.38 (1H) 4.18 (1H, dd, *J* = 3.5, 6.0)	65.5	4.09 (1H) 3.79 (1H, dd, *J* = 3.5, 6.0)	65.0	5.45 (1H, dd *J* = 3.5, 5.5)	102.5
16		177.1	4.72 (1H, bs)	98.9		176.3
17	0.85 (3H, d, *J* = 6.0)	17.8	0.90 (3H, d, *J* = 6.0)	17.1	0.84 (3H, d, *J* = 6.5)	17.5
18	0.95 (3H, s)	33.3	0.99 (3H, s)	33.3	0.93 (3H, s)	33.2
19	1.18 (3H, s)	24.9	1.21 (3H, s)	24.7	0.97 (3H, s)	23.9
20	1.25 (3H, s)	20.7	1.26 (3H, s)	20.5	1.23 (3H, s)	20.4

Compound **1** was obtained as white needles with a melting point of 149-151°C, $[\alpha]_D$ –0.35, and showed IR absorption bands at 2916 cm^{-1} and 1773 cm^{-1} indicating the presence of hydroxyl and carbonyl groups respectively. The ^{1}H NMR spectrum exhibited three oxygenated proton signals at δH 4.31 (1H-6, ddd), 4.38 (1H-15a, m), and 4.18 (1H-15b, m). Four methyl group proton signals resonated upfield in the ^{1}H NMR spectrum at δH 0.85 (3H-17, d, J = 6.8 Hz), δH 0.95 (3H-18, s), δH 1.18 (3H-19, s), and δH 1.25 (3H-20, s). The remaining proton signals resonated between δH 1.1 and 2.5 in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum showed the presence of twenty carbon signals, a carbonyl carbon signal at δC 177.1 (C-16), four oxygenated carbon signals at δC 95.5 (C-9), 83.3 (C-13), 68.0 (C-6) and 65.5 (C-15), and fifteen protonated carbon signals from δC 17.8 to 49.7. Interpretation of the full set of NMR data showed that the compound's molecular formula is $C_{20}H_{32}O_4$ and the compound is of the labdane-type diterpenoid class of compounds previously isolated from the *Leonotis spp*., and is very similar to the known compound **3** from

the *Vitex sp*. Compound **1** differed from compound **3** by the absence of the methoxy group at the C-15 position, and by the oxygenated substituent attached to C-6. The NOESY NMR spectrum showed correlations between the H-5 (δH 1.31) and H-6 (δH 4.31) signals indicating that these two are on the same plane. Since H-5 is α -orientated based on the biosynthetic pathway of labdanes, H-6 will also be α-orientated while the hydroxyl group attached to C-6 is β-orientated. The HMBC NMR spectrum showed correlations of the carbonyl carbon signal at δC 177.1 with the proton signals at δH 2.0 (1H-12b), 2.15 (1H-12a), 2.2 (1H-14b), 2.4 (1H-14a), 4.18 (1H-15a) and 4.38 (1H-15b), while the COSY NMR spectrum showed correlations between the proton signals at δH 2.2 (1H-14b), 2.4 (1H-14a), 4.18 (1H-15a) and 4.38 (1H-15b) indicating that C-14 and C-15 are adjacent to each other. Based on these correlations the lactone group is located in the 16,15 position.
Compound **2** was obtained as white powder with a melting point of 184-186°C, $[\alpha]_D$ – 1.32, and showed IR absorption bands at 2920 cm^{-1} and 1720 cm^{-1} indicating the presence of hydroxyl and carbonyl groups respectively. The ^{13}C NMR spectrum showed the presence of twenty carbons, five oxygenated carbon signals at δC 98.9 (C-16), 94.4 (C-9), 90.6 (C-13), 67.9 (C-6) and 65.0 (C-15), and fifteen protonated carbon signals from δC 17.1 to 50.0. The ^{1}H NMR spectrum showed the presence of four methyl group proton signals at δH 0.90 (3H-17, d, J = 6.6 Hz), 0.99 (3H-18, s), 1.21 (3H-19, s), and 1.26 (3H-20, s). The comparison of the structures of compounds **1** and **2** showed that compound **2** was possibly oxidised at the C-16 position during the isolation process to give compound **1**. This could clearly be seen by observing the absence of the carbonyl carbon signal and the presence of an extra oxygenated carbon signal in the ^{13}C NMR spectrum of compound **2**, which corresponded to the extra proton peak at δH 4.74 (1H-16) in the ^{1}H NMR spectrum of compound **2**.

