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LABDANE DITERPENES FROM *LEONURUS PERSICUS*

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Key Word Index—*Leonurus persicus*; Lamiaceae, motherwort; labdane diterpene; flavone; genkwanin.

Abstract—Eight new diterpenoids of labdane class, leopersin M-Q (**1–3**, **5**, **6**), 15-*epi*-leopersin O and Q (**4**, **7**) and 19-hydroxygaleopsin (**8**) were isolated from the aerial parts of *Leonurus persicus*, besides a flavone, genkwanin (**9**). Their structures were established by spectroscopic means, mainly by 1D and 2D NMR.

INTRODUCTION

Leonurus, commonly known as motherwort, is a small genus of Lamiaceae, which is represented by five species in the flora of Turkey [1]. In the course of our systematic phytochemical studies into the genus *Leonurus* [2], we have investigated *L. persicus* Boiss., which is rarely found in Eastern Anatolia. Recently, we have reported several labdane and *seco*-labdane type diterpenoids from the petrol extract of the aerial parts of this plant [3, 4]. As part of the continuing investigation of this species, we now wish to discuss the isolation and structure elucidation of eight further labdane diterpenes (**1–8**), and a known flavone, genkwanin (=apigenin 7-*O*-methyl ether) (**9**) obtained from the dichloromethane extract of the plant.

RESULTS AND DISCUSSION

All isolates were obtained from a methylene dichloride extract of air-dried powdered aerial parts of *Leonurus persicus* by a combination of VLC and HPLC as described in the Experimental.

Leopersin M (**1**) was obtained as a colourless oil of molecular formula $C_{22}H_{32}O_7$, determined by mass spectrometry. Its FTIR spectrum displayed diagnostic absorption bands at 3467 (hydroxy), 1785 (γ-lactone), 1724 (ester) and 1715 cm^{-1} (keto). The ^{13}C NMR spectrum of **1** contained 22 signals that included four methyl groups (δ 9.1, 18.7, 20.1, 27.3, all *q*), a keto carbonyl (δ 208.3 *s*), an acetate function (δ 21.2 *q*, 171.0 *s*), a carbinol (δ 75.6 *d*) and four additional oxygenated carbons (δ 78.0 *t*, 79.7 *d*, 97.0 *s*, 86.5 *s*). These data allowed the skeleton of **1** to be deduced as a labdane diterpene, which contained two extra C atoms in the form of an acetoxy function. The ^1H NMR data of **1**, recorded in $CDCl_3$, also confirmed this deduction revealing signals consistent with the presence of three tertiary methyls (δ 0.99 *s*, 1.47 *s*, 1.53 *s*), one secondary methyl group (δ 1.00, *d*, J = 6.5 Hz), acetoxy (δ 2.06 *s*), methine (δ 3.53 *q*, J = 6.5 Hz) and two oxymethine protons (δ 4.34 *m*, 4.44 *m*). The ^1H NMR spectrum further revealed two pairs of doublets at δ 2.46/2.85 (*d*, J = 17.0 Hz, H-14$_a$ and H-14$_b$) and at δ 4.14/4.27 (*d*, J = 9.1 Hz, H-16$_a$ and H-16$_b$), typical of a γ-lactone function in the side chain (C-15→C-16) [3, 4]. In the ^1H-^1H COSY spectrum of **1**, the H-$_3$-17 methyl doublet coupled to the one-proton methine quartet (H-8) which in turn correlated to the keto function at δ 208.3 in its HMBC spectrum. Further ^1H-^{13}C long range correlations (Table 3) between C-7/H$_3$-17, C-7/H-8 and C-9/H$_3$-17 indicated the attachment of the keto function at C-7. The assignments of the remaining secondary hydroxy and acetoxy functions were achieved by changing the NMR solvent from $CDCl_3$ to benzene-d_6 (Tables 1 and 2). In this solvent, the complex signal at δ 4.44 (*m*) was converged to a clearly resolved doublet of doublets at δ 4.59 (1H, J = 4.3, 11.6 Hz) and assigned to H-3, suggesting the presence of an OAc group rather than a hydroxy at C-3. Hence, the highfield signal at δ 4.13 (1H, *br s*) could readily be attributed to H-6. The proposed assignments were supported by ^1H-^1H homonuclear COSY spectrum, where H-3 showed a coupling to H$_2$-2 which in turn coupled to H$_2$-1, whereas H-6 coupled to H-5 (δ 1.55, *d*, J = 2.7 Hz).

