Further Labdane Diterpenoids Isolated from *Leonurus persicus*[1]

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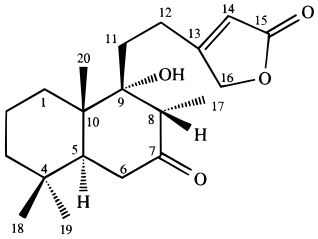
Seven new labdane diterpenoids, leopersin G−L (**1**−**4, 6**−**7**) and 15-*epi*-leopersin J (**5**), and two known ones, 13-hydroxyballonigrinolide (**8**) and ballotenol (**9**), were isolated from the aerial parts of *Leonurus persicus* along with β-sitosterol and stigmasterol. The structure determinations were mainly based on 1D and 2D NMR spectra. The stereochemical configuration of ballotenol (**9**) was reestablished by 2D ROESY spectroscopy and by single-crystal X-ray diffraction analysis.

In previous communications,[2,3] we have reported various labdane and *seco*-labdane diterpenoids obtained from the petroleum ether extract of the aerial parts of *Leonurus persicus* Boiss. (Lamiaceae). Herein we describe the isolation of nine additional labdanes, seven of which are new metabolites (**1**−**7**), obtained from the CH_2Cl_2 extract, in addition to β-sitosterol and stigmasterol, which were isolated from the petroleum ether extract of the same material.



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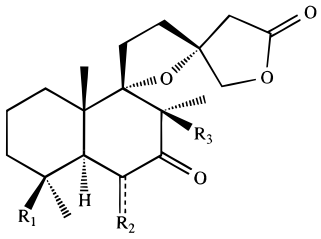
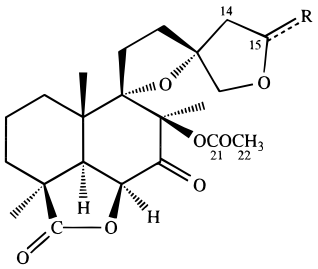
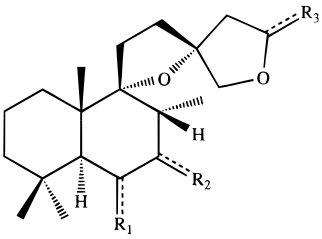


2 R_1=CH_2OH, R_2=H_α, OH_β, R_3=H
10 R_1=CH_3, R_2=H_2, R_3=$OCOCH_3$



3 R=O
11 R=H, $\Delta^{14,15}$



4 R_1=H_α, OH_β, R_2=O, R_3=H_α, OH_β
5 R_1=H_α, OH_β, R_2=O, R_3=H_β, OH_α
6 R_1=H_α, OH_β, R_2=O, R_3=O
12 R_1=O, R_2=H_α, OH_β, R_3=H_α, OH_β
13 R_1=O, R_2=H_α, OH_β, R_3=H_β, OH_α

Results and Discussion

The early vacuum−liquid chromatography (VLC) fractions of the petroleum-ether extract of the air-dried and powdered aerial parts of *Leonurus persicus* yielded β-sitosterol and stigmasterol as the main steroidal components of the extract (see Experimental Section). The CH_2Cl_2 extract of the same material was fractionated using VLC over Si gel. Purifications of the fractions by recrystallization or VLC followed by HPLC, afforded nine compounds. Compounds **2** and **4**/**5** were major metabolites, while **1**, **3** and **6**−**9** were minor components.

Leopersin G (**1**) was obtained as colorless oil, giving a positive test with Kedde reagent. HREIMS and ^{13}C-NMR data of **1** were in accordance with the molecular formula $C_{20}H_{30}O_4$. These data also indicated **1** to be a tricyclic molecule containing two carbon−oxygen double bonds and a carbon−carbon double bond. The IR spectrum of **1** exhibited characteristic absorption bands for hydroxyl (3450 cm^{-1}), conjugated γ-lactone (1770 cm^{-1}), keto (1720 cm^{-1}), and conjugated double-bond (1630 cm^{-1}) functionalities. Its ^{1}H-NMR spectrum confirmed the presence of four methyl groups, three tertiary (δ 0.90, 0.92, 1.21, each s), and one secondary (δ 1.09 d, J = 6.7 Hz), as well as an isolated methine resonance (δ 2.78 q, J = 6.7 Hz). Other functionalities that were apparent from the ^{1}H- and ^{13}C-NMR spectral data of **1** included an α,β-unsaturated γ-lactone group (δ_H 4.76 d, 5.86 t, J = 1.5 Hz; δ_C 73.1 t, 115.3 d, 170.1 s, 173.7 s), a free keto function (δ_C 211.2 s), and a tertiary hydroxy-bearing C atom (δ_C 81.3 s), the latter being initially recognized by a $[M - H_2O]^+$ fragment in the mass spectrum at m/z 316. The above features were in agreement with a labdane skeleton containing a β-substituted butenolide ring.

Analyzing the ^{1}H−^{1}H DQF-COSY spectrum of **1**, a one-proton quartet signal at δ 2.78 (J = 6.7 Hz) was correlated with a methyl doublet at δ 1.09 (d, J = 6.7 Hz, H_3-17) and was assigned to H-8. The proton resonance at δ 1.92 (dd, J = 3.8, 13.8) was coupled to a CH_2 group at δ 2.34 (t, J = 13.8, H-6_{ax}) and δ 2.44 (dd,

