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Sesterterpenoids and Other Constituents of *Salvia sahendica*

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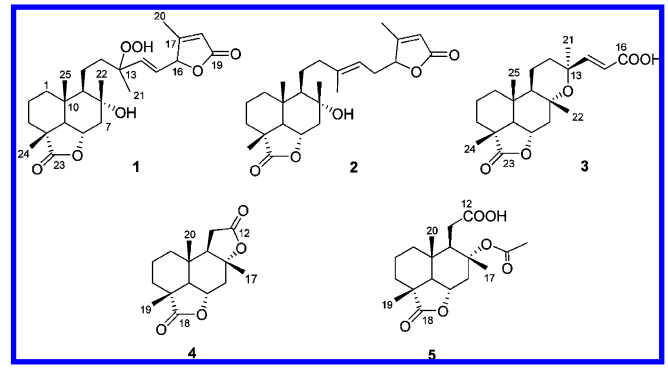
A new sesterterpene (**1**), a new norsesterterpene (**3**), and two new norditerpenes (**4**, **5**) were isolated from the aerial parts of *Salvia sahendica*, together with 12 known compounds, comprising a sesterterpene, a sesquiterpene, a diterpene, two triterpenes, two steroidal compounds, and five flavonoids. The structures of the new compounds were established by spectroscopic data interpretation, and in the case of **4**, its structure was confirmed by single-crystal X-ray analysis.

Salvia species are important medicinal and culinary plants, and they have been the subject of numerous chemical and biological studies. Apart from their common constituents (terpenoids and flavonoids) and their interesting biological activities, the genus *Salvia* is unusual, as it is the only genus in the Lamiaceae that produces sesterterpenes.[1] In contrast to di- and triterpenoids, sesterterpenoids are rare in nature and have been reported most commonly in marine sponges and algae. Among *Salvia* species, these rare and interesting compounds were isolated and identified for the first time from Iranian species, and this prompted us to undertake a systematic phytochemical investigation of members of this genus. To the best of our knowledge, sesterterpenoids have been isolated from only nine *Salvia* species, namely, *S. hypoleuca*,[2,3] *S. sahendica*,[4] *S. mirzayanii*,[5] *S. syriaca*,[6] *S. limbata*,[7] *S. palaestina*,[8] *S. yosgadensis*,[9,10] *S. aethiopis,*[11] and *S. dominica.*[12] It is interesting to note that seven of these species belong to the flora of Iran and the first three are endemic. The reported sesterterpenoids are bicyclic and mostly sesterterpene lactones. Therefore, Iranian *Salvia* species might contain sesterterpenoids as main constituents, a fact that could be of chemotaxonomic importance.

Salvia sahendica Boiss. & Buhse is one of 17 Iranian endemic *Salvia* species and grows in the northern hilly areas of Azerbaijan Province, preferentially around Mount Sahand. In a previous phytochemical study on the aerial parts of the plant, we have reported the presence of a sesterterpene, salvileucolide methyl ester, in addition to sclareol and salvigenin.[4] Furthermore, the absolute configuration of salvileucolide methyl ester was determined by X-ray crystallography.[13] Also, two new rearranged abietane diterpenoids together with three known derivatives have recently been isolated from the roots of the plant.[14]

In the peresent work, we have undertaken a further phytochemical investigation on the aerial parts of *S. sahendica*, which were collected from the same area as in our previous study, but in larger amounts, in order to identify minor sesterterpenoids that could not be identified previously. We report herein the isolation and structural elucidation of a new sesterterpene (**1**), a new norsesterterpene (**3**), and two new norlabdane diterpenes (**4**, **5**) on the basis of extensive spectroscopic data. In addition to sclareol and salvigenin, which have been previously isolated from the plant, the sesterterpenoid salvileucolide-6,23-lactone (**2**),[2] the terpenoid loliolide,[15] the triterpenoids oleanolic acid and maslinic acid,[16] the steroidal compounds β-sitosterol and daucosterol, and the four flavonoids eupatorin,[17] hispudulin,[18] ladanein,[19] and apigenin were isolated and are described here for *S. sahendica* for the first time. The ^{13}C NMR spectroscopic data of **2** are reported for the first time.



