Studies on the Constituents of *Leonurus sibiricus* L.

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Two new furanoditerpene-lactones, LS-1 (1) and LS-2 (2), were isolated along with four known furanoditerpene-lactones 3, 4, 5 and 6 from the aerial part of *Leonurus sibiricus* L. The structures of the new compounds were determined by spectroscopic means. Compounds 1–6 isolated here exhibited moderate cytotoxic activity (IC_{50} =50–60 μ g/ml) against leukemia cells (L 1210) in tissue culture.

Key words *Leonurus sibiricus* L.; Labiatae; furanoditerpene-lactone; cytotoxic activity

During the course of our studies on the isolation of biologically active substances from South American medicinal plants, we have studied the aerial part of *Leonurus sibiricus* L. (Labiatae), called erva-macae in Brazil, which has been used as a folk medicine for the treatment of bronchitis.¹⁾ We found that the ethanol extract of this plant exhibited cytotoxic activity against leukemia cells (L 1210) in tissue culture. Up to the present, there have been several reports on the isolation of furanoditerpene-lactone from other plants belonging to *Leonurus* species, such as *L. dysophylla*,²⁾ *L. nepetaefolia*,³⁾ and *L. dubia*.⁴⁾ In this paper, we report the isolation and structural elucidation of two new furanoditerpene-lactones, LS-1 (1) and LS-2 (2), along with four known diterpene-lactones, leonotinin (3),⁵⁾ leonotin (4),⁶⁾ dubiin (5)⁴⁾ and nepetaefuran (6)⁷⁾ from *L. sibiricus* L. (Fig. 1).

Results and Discussion

LS-1 (1) did not show a molecular ion peak but exhibited an $[M-H_2O+1]^+$ ion peak at m/z 347 in its FAB-MS spectrum. The ¹H-NMR spectrum of 1 indicated the presence of two tertiary methyls [δ 0.92 and 1.37 (3H each, s)], an oxymethine [δ 5.05 (1H, dt, J =6.4, 10.1 Hz)] and an oxymethylene [δ 4.26 and δ 4.38 (1H each, ABq, J =7.9 Hz)] groups, and three vinyl protons [δ 6.33 (1H, dd, J =0.9, 1.5 Hz), 7.27 (1H, dd, J =0.9, 1.5 Hz) and 7.37 (1H, t, J =1.5 Hz)], suggesting the presence of a β -substituted furan ring. Acetylation of 1 with acetic anhydride-pyridine gave a monoacetate [chemical ionization (CI)-MS: m/z 407 $[M+1]^+$] which exhibited proton signals [δ 4.43 and 4.66 (1H each, d, J =9.5 Hz)] due to oxymethylene protons, which had shifted downfield in the lower fields by 0.17 and 0.28 ppm as compared with those (δ 4.26, 4.38) of 1, indicating the presence of a primary alcohol moiety. The ¹³C-NMR spectrum of 1 showed very close similarity with that of leonotinin (3), except for the C-8, C-9 and C-17 signals (Table 2). These spectral data suggest that 1 is a hydroxymethyl derivative of 3, and the structural differences between 1 and 3 must be around C-8, C-9 and C-17. In the heteronuclear multiple bond connectivity (HMBC) spectrum of 1, the hydroxymethylene proton signals at δ 4.26 and 4.38 (H-17) showed cross peaks with the carbon signals at δ 73.7 (C-8), 94.9 (C-9) due to oxygen-bearing quaternary carbons, in addition to a methylene carbon signal at δ 35.9 (C-7). Thus, the partial structure around C-7–C-9 was confirmed to be as shown in Fig. 1.

The stereostructure of 1 was determined to be as shown in

Fig. 1, and the conformation of ring B should be a boat form by analysis of the nuclear Overhauser effect (NOE) difference spectrum which showed interactions between the following protons: H-5 and H-6, H-19; H-6 and H-5, H-7 α , H-17, H-19; H-7 α and H-6, H-17; H-11 and H-1 β , H-20; H-17 and H-5, H-6. The boat form conformation of ring B was further confirmed by the observation of W-shaped long range coupling between H-7 β and H-17 signals. Thus, the structure of LS-1 (1) was determined to be 1, as shown in Fig. 1.