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J = 3.8, 13.8, H-6_{eq}) and was readily attributed to H-5. Assignment of the quaternary C atoms and the position of the tertiary hydroxyl group as well as the butenolide ring were deduced by an HMBC experiment. In particular, $^1H-^{13}C$ correlations from C-7 to H_2-6, H-8, and H_3-17 enabled us to position the free keto function at C-7. Similar correlations from C-9 to H-8, H_2-11, H_3-17, and H_3-20 indicated that the tertiary hydroxyl group resided at C-9, which was also clear from the results observed in the HMQC spectrum (δ_C 81.3 s). On the basis of additional long-range interactions from both C-13 (δ_C 170.1 s) and C-14 (δ_C 115.3 d) to H_2-12/H_2-16 and from C-15 to H-14/H_2-16, it was possible to assign the olefinic proton at δ 5.86 (t, J = 1.5 Hz) to H-14 and a two-proton doublet at δ 4.76 (J = 1.5 Hz) to H_2-16, to generate the β-substituted butenolide ring at the side chain and to complete the planar structure of **1**. The relative stereoconfigurational structure of **1** was established by a 2D ROESY experiment. ROE interactions between H-5/H_3-17 and H-5/H_3-19 suggested that they are on the same face of the diterpene (α), while correlations between H-8/H_2-11, H-8/H_3-20, and H_3-18/H_3-20 indicated their β position. Based on the above results, the structure of leopersin G (**1**) was elucidated as 9α-hydroxy-15,16-epoxylabd-13-ene-7,15-dione.

Leopersin H (**2**) was isolated as an amorphous solid. Its molecular formula, $C_{20}H_{30}O_6$ as derived from HREIMS, indicated six degrees of unsaturation. The presence of resonances for two carbonyl C atoms in the ^{13}C-NMR spectrum and the absence of any other multiple bonds showed **2** to be a tetracyclic compound. Its IR spectrum had absorption bands typical for hydroxyl (3405 cm^{-1}), γ-lactone (1785 cm^{-1}), and keto (1725 cm^{-1}) functionalities. The ^1H- and ^{13}C-NMR spectra of **2** contained resonances for two tertiary methyl (δ_H 1.02, 1.45; δ_C 19.8, 26.7) and one secondary methyl (δ_H 0.96 d, J =6.6 Hz; δ_C 9.1 q) groups, eight methylene, three methine, one of which is oxygen-bearing (δ_C 74.1 d), and six quaternary C atoms, including two carbonyl (δ_C 174.7, 209.7) functions. Detailed examination of the 2D NMR spectra of **2** and the comparison of these data with those of leopersin A (**10**)[2] indicated these two compounds to be very similar in many respects. The significant difference between the two compounds was the absence of the acetoxyl group at C-8, the presence of a secondary hydroxyl group in ring B and the replacement of one of the methyl groups by a hydroxymethylene function in compound **2**, when compared with **10**. Therefore, the characteristic H-8 quartet appeared at δ 3.60 ($J_{8,17}$ = 6.6 Hz) in $^1H-^1H$ COSY spectrum of **2**. The cross peaks observed in a proton-detected 2D long-range spectrum between C-3/H-5, C-4/H-5, C-18/H-5, C-18/H_3-19, and C-5/H_2-18, and ROE interactions from H_2-18 (δ 3.20 d, 4.18 d, J = 11.6 Hz) to H_3-20 (δ 1.45 s) indicated that the primary hydroxyl group is unambiguously positioned at C-18. The location of the secondary hydroxyl group was determined by analyzing the $^1H-^1H$ COSY and HMBC spectra. Vicinal coupling between H-6 (δ 4.12 d, J = 1.8 Hz) and H-5 (δ 1.65 d, J = 1.8 Hz) and $^1H-^{13}C$ long-range correlations from C-7 (δ_C 209.7 s) to H-6 indicated the hydroxyl group to be at C-6. It was concluded that the 6-OH group must be β-positioned, based on ROE effects observed between H-5/H-6, H-5/H_3-19, and H-6/H_3-19, as well as inter-proton coupling between H-5 and H-6 ($J_{5,6}$ = 1.8 Hz). The relative stereochemistry of the remaining chiral centers was deduced to be the same as in **10** on the basis of the results of a 2D ROESY experiment. Hence, compound **2** is 6β,18-dihydroxy-9α,13α:15,16-bisepoxylabdane-7,-15-dione.

Leopersin I (**3**) gave the $[M + H]^+$ ion peak at m/z 421 analyzing for $C_{22}H_{28}O_8$ by DCIMS. Its IR spectrum revealed absorption bands for two lactone (1795 and 1780 cm^{-1}), one ester (1750 cm^{-1}), and one keto (1725 cm^{-1}) functionalities. The presence of resonances in the ^{13}C-NMR spectrum of **3** for four carbonyl double bonds (δ_C 168.5, 174.2, 178.9, 199.4, all s) indicated **3** to be pentacyclic. Comparison of all the spectroscopic data of **3** with those for (−)-leosibiricin (**11**)[2] and the results of $^1H-^1H$ and $^1H-^{13}C$ 2D NMR measurements showed that the two molecules were identical, except for the presence of a γ-lactone ring in the side chain of **3**. Thus, it was possible to assign the doublets at δ 2.51 and 2.81 (J = 17.4 Hz) to H_2-14 and δ 4.32 and 4.54 (J = 9.8 Hz) to H_2-16. This assignment was further substantiated by the observation of a strong ROE interaction between H_2-16 and H_3-17. Diagnostic interactions found in the ROESY spectrum indicated **3** to have the same relative stereochemistry as (−)-leosibiricin (**11**) at all corresponding centers. A literature survey revealed that compound **3** is a new natural product, but that it had previously been obtained by a Jones' reagent oxidation of leocardin,[4] a C-15 epimeric labdane diterpene, isolated from *Leonurus cardiaca*. Leopersin I (**3**) is 8β-acetoxyl-6β,18:9α,13α:15,16-trisepoxylabdane-7,15,18-trione.

