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Secondary metabolites from the aerial parts of *Salvia palaestina* Bentham

Giuseppina Cioffi [a], Ammar Bader [b], Anna Malafronte [a], Fabrizio Dal Piaz [a], Nunziatina De Tommasi [a,*]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy*
[b] *Faculty of Pharmacy, Al-Zaytoonah Private University of Jordan, P.O. Box 130, 11733 Amman, Jordan*

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Abstract

Three sesterterpenes (**1**–**3**), one triterpene (**4**) and five diterpenes (**5**–**9**) were isolated from the aerial parts of *Salvia palaestina* Bentham (Lamiaceae), together with two sesquiterpenes, 10 known diterpenes, three triterpenes, and rosmarinic acid. Their structural elucidation was accomplished by extensive spectroscopic methods including 1D (^{1}H, ^{13}C, ^{13}C DEPT, TOCSY, NOESY) and 2D NMR experiments (DQF-COSY, HSQC, HMBC, ROESY) as well as ESIMS analysis and chemical analysis.

Keywords: *Salvia palaestina*; Lamiaceae; Diterpenes; Sesterterpenes; Triterpenes

1. Introduction

The Lamiaceae family comprises about 200 genera and 3000 species. One of the largest genus of the family, *Salvia* L., is represented by over 900 species, and is widely distributed in various regions of the world. *Salvia* species have been used since ancient times for more than sixty different ailments ranging from aches to epilepsy, and mainly to treat colds, bronchitis, tuberculosis, haemorrhage, and menstrual disorders (Foster and Tyler, 2000; Steinegger and Hansel, 1988). The main secondary metabolite constituents of *Salvia* species are terpenoids and flavonoids. The aerial parts of these plants contain flavonoids, triterpenoids, and monoterpenes, particularly in the flowers and leaves, while diterpenoids are found mostly in the roots. However, a literature survey indicates that some American *Salvia* species also contain diterpenoids in the aerial parts, and in few *Salvia* species, triterpenoids and flavones are present in the roots (Bozan et al., 2002).

Salvia palaestina Benth. is widespread in south eastern Turkey where a preparation made from leaf extracts is commonly used in the folk medicine as a wound healer (Miski et al., 1983; Fiore et al., 2006). Previous phytochemical studies on the aerial parts of the plant reported the presence of modified abietane diterpenoids, ursolic acid, vergatic acid, lupane derivatives, and flavonoids (Miski et al., 1983; Hussein et al., 1997; Hussein and Rodriguez, 2000; Ulubelen et al., 1985).

The aim of our work was to carry out the phytochemical investigation of *S. palaestina* aerial parts collected in Jordan and herein we report the structural characterization of nine new terpenoids (**1**–**9**) from the acetone extract of the title plant, on the basis of extensive spectroscopic and spectrometric analysis, including 2D NMR and ESIMS spectra. Two sesquiterpenes, 10 known diterpenes, three triterpenes, and rosmarinic acid were also isolated and characterized.

* Corresponding author. Tel.: +39 089 969754; fax: +39 089 969602.
E-mail address: detommasi@unisa.it (N. De Tommasi).

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2. Results and discussion

The phytochemical study of *S. palaestina* aerial parts led to the isolation of three new sesterterpenes (**1**–**3**), one new triterpene (**4**), five new diterpenes (**5**–**9**) (see Fig. 1), together with the known aromadendrane-4β,10β-diol (Iijima et al., 2003), allospathulenol (Tringali et al., 1995), 13-*epi*-manoyloxide (Dolmazon et al., 1995), tarapacol (Zhou et al., 1995), labda-8(17),14-dien-13-ol (Barrero et al., 2005), aethiopinone (Lin et al., 1989), tarapacol-15-acetate (Zhou et al., 1996), 3-hydroxy-ambreinolide (Mugio et al., 1998), ambreinolide (Topcu and Ulubelen, 1996), 13-e*pi*-manoyloxide-18-oic acid (Duan et al., 1999), 14,15-dihydroxy-8,13-epoxy-labd-14-en-19-oic acid (San Feliciano et al., 1992), labda-7,14-dien-13-ol (Lee et al., 2005), 3β,23-dihydroxy-urs-12-en-28-oic acid (De Felice et al., 2006), 2α,3α-dihydroxy-24-nor-4(23),12-oleandien-28-oic acid (Ballesta-Acosta et al., 2002), hederagenin (Ye et al., 1999), and rosmarinic acid (Kelley et al., 1976).

Compounds **1** and **2** were isolated as amorphous powder, and both yielded a pseudomolecular ion peak in the negative HRMS spectrum at *m*/*z* 449.2560 $[M-H]^-$, consistent with a molecular formula of $C_{25}H_{38}O_7$, and equating to seven double bond equivalents.

