Terpenoids from *Microliabum polymnioides*

Oscar J. Díaz [a], Roberto Gil [b], Lázaro J. Novara [a], Virginia E. Sosa [c], Juana R. de la Fuente [a,*]

[a] *Consejo de Investigación, Universidad Nacional de Salta, 4400 Salta, Argentina*
[b] *Department of Chemistry, Carnegie Mellon University, 4400 Fifth Ave, Pittsburgh, PA 15213, USA*
[c] *Facultad de Ciencias Químicas, Instituto Multidisciplinario de Biología Vegetal, Universidad Nacional de Córdoba, IMBIV (CONICET-UNC), 5000 Córdoba, Argentina*

Abstract

The phytochemical study of *M. polymnioides* led to the isolation of two sesquiterpene lactones namely: 11αH-dihydrozaluzanin E and 1β-hydroxy-4-oxo-11βH-4-noreudesman-6,12-olide. Their structures were determined by spectroscopic methods. The relative stereochemistry was established by a combination of coupling constant analysis, NOESY correlations and molecular modeling. Three related known sesquiterpene lactones were also identified, and these data were used for chemotaxonomical purposes.
© 2004 Elsevier Ltd. All rights reserved.

1. Introduction

The tribe Liabeae was first described by Rydberg, 1927. Since then, modern taxonomists have made various revisions (Robinson and Brettell, 1973, 1974; Robinson, 1983, 1990). Recently, Cabrera et al. (1999), recognized 14 genera for this tribe. Three of these genera are found in Argentina: *Microliabum*, *Munnozia* and *Paranephelius*, and more recently *Liabum* (Gutíerrez, 2003) was reincorporated. The genus *Microliabum cabrera* comprises five species that grow in the southern part of South America. The geographical distribution of *Microliabum mulgediifolium* (Muschl.) H.Rob. (=*Liabum mulgediifolium* Muschl.) is restricted to Bolivia; while the other four species grow in northwest and central Argentina: *Microliabum polymnioides* (R.E.Fr.) H.Rob. (=*Liabum polymnioides* R.E.Fr., *Austroliabum*

polymniodes R.E.Fr.); *M. candidum* (Griseb.) H.Rob. (=*Liabum candidum* Griseb.); *M. eremophilum* (Cabrera) H.Rob. (=*Liabum eremophilum* Cabrera); *M. humile* (Cabrera) Cabrera.

Several species of *Liabum* were transferred to *Microliabum*. *Liabum* was previously transferred from the tribe Senecioneae to the tribe Liabeae (Robinson, 1983).

The chemistry of many species of the genus *Liabum* was investigated by Bohlmann (1977, 1980a, 1984) and Jakupovic et al. (1988b), leading to the isolation of several sesquiterpenes, and polyacetylenic hydrocarbons. The isolation of sesquiterpene lactones was consistent with Robinson's proposition.

As part of a chemotaxonomic project aimed to further examine Robinson's proposition regarding the position of the genus *Liabum* in the tribe Liabeae, we have performed a chemotaxonomical study of *M. polymnioides*. As a result we herein report the isolation and structural elucidation of two new sesquiterpene lactones, 11αH-dihydrozaluzanin E (**1**) and 1 β-hydroxy-4-oxo-11βH-4-noreudesman-6,12-olide (**2**), in addition to the

---

* Corresponding author. Fax: +54 387 4255363.
E-mail address: dlfuente@unsa.edu.ar (J.R. de la Fuente).

known lactones magnolialide (El-Feraly et al., 1979), artesin (Marco, 1989), 4β, 14,11β, 13-tetrahydro-3-dehydrozaluzanin C (Bohlmann et al., 1977), and the coumarin scopoletin were obtained. The known compounds were identified by comparison of their spectral properties with those reported in the literature.

The structures of the new compounds were unambiguously determined using a combination of modern spectroscopic techniques, and molecular modeling.

2. Results and discussion

The molecular formula of compound **1** was determinate as $C_{15}H_{20}O_4$ by HREIMS, and confirmed by ^{13}C NMR and DEPT analyses; examination of IR spectrum suggested the presence of carbonyl lactones (1774 and 1736 cm^{-1}) and a double bond (1639 cm^{-1}).