Leopersin J (**4**) and 15-*epi*-leopersin J (**5**) were isolated as an inseparable mixture (1:1) of two isomers. HREIMS of **4**/**5** gave a quasi-molecular ion $[M + H]^+$ at m/z 353.2332, which is consistent with a molecular formula of $C_{20}H_{32}O_5$, indicating five degrees of unsaturation. Its IR spectrum contained absorptions due to hydroxyl (3390 cm^{-1}) and keto (1700 cm^{-1}) functionalities. Both ^1H- and ^{13}C-NMR spectra contained two sets of resonances attributable to three tertiary and one secondary methyl groups; two oxygen-bearing methine resonances and a carbonyl function. Comparison of these data with those of leopersin C (**12**) and 15-*epi*-leopersin C (**13**), a C-15 epimeric mixture previously isolated from the same plant,[3] indicated **4**/**5** to be a structural isomer of **12**/**13**. Careful investigation of the 2D NMR spectra showed that **4**/**5** possessed a hydroxyl function at C-6 (δ_H 4.30 dd, J = 2.6, 3.5 Hz; δ_C 75.4, 75.6 d) and a keto group at C-7 (δ_C 2 × 210.3 s). This was supported by $^1H-^{13}C$ long-range correlations from C-7 to H-8, H_3-17, and H-6, which in turn geminally coupled to H-5 in the $^1H-^1H$ COSY spectrum. All ROEs observed for **4**/**5** in the 2D ROESY spectrum were comparable with those described for all chiral centers of **12**/**13**. Thus, the structures of **4**/**5**, which probably arise from **12**/**13** by a ketol isomerization, were identified as 6β,15β-dihydroxy-9α,13α:15,16-bisepoxylabdan-7-one and 6β,15α-dihydroxy-9α,13α:15,16-bisepoxylabdan-7-one, respectively.

Leopersin K (**6**) had a molecular formula consistent with $C_{20}H_{30}O_5$, as determined by HREIMS and NMR measurements. The NMR data of **6** were assigned with the aid of HMQC and $^1H-^1H$ COSY spectra and then compared with **4**/**5**. These comparisons revealed that the only difference between these molecules was the

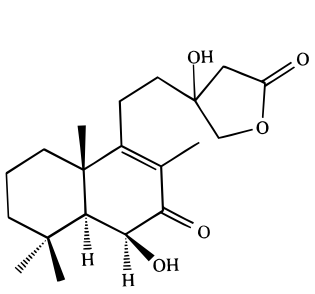
Table 1. ^{1}H-NMR Spectral Data of **1**−**9** (300 MHz, δ ppm, J Hz)

H	**1**[a]	**2**[a]	**3**[a]	**4/5**[a,e]	**6**[a]	**7**[a]	**8**[b]	**9**[c]
1	1.50[d]	1.35[d]	1.30−1.45[d]	1.38[d]/1.44[d]	1.26−1.44[d]	1.30−1.86[d]	1.67[d]	1.51−1.71[d]
2	1.60[d]	1.51[d]	1.61[d]	1.50[d]	1.56−1.75[d]	1.60−1.82[d]	1.64[d]	1.52−1.72[d]
3	1.19−1.48[d]	1.17−1.50[d]	2.25[d]	1.15−1.35[d]	1.19−1.40[d]	1.18−1.45[d]	1.40−1.95[d]	1.20−1.59[d]
5	1.92 (dd, 3.8, 13.8)	1.65 (d, 1.8)	2.76 (d, 6.1)	1.42[d]/1.59 (d, 2.6)	1.58[d]	1.54[d]	2.30 (d, 5.9)	1.95 (d, 2.0)
6	2.44 (6_{eq}, dd, 3.8, 13.8) 2.34 (6_{ax}, t, 13.8)	4.12 (d, 1.8)	5.00 (d, 6.1)	4.30 (dd, 2.6, 3.5)	4.36 m	4.32 m	4.88 (d, 5.9)	4.13 (d, 2.0)
8	2.78 (q, 6.7)	3.60 (q, 6.6)		3.55 (q, 6.6)	3.57 (q, 6.7)			3.61 (q, 6.7)
11	1.80−2.09[d]	1.92−2.24[d]	1.85−2.40[d]	1.85−2.20[d]	1.95−2.30[d]	2.45[d]	2.32[d]	1.91[d]
12	2.42[d]	2.10[d]	2.05[d]	1.88−2.10[d]/2.19[d]	2.00[d]	1.89[d]	1.71[d]	2.46−2.60[d]
14	5.86 (t, 1.5)	2.45 (d, 17.1) 2.82 (d, 17.1)	2.51 (d, 17.4) 2.81 (d, 17.4)	1.88−2.27[d]/ 1.90−2.29[d]	2.46 (d, 17.1) 2.85 (d, 17.1)	2.65 br s	2.35 (d, 16.9) 2.74 (d, 16.9)	6.32 br s
15				5.35 br s/ 5.51 (d, 4.5)				7.39 br s
16	4.76 (d, 1.5)	4.17 (d, 9.2) 4.32 (d, 9.2)	4.32 (d, 9.8) 4.54 (d, 9.8)	3.66 (d, 8.9), 4.12 (d, 8.9)/3.91 br s	4.16 (d, 9.1) 4.31 (d, 9.1)	4.21 (d, 10.0) 4.30 (d, 10.0)	4.11 (d, 9.3) 4.16 (d, 9.3)	7.29 br s
17	1.09 (d, 6.7)	0.96 (d, 6.6)	1.83 s	0.96/1.00 (d, 6.6)	1.00 (d, 6.7)	1.84 s	1.75 s	1.12 (d, 6.7)
18	0.92 s	3.20 (d, 11.6) 4.18 (d, 11.6)		1.28 × 2 s	1.27 s	1.31 s		3.34 (d, 12.0) 4.25 (d, 12.0)
19	0.90 s	1.02 s	1.33 s	0.99/1.00 s	1.02 s	1.06 s	1.23 s	1.05 s
20	1.21 s	1.45 s	0.75 s	1.43/1.45 s	1.45 s	1.41 s	0.88 s	1.53 s
22			2.17 (s)					
O*H*[f]		4.83 br s 6.29 br s		2.79, 2.88 (d, 3.5, 6-O*H*) 3.55, 3.67[d] (15-O*H*)			5.37 (s, 13-O*H*)	