Although neither compound **1** nor **2** showed activity against *M. tuberculosis,* their value as chemotaxonomic markers is apparent.

4. Chemotaxonomic significance

Labdane-type diterpenoids of the subclass currently isolated, have previously been identified only from representatives of *Leonotis* and *Vitex* (Lamiaceae; Viticoideae). Both **1** and **2** are very similar to compounds from *Vitex rotundifolia* L.f. (Ono et al., 1999; Ono et al., 2001), differing only in the absence of the oxygenated substituent at C-15 in **1** and **2**. Notably, most labdane-type diterpenoids found previously in *Leonotis* differ structurally in possessing the lactone between C-4 and C-6. These include compounds from *L. dubia* E.Mey (Eagle et al. 1978), *L*. *ocymifolia* (Burm.f.) Iwarsson var. *raineriana* (Visiani) Iwarsson (as *L. dysophylla* Benth.)*, L*. *ocymifolia* (Burm.f.) Iwarsson var. *ocymifolia* (as *L. leonitis* R.Br.) (Purushothaman et al., 1974; Eagle et al., 1978; Laonigro et al., 1979; Savona et al., 1984; Kruger et al., 1988; Habtemariam. et al., 1994; Rigano et al., 2006) and *L. nepetifolia* (L.) R.Br. (Manchand,1973; Purushothaman et al., 1974; Von Dreele et al., 1975; Blount et al., 1980; Habtemariam. et al., 1994; Ohsaki et al., 2005).

The Verbenaceae has been subject to considerable systematic research attention, with concomitant taxonomic flux. Nearly two thirds of the family in their traditional circumscription were transferred by Cantino et al. (1992) to the Lamiaceae, leaving a narrowly circumscribed Verbenaceae consisting of the subfamily Verbenoideae minus the tribe Monochileae. Chloroplast DNA studies (Cantino 1992a, 1992b) support this arrangement, and further reveal that Verbenaceae *s.str*. and Lamiaceae *s.l*. have originated in distant parts of the Scrophulariales (Atkins 2004). These phylogenetic studies revealed several genera, including *Vitex*, to be misplaced in Verbenaceae and better accommodated in Lamiaceae, albeit in different subfamilies. Parsimony analysis of *rbc*L sequences supports monophyly of the Lamiaceae *s.l.* (Wagstaff & Olmstead 1997), which includes the subfamilies Caryopteridoideae, Chloanthoideae and Viticoideae earlier transferred from the Verbenaceae to the Lamiaceae, and there variously accommodated (Cantino et al., 1992).

Consideration of classes of all isolates reported from Verbenaceae *s.str.*, with its residual 34 genera (Atkins, 2004), revealed that this family does not produce labdane-type diterpenoids (Dictionary of Natural Products, 2009). A single verbenaceous taxon yielded a clerodane diterpenoid. At a family level there is accordingly significant variance from the chemical profile of the Lamiaceae *s.l.* The

present findings of a novel labdane-type diterpenoid in *Leonotis* related to those reported from *Vitex* confirm this analysis, and provide chemotaxonomic support for recent phylogenetic assessments of the Lamiaceae and Verbenaceae, and subsequent transfers. Economic implications result: prior to these transfers the Verbenaceae *s.l.* would have been worth bioprospecting for medicinally-useful compounds related to forskolin; with Verbenaceae *s.str.* this is no longer the case.

Acknowledgements

The authors gratefully acknowledge the Department of Science and Technology of South Africa for the Innovation Fund award (Grant TM1002FP), and the Divisions of Medical Microbiology and Pharmacology at the University of Cape Town for screening the plant extracts, fractions and compounds in their *in vitro M. tuberculosis* assays. The staff of the Mary Gunn Library (SANBI) are thanked for facilitating access to literature.

References

Atkins, S., 2004. Verbenaceae. In: Kadereit, J.W. (Ed.). The families and genera of vascular plants. Vol. VII. Lamiales. Springer, Heidelber, pp. 449–468.

Blount, J.F., Manchand, P.S., 1980. J.C.S. Perkin 1, 264.

Bryant, A.T., 1970. Zulu medicine and medicine-men. Struik, Cape Town.

Cantino, P.D., 1992a. Ann. Miss. Bot. Gard. 79, 361–379.

Cantino, P.D., 1992b. Toward a phylogenetic classification of the Labiatae. In: Harley, R.M., Reynolds, T. (Eds). Advances in Labiate science. Royal Botanic Gardens, Kew, pp. 27–37.