The ^1H NMR spectrum of **1** exhibited signals for two secondary methyl groups at δ 1.17 (d, $J = 7.8$ Hz) and 1.51 (d, $J = 6.3$ Hz), two oxymethine protons at δ 4.53 and δ 3.92, and a pair of broad one-proton singlets at δ 4.86 and δ 5.09 for an exocyclic methylene group. This was supported by analyses of the ^{13}C NMR spectrum, which exhibited a total of 15 signals. The multiplicity of each carbon was achieved by DEPT analyses, which revealed the presence of six methines (including two oxymethines at δ 82,9 and δ 76.4), three methylenes, two methyls at δ 10.9 and 20.8, and three quaternary carbons which included two carbonyl signals at δ 170.7 and δ 178.7. The assignment of the signals of the attached protons was obtained by the analysis of the HETCOR experiment.

The signal at δ 3.92 (H-6β) in the ^1H NMR spectrum showed the typical multiplicity of a *trans* guaianolide (Jakupovic et al.,1988). The magnitude of the vicinal coupling between H-6/H-5 ($J_{5,6} = 8.3$ Hz) and H-6/H-7 ($J_{6,7} = 9.8$ Hz) indicated an axial orientation for these protons.

The three-proton doublet at δ 1.51 was assigned to CH_3-15 which showed COSY cross-correlation peaks with H-4 at δ 4.53, and in turn with H-5 at δ 2.09. The signal corresponding to H-1 at δ 3.00 showed cross-peaks with: (i) H-5; (ii) H-2β H-2α at δ 2.60; (iii) an allylic interaction with H-14b (δ 4.86). A W long-range coupling was observed between H-14a (δ 5.09), and H-9α (δ 2.12).

The complete ^1H and ^{13}C NMR spectra assignments for **1** are given in Section 3.

The relative stereochemistry of compound **1** was determined by comparison of experimental constants ($J_{4,5} = 8.4$, $J_{5,6} = 8.3$, $J_{6,7} = 9.8$, $J_{7,11} = 7.9$ Hz), with those calculated using the generalized equation proposed by Haassnoot et al., 1980): ($J_{4,5} = 10.7$, $J_{5,6} = 8.0$, $J_{6,7} = 9.3$, $J_{7,11} = 8.5$ Hz) and by the phase sensitive NOESY experiment.

NOESY cross-correlation peak between the pairs H-4/H-6 and H-13/H-6, suggested the β configuration for all these protons. Furthermore, the observed NOEs correlation of H-1 with H-5 and H-2 α indicated that these protons are on the same face of the molecule and established the *cis* fusion of the A/B rings.

Based on the above evidence, the structure of **1** was elucidated as 11α H-dihydrozaluzanin E.

It is noteworthy that the dehydrogenated lactone at C-11/C-13, zaluzanin E, was already published by Spring et al. (1995), as the first report of a modified guaianolide.

Compound **2** had a molecular formula of $C_{14}H_{20}O_4$ as shown by HREIMS, with 14 carbon resonances being deleted in the ^{13}C NMR spectrum and by DEPT analyses.

The IR and ^{13}C NMR spectra showed the presence of a carbonyl γ-lactone (1766 cm^{-1} and δ_C 178.7) a carbonyl ketone (1714 cm^{-1} and δ_C 206.5) and a tertiary hydroxyl by the sharp peak at 3454 cm^{-1}. Its ^1H NMR spectral data showed signals of two methyls at δ 1.21 (d, $J = 6.7$ Hz) and δ 0.87 (s), as well as two oxymethine groups at δ 4.14 (dd, $J = 10.5$ Hz) and δ 3.92 (dd, $J = 11.3$, 4.7 Hz).

The proton 6β (δ 4.14) showed COSY interactions with H-5 (δ 2.48) and H-7 (δ 1.55). The large coupling constants values involving these three protons (10.5 Hz), clearly indicate their *trans* diaxial relationship. In the HMBC spectrum the C-7 signal at δ 51.7 showed long-range correlation with H-5, H-11, H-9β, and H-13.

The chemical shift of H-13 (d, δ 1.21), agrees with its α-configuration as reported for a 4-oxonoreudesmanolide synthesized by ozonolysis of dihydro- β-cyclotulipinolide (Doskotch and El-Feraly, 1970). Besides, the proposed stereochemistry was supported by the NOE cross-peaks between the pairs H-13/H-7α and H-13/H-8α in the NOESY experiment.

The carbonyl ketone (δ_C 206.5), was located at C-4, based on the HMBC long-range correlation with H-5, H-3 α,and H-2α.