Compound **1** was isolated as a colorless gum. It gave a molecular formula of $C_{25}H_{36}O_7$ as deduced from HRESIMS at *m*/*z* 466.2798 $[M + NH_4]^+$ (calcd 466.2799). Its IR spectrum showed the presence of hydroxy (3430 cm^{-1}) and γ-lactone (1770 cm^{-1}) groups. The ^{13}C NMR data (Table 1) showed signals for a disubstituted double bond (δ_C 124.4, 139.8), a trisubstitued double bond (δ_C 116.3, 168.3), and two carbonyl carbons (δ_C 173.4, 181.6). According to the degree of unsaturation, the structure of **1** must be tetracyclic due to the absence of any other sp or sp^2 carbon signals. The ^{1}H NMR spectrum of **1** (Table 1) showed resonances of five methyl singlets at δ_H 0.84, 1.14, 1.18, 1.30, and 1.99, three vinyl protons at δ_H 5.39 (dd, J = 8.0, 16.0 Hz), 5.80 (brs), and 5.92 (d, J = 16.0 Hz), and a broad doublet at δ_H 5.16 (d, J = 8.0 Hz). A multiplet at δ_H 4.18 (ddd, J = 4.5, 11.0, 11.0 Hz) indicated an oxygen-bearing carbon situated between a methine and a methylene group. Such a group could only be placed at either C-6 or C-11. Comparison of the signal with those of sesterterpenoids previously isolated from *S. hypoleuca*[2,3] indicated the position of this functionality at C-6. The downfield shift of H-6 from 3.6 ppm in salvileucolide methyl ester to 4.18 ppm indicated that **1** is most likely the lactone of a

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **1** and **3** and ^{13}C NMR Spectroscopic Data of Compound **2** ($CDCl_3$, 500 MHz)[a]

	1		**2**	**3**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_C	δ_H (J in Hz)
1	40.4	0.96, m	41.1	40.3	0.94, m
		1.67[b]			1.70[b]
2	18.1	1.65[b]	19.1	19.1	1.70[b]
3	32.7	1.38, m	33.2	33.6	1.43, m
		1.78, brd (13.0)			1.88, brd (13.0)
4	41.4		41.8	41.5	
5	60.2	1.45, d (11.0)	60.6	61.1	1.56[b]
6	74.4	4.18, ddd (4.5, 11.0, 11.0)	74.6	74.9	4.25, ddd (4.5, 11.0, 11.0)
7	47.7	1.62[b]	48.3	46.4	1.51[b]
		2.39, dd (4.5, 11.0)			2.39, dd (4.5, 11.0)
8	74.9		74.9	77.1	
9	60.7	1.34[b]	60.1	58.7	1.38, m
10	36.4		36.9	35.3	
11	18.7	1.65[b]	23.4	16.4	1.53[b]
12	38.7	1.65[b]	41.7	35.5	1.53[b]
		1.87, dt (3.5, 10.0)			2.23, brd (13.0)
13	83.8		141.2	73.6	
14	139.8	5.92, d (16.0)	116.6	159.4	7.18, d (16.5)
15	124.4	5.39, dd (8.0, 16.0)	30.5	116.5	5.75, d (16.5)
16	85.5	5.16, d (8.0)	84.8	171.7	
17	168.3		169.1		
18	116.3	5.80, brs	117.6		
19	173.4		173.8		
20	13.9	1.99, brs	14.3		
21	22.8	1.30, s	16.7	31.8	1.25, s
22	24.5	1.18, s	24.8	24.7	1.21, s
23	181.6		181.9	181.7	
24	15.7	1.14, s	16.2	15.8	1.17, s
25	15.3	0.84, s	15.8	15.6	0.82, s

[a] δ values were established from HMBC, COSY, and HMQC experiments. [b] Overlapping signals.