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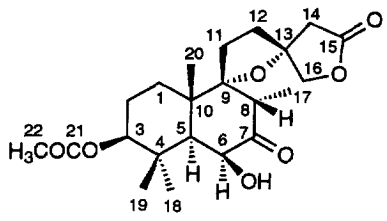

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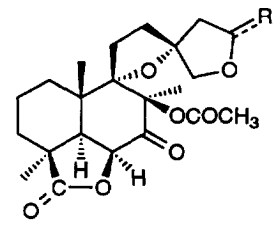
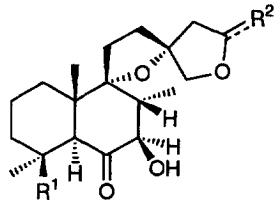
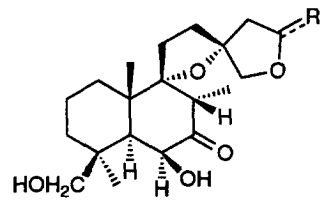


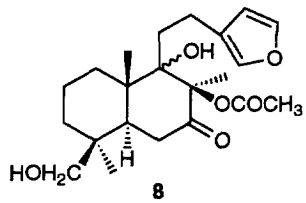
2 R=Hβ, OMeα
10 R=H, $\Delta^{14,15}$



3 R^1=CH_2OH, R^2=Hα, OHβ
4 R^1=CH_2OH, R^2=Hβ, OHα
5 R^1=CH_2OH, R^2=O
11 R^1=CH_3, R^2=Hα, OHβ
12 R^1=CH_3, R^2=Hβ, OHα



6 R=Hα, OHβ
7 R=Hβ, OHα



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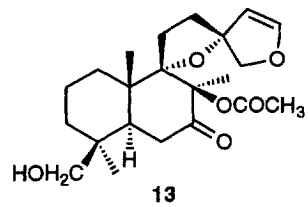

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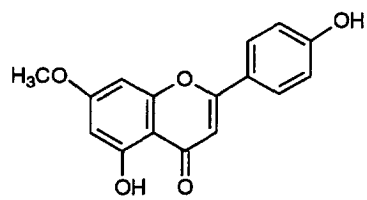

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A two-bond isotope effect observed from a D_2O shake experiment definitively evidenced that C-6 was associated with an alcohol group, as the C-6 signal at δ 75.7 showed an upfield shift of 0.16 ppm when OH was converted to OD (Table 2) while C-3 was essentially unchanged. The stereochemical configurations of the acetoxy and the hydroxy groups were determined on the basis of coupling constant analysis and the results of a 2D ROESY experiment. The *J* values exhibited by H-3 (4.3 and 11.6 Hz) indicated axial-axial and axial-equatorial relationships with its neighbouring protons (H_2-2). Therefore, H-3 had to be α-oriented. The connectivities inferred from the ROESY spectrum between H-3/H-5 and H-3/H_3-18 confirmed these assignments unambiguously. Similar correlations were also obtained between H-6/H_3-18, H-5/H_3-18 and H-6/H-5. This latter correlation together with a $J_{5,6}$ value of 2.7 Hz suggested the α-configuration of

Table 1. ^{1}H NMR data of compounds **1**–**8** ($CDCl_3$, δ ppm, J in Hz, 300 MHz)*

