Sesquiterpene Lactones from *Lychnophora ericoides*

Humberto T. Sakamoto,[†] Douglas Flausino,[‡] Eduardo E. Castellano,[§] Christian B. W. Stark,[⊥] Paul J. Gates,[⊥] and Norberto P. Lopes*[,‡]

Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, CEP 14.040-903, Ribeirão Preto-SP, Brazil, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Via do Café S/N, CEP 14.040-903, Ribeirão Preto-SP, Brazil, Instituto de Física e Química de São Carlos, Universidade de São Paulo, C.P. 369, CEP 13.560, São Carlos-SP, Brazil, and Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, U.K.

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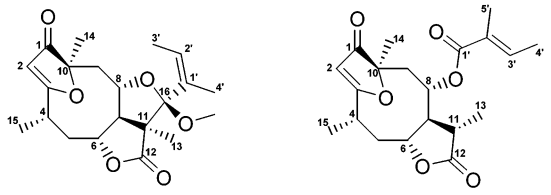
Two new sesquiterpene lactones, (4*S*,6*R*,7*S*,8*S*,10*R*,11*S*,16*R*)-1-oxo-3(10),8(16)-diepoxy-16-methylprop-1*Z*-enyl-16-methoxygermacra-2-en-6(12)-olide (**1**) and (4*S*,6*R*,7*S*,8*S*,10*R*,11*S*)-1-oxo-3,10-epoxy-8-angeloyloxygermacra-2-en-6(12)-olide (**2**), were isolated from *Lychnophora ericoides*. Their structures, including absolute configuration, were established by spectroscopic methods, including single-crystal X-ray analysis. Monitoring the furanoheliangolide metabolism of *L. ericoides* revealed an increase in biosynthesis during the plant flowering period.

Species of *Lychnophora* (Asteraceae) have emerged as a rich source of furanoheliangolides, which often exhibit trypanocidal and antiinflammatory activities.[1−4] Some of these compounds modulate the antiinflammatory process by inhibition of transcription factor NF-κB by selectively alkylating its p65 subunit. The most potent furanoheliangolides normally belong to the goyazensolide class of compounds. They possess two functionalities in the form of an α-methylene-γ-lactone and an $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl group.[5] The intramolecular stereospecific Michael addition of the goyazensolide moiety produces an eremantholide, normally resulting in the loss of antiinflammatory activity.[5] The species *Lychnophora ericoides* Mart., popularly known as "Arnica", is used in folk medicine as an analgesic and antiinflammatory agent.[6] Microscopic investigation of the surface of *L. ericoides* leaves has revealed the presence of glandular trichomes that store sesquiterpene lactones, similar to other species of Asteraceae.[7,8] Commercial preparations (by natural botanical companies) from intact leaves of *L. ericoides* consist of a complex mixture of compounds from the leaves.[9] Until now, there has existed insufficient information about the chemical composition of such extracts. There has only been phytochemical investigations of the total powdered aerial parts of the plants which report the presence of furanoheliangolides and flavonoids.[10] As part of our program devoted to the investigation of commercial phytomedicines, we prepared and analyzed a commercial glandular extract of *L. ericoides*. The pure isolated standards were used to monitor the most important biosynthetic conversions over the period of one year at different time intervals. Also, the optimal period to collect the plant leaves (i.e., the period with highest amounts of antiinflammatory sesquiterpenes lactones present) was defined.

The extract yielded 18 furanoheliangolides (see Experimental Section), three flavonoids, and four triterpenoids in addition to two new sesquiterpenes (**1** and **2**). Compounds **1** and **2** were crystallized, and their molecular formulas were found to be $C_{21}H_{28}O_6$ and $C_{20}H_{26}O_6$, respectively. The IR spectrum of **1** showed absorptions at 1771 cm^{-1} (γ-lactone), 1705 and 1588 cm^{-1} (furanone), which are indicative of a furanoheliangolide.[1,3,10] The ^1H and ^{13}C NMR spectra of **1** revealed the presence of a 16β-methoxy group that was characterized by a singlet at δ 3.04 (^1H NMR) and at δ 49.1 (^{13}C NMR), in addition to the characteristic eremantholide signals. These chemical shifts of the 16β-methoxy group have previously been observed.[11] The ^1H NMR spectrum of **1** also exhibited signals at δ 1.37 (3H, d, J = 7.1 Hz, H-15), 2.05 (1H, ddd, J = 10.7, 11.0, 13.7 Hz, H-5α), 2.41 (1H, br dd, J = 2.1, 13.7 Hz, H-5β), 4.08 (1H, br dd, J = 6.6, 11.0 Hz, H-6), and 3.02 (1H, m, H-4), which are indicative of a 4,5-dihydro derivative, resulting in an additional chiral center at C-4 (Figures 1 and 2). X-ray analysis of compound **1** was used to finally elucidate its structure as (4*S*,6*R*,7*S*,8*S*,10*R*,11*S*,16*R*)-1-oxo-3(10),8(16)-diepoxy-16-methylprop-1*Z*-enyl-16-methoxygermacra-2-en-



