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DITERPENOIDS FROM *LEONURUS SIBIRICUS*

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Key Word Index—*Leonurus sibiricus*; Labiatae; labdane diterpenoids; furanic and prefuranic derivatives.

Abstract—From the aerial part of *Leonurus sibiricus* three new labdane diterpenoids have been isolated. Their structures were established mainly by spectroscopic means and shown to be 3β, 19-diacetoxy-15,16-epoxy-6β,9α-hydroxylabda-13(16),14-dien-7-one(leosibirin), 3β,19-diacetoxy-15,16-epoxy-7,9α-dihydroxylabda-13(16), 14-dien-6-one(isoleosibirin) and 8-acetoxy-9α, 13; 15,16-diepoxy-7-ketolabda-14-en-19,6β-olide(leosibiricin).

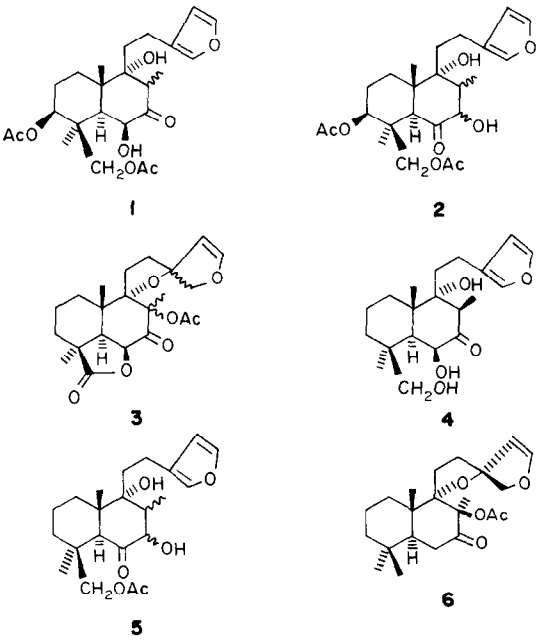
INTRODUCTION

In our search for new natural diterpenoids in the Labiatae family [1–4], we have examined the aerial part of *Leonurus sibiricus*. From this plant three new labdane derivatives have been isolated. Their structures have been shown to be 3β, 19-diacetoxy-15, 16-epoxy-6β,9α-dihydroxylabda-13(16), 14-dien-7-one (**1**, leosibirin), 3β,19-diacetoxy-15,16-epoxy-7,9α-dihydroxylabda-13(16),14-dien-6-one (**2**, isoleosibirin), and 8-acetoxy-9α,13;15,16-diepoxy-7-ketolabda-14-en-19,6β-olide (**3**, leosibiricin).

RESULTS AND DISCUSSION

The more polar chromatographic fraction obtained from the acetone extract of *L. sibiricus* showed a unique spot on TLC, but its ^{1}H NMR spectrum revealed the presence of a mixture of prefuranic diterpenes. Therefore this mixture was treated with Amberlite IR-120 (H^+ form) yielding leosibirin(**1**) and isoleosibirin (**2**), both oils, after CC.

Leosibirin(**1**) had the molecular formula $C_{24}H_{34}O_8$ and its structure was easily established from its ^{1}H and ^{13}C NMR data (Tables 1 and 2) compared with those of the very related compound ballotenol(**4**) [5]. Leosibirin contained a β-substituted furan ring, two acetoxyl groups on C-19 (axial, AB system centred at δ 4.79, $J_{gem} = 11.5$ Hz) and on C−3 in equatorial configuration (axial geminal proton at 4.58, doublet of doublets, $J_{3\alpha,2\beta} = 8.5$ Hz, $J_{3\alpha,2\alpha} = 6$ Hz), two tertiary methyl groups, a secondary methyl group (d, J = 7 Hz) whose vicinal proton (3.53, q, $J = 7$ Hz) was indicative of the partial structure –C(H)–CH(CH$_3$)–CO–, and finally a secondary alcohol function (geminal proton at 4.34, d, $J = 3$ Hz) which must be axially oriented and placed between a carbonyl group and a methyne group. In particular, the ^{13}C NMR data of compound **1** (Table 2) are identical with those reported for ballotenol(**4**) [5] taking into account the shifts caused by acetylation of its C-19 hydroxy group and by introduction of an equatorial acetoxy function on C-3 [6]. The closeness of these data allowed us to attribute tentatively the 8β-CH$_3$ axial configuration in leosibirin as in the case of ballotenol.



