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# Leonurenones A–C: Labdane diterpenes from *Leonotis leonurus*

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ABSTRACT

Labdanes, leonurenones A–C, two known labdanes, luteolin 7-*O*-β-glucoside and luteolin were isolated and characterized from a commercial source of *Leonotis leonurus*. Genetic methods allowed for identification of the plant material. The leonurenones contain an uncommon α,β-unsaturated enone moiety in ring B, and leonurenones A and B were evaluated in a competitive inhibition assay at the GABA A neuroreceptor site.



## 1. Introduction

*Leonotis leonurus* R. Br. (Lamiaceae) is a shrub 2–5 m in height that is native to South Africa. In traditional medicine, decoctions have been used externally for dermatological problems (rashes, boils and eczema) and internally to treat coughs, fever, headaches and hypertension (Scott et al., 2004). Leaves of the plant are smoked for its anti-epileptic effects (Bienvenu et al., 2002). Aqueous leaf preparations have been reported to possess anticonvulsant, antinociceptive, anti-inflammatory and antidiabetic properties in rodents (Bienvenu et al., 2002; Ojewole, 2005; Oyedemi et al., 2011). Additionally, crude aqueous extracts have been shown to possess antihelminthic activity (Maphosa and Masika, 2012; Maphosa et al., 2010).

In addition to folkloric uses mentioned above, *L. leonurus* reportedly produces marijuana-like effects (Stafford et al., 2008). The plant is commercially available and is marketed largely for its psychoactive effects. Some internet websites claim that this activity is attributable to an alkaloid, leonurine. There is some doubt about the validity of this claim since leonurine has never been reported to occur in the plant (although its presence in related species is documented) (Chen and Kwan, 2001; Hayashi, 1962; Kong et al., 1976; Luo, 1985). Thus the component(s) responsible for the reputed psychoactive effects are scientifically unverified at this time. Prior phytochemical investigations of various extracts of the plant have uncovered a number of labdane diterpenes, an iridoid glycoside and phenolic compounds, primarily of the flavonoid class (Agnihotri et al., 2009; El-Ansari et al., 2009; Kaplan and Rivett, 1968; Kruger and Rivett, 1988; Laonigro et al., 1979; McKenzie et al., 2006; Naidoo et al., 2011; Obikeze et al., 2008; Piozzi et al., 2007; Rivett, 1964).

As part of our program to identify natural products with central nervous system (CNS) activity from *Leonotis* plants, an investigation of a commercially available source of *L. leonurus* was carried out. Verification of the taxonomic identity of the plant was achieved via molecular methods. Details of the taxonomic identification as well as our chemical, spectroscopic and biological studies are reported herein.

## 2. Results and discussion

Since the plant material procured was not of sufficient quality to permit positive morphological identification (i.e. as distinct from other similar *Leonotis* species), identification of the plant using genetic methods was done.

Molecular markers have proved a powerful tool in diagnostics of species and varieties of various commercial products in order to identify and delimiting closely related species and to ensure quality control. Both DNA fingerprinting techniques and DNA sequence information have been employed, even from degraded and unrecognizable plant material (Martellossi et al., 2005; Zerega et al., 2002). The application, sometimes referred to as "DNA barcoding", can assist in the process of identifying unknown plant specimens to known species. (Hollingsworth et al., 2011) For example, this method has been suggested as useful for an accurate and rapid authentication of medicinal plant products and their adulterants (Chen et al., 2010).

