

SESQUITERPENES AND FLAVONOIDS OF *Anthemis odontostephana* var. *odontostephana*

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Phytochemical investigation of the flowers of Anthemis odontostephana Boiss. var. odontostephana (Asteraceae) resulted in the isolation of a natural cyclohexenone sesquiterpenoid with a bisabolene skeleton, 1-hydroxydelobanone (1), for the first time as a natural product, and a previously reported cyclohexenone derivative, antheminone A (2), along with two known flavonoids, pectolinarigenin (3), and salvigenin (4). The stereochemistry at positions C-4 and C-5 of the cyclohexenone ring of compound 2 was established based on ¹H–¹H spin-spin coupling constants and ROESY spectra. Compounds 2 and 3 were reported previously as antileishmanial and cytotoxic agents by other authors. The structures of the compounds were elucidated using NMR spectroscopic methods including ¹H NMR, ¹³C NMR, APT, COSY, HSQC, and HMBC, and mass spectrometry.

Keywords: *Anthemis odontostephana* var. *odontostephana*, antheminone, sesquiterpenoid, cyclohexenone, salvigenin, pectolinarigenin.

The genus *Anthemis* belongs to the Asteraceae family and consists of about 200 species [1] of which 39 are found in Iran, with 15 endemic species showing a rate of endemism of ca. 38% [2]. The most important biological activity of plants of the genus *Anthemis* is their use in gastrointestinal ailments [3]. *Anthemis* spp. or their constituents have shown antimicrobial [4–6], anti-inflammatory, sedative [7], and antiprotozoal [8] effects. In Iranian traditional medicine, some *Anthemis* spp. are regarded as appetizers and as wound healers [3].

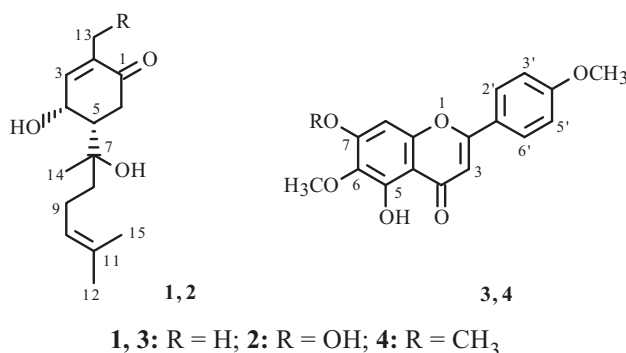
The essential oil of *A. odontostephana* var. *odontostephana* has been investigated previously, and 47 compounds have been identified of which spathulenol, hexadecanoic acid, and germacrene D were the most abundant compounds [9]. The amounts of the first two compounds reported in the oil of *A. odontostephana* were more than what have been reported from *Anthemis* spp. so far [10]. Different solvent extracts of *A. odontostephana* showed antileishmanial effect against *Leishmania* promastigotes, among which the hexane extract was the most potent extract [11]. In this paper, we report a bisabolol sesquiterpenoid with cyclohexenone functionality, 1-hydroxydelobanone (1), and its known hydroxylated derivative antheminone A (2), in addition to two flavonoids (3 and 4).

Using a series of normal and reversed-phase (RP) column chromatographies and HPLC purifications, we isolated two sesquiterpene cyclohexenones (1 and 2) and two flavonoids (3 and 4) and elucidated their structures by extensive NMR spectroscopy and mass spectrometry experiments.

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TABLE 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data of 1-Hydroxydelobanone (**1**) and Anthemione A (**2**) (CDCl_3 , δ , ppm, J/Hz)

C atom	1		2
	δ_{C}	δ_{H}	δ_{H}
1	200.4 s	–	–
2	137.5 s	–	–
3	141.9 d	6.75 (dd, J = 1.4, 6.0)	6.97 (dd, J = 1.6, 5.9)
4	65.0 d	4.68 (dd, J = 3.0, 6.0)	4.78 (dd, J = 3.0, 5.9)
5	44.2 d	1.98 (ddd, J = 3.0, 3.7, 13.5)	2.01 (ddd, J = 3.0, 3.7, 13.5)
6 _{ax}	33.4 t	2.87 (dd, J = 13.5, 16.5)	2.89 (dd, J = 13.5, 17.0)
6 _{eq}		2.52 (dd, J = 3.7, 16.5)	2.55 (dd, J = 3.7, 17.0)
7	74.9 s	–	–
8	40.2 t	1.55 (ddd, J = 1.5, 6.6, 9.2)	1.56 (ddd, J = 1.5, 6.6, 9.2)
9	22.5 t	1.95 m	1.98 m
10	123.5 d	5.10 (br.t, J = 7.5)	5.11 (t, J = 7.5)
11	132.5 s	–	–
12	25.6 q	1.69 s	1.72 s
13	15.6 t	1.82 (br.d, J = 1.4)	4.16 (d, J = 14.5)
			4.13 (d, J = 14.5)
14	25.0 q	1.41 s	1.45 s
15	17.7 q	1.61 s	1.63 s