1 **2**

Figure 1. New sesquiterpene lactones from *L. ericoides*.

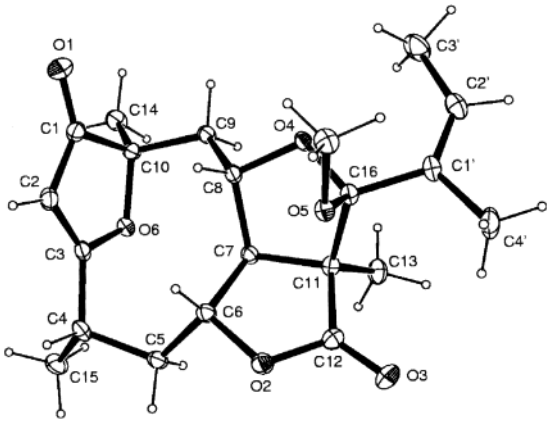


Figure 2. ORTEP projection of compound **1**.

* To whom correspondence should be addressed. Tel: 0055 16 6024168. Fax: 00 55 16 6332960. E-mail: npelopes@fcfrp.usp.br.
[†] Faculdade de Filosofia Ciências e Letras de Ribeirão Preto.
[‡] Faculdade de Ciências Farmacêuticas de Ribeirão Preto.
[§] Instituto de Física e Química de São Carlos.
[⊥] University of Cambridge.

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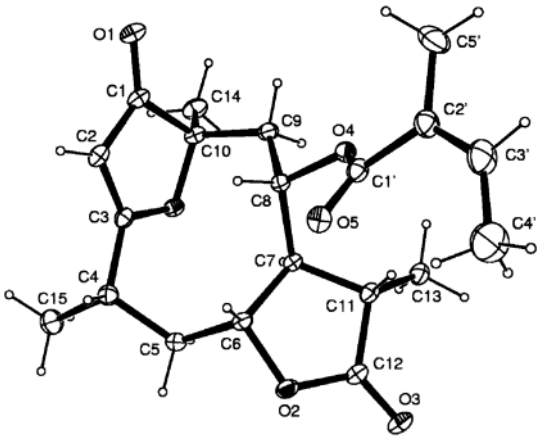

Figure 3. ORTEP projection of compound **2**.

6(12)-olide. Figure 2 shows the ORTEP projection of this new eremantholide.

The IR spectrum of **2** also showed characteristic bands for furanone and γ-lactone groups. The ^{1}H NMR spectrum of **2** was similar to that of 4,5-dehydro-15α-lychnopholide.[12,13] However, the vinylic H-13a and H-13b resonances of 4,5-dehydro-15α-lychnopholide were missing in **2** and there was a methyl doublet at δ 1.31 (J = 6.6 Hz) indicating an unusual reduction of the $\Delta^{11(13)}$ exocyclic double bond, thus producing a new chiral center at C-11. The relative stereochemistry of the C-4, C-6, C-7, C-8, and C-10 centers could be deduced by comparison of both chemical shifts and coupling constants with previously reported data.[14] The ^{13}C NMR and HMQC spectra were in full agreement with the proposed structure of **2**. Moreover, X-ray analysis was used to define the absolute configuration of the new stereocenter (C-11), and compound **2** was unequivocally confirmed as (4*S*,6*R*,7*S*,8*S*,10*R*,11*S*)-1-oxo-3,10-epoxy-8-angeloyloxygermacra-2-en-6(12)-olide. Figure 3 shows the ORTEP projection of compound **2**.

