

A New Labdane Diterpene and Other Constituents from *Marrubium deserti* Noe ex coss.

Hocine Dendougui¹, Souad Seghir¹, Zahia Belloum¹, Fadila Benayache²,
Francisco Leon³, Ignacio Brouard³, Jaime Bermejo³ and Samir Benayache^{*1}

¹Laboratory of Valorization of Natural Resources, University Mentouri of Constantine,
Algeria.

²Laboratory of Phytochemistry and Physico-Chemical and Biological Analysis, University
Mentouri of Constantine, Algeria.

³Instituto Productos Naturales y Agrobiología- Instituto Universitario de Bioorgánica A. G.,
Tenerife, Spain

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Abstract: The phytochemical study of the chloroform soluble part of the hydroalcoholic extract of *Marrubium deserti* allowed us to describe a new labdane diterpene, 6-dehydroxy-19-acetyl-marrubenol (**3**) beside three other diterpenes : 19-acetyl-marrubenol (**6**) , 6-acetyl-marrubenol (**7**) and 16-epoxy-9-hydroxy-labda-13(16), 14- diene (**1**). This latter derivative is described for the first time as natural compound. Phytol (**2**), and three sterols: β -sitosterol (**4**), stigmasterol (**5**) and β -sitosterol 3-*O*-glucoside (**8**) were also isolated from this species. Structure elucidation of the isolated compounds was accomplished by means of spectroscopic techniques, especially NMR spectroscopy and mass spectrometry.

Keywords: Lamiaceae; *Marrubium deserti*; diterpenoids, sterols; phytol.

1. Plant Source

Endemic plant *Marrubium deserti* Noe ex Coss is distributed in Algerian and Moroccan Sahara in septentrional and central Sahara [1-2] . The present sample was collected in April 2007 from flowering plants from the Daya of Mogheul at twenty km from Bechar in the south-west of Algeria, and identified by Professor M. Kabeche of University of Setif and M. Benabdelhakem from the National Agency of Preservation of Natural Resources of Bechar. Voucher specimens are kept in the Herbarium of Constantine University (MDB-N°206-2007).

2. Previous Studies

The genus *Marrubium* belongs to the Lamiaceae family. It comprises about 30 species indigenous to Europe, the Mediterranean area and Asia [3].

The main chemical constituents of *Marrubium* species are diterpenes, β - sitosterol, flavonoids and phenyl propanoids [4-8]. *Marrubium vulgare* which is the most representative species has several traditional uses such as expectorant, antispasmodic properties, antiasthmatic, anti-infective agent and

* Corresponding author: E-Mail: sbenayache@yahoo.com

externally, it has been used in ulcers and wounds [9-10]. *Marrubium deserti* is used traditionally for colics, colds, cough, fever, digestive troubles, helminthiasis and nausea [11-13]. Phytochemically, only one study on the composition of the essential oil of *Marrubium deserti* was previously reported in the literature [14].

3. Present Study

Air-dried leaves and flowers (1450 g) of *Marrubium deserti* Noe ex Coss (Lamiaceae) were macerated at room temperature with EtOH-H₂O (80:20 v/v) for 48 h, three times. After filtration, the filtrate was concentrated (600 mL) and dissolved in H₂O (700 mL). The resulting solution was extracted with CHCl₃. The organic phase was dried with Na₂SO₄, filtered and concentrated *in vacuo* at room temperature. The chloroform extract (32 g) was fractionated by CC (silica gel; Hexane/EtOAc step gradients and then with increasing percentages of MeOH) to yield 105 fractions (1-105). The obtained fractions were combined on the basis of TLC analysis. Fractions 6-9 (490 mg) (Hexane/EtOAc 90:10) were combined and rechromatographed on a silica gel column (Hexane/EtOAc with increasing polarity), to yield four subfractions (A₁-A₄). A₄ (180 mg) (Hexane/EtOAc 80:20) was submitted to preparative TLC (Hexane/EtOAc 95:5) to afford 15, 16-epoxy-9-hydroxy-labda-13(16), 14-diene (7.8 mg), (**1**) [15]. Fractions 26-38 (1.2 g) (Hexane/EtOAc 85:15) were combined and rechromatographed on a silica gel column (Hexane/CHCl₃) with increasing amounts of CHCl₃ to yield four subfractions (D₁-D₄). D₁ (Hexane/CHCl₃ 6 : 4) (374 mg) was rechromatographed on silica gel column (Toluene/CH₂Cl₂/Et₂O 16:1:1) to give phytol (**2**), (19.4 mg) [16] and the new compound (75.2 mg) (**3**). Fractions 39-41 (Hexane/EtOAc 80:20) (962 mg) gave after the purification by crystallization Hexane/EtOAc a mixture of β -sitosterol and stigmasterol [17-18] 1:1 (440 mg) (**4**) and (**5**). Fractions (51-54) (Hexane/EtOAc 80:20) (2.8 g) were rechromatographed on silica gel column eluted with (Hexane/CHCl₃/ EtOAc 2:2:1) to give 19-acetyl- marrubenol (246.7 mg) (**6**) and 6-acetyl-marrubenol (740 mg) (**7**) which was purified by crystallization. These two latter derivatives have been isolated previously from *M. sericeum* [4], as it was described; 6-acetyl-marrubenol (**7**) was highly instable and isomerized into 19-acetyl-marrubenol (**6**) which was more stable. Fractions 102-105 (AcOEt/MeOH 95:5) afforded β -sitosterol-3-*O*-glucoside (**8**) (55.8 mg) [19]. The known compounds were identified by their physical and spectroscopic data and comparison with literature data.

