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New cytotoxic saturated and unsaturated cyclohexanones from *Anthemis maritima*

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Abstract—Two new cyclohexenones (antheminones A and B) and a new cyclohexanone, (antheminone C) along with five known compounds were isolated from the leaves of *Anthemis maritima* L. The structures were mainly deduced from extensive 1D and 2D NMR spectroscopy and mass spectrometry. The new compounds were tested in vitro for their cytotoxic activity against adherent and non-adherent cancer cell lines. Antheminones A and C exhibited significant antiproliferative activity against leukemia cells with IC_{50} values ranging from 3.2 to 14 μM.

The genus *Anthemis* is represented by 130 accepted taxa and is known to contain sesquiterpene lactones and flavonoids.[1] *Anthemis maritima* L. (Asteraceae) is an aromatic herb which grows on sandy beaches along the western Mediterranean coasts.[2] There are no reports in the literature regarding the chemical constituents of this plant. As part of a continuing search aimed at the discovery of novel cytotoxic compounds from Sardinian plants belonging to the Anthemideae tribe,[3] it was found that the EtOAc extract of the leaves of *A. maritima* showed cytotoxic activity. The phytochemical analysis resulted in the isolation of two new cyclohexenones (**1** and **2**) and a new cyclohexanone (**3**). From the petroleum ether extract three known flavonoids, salvigenin (**4**), cirsimaritin (**5**), and eupatilin (**6**) and the triglyceride 2-*trans*,*trans*-sorbo-1,3-dimyristin (**7**) were also isolated.

The dried and powered leaves of *A. maritima*[4] (580 g) were ground and extracted with petroleum ether (5 L) by percolation. The remaining plant material was then extracted with EtOAc (4 L) giving 27.9 g of dried extract. An aliquot (20 g) of the EtOAc extract was subjected to VLC (silica gel) using a step gradient of petroleum ether–CH_2Cl_2–EtOAc (9:1:0–0:1:9, 500 mL each) to yield 53 fractions. Homogeneous fractions were pooled to give seven major fractions (F1–F7). A portion of fraction F2 (0.5 g) was subjected to open-column chromatography over Sephadex LH-20 using methanol as eluent to give a mixture of two compounds. Subsequent purification by semi preparative RP HPLC with water–acetonitrile (60:40) as eluent yielded compounds **1** (8.4 mg) and **2** (26.9 mg). Fraction F2 (0.6 g) was fractionated by Sephadex LH-20 using methanol as eluent and then with RP HPLC using a mixture of water–acetonitrile–methanol (50:40:10) to give compound **3** (8.3 mg). From the petroleum extract, by using similar fractionation procedure, the known compounds **4**–**7** were isolated. Compounds **4**–**7** were identified by comparing their physical and spectroscopic data with those reported in the literature.[5] ^{13}C NMR data for compound **7** are reported here for the first time.[6]

The ^{13}C NMR (Table 1) spectrum of compound **1** showed 15 carbon signals, which were sorted by DEPT 90 and 135 experiments into three CH_3, four CH_2, four CH, and four quaternary carbons. This corresponds to a molecular formula of $C_{15}H_{24}O_4$, in agreement with a $[M+H+Na]^+$ at *m*/*z* 291 in the ESI-MS. Elemental analysis confirmed the proposed empirical formula giving C = 66.98% (theoretical = 67.14%) and H = 9.02%

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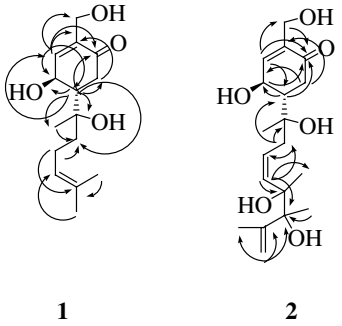
Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHZ) NMR spectral data of antheminones A–C (CD_3OD, δ in ppm)