Centratherin, 15-desoxigoyazensolide, lychnopholide, eremantholide C, and 15-desoxieremantholide C were the major sesquiterpene lactones isolated. Centratherin, 15-desoxigoyazensolide, and lychnopholide have an α-methylene-γ-lactone and an α,β,γ,δ-unsaturated carbonyl group, which are responsible for the NF-κB inhibition resulting in antiinflammatory effects. Centratherin and the minor compound goyazensolide also have a α,β,γ,δ-unsaturated acyl side-chain group that is considered to be a third reaction center.[5] These two compounds are the most potent antiinflammatory members of this class of molecules,[5] supporting the use of *L. ericoides* in folk medicine. Finally, a detailed investigation of the metabolite production by HPLC[7,8] revealed that the highest degree of biosynthesis of the five major compounds was during the flowering period (see Supporting Information).

Experimental Section

General Experimental Procedures. All solvents were redistilled. Merck silica gel 60 (230−240 mesh) and silica gel 60 GF_{254} were used for vacuum flash chromatography, circular chromatography (chromatotron), and thin-layer chromatography. HPLC (Shimadzu LC-6A apparatus) was performed using a UV detector (266 nm) and a Shim-Pack ODS (5 μm) column. The optical rotation was recorded on a JASCO DIP-370 polarimeter (*c* g/mL) set to the Na wavelength (589 nm). The UV spectra were recorded on a Hitachi U-3501 diode array spectrophotometer, while the IR spectra were obtained on a Nicole Protégé 460. All ^{1}H NMR spectra were recorded at 500 MHz and the ^{13}C NMR spectra at 125 MHz (Bruker DPX-300). HRESIMS analyses were performed on a Bruker Daltonics 4.7 T Fourier transform ion cyclotron resonance mass spectrometer (BioApex II).

Plant Material. The leaves of *Lychnophora ericoides* were collected from Ibiraci, Minas Gerais, Brazil, in February 1999 and were identified by João Semir, University of Campinas, Brazil, where a voucher specimen is deposited (NPL-122).

Extraction and Isolation. Intact air-dried leaves (479 g) were washed with dichloromethane at room temperature and furnished 44.8 g of the glandular extract. A part of this extract (20.0 g) was submitted to reversed-phase (C_{18}) vacuum-liquid chromatography, eluted with MeOH−H_2O (1:1, 6:4, 7:3, and 100%), and yielding 0.9, 9.4, 7.5, and 0.75 g of material, respectively. The methanolic fraction was analyzed by IR, GC−MS, and ^{1}H and ^{13}C NMR, showing the presence of the triterpenes fridelanol, lupeol, and α- and β-amyrin as the major compounds. The MeOH−H_2O (1:1) fraction was submitted to preparative HPLC (ODS-Shimadzu, 5.0 × 250 mm column, 50:50 MeOH−H_2O, λ = 280 nm, flow 8 mL/min), yielding goyazensolide (17 mg) and centratherin (230 mg). Fraction 6:4 (8 g) was submitted to flash column chromatography eluted with hexane−EtOAc at increasing polarities, producing five pooled fractions. Fraction A (2 g) was purified by column chromatography ($CHCl_3$−MeOH, 97:3), yielding eremantholide A (20 mg), 16α-(1-methylprop-1*Z*-enyl)eremantholide (230 mg), lichnopholide (200 mg), and a mixture of pinocembrin and pinobankisin (200 mg). Fraction B (223 mg) was submitted to preparative TLC (CH_2Cl_2−acetone, 8:2), yielding 4,5-dihydro-[8α-(2-methylbut-2*Z*-enoxy)]-15α-desoxygoyazensolide (45 mg), 4,5-dihydro-15α-lichnopholide (10 mg), and eremantholide C (30 mg). Portions of fractions C (500 mg), D (1.0 g), and E (650 mg) were purified by preparative HPLC (ODS-Shimadzu, 5.0 × 250 mm column, MeOH−H_2O 60:40, λ = 280 nm, flow 8 mL/min), yielding 4,5-dihydro-15α-desoxygoyazensolide (19 mg), **2** (20 mg), centratherin (200 mg), goyazensolide (150 mg), 4,5-dihydro-15β-eremantholide C (50 mg), 4,5-dihydro-15α-eremantholide C (300 mg), 4,5-dihydro-15β-[16α(1-methylprop-1*Z*-enyl)]eremantholide (12 mg), 4,5-dihydro-15α-[16α(1-methylprop-1*Z*-enyl)]eremantholide (63 mg), 15-hydroxy[16α-(1-methylprop-1*Z*-enyl)eremantholide (10 mg), and 16α-(1-methylprop-1*Z*-enyl)eremantholide (230 mg). The 7:3 fraction (700 mg) was submitted to preparative HPLC (ODS-Shimadzu, 5.0 × 250 mm column, 70:30 MeOH−H_2O, λ = 280 nm, flow 8 mL/min) and resulted in the isolation of 4,5-dihydro-15β-desoxygoyazensolide (17 mg), 15-hydroxy[16α-(1-methylprop-1*Z*-enyl)]eremantholide (10 mg), **1** (20 mg), 16α-(1-methylprop-1*Z*-enyl)- and 16β-(methoxy)eremantholide (35 mg), and pinostrombin (40 mg).