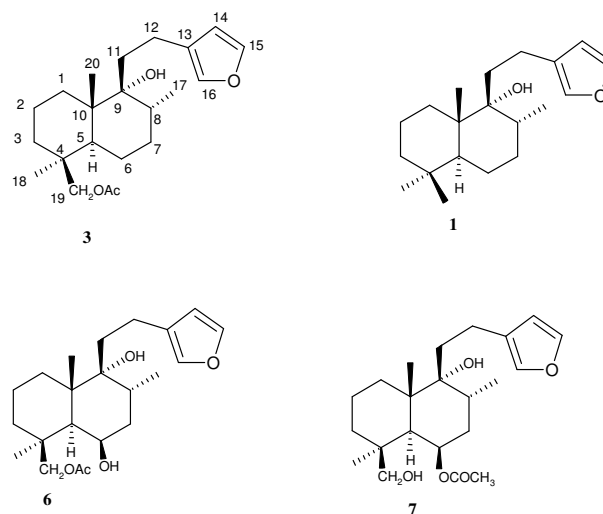


Figure 1. Labdane diterpenes isolated from *Marrubium deserti*

The high resolution mass spectrum of the new compound (**3**) showed an $[M]^+$ ion at m/z 362.2464 indicating a molecular formula of $C_{22}H_{34}O_4$ (calc 362.2457) of a functionalized diterpene. The ^{13}C and DEPT NMR data ($CDCl_3$) indicated the presence of four methyls (δ 16.24, 16.95, 20.94 and 26.76), eight methylenes (δ 18.16, 22.57, 22.59, 31.25, 31.61, 34.97, 35.70 and 67.10), five methines (δ 36.60, 47.05, 110.80, 138.40 and 142.80) and five quaternary carbons (δ 36.94, 43.02, 76.90, 125.50 and 171.40). The EIMS spectrum showed a fragment peak at m/z 344 indicating the presence of a hydroxyl group (M-H₂O) and an acetyl group m/z 302 (M-CH₃COOH), m/z 289 (M-CH₂OCOCH₃) and m/z 272 (M-H₂O-CH₂OCOCH₃) respectively. The presence of the acetyl group was also confirmed by the signal of the quaternary carbon at δ 171, 40. The EIMS spectrum showed also fragments peaks at m/z 81 and 95 indicating the presence of a β -monosubstituted furan ring. This was confirmed by the 1H NMR spectrum ($CDCl_3$) which showed typical signals of a β -monosubstituted furan ring at δ 6.25 (1H, d, H-14), 7.32 (1H, d, H-15) and 7.20 (1H, s, H-16). The molecular formula indicated the presence of six unsaturations in the structure, three were due to the furan ring, one of them was due to the acetate group. The absence of any signals corresponding to other multiple bonds in the 1H or ^{13}C NMR spectrum indicated that the remaining two unsaturations should be due to the presence of two cycles. All these data agreed with the presence of a furanolabdane diterpene.

Table 1. 1H - ^{13}C NMR (δ , J in Hz in parentheses)
Data of Compound **3**

	δ_H	δ_C^a
	3^a	
1	1.28-1.32 m ^b 1.52-1.55 m ^b	31.70 t
2	1.39 ddd (3.0; 6.6; 13) 1.52-1.55 m ^b	18.60 t
3	0.90-0.95 m ^b 1.78-1.80 m ^b	36.10 t
4		37.30 s
5	1.68 d (2.6)	43.10 d
6	1.11-1.14 m ^b 1.51-1.54 m ^b	21.90 t
7	1.22-1.27 m ^b 1.50-1.54 m ^b	31.40 t
8	1.41-1.44 m ^b	36.90 d
9		76.70 s
10		43.10 s
11	1.42-1.44 m ^b 1.72-1.75 m ^b	35.30 t
12	2.33 d br (8.4) 2.35 d br (8.8)	21.90 t
13		125.90 s
14	6.10 d (1.7)	111.20 d
15	7.15 m ^b	143.10 d
16	7.10 s	138.8 d
17	0.75 d (6.6)	16.39 q
18	0.98 s	27.63 q
19	3.97 d (11) 4.42 d (11)	66.80 t
20	0.73 s	16.92 q
OAc	1.71 s	20.50 q
		170.40 s

^aBased on COSY, HSQC, HMBC, and ROESY experiments and recorded in benzene-*d*₆. ^b Signals are overlapped.

