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Elephantopus-type sesquiterpene lactones from a second *Vernonanthura* species, *Vernonanthura lipeoensis*

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1. Subject and source

Aerial parts of *Vernonanthura lipeoensis* (Spreng.) H. Robinson were collected at the end of the flowering stage (seed formation) on 14 September 1999 on the road between Los Toldos and Lipeo, Jujuy province, Argentina. A voucher specimen (LIL 604871) is on deposit in the herbarium of the Instituto Miguel Lillo, Tucumán.

2. Previous work

V. lipeoensis (Spreng.) H. Robinson, an endemic of northwestern Argentina (Cabrera, 1978), is one of nearly seventy members of the New World genus formerly included in *Vernonia* (Robinson, 1992, 1995, 1999). Earlier chemical studies of *Vernonanthura* species, frequently under the old *Vernonia* binomials, resulted in the isolation of flavonoids and sesquiterpene lactones similar to or identical with such compounds found in other Vernonieae (Wagner et al., 1972; Mabry et al., 1975; Bohlmann and Zdero, 1977, 1988; Maldonado et al., 1980; Bohlmann et al.,

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1981a,b, 1983; Jakupovic et al., 1986, 1987a, b; Catalán et al., 1986, 1988; Stutts, 1988; Bardón et al., 1988, 1992; Budesinsky et al., 1994; Borkosky et al., 1997; Bazon et al., 1997; Kotowicz et al., 1998), while in one instance the occurrence of pimarane and kaurane derivatives was reported (Borkosky et al., 1997). However, in a recent article (Pollora et al., 2003) our groups described isolation from the Argentine endemic *Vernonanthura nebularum* of seven new sesquiterpene lactones closely related to lactones previously found only in *Elephantopus mollis*, a member of subtribe Elephantopodiinae of the Vernoniaceae.

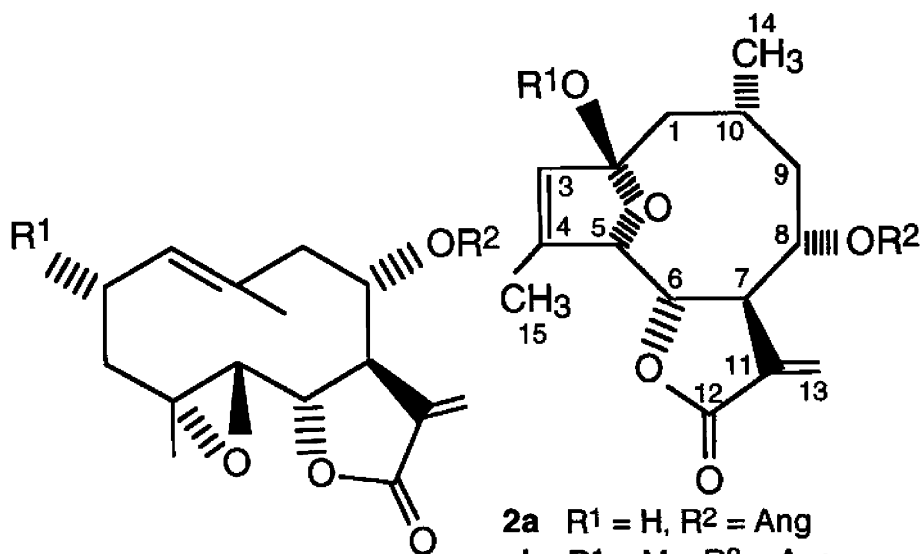
3. Present work

3.1. General procedures

For HPLC the column used was a Beckman ultrasphere C 18 (10 × 250 mm). Retention times were measured from the solvent peak. ¹H NMR spectra were run on Varian Inova 500 MHz or Varian Gemini 300 MHz NMR spectrometers, mass spectra were run on a JEOL MS Route 600 H instrument.