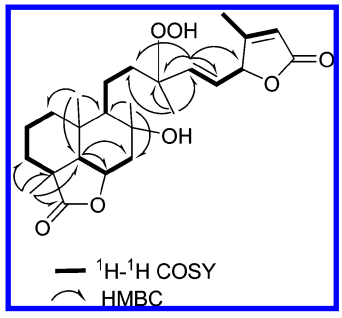

Figure 1. Key COSY and HMBC correlations of **1**.

hydroxy acid that corresponds to an ester. COSY correlations (Figure 1) confirmed the relationship between H-6 and H-7β (δ_H 2.39, dd, J = 4.5, 11.0 Hz) and H-7α (δ_H 1.62, m), as well as between H-6 and H-5 (δ_H 1.45, d, J = 11.0 Hz). The relative configuration of the lactonized group at C-6 was determined as α on the basis of the magnitude of the coupling constants of H-6. In addition, in the NOESY spectrum, H-6 was found to show NOEs with H-22, H-24, and H-25, all with β-orientations. Moreover, the observation of NOE correlations between H-5 and H-9 was used to place them on the same face of the molecule.[5] The HMBC and COSY spectra of **1** were particularly useful in determining the partial structure of the side chain (Figure 1). Thus, H-21 (δ_H 1.30, s) showed long-range correlations to C-13 (δ_C 83.8), C-12 (δ_C 38.7), and C-14 (δ_C 139.8). On the other hand, H-15 (δ_H 5.39 dd) could be related via long-range correlations to C-13 (δ_C 83.8), C-16 (δ_C 85.5), and C-17 (δ_C 168.3). Additionally, the COSY experiment confirmed the relationship between H-15 and H-16. The molecular formula resulting from the HRESIMS, together with the large chemical shift of the nonacetoxy oxygen-bearing carbon (δ_C 83.8), indicated the presence of a hydroperoxy group. According to the above connectivities, this hydroperoxide function could be located at C-13. Several hydroperoxide sesterterpenes were previously isolated from *S. hypoleuca*.[3] The *E*-configuration of the Δ^{14} double bond was deduced from the magnitude of the coupling constant (J = 16.0 Hz). However, the relative configurations at C-13 and C-16 in **1** remain unassigned. Therefore, the structure of **1** was determined as 8α-hydroxy-13-hydroperoxylabd-14,17-dien-19,16;23,6α-diolide.

Compound **2** was isolated as a colorless gum, and its ^{1}H NMR and other data were used to identify it as salvileucolide-6,23-lactone, which was obtained from *S. hypoleuca* several years ago.[2] The ^{13}C NMR spectroscopic data are reported for this compound for the first time (Table 1).

Compound **3** was isolated as an amorphous powder. Its molecular weight was established by HRESIMS, and this compound showed a molecular ion peak at m/z 363.2170 $[M + H]^+$ (calcd 363.2166), indicating a molecular formula of $C_{21}H_{30}O_5$. The IR spectrum displayed a hydroxy group absorbance between 3500 and 3200 cm^{-1}, suggesting the probable presence of a carboxylic acid group, along with two carbonyl absorptions at 1760 cm^{-1} (γ-lactone) and 1690 cm^{-1} (α,β-unsaturated carboxylic acid). The molecular formula accounted for seven degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) exhibited four methyl singlets at δ_H 0.82, 1.17, 1.21, and 1.25, two vinyl protons at δ_H 7.18 (d, J = 16.5 Hz) and 5.75 (d, J = 16.5 Hz), and a carbinol methine signal at δ_H 4.25 (ddd, J = 4.5, 11.0, 11.0 Hz). The ^{13}C NMR data (Table 1) and HMQC spectra showed the presence of four methyls, six methylenes, and five methine groups, as well as six quaternary carbons. The ^{13}C NMR chemical shift of three of these (δ_C 73.6, 74.9, and 77.1) indicated they are linked to oxygen. It was rationalized that the oxygen atom must be part of an ether bridge connecting two quarternary carbon atoms, and the remaining oxygen was situated between a methine and a methylene group. The location of this oxygen at C-6 and the connectivity between H-5, H-6, and H-7 were deduced from the COSY spectrum as in compound **1**. From the above data, **3** was suggested to possess the manoyl oxide skeleton in the A, B, and C rings. The rest of the molecule forms a side chain containing three carbons, in which a conjugated acid could be assigned in accordance with the HMBC correlations. Thus,

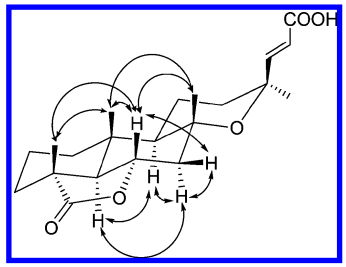

Figure 2. Key NOESY correlations of **3**.