Structure **2** was unambiguously assigned to isoleosibirin by comparison of its ^{1}H NMR and ^{13}C NMR data with those of the closely related ballotenol(**4**) [5] and isoballotenol acetate(**5**) [5] (Tables 1 and 2). The ^{1}H NMR spectrum of **2** was identical with the spectrum of leosibirin(**1**) except for the signals of H-5 (singlet at δ 3.29) and H-7 (doublet at 3.97, J = 11 Hz, with a *trans* relationship between H-7 and H-8). In this case, as with isoballotenol acetate, the possibility of a boat conformation prevents a positive assignment of the configurations at C-7 and C-8.

It is possible that isoleosibirin(**2**) may be an artifact arising from leosibirin(**1**), already at the level of prefuranic compounds, by a ketol isomerization. As in the case of ballotenol acetate and isoballotenol acetate, the driving force is probably the relief of the diaxial interactions between 6β–OH, 10β–Me and 4β–CH$_2$OAc.

Table 1. ^{1}H NMR chemical shifts* and coupling constants (in Hz) of compounds **1–6**

	1	**4**†	**2**	**5**	**3**	**6**
H-3	4.58 *dd* $J_{3\alpha,2\beta}=8.5$ $J_{3\alpha,2\alpha}=6$		4.53 *dd* $J_{3\alpha,2\beta}=9$ $J_{3\alpha,2\alpha}=6$			
H-5		2.21 *d* $J_{5\alpha,6\alpha}=3$	3.29 *s*	3.20 *s*	2.80 *d*‡ $J_{5\alpha,6\alpha}=6$	
H-6	4.34 *d* $J_{6\alpha,5\alpha}=3$	4.49 *d* $J_{6\alpha,5\alpha}=3$			5.00 *d*‡ $J_{6\alpha,5\alpha}=6$	
H-7			3.97 *d* $J_{7,8}=11$	3.90 *d* $J_{7,8}=9$		
H-8	3.53 *q*‡ $J_{8,17}=7$	3.81 *q* $J_{8,17}=7$				
H-14	6.32 *m*	6.38 *m*	6.33 *m*	6.27 *m*	5.13 *d*‡ $J_{14,15}=3$	5.11 *d* $J_{14,15}=2.5$
H-15	7.40 *m*	7.50 *m*	7.43 *m*	7.35 *m*	6.55 *d*‡ $J_{15,14}=3$	6.41 *d* $J_{15,14}=2.5$
H-16	7.30 *m*	7.40 *m*	7.32 *m*	7.25 *m*	4.55 *d*‡ $J_{gem}=10.5$	4.48 *d* $J_{gem}=10.5$
H'-16					4.17 *d*† $J_{gem}=10.5$	4.02 *d* $J_{gem}=10.5$
3H-17	1.12 *d*‡ $J_{17,8}=7$	1.29 *d* $J_{17,8}=7$	1.27 *d* $J_{17,8}=7$	1.28 *d* $J_{17,8}=7$	1.83 *d*	1.34 *s*
3H-18	1.05 *s*	1.05 *s*	0.92 *s*	0.90 *s*	1.30 *s*	0.87 *s*
2H-19	4.73/4.85 *d* $J_{gem}=11.5$	3.43/4.40 *d* $J_{gem}=12$	4.77/4.83 *d* $J_{gem}=12$	4.48/4.88 *d* $J_{gem}=15$		0.83 *s*(3H)
3H-20	1.45 *s*	1.62 *s*	1.17 *s*	1.08 *s*	0.72 *s*	1.15 *s*
$OCOCH_3$	2.05/2.10	2.05/2.10		2.05 *s*	2.17 *s*	2.05 *s*

*In δ ppm relative to int. TMS and in $CDCl_3$ solution.
†In pyridine.
‡Assignments confirmed by double resonance experiments.

Table 2. ^{13}C NMR chemical shifts of compounds **1**, **3**, **4**, **6** (ppm from int. TMS, $CDCl_3$ solution).