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Recent molecular work in the Lamiaceae subfamily Lamioideae, to which *Leonotis* belongs, has produced a large database of chloroplast DNA sequences. (Scheen and Albert, 2009) A diagnostic assay was thus employed based on two chloroplast intron gene sequences (trnL intron and rps16 intron) that provide sufficient sequence variation to distinguish members of the group *Leonotis* belongs to, including closely related *Leonotis* and *Leucas* R.Br. species. After sequencing these gene regions from the procured *Leonotis* plant material several approaches were taken to ensure a correct identification. First, the sequences were analyzed using the BLAST search tool against sequences in the public database, GenBank, held at the National Center for Biotechnology Information (NCBI). Secondly, the DNA sequences were compared to the large data set of several hundred lamioid sequences, which clearly confirmed a large diagnostic "gap" in the rps16 sequence, which was shared with the *L. leonurus* sample in the data set. Finally, phylogenetic reconstruction was performed verifying that the obtained plant material grouped with the other *L. leonurus* in the data set. Together, these results clearly confirm the obtained material to belong to *Leonotis leonurus*.

The aqueous extracts of the aerial parts of the plant have not been subjected to phytochemical study and given the pharmacological activity attributed to this extract, it was considered worthy of such an investigation.

Repeated purification procedures (HPLC and flash column chromatography) on an aqueous extract of aerial parts of *L. leonurus* gave compounds **1**, **2**, **6** and **7** (Fig. 1). Similarly, repeated chromatography of an acetone extract afforded compounds **3**–**5**.

The initial diterpenoid nature of **1** came from its $^{13}C$ NMR spectrum where 20 carbons were observed. The HRESIMS spectrum showed a molecular ion peak at *m/z* 336.2380. This implied a molecular formula of $C_{20}H_{32}O_4$ in agreement with the $^{13}C$ NMR spectroscopic data. In the $^{13}C$ NMR spectrum, signals typical of the quaternary C-9 and C-13 positions of a spiroether labdane framework were observed ($\delta_C$ 95.9 and 85.7 ppm, respectively). Other low field quaternary signals were observed at $\delta_C$ 200.0 and 172.4 ppm. The former resonance was placed at position 7 of the labdane core based on HMBC correlations with the C-17 methyl protons ($\delta_H$ 1.21, d). The resonance at $\delta_C$ 172.4 showed HMBC cross-peaks to the C-18 and C-19 methyl protons as well as the C-20 methyl protons. This signal was thus attributed to C-5 of the diterpene skeleton. A vinylic system appeared to be present based on the signal at $\delta_C$ 123.1 (CH). The proton attached to this vinylic carbon ($\delta_H$ 6.06) showed HMBC correlations to C-8 ($\delta_C$ 47.7) and C-10 ($\delta_C$ 45.2). On the basis of the preceding, it became evident that the resonances at $\delta_C$ 200.0, 172.4 and 123.1 were due to the presence of an α,β-unsaturated enone system in ring B. The presence of two oxymethylene carbons was inferred from DEPT-135 and HSQC data ($\delta_C$ 66.9 and 59.2). That at $\delta_C$ 66.9 was attached to mutually coupled doublets at $\delta_H$ 3.50 and 3.56, thereby placing this carbon at position 16. Further HMBC correlations from the proton at $\delta_H$ 3.56 to C-12, C-13 and the C-14 methylene carbon supported this assignment. The oxymethylene carbon at $\delta_C$ 59.2 was assigned to position 15. HMBC cross-peaks were observed for the H-15 protons to C-13 and C-14. The above established the gross structure of compound **1**. The relative stereochemical assignments for **1** were made on the basis of NOESY spectroscopic data. Here cross-peaks were seen for H-8 to the C-20 and C-11 protons, thereby establishing that these groups were on the same side of the labdane structure. H-20 in turn showed NOESY cross-peaks to H-16 while H-17 showed cross-peaks to H-14. The α,β-unsaturated ketone system in ring B seen in **1** is very rare in the *Leonotis* genus having only been reported once in the literature (Kaplan and Rivett, 1968).

