Journal of Natural Products
Vol. 58, No. 10, pp. 1543-1554, October 1995

DETAILED ^{1}H- AND ^{13}C-NMR INVESTIGATIONS OF SOME DITERPENES ISOLATED FROM *LEONURUS PERSICUS*

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ABSTRACT.—Five new labdane diterpenoids, leopersin A [**1**], 8-deacetoxyleopersin A [**2**], leopersin B [**3**], 15-*epi*-leopersin B [**4**], and 4β-hydroxymethylpregaleopsin [**5**] were isolated from the aerial parts of *Leonurus persicus*, together with the known compounds pregaleopsin [**7**], its rearrangement product, galeopsin [**8**], and the optical antipode of (+)-leosibiricin [**9**]. Leopersin B [**3**] and 15-*epi*-leopersin B [**4**] were obtained as a C-15 epimeric mixture and their structures were elucidated on the basis of spectroscopic and chemical evidence. 4β-Hydroxymethylpregaleopsin [**5**] was isolated as its *p*-bromobenzoate derivative [**6**] and its absolute configuration established by single-crystal X-ray crystallographic analysis. Complete nmr assignments for the known compounds were made using extensive 1D and 2D homonuclear and heteronuclear ^{1}H-^{13}C-nmr spectroscopic methods. For pregaleopsin [**7**], the ^{1}H- and ^{13}C-nmr data were reassigned, primarily on the basis of results of 2D nmr INADEQUATE measurements.

Leonurus is a widespread genus of the family Lamiaceae, which is represented by more than 20 species in the world's flora. Various preparations of some plants of this genus have been used for the treatment of cardiovascular diseases, and for hypotensive, sedative, and uterotonic effects in both Europe and China (1–6). In Turkey, *L. cardiaca* is used for its cardiotonic, expectorant, astringent, and euphoric effects (7). Chemically, this genus is probably best known for its alkaloid components, particularly for the guanidine alkaloid, leonurine, which is the main uterotonic component of several *Leonurus* species (2,3,8). Other chemical constituents comprise flavonoids, iridoids, phenylpropanoid glycosides, proteins, sterols, and fatty acids, as well as clerodane- and/or labdane-type diterpenoids, some of which show anti-platelet aggregation activity (9,10). In a continuation of our phytochemical investigations on *Leonurus* species found in the flora of Turkey (11,12), *Leonurus persicus* Boiss. has been examined. The current report describes the isolation and structure elucidation of the new compounds **1–5** and the complete nmr data and stereochemical assignments of the known diterpenoids **7–9**, isolated from the petroleum ether extract of the aerial parts of the plant.

RESULTS AND DISCUSSION

The petroleum ether extract of the air-dried and powdered aerial parts of *Leonurus persicus* was separated using normal-phase vlc. Further purification of the resulting fractions by normal- or reversed-phase hplc led to the isolation of the eight compounds, **1–5** and **7–9**.

Compound **1** was found to have the molecular formula $C_{22}H_{32}O_6$, by hreims and ^{13}C-nmr spectroscopy. The presence of resonances for three carbonyl carbons in the ^{13}C-nmr

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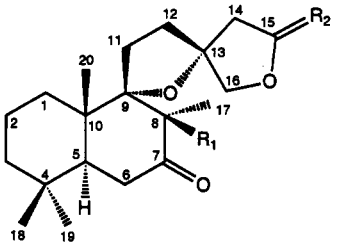
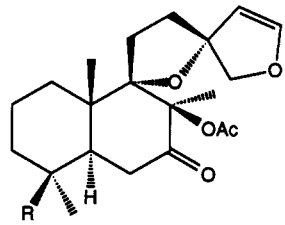
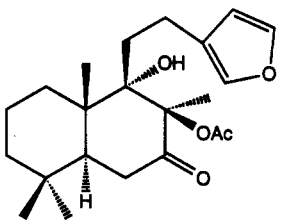


1 R_1=OAc, R_2=O
2 R_1=OH, R_2=O
3 R_1=OAc, R_2=Hα, OHβ
4 R_1=OAc, R_2=Hβ, OHα



5 R=CH_2OH
6 R=CH_2O-CO-C_6H_4Br
7 R=CH_3



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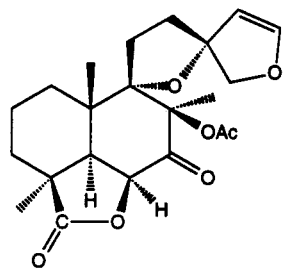

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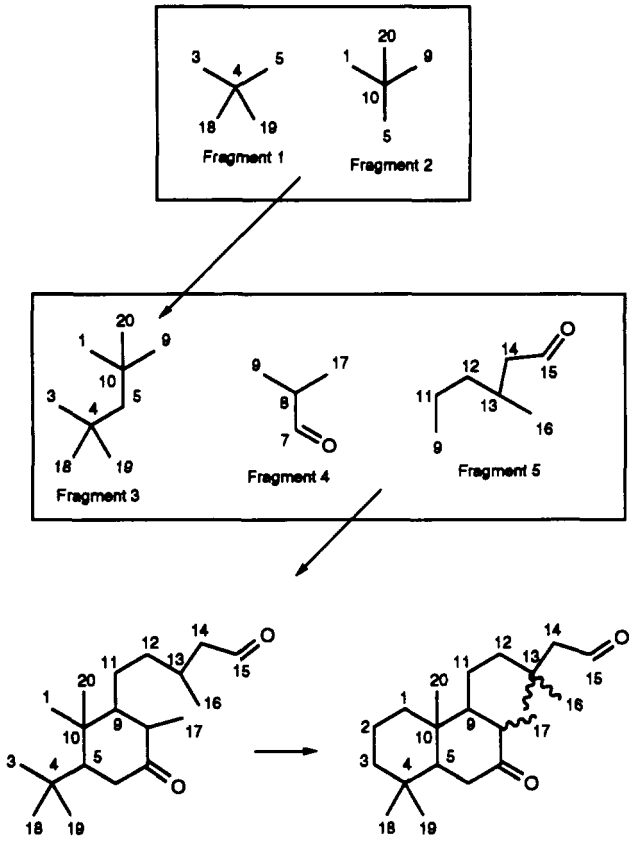
spectrum of **1** and the absence of resonances for any other multiple bonds indicated **1** to be a tetracyclic compound. Its ir spectrum contained absorption bands characteristic of γ-lactone (1790 cm^{-1}), ester (1740 and 1245 cm^{-1}), and keto (1725 cm^{-1}) functionalities. The ^1H- and ^{13}C-nmr spectra exhibited resonances associated with an acetoxyl moiety (δ_H 2.07 s; δ_C 21.3 q, 169.0 s), a keto (s, 205.5 ppm) group, an ester (s, 173.5 ppm) function, four tertiary methyl (δ_H 0.83 s, 0.89 s, 1.18 s, 1.32 s; δ_C 15.7 q, 18.0 q, 21.5 q, 32.7 q) groups, eight CH_2 groups and one CH group, as well as resonances for six fully substituted carbon atoms. It was also evident from these data that the oxygen-containing functionality within **1** must include an epoxy function, probably a furan (s, 87.0 ppm; s, 98.0 ppm), and an apparent γ-lactone (δ_C 42.8 t, 77.7 t, 173.5 s) function.