Immediately identifiable from NMR spectroscopic data for **1** (Table 1) were resonances consistent with two trisubstituted double bond (δ_C 112.3, 117.6, 152.8, 158.0), as well as two carboxylic carbon (δ_C 172.0, and 188.0). In the absence of any other sp or sp^2 carbon, the structure of **1** must be tricyclic. The ^1H NMR spectrum (Table 1) of **1** exhibited four methyls singlets at δ 0.85, 1.10, 1.31, 1.34, a vinylic methyl doublet at δ 2.22 ($J = 1.0$ Hz), a carbinol methine signal at δ 4.34 (1H, *d*, $J = 9.5$ Hz, H-14) coupled with a vinyl proton a δ 5.52 (1H, *d*, $J = 9.5$ Hz, H-15), another vinyl proton was observed at δ 6.11 (1H, *d*, $J = 1.0$ Hz, H-18). COSY and 1D TOCSY experiments showed the relation between H-14 and H-15, and Me-20 and H-18. The carbonyl resonance at C-19 (172.0 ppm) was assigned basing on HMBC correlations between H-18 and C-19, Me-20 and C-19. A signal at δ 2.22 was consistent with the presence of a methyl group at C-17 and this was supported by HMBC correlations between Me-20 (δ 2.22) and C-17 (δ 158.0); Me-20 also showed long-range COSY correlations to H-18 (δ 6.11), supporting the presence of an α,β,γ-unsaturated butenolide moiety (Topcu et al., 1996).

Results obtained from 1D TOCSY and COSY experiments established the correlations of protons H-9–H-11–H-12, H-14–H-15 confirmed by HMBC cross peaks between H-12 and C-9 and C-14.

The remaining part of the molecule as inferred by the molecular formula and inspection of ^{13}C and ^{13}C DEPT NMR spectra, had to be composed only sp^3 hybridized carbon atom and must be bicyclic.

1D TOCSY and COSY experiments provided evidences for the presence in the molecule of segment C-1–C-3, C-5–C-7. HSQC and HMBC experiments provided unambiguous assignments of all the proton and carbon resonances. Long-range correlations were observed between Me-24 and C-23, C-3, C-5; between Me-25 and C-1, C-9, C-5; between Me-22 and C-9, C-8, C-7; between H-9 and C-10, C-12, C-7; between Me-21 and C-12, C-13, C-15. The relative stereochemistry of **1** was determined through analysis of proton coupling constants and correlations observed in ROESY spectrum. The coupling constant observed for H-5, $J = 11$ and 4.5 Hz suggested that H-5

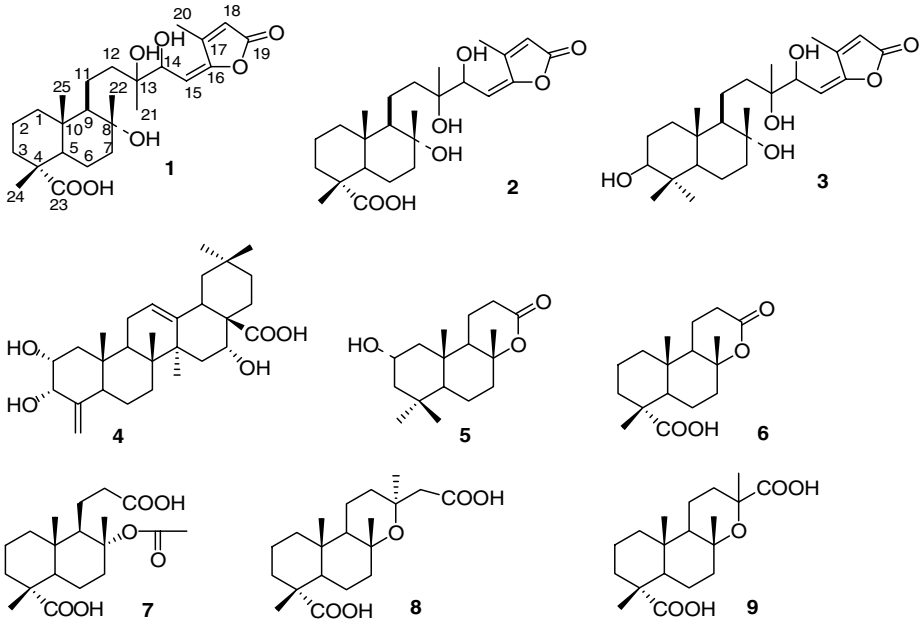

Fig. 1. Structures of compounds **1**–**9**.