[a] Measured in $CDCl_3$. [b] Measured in DMSO-d_6. [c] Measured in CD_3OD. [d] ^{1}H-NMR chemical shifts assigned on the basis of a ^{13}C−^{1}H COSY experiment. Multiplicity of the signals are unclear due to overlapping. [e] Signal pairs given together are separated by /. [f] Signals exchange upon the addition of D_2O.

presence of a γ-lactone carbonyl function in **6** at C-15 (δ_C 174.4 s), as determined by the IR spectrum (1785 cm^{-1}), instead of the hemiacetalic function found in **4**/**5**. Thus, the typical H-15 signals of a C-15 epimeric mixture were absent, and both H_2-14 and H_2-16 signals were replaced by two AB systems at δ 2.46; 2.85 (d, J = 17.1 Hz) and δ 4.16; 4.31 (d, J = 9.1 Hz), respectively. The relative stereochemistry of **6** was deduced from a ROESY study and suggested the overall structure to be as shown. Compound **6** is 6β-hydroxy-9α,13α:15,16-bisepoxylabdane-7,15-dione.

Leopersin L (**7**) was found to have the molecular



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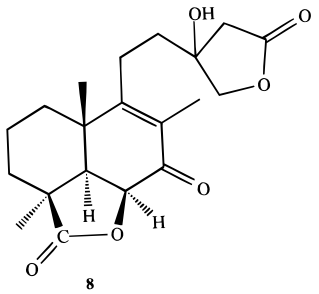

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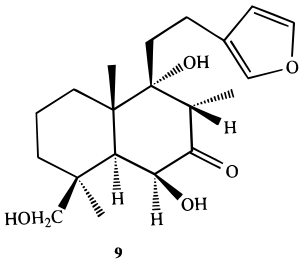

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formula $C_{20}H_{30}O_5$, giving a molecular ion peak at m/z 350.2103 (HREIMS), the same as that of **6**. Of the six units of unsaturation, two were present as carbon−oxygen double bonds and one carbon−carbon double bond, indicating that compound **7** was a tricyclic molecule. The ^{1}H- and ^{13}C-NMR data of **7** showed remarkable similarities to those of **6**. The existence of the absorption bands in the IR spectrum for an α,β-unsaturated keto functionality (1715 and 1650 cm^{-1}) and long-range correlations derived from the HMBC spectrum (between C-7/H_3-17, C-8/H_2-11, C-8/H_3-17, C-9/H_3-17, and C-9/H_3-20), suggested the presence of a $\Delta^{8,9}$ double bond in **7**, which differs from **6**. The formation of this double bond requires the cleavage of the C9−C13 ether linkage and migration of the 9-OH group to C-13 (δ_C 77.2 s). The absence of any ^{1}H−^{1}H couplings to C-13 from either H_2-14 or H_2-16 in the COSY spectrum of **7** further supported the existence of this transformation. A ROESY experiment showed that **7** has the same stereochemical configurations at centers C-5, C-6, and C-10 as in **6**. Therefore, **7** was identified as 6β,13ξ-dihydroxy-15,16-epoxylabd-8-ene-7,15-dione.

In addition to the new compounds, two known labdanes, **8** and **9** were also isolated. Compound **8** was identified as 13-hydroxyballonigrinolide by comparison of its physical and spectroscopic data with those of an authenthic sample.[5] For **8**, we report the complete ^{1}H- and ^{13}C-NMR data (Tables 1, 2), which are lacking or ambiguous in the literature.[5] Compound **9** was characterized as ballotenol.[6] Its IR, MS, and NMR data were in agreement with those of the original compound,[6] however, on the basis of the results of a 2D ROESY experiment, the relative configuration previously assigned to C-8 seemed to be incorrect. Key ROE correlations between H_3-17/H-5, H_3-17/H-6, H-8/H_2-11, and H-8/H_3-20, as well as the interproton coupling pattern of H-8 (δ 3.61 q, J = 6.7 Hz) suggested that **9** has the same stereochemistry at this center as compounds **1**, **2**, and **4**−**6**. In order to prove this assumption, a low-temperature single-crystal X-ray crystallographic analysis of **9** was undertaken. This experiment indicated the presence of two independent molecules in the asymmetric unit. These molecules differ only in the orientation of the side chain owing to twists about the C(9)−C(11) and C(12)−C(13) bonds, of approximately 143 and 108°, respectively, as shown in Figure 1. The absence

Table 2. ^{13}C-NMR Spectral Data of **1**−**9** (75.5 MHz, δ ppm)