Compound **2** was isolated as colorless oil and showed a molecular ion peak in its HRESIMS spectrum (*m/z* 332.1992) corresponding to the molecular formula $C_{20}H_{28}O_4$. The structural similarity to compound **1** was evident upon inspection of the $^{13}C$ NMR and $^{1}H$ NMR spectroscopic data. The $^{1}H$ NMR spectrum showed 3 methyl singlets and a methyl doublet as seen in **1**. Typical $^{13}C$ NMR signals for the α,β-unsaturated enone in ring B ($\delta_C$ 174.6, C-5; 122.3, C-6; 201.1, C-7) and the quaternary C-9 and C-13 positions ($\delta_C$ 96.5 and 86.6, respectively) were also observed. The main difference in the $^{1}H$ and $^{13}C$ NMR spectra of the **2**, as compared to **1**, was the absence of additional signals corresponding to an oxymethylene group. This oxymethylene group was apparently replaced by an ester-like carbonyl functionality ($\delta_C$ 175.8). A pair of coupled oxymethylene doublets ($\delta_H$ 4.30 and 4.23; $J$ = 9.0 Hz) was reminiscent of similar signals for C-16 of **1**. The resonance at $\delta_H$ 4.23 correlated to C-13 as well as $\delta_C$ 175.8. These data support the presence of a γ-butyrolactone moiety comprising C-13 to C-16. In the NOESY spectrum, correlations from H-8 to H-20 and H-11 were seen, placing these groups on the same face of the diterpene scaffold. Further NOESY cross-peaks between H-14 and H-17 and H-16 and H-12 established the 13*S* relative stereochemistry depicted. It is interesting that **2** being relatively non-polar was isolated from the aqueous extract; we cannot exclude the possibility that **2** is an artifact being formed via lactonization of a more polar C15 hydroxyl, C16 carboxylate intermediate during the isolation process.

The HRESIMS of compound **3** indicated a molecular formula of $C_{22}H_{34}O_5$ (*m/z* 378.2407). The previously described compounds **1** and **2** were obviously similar to **3** based on the NMR spectroscopic data accumulated. Assignments for C-1 to C-13 (particularly the now familiar α,β-unsaturated enone moiety) could readily be made by comparison of their $^{1}H$ NMR and $^{13}C$ NMR spectra in tandem with HMBC analysis. Protons at $\delta_H$ 3.52 and 3.40 (both doublets, $J$ = 11.3 Hz) were correlated to C-13 ($\delta_C$ 85.2) and C-12 in the HMBC spectrum. These protons were assigned to the oxymethylene group at C-16. HMBC cross-peaks were observed from protons at $\delta_H$ 1.91, 2.15, 4.06 and 4.16 to C-13. Signals at $\delta_H$ 4.06 and 4.16 were attached to $\delta_C$ 61.5. Their downfield chemical shift suggested attachment to an acyloxy group. Indeed, presence of an acetoxyl group was established via resonances at $\delta_H$ 2.05 (3H, s); $\delta_C$ 21.0 ($CH_3$) and $\delta_C$ 171.1 (C). Thus the carbon at $\delta_C$ 61.5 was assigned to C-15 enabling complete assignments for the molecule and elucidation of the structure proposed. The NOESY data acquired suggested that

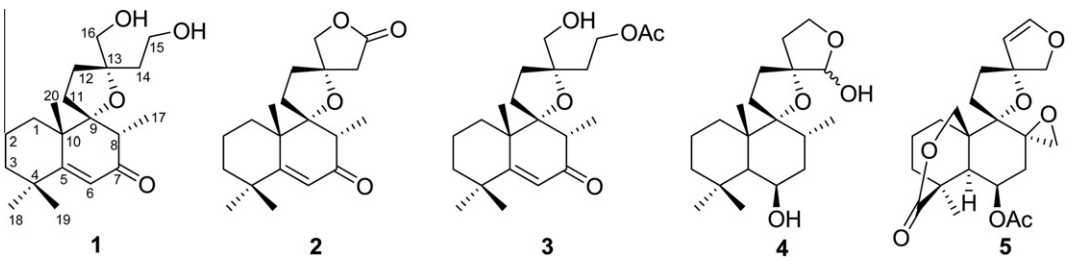


**Fig. 1.** Structures of isolated compounds **1**–**5**.