Table 1
^{1}H and ^{13}C NMR data of compounds **1**–**3** (CD_3OD, 600 MHz)[a]

Position	**1**		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	40.3	1.05 *ddd* (13.0, 12.5, 4.5) 1.71 *m*	40.0	1.04 *ddd* (13.0, 12.5, 4.5) 1.73 *m*	31.7	1.53 *ddd* (13.0, 12.5, 4.5) 1.18 *m*
2	19.2	1.69[b] 1.56[b]	19.0	1.71 *o* 1.57[b]	26.9	2.00 1.56
3	39.3	1.51[b] 1.83 *m*	38.9	1.53[b] 1.83 *m*	75.5	3.38 *dd* (11.5, 4.5)
4	50.4	–	50.4	–	38.5	–
5	53.2	1.89 *dd* (11.0, 4.5)	53.0	1.84 *dd* (11.0, 4.5)	48.7	1.50[b]
6	16.5	1.69 *m*	16.5	1.69 *m*	1.2	1.60 *m*
7	43.8	1.51 *m* 1.73 *m*	43.6	1.60 *m* 1.51 *m* 1.72 *m*	43.0	1.39 *m* 1.78 *m* 1.48[b]
8	78.0	–	77.6	–	75.9	–
9	60.0	1.22 *dd* (5.0, 6.5)	59.8	1.28 *dd* (5.0, 6.5)	58.3	1.29 *dd* (5.0, 6.5)
10	38.1	–	38.0	–	38.5	–
11	23.5	1.49 *m* 1.53[b]	23.5	1.49 *m* 1.31 *m*	24.0	1.35 *m*
12	34.2	1.68[b] 1.63 *m*	33.6	1.55[b] 1.90 *m*	32.5	1.87 *m* 1.54[b]
13	78.0	–	77.0	–	74.3	–
14	75.2	4.34 *d* (9.5)	74.3	4.25 *d* (9.5)	73.5	4.26 *d* (9.5)
15	112.3	5.52 *d* (9.5)	112.3	5.55 *d* (9.5)	111.5	5.56 *d* (9.5)
16	152.8	–	152.6	–	152.4	–
17	158.0	–	157.3	–	157.0	–
18	117.6	6.11 *d* (1.0)	117.6	6.09 *d* (1.0)	116.3	6.10 *d* (1.0)
19	172.0	–	172.0	–	173.0	–
20	12.0	2.22 *d* (1.0)	11.8	2.23 *d* (1.0)	10.8	2.32 *d* (1.0)
21	24.3	1.31 *s*	24.8	1.24 *s*	23.8	1.24 *s*
22	25.7	1.34 *s*	25.7	1.33 *s*	24.9	1.33 *s*
23	188.0	–	188.0	–	27.2	0.96 *s*
24	17.8	1.10 *s*	17.2	1.14 *s*	21.2	0.86 *s*
25	16.3	0.85 *s*	16.6	0.86 *s*	15.6	0.94 *s*

[a] δ values were established from the 1D TOCSY, COSY and HSQC experiments.
[b] Overlapped signals.

was axial, which was further confirmed by ROE correlation between H-5 and H-9. In addition, the observation of ROE correlations between Me-24 and H-2ax, and Me-25 and Me-22 placed them on the same face of the molecule (Moghaddam et al., 1998).

The NMR data of compound **2** was quite similar to that of **1** (Table 1), the main differences being in the resonances of H_2-12, H-14, Me-21, and C-13, C-14, C-21. This suggested the presence of a C-13/C-14 epimer. The relative configuration of the 1,2-diol C-13/C-14 in **1** and **2** was determined with the following methods. First of all, in order to obtain the relative configuration of C-13 and C-14, the acetonide derivative was prepared and the ^{1}H NMR chemical shift of the acetonyl methyl groups was observed. If the configuration of the vicinal diol is *threo*, the two acetonyl proton methyl signals will appear together as a six-proton singlet; and if the configuration is *erythro*, the acetonyl proton methyl signals will appear as two separated three-proton singlets.

The ^{1}H NMR signals of the acetonyl methyl groups in **1** appeared as six-proton singlet at ~1.29 ppm, showing their equivalence and demonstrating that the vicinal diol at C-13/C-14 was *threo*; while the acetonyl methyl signals in **2** appeared as two separated three-proton singlets at δ 1.30 and 1.56 and demonstrating that the configuration of the 1,2 vicinal diol in **2** was *erythro* (Braca et al., 2003).

Therefore, the structure of compounds **1** and **2** were determined as 8α,13,14-*threo*-trihydroxy-labd-15,17-dien-16,19-olide-23-oic acid, and as 8*a*,13,14-*erythro*-trihydroxy-labd-15,17-dien-16,19-olide-23-oic acid, respectively.