Table 2. 1H - ^{13}C NMR (δ , J in Hz in parentheses)
Data of Compound **3**

	δ_H	δ_C^a
	3^a	
1	1.15-1.32 m ^b 1.61-1.69 m ^b	31.61 t
2	1.39-1.51	18.16 t
3	0.99 1.58-1.65 m ^b	35.70 t
4		36.94 s
5	1.58-1.65 m ^b	47.05 d
6	1.41-1.46 m ^b 1.51-1.54 m ^b	22.57 t
7	1.39-1.43 m ^b 1.50-1.54 m ^b	31.25 t
8	1.62 m	36.60 d
9		76.90 s
10		43.02 s
11	1.58-1.62 m ^b 1.82-1.85 m ^b	34.97 t
12	2.45 br d (8.8) 2.48 br d (8.8)	22.59 t
13		125.50 s
14	6.25 d (1.7)	110.80 d
15	7.32 d (1.7)	142.80 d
16	7.20 s	138.40 d
17	0.92 d (6.6)	16.24 q
18	0.93 s	26.76 q
19	3.97 d (11) 4.42 d (11)	67.10 t
20	0.95 s	16.95 q
OAc	2.08 s	20.94 q
		171.40 s

^aBased on COSY, HSQC, HMBC, and ROESY experiments and recorded in $CDCl_3$. ^b Signals are overlapped.

The proton NMR spectrum (CDCl_3) confirmed this assumption by exhibiting beside the characteristic furan protons (H-14, H-15 and H-16), an AB system at δ 3.97 and 4.42 corresponding to the CH_2OAc group, the presence of a secondary methyl at δ 0.92 (H₃-17) and two C-methyls at δ 0.93 and 0.95 respectively. The spectrum exhibited also the deshielded protons H-12 at δ 2.45 and δ 2.48, the methyl of the acetate group at δ 2.08 and the remaining protons of the methylenes of the two cyclohexane rings of the labdane skeleton between δ 0.99 and 1.69 ppm. The COSY, HSQC and HMBC spectra agreed with a furano labdane skeleton bearing a hydroxyl group at C-9 and an acetyl group at C-19. The HMBC spectra showed clearly in particular the coupling between the hydroxylated carbon C-9 and the methyl protons doublet H₃-17, the methyl protons H₃-20, and the methylene protons H₂-11 and H₂-12 (Figure 2). These spectra also showed the coupling between the oxygenated methylene C-19 and the methyne proton H-5, the methyl protons H₃-18 and the methyl protons H₃-22 of the acetate group and the coupling between the carbonyl C-21 of this acetate and the AB system protons H-19. The relative stereochemistry of the chiral centers C-4, C-9, C-5 and C-10 was deduced by the ROESY spectrum (Figure 3). The NMR data are relatively close to that of compound (6), 19-acetyl marrubenol except the lack of the methine carbinol H-6 at δ 4.5 in the ^1H spectrum and the oxygenated methine at δ 66.71 with the appearance of an additional CH_2 group in ^{13}C spectrum of compound (3). Thus, all the NMR data of this new compound agreed with the structure 6-dehydroxy-19-acetyl-Marrubenol. ^1H NMR (Bruker DRX 500 MHz, C_6D_6 and CDCl_3) Table 1 and table 2 respectively. The optical rotation of compound (3) was measured on a Perkin Elmer model 343 polarimeter, $[\alpha]_D^{22} = +5.4^\circ$ (c 0.0055, CHCl_3).

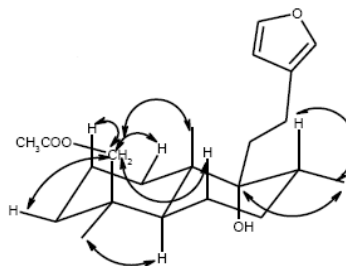
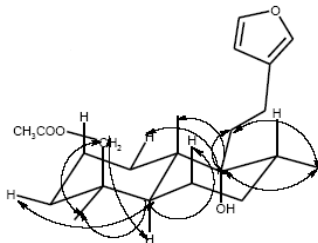


Figure 2. Key HMBC couplings of compound (3) **Figure 3.** Key ROESY correlation observed for compound (3)

Furanic labdane diterpenes are found in the genus *marrubium*, the labdane skeleton is a typical marker of this genus. In this study four labdane diterpenes (Figure 1), phytol, β -sitosterol, β -sitosterol 3-*O*-glucoside, and stigmasterol were obtained from *M. deserti* for the first time, of which compound (3), 6-dehydroxy-19-acetyl marrubenol is a new labdane diterpene. It is also the first time that compound (1) has been isolated from a natural source. It was previously obtained from hispanolone by tosylation and reduction with LiAlH_4 [15]. 19-acetyl-marrubenol (6) and 6-acetyl-marrubenol (7) have been isolated only from *M. sericeum* [4]. Phytol (2) and sterols (4, 5 and 8) were widely distributed as natural compound and cannot be considered as specific markers of *marrubium* species or Lamiaceae. But, it is interesting to notice that β -sitosterol 3-*O*-glucoside was not reported before from this genus.

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