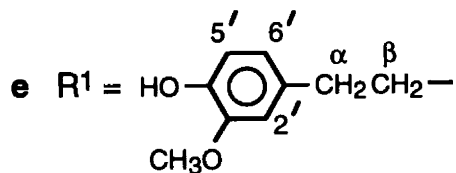
3.2. Extraction and isolation of constituents

Flowers and leaves (250 g) of *V. lipoensis* were extracted with CHCl₃ (3 × 2.5 l) at rt for several days to give 7 g (2.8%) of crude extract which was suspended in EtOH (60 ml) at 60 °C, diluted with H₂O (46 ml) and extracted successively with hexane (3 × 70 ml) and benzene (3 × 70 ml). Evaporation of the hexane extract at reduced pressure gave 4 g (1.6%) of residue which was not investigated further. The benzene extract on evaporation at reduced pressure furnished 2.7 g of residue (1.8%) which was subjected to CC over Si gel (Merck 230–400 mesh) using hexane–EtOAc–MeOH mixtures (85:15:0, 80:20:0, 70:30:0, 60:40:0, 60:39:1, 60:38:2 and 60:35:5) to yield 20 fractions. Frs 7, 8, 13 and 14 which exhibited strong lactone absorption near 1765 cm⁻¹ were subjected to HPLC and eluates were analyzed by ¹H NMR spectrometry. Fr 7 (50.5 mg) from the mother column was processed by HPLC (MeOH–H₂O 3:1, 2 ml min⁻¹) to give 2.8 mg of a mixture of **1b** and **3a** (*R*_t 3.5 min) as shown by ¹H NMR analysis, 18.2 mg of **1a** (*R*_t 7 min, mp 157–158 °C) and 3.3 mg of a mixture of **2b** (major) and **2a** (minor), (*R*_t 15 min). Fr 8 (40 mg) on HPLC afforded 4.5 mg of **1b** (*R*_t 6.5 min, mp 135–137 °C), 1.5 mg of a mixture of **2b** (major) and **2a** (minor), (*R*_t 10.5 min), 1.7 mg of a 1:1 mixture of **2d** and **2f** (*R*_t 16.5 min), 2 mg of impure **2c** (*R*_t 18 min) and 1.5 mg of **2e** containing a trace of **2b** (*R*_t 26.5 min). HPLC of a 170 mg portion of fr 13 (398 mg) from the mother column gave 1.2 mg of **1a** (*R*_t 7 min), a trace of **2c** (*R*_t 10 min), 4.7 mg of **2c** containing some **2d** (*R*_t 13.5 min), 1.3 mg of a mixture of lactones (*R*_t 18.5 min), 18.9 mg of **2a** containing some **2b** (*R*_t 22.5 min) 20 mg of **2d** containing some **2c** (*R*_t 31.5 min), 5.9 mg of **3b** containing a little **3a** (*R*_t 42 min) and 48.6 mg of **2b** (*R*_t 50 min). HPLC of a 250 mg portion of fr 14 (589 mg) from the mother column gave 25.6 mg of **4a** (*R*_t 3.5 min), 19.4 mg of a 1:1 mixture of **2c** and **2d** (*R*_t 5 min), 13 mg of **2b** containing a little **2a** (*R*_t 8.5 min), 46 mg of **2d** containing a little **2c** (*R*_t 13 min) and 29.6 mg of **2b** containing a little **2a** (*R*_t 20 min).

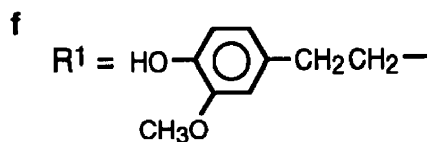


- 1a** R¹ = H, R² = Ang
b R¹ = H, R² = MeAcr
c R¹ = OH, R² = Ang
d R¹ = OH, R² = Sen

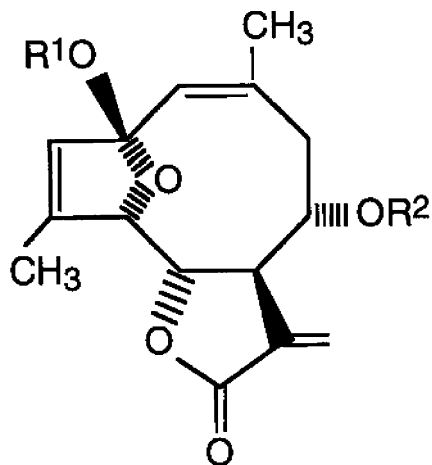
- 2a** R¹ = H, R² = Ang
b R¹ = Me, R² = Ang
c R¹ = H, R² = MeAcr
d R¹ = Me, R² = MeAcr