The position of the OH group at C-1 was determined from the COSY correlation of H-1 (δ 3.92) with both H-2α, and H-2β protons. Additional evidence was given by the HMBC long-range correlation of H-1 with H-14 (δ_C 12.3), and C-3 (δ_C 38.7). The β configuration of the C-1 OH group was determined from the NOESY cross-peaks of H-1α with H-5α, H-2α, and H-9α, indicating that these four protons are located on the same face of the molecule.

The signal at δ_H 0.87, was assigned to H-14 due to its HMBC long range correlation with C-9, C-10, C-5, and C-1, and its β orientation was proposed on the basis of the observed NOEs between H-14/H-6β, H-14/H-2β, H-14/H-8β and H-14/9β.

The relative stereochemistry of **2** was also supported by molecular modeling, and by comparison of the ob-

served coupling constants using the Karplus generalized equation proposed by Haassnoot et al., 1980).

On the basis of these results, compound **2** was characterized as 1 β-hydroxy-4-oxo-11β H-4-noreudesman-6,12-olide.

To the present date sesquiterpene lactones, mainly guaianolides and eudesmanolides, have been isolated from several species of *Liabum* (Bohlmann et al., 1977, 1980a, 1984; Jakupovic et al., 1988b). Related guaianolides and eudesmanolides have also been found in other genus of the tribe Liabeae such as *Bishopanthus* (Singh et al., 1985), *Cacosmia* (Bohlmann et al., 1980b) and *Ferreyranthus* (Bohlmann et al., 1977; Jakupovic et al., 1988a).

The structures of the sesquiterpene lactones present in *M. polymnioides* shows that they are not only related to those isolated from species of genus *Liabum* but also to those obtained from other genus of the tribe Liabeae. Consequently, these results are in complete agreement with the placement of the genus *Liabum* and *Microliabum* in the tribe Liabeae.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were measured at 200.13 and 50.3 MHz in a Bruker AC 200 NMR spectrometer, using CDCl$_3$ as solvent and TMS as int standard. ^{1}H NMR of compound **2** was also recorded in a Bruker Advance at 400 MHz (including COSY-90, HSQC, HMBC, and NOESY spectra). HREIMS were recorded on a VG-7070 EHF spectrometer, and EIMS were obtained in a TRIO-2 VG MASS LAB spectrometer (70 eV). IR spectra were recorded in an IR-FT Bruker.

Chromatographic separations were achieved by CC and flash chromatography: silica gel (Merck 70–230 mesh, 230–400 mesh, ASTM). Analytical TLC was performed on precoated silica gel 60 F 254 plates (Merck).

3.2. Plant material

M. polymnioides was collected in April 1999, in highway 9, km 1614, Salta Province, Argentina. A voucher specimen is deposited in the Herbarium of the Museum of the Facultad de Ciencias Naturales, Universidad Nacional de Salta under No. 11279 (L. Novara and S. Bruno).

3.3. Extraction and isolation

The dried aerial parts of *M. polymnioides* (1.5 kg) were steeped in hexane, the rinsed material was extracted three times with CHCl$_3$ (3 × 4.0 l), under heat-ing. The resulting extract was conc. under reduced pressure and was dissolved in hot EtOH (400 ml), and a soln. of 4% Pb(CH$_3$COO)$_2$ was added. The EtOH solubles were evapd with the aq. soln. resulting in vacao extracted with CHCl$_3$. The organic solvent was evapd under reduced pressure, and the residue (4.5 g) was fractionated by CC (85 × 2.5 cm) on silica gel in a stepwise fashion using solvent mixtures of increasing polarity from C$_6$H$_6$ through CHCl$_3$, EtOAc, and Me$_2$CO.

Selected fractions were obtained and then submitted to additional flash chromatography as follows: fractions from the CHCl$_3$–EtOAc eluates (1:1; 2:3; 3:7) were combined (178.3 mg), and eluted with C$_6$H$_6$–EtOAc (9.5:0.5) to afford 8 mg of 4β, 14,11β, 13-tetrahydro-3-dehydrozaluzanin C.

Fraction CHCl$_3$–EtOAc 1:4 (634.0 mg) was purified using hexane–EtOAc 8:2 (120 ml) and C$_6$H$_6$–EtOAc 8:2 (400 ml); a total of eight subfractions (fraction size 65 ml) were collected and combined on the basis of analyses of their TLC profiles and IR spectra. Subfraction 5 (12.5 mg) was subjected to further s. gel cc with C$_6$H$_6$–EtOAc 9:1 as eluent to yield a mixture (4.0 mg) of magnolialide and artesin. Subfraction 6 (30 mg) after crystallization on MeOH afforded 11 αH-dihydrozaluzanin E (**1**). Subfraction 7 (15 mg) yielded (3 mg) scopoletin.