H-14 showed long-range couplings with the C-13 tertiary, the C-16 carbonyl, and the C-21 methyl carbons. In turn, H-15 could be related via long-range correlations to C-13 and C-16. The relative configuration of **3** was assigned from NOESY correlations (Figure 2), which showed relationships between H-6β and H-22, H-24, H-25, and H-7β as well as between H-5α and H-9α, again as in **1**. The relative configuration of Me-21 was not deduced in this manner. However, when H-22 was irradiated in a 1D NOE experiment, only H-25 was enhanced and H-21 remained unchanged. The absence of a NOE between H-22 and H-21 suggested the α-orientation of H-21 at C-13. Comparison of the chemical shifts of C-21 and C-22 in **3** (δ_C 31.8 and 24.7) with those of C-16 and C-17 in manoyl oxide (δ_C 28.4 and 25.4)[20] and 13-*epi*-manoyl oxide (δ_C 32.7 and 24.0)[21] and their derivatives[11,22] was used to confirm this orientation. Thus, **3** is a tetranorsesterterpene containing the partial structure of 13-*epi*-manoyl oxide, with the structure 17,18,19,20-tetranor-13-*epi*-manoyloxide-14-en-16-oic acid-23,6α-olide. To our knowledge, this is the first tetranorsesterterpene to have been purified from the genus *Salvia*.

Compound **4** was obtained as colorless crystals. The HRESIMS showed a pesk at *m/z* 279.1592 $[M + H]^+$ (calcd 279.1591), in agreement with the elemental formula $C_{16}H_{22}O_4$. The IR spectrum displayed an absorption band at 1765 cm^{-1}, indicating the presence of a γ-lactone moiety. The ^{13}C NMR and HMQC spectra (Table 2) showed the occurrence of three methyls, five methylenes, and three methine groups, as well as five quaternary carbons. The ^1H NMR spectrum exhibited three methyl singlets at δ_H 1.08, 1.26, and 1.41 and a methine proton at δ_H 4.38 (ddd, $J = 4.5$, 11.0, 11.0 Hz). The NMR data of **4** showed great similarity to those of 6α-hydroxynorambreinolide isolated from *S. yosgadensis*.[10] In the ^{13}C NMR spectrum of **4**, one methyl signal at 36.2 ppm was replaced by another signal at 180.6 ppm. The chemical shifts of C-3, C-4, and C-6 were observed at δ_C 33.7, 41.1, and 74.5, respectively, with downfield shifts of ca. 10, 7, and 5 ppm relative to 6α-hydroxynorambreinolide, while the chemical shift of Me-19 was observed at δ_C 15.2 with an upfield shift of ca. 6 ppm. These observations suggested the replacement of the C-18 methyl with a carbonyl group. In the ^1H NMR spectrum of **4**, the multiplet at 3.99 ppm was shifted to 4.38 ppm. This observation, along with the loss of the absorption of the hydroxy group in the IR spectrum, indicated that **4** is the lactone of a hydroxy acid. Unambiguous assignments of ^1H and ^{13}C NMR data were enabled by a combination of HMQC, $^1H-^1H$ COSY, and HMBC experiments. The relative configuration of the molecule was determined from NOESY correlations. Definitive support for the proposed structure of **4** was provided by the X-ray crystallographic analysis of colorless crystals (Figure 3). Compound **4** was therefore established structurally as norambreinolide-18,6α-olide.

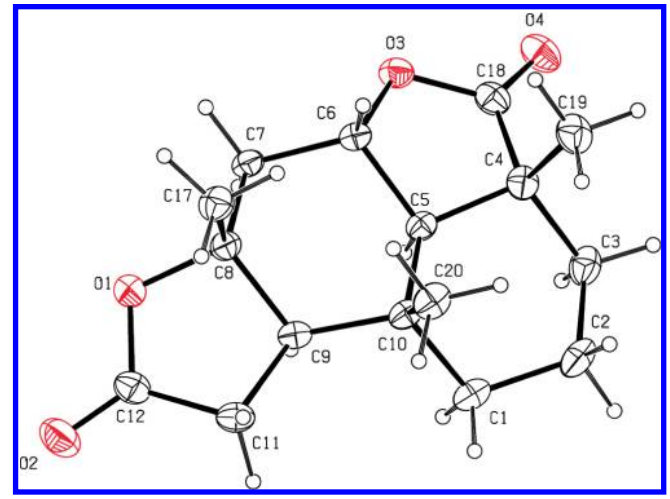

Figure 3. Thermal ellipsoid plot of **4**.