(4*S*,6*R*,7*S*,8*S*,10*R*,11*S*,16*R*)-1-Oxo-3(10),8(16)-diepoxy-16-methylprop-1*Z*-enyl-16-methoxygermacra-2-en-6(12)-olide (1): transparent crystals; $[\alpha]^{20}_D$ +140° (*c* 0.04 EtOH); UV (MeOH) λ_{max} (log ϵ) 212 (3.75), 262 (4.05) nm; IR (CH_3Cl) ν_{max} 2977, 2935, 1771, 1705, 1588, 1446, 1380, 1233, 1179, 1147, 1132 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.61 (1H, s, H-2), 3.02 (1H, m, H-4), 2.05 (1H, ddd, J = 10.7, 11.0, 13.7 Hz, H-5α), 2.41 (1H, br dd, J = 2.1, 13.7 Hz, H-5β), 4.08 (1H, br dd, J = 6.6, 11.0 Hz, H-6), 2.52 (1H, dd, J = 4.6, 6.6 Hz, H-7), 3.70 (1H, ddd, J = 2.0, 4.6, 12.0 Hz, H-8), 2.11 (1H, dd, J = 12.0, 13.4 Hz, H-9α), 2.46 (1H, dd, J = 2.0, 13.4 Hz, H-9β), 1.22 (3H, s, H-13), 1.46 (3H, s, H-14), 1.37 (3H, d, J = 7.1 Hz, H-15), 5.69 (1H, dq, J = 1.1, 7.1 Hz, H-2′), 1.75 (3H, dd, J = 1.1, 7.1 Hz, H-3′), 1.73 (3H, d, J = 1.1 Hz, H-4′), 3.04 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 205.7 (s, C-1), 193.6 (s, C-3), 175.9 (s, C-12), 130.1 (s, C-1′), 128.7 (d, C-2′), 112.5 (s, C-16), 104.1 (d, C-2), 90.0 (s, C-10), 79.5 (d, C-6), 77.2 (d, C-8), 66.6 (d, C-7), 61.8 (s, C-11), 49.1 (q, OCH_3), 44.4 (t, C-9), 41.3 (t, C-5), 30.9 (d, C-4), 21.8 (q, C-13), 21.2 (q, C-14), 20.8 (q, C-15), 16.6 (q, C-3′), 14.9 (q, C-4′), HRESIMS *m/z* 399.1789 [M + Na]$^+$; (calcd for $C_{21}H_{28}O_6Na$ 399.1778); *anal.* C 67.11%, H 7.40%, calcd for $C_{21}H_{28}O_6$ C 67.02%, H 7.45%.

X-ray Crystal Structure Analysis of Compound 1. An octant of Bragg intensities was measured at 293(2) K on a CAD4 Enraf-Nonius diffractometer equipped with graphite-monochromatized Cu Kα radiation (λ = 1.54178 Å). Altogether,

2063 reflections were measured up to $\theta = 67°$, all of which were unique ($R_\sigma = 0.015$).The intensities were corrected by Lorentz, polarization, and absorption effects. The space group was $P2_12_12_1$ with unit cell parameters $a = 11.578(6)$ Å, $b = 11.711(5)$ Å, and $c = 14.994(3)$ Å. The structure was solved by direct methods with SHELXS-97.[15] The H atoms were positioned stereochemically and were refined with fixed individual displacement parameters [U_{iso}(H) = $1.3U_{eq}$(C) or $1.3U_{eq}$($C_{methoxy}$)] using a riding model with C−H = 0.99 Å. The model was refined by full-matrix least-squares procedures on F^2 using SHELXL-97 to a value of $R_1 = 0.0376$ for 245 parameters and 2065 reflections with $I > 2\sigma(I)$.[15] The program WINGX was used to analyze and prepare the data for publication.[16] ORTEP-3 for Windows[17] was used to prepare the molecular graphics.[17,18]