The HR-ESI-MS suggested the molecular formula $\text{C}_{15}\text{H}_{24}\text{O}_3$ for compound **1** by virtue of an ion at m/z 252.1733 (calcd 252.1725). The results of the attached proton test (ATP) and the ^{13}C NMR and ^1H NMR (Table 1) data agreed with those reported for anthemione A (**2**) previously isolated from *Anthemis maritime* [12]. The planar structure of compound **1** was determined by comparing the NMR and MS data with those previously reported [12]. In addition, HSQC and HMBC experiments were performed to correlate the protons to their corresponding carbon atoms and to establish the ^1H – ^{13}C long-range correlations (Table 1, Fig. 1). The ^1H NMR signals at δ 6.75 (1H, dd, J = 1.4, 6.0 Hz, H-3), 4.68 (1H, dd, J = 3.0, 6.0 Hz, H-4), 1.98 (ddd, J = 3.0, 3.7, 13.5 Hz, H-5), 2.87 (dd, J = 13.5, 16.5 Hz, H-6_{ax}), and 2.52 (dd, J = 3.7, 16.5 Hz, H-6_{eq}) are attributed to the cyclohexenone ring spin system by virtue of the ^1H – ^1H coupling constants and ^1H – ^1H COSY cross signals. The position of the exo-methyl group (Me-13) was established at C-2 by the observation of cross peaks between the signal at δ_{H} 1.82 (3H, br.d, J = 1.4 Hz, H-13) and the carbonyl signal C-1 (δ_{C} 200.4), and the olefinic carbons C-2 (δ 137.5) and C-3 (δ 141.9) in the HMBC spectrum (Fig. 1). The two small coupling constants of $J_{\text{H-4-H-5}} = 3.0$ Hz and $J_{\text{H-5-H-6eq}} = 3.7$ Hz indicated that the alkenyl side chain at C-5 and the hydroxyl at C-4 are in pseudoequatorial and pseudoaxial orientation, respectively (*cis*-configuration), while H-5_{ax} and H-6_{ax} are in *trans* antiparallel orientation, having a large coupling constant $J_{\text{H-5ax-H-6ax}} = 13.5$ Hz. The above conclusion concerning the relative configuration was supported by the observation of cross peaks of H-4 with H-5 and H-3 in the ROESY spectrum (Fig. 1). The two signals of the olefinic methyl groups at δ_{H} 1.69 (3H, s, H-12) and 1.61 (3H, s, H-15) and one signal of a methyl group attached to the hydroxylated quaternary carbon atom (C-7) at δ 1.41 (3H, s, H-14) were attributed to an isoprenoid moiety attached to C-5. The HMBC cross peaks confirmed the connectivity of H-14 and C-7 and H-12 and H-15 to C-10 (Fig. 1). Based on ^1H – ^1H -COSY data, the proton signals at δ 1.55 (2H, ddd, J = 1.5, 6.6, 9.2, H-8), 1.95 (2H, m, H-9), and 5.10 (br.t, J = 7.5 Hz, H-10) are connected to each other in a three-spin system.

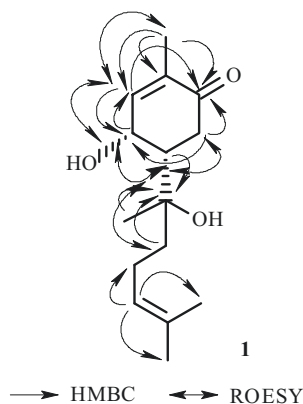


Fig. 1. Selected HMBC and ROESY correlations in 1-hydroxydelobanone (**1**).

The connection of the alkenyl side chain to the cyclohexenone ring was confirmed by observation of cross peaks between H-5 (δ_{H} 1.98) and C-7 (δ 74.9) in the HMBC spectrum (Fig. 1). From that data, compound **1** was identified to be 1-hydroxydelobanone, a compound reported as a hydrolysis product of 1-acetoxidelobanone [13]. 1-Hydroxydelobanone (**1**) was found here from *Anthemis odontostephana* for the first time as a natural product.

Anthemione A (**2**) is a 13-hydroxy derivative of compound **1** showing two doublets at δ 4.13 (d, $J = 14.5$ Hz, H-13a) and 4.16 (d, $J = 14.5$ Hz, H-13b) in the ^1H NMR spectrum, while the rest of the signals are similar to those recorded for compound **1** (Table 1) and those reported previously [12]. Although we found similar ^1H NMR data for anthemione A (**2**) (Table 1), as reported by [12], we concluded that the stereocenters C-4 and C-5 have the opposite *trans*-configuration. The stereochemistry at these positions is confirmed by observing the same spin system at δ 4.78 (1H, dd, $J = 3.0, 5.9$ Hz, H-4), 2.01 (ddd, $J = 3.0, 3.7, 13.5$ Hz, H-5), 2.89 (dd, $J = 13.5, 17.0$ Hz, H-6_{ax}), and 2.55 (dd, $J = 3.7, 17.0$ Hz, H-6_{eq}) as we already described for compound **1**. The configuration proposed here was compatible with that suggested for compound **1** by [13]. The absolute configuration of acetoxidelobanone was determined by ORD [13].