H	1	1‡	2	3/4§	5	6/7§	8
1	1.50†	1.10†	1.30–1.51†	1.58†	1.51†	1.45†/1.51†	1.47†
2	1.80†	1.68 *m*	1.52†	1.52†	1.53†	1.55†	1.49–1.55†
3	4.44 *m*	4.59 (*dd*, 4.3, 11.6)	1.45–2.20†	1.05–1.70†	0.99-1.79†	1.21–1.54†	0.99–1.72†
5	1.62 (*d*, 2.9)	1.55 (*d*, 2.7)	2.80 (*d*, 6.1)	3.00 *s*/3.08 *s*	2.94 *s*	1.60 (*d*, 2.0,)/1.76 (*d*, 2.0)	1.89 (*dd*, 2.2, 14.2)
6	4.34 *m*	4.13 *br s*	4.97 (*d*, 6.1)			4.19 (*dd*, 2.0, 4.8)	2.69 (*dd*, 11.9, 14.2) 2.45 (*dd*, 2.2, 11.9)
7				4.02 (*d*, 11.8)	3.92 (*dd*, 0.4, 10.6)		
8	3.53 (*q* 6.5)	3.13 (*q*, 6.7)		2.47 *m*	1.87 *m*	3.62 (*m*, 6.7)	
11	2.02†	1.79†	1.80–2.40†	1.15–1.25†/1.85–2.14†	1.95–2.20†	2.00–2.20†	2.10†
12	2.15†	1.79†	2.00†	2.02–2.24†/2.30†	2.20†	1.90–2.16†/2.21†	2.50†
14	2.46 (*d*, 17.0)	1.78 (*d*, 16.5)	2.07 (*d*, 4.5)	2.19–2.41†/1.89–2.07†	2.60 (*d*, 17.0)	1.89–2.29†/1.90–2.30†	6.31 *br s*
	2.85 (*d*, 17.0)	2.02 (*d*, 16.5)			2.98 (*d*, 17.0)		
15			5.00 (*t*, 4.5)	5.67 (*dd*, 1.2, 4.1)/5.19 *m*		5.38 (*d*, 3.8)/ 5.55 (*dd*, 1.2, 5.2)	7.39 *br s*
16	4.14 (*d*, 9.1)	3.52 (*d*, 8.7)	3.73 (*d*, 8.6)	3.74 (*d*, 8.9), 4.27 (*d*, 8.9)/	4.26 (*d*, 9.1)	3.70 (*d*, 9.0), 4.18 (*d*, 9.0)/	7.27 *br s*
	4.27 (*d*, 9.1)	3.76 (*d*, 8.7)	4.15 (*d*, 8.6)	4.03 (*d*, 8.9), 4.10 (*d*, 8.9)	4.45 (*d*, 9.1)	3.97 (*d*, 2.1)	
17	1.00 (*d*, 6.5)	0.74 (*d*, 6.7)	1.76 *s*	1.15/1.18 (*d*, 6.7)	1.14 (*d*, 6.5)	1.00/1.02 (*d*, 6.7)	1.50 *s*
18	0.99 *s*	0.91 *s*	1.30 *s*	1.02 × 2 *s*	1.02 *s*	1.05 × 2 *s*	1.01 *s*
19	1.47 *s*	1.38 *s*		3.40 (*d*, 11.7), 4.26 (*d*, 11.7)/	3.41 (*d*, 11.5)	3.28 (*d*, 11.5), 4.27 (*d*, 11.5)/	3.57 (*d*, 10.9)
				3.41 (*d*, 11.7), 4.26 (*d*, 11.7)/	4.26 (*d*, 11.5)	3.30 (*d*, 11.5), 4.27 (*d*, 11.5)	3.69 (*d*, 10.9)
20	1.53 *s*	1.18 *s*	0.73 *s*	0.89 *s*/ 0.90 *s*	0.88 *s*	1.48 *s*/1.50 *s*	1.23 *s*
22	2.06 *s*	1.76 *s*	2.15 *s*				2.09 *s*
OCH_3			3.41 *s*				
OH		6.20 *s*					

*^{1}H NMR chemical shifts were assigned on the basis of ^{1}H-^{1}H DQFCOSY and ^{1}H-^{13}C HMQC experiments.
†Signal pattern unclear due to overlapping.
‡Measured in D_6D_6.
§Signal pairs are separated by /.

Table 2. ^{13}C NMR data of compounds **1–8** (δ ppm, 75.5 MHz, $CDCl_3$)

C	1	1†	2	3/4‡	5	6/7‡	8
1	31.5 *t*	31.5 *t*	30.8 *t*	32.5/32.8 *t*	32.7 *t*	34.3/34.7 *t*	32.2 *t*
2	23.7 *t*	24.0 *t*	17.7 *t*	17.8 × 2 *t*	17.8 *t*	18.6 × 2 *t*	17.6 *t*
3	79.7 *d*	79.8 *d*	29.2 *t*	39.2/39.4 *t*	38.5 *t*	40.5 × 2 *t*	35.5 *t*
4	39.1 *s*	39.2 *s*	41.5 *s*	37.3/37.4 *s*	37.7 *s*	39.8 × 2 *s*	39.2 *s*
5	49.1 *d*	49.3 *d*	47.0 *d*	58.9/59.0 *d*	58.7 *d*	51.6/52.3 *d*	49.9 *d*
6	75.6 *d*	75.7 *d*¶	75.9 *d*	215.5/215.9 *s*	213.9 *s*	74.3/74.5 *d*	36.3 *t*
7	208.3 *s*	206.6 *s*	200.1 *s*	77.5/77.6 *d*	77.3 *d*	209.5/210.1 *s*	206.9 *s*
8	44.8 *d*	44.6 *d*	89.5 *s*	47.6/47.7 *d*	47.3 *d*	45.5 × 2 *d*	88.3 *s*
9	97.0 *s*	96.2 *s*	96.0 *s*	92.3/93.1 *s*	93.7 *s*	97.0/98.4 *s*	81.7 *s*§
							81.8 §
10	42.7 *s*	42.7 *s*	40.9 *s*	49.2/49.3 *s*	48.8 *s*	43.1/43.2 *s*	44.5 *s*
11	29.5 *t*	29.5 *t*	29.2 *t*	29.1/29.5 *t*	29.2 *t*	29.7/29.9 *t*	30.7 *t*
12	37.9 *t*	37.5 *t*	38.7 *t*	36.5/38.3 *t*	37.8 *t*	35.1/38.7 *t*	21.2 *t*
13	86.5 *s*	86.2 *s*	89.3 *s*	91.0 × 2 *s*	87.1 *s*	90.5/90.8 *s*	124.6 *s*
14	42.6 *t*	42.1 *t*	45.6 *t*	46.5/47.8 *t*	42.7 *t*	45.8/47.6 *t*	110.8 *d*
15	173.8 *s*	172.5 *s*	105.1 *d*	98.9/99.0 *d*	173.9 *s*	99.1/99.3 *d*	143.2 *d*
16	78.0 *t*	76.9 *t*	73.8 *t*	76.7/78.1 *t*	78.2 *t*	77.1/78.2 *t*	138.8 *d*
17	9.1 *q*	9.2 *q*	22.8 *q*	13.0/13.1 *q*	13.2 *q*	9.2/9.5 *q*	15.1 *q*
18	27.3 *q*	27.3 *q*	26.7 *q*	26.3 × 2 *q*	26.3 *q*	26.7/26.9 *q*	26.8 *q*
19	18.7 *q*	18.9 *q*	179.3 *s*	67.2/67.3 *t*	66.4 *t*	68.1/68.2 *t*	64.8 *t*
20	20.1 *q*	19.8 *q*	17.5 *q*	19.8 × 2 *q*	19.9 *q*	20.0/20.1 *q*	17.1 *q*
21	171.0 *s*	169.8 *s*	168.7 *s*				169.1 *s*
22	21.2 *q*	20.7 *q*	22.0 *q*				21.4 *q*
OCH_3			55.3 *q*				