Compound **5** was obtained as an amorphous powder, and its molecular formula $C_{18}H_{26}O_6$ was established by HRESIMS ($[M + H]^+$, *m/z* 339.1805, calcd 339.1802). The IR spectrum showed a large hydroxy band at 3200−3400 cm^{-1} and three carbonyl absorptions at 1705 cm^{-1} (carboxylic acid), 1730 cm^{-1} (acetyl), and 1760 cm^{-1} (γ-lactone). The NMR spectroscopic data (Table

Table 2. ^1H and ^{13}C NMR Spectroscopic Data of Compounds **4** and **5** ($CDCl_3$, 500 MHz)[a]

	4		**5**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	40.3	1.12, ddd (6.0, 12.0, 13.0)	40.1	1.13, m
		1.68, dt (3.0, 13.0)		1.66[b]
2	18.6	1.79, m	19.0	1.70, m
3	33.7	1.57, ddd (5.0, 13.0, 13.0)	33.0	1.46, m
		1.95, dt (3.0, 13.0)		1.85, brd (13.0)
4	41.1		41.7	
5	61.2	1.70, d (11.0)	59.9	1.63, d (11.0)
6	74.5	4.38, ddd (4.5, 11.0, 11.0)	73.5	4.22, ddd (4.0, 11.0, 11.0)
7	42.6	1.89, t (11.0)	42.7	2.00, t (11.0)
		2.78, dd (4.5, 11.0)		3.28, dd (4.0, 11.0)
8	85.0		84.8	
9	59.4	2.15, dd (6.5, 14.5)	55.0	2.45[b]
10	34.5		36.3	
11	28.2	2.38, dd (6.5, 16.0)	30.5	2.30, dd (8.0, 10.0)
		2.52, dd (14.5, 16.0)		2.45[b]
12	175.5		179.5	
17	22.9	1.41, s	21.5	1.53, s
18	180.6		181.2	
19	15.2	1.26, s	16.1	1.18, s
20	15.1	1.08, s	16.0	0.92, s
CH$_3$CO			22.6	1.88, s
CH$_3$*CO*			169.9	

[a] δ values were established from HMBC, COSY, and HMQC experiments. [b] Overlapping signals.

2) of **5** were very similar to those of 6α-hydroxy-8α-acetoxy-13,14,15,16-tetranorlabdan-12-oic acid, isolated from *S. yosgadensis*,[10] indicating that they are structurally related. Comparison of the ^{13}C NMR data of these two compounds indicated the absence of a signal for one methyl group but showed an additional signal at $\delta_C = 181.2$ ppm, suggesting that in **5** one of the methyl groups was replaced by a carbonyl group. As in **4**, the most significant features of the NMR spectra of **5** suggested the location of a carbonyl group at C-18 from the downfield shifts of C-3, C-4, and C-6 and the upfield shift of Me-19 (Table 2).[10] In the ^1H NMR spectrum, the multiplet at 3.80 ppm was shifted to 4.22 ppm, indicating the lactonization of the hydroxy group. The connectivity of H-9 and H-11, as well as of H-5, H-6, and H-7, was confirmed by their correlations in the COSY spectrum. The NOE correlations of H-6 with all β-oriented methyl groups [δ_H 1.18 (C-17), 0.92 (C-19), and 1.53 (C-20)] confirmed that both the acetoxy group on C-8 and the oxygen function on C-6 have an α-orientation. The HMQC and HMBC experiments allowed the assignment of all protons and carbons, and thus the structure of **5** was deduced as 8α-acetoxy-13,14,15,16-tetranorlabdan-12-oic acid-18,6α-olide.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet Magna 550 spectrometer. NMR spectra were recorded on Bruker DRX 500 and ARX 400 spectrometers, using the residual $CDCl_3$ (δ_H 7.27/δ_C 77.0) and pyridine-d_5 (δ_H 8.71/δ_C 149.8) signals as references. The 2D NMR experiments ($^1H-^1H$ COSY, HMQC, HMBC, NOESY) were performed using standard Bruker software. HRESIMS were acquired on a Micromass Q-TOF2 mass spectrometer. Silica gel (70−230 and 230−400 mesh, Merck) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography. Preparative TLC was performed on silica gel 60 GF_{254} (Merck). Spots were detected on TLC under UV or by heating after spraying with 5% phosphomolibdic acid in C_2H_5OH.