These results, combined with the results of 2D shift-correlated ^1H-^{13}C nmr HMQC (J=150 Hz), HMBC (J=8.3 Hz) and ^1H-^1H COSY90 experiments, allowed the planar structure of **1** to be established. Thus, from the HMBC and HMQC spectra of **1**, it was possible to develop seven molecular fragments (Scheme 1). The first fragment was established by long-range ^1H-^{13}C correlations observed from C-3 (t, 41.2 ppm), C-4 (s, 34.4 ppm), and C-5 (d, 50.2 ppm) to H_3-18 and H_3-19 (δ 0.83 s and δ 0.89 s). Further, the ^1H-^{13}C couplings of both C-18 to H_3-19 and C-19 to H_3-18, and the fact that both proton resonances for these groups were singlets, allowed C-18 and C-19 to be positioned at C-4. HMBC correlations were observed between H_3-20 (δ 1.18 s) and C-1 (t, 33.4 ppm), C-5, C-9 (s, 98.0 ppm) and C-10 (s, 43.5 ppm). As Me-20 was a singlet resonance in the ^1H-nmr spectrum, it must reside at either C-9 or C-10. Because C-9 is oxygen-bearing (s, 98.0 ppm) and further connected to H_2-11 (see below), it was thus concluded that C-20 is attached to C-10, hence establishing the second fragment of the molecule. As C-5 was common to both of these fragments, they could be connected to generate fragment 3. The fourth fragment was composed of C-7, C-8, C-9, and C-17, as shown in Scheme 1, on the basis of long-range correlations observed from C-7 (s, 205.5 ppm), C-8 (s, 87.2 ppm) and C-9 to H_3-17 (δ 1.32, s).

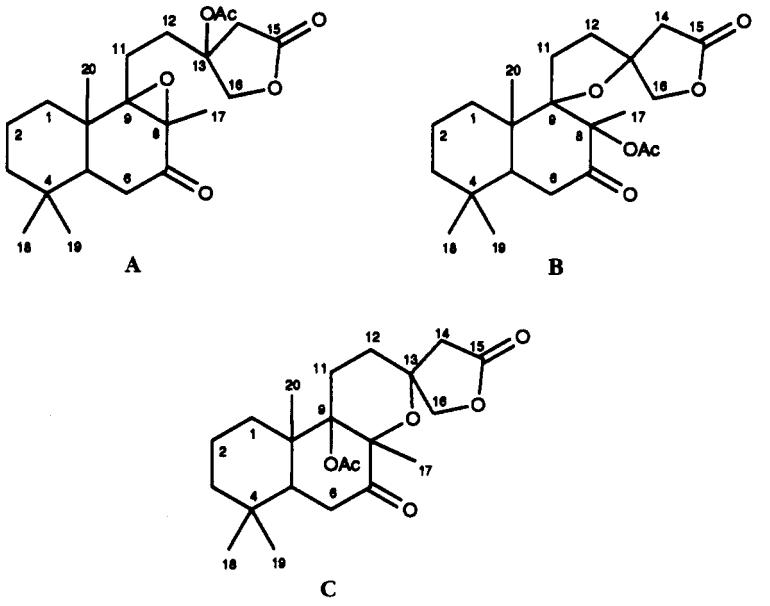


SCHEME 1. Fragments of **1** deduced from 2D nmr measurements.

Further ^{1}H-^{13}C couplings in the HMBC spectrum between C-9, H_2-11; C-11, H_2-12; C-12, H_2-11 and H_2-16; C-13, H_2-12; C-13, H_2-14; C-13, H_2-16, and C-15, H_2-14 enabled fragment 5 to be deduced. Fragments 3–5 could be united to generate fragment 6 on the basis of the common C-9 atom. This left two methylene groups, an acetoxyl function, and two oxygen atoms to be positioned.

From the results contained in the ^{1}H-^{1}H COSY90 spectrum of **1** it was evident that the methylene protons with resonances at δ 2.50 (dd, J=11.8 and 14.0 Hz) and δ 2.37 (dd, J=2.6 and 11.8 Hz), which showed geminal coupling and also coupled to H-5 (δ 1.65, J=2.6 and 14.0 Hz, dd), had to reside at C-6 and thus could be attributed to H_{ax}-6 and H_{eq}-6, respectively. The final CH_2 group was positioned between C-1 and C-3 on the basis of observed inter-proton coupling between H_2-1, H_2-2, and H_2-3, and gave rise to fragment 7 (Scheme 1).

The remaining oxygen-containing functionalities were positioned in the following way: as the ^{13}C-nmr resonance for C-15 (173.5 ppm) was that of an ester (lactone), one of the oxygen atoms had to be attached to it. This O atom must bond to C-16 as this is the only possibility of generating a γ-lactone. The remaining two structural elements (O and OAc) could be positioned in three possible ways (see planar structures **A**, **B**, and **C**, Scheme 2). The one containing the three-membered ring epoxide (**A**) was immediately eliminated as a possibility because the ^{13}C-nmr shifts for this moiety were not consistent with the data. To distinguish between the other two possibilities (**B** and **C**), it was



SCHEME 2. Possible planar structures for **1**.

necessary to evaluate to the NOESY spectrum of **1**, which clearly showed cross-peaks between the H_3-17 and H_2-16 protons, which could only occur in **B**.