*Multiplicities determined by DEPT sequences.
†Measured in C_6D_6.
‡Signal pairs are separated by /.
§Signals for the epimeric center at C-9.
¶Signal shifts to upfield upon the addition of D_2O.

both protons and also the *trans*-junction of the decalin ring. The relative stereochemistry of the remaining chiral centers at C-8, C-9, C-10 and C-13 were also deduced by interpretation of the ROESY spectrum. Key ROE correlations between H-8/H_2-11, H-8/H_3-20 and H_2-11/H_3-20 indicated their close proximity (β-

Table 3. ^1H-^{13}C long range correlations of compounds **1** and **2** obtained from HMBC experiments

Proton	Compound **1** (C, δ in ppm)	Compound **2** (C, δ in ppm)
1	20.1 (C-20)	17.5 (C-20)
2		30.8 (C-1), 29.2 (C-3)
3	18.7 (C-19), 27.3 (C-18)	30.8 (C-1), 17.7 (C-2), 26.7 (C-18)
5	39.1 (C-4), 42.7 (C-10), 18.7 (C-19), 27.3 (C-18), 20.1 (C-20)	41.5 (C-4), 75.9 (C-6), 96.0 (C-9) 40.9 (C-10) 179.3 (C-19), 26.7 (C-18), 17.5 (C-20)
6		47.0 (C-5), 200.1 (C-7), 40.9 (C-10)
8	208.3 (C-7), 9.1 (C-17)	
11	37.9 (C-12)	40.9 (C-10), 38.7 (C-12)
12	29.5 (C-11), 42.6 (C-14), 78.0 (C-16)	29.2 (C-11), 45.6 (C-14), 73.8 (C-16)
14	37.9 (C-12), 86.5 (C-13), 173.8 (C-15), 78.0 (C-16)	38.7 (C-12), 89.3 (C-13), 105.1 (C-15), 73.8 (C-16)
15	42.6 (C-14), 78.0 (C-16)	45.6 (C-14), 73.8 (C-16), 55.3 (OCH_3)
16	37.9 (C-12), 86.5 (C-13), 42.6 (C-14), 173.8 (C-15)	38.7 (C-12), 89.3 (C-13), 45.6 (C-14), 105.1 (C-15)
17	208.3 (C-7), 44.8 (C-8), 97.0 (C-9)	200.1 (C-7), 89.5 (C-8), 96.0 (C-9)
18	79.7 (C-3), 39.1 (C-4), 49.1 (C-5), 18.7 (C-19)	39.2 (C-3), 41.5 (C-4), 47.0 (C-5), 179.3 (C-19)
19	79.7 (C-3), 39.1 (C-4), 49.1 (C-5), 27.3 (C-18)	
20	31.5 (C-1), 49.1 (C-5), 97.0 (C-9), 42.7 (C-10)	30.8 (C-1), 47.0 (C-5), 96.0 (C-9), 40.9 (C-10)
22	171.0 (C-21)	168.7 (C-21)
OCH_3		105.1 (C-15)

disposition). The ROESY spectrum further revealed an interaction between H_3-17 and H_2-16 and thus supported the regiochemistry of the γ-lactone function as shown in **1**. Considering these spectroscopic observations, the structure of leopersin M (**1**) was determined as 3β-acetoxy-9α,13-epoxy-6β-hydroxy-7-oxo-labdan-15,16-olide.

