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An unique n-propyl sesquiterpene from *Eryngium creticum* L. (Apiaceae)

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The unique 1-*n*-propyl-perhydronaphthaline 1,2,4a,5,6,7,8,8a-octahydro-4-methyl-1-propyl-naphthalene-7-carbaldehyde, a compound which possesses an unusual sesquiterpene carbon skeleton, was isolated and identified, together with the new natural methyl ketone eicos-8,11-dien-18-ol-2-one, from the hexane : ether extract of the aerial parts of *Eryngium creticum* growing in Sinai, Egypt. The structures were established by conventional methods of analysis and confirmed by DEPT, COSY, HMQC and HMBC.

1. Introduction

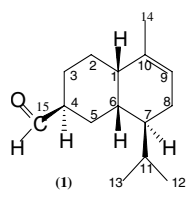
The genus *Eryngium* (Apiaceae) which embraces approximately 250 species is native to the tropical and temperate regions of east and southeast Asia and occurs in most parts of Europe as well [1]. Plants of this genus have been reported to have different therapeutic uses in folk medicine. A decoction of them is used as a diuretic, appetiser, laxative and anti-inflammatory [2]. In Jordan, *E. creticum* is used as a remedy for scorpion stings [3]. Previous studies on the secondary metabolites of this plant reported the isolation and characterization of triterpenes [4] and coumarins [5] and also reported the identification of the muurolane derivative, muurol-9-en-15-al, **1,** isolated from *Eryngium maritimum* [6]. It is well known that the sesquiterpenes, cadinanes and muurolanes, bear an isopropyl substituent connected to their perhydronaphthalene cores. In a continuation of our previous studies [6–8] on the essential oils of *Eryngium* we describe herein the isolation, from *E. creticum*, of a unique perhydronaphthaline derivative with an *n*-propyl substituent at its no. 1 position. It was identified as 1,2,4a,5,6,7,8,8a-octahydro-4-methyl-1-propyl-naphthalene-7-carbaldehyde (**2**). The compound possesses a new sesquiterpene carbon skeleton for which we suggest the name eryngane (**3**). The isolated terpenoid **2** itself is therefore, renamed, eryng-9-en-15-al. In addition, we were also, able to isolate the new natural methyl ketone eicos-8,11-dien-18-ol-2-one (**4**). Structures of compounds **2** and **4** were established by conventional methods of analysis and confirmed by DEPT, COSY, HMQC and HMBC spectral analysis.

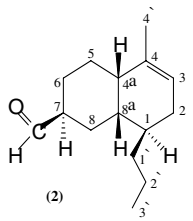
2. Investigations, results and discussion

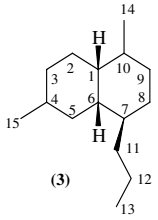
The species *Eryngium creticum* L. is a glaucous perennial plant (20–50 cm) with flattened, blade ovate, crenate leaves, growing in the East Mediterranean region. The hexane : ether (1 : 1) extract of the aerial parts of *E. creticum* was fractionated over a Silica Gel Woelm column, using pentane : ether as an eluent [9]. GC analysis of the products proved that compound **2** is the major constituent of II, while compound **4 is** predominant in fraction IV (positive test with 2,4 dinitro-phenyl hydrazine, specific for ketones). Purification of **2** and **4** was achieved by applying the corresponding fractions (II and IV), individually, to a preparative GC column, using a SE 30 packed column, whereby pure samples of **2** (12 mg) and **4** (25 mg) were obtained.

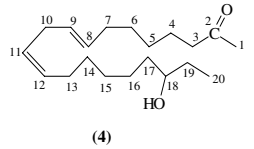
Compound **2,** isolated as a faint yellow oil was found to appear on TLC under UV light as a dark purple spot with R_f value [0.50, solvent hexane : ether (8 : 2)]. GC/MS analysis of **2** showed a molecular ion M^+ at 220 *m/z* in addition to an ion at *m/z* 159 (100%), indicating an oxygenated sesquiterpene with a molecular formula of $C_{15}H_{24}O$.