The relative stereochemistry of the five chiral centers within **1** was proposed on the basis of the results of a NOESY measurement. Diagnostic nOes between H_{ax}-6/H_3-18, H_{ax}-6/H_3-20, H_{ax}-6/H_3-OCOCH_3, H_3-18/H_3-20, H_3-20/H_2-11, and H_3-20/H_3-OCOCH_3 indicated them to be all on the same side (β), while interactions between H-5/H_{eq}-6, H-5/H_3-19, H_{eq}-6/H_3-17, and H_{eq}-6/H_3-19 revealed these to be on the opposite side (α). These data, in addition to the magnitude of the coupling constant between H-5 and H_{ax}-6 ($J_{5,6ax}$=14.0 Hz), established the α and axial configuration of H-5 and also the trans junction of the A and B rings. The nOe observed between the H_3-17 and H_2-16 protons supported the configuration shown in **1**. The trivial name of leopersin A for **1** is proposed.

Compound **2** was deduced to have a molecular formula of $C_{20}H_{30}O_5$ by hreims. Its ^{13}C-nmr spectrum showed the presence of resonances for two carbon-oxygen double bonds and for no further sp^2 or sp hybridized carbon atoms; **2** was thus a tetracyclic compound. Its ir spectrum contained absorption bands typical for hydroxyl (3440 cm^{-1}), γ-lactone (1780 cm^{-1}), and keto (1715 cm^{-1}) functionalities, similar to those found in **1**. A close comparison of the ^{1}H- and ^{13}C-nmr data of **2** with those of **1** revealed the only significant differences between the two molecules to be in the region around C-8; C-7 (215.9 (s) for **2**, 205.5 (s) for **1**); C-8 (80.2 (s) for **2**, 87.2 (s) for **1**) and C-17 (27.1 (q) for **2**, 15.7 (q) for **1**, H_3-17 δ 1.22 (s) for **2**, δ 1.32 (s) for **1**). These differences were consistent with the presence of a hydroxyl group at C-8, instead of the acetoxyl function found in **1**. The configuration at C-8 was shown to be the same as that of **1** on the basis of nOe data. Thus, compound **2** is 8-deacetoxyleopersin A.

Compound **3–4** had a molecular formula of $C_{22}H_{34}O_6$, as determined by accurate mass measurement. Of the six degrees of unsaturation, two were present as carbon-oxygen double bonds. As there were no other multiple bonds, **3–4** had to be tetracyclic. From the ^{1}H- and ^{13}C-nmr data, it was evident that there were far too many resonances, in both spectra, to be consistent with the calculated molecular mass and, as the "purity"

of **3–4** was not in doubt, it seemed likely that **3–4** was an equilibrium mixture of two isomers. All of the data were treated as such and attempts to resolve the structure/structures were made on this basis. The ^{1}H- and ^{13}C-nmr data indicated the presence of duplicate resonances for four tertiary methyl groups (3H each, δ_H 0.83×2; 0.88×2; 1.17, 1.19; 1.31, 1.35; all s; δ_C 15.7, 15.9; 18.1×2; 21.5×2, and 32.8, 32.9; all q), eight CH_2 groups, two CH groups as well as six C atoms, including carbonyl (205.1 and 206.1 ppm, both s) and acetoxyl (δ_H 2.07×2 s; δ_C 21.4×2 q; 169.0 s, 169.1 s) functions. Detailed examination of the ^{1}H- and ^{13}C-nmr data indicated **3–4** to be closely related to **1**, in particular from C-1 to C-13. The major difference between the compounds appeared to be due to the presence of a secondary hydroxyl function in **3–4**, as determined by ^{1}H- and ^{13}C-nmr, ir (ν 3435 cm^{-1}) and ms data, instead of the lactone carbonyl function found in **1**. From the 2D ^{1}H-^{1}H and ^{1}H-^{13}C nmr spectra (HMQC, J=150 Hz), it was possible to assign the AB quartets at δ 3.65, 4.16, and δ 3.93, 4.02 (J=8.7 Hz, d) to H_2-16. The methylene protons at C-14 coupled geminally and further coupled to H-15. Thus, the signals at δ 5.39 (1H, J=3.1 and 5.7 Hz, dd) and δ 5.53 (1H, J=5.0 Hz, d) could be assigned to H-15. These data clearly indicated this center to be the one that was epimeric. To confirm this deduction, **3–4** was oxidized to yield **1**. Thus, **3–4** was established as the 1:1 equilibrium mixture of leopersin B [**3**] and 15-*epi*-leopersin B [**4**].

Compound **5** could not be obtained in a pure form, even after many attempts at purification using normal- or reversed-phase hplc with various solvent systems, and was eventually isolated as its *p*-bromobenzoate derivative [**6**]. Based on its hreims, the molecular formula of **6** was determined to be $C_{29}H_{35}O_7Br$ (corresponding to $C_{22}H_{32}O_6$ for **5**), revealing the presence of twelve degrees of unsaturation, five of which came from the *p*-bromobenzoate. The resonances observed in the ^{13}C-nmr spectrum for four carbon-carbon double bonds and three carbon-oxygen bonds showed the molecule to be pentacyclic. Closer examination of these data revealed **5** (**6**) to be very similar to both **1** and **3–4**, the obvious differences between them being the presence of a primary hydroxyl function and a $\Delta^{14,15}$ double bond in **5** (**6**) and the absence of the oxygen-containing functionality of **1** and **3–4** at C-15. On the basis of nOe cross-peaks observed between H_2-18/H_{ax}-6, H_2-18/H_3-20 and H_2-18/H_3-OCOCH_3, the primary alcohol was assigned to C-18.