EXPERIMENTAL

General Experimental Procedures. High-performance liquid chromatography (HPLC) was performed on a Waters apparatus equipped with a pump module 600 and a dual wavelength (254, 366 nm) UV detector using a Shimpack Si 20 \times 250 mm column.

The ^1H and ^{13}C NMR were recorded on a Bruker DRX 500 and a Bruker AV 400 MHz, respectively. CDCl_3 and CD_3OD were used as a solvent and TMS as an internal standard. Homonuclear ^1H - ^1H connectivity was determined by the COSY experiment. One-bond heteronuclear ^1H - ^{13}C connectivity was determined with the HSQC experiment. Through-space ^1H - ^1H connectivity was established using ROESY experiments with a mixing time of 300 ms. Two- and three-bond ^1H - ^{13}C connectivity was determined by gradient 2D HMBC experiments optimized for $J_{\text{H-C}} = 10$ Hz. Electron-impact mass spectra (EI-MS) were recorded on a MasSpec sector field mass spectrometer (Micromass Ltd., Manchester, UK) with a direct insertion of probe.

Silica gels in different particle sizes (70–230 mesh) were used for gravity column chromatography. TLC plates (silica gel 60 GF₂₅₄ pre-coated plates, Merck) were visualized by UV observation at 254 and 365 nm and by spraying with cerium sulfate-sodium molybdate reagent. All reagent solid materials and solvents were purchased from Merck (Germany).

Plant Material. Aerial parts of *A. odontostephana* var. *odontostephana* were collected from Mazraehno Village, Yazd, Iran, in May 2009 at an altitude of 1370 m above sea level. The plant was identified by Prof. Asghar Mosleh-Arani, Department of Agriculture and Natural Resources, Yazd University, Yazd, Iran, and a voucher specimen of the source plant was kept (No. 2411) at the School of Pharmacy, Isfahan, Iran. Flowers were separated, shadow dried, and well grounded.

Extraction and Isolation of Compounds. Grounded flowers (335 g) were extracted with acetone (3 \times 3.3 L) with constant stirring for 2 days to render Ext-1 (20.7 g). The acetone extract was suspended in MeOH and kept at -20°C ; after 2 days it was chilled filtered, and the filtrate was dried in vacuum to get Ext-2 (10.8 g). The latter extract was eluted on silica

gel using EtOAc to obtain Ext-3 (7.5 g), which was then fractionated on RP18 using MeOH–H₂O (5:5 to 10:0) to get four fractions (F1–F4). Fraction F2 was then further purified using normal-phase chromatography [silica gel, heptane–EtOAc (6:4 to 3:7)] to get pure compound **1** (257 mg) and a nonpolar fraction that yielded compound **2** (2 mg) after finally purifying by HPLC (silica gel column, CH₂Cl₂–MeOH, 99:1 to 90:10, flow 10 mL/min). Fraction F3 was further fractionated on a silica gel open column eluted with heptane–EtOAc (8:2 to 3:7, v:v) and then purified by HPLC in the aforementioned conditions to get pure compounds **3** (2 mg) and **4** (13 mg).

Compound 1. Pale yellow liquid. UV quenching and carbonized with CeSO₄/Na₂MoO₄ reagent on TLC. EI-MS *m/z* 252 [M]⁺, HR-EI-MS *m/z* 252.1733 [M]⁺ (calcd for C₁₅H₂₄O₃, 252.1725). For ¹H and ¹³C NMR (500 and 125 MHz, respectively, CDCl₃) data, see Table 1.

Compound 2. Pale yellow liquid. For ¹H NMR (500 MHz, CDCl₃) data, see Table 1.

Pectolinarigenin (3). Pale yellow crystals. ¹H NMR (400 MHz, CD₃OD, δ, ppm, J/Hz): 3.93, 4.08 (each 3H, s, OMe), 6.58 (1H, s, H-8), 6.62 (1H, s, H-3), 7.04 (2H, d, J = 9.0, H-3', 5'), 7.87 (2H, d, J = 9.0, H-2', 6'), 13.3 (1H, s, OH).

Salvigenin (4). Pale yellow crystals. MS [M⁺] 328. ¹H NMR (400 MHz, CD₃OD, δ, ppm, J/Hz): 3.92, 3.96, 4.00 (each 3H, s, OMe), 6.57 (1H, s, H-8), 6.61 (1H, s, H-3), 7.05 (2H, d, J = 9.0, H-3', 5'), 7.86 (2H, d, J = 9.0, H-2', 6').

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