^{1}H NMR spectral analysis (room temperature and $CDCl_3$) was employed to unravel the structural problem of compound **2**. The recorded spectrum revealed the presence of a lowfield resonance at δ ppm: 9.63 (d, J = 1.5 Hz), assignable to an aldehydic proton, as well as an olefinic proton resonance at δ ppm: 5.35 (broad s, $\Delta\nu_{1/2} = 4$ Hz)









proving the presence of at least one olefinic bond in the molecule of **2**. The spectrum also showed resonance of a terminal propyl methyl group at δ ppm: 0.92 (t, J = 6 Hz), in addition to a methyl proton singlet located at δ ppm 1.66 assignable to the protons of an olefinic methyl group. These data when combined with the results of GC/MS analysis proved that compound **2** is a sesquiterpene aldehyde containing one olefinic bond. In general the chemical shift values and mode of splitting of the proton resonances in the spectrum obtained were found consistent with the muurol-9-en-15-al structure [6] except that the group of resonances of the isopropyl moiety in muurol-9-en-15-al at [δ ppm 1.75 (m, H-7); δ ppm 0.92 (d, J = 5 Hz, CH_3-12); δ ppm 0.78 (d, J = 5 Hz, CH_3-13)] was quite different, with respect to their chemical shifts and mode of splitting, from those recorded for the 1-substituent in compound **2** [resonances at δ ppm 0.92 (t, J = 6 Hz, CH_3-3′); δ ppm 1.45 (m, CH_2-1′ and CH_2-2′)]. The group of resonances, in **2** is, therefore, best interpreted in terms of an *n*-propyl substituent. These NMR data proved that the H-1 and H-7 protons are equatorially configured, thus keeping the propyl moiety as well as the aldehydic group in an axial configuration. It should be borne in mind that an axial configuration for the H-1 and H-7 protons would bring their resonances upfield when compared with those recorded for the same protons in the spectrum of compound **2** [10]. This conclusion was confirmed by ^{1}H NMR NOESY and COSY experiments.

The ^{13}C NMR spectrum of compound **2** exhibited 14 individual carbon resonances, of which the two most upfield at δ ppm: 14.43 and 21.46 were assigned to the carbons of the CH_3-3′ and CH_3-4′, respectively. The three most down field signals at δ ppm: 205.18, 137.10 and 120.85 were attributed to the carbons of the aldehydic carbonyl C7-CHO, the olefinic quaternary carbon No. 4 and the protonated olefinic carbon No. 3, respectively. The assignment of the remaining eight carbon resonances was aided by the data obtained from a DEPT experiment, which showed six methylenic carbon signals. Two of them, resonating at the same chemical shift value δ ppm 26.95, were assigned to C-8 and C-2. Resonances at δ ppm 26.32 and 24.77 were assigned to C-6 and C-5, respectively and those at δ ppm 21.60 and 35.37 were assigned to C-2′ and C-3′ of a propyl group, respectively. Unambiguous assignments of the remaining five methine carbons could be achieved only by measuring HMQC, which proved that the downfield signals at δ ppm: 45.36 and 41.96 were assignable to C-7 and C-4a, respectively. Resonances located at δ ppm: 34.98 and 34.25 are attributable to C-8a, C-1, respectively. Confirmation of the assignment given above was then achieved through measurement of HMQC and HMBC. From these data it becomes evident that compound **2** is the new natural sequiterpene 1,2,4a,5,6,7,8,8a-octahydro-4-methyl-1-propyl-naphthalene-7-carbaldehyde or eryng-9-en-15-al.

Compound **4**, the new unsaturated methyl ketone isolated as a colourless oil, exhibited, in EI/MS a Mr 308 (5%) and a molecular formula of $C_{20}H_{36}O_2$. Among the fragment ions recorded in this spectrum, the ions at *m/z* 265 (18%), 247 (7%), 58 (4%), 43 (68%) are consistent with an aliphatic chain with a terminal methyl ketone function [M-43 = 265], which might bear an alcoholic OH [M-43-18 = 247]. However the low intensity of fragment ion 58 was quite unexpected, as it should appear at about 80–100% relative intensity according to the expected McLafferty rearrangement [11]. The presence of the olefinic double bonds in the molecule of **4** could be the factor which inhibited this arrangement. A similar result has been briefly discussed by Kubeczka et al. [7]. It followed from the fragment ions at *m/z* 110 (30%), 96 (10%) and 95 (60%), respectively that the compound contains $[(CH_2)_6-CH=CH]$, $[(CH_2)_5-CH=CH]$ and $\{[(CH_2)_5-CH=CH]-H\}$ moieties. These three ions, together with the fragment ions recognized at *m/z* 81 (78%), 67 (100%) and 53 (23%), reflect the sequential losses of four CH_2 fragments from the parent ion at *m/z* 95, thus confirming the proposed identity of this moiety, and suggest, therefore, that **4** is eicos-8,11-dien-18-ol-2-one.