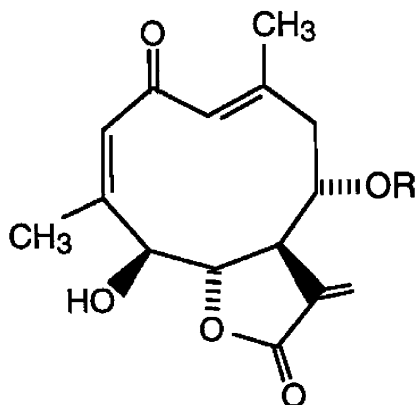
R² = Angelate



R² = Methacrylate



- 3a** R¹ = H, R² = Ang
b R¹ = Me, R² = Ang
c R¹ = H, R² = MeAcr
d R¹ = Me, R² = MeAcr



- 4a** R = Angelate
b R = Methacrylate
c R = Senecioate

¹H NMR, mass spectra and mp of lactone **1a** corresponded to data previously reported for deltoidin B from *Ageratina deltoidea* (Jacq.) King and Robinson (described as *Eupatorium deltoideum*) by Quijano et al. (1980); the same substance was subsequently reported from *Anthemis cupaniana* by Bruno et al. (1991) while both **1a** and **1b** have been isolated previously from *Helipterum propinquum* (Zdero et al., 1989). The ¹H NMR spectrum and mp of **1b** corresponded to the data reported by the German workers. Lactones **2a** and **2c** were identical with 1,10-dihydrohemiacetal derivatives of molephantin (**4b**) and molephantinin (**4c**) encountered by us earlier in *V. nebularum* (Pollora et al., 2003) and, as found previously, were apparently in equilibrium with ethers **2b** or **2d**, respectively, presumably because of the MeOH–H₂O solvent mixture used for the HPLC separations. Lactone **4a**, the angelate analog of molephantin and molephantinin (Lee et al., 1973, 1975, 1980; McPhail et al., 1974), has also been isolated by us earlier from *V. nebularum* (Pollora et al., 2003). Lactones **2e** and **2f**, each slightly contaminated by the other, are new as is the angelate analog **3b** of **3c** (But et al., 1996) and **3d** (phantomolin, Lee et al., 1980; Banerjee et al., 1986) which have been isolated from *E. mollis*. Angelate **3b** was contaminated by hemiacetal **3a** and may conceivably be an artifact formed from **3a** during the elution process; its ¹H NMR spectrum is included in Table 1.

Table 1
 ^1H NMR spectra of compounds **2e**, **f** and **3b** (500 MHz, CDCl_3)