(4*S*,6*R*,7*S*,8*S*,10*R*,11*S*)-1-Oxo-3,10-epoxy-8-angeloyloxygermacra-2-en-6(12)-olide (2): transparent crystals; $[\alpha]^{20}_D$ +55° (*c* 0.05 EtOH); UV (MeOH) λ_{max} (log ϵ) 218 (3.92), 262 (4.04) nm; IR (CH_3Cl) ν_{max} 2975, 2932, 1772, 1707, 1600, 1454, 1372, 1229, 1190, 1154, 1133 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.73 (1H, s, H-2), 3.11 (1H, m, H-4), 2.06 (1H, br d, $J = 12.8$ Hz, H-5α), 2.39−2.31 (1H, m, H-5β), 4.39 (1H, br d, $J = 7.6$, Hz, H-6), 2.39−2.31 (1H, m, H-7), 4.83 (1H, br d, 11.1 Hz, H-8), 2.15 (1H, br d, $J = 14.4$ Hz, H-9α), 2.39−2.31 (1H, m, H-9β), 2.39−2.31 (1H, m, H-11), 1.31 (3H, d, $J = 6.6$ Hz, H-13), 1.47 (3H, s, H-14), 1.34 (3H, d, $J = 8.4$ Hz, H-15), 6.18 (1H, q, $J = 7.1$ Hz, H-3′), 1.98 (3H, d, $J = 7.1$ Hz, H-4′), 1.83 (3H, s, $J = 1.1$ Hz, H-5′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.9 (s, C-1), 193.1 (s, C-3), 177.2 (s, C-12), 166.5 (s, C-1′), 141.5(s, C-3′), 126.2 (s, C-2′), 105.3 (d, C-2), 89.5 (s, C-10), 81.8 (d, C-6), 67.6 (d, C-8), 59.2 (d, C-7), 46.5 (t, C-9), 42.6 (t, C-5), 38.4 (s, C-11), 33.2 (d, C-4), 20.6 (q, C-14), 20.3 (s, C-5′) 19.4 (q, C-15), 16.2 (q, C-13), 15.9 (q, C-4′); HRESIMS *m*/*z* $[M + Na]^+$ 385.1638 (calcd for $C_{20}H_{26}O_6Na$ 385.1621); *anal*. C 66.19%, H 7.21%, calcd for $C_{20}H_{26}O_6$ C 66.29%, H 7.18%.

X-ray Crystal Structure Analysis of Compound 2. The X-ray measurements were made on an Enraf-Nonius Kappa-CCD diffractometer with graphite-monochromated Mo Kα (λ = 0.71073 Å) radiation at 293(2) K. Data were collected up to 26.73° in θ. The final unit cell parameters were based on all 11 516 reflections measured, 4180 of which were independent (R_{int} = 0.0264). The intensities were corrected by Lorentz, polarization, and absorption effects. The space group was $P2_12_12_1$ with unit cell parameters $a = 7.6723(4)$ Å, $b = 13.2748(7)$ Å, and $c = 19.606(1)$ Å. Data collections were made using the *COLLECT* program,[19] and integration and scaling of the reflections were performed with the HKL Denzo-Scalepack system of programs.[20] The structures were solved by direct methods with SHELXS-97.[15] The H atoms were positioned stereochemically and were refined with fixed individual displacement parameters [U_{iso}(H) = $1.3U_{eq}$(C) or $1.3U_{eq}$($C_{methoxy}$)] using a riding model with C−H = 0.99 Å. The model was refined by full-matrix least-squares procedures on F^2 using SHELXL-97 to a value of $R_1 = 0.0437$ for 241 parameters and 4180 reflections with $I > 2\sigma(I)$. The program WINGX was used to analyze and prepare the data for publication.[16] ORTEP-3 for Windows[17] was used to prepare the molecular graphics.[17,18]

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Supporting Information Available: Graphic of the seasonal variation of the goyazensolide and eremantholide lactones in *Lychnophora ericoides*. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Crystallographic data of compounds **1** and **2** have been deposited at the Cambridge Crystallographic Data Center, Cambridge U.K., under the reference numbers CCDC-200250 and CCDC-200251. These data may be obtained on application to the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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