Positive HRMS spectrum of **3** showed a single ion peak at 459.2730 $[M+Na]^+$, indicative of the molecular formula $C_{25}H_{40}O_6$. Analysis of the COSY and HSQC experiments of **3** indicated the presence of the same partial structure in side chain and an α,β,γ-unsaturated butenolide moiety as that of **2**, but showed that rings A and B were different. Analysis of the DQF-COSY and 1D TOCSY spectra led to the determination of the spin systems of rings A and B (Table 1). This was confirmed from HSQC and HMBC data. The β-orientation of the hydroxyl group at C-3 was derived from the coupling constant values of the H-3 axial at δ 3.38 (1H, *d*, $J = 11.5$ and 4.5 Hz).

The NMR spectra of **3** contained one less carboxyl, and one more methyl (δ_H 0.96, δ_C 27.2) than those of **2**,

suggesting that in **3** the carboxylic group was replaced by a methyl group. The stereochemistry of C-13/C-14 diol was determined as compounds **1** and **2**. The ^{1}H NMR signals of the acetonyl methyl groups in **3** appeared as two separated three-proton singlets at δ 1.29 and 1.56, demonstrating that the configuration of the 1,2 vicinal diol in **3** was *erythro*.

Therefore, compound **3** was determined as 3β,8α,13,14-*erythro*-tetrahydroxy-labd-15,17-dien-16,19-olide.

The molecular formula of **4** was determined as $C_{29}H_{44}O_5$ by HRMS at *m/z* 471.3120 $[M-H]^-$ as well as from its ^{13}C DEPT data. The ^{1}H NMR, 1D TOCSY and COSY spectra of **4** revealed the presence of five methyl singlets (δ 0.85, 0.93, 0.94, 0.98, 1.35), a trisubstituted olefinic double bond (δ 5.30 1H, *t*, $J = 3.5$ Hz), an exocyclic methylene group (δ 4.70, 5.08), a (C)–CH_2–CHOH–CHOH–(C) structural moiety (δ 1.33 *dd*, $J = 12.8$ and 11.6. Hz; δ 1.74 *dd*, $J = 12.8$ and 4.5 Hz; δ 3.70 *ddd*, $J = 11.6$, 4.5 and 4.0 Hz; δ 4.17 *d*, $J = 4.0$ Hz), and a carbinol proton at δ 3.95 (*brs*). The 3α,2α-OH substitutions were evident from the chemical shifts and *J* values of the protons assigned to C-2 and C-3 (Table 2). The signal at δ 3.95 *brs* indicated the presence of a C-16α hydroxyl group, a conclusion that was further supported by ^{13}C NMR data (Table 2) and by the C-27 methyl, which resonated at δ 1.35, downfield from its usual position (Cioffi et al., 2006).

The observed coupling constant values for the H-1α, H-1β, H-2β, H-3β protons of **4** are compatible only with a spatial arrangement in which the H-1α, H-2β are axial substituent and H-1β, H-3β protons are in equatorial configuration.

This was supported by 1D NOESY experiments because irradiating the H-2β, at δ 3.70 caused nOe effect at H-1β, and Me 25 protons, whereas on irradiating at δ 4.17 (H-3β) only the signals of H-2β, H_b-23 (δ 5.08) protons were affected. The NOE observed between H-3β and H_b-23 protons allowed the assignments of both C-23 methylene hydrogens, being the H_b-23 proton (δ 5.08) the pro-*Z* hydrogen. All the observed data can be accommodated only on a C-24-nor-olean-12-ene triterpenoid structure for compound **4** with three secondary hydroxyl groups at C-2, C-3 and C-16 positions. The HSQC and HMBC spectra of **4** confirmed the above deductions. Consequently, the new compound **4** can be formulated as 2α,3α,16α-trihydroxy-olean-24-nor-4(23),12-dien-28-oic acid.

Negative HRMS analysis performed on compound **5** showed an intense signal at *m/z* 279.1968 $[M-H]^-$, in agreement with a $C_{17}H_{28}O_3$ formula. The ^{13}C NMR spectrum of compound **5** (Table 3) displayed four methyls, six methylenes, three methines, and four quaternary carbons. The ^{1}H NMR spectrum exhibited four methyl singlets at δ 0.90, 0.93, 0.99, 1.38, and a methine proton a δ 3.76 (1H, *br ddd*, $J = 13.0$, 11.0 and 5.0 Hz). The placement of hydroxyl group on C-2 was deduced by the following observations: the chemical shifts of C-1 and C-3 were observed at 44.9 and 45.6 ppm, respectively, with a downfield shift ~10 ppm relative to ambreinolide, while the chemical shift of C-4 and C-10 were observed at 29.5 and 34.0 ppm, respectively, with a upfield shift ~3 ppm relative to ambreinolide (Topcu and Ulubelen, 1996). Analysis of the 1D TOCSY and COSY data starting from H-2 signal suggested the sequence H-1–H-3 and confirmed the C-2 hydroxyl substitution. The stereochemistry of hydroxyl group at C-2 was determined as α on the basis of the coupling constant of H-2 and the 1D NOESY results. Thus, H-2 was found to show NOE with Me-19 and Me-20, Me-20 with Me-17, all oriented at β positions. All carbon signals could be assigned by HSQC and HMBC experiments. Compound **5** was therefore established as a trinorlabdane, 2α-hydroxyambreinolide (Topcu and Ulubelen, 1996).