As high quality crystals of **6** could be grown readily, a low-temperature single-crystal X-ray crystallographic analysis of this compound was undertaken in order to determine its absolute configuration. The crystals of **6** were grown from a $CHCl_3$ solution and the crystal lattice incorporated molecules of $CHCl_3$ which are disordered about a crystallographic two-fold axis, thus giving a ratio of **6** to solvent molecules of 2:1. The determination of the structure presented special difficulties because of the extremely long *c*-axis of the unit cell, which required careful data collection conditions in order to minimize reflection overlap (see Experimental). These difficulties have reduced the accuracy of the atomic parameters to slightly below normal, however, the structure of the molecule is clearly defined and the absolute configuration has been confidently determined (Figure 1). The bond lengths and angles fall within the normally expected ranges.

Together with the new compounds, the previously reported diterpenes, **7–9**, were also obtained and characterized. Compound **7** (pregaleopsin) (13) was isolated as the major component of the petroleum ether extract. Its ^{1}H- and ^{13}C-nmr assignments in the literature (13) were found to be either ambiguous or incorrect and so detailed nmr studies of **7** employing 2D ^{1}H-^{1}H (COSY), ^{1}H-^{13}C (HMQC, J=150 Hz), and HMBC (J=8.3 Hz) experiments were performed (Tables 1 and 2). These data were not sufficient to assign completely all of the ^{1}H- and ^{13}C-nmr resonances and so an INADEQUATE

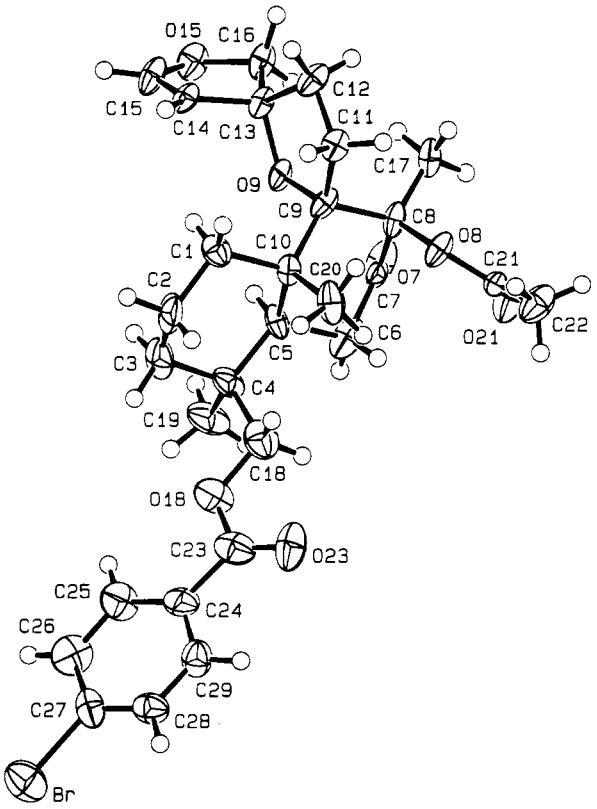


FIGURE 1. ORTEP drawing of **6** at 115°K, with 50% ellipsoids. H atoms are represented by circles of arbitrary radius.

spectrum (J=60 Hz) was recorded (available as supplementary data) so as to facilitate this.

After storage at +4° for 2–3 months, it was found that pregaleopsin [**7**] had quantitatively converted to galeopsin [**8**], thus supporting the proposition of Rodriguez and Savona (13). The ^{1}H- and ^{13}C-nmr data of **8** were also reassigned on the basis of the results of HMQC (J=150 Hz) and HMBC (J=8.7 Hz) measurements as well as from the comparison with the 1D and 2D nmr data of pregaleopsin [**7**].

Compound **9** was identified as the optical antipode of (+)-leosibiricin, which was first isolated from *Leonurus sibiricus* (14). Ir, ms, and ^{1}H-nmr data of **9** were in agreement with those of (+)-leosibiricin, but the ^{13}C-nmr data (14) required some reassignment. In contrast to (+)-leosibiricin, which was reported to be an extremely unstable oil, (−)-leosibiricin was isolated as an amorphous powder that was stable under storage at +4° for many months. Prior to storage, extensive 2D nmr measurements [2D ^{1}H-^{1}H (COSY), ^{1}H-^{13}C (HMQC, J=150 Hz), and 2D-NOESY] experiments were performed. These data, together with the comparison of the nmr data of **9** with those of **6–8**, permitted the complete assignment of all ^{1}H- and ^{13}C-nmr resonances.

In conclusion, pregaleopsin [**7**] was isolated from the genus *Leonurus* for the first time. This is only the second report of **7** as a natural product. Although it was reported (10,14) to be of taxonomic interest that *Leonurus sibiricus* and *Leonurus heterophyllus* contained labdane diterpenoids while other *Leonurus* species contained only clerodane diterpenoids, the present work indicates this not to be the case. The determination of the

TABLE 1. ^{1}H-Nmr Data of **1–4** and **6–9** ($CDCl_3$, 300 MHz, δ, *J* Hz).