Plant Material. The aerial parts of *Salvia sahendica* were collected between Tabriz and Bostanabad, East Azerbaijan Province, Iran, in June 2006 and identified by Dr. G. R. Amin. A voucher specimen (No. 6514-TEH) was deposited at the herbarium of the Faculty of Pharmacy, Tehran University of Medical Science.

Extraction and Isolation. The air-dried, powdered aerial parts of *S. sahendica* (3 kg) were extracted with Me_2CO (7 × 5 L) by maceration at room temperature for seven days. The combined solutions were concentrated to dryness, and the residue (200 g) was triturated with MeOH to remove waxy components. The MeOH-soluble portion (180 g) was subjected to silica gel column chromatography (230−400 mesh, 800 g), eluted with a gradient of *n*-hexane−EtOAc (100:0 to 0:100), followed by increasing concentrations of MeOH (up to 5%). After screening by TLC, fractions with similar compositions were pooled, to yield 27 combined fractions. From fraction 7 [7 g, eluted with hexane−EtOAc (70:30)] crude crystals were obtained, which were recrystallized from Me_2CO to afford β-sitosterol (1.5 g). A part (3 g) of fraction 12 [9.2 g, eluted with hexane−EtOAc (60:40)] was separated over a silica gel column (70−230 mesh, 90 g), eluting with $CHCl_3$ followed by increasing concentrations of MeOH (up to 5%), to afford oleanolic acid (700 mg) and sclareol (950 mg). Fraction 14 [11 g, eluted with hexane−EtOAc (60:40)] was triturated with Me_2CO to separate an insoluble solid, which was recrystallized from Me_2CO to afford salvigenin (3.2 g). Fraction 16 [2.1 g, eluted with hexane−EtOAc (50:50)] was subjected to silica gel column chromatography (70−230 mesh, 100 g), eluted with a gradient of $CHCl_3$−MeOH (100:0, 98:2, 95:5), to afford four fractions (16a−16d). Fraction 16a (100 mg) was recrystallized from Me_2CO to afford maslinic acid (15 mg). Fraction 16b (180 mg) was subjected to passage over a Sephadex LH-20 column, eluted with hexane−$CHCl_3$−MeOH (10:30:1), then purified by preparative TLC [developed with $CHCl_3$−MeOH (15:1)], to afford **4** (10 mg, $R_f = 0.59$). Fraction 17 [1.2 g, eluted with hexane−EtOAc (50:50 and 40:60)] was applied to a silica gel column (70−230 mesh, 50 g), eluted with $CHCl_3$ followed by increasing amounts of MeOH (up to 10%), to separate four fractions (17a−17d). Fraction 17a (160 mg) was further separated over Sephadex LH-20, using hexane−$CHCl_3$−MeOH (10:30:1) as eluent, to give a crude solid, which was recrystallized from Me_2CO to afford **3** (12 mg). Fraction 17b (310 mg) was purified by preparative TLC [developed with $CHCl_3$−MeOH (95:5)] to afford hispudulin (58 mg, $R_f = 0.48$). Fraction 17c (80 mg) also was purified by preparative TLC [developed with $CHCl_3$−MeOH (95:5)] to give **5** (10 mg, $R_f = 0.36$). Fraction 18 [1.6 g, eluted with hexane−EtOAc (40:60)] was chromatographed on a silica gel column (70−230 mesh, 60 g), eluted with $CHCl_3$ followed by a gradient of MeOH (up to 15%), to give five fractions (18a−18e). Fraction 18a (420 mg) was triturated with Me_2CO to yield an insoluble solid, which was recrystallized from Me_2CO to afford eupatorin (110 mg). Fraction 18b (250 mg) also contained a crude solid, which was triturated with Me_2CO to yield ladanein (45 mg). The Me_2CO-soluble part was further purified by preparative TLC [developed with $CHCl_3$−MeOH (95:5)] to give **2** (15 mg, $R_f = 0.46$). Fraction 18c (90 mg) was applied to a Sephadex LH-20 column, with hexane−$CHCl_3$−MeOH (10:30:1) as eluent, and then purified by preparative TLC [developed with $CHCl_3$−MeOH (95:5)] to afford **1** (10 mg, $R_f = 0.39$). Fraction 18d (170 mg) was purified by preparative TLC [developed with $CHCl_3$−MeOH (90:10)] to give apigenin (37 mg, $R_f = 0.57$). Fraction 19 [1.3 g, eluted with hexane−EtOAc (30:70)] was separated on a silica gel column (70−230 mesh, 50 g), eluted with $CHCl_3$ followed by a gradient of $CHCl_3$−MeOH (up to 15% MeOH), to give three fractions (19a−19c). Fraction 19b (135 mg) contained a crude solid, which was recrystallized from Me_2CO to afford loliolide (25 mg). From fraction 27 [11 g, eluted with EtOAc−MeOH (98:2)] a crude solid was obtained, which was triturated with MeOH to give daucosterol (1.9 g).