Leopersin N (**2**) was assigned the protonated molecular formula of $C_{23}H_{32}O_8$ from its HREI mass spectrum ($[M+H]^+$ *m/z* 437.2173. Calcd. 437.2175). Its IR absorptions at 1783, 1763 and 1742 cm^{-1} and ^{13}C NMR resonances at δ 179.3 (*s*), 168.7 (*s*) and 200.1 (*s*) indicated the presence of a γ-lactone, an ester and a free keto function. The ^1H NMR spectrum of **2** exhibited an acetate methyl singlet at δ 2.15 (3H, *s*), a methoxy singlet at δ 3.41, three singlet resonances for methyl groups (δ 0.73, 1.30 and 1.76, all 3H) and two AB systems appearing at δ 2.80/4.97 (both *d*, $J = 6.1$ Hz) and δ 3.73/4.15 (both *d*, $J = 8.6$ Hz), respectively. The above data suggested the presence of eight degrees of unsaturation and **2** was thereby deduced as being a pentacyclic diterpenoid. The ^{13}C NMR chemical shift values of **2** were assigned by means of HMQC (Table 2) and HMBC (Table 3) experiments. Comparison of these data with those of (−)-leosibiricin (**10**) [3], previously obtained from the same plant, strongly suggested that **2** differed from **10** only in the presence of a methoxy function in ring D, instead of the double bond ($\Delta^{14,15}$) found in **10**. The methoxy function was considered to be positioned at C-15 on the basis of the results of a homonuclear DQFCOSY experiment; oxymethine proton (H-15, δ 5.00 *t*, $J = 4.5$ Hz) was coupled to H_2-14 (2H, δ 2.07 *d*, $J = 4.5$ Hz) while H_2-16 only intercoupled (δ 3.73/4.15 *d*, $J = 8.6$ Hz). ^1H-^{13}C long range correlations observed from H-15 to OCH_3, C-14, C-16; from H_2-14 to C-12, C-13, C-15, C-16 as well as from H_2-16 to C-12, C-13, C-14 and C-15 conclusively proved this assumption.

The relative stereochemistry of the asymmetric carbons within **2** was accomplished by means of 2D ROESY measurement which indicated **2** to have the same configurations as (−)-leosibiricin (**10**) at the stereocenters C-4, C-5, C-6, C-8, C-9, C-10 and C-13. The observation of ROE interactions between H-15 and H_2-12 signals suggested the β-orientation of H-15. In order to ascertain this deduction, a NOE difference experiment was undertaken. Irradiation of the doublet at δ 4.15 (H-16$_\alpha$) caused a significant enhancement of the OMe signal whereas irradiation of the signal at δ 3.73 (H-16$_\beta$) produced no NOE enhancement of OMe signal but a significant effect on H_2-12. These data together with the inspection of Dreiding models showed that only an α-positioned methoxy group at C-15 permits these interactions. The structure of **2** was thus deduced as 8β-acetoxy-9α, 13; 15,16-diepoxy-15α-methoxy-7-oxo-labdan-19,6β-olide.

Other related metabolites, leopersin O and 15-*epi*-leopersin O (**3**, **4**), were isolated as an epimeric pair. TLC analysis (silica gel) **3**, **4**, developed with different solvent systems, always afforded one spot and repeated reversed-phase and normal-phase HPLC similarly gave a sharp single peak. The HREI mass spectrum indicated the molecular formula of $C_{20}H_{32}O_6$, and an identical carbon skeleton for **3** and **4**. The ^{13}C NMR spectrum, coupled with the results of the DEPT 135 experiment, contained duplicate resonances (1:1) for twenty C atoms, comprising two tertiary and one secondary methyl groups, eight methylene, four methine and five quaternary C atoms. Additional functionalities deduced from ^1H NMR and ^{13}C NMR spectra were attributable to a primary hydroxy group (δ_H 3.40, 4.26 *d*, $J = 11.7$ Hz/3.41, 4.26 *d*, $J = 11.7$ Hz; δ_C 67.2/67.3 *t*), one ether type methylene group (δ_H 3.74, 4.27 *d*, $J = 8.9$ Hz/4.03, 4.10 *d*, $J = 8.9$ Hz; δ_C 76.7/78.1 *t*), one oxymethine (δ_H 4.02 *d*, $J = 11.8$ Hz, δ_C 77.5/77.6 *d*), and one hemiacetal group (δ_H 5.67 *dd*, $J = 1.2$, 4.1 Hz/5.19 *m*; δ_C 98.9/99.0 *d*) as well as a carbonyl function (δ_C 215.5/215.9 *s*). These data coupled with a consideration of the compounds isolated so far from *Leonurus persicus*, led to the assumption that compounds **3** and **4** are a C-15 epimeric mixture of two labdane diterpenes. Indeed, detailed comparison of the NMR data of **3**, **4** with those for leopersin C (**11**) and 15-*epi*-leopersin C (**12**) [4] revealed that they were identical with the exception of the resonances observed for the hydroxymethylene function, instead of a methyl group. The deshielding of C-4 (δ_C 37.3/37.4 in **3**, **4**, δ_C 32.4 × 2 in **11**, **12**) clearly indicated the hydroxymethylene group to reside at C-4. Further proof for this deduction came from HMBC and 2D-ROESY spectra. ^1H-^{13}C long-range correlations between C-19 and H_2-3, H-5 and H_3-18 in addition to the ROE interactions from H_2-19 to H_3-20 and from H_3-18 to H-5 allowed the primary hydroxy function to be assigned to C-19. The relative configurations of the remaining chiral carbons were also proved by the latter experiment and **3** and **4** were found to be identical to **11** and **12** at all corresponding centers. Based on the above results, compounds **3** and **4** were identified as 9α,13;15,16-diepoxy-7β,15β,19-trihydroxy-labdan-6-one and 9α, 13; 15,16-diepoxy-7β,15α,19-trihydroxy-labdan-6-one, respectively.