Proton(s)	Compound						
	1	**2**	**3–4**[c]	**6**[d]	**7**	**8**	**9**
1	1.28–1.41[a]	1.33–1.56[a]	1.46[a]	1.45–1.69[a]	1.39–1.60[a]	1.45[a]	1.26–1.52[a,b]
2	1.56–1.70[a]	1.53[a]	1.58[a]	1.59–1.69[a]	1.50–1.65[a]	1.47–1.68[a]	1.57[a]
3	1.44–1.50[a]	1.19–1.44[a]	1.15–1.41[a]	1.15–1.76[a]	1.20–1.40[a]	1.10–1.40[a]	1.26–2.22[a,b]
5	1.65 (dd, 2.6,14.0)	2.11 (dd, 8.9,10.7)	1.61 (dd, 2.7,14.0)/ 1.69 (dd, 2.7,14.0)	1.89 (dd, 2.8,14.4)	1.70 (dd, 2.6,14.0)	1.75 (dd, 2.5,14.0)	2.79 (d, 6.2)
6_{ax}	2.50 (dd, 11.8,14.0)	2.48 (dd, 10.7,17.8)	2.50 (dd, 11.7,14.0)/ 2.50 (dd, 11.7,14.0)	2.64 (dd, 11.7,14.4)	2.49 (dd, 11.7,14.0)	2.51 (dd, 11.6,14.0)	
6_{eq}	2.37 (dd, 2.6,11.8)	2.56 (dd, 8.9,17.8)	2.28–2.39[a]	2.51 (dd, 2.8,11.7)	2.33 (dd, 2.6,11.7)	2.29 (dd, 2.5,11.6)	4.96 (d, 6.2)
8 (OH)		3.84 s					
11	2.32[a]	1.82 (ddd, 7.1,11.7, 18.7) 2.37 (ddd, 2.4,7.1, 12.6)	2.26[a]	2.16–2.26[a]	2.20[a]	2.10[a]	1.85–2.35[a]
12	2.20[a]	1.99 (ddd, 2.4,7.1, 12.6) 2.20 (ddd, 7.1, 11.7, 18.7)	1.97–2.16[a] 2.20–2.38[a]	2.13[a]	2.10[a]	2.51[a]	2.02[a]
14	2.50 (d, 16.9) 2.90 (d, 16.9)	2.47 (d, 17.3) 2.89 (d, 17.3)	2.05–2.32[a] 1.97–2.32[a]	5.13 (d, 2.6)	5.12 (d, 2.6)	6.28 br s	5.09 (d, 2.6)
15			5.53 (d, 5.0)/ 5.39 (dd, 3.1,5.7)	6.43 (d, 2.6)	6.41 (d, 2.6)	7.34 br s	6.51 (d, 2.6)
16	4.17 (d, 9.0) 4.35 (d, 9.0)	4.29 (d, 9.3) 4.52 (d, 9.3)	3.65 (d, 8.7) 4.16 (d, 8.7)/ 3.93 (d, 8.7) 4.02 (d, 8.7)	4.07 (d, 10.6) 4.50 (d, 10.6)	4.05 (d, 10.6) 4.49 (d, 10.6)	7.23 br s	4.12 (d, 10.9) 4.52 (d, 10.9)
17	1.32 s	1.22 s	1.31/1.35 s	1.35 s	1.35 s	1.46 s	1.81 s
18	0.83 s	0.92 s	2×0.83 s	4.12 (d, 11.0) 4.43 (d, 11.0)	0.82 s	0.83 s	
19	0.89 s	0.88 s	2×0.88 s	1.09 s	0.88 s	0.86 s	1.28 s
20	1.18 s	0.75 s	1.17/1.19 s	1.20 s	1.14 s	1.16 s	0.71 s
22	2.07 s		2×2.07 s	2.00 s	2.08 s	2.06 s	2.15 s

[a]Multiplicity of the signals are unclear due to overlapping.
[b]Signals can be interchanged.
[c]Signal pairs are given together separated by "/".
[d]Additional signals due to *p*-bromobenzoate: 7.58 (dd, *J*=1.9, 4.8), 7.85 (dd, *J*=1.9, 4.8).

TABLE 2. ^{13}C-Nmr Data of **1–4** and **6–9** ($CDCl_3$, 75.5 MHz, ppm).

Carbon	Compound						
	1	**2**	**3–4**[a]	**6**	**7**	**8**	**9**
1	33.4 t[b]	33.4 t	33.3/33.8 t	33.7 t	33.4 t	32.0 t	30.9 t[c]
2	17.9 t	18.1 t	2×18.0 t	17.8 t	17.8 t	17.7 t	17.7 t
3	41.2 t	41.3 t	41.3/41.4 t	36.2 t	41.0 t	41.0 t	29.1 t[c]
4	34.4 s	34.0 s	2×34.4 s	38.2 s	34.2 s	34.2 s	41.5 s[d]
5	50.2 d	43.2 d	50.2/50.4 d	50.6 d	50.2 d	49.5 d	47.0 d
6	35.9 t	34.5 t	2×36.0 t	35.9 t	35.5 t	35.9 t	76.0 d
7	205.5 s	215.9 s	205.1/206.1 s	205.0 s	205.7 s	207.2 s	200.2 s
8	87.2 s	80.2 s	87.5/87.6 s	87.5 s	87.3 s	88.4 s	89.5 s
9	98.0 s	97.2 s	96.7/97.8 s	96.6 s	96.8 s	81.9 s	96.2 s
10	43.5 s	42.9 s	43.5/43.6 s	42.9 s	42.8 s	44.5 s	40.8 s[d]
11	28.2 t	28.8 t	28.3/28.7 t	28.8 t	28.4 t	30.6 t	29.6 t
12	38.8 t	37.2 t	37.2/39.9 t	38.4 t	38.1 t	21.0 t	36.2 t
13	87.0 s	86.1 s	91.0/91.4 s	94.8 s	94.2 s	124.7 s	93.1 s
14	42.8 t	41.6 t	46.7/47.5 t	106.8 d	106.7 d	110.7 d	105.8 d
15	173.5 s	174.6 s	2×98.6 d	148.6 d	147.9 d	142.9 d	148.9 d
16	77.7 t	76.8 t	75.8/78.2 t	80.3 t	79.9 t	138.6 d	77.5 t
17	15.7 q	27.1 q	15.7/15.9 q	15.8 q	15.5 q	15.0 q	23.6 q
18	21.5 q	21.9 q	2×21.5 q	68.0 t	21.3 q	21.4 q	179.3 s
19	32.7 q	32.5 q	32.8/32.9 q	27.1 q	32.4 q	32.9 q	26.5 q
20	18.0 q	17.2 q	2×18.1 q	18.2 q	17.3 q	16.5 q	17.5 q
21	169.0 s		169.0/169.1 s	169.0 s	168.8 s	169.1 s	168.7 s
22	21.3 q		2×21.4 q	21.3 q	21.1 q	21.3 q	22.0 q
23				166.0 s			
24				129.0 s[c]			
25,29				2×131.8 d[d]			
26,28				2×131.0 d[d]			
27				128.2 s[c]			

[a]Signal pair are given together separated by "/".
[b]Multiplicity by DEPT.
[c–d]Signals can be mutually interchanged.