8α-Hydroxy-13-hydroperoxylabd-14,17-dien-19,16;23,6α-diolide (1): colorless gum; $[\alpha]^{20}_D$ −26.8 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3430, 2940, 1770, 1645, 1460, 1390, 1280, 1150 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 466.2798 $[M + NH_4]^+$ (calcd for $C_{25}H_{36}O_7N_1$, 466.2799).

Salvileucolide-6,23-lactone (2): ^{13}C NMR data, see Table 1.

17,18,19,20-Tetranor-13-*epi*-manoyloxide-14-en-16-oic acid-23,6α-olide (3): white, amorphous powder; $[\alpha]^{20}_D$ +3.4 (*c* 0.8, $CHCl_3$); IR (KBr) ν_{max} 3500−3200, 2940, 1760, 1690, 1645, 1460, 1380, 1280, 1155 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 363.2170 $[M + H]^+$ (calcd for $C_{21}H_{31}O_5$, 363.2166).

Norambreinolide-18,6α-olide (4): colorless crystals; $[\alpha]^{20}_D$ +12.3 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 2930, 2850, 1765, 1460, 1395, 1270, 1060 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 279.1592 $[M + H]^+$ (calcd for $C_{16}H_{23}O_4$, 279.1591).

8α-Acetoxy-13,14,15,16-tetranorlabdan-12-oic acid-18,6α-olide (5): white, amorphous powder; $[\alpha]^{20}_D$ +16.1 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3400−3200, 2930, 1760, 1730, 1705, 1460, 1380, 1285, 1155 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 339.1805 $[M + H]^+$ (calcd for $C_{18}H_{27}O_6$, 339.1802).

X-ray Crystal Structure Analysis of Norambreinolide-18,6α-olide (4). [23] The X-ray diffraction measurements were made on a STOE IPDS-II diffractometer with graphite-monochromated Mo Kα radiation. For norambreinolide-18,6α-olide (**4**), a colorless prismatic crystal with dimensions of 0.50 × 0.40 × 0.25 mm was chosen and mounted on a glass fiber and used for data collection. Cell constants and an orientation matrix for data collection were obtained by least-squares refinement of diffraction data from 6677 unique reflections. Data were collected at a temperature of 298(2) K to a maximum 2θ value of 58.56° for **4** and in a series of ω scans in 1° oscillations and integrated using the Stoe X-ARE[24] software package. The numerical absorption coefficient, μ, for Mo Kα radiation is 0.091 mm^{-1}, and the data were corrected for Lorentz and polarizing effects. The structures were solved by direct methods[25] and subsequent difference Fourier maps and then refined on F^2 by a full-matrix least-squares procedure using anisotropic displacement parameters.[26] All hydrogen atoms were located in ideal positions and then refined isotropically. Subsequent refinement was then converged with R1 = 0.0529 [$I > 2.00\sigma(I)$] and wR2 = 0.1355. The Flack parameter for this compound is 2.3(14). Atomic factors are from International Tables for X-ray Crystallography.[27] All refinements were performed using the X-STEP32 crystallographic software package.[28]