LS-2 (2) showed a molecular ion peak at m/z 389 $[M+1]^+$ in its FAB-MS spectrum. The ¹H-NMR spectrum of 2 indicated the presence of a tertiary methyl [δ 1.14 (3H, s)], an oxymethine [δ 5.19 (1H, m)], an oxymethylene [δ 4.10 and 4.47 (1H each, ABq, J =11.3 Hz)], an exomethylene [δ 4.95 and 5.14 (1H each, d, J =1.0 Hz)] groups, and a β -substituted furan ring [δ 6.29 (1H, dd, J =0.9, 1.8 Hz), 7.26 (1H, dd, J =0.9, 1.5 Hz) and 7.39 (1H, dd, J =0.9, 1.5 Hz)]. The ¹³C-NMR spectral data of 2 were very similar to those of dubiin (5) and nepetaefuran (6), except for the C-8 and C-17 signals (Table 2). In the HMBC spectrum of 2, the exomethylene proton signals (δ 4.95, 5.14) showed cross peaks with the carbon signals at δ 76.8 (C-9) and 35.7 (C-7) due to an oxygen-bearing quaternary carbon and methylene carbon, suggesting the presence of an exomethylene group at C-8. This was further confirmed by observation of the long range couplings between exomethylene proton signals and the H-7 signals (δ 2.10, 2.54). All spectral data, including HMBC and nuclear Overhauser effect spectroscopy (NOESY) for 2, were explained satisfactorily by the structure of 2 (Tables 1, 2).

Compounds 1–6 isolated here exhibited moderate cytotoxic activity (IC_{50} =50–60 μ g/ml) against leukemia cells (L 1210) in tissue culture.

Fig. 1

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Table 1. ^1H -NMR Spectral Data for **1**, **2** [500 MHz, CDCl_3 , δ]

H	1	2
1	1.30 m, 1.57 m	1.88m, 2.00m
2	1.48 m, 1.68 m	1.81m (2H)
3	1.50 m, 2.01 m	1.55m, 1.84 m
5	2.59 d (6.4)	2.17 dd (2.4, 3.4)
6	5.05 dt (6.4, 10.1)	5.19 q (3.4)
7	2.10 ddd (0.9, 6.4, 15.0), 2.54 dd (10.1, 15.0)	2.44 dd (3.4, 14.3), 2.90 dd (3.4, 14.3)
11	1.86 m, 2.06 m	1.70 m, 2.13 m
12	2.77 m, 3.01 m	2.56 m (2H)
14	6.33 dd (0.9, 1.5)	6.29 dd (0.9, 1.8)
15	7.37 t (1.5)	7.39 t (1.8)
16	7.27 dd (0.9, 1.5)	7.26 dd (0.9, 1.8)
17	4.26 d (7.9), 4.38 dd (0.9, 7.9)	4.95 d (1.0), 5.14 d (1.0)
19	1.37 s (3H)	1.14 s (3H)
20	0.92 s (3H)	4.10 dd (1.2, 11.3), 4.47 dd (2.4, 11.3)
$\text{CH}_3\text{C}=\text{O}$		2.04 s (3H)

The numbers in parentheses are *J* values in Hz.

Table 2. ^{13}C -NMR Data for **1**, **2** [125 MHz, CDCl_3 , δ]

C	1	2
1	29.0	31.8
2	17.5	20.5
3	28.3	39.8
4	42.2	41.2
5	44.3	46.9
6	74.1	69.9
7	35.9	35.7
8	73.7	142.7
9	94.9	76.8
10	39.6	41.6
11	29.6	30.2
12	20.1	19.0
13	125.4	124.3
14	111.1	110.6
15	142.7	143.3
16	138.8	138.7
17	78.3	115.0
18	182.7	175.9
19	24.4	22.5
20	21.0	75.2
$\text{C}=\text{O}$		170.1
CH_3		20.9

Experimental

The ^1H - and ^{13}C -NMR spectra were measured on a JEOL JNM- α 500 spectrometer in CDCl_3 containing tetramethylsilane (TMS) as an internal standard. The mass spectra were obtained on a JEOL JMS-D 300 instrument. Diaion HP-20 and Wako-gel C-200 were used for column chromatography. The optical rotations were measured on a JASCO DIP-370 polarimeter. Senshu pack (Silica 4201-N, $10\phi \times 200$ mm) column was used for HPLC.

The plant material was purchased from Laboratório Farmaervas Ltda. in São Paulo, Brazil, and was identified by Pharmacist Gilbert Rubens Biancalana.