Compound **6** had molecular weight 294.1840 and molecular formula $C_{17}H_{26}O_4$, derived from HRMS and ^{13}C NMR analyses. Comparison of the NMR data of **6** with those of **5** indicated that **6** is ambreinolide derivative.

Table 2
^{1}H and ^{13}C NMR data of compound **4** (CD_3OD, 600 MHz)[a]

Position	**4**	
	δ_H (*J* in Hz)	δ_C
1	1.33 *dd* (12.8, 11.6) 1.74 *dd* (12, 4.5)	43.6
2	3.70 *ddd* (11.6, 4.5, 4.0)	70.0
3	4.17 *d* (4.0)	77.1
4	–	150.0
5	1.80 *dd* (11.0, 3.0)	46.6
6	1.29 *m* 1.50 *m*	19.9
7	1.60 *m* 1.40 *m*	32.5
8	–	41.0
9	1.86 *dd* (2.3, 11.8)	52.7
10	–	39.1
11	1.98 *m*	24.9
12	5.30 *t* (3.5)	123.0
13	–	147.0
14	–	40.3
15	1.37 *dd* (13.2, 2.0) 1.98 *dd* (13.2, 5.5)	35.2
16	3.95 *brs*	74.2
17	–	52.5
18	2.94 *dd* (13.8, 4.5)	42.4
19	1.24[b] 1.78[b]	47.8
20	–	32.5
21	1.80[b] 1.54[b]	34.4
22	1.40[b] 1.20[b]	35.5
23	5.08 *dd* (1.4, 1.2) 4.70 *t* (1.4)	110.6
25	0.85 *s*	15.9
26	0.94 *s*	18.5
27	1.35 *s*	26.4
28	–	181.8
29	0.93 *s*	33.9
30	0.98 *s*	24.4

[a] δ values were established from the 1D TOCSY, COSY and HSQC experiments.
[b] Overlapped signals.

Table 3
^{1}H and ^{13}C NMR data of compounds **5**–**7** (CD_3OD 600 MHz)[a]

Position	**5**		**6**		**7**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	44.9	1.30 *dd* (13.0, 11.0) 1.81 *m*	40.0	1.47[b] 1.26 *m*	38.8	1.66[b] 1.24 *m*
2	70.6	3.76 *ddd* (13.0, 11.0, 5.0)	19.5	1.35 *m* 1.49[b]	17.6	1.55[b] 1.68[b]
3	45.6	1.47[b] 1.56 *dd* (13.0, 5.0)	38.7	1.52[b] 1.76 *m*	37.3	1.67[b] 1.87 *m*
4	29.5	–	50.5	–	47.8	–
5	57.1	1.17 *dd* (11.0, 4.0)	51.2	1.94 *dd* (11.0, 4.0)	50.1	1.96 *dd* (11.0, 4.0)
6	19.9	1.38 *m* 1.48[b]	18.0	1.80 *m* 1.54[b]	22.2	1.68[b] 1.56[b]
7	34.4	2.09 *br dd* (13.0, 4.5) 1.74 *m*	39.4	2.08 *dd* (13.0, 4.5) 1.74 *m*	38.9	2.66 *dd* (13.0, 4.5) 1.83 *m*
8	87.0	–	88.0	–	87.2	–
9	59.9	2.06 *dd* (9.5, 5.0)	57.4	2.14 *dd* (9.5, 4.5)	55.9	2.37 *dd* (9.5, 5.0)
10	34.0	–	37.0	–	38.0	–
11	27.1	1.60 *m*	30.0	1.50 *m*	31.4	2.34 *m*
12	28.0	2.59 *t* (13.0) 2.07 *dd* (13.0, 5.0)	28.4	2.57 *t* (13.0) 2.26 *dd* (13.0, 5.0)	38.8	2.17 *br d* (12.5) 1.85 *m*
13	173.0	–	179.4	–	178.9	–
17	21.7	1.38 *s*	20.0	1.35 *s*	119.5	1.52 *s*
18	33.5	0.93 *s*	185.0	–	182.2	–
19	20.9	0.90 *s*	16.0	1.15 *s*	15.7	1.15 *s*
20	14.8	0.99 *s*	16.2	0.99 *s*	16.1	0.92 *s*
CH$_3$CO	–	–	–	–	21.4	1.91 *s*
CH$_3$*CO*	–	–	–	–	171.3	–

[a] δ values were established from the 1D TOCSY, COSY and HSQC experiments.
[b] Overlapped signals.