absolute configuration of **6**, and hence **5**, appears to be the first reliable data for this type of diterpenoid. The data contained in this paper suggest that a great deal of early spectral assignment work (13,14), particularly ^{1}H- and ^{13}C-nmr, for this class of compound should be viewed with some caution.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Mettler FP5 apparatus connected to a FP52 hot stage. Optical rotations were recorded with a Perkin-Elmer 241 polarimeter using $CHCl_3$ as solvent. Ir spectra were measured on a Perkin-Elmer 2000 Ft-ir spectrometer as liquid films on NaCl tablets or as pressed KBr disks. Uv spectra were recorded in MeOH or EtOH using an Uvikon 930 spectrophotometer. Eims spectra were measured on a Hitachi-Perkin-Elmer-RMUGM mass spectrometer at 70 eV. ^{1}H- and ^{13}C-nmr spectra were recorded using a Bruker AMX-300 spectrometer operating at a basic frequency of 300 MHz, with residual $CHCl_3$ in $CDCl_3$ (δ 7.26) and $CDCl_3$ (77.0 ppm) as references. Hplc separations were performed with a Waters model 590 pump connected to a Rheodyne hplc injector and a Knauer differential refractometer. Hplc columns were from Knauer (LiChrosorb Si 60 and Spherisorb ODS II, both 250×8 mm, 5 μm). Silica (Si gel 60F_{254}, 5-40 μm, Merck) was used for vlc (column 6.5×20 cm, vacuum by H_2O aspiration). Si gel 60 F_{254} precoated Al sheets (0.2 mm, Merck) were used for tlc controls. All solvents were hplc grade.

PLANT MATERIAL.—*Leonurus persicus* was collected from Turkey, East Anatolia, in the Tekman province of Erzurum, in early August 1992. Voucher specimens (HUEF 92111) are deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

EXTRACTION AND ISOLATION.—Air-dried and powdered aerial parts of the plant (900 g) were successively extracted with petroleum ether, CH_2Cl_2, EtOAc, MeOH, and 5:1 and 1:1 MeOH-H_2O

mixtures, respectively, to afford 27.8 g of petroleum ether-soluble material. A 24-g quantity of this extract was applied to vlc, as four separate portions, 6 g each. Elution with hexane containing increasing amounts of EtOAc and final washing with MeOH yielded 18 fractions of 100 ml each. Based on the tlc similarities, various fractions were combined to give 12 fractions in all.

Altogether, 2 g of recombined vlc fractions 8 and 9 were separated by vlc over Si gel, using hexane-$CHCl_3$-MeOH (175:95:5 to 0:30:70) mixtures as eluents. Tlc and ^{1}H-nmr investigations of these fractions indicated fractions 1–5 to be of further interest. Hplc separation of combined fractions 1–5, over normal-phase silica using $CHCl_3$-MeOH-hexane (95:5:270) as eluent gave 10 fractions. Of these, fractions 5 and 6 were chosen for further separation. Reversed-phase hplc purification of fraction 6 with MeCN-*i*-PrOH-H_2O (7:1:8.5) as eluent yielded **1**.

Leopersin A [**1**].—White amorphous powder (60 mg, 0.007%): $[\alpha]^{22}D$ −29.0° (*c*=0.79, $CHCl_3$); ir ν max (film) 2995, 1790, 1740, 1725, 1245, 1110, 1030, 755 cm^{-1}; eims *m/z* $[M]^+$ 392 (<1), 375 (<1), 350 (8), 333 (12), 195 (9), 149 (55), 123 (30), 109 (36), 97 (34), 95 (44), 81 (56), 43 (100); hreims *m/z* 392.2190 (calcd for $C_{22}H_{32}O_6$ 392.2198); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

Fraction 5 (see above) was chromatographed by normal-phase hplc employing hexane-Me_2CO-MeOH (81:20:2) as the eluent, to yield 10 fractions. Purification of fraction 5 by reversed-phase- hplc with MeCN-*i*-PrOH-H_2O (7:1:7) as the eluent gave **2**.

8-Deacetoxyleopersin A [**2**].—White amorphous powder (6.5 mg, 0.0007%): $[\alpha]^{20}D$ −10.0° (*c*=0.61, $CHCl_3$); ir ν max (film) 3440, 2985, 1780, 1715, 1460, 1170, 1025, 595 cm^{-1}; eims *m/z* $[M+H]^+$ 351 (15), $[M]^+$ 350 (56), 322 (6), 304 (9), 289 (6), 279 (11), 249 (18), 213 (32), 195 (22), 181 (23), 167 (32), 149 (100), 123 (48), 109 (37), 97 (41), 95 (27), 83 (37), 81 (25), 43 (54); hreims *m/z* 350.2100 (calcd for $C_{20}H_{30}O_5$ 350.2094); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

Hplc separation of vlc fraction 10 (96.5 mg) over normal-phase silica with $CHCl_3$-hexane-MeOH (95:55:5) followed by reversed-phase hplc, employing MeCN containing 55% H_2O afforded **3–4** (18 mg). Vlc fraction 11 (404 mg) was further fractionated by normal-phase hplc using hexane-EtOAc-MeOH (75:25:10) as eluent to yield two main fractions, A and B. Hplc separation of fraction A over RP-18 material using MeOH containing 35% H_2O as eluent yielded further **3–4** (20 mg).

Leopersin B and 15-epi-*leopersin B* [**3–4**].—White amorphous powder (38 mg, 0.004%): ir ν max (film) 3435, 2975, 1740, 1720, 1250, 1105, 1050, 790 cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; eims *m/z* $[M]^+$ 394 (<1), 352 (<1), 335 (4), 317 (<1), 186 (6), 179 (2), 149 (4), 109 (13), 95 (13), 82 (13), 81 (30), 43 (100); hreims *m/z* 394.2339 (calcd for $C_{22}H_{34}O_6$ 394.2355).

OXIDATION OF **3–4** TO **1**.—A few drops of Jones reagent were added to a solution of **3–4** (10 mg) in Me_2CO at room temperature. After 20 min, the reaction was quenched with *i*-PrOH (5 ml). The solution was evaporated to dryness, dissolved in 5 ml of H_2O, and was extracted with 4×5 ml of CH_2Cl_2. The combined CH_2Cl_2 solubles were purified by normal-phase hplc using $CHCl_3$-MeOH-hexane (9:1:22) as eluent to give **1** (6 mg).