Compound **5**, molecular formula $C_{20}H_{30}O_6$, was found to be spectroscopically very similar to **3** and **4**. Close examination of the IR spectrum of **5** clearly revealed the presence of a γ-lactone function (1785 cm^{-1}), as did the ^{13}C NMR data (δ_C 173.9 *s*), when compared with those for **3**, **4** (Table 2). The lack of the C-15 hemiacetal signals near δ_C 100 and the agreement of the carbon signals of C-11 to C-16 with those of **1** suggested the presence of a carbonyl moiety at C-15. A ROE correlation observed in the ROESY spectrum of **5** between H_3-17 and H_2-16 further secured this deduction. All the remaining spectroscopic features of **5**, as determined by HMQC, HMBC and ROESY experiments, were consistent with it being the C-15 lactone analogue of **3** and **4**. In view of these observations, compound **5** is 9α,13-epoxy-7β,19-dihydroxy-6-oxo-labdan-15,16-olide.

Like compounds **3** and **4**, leopersin Q (**6**) and 15-*epi*-leopersin Q (**7**) were also a 1:1 mixture of the C-15 epimers of 15,16-hemiacetal, possessing the same molecular formula as **3** and **4**, $C_{20}H_{32}O_6$. The ^1H NMR spectrum of **6**, **7** was almost identical to that of **3** and **4**, but H-8 resonated at lower field (δ_H 3.62 *m*, $J = 6.7$ Hz in **6**, **7**, δ_H 2.47 *m*, $J = 6.7$ Hz in **3**, **4**) and the typical H-5 singlets were absent. These differences were in agreement with **6** and **7** being positional isomers of **3** and **4** at C-6 and C-7. Thus, in **6**, **7** the H-5 (δ_H 1.60, 1.76 *d*, $J = 2.0$ Hz) coupled to H-6 (δ_H 4.19, *dd*, $J = 2.0$, 4.8 Hz) and H-8 coupled only to H_3-17 (δ_H 1.00, 1.02 *d*, $J = 6.7$ Hz). Additional HMBC correlations from C-7 to H-6, H-8, H_3-17 along with strong ROE interactions between H-5 and H-6 established the structure depicted in **6** and **7**. Consequently, compounds **6** and **7** are 9α,13;15,16-diepoxy-6β,15β,19-trihydroxy-labdan-7-one and 9α,13;15,16-diepoxy-6β,15α,19-trihydroxy-labdan-7-one, respectively.

The last diterpenoid, 19-hydroxygaleopsin (**8**) exhibited a $[M]^+$ peak at *m/z* 392.2197 (Calcd. 392.2194) in the HREI mass spectrum, corresponding to a molecular formula of $C_{22}H_{32}O_6$. From these data and its ^{13}C NMR spectrum, **8** was deduced to be a tricyclic molecule containing two carbon-carbon double bonds (δ 124.6 *s*, 110.8 *d*, 143.2 *d*, 138.8 *d*) and two carbonyl moieties (δ 206.9 *s*, keto; δ 169.1 *s*, acetoxy). The ^{13}C NMR spectrum contained additional signals for three tertiary methyl groups and two hydroxy-bearing C atoms; one primary and one tertiary, which was supported by its IR spectrum (3500 cm^{-1}, broad). Inspection of the spectral data of **8** and comparison with earlier published data for *Leonurus persicus* metabolites [3] showed **8** to have many structural similarities with 19-hydroxypregaleopsin† (**13**), except that the β,β-disubstituted dihydrofuran ring found in the side chain of **13** was replaced in **8** by a β-substituted furan ring (δ 6.31 *br s*, 7.39 *br s*, 7.27 *br s*, H-14, H-15 and H-16, respectively). At this point it became obvious that **8** is the rearrangement product of **13**, as the 9-hydroxyfuranolabdanes are regarded to arise from their C-9/C-13-epoxyprefuranoid progenitors during the isolation process [3, 4]. In the ^{13}C NMR spectrum, however, C-9 showed doubling of the signals (δ 81.7 and 81.8, both *s*). This phenomenon is consistent with the presence of epimerism at this center. The relative stereoconfigurations of the other chiral centers were elucidated by performing a 2D ROESY experiment on **8**. Detailed interpretation of the cross peaks observed in this spectrum fully supported the proposed structure for **8**. Thus, compound **8** is 8β-acetoxy-15,16-epoxy-9ξ,19-dihydroxy-labd-13(16),14-dien-7-one.