This view was supported by NMR spectral analysis of **4**. The ^{1}H NMR spectrum ($CDCl_3$, room temperature) revealed the presence of a proton pattern of resonances typical of an aliphatic ketonic structure possessing a terminal acetyl group (δ ppm 2.18, s, 3 H, CH_3-1). It followed from the multiplet located in this spectrum at δ ppm 5,32 (4 H, H-8, H-9, H-11, H-12) and from the broad triplet detected in the aliphatic region at δ ppm 2.77 (2 H, J = 4.2 Hz, H-10 methylinic protons) that the molecule of **4** contains two double bonds separated by only one aliphatic methylene group. The presence of an OH-function attached to an sp3 carbon was deduced from the proton multiplet resonances (in the form of a quartet) recognised at δ ppm 4.18, and assignable to the methinic proton geminal to the OH function. The remaining resonances in this spectrum exhibited chemical shift values and modes of splitting which agree well with the structure of **4** as a eicos-8,11-dien-18-ol-2-one, a conclusion which was further confirmed by the results of ^{1}H-^{1}H COSY investigation of **4**. The cross peaks recognized in this spectrum correlated the terminal methyl protons (CH_3-20) to the α-methylenic (CH_2-19) located at δ ppm 1.29 and correlated the latter to the methinic proton (CH-18) at δ ppm 4.18, thus confirming the site of attachment of the hydroxyl group to be at the carbon β-to the terminal methyl (CH_3-20).

Final confirmation of the structure **4** was then obtained from the ^{13}C NMR analysis. The recorded spectrum ($CDCl_3$, room temperature) showed a characteristic aliphatic ketone carbon resonance at δ ppm 212.6 (C-2), olefinic carbon resonances at δ ppm 130.20, 127.89, 127.86, 130.02 (C-8, C-9, C-11, C-12); two terminal methyl carbon resonances at δ ppm 29.54 and 14.03 (C-1 and C-20); an oxygenated aliphatic carbon resonance at δ ppm 74.54 (C-18) and a unique methylinic carbon resonance at δ ppm 25.17 (C-10). The multiplicity showed by DEPT and ^{1}H- ^{13}C NMR correlation showed by HMQC studies confirmed the assignments given above. ^{13}C, DEPT and HMQC data for the remaining carbon resonances fit perfectly with the structure of **4** as eicos-8,11-dien-18-ol-2-one, Compound **4**, therefore represents a novel natural product.

3. Experimental

3.1. Gas chromatography

Analysis of Fractions II and IV was performed using an HP 5890 Series II gas chromatograph equipped with a FID and a 30 m DB5 (J & W) fused-silica capillary column (0.25 mm i.d.; 0.25 μm film thickness). Operating conditions: Linear temperature program from 45 °C to 220 °C, 3 °C/min; injector and detector temperatures 220 °C; carrier gas nitrogen at a flow rate of 1.5 ml/min.

3.2. Preparative gas chromatography

Isolation of compounds **2** and **4** was performed using a Varian Aerograph 1400 equipped with a 2m × 4 mm i.d. packed column with 20% silicon GE SE-30 on 80–100 mesh Volaspher A2 in connection with an outlet

Table 1: ^{1}H and ^{13}C NMR data of compound 2

^{1}H		^{13}C	
H-1	1.78 m	C-1	34.51
H-2	1.83 m	C-2	26.95
H-3	5.35 s	C-3	121.24
–	–	C-4	137.49
H-4a	1.88 m	C-4a	42.35
H-5	1.35 m	C-5	24.77
H-6	1.95 m	C-6	26.32
H-7	2.30 m	C-7	45.75
H-8 ax	1.21 m	C-8	26.95
H8 eq	2.05 m		
H-8a	1.85 m	C-8a	26.95
CH_2-1′	1.45 m	CH_2-1′	35.37
CH_2-2′	1.45 m	CH_2-2′	21.60
CH_3-3′	0.92 (t, J = 6 Hz)	CH_3-3′	14.43
CH_3-4′	1.66 s	CH_3-4′	21.46
CHO	9.63 (d, J = 1.5 Hz)	CHO	205.55

splitter/FID (split ratio 1 : 10). Column temperature: 190 °C isothermal, carrier gas nitrogen at flow rate 190 ml/min.