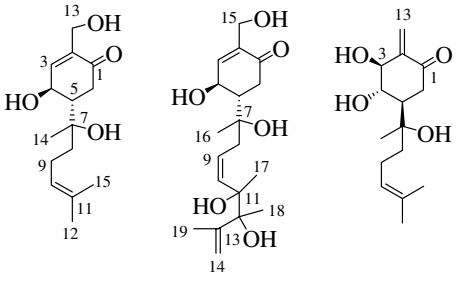
Position	Antheminone A (**1**)		Antheminone B (**2**)		Antheminone C (**3**)	
	δ_C, multiplicity[a]	δ_H, multiplicity[b]	δ_C, multiplicity[a]	δ_H, multiplicity[b]	δ_C, multiplicity[a]	δ_H, multiplicity[b]
1	202.4, s		202.4, s		201.2, s	
2	140.4, s		140.4, s		147.8, s	
3	143.6, d	7.05, dt (1.6, 6)	143.7, d	7.02, dt (1.5, 6)	85.8, d	4.68, d (1.6)
4	65.1, d	4.75, dd (3.6, 6)	65.1, d	4.71, dd (3.5, 6)	80.8, d	4.46, dd, br (1, 1.6)
5	46.0, d	2.18, dt (3.6, 13.6)	46.1, d	2.13, dt (3.5, 13.6)	48.4, d	2.52, dt (2.8, 4.1)
6_{ax}	35.2, t	2.88, dd (13.6, 16.7)	35.1, t	2.88, dd (13.6, 16.8)	43.5, d	2.80, dd (2.8, 19.2)
6_{eq}		2.53, dd (3.6, 16.7)		2.55, dd (3.5, 16.8)		2.61, dd (4, 19.2)
7	75.4, s		75.0, s		87.2, s	
8	41.2, t	1.64, m	44.4, t	2.38, m	42.4, t	1.49, m
9	23.7, t	2.10, m	126.5, d	5.72, m	25.2, t	2.15, m
10	125.6, d	5.22, t (7.5)	139.7, d	5.74, dt (7.2)	125.3, d	5.17, dt (7.2)
11	132.8, q		82.7, s		133, s	
12	26.1, q	1.80, s	90.8, s		26.1, q	1.76, s
13_a	59.9, t	4.31, dd (14.5, 1.6)	146.0, s		122.8, t	5.98, d (1.2)
13_b		4.32, d (14.5)				5.45, d (1.2)
14_a	25.1, q	1.46, s	114.5, t	4.96, br s	26.9, q	1.60, s
14_b				5.01, br s		
15_a	18.0, q	1.71, s	59.9, t	4.29, dd (1.6, 14.5)	18.0, q	1.69, s
15_b				4.31, d (14.5)		
16			25.1, q	1.45, s		
17			25.5, q	1.44, s		
18			25.2, q	1.38, s		
19			17.5, q	1.67, s		

[a] Multiplicity was determined by analysis of the DEPT spectra.
[b] J values (Hz) in parentheses.

(theoretical = 9.01%). Infrared absorption bands at 3420 and 1682 cm^{-1} suggested the presence of a hydroxyl group and α,β-unsaturated ketone, respectively. In the ^{1}H NMR (Table 1) spectrum the methine signals at δ_H 7.05 (1H, dt, $J = 1.6$, 6 Hz), and 5.22 (1H, t, $J = 7.5$ Hz) were assigned as olefinic protons whereas the methine at 4.75 (1H, dd, $J = 3.6$, 6 Hz) ppm must possess an oxygen substituent (δ_C 65.1). In addition to these signals, the ^{1}H NMR spectrum exhibited resonances for one hydroxymethylene function [δ_H 4.31 (dd, $J = 14.5$, 16 Hz) and 4.32 (d, $J = 14.5$ Hz)] and alkenyl chain. A HSQC experiment was utilized to assign the protons to their attached carbons. In the DQF-COSY spectrum, H-5 (δ_H 2.18 [dt, $J = 13.6$, 3.6 Hz)] showed cross-peaks with H-6 methylene protons [δ_H 2.88 (dd, $J = 13.6$, 16.7 Hz) and 2.53 (dd, $J = 3.6$, 16.7 Hz)] and H-4 (δ_H 4.75) while H-4 correlated with H-5 and H-3 (δ_H 7.05). HMBC interactions between H-3 and C-1 (δ_C 202.4), C-2 (δ_C 140.4), C-4 (δ_C 65.1), C-5 (δ_C 46.0) and between H-5 and C-1, C-3 (δ_C 143.6), C-4, and C-6 (δ_C 35.2), (Fig. 1), suggested the presence of a cyclohexenone ring. The structure of the alkenyl chain and its position on the cyclohexanone moiety were unambiguously established by HMBC experiments. In particular, HMBC correlations between the methine proton at δ_H 2.18 and C-4, C-6, C-7 (δ_C 75.4), C-8 (δ_C 41.2), and C-14 (δ_C 25.1) fixed the hexenyl chain at position 5 of the cyclohexenone. The key HMBC connectivities are displayed in Figure. 1. The relative stereochemistry of compound **1** was determined by ROESY experiments and analyzing scalar ($^{3}J_{HH}$) coupling of the protons. Namely, from the coupling patterns of adjacent proton signals in the ^{1}H NMR spectrum, the coupling constants values of $J_{6ax\text{-}5}$ and $J_{4\text{–}5}$ were calculated to be 13.6 and 3.6 Hz, respectively. Therefore, the hexenyl chain and the hydroxyl group at the 5- and 4-positions must be in the pseudoequatorial and pseudoaxial orientation, respectively. This observation was supported on the basis of the ROESY spectrum, which showed correlations between H-4 and H-5. Consequently, the structure of compound **1** was established as, 4-hydroxy-5-(1-hydroxy-1,5-dimethyl-4-hexenyl)-2 (hydroxymethyl)-2-cycloh-



1 **2**



1 **2** **3**

Figure 1. Main HMBC correlations of antheminones A (**1**) and B (**2**).