Fraction CHCl$_3$–EtOAc 1:9 (172 mg) eluted during the initial CC was subjected also to flash chromatography using hexane–EtOAc 4:1, 1:1 and EtOAc. From the EtOAc residue, 10 mg of pure 1 β-hydroxy-4-oxo-11β H-4-noreudesman-6,12-olide (**2**) were isolated.

3.4. 11αH-dihydrozaluzanin E (1)

Colourless needles from EtOAc, m.p. 147–148 °C. IR (KBr) v_{max} cm^{-1}: 1774, 1736 (>C=O lactone), 1639 (>C=C<), 1216, 1201 (>C–O). HREIMS m/z 264.1355 [M]$^+$, calc. for C$_{15}$H$_{20}$O$_4$, 264.1362. EIMS 70 eV m/z (rel. int.): 264 [M]$^+$ (3), 222 (18), 176 (95), 151 (40), 134 (100), 122 (58), 107 (55). ^{1}H NMR (200.13 MHz, CDCl$_3$): δ 1.17 (3H, d, $J_{13\beta,11\alpha}$ = 7.8 Hz, H-13), 1.41 (1H, m, H-8b), 1.51 (3H, d, $J_{15\alpha,4\beta}$ = 6.3 Hz, H-15), 1.96 (1H, m, H-8a), 2.09 (1H, m, H-5), 2.12 (1H, m, H-9α),2.54 (1H, m, H-9 β) 2.56 (1H, m, H-7) 2.60 (1H, m, H-2α) 2.69 (1H, m, H-2β), 2.71 (1H, dq, $J_{11\alpha,7\alpha}$ = 7.9 Hz, $J_{11\alpha,13\beta}$ = 7.8 Hz, H-11), 3.00 (1H, ddd, $J_{1\alpha,2\alpha,1\alpha,2\beta,1\alpha,5\alpha} \cong$ 8.4 Hz, H-1), 3.92 (1H, dd, $J_{6\beta,5a}$ = 8.3 Hz, $J_{6\beta,7a}$ = 9.8 Hz, H-6), 4.53 (1H, dd, $J_{4\beta,5\alpha}$ = 8.4 Hz, $J_{4 \ \beta,15\alpha}$ = 6.3 Hz, H-4), 4.86 (1H, brs, H-14b), 5.09 (1H, brs, H-14a). ^{13}C NMR (50.3 MHz, CDCl$_3$): δ 10.9 (q, C-13), 20.8 (q, C-15), 27.7 (t, C-8), 33.6 (t, C-2), 36.5 (t, C-9), 37.5 (d, C-1), 39.1 (d, C-11), 43.2 (d C-7), 46.0 (d, C-5), 76.4 (d, C-4), 82.9 (d, C-6), 114. 7 (t, C-14), 147.5 (s, C-10), 170.7 (s, C-3), 178.7 (s, C-12).

3.5. 1β-Hydroxy-4-oxo-11βH-4-noreudesman-6,12-olide (2).

Gum. IR (KBr) v_{max} cm^{-1}: 3454 (–OH), 1766 (>C=O lactone), 1714 (>C=O ketone), 1664 (>C=C<). HREIMS m/z 252.1358 [M]$^+$ calcd for $C_{14}H_{20}O_4$,252.1361. EIMS 70 eV, m/z (rel. int.): 252 [M]$^+$ (45), 220 (5), 193 (55), 164 (60), 149 (100). ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.87 (3H, s, H-14), 1.21 (3H, d, $J_{13a,11\beta}$ = 6.7 Hz, H-13), 1.42 (1H, m, H-9α), 1.50 (1H, m, H-8β), 1.55 (1H, m, H-7), 1.90 (1H, m, H-2β), 1.90 (1H, m, H-8α), 2.01 (1H, m, H-9β), 2.18 (1H, m, H-2α), 2.30 (1H, dq, $J_{11\beta,7\alpha}$ = 10.3 Hz, $J_{11\beta,13\alpha}$ = 6.7, H-11), 2.39 (1H, m, H-3α), 2.47 (1H, m, H-3β), 2.48 (1H, d, $J_{5\alpha,6\beta}$ = 10.5 Hz, H-5), 3.92 (1H, dd, $J_{1\alpha,2\beta}$ = 11.3 Hz, $J_{1\alpha,2\alpha}$ = 4.7 Hz, H-1), 4.14 (1H, dd, $J_{6\beta,5\alpha}$, $J_{6\beta,7\alpha}$ = 10.5 Hz, H-6). ^{13}C NMR (50.3 MHz, CDCl$_3$): δ 12.3 (q, C-14), 12.5 (q, C-13), 22.6 (t, C-8), 30.6 (t, C-2), 36.5 (t, C-9), 38.7 (t, C-3), 40.6 (d, C-11), 51.7 (d, C-7), 58.1 (d, C-5), 76.3 (d, C-6), 76.8 (d, C-1), 178.7 (s, C-12), 206.5 (s, C-4).