In particular, protons and carbons due to rings B and C resonated at almost the same frequencies as the corresponding signals in **5**, while the ring A NMR signals were observed at somewhat different positions. The NMR spectra of **6** contains one less methyl, one less methine, and one more signal (185.0 ppm) than those of **5**, suggesting that compound **6** lacked of C-2 hydroxyl group and that in **6** one of the Me group was replaced by a carboxylic group. The most significant features of the NMR spectra of **6**, which suggested the location of the carboxyl group at C-18, were the downfield shifts exhibited by C-4, and the upfield shift of C-3 and C-5, and Me-24 (Table 3) (Topcu and Ulubelen, 1996). Therefore, the structure of **6** was elicited as a trinorlabdane, ambreinolide-18-oic acid.

The molecular formula of **7** was determined as $C_{19}H_{30}O_6$ by HRMS at *m*/*z* 353.1970 $[M-H]^-$ as well as from its ^{13}C DEPT NMR data. The ^{13}C NMR spectrum displayed signals for four methyls, seven methylenes, two methines, and six quaternary carbons. The presence of carbonyl groups were substantiated by three carbonyl signals present at δ 171.3, 178.9, and 182.2 as well as an acetyl methyl signal at δ 21.4 in its ^{13}C NMR spectrum. The ^{1}H NMR (Table 3) gave four methyl singlets at δ 0.92, 1.15, 1.52, and 1.91, the last assigned to an acetyl methyl. Connectivity of H-5 and H-7, as well as H-9 and H-12 was confirmed by 1D TOCSY and COSY experiments. The stereochemistry of acetoxyl group at C-8 was determined as α on the basis of the 1D NOESY, while Me-20 showed NOE with Me-17 and Me-19 demonstrating the β-orientation of methyl groups.

The HSQC and HMBC experiments allowed the assignments of all carbons and protons, and the structure of **7** was deduced as 8α-acetoxy-14,15,16-trinorlabdan-12,18-dioic acid.

Compound **8** had molecular weight 352.2258 and molecular formula $C_{20}H_{32}O_5$, derived from HRMS and ^{13}C NMR analyses. Comparison of the NMR data of **8** with those of 13-e*pi*-manoyloxide-18-oic acid indicated that **8** is manoyloxide derivative (Tsichritzis and Jakupovic, 1991). In particular, protons and carbons due to rings A–C resonated at almost the same frequencies as the corresponding signals in 13-e*pi*-manoyloxide-18-oic acid, while the side chain NMR signals were observed at different positions. The major difference in the ^{1}H NMR spectra of **8** respect to 13-e*pi*-manoyloxide-18-oic acid was the absence of the signals for –CH=CH_2 group and the presence of a signals for a –CH_2COOH group (Table 4). These data, together with MS data were consistent with **8** being 13-e*pi*-manoyloxide-15,18-dioic acid.

The HRMS of compound **9** ($C_{19}H_{30}O_5$) showed a molecular weight 338.2098 that differed from that of compound **8** by 14 a.m.u. Its NMR spectral data (Table 4), when compared to that of **8**, showed the absence of the

signal of a methylene group at 44.1 ppm (Table 4). These data, together with MS data were consistent with **9** being 15-nor-13-e*pi*-manoyloxide-14,18-dioic acid.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. All the 2D NMR spectra were acquired in CD_3OD in the phase-sensitive mode with the transmitter set at the solvent resonance and Time Proportional Phase Increment (TPPI) used to achieved frequency discrimination in the ω_1 dimension. The standard pulse sequence and phase cycling were used for DQF-COSY, TOCSY, HSQC, HMBC, NOESY and ROESY experiments. The NMR data were processed on a Silicon Graphic Indigo2 Workstation using UXNMR software. Column chromatographies were performed over silica gel (63–200 μm, Merck, Darmstadt, Germany); high resolution mass spectra were acquired on a Q-Tof Premier instrument (Waters, Milford, MA), equipped with a nanospray ion source; to achieve high accuracy mass measurements, both external and internal calibrations of the spectrometer were performed using quercetin (molecular mass 302.0427) or amentoflavone (molecular mass 538.0900) as standards. HPLC separations were conducted on a Waters 590 system equipped with a Waters R401 refractive index detector, and with a Waters μ-Bondapak C_{18} column (Waters, Milford, MA). TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck, Darmstadt, Germany); compounds were detected by $Ce(SO_4)_2/H_2SO_4$ (Sigma–Aldrich, Milano, Italy) solution.