	1	**4**	**3**	**6**
C-1	35.1 *t*	35.5 *t*	36.1 *t*	35.9 *t*
C-2	23.9 *t*	18.9 *t*	17.7 *t*	18.2 *t*
C-3	79.5 *d*	39.3 *t*	29.1 *t*	41.4 *t*
C-4	43.4 *s*	44.2 *s*	41.4 *s*	33.1 *s*
C-5	49.7 *d*	51.4 *d*	46.9 *d*	50.4 *d*
C-6	74.8 *d*	74.9 *d*	75.9 *d*	38.5 *t*
C-7	209.2 *s*	213.0 *s*	199.9 *s*	205.6 *s*
C-8	45.8 *d*	46.0 *d*	89.2 *s*	87.6 *s*
C-9	81.2 *s*	82.9 *s*	95.9 *s*	97.0 *s*
C-10	42.7 *s*	40.1 *s*	40.8 *s*	43.2 *s*
C-11	31.1 *t*	34.5 *t*	30.9 *t*	33.8 *t*
C-12	21.4 *t*	22.0 *t*	29.5 *t*	28.7 *t*
C-13	124.6 *s*	126.0 *s*	92.8 *s*	94.5 *s*
C-14	110.5 *d*	111.4 *d*	105.6 *d*	106.9 *d*
C-15	142.9 *d*	143.3 *d*	148.6 *d*	148.1 *d*
C-16	138.5 *d*	138.8 *d*	77.3 *t*	80.3 *t*
C-17	8.3 *q*	8.7 *q*	17.4 *q*	15.9 *q*
C-18	21.8 *q*	27.8 *q*	26.5 *q*	32.2 *q*
C-19	64.8 *t*	67.0 *t*	179.1 *s*	21.3 *q*
C-20	19.4 *q*	20.1 *q*	23.5 *q*	17.7 *q*
$OCOCH_3$	170.2 *s*	—	168.3 *s*	168.9 *s*
	170.5 *s*	—	—	—
$OCOCH_3$	21.0 *q*	—	21.9 *q*	21.6 *q*
	21.1 *q*	—	—	—

From the less polar chromatographic fraction obtained from the extract of *L. sibiricus* we have isolated another diterpenic compound, leosibiricin(**3**), the structure of which was established as follows.

Leosibiricin was an extremely unstable compound with a molecular formula $C_{22}H_{28}O_7$. Its IR spectrum showed no hydroxyl absorption but typical bands for a γ-lactone (1770 cm^{-1}), acetoxyl (1730 and 1260 cm^{-1}) and ketone (1720 cm^{-1}) groups. The ^1H NMR spectrum of leosibiricin(**3**) (Table 1) was consistent with a prefuranic partial structure (doublets at δ 5.13 and 6.55, $J = 3$ Hz, and an AB system at 4.17 and 4.55, $J_{gem} = 10.5$ Hz) [7] and also with a C-19/C-6 lactone ring with a keto group in the C-7 position (6α-H 5.00, *d*, $J_{6\alpha,5\alpha} = 6$ Hz; 5α-*H* 2.80, *d*, $J_{5\alpha,6\alpha} = 6$ Hz) of a labdane skeleton [8]. Moreover, the presence of a tert. acetoxy group and of three C-CH_3 singlets suggested that position C-8 is the site of attachment of this acetoxy group. On the other hand, the ^{13}C NMR spectrum of leosibiricin(**3**) (Table 2) was also consistent with the proposed structure [7, 8].

A careful study was undertaken of the ^1H NMR and ^{13}C NMR spectra of leosibiricin (**3**) and pregaleopsin(**6**), a prefuranic diterpenoid whose structure [7] is firmly established. The differences of the chemical shifts observed for C-11, C-12, C-13, C-14 and C-16 could be interpreted on the basis of a reversed configuration at C-13, i.e. (13*S*)-configuration for leosibiricin whereas pregaleopsin has the (13*R*)-configuration. However, the small ^1H NMR spectral differences between the C-13 epimeric series and the lack of NOE experiments due to the shortage of leosibiricin, do not allow us to attribute the configurations at C-8 and C-13. The high field value (δ 0.72) attributed to the C-20 methyl group arises from the strong deshielding effect of the C-19/C-6 lactone and of the C-7 ketone function [8]. Also the value (δ 1.83) of the C-17 methyl group differs from the one of pregaleopsin, thus casting further doubts on the configurations at C-8 and C-13.

From a taxonomic point of view, it is important to note that *Leonurus sibiricus* contains only labdanic diterpenoids, whereas from other *Leonurus* species only clerodanic diterpenoids have been isolated [9–11].

EXPERIMENTAL

For general experimental details see refs [1–4, 7]. Seeds of *L.sibiricus* L. were supplied by the Botanic Garden of Jena, D.D.R. and cultivated in the Botanic Garden of Palermo, Italy. Plants were collected in July 1980, and voucher specimens were deposited in the herbarium of this centre.

Extraction and isolation of the diterpenoids. Dried and finely powdered aerial parts (270 g) were extracted with Me_2CO (3 l.) at room temp. for 1 week. After filtration the solvent was evapd yielding a gum which was subjected to dry CC over Si gel (400 g, Merck No. 7734, deactivated with 15% H_2O). Elution with petrol–EtOAc mixtures gave leosibiricin (**3**, less polar component, 80 mg) and an inseparable mixture of prefuranic diterpenoids (170 mg). A suspension of this mixture, EtOH (5 ml) and Amberlite IR-120 (H^+ form, 100 mg) was stirred at room temp. for 2 hr. The soln was filtered, the solvent removed and the residue (140 mg) subjected to dry CC over Si gel. Elution with petrol–EtOAc (1:1) yielded isoleosibirin (**2**, less polar component, 8 mg) and leosibirin (**1**, 106 mg).