References

Bohlmann, F., Grenz, M., Zdero, C., 1977. Inhaltsstoffe der *Liabum*-gruppe. Phytochemistry 16, 285–286.

Bohlmann, F., Le Van, Ngo, 1977. Sesquiterpenlactone und polyine aus der gattung *Arctotis*. Phytochemistry 16, 487–488.

Bohlmann, F., Zdero, C., Bohlmann, R., King, R.M., 1980a. Neue sesquiterpene aus *Liabum-arten*. Phytochemistry 19, 579–582.

Bohlmann, F., Knoll, K.-H., Robinson, H., King, R.M., 1980b. Neue guaianolide aus *Cacosmia rugosa*. Phytochemistry 19, 599–602.

Bohlmann, F., Umemoto, K., Jakupovic, J., King, R.M., Robinson, H., 1984. Sesquiterpenes from *Liabum floribundum*. Phytochemistry 23, 1800–1802.

Cabrera, A.L., Freire, S.E., Ariza Espinar, L., 1999. Flora Fanerogámica Argentina. Asteraceae. Parte 13. Tribu VIII bis. *Liabeae* : 165–180.

Doskotch, R.W, El-Feraly, F.S., 1970. The structure of tulipinolide and epitulipinolide. cytotoxic sesquiterpenes from *Liriodendron tulipifera*. L. J. Org. Chem. 35, 1928–1936.

El-Feraly, F.S., Chan, Y.M., Benigni, D.A., 1979. Magnolialide: a novel eudesmanolide from the root bark of *Magnolia grandiflora*. Phytochemistry 18, 881–882.

Gutíerrez, D., 2003. Reincorporación del género *Liabum* (Asteraceae, Liabeae) a la flora Argentina y primer registro de *L. acuminatum* para el pais. Darwiniana 41, 55–59.

Haassnoot, C.A.G., de Leeuw, F.A.A.M., Altona, C., 1980. The relationship between proton–proton NMR coupling-constant and substituent electronegativities. 1. An empirical generalization of the Karplus equation. Tetrahedron 36, 2783–2792.

Jakupovic, J., Shuster, A., Bolhmann, F., Dillon, M.O., 1988a. Lumi-yomogin, ferreyrantholide, fruticolide and other sesquiterpene lactones from *Ferreyranthus fructicosus*. Phytochemistry 27, 1113–1120.

Jakupovic, J., Shuster, A., Bolhmann, F., Dillon, M.O., 1988b. Guaianolides and other constituents from *Liabum floribundum*. Phytochemistry 27, 1771–1775.

Marco, J.A., 1989. Sesquiterpene lactones from *Artemisia herba-alba* subsp. *herba-alba*. Phytochemistry 28, 3121–3126.

Robinson, H., Brettell, R.D., 1973. Studies in the Liabeae (Asteraceae) III. A new tribe, *Liabeae*. Phytologia 25, 404–407.

Robinson, H., Brettell, R.D., 1974. Studies in the Liabeae (Asteraceae) II. Preliminary survey of the genera. Phytologia 28, 43–63.

Robinson, H., 1983. A generic review of the tribe Liabeae (Asteraceae). Smithsonian Contr. Bot. 54, 1–69.

Robinson, H., 1990. A redelimitation of *Microliabum* Cabrera (Asteraceae: Liabeae). Syst. Bot. 15, 736–744.

Rydberg, P.A., 1927. (Carduales) Carduaceae. Liabeae. Neurolaeneae. Senecioneae. N. Am. Flora 34, 289–360.

Singh, P., Jakupovic, J., Bohlmann, F., 1985. Highly oxygenated guaianolides from *Bishopanthus soliceps*. Phytochemistry 24, 2110–2112.

Spring, O., Buschmann, H., Vogler, B., Shilling, E.E., Spraul, M., Hoffmann, M., 1995. Sesquiterpene lactone chemistry of *Zaluzania grayana* from on-line lc-nmr measurements. Phytochemistry 39, 609–612.