Compound **9** was identified as genkwanin (=apigenin 7-*O*-methyl ether) by comparison of its spectral data with those reported in the literature [5–8]. Although it is a widespread flavone, genkwanin was successfully used as a chemotaxonomic marker within the genus *Leonurus*, which is taxonomically very complex. It was shown that compound **9** is universally present in section *Leonurus* of this genus [9], where *L. persicus* also belongs. This is the second isolation of genkwanin from the genus *Leonurus*.

The results of this and the earlier investigations of *Leonurus persicus* [3, 4] indicated the occurrence of a greater variety and abundance of the labdane-type diterpenoids in this plant. Noteworthy is the co-occurrence of epimeric C-15 hydroxylabdanes and their corresponding C-15 lactone analogues, in reasonable yields. Moreover, as the current study revealed, C-15 methylacetal derivatives are also present in this plant species. Therefore, it is possible that C-9/C-13 epoxy C-15/C-16 tetrahydrofuran type of C-15 hemiacetals represent the early steps in the catabolism of the C-15 oxygenated labdane diterpenes which are rare in the family Lamiaceae. To our knowledge, leopersin N (**2**) is the first methoxy containing labdane diterpene obtained from the genus *Leonurus*.

EXPERIMENTAL

For general details on methods and plant material, see Refs [3] and [4].

Extraction and isolation

Details of the extraction procedure of the plant have previously been reported [3]. An aliquot (36 g) of the CH_2Cl_2 extract (38.4 g, 4.27%) of the plant was fractionated over silica gel (VLC), using hexane containing increasing proportions of EtOAc as eluent to afford 18 frs. Rechromatography of VLC fr. 9 by reversed-phase VLC, employing a MeOH-water gradient yielded 17 further frs. Frs. 16 and 17 were combined and subjected to normal-phase HPLC (250 × 8 mm, 5 μm, LiChrosorb Si60 column, RI detection) with $CHCl_3$-MeOH (3:2) mixture as mobile phase followed by RP-HPLC (250 × 8 mm, 5 μm, Spherisorb ODS II column, RI detector). Elution of the diterpenoid fraction with MeCN-H_2O (3:7) led to the isolation of **1** (3.8 mg) and **2** (19.8 mg).

A portion (0.9 g) of the initial VLC fr. 10 (2.35 g) was submitted to normal phase VLC and additionally to reversed phase-HPLC with MeCN-H_2O (3:7) to give 11 frs. Of these, fr. 5 was further purified by RP-HPLC (MeCN-H_2O, 2:8) and fr. 11 by silica gel HPLC (*n*-hexane-EtOAc, 7:3) to yield **5** (8.7 mg) and **8** (10.3 mg), respectively.

Recombination and chromatographic separation of VLC frs. 12 and 13 over RP-18 material (VLC) with gradient mixtures of MeOH-H_2O afforded **6**, **7** (23 mg) and 33 additional frs. Open CC of fr. 18 with $CHCl_3$-*iso*-PrOH (99:1 to 97:3) gave **3**, **4** (21.4 mg) and **5** (4.5 mg).

† This compound was incorrectly referred to as 4β-hydroxymethylpregaleopsin in Ref. [3]. The correct name would be 19-hydroxypregaleopsin.

VLC frs. 14 and 15 were also combined and subjected to VLC using RP-18 material as stationary phase. Genkwanin (**9**) was readily precipitated (10 mg) from the 60% MeOH fraction by the addition of cold MeOH.

Leopersin M (**1**). Colourless oil. $[\alpha]_D^{20}$ −17.6° ($CHCl_3$, *c* 0.25). UV λ_{max}^{MeOH} 206 nm. IR ν_{max}^{film} cm $^{-1}$: 3467, 2824, 1785, 1724, 1715, 1250. EIMS 70 eV, *m/z* (rel. int.) 409 $[M+H]^+$ (<1), 408 $[M]^+$ (<1), 390 $[M-H_2O]^+$ (<1), 348 $[M-HOAc]^+$ (9), 208 (22), 197 (100), 123 (29), 109 (10), 81 (5). ^1H NMR (300 MHz, $CDCl_3$ and C_6D_6) Table 1. ^{13}C NMR (75.5 MHz, $CDCl_3$ and C_6D_6) Table 2.