Table 4
^1H and ^{13}C NMR data of compounds **8** and **9** (CD_3OD, 600 MHz)[a]

Position	**8**		**9**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	38.8	0.99[b]	38.9	0.99[b]
		1.69 *m*		1.60 *m*
2	18.6	1.50 *m*	18.5	1.51 *m*
		1.66[b]		1.62[b]
3	32.7	1.80[b]	38.2	1.84
		1.50 *m*		1.45
4	48.3	–	48.9	–
5	51.4	1.82 *dd* (11.5, 4.0)	52.0	1.84 *dd* (11.3, 4.2)
6	16.5	1.67 *m*	16.0	1.60 *m*
				1.57
7	42.7	1.46	44.0	1.45
		1.67		1.69
8	75.4	–	74.0	–
9	58.8	1.20	60.0	1.20
10	36.8	–	37.5	–
11	21.8	1.41	23.4	1.41
		1.32		1.30
12	31.2	1.90 *m*	30.2	1.89 *m*
		1.56 *m*		1.30 *m*
13	74.3	–	76.0	–
14	44.1	2.09 *d* (13.0)	176.6	–
		2.23 *d* (13.0)		
15	178.6	–	–	–
16	23.7	1.30 *s*	28.1	1.32 *s*
17	23.9	1.32 *s*	26.1	1.27 *s*
18	184.0	–	183.0	–
19	16.0	1.10 *s*	17.3	1.08 *s*
20	15.4	0.84 *s*	15.9	0.75 *s*

[a] δ values were established from the 1D TOCSY, COSY and HSQC experiments.
[b] Overlapped signals.

3.2. Plant material

The aerial parts of *S. palaestina* Bentham were collected in April 2005 in Jordan, and identified by Dr. Ammar Bader. A voucher specimen number Jo-It-2003/2 has been deposited in the Herbarium of Laboratory of Pharmacognosy and Phytochemistry at Al-Zaytoonah Private University of Jordan.

3.3. Extraction and isolation

The dried aerial parts of *S. palaestina* (500 g) were finely powdered and exhaustively extracted with hexane (5.74 g) and acetone (10.0 g) by maceration at room temperature. Part of acetone extract (6.0 g) was subjected to column chromatography using silica gel and eluting with dichloromethane followed by increasing concentrations of chloroform (between 10% and 100%), followed by increasing concentrations of MeOH in $CHCl_3$ (between 1% and 50%). Fractions of 40 ml were collected, analysed by TLC (silica gel plates, in $CHCl_3$ or mixtures $CHCl_3$–MeOH 99:1, 98:2, 97:3, 9:1, 4:1), and grouped into 16 fractions (A–O). Fraction B contained pure aethiopinone (10 mg). Fraction O contained pure rosmarinic acid (7 mg). Fractions C and D (100 mg) were subjected to RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) with MeOH–H_2O (4:1) to give pure compounds labda-8(17),14-dien-13-ol (3 mg, $t_R = 33$ min), labda-7,14-dien-13-ol (3.5 mg, $t_R = 30$ min), 3-hydroxy-ambreinolide (2.5 mg, $t_R = 13$ min), ambreinolide (5 mg, $t_R = 14$ min). Fraction E (40 mg) was subjected to RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) with MeOH–H_2O (75:25) to give pure compound **5** (1.5 mg, $t_R = 25$ min), aromadendrane-4β,10β-diol (10 mg, $t_R = 32$ min). Fraction G (75 mg) was subjected to RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) with MeOH–H_2O (7:3) to give pure compound **9** (1.5 mg, $t_R = 20$ min). Fraction H (250 mg) was subjected to RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) with MeOH–H_2O (7:3) to give pure compounds 13-*epi*-manoyloxide (25 mg, $t_R = 36$ min), tarapacol (6 mg, $t_R = 29$ min), and tarapacol-15-acetate (4 mg, $t_R = 8$ min). Fraction I (190 mg) was subjected to RP-HPLC

on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) with MeOH–H_2O (34:16) to give pure compounds **6** (4 mg, $t_R = 4$ min), **2** (10 mg, $t_R = 17$ min), **4** (4 mg, $t_R = 23$ min), and 2α,3α-dihydroxy-24-nor-4(23),12-oleandien-28-oic acid (1 mg, $t_R = 29$ min). Fraction L (260 mg) was subjected to RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) with MeOH–H_2O (34:16) to give pure compounds **8** (5 mg, $t_R = 7.5$ min), **3** (2 mg, $t_R = 12$ min), **1** (4 mg, $t_R = 13$ min), 13-*epi*-manoyloxide-18-oic acid (10 mg, $t_R = 22$ min), and tarapacol (5 mg, $t_R = 25$ min).