| Position | 2e | 2f^a | 3b^b |
|------------------|-----------------------------------|-----------------------------------|---------------------------|
| 1a | 1.73 <i>m</i> | 1.73 <i>m</i> | 5.46 <i>s</i> |
| 1b | 1.73 <i>m</i> | 1.73 <i>m</i> | – |
| 3 | 5.41 <i>brs</i> | 5.41 | 5.61 <i>brs</i> |
| 5 | 5.14 <i>brs</i> | 5.15 | 5.26 <i>d</i> (4) |
| 6 | 4.45 <i>dd</i> (7.5, 2.6) | 4.47 | 4.64 <i>dd</i> (6,4) |
| 7 | 3.25 <i>dddd</i> (11,7.5,3.5,2.6) | 3.28 | 3.11 <i>dddd</i> |
| 8 | 5.12 <i>ddd</i> (11, 4, 2) | 5.11 | 5.22 <i>m</i> |
| 9a | 2.35 <i>brdd</i> (15,2) | 2.34 | 2.22 <i>dd</i> (14.1, 4) |
| 9b | 1.67 <i>m</i> | 1.67 | 3.70 <i>brd</i> (14) |
| 10 | 1.69 <i>m</i> | 1.69 | – |
| 13a | 6.26 <i>d</i> (3.5) | 6.27 | 6.31 <i>d</i> (3.4) |
| 13b | 5.75 <i>d</i> (2.6) | 5.75 | 5.78 <i>d</i> (2.7) |
| 14 ^c | 0.88 <i>d</i> (6.5) | 0.88 | 1.72 <i>brs</i> |
| 15 ^c | 1.73 <i>brs</i> | 1.74 | 1.78 <i>brs</i> |
| H- α^d | 2.74 <i>brt</i> (7) | 2.74 | |
| H- β^d | 3.50 <i>q</i> , 3.39 <i>q</i> (7) | 3.49, 3.39 | |
| 2' | 6.71 <i>d</i> (1.8) | 6.70 | |
| 5' | 6.67 <i>dd</i> (8,1.8) | 6.67 | |
| 6' | 6.81 | 6.81 | |
| OMe ^c | 3.79 <i>s</i> | 3.85 | 3.19 <i>s</i> |
| 3'' | 6.17 <i>qq</i> (7.2, 1.4) | 6.16 <i>brs</i> , 5.66 <i>brs</i> | 6.17 <i>qq</i> (7.4, 1.4) |
| 4'' ^c | 2.01 <i>dq</i> (7.2, 1.4) | 1.95 <i>brs</i> | 2.00 <i>dq</i> (7.4, 1.4) |
| 5'' ^c | 1.93 <i>quint</i> (1.5) | | 1.94 <i>quint</i> (1.4) |

^a Coupling constants correspond to those give for **2c**.

^b Contaminated by **3a**.

^c Intensity 3 protons.

^d Intensity 2 protons.

(2*S*^{*},5*S*^{*},6*S*^{*},7*R*^{*},8*S*^{*},10*R*^{*})-2-(3-methoxy-4-hydroxyphenylethoxy)-2,5-epoxy-8-angeloxygermacra-3*Z*,11(13)-dien-6,12-olide (**2e**). Gum; MS (FAB, Na, NBA) 535.2305; Calcd for $\text{C}_{29}\text{H}_{36}\text{O}_8 + \text{Na}$, 535.2308; ^1H NMR spectrum in Table 1.

(2*S*^{*},5*S*^{*},6*S*^{*},7*R*^{*},8*S*^{*},10*R*^{*})-2-(3-methoxy-4-hydroxyphenylethoxy)-2,5-epoxy-8-methacryloxy-3*Z*,11(13)-dien-6,12-olide (**2f**). Gum; MS (FAB, Na, NBA) 521.2142; Calcd for $\text{C}_{28}\text{H}_{34}\text{O}_8 + \text{Na}$, 521.2151; ^1H NMR spectrum in Table 1. The low and high resolution mass spectra indicated contamination by some **2e** as did the ^1H NMR spectrum of **2f**.

(2*S*^{*},5*S*^{*},7*R*^{*},8*S*^{*},10*R*^{*})-2-(3-methoxy-8-angeloxygermacra-1(10),3*Z*,11,13-trien-6,12-olide (**3b**); MS (FAB, Na, NBA) 397.1621; Calcd for $\text{C}_{21}\text{H}_{26}\text{O}_6 + \text{Na}$, 397.1627; ^1H NMR spectrum in Table 1. The low and high resolution mass spectra indicated contamination by previously known **3a** as did the ^1H NMR spectrum.

4. Chemotaxonomic significance

The sesquiterpene lactone chemistry of *V. lipeoensis* resembles that of its fellow Argentine endemic *V. nebularum* from the same geographical area but differs from

the sesquiterpene lactone chemistry of other *Vernonanthura* species for which chemical records are extant. As has been pointed out previously (Pollora et al., 2003) the chemistry of these two species is similar to the sesquiterpene lactone chemistry of *E. mollis* of Elephantopodiinae. Lactones **1a** and **1b** from *V. lipeoensis* are also analogs of lactone **1c** from *Elephantopus angustifolius* (Jakopovic et al., 1987a) and may be biological precursors of **3a** and **3c**. The taxonomic implications, if any, of the similarities in secondary metabolites between the two *Vernonanthura* species and the *Elephantopus* taxa are not clear.

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