Leosibirin (**1**). An oil, $[\alpha]_D^{20°} - 0.7°$, $[\alpha]_{365}^{20°} - 24.7°$ ($CHCl_3$, *c* 0.30); IR ν_{max}^{NaCl} cm^{-1}: 3490, 3150, 3140, 2960, 2890, 1725, 1715, 1505, 1465, 1380, 1370, 1260, 1160, 1030, 940, 915, 880. ^1H NMR (90 MHz, $CDCl_3$) see Table 1. ^{13}C NMR (25.2 MHz, $CDCl_3$) see Table 2. EIMS (direct inlet, 10 eV) *m/z* (rel.int.): 450 $[M]^+$(12), 432 (40), 394 (27), 393 (42), 391 (65), 390 (26), 375 (21), 349 (30), 333 (100), 331 (34), 304 (26), 304 (26), 267 (31), 249 (25), 181 (75), 150 (21), 133 (7), 123 (42), 96 (10), 82 (10), $C_{24}H_{34}O_8$ MW 450.

Isoleosibirin (**2**). An oil, $[\alpha]_D^{28°} + 7.1°$ ($CHCl_3$, *c* 0.63); IR $\nu_{max}^{CCl_4}$ cm^{-1} 3620, 3570, 3490, 2980, 2940, 2880, 1740, 1713, 1500, 1470, 1400, 1380, 1370, 1250, 1090, 1045, 1030, 975, 950, 910, 878. ^1H NMR (90 MHz, $CDCl_3$) see Table 1. EIMS (direct inlet, 75 eV) *m/z* (rel. int.): 450 $[M]^+$ (2), 432 (5), 390 (5), 349 (4), 333 (6), 289 (5), 267 (12), 249 (10), 225 (14), 209 (10), 207 (10), 189 (12), 181 (46), 170 (12), 167 (16), 149 (34), 121 (93), 107 (26), 105 (12), 95 (48), 93 (24), 81 (100). $C_{24}H_{34}O_8$ MW 450.

Leosibiricin (**3**). An oil, $[\alpha]_D^{19°} + 33°$ ($CHCl_3$, *c* 0.088); IR ν_{max}^{NaCl} cm^{-1}: 3150, 3140, 2950, 2890, 1770, 1730, 1720, 1505, 1430, 1365, 1260, 1120, 1040, 940, 880. ^1H NMR (90 MHz, $CDCl_3$) see Table 1. ^{13}C NMR (25.2 MHz, $CDCl_3$) see Table 2. EIMS (direct inlet, 75 eV) *m/z* (rel.int.): 404 $[M]^+$ (20), 362 (90), 296 (38), 249 (45), 221 (60), 193 (100), 109 (81), 81 (79). $C_{22}H_{28}O_7$ MW 404.

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REFERENCES

1. Martinez-Ripoll, M., Fayos, J., Rodriguez, B., Garcia-Alvarez, M. C., Savona, G., Piozzi, F., Paternostro, M. P. and Hanson, J. R. (1981) *J. Chem. Soc. Perkin Trans.* 1, 1186.
2. Eguren, L., Perales, A., Fayos, J., Savona, G., Paternostro, M. P., Piozzi, F. and Rodriguez, B. (1981) *J. Org. Chem.* **46**, 3364.
3. Bruno, M., Savona, G., Pascual, C. and Rodriguez, B. (1981) *Phytochemistry* **20**, 2259.
4. Savona, G., Garcia-Alvarez, M. C. and Rodriguez, B. (1982) *Phytochemistry* **21**, 721.
5. Savona, G., Piozzi, F., Hanson, J. R. and Siverns, M. (1977) *J. Chem. Soc. Perkin Trans.* 1, 497.
6. Wenkert, E., Raju, M. S., Ceccherelli, P., Curini, M., Tingoli, M. and Pellicciari, R. (1980) *J. Org. Chem.* **45**, 741.
7. Rodriguez, B. and Savona, G. (1980) *Phytochemistry* **19**, 1805, and references therein.
8. Savona, G., Piozzi, F., Hanson, J. R. and Siverns, M. (1976) *J. Chem. Soc. Perkin Trans.* 1, 1607.
9. Laonigro, G., Lanzetta, R., Parrilli, M., Adinolfi, M. and Mangoni, L. (1979) *Gazz. Chim. Ital.* **109**, 145.
10. Tschesche, R. and Plenio, H. U. (1973) *Chem. Ber.* **106**, 2929.
11. Brieskorn, C. H. and Hofmann, R. (1979) *Tetrahedron Letters* 2511.