C[a]	**1**[b]	**2**[b]	**3**[b]	**4/5**[b,e]	**6**[b]	**7**[b]	**8**[c]	**9**[d]
1	31.9 t	34.3 t	31.2 t	34.1/34.6 t	34.4 t	37.7 t	29.8 t	35.4 t
2	18.4 t	18.4 t	17.6 t	18.8 × 2 t	18.7 t	18.7 t	18.2 t	19.7 t
3	41.2 t	40.0 t	29.1 t	43.8 × 2 t	43.7 t	43.2 t	28.1 t	40.3 t
4	33.7 s	39.8 s	41.5 s	34.9/35.0 s	35.0 s	34.1 s	42.7 s	40.9 s
5	46.9 d	51.7 d	47.2 d	50.0/50.7 d	50.1 d	53.3 d	48.8 d	52.3 d
6	39.3 t	74.1 d	75.7 d	75.4/75.6 d	75.8 d	70.9 d	76.8 d	75.9 d
7	211.2 s	209.7 s	199.4 s	210.3 × 2 s	209.3 s	198.9 s	195.2 s	213.5 s
8	51.4 d	45.0 d	89.0 s	45.5 × 2 d	45.1 d	128.7 s	130.8 s	46.6 d
9	81.3 s	98.4 s	97.5 s	96.7/98.0 s	98.0 s	168.3 s	169.0 s	83.6 s
10	43.6 s	43.0 s	40.9 s	43.0 × 2 s	43.1 s	41.3 s	37.1 s	45.1 s
11	31.4 t	29.4 t	29.5 t	29.6/29.8 t	29.4 t	23.9 t	24.2 t	36.5 t
12	25.2 t	37.4 t	35.8 t	35.0/38.5 t	37.7 t	37.2 t	36.4 t	22.6 t
13	170.1 s	86.4 s	86.4 s	90.3/90.6 s	86.4 s	77.2 s	77.0 s	126.7 s
14	115.3 d	42.5 t	41.2 t	47.5/50.0 t	42.6 t	42.1 t	42.2 t	111.7 d
15	173.7 s	174.7 s	174.2 s	99.0/99.3 d	174.4 s	174.6 s	178.0 s	144.1 d
16	73.1 t	78.3 t	76.0 t	77.0/78.0 t	78.2 t	78.4 t	79.0 t	139.7 d
17	8.3 q	9.1 q	23.5 q	9.1/9.4 q	9.2 q	11.6 q	12.3 q	8.6 q
18	21.4 q	67.7 t	178.9 s	24.5/24.6 q	24.6 q	23.9 q	182.5 s	68.0 t
19	33.1 q	26.7 q	26.7 q	32.6/32.8 q	32.7 q	32.4 q	24.7 q	28.2 q
20	16.2 q	19.8 q	17.6 q	20.0 × 2 q	20.0 q	22.2 q	28.4 q	20.5 q
21			168.5 s					
22			22.1 q					

[a] Multiplicity by DEPT. [b] Measured in $CDCl_3$. [c] Measured in DMSO-d_6. [d] Measured in CD_3OD. [e] Signal pairs given together are separated by /.

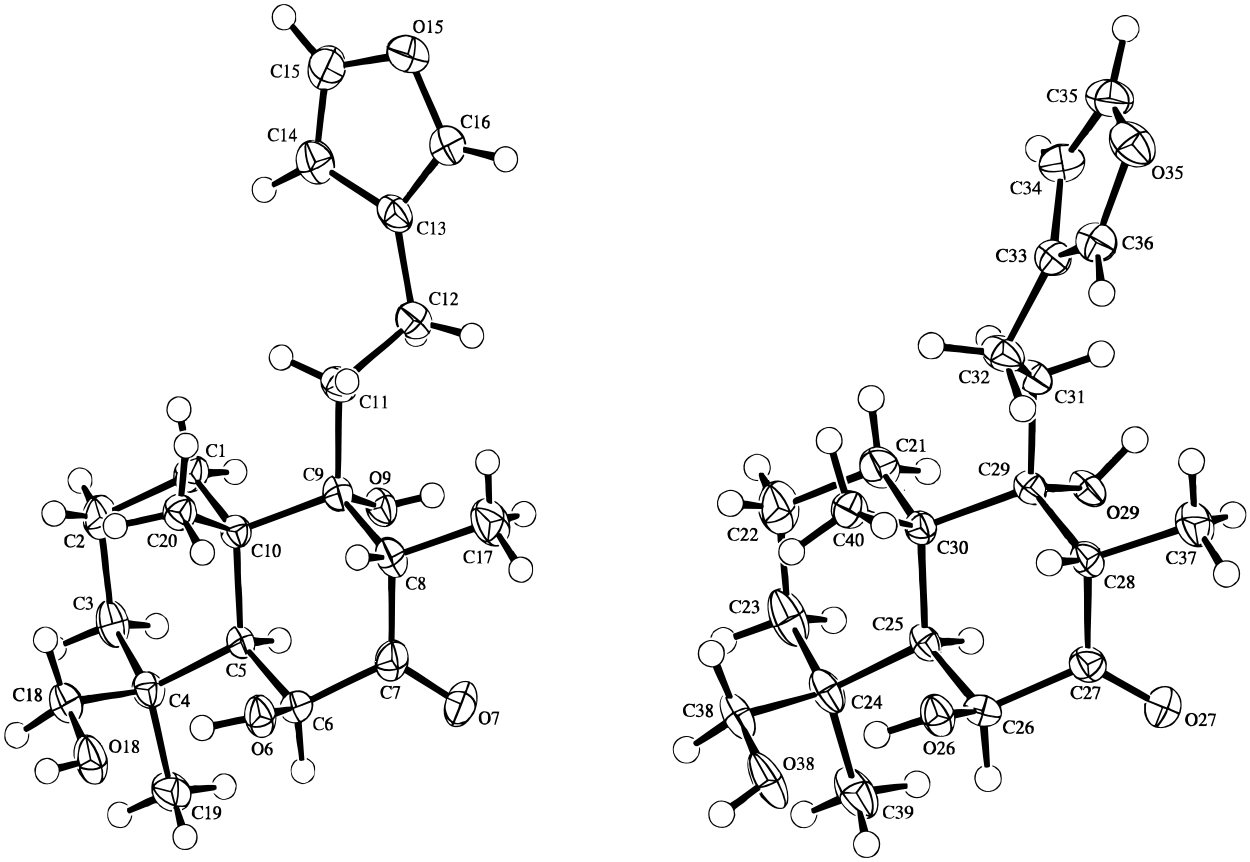

Figure 1. ORTEP[10] drawing of the two independent molecules of **9** at 173 K, with 50% probability ellipsoids. H atoms are represented by circles of arbitrary radius.

of double signals in the 2D ROESY spectrum indicate that such specific conformations do not exist in solution. The crystal structure of **9** confirms that the relative configuration at C-8 is indeed opposite to that previously proposed. The absolute configuration of the molecule has, however, not been determined. The complete NMR data of this compound are also given in Tables 1 and 2.