Fraction M (60 mg) was subjected to RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) with MeOH–H_2O (34:16) to give pure compounds **7** (12 mg, $t_R = 5$ min), hederagenin (3 mg, $t_R = 25$ min), and 14,15-dihydroxy-8,13-epoxy-labd-14-en-19-oic acid (4 mg, $t_R = 34$ min).

Fraction N (200 mg) was subjected to RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) with MeOH–H_2O (65:35) to give pure compounds 13-*epi*-manoyloxide-18-oic acid (5 mg, $t_R = 9$ min), **7** (10 mg, $t_R = 3$ min), allospathulenol (1 mg, $t_R = 5$ min), and 3β,23-dihydroxy-urs-12-en-28-oic acid (4 mg, $t_R = 8$ min).

3.3.1. 8α,13,14-Threo-trihydroxy-labd-15,17-dien-16,19-olide-23 oic acid (*1*)

White amorphous powder, $[\alpha]_D^{25}$: 8.80° (*c* 0.1, MeOH); ESIMS negative ion mode (*m/z*): 449.2560 $[M-H]^-$; for ^1H and ^{13}C NMR spectroscopic data, see Table 1.

3.3.2. 8α,13,14-Erythro-trihydroxy-labd-15,17-dien-16,19-olide-28-oic acid (*2*)

White amorphous powder, $[\alpha]_D^{25}$: +19.6° (*c* 0.1, MeOH); ESIMS in negative ion mode (*m/z*): 449.2565 $[M-H]^-$; for ^1H and ^{13}C NMR spectroscopic data, see Table 1.

3.3.3. 3α,8α,13,14-Erythro-tetrahydroxy-labd-15,17-dien-16,19-olide (*3*)

White amorphous powder, $[\alpha]_D^{25}$: 23.0° (*c* 0.1, MeOH); ESIMS in positive ion mode (*m/z*): 459.2730 $[M+Na]^+$; for ^1H and ^{13}C NMR spectroscopic data, see Table 1.

3.3.4. 2α,3α,16α-Trihydroxy-24-nor-4(23),12-oleandien-28-oic acid (*4*)

White amorphous powder, $[\alpha]_D^{25}$: +16.7° (*c* 0.1, MeOH); ESIMS in negative ion mode (*m/z*): 471.3120 $[M-H]^-$; for ^1H and ^{13}C NMR spectroscopic data, see Table 2.

3.3.5. 2α-Hydroxyambreinolide (*5*)

White amorphous powder, $[\alpha]_D^{25}$: +12.9° (*c* 0.1, MeOH); ESIMS in negative ion mode (*m/z*): 279.1968 $[M-H]^-$; for ^1H and ^{13}C NMR spectroscopic data, see Table 3.

3.3.6. Ambreinolide-18-oic acid (*6*)

White amorphous powder, $[\alpha]_D^{25}$: +9.4° (*c* 0.1, MeOH); ESIMS in negative ion mode (*m/z*): 293.1770 $[M-H]^-$; for ^1H and ^{13}C NMR spectroscopic data, see Table 3.

3.3.7. 8α-Acetoxy-14,15,16-trinorlabdan-13-oic acid (*7*)

White amorphous powder, $[\alpha]_D^{25}$: +13.7° (*c* 0.1, MeOH); ESIMS in negative ion mode (*m/z*): 353.1970 $[M-H]^-$; for ^1H and ^{13}C NMR spectroscopic data, see Table 3.

3.3.8. 13-epi-Manoyloxide-15,18-dioic acid (*8*)

White amorphous powder, $[\alpha]_D^{25}$: +26.5° (*c* 0.1, MeOH); ESIMS in negative ion mode (*m/z*): 351.2180 $[M-H]^-$; for ^1H and ^{13}C NMR spectroscopic data, see Table 4.

3.3.9. 15-Normanoyloxide-14,18-dioic acid (*9*)

White amorphous powder, $[\alpha]_D^{25}$: +21.2° (*c* 0.1, MeOH); ESIMS in negative ion mode (*m/z*): 337.2020 $[M-H]^-$; for ^1H and ^{13}C NMR spectroscopic data, see Table 4.

3.4. Preparation of acetonide derivatives

A suspension of compounds **1**–**3** (2.0 mg) in THF (2.0 ml) was separately treated with 2,2-dimethoxypropane (0.5 ml), followed by a catalytic amount of anhydrous *p*-TsOH at 25 °C. After 1 h of stirring, few drops of Et_3N were added, and the mixture was concentrated *in vacuo*. The residue was partitioned between $CHCl_3$ and a saturated solution of $NaHCO_3$ and the chloroform part was concentrated *in vacuo*, affording the corresponding acetonides; ^1H NMR spectra of the acetonides were recorded.

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