Cantino, P.D., Harley, R.M., Wagstaff, S.J., 1992. Genera of Labiatae: status and classification. In: Harley, R.M., Reynolds, T. (Eds). Advances in Labiate science. Royal Botanic Gardens, Kew, pp. 511–522.

Dictionary of Natural Products on DVD, 2009. Chapman Hall/CRC Press/Hampden Data Services, Ltd.

Eagle, G.A., Rivett, D.E.A., 1978, J.C.S. Perkin 1, 994.

Felhaber, T., 1997. South African traditional healers' primary health care handbook. Kagiso Publishers, Cape Town.

Gerstner, J., 1941. Bantu Studies 15, 277–301.
Githens, T.S., 1949. Drug plants of Africa. African handbooks 8. University of Pennsylvania Press, Philadelphia.
Habtemariam, S., Gray, A.I., Waterman, P.G., 1994. J. Nat. Prod. 57, 1570–1574.
Harley, R.M., Atkins, S., Budantsev, A.L., Cantino, P.D., Conn, B.J., Grayer, R., Harley, M.M., De Kok, R., Krestovskaja, T., Morales, R., Paton, A.J., Ryding, O., Upson, T., 2004. Labiatae. In: Kadereit, J.W. (Ed.). The families and genera of vascular plants. Vol. VII. Lamiales. Springer, Heidelberg, pp. 167–275.
Hulme, M.M., 1954. Wild flowers of Natal. Shuter & Shooter, Pietermaritzburg.
Iwarsson, M., 1985. Leonotis. In: leistner, O.A. (Ed.). Flora of Southern Africa 28, 31–37.
Kamatou, G.P.P., Viljoen, A.M., van Vuuren, S.F., van Zyl, R.L., 2006. S. Afr. J. Bot. 72, 634–636.
Kruger, G.J., Rivett, D.E.A., 1988. S. Afr. J. Chem. 41, 124–125.
Lall N., Meyer J.J.M., 1999. J. Ethnopharm. 66, 347–354.
Laonigro, G., Lanzetta, R., Parrilli, M., Adolfini, M., Mangoni, L., 1979. Gazz. Chim. Ital. 109, 145–150.
Lukhoba, C.W., Simmonds, M.S.J., Paton, A.J., 2006. J. Ethnopharm. 103, 1–24.
Manchand, P.S., 1973. Tet. Lett. 14, 1907–1908.
McKenzie, J.M., Green, I.R., Mugabo, P., 2006. S. Afr. J. Chem. 59, 114–116.
Ohsaki, A., Kishimoto, Y., Isobe, T., Fukuyama, Y., 2005. Chem. Pharm. Bull. 53, 1577–1579.
Ojewole, J.A., 2005. Methods Find Exp. Clin. Pharmacol. 27, 257–64.
Ono, M., Yamamoto, M., Masuoka, C., Ito, Y., Yamashita, M., Nohara, T., 1999. J. Nat. Prod. 62, 1532–1537.
Ono, M., Yamamoto, M., Yanaka, T., Ito, Y., Nohara, T., 2001. Chem. Pharm. Bull. 49, 82–86.
Ono, M., Yanaka, T., Yamamoto, M., Ito, Y., Nohara, T., 2002. J. Nat. Prod. 65, 537–541.
Pappe, L., 1868. Florae Capensis medicae prodromus. W. Brittain, Cape Town.
Purushothaman, K.K., Vasanth, S., Connolly, J.D., 1974. J.C.S. Perkin 1, 2661–2663.
Rigano, D., Grassia, A., Bruno, M., Rosselli, S., Piozzi, F., Formisano, C., Arnold, N.A., Senatore, F., 2006. J. Nat. Prod. 69, 836–838.

Sagitdinova, G.B., Makhmudov, M.K., Tashkhodzhaev, B., Mal'tsev, I.I., 1996. Chem. Nat. Compd. 32, 43–46.

Savona, G., Bruno, M., Rodríguez., B., 1984. Phytochem. 23, 191.

Tasdemir, D. Çalis, I., Sticher, O., 1998. Phytochem. 49, 137–143.

Tasdemir, D., Sticher, O., Çalis, I., Linden, A., 1997. J. Nat. Prod. 60, 874–879.

Von Dreele, R.B., Pettit, G.R., Ode, R.H., Perdue Jr., R.E., White, J.D., Manchand, P.S., 1975. J.A.C.S. 97, 6236–6240.

Wagstaff, S.J., Olmstead, R.G., 1997. Syst. Bot. 22, 165–179.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. Medicinal and poisonous plants of southern and eastern Africa. E & S Livingstone Ltd, London and Edinburgh.