Isolation of Compounds 1–6 The dried aerial parts of *Leonurus sibiricus* L. (3.5 kg), collected in São Paulo, Brazil, were extracted with ethanol (4.01×3) under ultrasonication. The ethanol solution was concentrated *in vacuo* to give 56.0 g of concentrate. The ethanol extract was suspended into H_2O , and then extracted successively with hexane and AcOEt. The AcOEt extract was concentrated *in vacuo* to give 18.6 g of oily material which was chromatographed on a Diaion HP-20 (Nippon Rensui) column eluted successively with 2 l each of H_2O (Fr. 1, 2.1 g), $\text{H}_2\text{O}:\text{MeOH}=1:1$ (Fr. 2, 0.8 g), $\text{H}_2\text{O}:\text{MeOH}=1:3$ (Fr. 3, 4.4 g), $\text{H}_2\text{O}:\text{MeOH}=1:4$ (Fr. 4, 2.2 g), $\text{H}_2\text{O}:\text{MeOH}=1:9$ (Fr. 5, 2.3 g), MeOH (Fr. 6, 3.1 g) and AcOEt (Fr. 7, 2.5 g). Fr. 3 (2.0 g) was chromatographed on a silica gel column eluted with hexane:AcOEt=3:2 to give eight fractions (Fr. 3-1—Fr. 3-8). Then, Fr. 3-5 was purified by HPLC (hexane:AcOEt=3:2, flow rate: 3.0 ml/min) to give **1** (8.8 mg, retention time: 20.5 min). Fr. 3-2 was purified by HPLC (hexane:AcOEt=2:1, flow rate: 4.0 ml/min) to give **2** (7.7 mg, retention time: 13.0 min) and **5** (9.9 mg, retention time: 12.0 min). Fr. 3-3 was purified by HPLC (hexane:AcOEt=2:1, flow rate: 4.0 ml/min) to give **4** (66.8 mg, retention time: 16.0 min). Fr. 3-6 was separated by HPLC (hexane:AcOEt=2:1, flow rate: 4.0 ml/min) to give **3** (15.6 mg, retention time: 27.0 min). Fr. 3-8 was further purified by HPLC (hexane:AcOEt=3:2, flow rate: 3.0 ml/min) to give **6** (11.9 mg, retention time: 29.0 min). Compounds **3**—**6** were identified as leonotinin (**3**), leonotin (**4**), dubiin (**5**) and nepetaefuran (**6**), respectively, in comparison of their spectral data with those described in the literature.

Compound **1**: Amorphous. $[\alpha]_{\text{D}}^{20} +22.0^\circ$ ($c=0.6$, MeOH). ^1H - and ^{13}C -NMR see Tables 1 and 2. FAB-MS m/z : 347 $[\text{M}-\text{H}_2\text{O}+1]^+$. HR-FAB-MS m/z : 347.1867 (Calcd for $\text{C}_{20}\text{H}_{28}\text{O}_6-\text{H}_2\text{O}+1$: 347.1858).

Acetylation of 1 A mixture of **1** (2 mg), pyridine (0.5 ml) and acetic anhydride (0.5 ml) was stirred at room temperature for 1 h. The usual work-up gave a monoacetate (2 mg). CI-MS m/z : 407 $[\text{M}+1]^+$. ^1H -NMR (270 MHz, CDCl_3) δ : 0.97 (3H, s), 1.30 (1H, m), 1.37 (1H, m), 1.38 (3H, s), 1.50 (1H, m), 1.70 (1H, m), 1.88 (1H, m), 2.0—2.2 (4H, m), 2.12 (3H, s, -OAc), 2.57 (1H, m), 2.61 (1H, m), 2.79 (1H, m), 3.18 (1H, m), 4.43 (1H, d, $J=9.4$ Hz), 4.66 (1H, dd, $J=1.8, 9.4$ Hz), 5.06 (1H, m), 6.31 (1H, s), 7.26 (1H, s), 7.38 (1H, s).

Compound **2**: Amorphous. $[\alpha]_{\text{D}}^{20} +18.0^\circ$ ($c=0.5$, MeOH). ^1H - and ^{13}C -NMR see Tables 1 and 2. FAB-MS m/z : 389 $[\text{M}+1]^+$. HR-FAB-MS m/z : 389.1959 (Calcd for $\text{C}_{22}\text{H}_{28}\text{O}_6+1$: 389.1964).

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