The results of the current report, together with the findings of our previous investigations,[2,3] clearly show that *Leonurus persicus* is capable of producing a large number of labdane diterpenes with great variations in functionalities, particularly in ring B and in the side chain. It is known that the prefuranoid labdanes rearrrange to the corresponding 9-OH furanolabdanes,[2] during or after the isolation process, by cleavage of the 9−13 epoxide bridge; however, the co-occurrence of 9-OH, 13→16 butenolide (compound **1**) and 13-OH, 13→16-γ-lactone rings (compounds **7**, **8**) in the same plant indicate the presence of alternative transformation patterns within this class of metabolites.

To our knowledge, 13-hydroxyballonigrinolide (**8**) and ballotenol (**9**), both previously reported from *Ballota* species,[5,6] have been isolated for the first time from the genus *Leonurus*. The additional results concerning the chemistry of *L. persicus* indicate a close relationship between the genera *Ballota* and *Leonurus*, both having a labdane skeleton with an oxygen function at C-7. It is noteworthy that the genus *Leonurus* contains both prefuranoid/furanoid and C-15 oxygenated-type labdanes, while *Ballota* species are rather rich in the former ones. Oxygenation of C-15 may present another feature for the chemotaxonomical classification within the tribe Stachydeae (Lamiaceae), where both genera are placed.

Experimental Section

General Experimental Procedures. Details have been published previously.[2,3]

Plant Material. For information regarding plant material used, see Tasdemir *et al.*[2,3]

Extraction and Isolation. The detailed procedure for the extraction of the plant and the preliminary fractionation of the petroleum-ether extract has been reported previously.[2,3] In the course of the above investigations, the steroid-rich VLC fraction 4 (195 mg) was subjected to a reversed-phase HPLC (250 × 8 mm, 5 μm, Spherisorb ODS II column) with MeOH−*i*-PrOH−H_2O (70:35:11) to obtain β-sitosterol (30.8 mg) and stigmasterol (19.3 mg), identified by comparison of their ^{1}H- and ^{13}C-NMR data with authentic samples.

A 36-g quantity of the CH_2Cl_2-soluble fraction (38.4 g) of the plant extract was separated by silica VLC by using sequential mixtures of *n*-hexane and EtOAc to give 18 fractions. VLC fraction 8 was refractionated by a second silica VLC yielding 13 additional fractions. Of these, fraction 2 was subjected to a semi-preparative normal-phase HPLC (250 × 8 mm, 5 μm, LiChrosorb Si60 column) with $CHCl_3$−MeOH−*n*-hexane (97:3:180) as eluent to afford compound **6**. Chromatography of fraction 3 under the same conditions yielded **7** and 14 additional fractions. Fractions 12, 13, and 14 of these were further purified by HPLC using RP-18 material with a MeCN−H_2O (35:65) mixture to give **9**, **1**, and **4**/**5**, respectively.

Of the initial VLC fraction 10 (see above), 0.9 g was submitted to a normal-phase VLC followed by a reversed-phase HPLC eluting with MeCN-H_2O (30:70) to yield **2** and **3**. Compound **8** was obtained from the initial VLC fraction 12 after recrystallization with *n*-hexane−EtOAc (1:1) mixture as white crystals.

Leopersin G (1): colorless oil (3.0 mg): $[\alpha]^{20}_D$ +1.8° (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3450, 2920, 1770, 1720, 1630, 1450, 1245 cm^{-1}; UV λ^{MeOH}_{max} 220 nm; HREIMS *m*/*z* 334.2143 (calcd for $C_{20}H_{30}O_4$ 334.2144); EIMS *m*/*z* (rel int.) 335 $[M + H]^+$ (20), 334 $[M]^+$ (17), 319 (11), 316 $[M - H_2O]^+$ (2), 212 (16), 211 (100), 210 (85), 195 (42), 168 (59), 123 (69), 109 (76), 95 (10), 81 (9); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75.5 MHz, $CDCl_3$), see Table 2.

Leopersin H (2): white amorphous powder (200 mg), $[\alpha]^{20}_D$ +2.97° (*c* 0.37, MeOH); IR (KBr) ν_{max} 3405, 3280, 2950, 1785, 1725, 1470, 1285 cm^{-1}; UV λ^{MeOH}_{max} 212 nm; HREIMS *m*/*z* 366.2066 (calcd for $C_{20}H_{30}O_6$ 366.2042); EIMS *m*/*z* (rel int) 366 $[M]^+$ (1), 348 $[M - H_2O]^+$ (3), 318 (4), 219 (6), 209 (43), 197 (40), 123 (91), 109 (71), 95 (14), 81 (18), 55 (100); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75.5 MHz, $CDCl_3$), see Table 2.