Leopersin N (**2**). Colourless oil. $[\alpha]_D^{20}$ −63.6° ($CHCl_3$, *c* 0.26). UV λ_{max}^{MeOH} 217 nm. IR ν_{max}^{film} cm $^{-1}$: 2934, 1783, 1763, 1742, 1213. HREIMS obsd, *m/z* 437.2173, $C_{22}H_{33}O_6$ requires 437.2175. EIMS 70 eV, *m/z* (rel. int.) 437 $[M+H]^+$ (<1), 405 $[M-OCH_3]^+$ (4), 377 $[M-HOAc+H]^+$ (8), 362 (10), 223 (30), 197 (29), 193 (48), 165 (33), 123 (29), 109 (100), 95 (44), 81 (15), 80 (42). ^1H NMR (300 MHz, $CDCl_3$) Table 1. ^{13}C NMR (75.5 MHz, $CDCl_3$) Table 2.

Leopersin O and 15-epi-leopersin O (**3**, **4**). Colourless oil. UV λ_{max}^{MeOH} 221 nm. IR ν_{max}^{film} cm $^{-1}$: 3354 (broad), 2947, 1705, 1261, 1029. HREIMS obsd, *m/z* 369.2264, $C_{20}H_{33}O_6$ requires 369.2277. EIMS 70 eV, *m/z* (rel. int.) 369 $[M+H]^+$ (17), 351 $[[M-H_2O+H]^+$ (26), 333 $[M-2H_2O+H]^+$ (11), 209 (55), 199 (100), 180 (35), 151 (30), 123 (63), 109 (28), 95 (25), 81 (26). ^1H NMR (300 MHz, $CDCl_3$) Table 1. ^{13}C NMR (75.5 MHz, $CDCl_3$) Table 2.

Leopersin P (**5**). White amorphous powder. $[\alpha]_D^{20}$ +39.8° ($CHCl_3$, *c* 0.51). UV λ_{max}^{MeOH} 209 nm. IR ν_{max}^{film} cm $^{-1}$: 3528, 3453, 2930, 1785, 1709, 1289. HREIMS obsd, *m/z* 366.2042, $C_{20}H_{30}O_6$ requires 366.2042. EIMS 70 eV, *m/z* (rel. int.) 367 $[M+H]^+$ (44), 366 $[M]^+$ (4), 349 $[M-H_2O+H]^+$ (35), 348 $[M-H_2O]^+$ (8), 331 $[M-2H_2O+H]^+$ (5), 317 (6), 209 (100), 197 (63), 180 (37), 149 (18), 123 (48), 109 (33), 95 (20), 81 (23). ^1H NMR (300 MHz, $CDCl_3$) Table 1. ^{13}C NMR (75.5 MHz, $CDCl_3$) Table 2.

Leopersin Q and 15-epi-leopersin Q (**6**, **7**). Colourless oil. UV λ_{max}^{MeOH} 213 nm. IR ν_{max}^{film} cm $^{-1}$: 3320 (broad), 2926, 1722, 1470, 1020. EIMS 70 eV, *m/z* (rel. int.) 369 $[M+H]^+$ (<1), 368 $[M]^+$ (<1), 351 $[M-H_2O+H]^+$ (17), 350 $[M-H_2O]^+$ (10), 333 $[M-2H_2O+H]^+$ (8), 332 $[M-2H_2O]^+$ (7), 209 (55), 199 (77), 123 (100), 109 (44), 95 (39), 81 (27). ^1H NMR (300 MHz, $CDCl_3$) Table 1. ^{13}C NMR (75.5 MHz, $CDCl_3$) Table 2.

19-Hydroxygaleopsin (**8**). White amorphous powder. $[\alpha]_D^{20}$ +15.3° ($CHCl_3$, *c* 1.03). UV λ_{max}^{MeOH} 228 nm. IR ν_{max}^{film} cm $^{-1}$: 3501 (broad), 2923, 1739, 1716, 1504, 1470. HREIMS obsd, *m/z* 392.2197, $C_{22}H_{32}O_6$ requires 392.2194. EIMS 70 eV, *m/z* (rel. int.) 392 $[M]^+$ (3), 350 (15), 332 $[M-HOAc]^+$ (4), 314 $[M-HOAc-H_2O]^+$ (11), 305 (19), 251 (16), 209 (10), 191 (100), 123 (28), 109 (22), 80 (14). ^1H NMR (300 MHz, $CDCl_3$) Table 1. ^{13}C NMR (75.5 MHz, $CDCl_3$) Table 2.

Genkwanin (= *apigenin 7-O-methyl ether*) (**9**). Yellow amorphous powder. EIMS, ^1H NMR and ^{13}C NMR data were identical with those reported in the literature [5–8].

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