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Supporting Information Available: HRESIMS and 1D and 2D NMR spectra are reported for the new compounds (**1**, **3**, **4**, **5**) and

compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Rustaiyan, A.; Masoud, S.; Anaraki, M. T. *Nat. Prod. Rep.* **2007**, *10*, 1031–1042.
(2) Rustaiyan, A.; Niknejad, A.; Nazarians, L.; Jakupovic, J.; Bohlmann, F. *Phytochemistry* **1982**, *21*, 1812–1813.
(3) Rustaiyan, A.; Koussari, S. *Phytochemistry* **1988**, *27*, 1767–1769.
(4) Moghaddam, F. M.; Zaynizadeh, B.; Ruedi, P. *Phytochemistry* **1995**, *39*, 715–716.
(5) Moghaddam, F. M.; Amiri, R.; Alam, M.; Hossain, M. B.; Van Der Helm, D. *J. Nat. Prod.* **1998**, *61*, 279–281.
(6) Rustaiyan, A.; Sadjadi, A. *Phytochemistry* **1987**, *26*, 3078–3079.
(7) Ulubelen, A.; Topcu, G.; Sonmez, U.; Eris, C.; Ozgen, U. *Phytochemistry* **1996**, *43*, 431–434.
(8) Gioffi, G.; Bader, A.; Malafronte, A.; Piaz, F. D.; DeTommasi, N. *Phytochemistry* **2008**, *69*, 1005–1012.
(9) Topcu, G.; Ulubelen, A.; Tam, T. C. M.; Che, C. T. *Phytochemistry* **1996**, *42*, 1089–1092.
(10) Topcu, G.; Ulubelen, A.; Tam, T. C. M.; Che, C. T. *J. Nat. Prod.* **1996**, *59*, 113–116.
(11) Gonzalez, M. S.; San Segundo, J. M.; Grande, M. C.; Medarde, M.; Bellido, I. S. *Tetrahedron* **1989**, *43*, 3575–3582.
(12) Piaz, F. D.; Vassallo, A.; Lepore, L.; Tosco, A.; Bader, A.; DeTommasi, N. *J. Med. Chem.* **2009**, *52*, 3814–3828.
(13) Linden, A.; Juch, M.; Moghaddam, F. M.; Zaynizadeh, B.; Ruedi, P. *Phytochemistry* **1996**, *41*, 589–590.
(14) Jassbi, A. R.; Mehrdad, M.; Eghtesadi, F.; Ebrahimi, S. N.; Baldwin, I. T. *Chem. Biodiversity* **2006**, *3*, 916–921.
(15) Hodges, R. *Tetrahedron* **1964**, 1463–1467.
(16) Taniguchi, S.; Imayoshi, Y.; Kobayashi, E.; Takamatsu, Y.; Ito, H.; Hatano, T.; Sakagami, H.; Tokuda, H.; Nishino, H.; Sugita, D.; Shimura, S.; Yoshida, T. *Phytochemistry* **2002**, *59*, 315–323.
(17) Moghddam, F. M.; Farimani, M. M.; Taheri, S.; Tafazoli, M.; Amin, G. *Chem. Nat. Compd.* **2008**, *44*, 518–519.
(18) Becchi, M.; Carrier, M. *Planta Med.* **1980**, *38*, 267–268.
(19) Barberan, F. A. T.; Hernandez, L.; Ferreres, F.; Tomas, F. *Planta Med.* **1985**, *51*, 452–454.
(20) Buckwalter, B. L.; Burfitt, I. R.; Nagel, A. A.; Wenkert, E.; Naf, F. *Helv. Chim. Acta* **1975**, *58*, 1567–1573.
(21) Rodriguez, B.; Gadea, F. F.; Savona, G. *Phytochemistry* **1984**, *23*, 1805–1806.
(22) Tsichritzis, F.; Jakupovic, J. *Phytochemistry* **1991**, *30*, 211–213.
(23) Crystallographic data for **4** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 776855). 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 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
(24) Stoe & Cie. *X-AREA, vesion 1.30: Program for the Acquisition and Analysis of Data*; Stoe & Cie GmbH: Darmatadt, Germany, 2005.
(25) Sheldrick, G. M. *SHELX97: Program for Crystal Structure Solution*; University of Göttingen: Göttingen, Germany, 1997.
(26) Sheldrick, G. M. *SHELX97: Program for Crystal Structure Refinement*; University of Göttingen: Göttingen, Germany, 1997.
(27) *International Tables for X-ray Crystallography, Vol. C*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995.
(28) Stoe & Cie. *X-STEP32, Version 1.07b: Crystallographic Package*; Stoe & Cie GmbH: Darmstadt, Germany, 2000.

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