Table 3. Crystallographic Data for **9**

empirical formula	$C_{20}H_{30}O_5$
formula weight	350.45
crystal color, habit	colorless, plate
crystal dimensions [mm]	0.10 × 0.38 × 0.45
temperature [K]	173 (1)
crystal system	monoclinic
space group	$P2_1$
Z	4
reflections for cell determination	25
2θ range for cell determination [°]	31−40
a [Å]	10.528 (1)
b [Å]	11.819 (3)
c [Å]	15.189 (1)
β [°]	94.771 (9)
V [Å^3]	1883.5 (6)
F(000) [e]	760
D_x [g cm^{-3}]	1.236
μ(Mo K_α) [mm^{-1}]	0.0872
$2\theta_{(max)}$ [°]	55
reflection ranges	$0 < h < 13$; $0 < k < 15$; $-19 < l < 19$
total reflections measured	4782
symmetry independent reflections	4539
reflections used [$I > 2\sigma(I)$]	3671
parameters refined	475
R	0.0415
R_w	0.0364
goodness of fit	1.531
secondary extinction coefficient	1.40 × 10^{-6}
final Δ_{max}/σ	0.0004
$\Delta\rho$ (max; min) [e Å^{-3}]	0.26; −0.23

Leopersin I (3): colorless oil (3.0 mg); $[\alpha]^{20}_D$ +13.0° (*c* 0.17, $CHCl_3$); IR (film) ν_{max} 2925, 1795, 1780, 1750, 1465, 1260 cm^{-1}; UV λ^{MeOH}_{max} 223 nm; EIMS $[M]^+$ absent; DCIMS (NH_3) *m*/*z* (rel int) 438 $[M + NH_4]^+$ (100), 421 $[M + H]^+$ (53), 379 $[M - HOAc + NH_4 + H]^+$ (26), 378 $[M - HOAc + NH_4]^+$ (47), 361 $[M - HOAc + H]^+$ (17), 335 (11), 181 (6), 109 (11); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75.5 MHz, $CDCl_3$), see Table 2.

Leopersin J and 15-*epi*-leopersin J (4/5): colorless oil (99.0 mg); IR (film) ν_{max} 3390, 2930, 1700, 1470, 1360, 1260 cm^{-1}; UV λ^{MeOH}_{max} 221 nm; HREIMS *m*/*z* 353.2332 (calcd for $C_{20}H_{33}O_5$ 353.2329); EIMS *m*/*z* (rel int) 352 $[M]^+$ (<1), 334 $[M - H_2O]^+$ (5), 228 (15), 200 (12), 199 (100), 197 (58), 123 (36), 109 (19), 95 (25), 81 (22); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75.5 MHz, $CDCl_3$), see Table 2.

Leopersin K (6): colorless oil (6.0 mg); $[\alpha]^{20}_D$ −17.6° (*c* 0.37, $CHCl_3$); IR (film) ν_{max} 3405, 2950, 1785, 1725, 1470, 1285 cm^{-1}; UV λ^{MeOH}_{max} 218 nm; HREIMS *m*/*z* 350.2101 (calcd for $C_{20}H_{30}O_5$ 350.2094); EIMS *m*/*z* (rel int) 351 $[M + H]^+$ (8), 350 $[M]^+$ (8), 333 $[M - H_2O + H]^+$ (13), 226 (37), 208 (17), 197 (100), 195 (46), 109 (20); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75.5 MHz, $CDCl_3$), see Table 2.

Leopersin L (7): colorless oil (2.1 mg); $[\alpha]^{20}_D$ +32.2° (*c* 0.09, $CHCl_3$); IR (film) ν_{max} 3430, 2920, 1780, 1715, 1650, 1283, 1033 cm^{-1}; UV λ^{MeOH}_{max} 208 and 251 nm; HREIMS *m*/*z* 350.2103 (calcd for $C_{20}H_{30}O_5$ 350.2094); EIMS *m*/*z* (rel int) 351 $[M + H]^+$ (15), 350 $[M]^+$ (9), 333 $[M - H_2O + H]^+$ (8), 332 $[M - H_2O]^+$, 237 (39), 221 (100), 123 (10); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75.5 MHz, $CDCl_3$), see Table 2.

13-Hydroxyballonigrinolide (8): white crystals (15.0 mg) mp 229−232 °C (lit.[5] 227 °C), $[\alpha]^{20}_D$ +3.1° (*c* 0.35, MeOH) (lit.[5] $[\alpha]^{20}_D$ +15.0°, *c* 0.25, MeOH); IR and

Table 4. Fractional Atomic Coordinates and Equivalent Isotropic Displacement Parameters (Å^2) for **9** (with standard uncertainties in parentheses)