Fraction B (see above) was subjected to reversed-phase hplc eluting with MeOH containing 35% H_2O followed by normal-phase hplc separation using hexane-EtOAc-*i*-PrOH (8:1:1) as eluent to give impure **5** (23 mg).

PREPARATION OF THE *P*-BROMOBENZOATE DERIVATIVE [**6**] OF 4β-HYDROXYMETHYLPREGALEOPSIN [**5**].—A 15-mg quantity of impure **5** was dissolved in $CHCl_3$ (1 ml). 12.2 mg of 4-bromobenzoyl chloride (in 2 ml $CHCl_3$) and a catalytic amount of DMAP (dimethylaminopyridine) were added dropwise to this solution during stirring at room temperature. After 9 h, the reaction was quenched with H_2O (3 ml) and the aqueous portion of the solution was extracted with 3×3 ml $CHCl_3$. The combined $CHCl_3$ extracts were chromatographed (normal-phase hplc with hexane containing 25% EtOAc as eluent) to yield **6** (13 mg).

COMPOUND **6**.—Colorless prisms (13 mg): $[\alpha]^{25}D$ −56.0° (*c*=0.1, $CHCl_3$); mp 159–162°; uv λ max (EtOH) 204 (ε 315), 244 (ε 195) nm; ir ν max (film) 2925, 1735, 1725, 1615, 1590, 1465, 1370, 1270, 1070, 755 cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; eims *m/z* $[M]^+$ 576 (7), $[M]^+$ 574 (7), 535 (9), 533 (9), 534 (30), 532 (29), 517 (7), 516 (6), 489 (6), 419 (8), 332 (14), 315 (4), 210 (15), 191 (74), 185 (97), 183 (100), 149 (36), 109 (54), 95 (30), 81 (52), 43 (38); hreims *m/z* 574.1599 (calcd for $C_{29}H_{35}O_7{}^{79}Br$ 574.1568).

A portion of vlc fraction 7 (580 mg) was chromatographed over normal-phase hplc employing hexane containing 20% Me_2CO as eluent followed by hplc using RP-18 material with MeCN-*i*-PrOH-H_2O (7:1:6.5) to yield **9**.

(−)-*Leosibiricin* [**9**].—A white, amorphous powder (19 mg, 0.002%): $[\alpha]^{25}D$ −39.0° (*c*=0.74, $CHCl_3$) for (+)-leosibiricin, $[\alpha]^{19}D$ +33.0° (14); ir and eims data were identical with those previously reported (14). ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

Vlc fraction 6 after recrystallization with hexane-Me_2CO (4:1) afforded **7** (900 mg, 0.1%) as white crystals. This compound quantitatively converted to **8** during storage at +4° for 2–3 months. For compounds **7** and **8**, complete and unambiguous ^{1}H- and ^{13}C-nmr data are reported (see Tables 1 and 2). All other physical and spectral data were compatible with those previously reported (13).

SINGLE CRYSTAL X-RAY ANALYSIS OF **6**.[2]—Crystal data: $C_{29}H_{35}BrO_7 \cdot 1/2CHCl_3$, FW=635.18, tetragonal, space group $P4_12_12$, a=8.150(1), c=87.67(2)^2 Å, V=5824(2) Å^3, Z=8, Dc=1.449 g cm^{-3}, MoKα radiation (graphite-monochromated), λ=0.71069 Å, μ(MoKα)=1.598 mm^{-1}, T=173(1)° K, crystal size 0.33×0.37×0.42 mm. Intensity data were measured on a Rigaku AFC5R diffractometer using ω-scans with a narrow scan width of (0.5+0.35 tanθ)°. The ω scan-speed was constant at 16° min^{-1}. The weak reflections [$I<10\sigma(I)$] were rescanned up to a maximum of 4 scans and the counts were accumulated. Of the 6075 reflections which were measured in the range $0<h<9$, $0<k<9$, $-1<l<104$ and $2\theta<50°$, 5146 were unique and 2568 reflections with $I>2\sigma(I)$ were used in the refinement. The crystal was very weakly diffracting when $2\theta>50°$.

The extremely long c-axis caused difficulties with the data collection because of partial overlap of neighboring reflections. The reflections along the c-axis could be cleanly resolved because of the systematic absence condition $00l$: $l\neq 4n$. Profile analysis of a series of general reflections showed that data collection using very narrow width ω-scans minimized the overlap problem, although it was apparent that such a narrow scan-width caused some background measurements to be made in the tail of the reflection. However, broader scan-widths caused more severe problems with some of the backgrounds being measured at positions where there was significant intensity from a neighboring reflection. This reflection overlap problem also appeared to prevent the successful refinement of the unit cell parameters from high-angle reflections. The data were corrected for Lorentz and polarization effects and an empirical absorption correction was applied (15) (range of correction coefficients 0.365–1.128). The extreme values for these correction coefficients and the high value for R_{int} (0.155) probably result from small errors in the intensities caused by reflection overlap or intensity cutoff from the narrow scan-width. Thus, the absorption correction is correcting for both absorption and errors in the data. Under the circumstances such a correction is considered to be a useful means of reducing the severity of the errors in the data. A comparison of the refinement results using corrected and uncorrected data showed that the correction did not introduce any undesirable features.

The structure was solved by direct methods (16). The crystal lattice contains chloroform solvent molecules disordered about a crystallographic twofold axis. The three Cl-atom positions are common to both orientations of the molecule; one lying on the twofold axis, and the other two Cl-atoms being related by the twofold axis, while the C-atom position is not common to both orientations. The large thermal parameters for the Cl atoms suggest that perfect overlap of the two orientations is not occurring, but it was not possible to resolve this separation, because the overlap of the electron density from the closely adjacent sites forms single maxima. Nonetheless anisotropic refinement of the chloroform atoms was successful. Refinement was carried out on F using full-matrix least-squares procedures, which minimized the function $\Sigma w(|F_o|-|F_c|)^2$, with weights $w=[\sigma^2(F_o)+(0.005F_o)^2]^{-1}$. All non-hydrogen atoms were refined anisotropically. All of the hydrogen atoms were fixed in geometrically calculated positions with a C-H distance of 0.95 Å and were assigned fixed isotropic temperature factors with a value of 1.2 B_{eq} of the parent C-atom. Final R=0.0693, R_w=0.0609, goodness-of-fit=2.145, 357 variables, 2568 reflections with $I>2\sigma(I)$, max and min residual electron densities were 0.69, −0.53 e Å^{-3}, largest shift/error in final cycle 0.07. A correction for secondary extinction was not applied. All calculations were carried out using the TEXSAN software package (17).