3.3. GC-MS system

HP 5890 A Series II; MSD HP 5970 B; data system: HP-UX series 9000, Mod. 340; a 60 m DB-Wax (J & W) fused silica capillary (0.25 mm i.d.; 0.25 µm film thickness) was used. Operating condition: Linear temperature program from 45 °C to 220 °C, 3 °C/min; injector 220 °C (split 1 : 17); carrier gas helium at a flow rate of 0.9 ml/min.; ionization energy: 70 eV.

3.4. NMR-spectroscopy

^{1}H NMR spectra were measured on a Bruker AMX 400, relative to TMS. ^{13}C NMR were measured at 100 MHz, relative to $CDCl_3$ and converted to the TMS values by adding 77. Typical conditions = 6000 Hz for ^{1}H and 22 000 Hz for ^{13}C, 32 k data points and a flip angle of 45°.

3.5. Plant material

Aerial parts of *E. creticum* were collected in March 2000 from Sinai (Egypt) and authenticated by Prof. Dr. Nabil El-Hadidi, Department of Botany, Faculty of Science, Cairo University, Egypt. A voucher specimen has been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

3.6. Isolation and identification

Fresh aerial parts (500 g) were extracted with a mixture of *n*-hexane : diethylether (1 : 1). The extract was filtered and the solvent was removed in vacuo, at about 40 °C. The resultant oily extract (2 g), was fractionated by silica gel dry CC using *n*-pentane and a mixture of *n*-pentane-Et_2O (1 : 4) as eluents [9]. Fractions II and IV were analysed over a DB5 capillary column then subjected separately to preparative GC, using an SE-30 column, to give finally pure samples of compounds **2** (12 mg) and **4** (25 mg).

3.6.1. 1,2,4a,5,6,7,8,8a-Octahydro-4-methyl-1-propyl-naphthalene-7-carbaldehyde, 2 or erng-9-en-15-al

EI/MS (*m/z* rel. int.): 220 $[M]^+$ (10), 159 (100), 91 (32); ^{1}H and ^{13}C NMR (Table 1).

Table 2: ^{1}H and ^{13}C NMR data of compound 4

^{1}H		^{13}C	
Me-1	2.18 (sharp s)	CH_3-1	25.61 q
H-2	–	C-2	206.62 s
H-3	2.43 m	C-3	37.50 t
H-4	1.29 m	C-4	23.57 t
H-5	1.29 m	C-5	29.04^{***} t
H-6	1.29 m	C-6	22.54 t
H-7	2.01 m	C-7	27.17 t
H-8	5.32 m	C-8	130.20^{*} d
H-9	5.32 m	C-9	127.89^{**} d
H-10	2.77 (t, J = 4.2 Hz)	C-10	25.61 t
H-11	5.32 m	C-11	127.86 d
H-12	5.32 m	C-12	130.02^{*} d
H-13	2.01 m	C-13	27.17^{**} t
H-14	1.29 m	C-14	22.54 t
H-15	1.29 m	C-15	29.16^{***} t
H-16	1.29 m	C-16	19.80 t
H-17	2.25 m	C-17	33.53 t
H-18	4.18 m	C-18	72.54 d
H-19	1.35 m	C-19	31.50 t
Me-20	0.89 t	Me-20	14.03 q

3.6.2. Eicos- 8,11-dien-18-ol-2-one **(4)**

EI/MS (*m/z* rel. int.): 308 $[M]^+$ (5), 265 (18), 247 (7), 110 (30), 96 (10), 95 (60), 81 (78), 67 (100), 58 (4), 53 (23), 43 (68); ^{1}H and ^{13}C NMR (Table 2).

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