atom	*x*	*y*	*z*	U_{eq}[a]
O(6)	0.5863(2)	1.16190[†]	0.2011(1)	0.0290(7)
O(7)	0.7901(2)	1.2411(3)	0.3653(2)	0.0413(9)
O(9)	0.7468(2)	0.9915(3)	0.4489(1)	0.0257(7)
O(15)	1.0492(2)	0.5224(3)	0.4054(2)	0.0446(9)
O(18)	0.3405(2)	1.1283(3)	0.1821(2)	0.0359(8)
C(1)	0.5305(3)	0.8661(3)	0.3921(2)	0.026(1)
C(2)	0.3850(3)	0.8695(4)	0.3875(2)	0.031(1)
C(3)	0.3366(3)	0.9884(4)	0.4043(2)	0.030(1)
C(4)	0.3808(3)	1.0774(3)	0.3394(2)	0.0235(9)
C(5)	0.5307(3)	1.0705(3)	0.3435(2)	0.0192(8)
C(6)	0.5955(3)	1.1669(3)	0.2960(2)	0.0243(9)
C(7)	0.7381(3)	1.1633(3)	0.3252(2)	0.028(1)
C(8)	0.8031(3)	1.0541(4)	0.3050(2)	0.027(1)
C(9)	0.7386(3)	0.9584(3)	0.3572(2)	0.0227(9)
C(10)	0.5907(3)	0.9508(3)	0.3292(2)	0.0210(9)
C(11)	0.8049(3)	0.8425(3)	0.3458(2)	0.028(1)
C(12)	0.9078(3)	0.8082(4)	0.4185(2)	0.033(1)
C(13)	0.9421(3)	0.6853(4)	0.4130(2)	0.028(1)
C(14)	0.8563(3)	0.5918(4)	0.4200(2)	0.033(1)
C(15)	0.9246(4)	0.4975(4)	0.4156(2)	0.037(1)
C(16)	1.0558(3)	0.6386(4)	0.4039(3)	0.040(1)
C(17)	0.9486(3)	1.0635(4)	0.3244(2)	0.041(1)
C(18)	0.3045(3)	1.0545(4)	0.2508(2)	0.029(1)
C(19)	0.3417(3)	1.1949(4)	0.3720(2)	0.034(1)
C(20)	0.5704(3)	0.9063(3)	0.2336(2)	0.0252(9)
O(26)	0.8588(2)	0.5704(3)	−0.0624(1)	0.0262(7)
O(27)	0.7131(2)	0.3204(3)	−0.0978(1)	0.0344(8)
O(29)	0.7640(2)	0.2815(3)	0.1143(1)	0.0239(7)
O(35)	0.3311(2)	0.6016(3)	0.3090(2)	0.0359(8)
O(38)	1.1003(2)	0.6120(3)	−0.0525(2)	0.0404(9)
C(21)	0.9791(3)	0.3878(4)	0.1947(2)	0.031(1)
C(22)	1.1205(3)	0.4165(4)	0.1980(2)	0.041(1)
C(23)	1.1761(3)	0.3803(4)	0.1132(3)	0.039(1)
C(24)	1.1098(3)	0.4330(3)	0.0286(2)	0.026(1)
C(25)	0.9616(3)	0.4147(3)	0.0309(2)	0.0196(8)
C(26)	0.8794(3)	0.4508(3)	−0.0533(2)	0.0244(9)
C(27)	0.7498(3)	0.3961(3)	−0.0483(2)	0.0245(9)
C(28)	0.6779(3)	0.4386(3)	0.0269(2)	0.0226(9)
C(29)	0.7574(3)	0.4037(3)	0.1144(2)	0.0191(8)
C(30)	0.8998(3)	0.4472(3)	0.1181(2)	0.0202(8)
C(31)	0.6849(3)	0.4376(3)	0.1951(2)	0.0234(9)
C(32)	0.6314(3)	0.5582(3)	0.2027(2)	0.029(1)
C(33)	0.5199(3)	0.5605(3)	0.2587(2)	0.0235(9)
C(34)	0.5108(3)	0.5046(4)	0.3416(2)	0.031(1)
C(35)	0.3967(3)	0.5318(4)	0.3685(2)	0.036(1)
C(36)	0.4103(3)	0.6167(3)	0.2425(2)	0.029(1)
C(37)	0.5399(3)	0.3958(4)	0.0199(2)	0.037(1)
C(38)	1.1605(3)	0.5547(3)	0.0217(2)	0.030(1)
C(39)	1.1543(3)	0.3685(4)	−0.0512(3)	0.039(1)
C(40)	0.9020(3)	0.5759(3)	0.1364(2)	0.0266(9)

[a] U_{eq} is defined as one-third of the trace of the orthogonalized $\mathbf{U}^{ij}$ tensor. [b] Origin defined by fixing the *y* coordinate of O(6).

EIMS data were identical with those previously reported,[5] ^{1}H-NMR (300 MHz, DMSO-d_6), see Table 1; ^{13}C-NMR (75.5 MHz, DMSO-d_6), see Table 2.

Ballotenol (9): a white amorphous powder (12 mg) $[\alpha]^{20}_D$ +14.5° (*c* 0.28, pyridine) (lit.[6] $[\alpha]^{20}_D$ 0°, *c* 0.38, pyridine); IR and EIMS data were identical with those previously reported;[6] ^{1}H-NMR (300 MHz, CD_3OD), see Table 1; ^{13}C-NMR (75.5 MHz, CD_3OD), see Table 2.

Single Crystal X-Ray Analysis of 9.[7] Crystals of $C_{20}H_{30}O_5$, obtained from $CHCl_3$−hexane, were used for a low-temperature X-ray structure determination. All measurements were made on a Rigaku AFC5R diffractometer using graphite-monochromated Mo K_α radiation (λ = 0.71069 Å) and a 12 kW rotating anode generator. The intensities were collected using $\omega/2\theta$ scans, and three frequently measured standard reflections remained stable throughout the data collection. The intensities were corrected for Lorentz and polarization effects, but not for absorption. The space group was determined from the systematic absences and packing considerations. The structure was solved by direct methods using SHELXS-86,[8] which revealed the positions of all non-hydrogen atoms. There are two independent molecules in the asymmetric unit. Both have the same configuration, but there are significant differences in the orientation of the chain connecting the five-membered ring to the fused rings. The non-hydrogen atoms were refined anisotropically. All of the H-atoms were located in a difference electron density map. Those of the hydroxyl groups were allowed to refine together with individual isotropic displacement parameters. The positions of the remaining H-atoms were geometrically idealized [d(C − H) = 0.95 Å] and held fixed. These H-atoms were assigned fixed isotropic displacement parameters with values equal to $1.2U_{eq}$ of the parent C-atoms. Refinement of the structure was carried out on *F* using full-matrix least-squares procedures, which minimized the function $\Sigma w(|F_o| - |F_c|)^2$, where $w = [\sigma^2(F_o) + (0.005F_o)^2]^{-1}$. A correction for secondary extinction was applied. Data collection and refinement parameters are given in Table 3, and the fractional atomic coordinates and equivalent isotropic displacement parameters are in Table 4. A view of the molecule is shown in Figure 1. All calculations were performed using the TEXSAN crystallographic software package.[9] The absolute configuration has not been determined. The enantiomorph was chosen arbitrarily.

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References and Notes

(1) Parts of this work were presented at The 37th Annual Meeting of the American Society of Pharmacognosy, University of California, Santa Cruz, 27−31 July 1996, and The 44th Annual Meeting of the Society for Medicinal Plant Research and a Joint Meeting with the Czech Biotechnology Society, Prague, 3−7 September 1996.
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(7) Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [FAX: +44-(0)1223-336033 or email: teched@ccdc.cam.ac.uk].
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