The absolute configuration was tested by two methods. First, the Hamilton R-test was employed (18). Refinement of the mirror image structure under identical conditions in the space group $P4_32_12$ yielded R=0.084, R_w=0.069. At the 3σ confidence limit these R-factors are statistically significantly higher than those obtained from the original refinement. The second test involved refinement of the enantiopole, or Flack's x, parameter (19), which describes the structure as an inversion twin and indicates the fraction of the enantiomorph defined by the refined coordinates, 1-x, and its inverse, x, in the sample. Refinement of the structure and the enantiopole parameter with the CRYSTALS program (20) yielded x=0.00 (3). The low quality of the data for this structure, caused by the reflection overlap problem, as well the fact that only a relatively small number of Bijvoet pairs of reflections were recorded, means that the results of the absolute configuration tests should be treated cautiously, although the presence of a bromine atom in the molecule makes this test very sensitive. The results strongly favor the absolute configuration that is defined by the atomic coordinates listed in Table 3 and shown by the ORTEP representation in Figure 1 (21).

[2]Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

TABLE 3. Final Atomic Coordinates and Equivalent Isotropic Thermal Parameters for **6**. (estimated standard deviations in parentheses).

Atom	*x*	*y*	*z*	U_{eq} (Å^2)[a]
Br	0.5306 (2)	−0.0702 (2)	0.55475 (1)	0.0664 (5)
O-7	0.0627 (8)	0.0489 (8)	0.40976 (8)	0.045 (3)
O-8	0.2849 (8)	−0.3138 (7)	0.40769 (8)	0.032 (3)
O-9	0.4580 (8)	0.0967 (7)	0.40583 (7)	0.030 (2)
O-15	0.5484 (9)	0.4305 (8)	0.38924 (8)	0.043 (3)
O-18	0.392 (1)	−0.0967 (9)	0.47800 (9)	0.054 (3)
O-21	0.0075 (9)	−0.3318 (9)	0.4083 (1)	0.052 (4)
O-23	0.360 (1)	−0.366 (1)	0.4831 (1)	0.065 (4)
C-1	0.664 (1)	−0.015 (1)	0.4305 (1)	0.036 (4)
C-2	0.694 (1)	0.005 (2)	0.4477 (1)	0.054 (5)
C-3	0.559 (2)	0.099 (1)	0.4555 (1)	0.052 (5)
C-4	0.385 (1)	0.025 (1)	0.4526 (1)	0.039 (4)
C-5	0.357 (1)	−0.005 (1)	0.4357 (1)	0.034 (4)
C-6	0.187 (1)	−0.076 (1)	0.4312 (1)	0.045 (5)
C-7	0.160 (1)	−0.048 (1)	0.4148 (1)	0.029 (4)
C-8	0.281 (1)	−0.137 (1)	0.4043 (1)	0.031 (4)
C-9	0.460 (1)	−0.076 (1)	0.4090 (1)	0.030 (3)
C-10	0.497 (1)	−0.099 (1)	0.4264 (1)	0.028 (4)
C-11	0.592 (1)	−0.150 (1)	0.3983 (1)	0.033 (4)
C-12	0.622 (1)	−0.020 (1)	0.3863 (1)	0.037 (4)
C-13	0.577 (1)	0.143 (1)	0.3938 (1)	0.029 (4)
C-14	0.714 (1)	0.244 (1)	0.4007 (1)	0.030 (4)
C-15	0.686 (1)	0.399 (1)	0.3977 (1)	0.039 (5)
C-16	0.499 (1)	0.273 (1)	0.3834 (1)	0.034 (4)
C-17	0.239 (1)	−0.114 (1)	0.3877 (1)	0.040 (4)
C-18	0.366 (1)	−0.134 (1)	0.4622 (1)	0.052 (5)
C-19	0.261 (2)	0.151 (1)	0.4592 (1)	0.055 (5)
C-20	0.512 (1)	−0.284 (1)	0.4309 (1)	0.046 (4)
C-21	0.140 (2)	−0.395 (1)	0.4085 (1)	0.035 (4)
C-22	0.171 (1)	−0.574 (1)	0.4106 (1)	0.052 (5)
C-23	0.388 (1)	−0.229 (2)	0.4878 (1)	0.050 (5)
C-24	0.424 (1)	−0.181 (1)	0.5037 (1)	0.046 (5)
C-25	0.451 (1)	−0.022 (1)	0.5083 (1)	0.055 (5)
C-26	0.484 (1)	0.013 (1)	0.5237 (1)	0.058 (5)
C-27	0.484 (1)	−0.116 (1)	0.5339 (1)	0.045 (5)
C-28	0.462 (1)	0.274 (1)	0.5294 (1)	0.044 (4)
C-29	0.426 (1)	−0.307 (1)	0.5148 (1)	0.045 (4)
Cl-30	−0.1744 (6)	−0.1198 (6)	0.48412 (5)	0.119 (2)
Cl-31[b]	0.0591 (6)	0.0591 (6)	0.5	0.163 (4)
C-30[b]	−0.127 (3)	−0.032 (3)	0.5017 (5)	0.07 (1)

[a] $U_{eq}=(1/3)\Sigma_i\Sigma_j U_{ij}a_i^*a_j^*a_i\cdot a_j$.
[b] Atom with site occupation factor of 0.5.

ACKNOWLEDGMENTS

We thank Dr. E. Zass, ETH Chemistry Department, for performing literature searches, and Mr. Oswald Greter and Dr. Walter Amrein, ETH Chemistry Department Mass Spectral Service, for recording all mass spectra and making accurate mass measurements.

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Received 29 March 1995