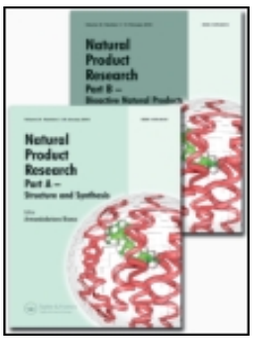

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DITERPENES FROM *SIDERITIS TROJANA*

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Six known *ent*-kaurene, a new *ent*-kaurane and a new pimarane diterpenes were isolated from *Sideritis trojana*. The structures of new compounds were determined as *ent*-7α-15β,16β-epoxykaurane (**1**), and *ent*-2α-hydroxy-8(14),15-pimaradiene (**2**) along with the known compounds siderol (**3**), sideridiol (**4**), 7-epicandicandiol (**5**), isocandol B (**6**), candol A acetate (**7**) *ent*-7α-acetoxykaur-15-ene (**8**) by IR, 1D and 2D NMR techniques and HRMS.

Keywords: Labiatae; *Sideritis trojana*; Diterpenoids; *Ent*-kaurenes; *Ent*-kaurane; Pimarane

INTRODUCTION

Sideritis species are widely distributed in Turkey with 45 species mainly in Marmara and Aegean regions [1]. In continuation of our studies on *Sideritis* species, we now report here a study on *Sideritis trojana* which is collected from southwest of Turkey. *S. trojana* are being used as a folk medicine, particularly in infusion forms of the herbal tea [2,3]. In previous studies, we have investigated two *Sideritis* species *S. athoa* [4] and *S. argyrea* [5], and obtained some new and known *ent*-kaurenes. In addition, from *S. argyreae*, a new *ent*-labdane and a known beyerene diterpenes were isolated, but, the identification of the latter compound (*ent*-7α,18-dihydroxybeyer-15-ene) [6] was completed after the study was sent for publication [4], therefore, it is informed herein.

RESULTS AND DISCUSSION

From the whole plant extract of *S. trojana*, a new *ent*-kaurane (*ent*-7α-acetoxy-l5β,16β-epoxykaurane) **1** and a new *ent*-pimarane (*ent*-2α-hydroxy-8(14),15-pimaradiene) **2** were isolated along with six known *ent*-kaurenes. The known kaurene diterpenes

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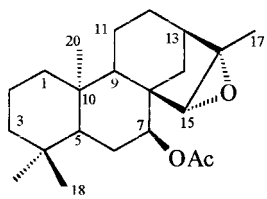
were identified as siderol **3** [7,8] sideridiol **4** [9], 7-epicandicandiol **5** [10] isocandol B **6** [11–13], candol A acetate **7** [14,15], *ent*-7α-acetoxykaur-15-ene **8** [14,15]. Particularly siderol and sideridiol were isolated in high yield 0.2 and 0.17% (dry weight), respectively. All the structures of compounds were identified based on IR, ^{1}H- and ^{13}C-NMR and mass spectroscopic techniques.

The IR spectrum of compound **1** showed the presence of an acetyl group with the absorption bands at 1725 and 1270 cm^{-1}, and epoxy group at 1085 cm^{-1} and no hydroxyl group was observed. In the HRMS spectrum, the compound **1** gave a molecular ion peak at m/z 346.2469 accounting for a molecular composition $C_{22}H_{32}O_2$. In the ^{1}H-NMR spectrum, three methyl signals for four methyl groups were observed at δ 0.78 (6H, s), 1.02 (3H, s) and 1.42 (3H, s). In addition, there was an acetyl methyl signal at δ 2.09 which was corroborated with a signal at δ 4.85 as a narrow triplet ($J = 2$ Hz) attributing to C-7 α proton. The presence of a signal at δ 2.97 as a singlet was indicative of a characteristic H-15β-epoxy proton as observed in similar kaurane diterpenes. *Ent*-7α,18-dihydroxy-15β,16β-epoxykaurane have similar spectral features to those of **1**, which showed epoxy group at δ 2.84 as a singlet in the ^{1}H-NMR spectrum [16]. The ^{13}C-NMR spectrum (by APT technique) revealed 22 carbon signals consisting of five methyl, seven methylene, five methine and five quaternary carbon atoms. A methine carbon at δ 74.89 was assigned to C-7, another methine carbon at δ 62.29 attributed to the epoxy methine carbon (C-15), while quaternary carbon of this epoxy group was observed at δ 78.05 (Table I), the assignments of protonated carbon signals were realized by a HETCOR experiment.

Thus, the structure of this diterpene **1** was elucidated as *ent*-7α-acetoxy-15β,16β-epoxykaurane which was isolated for the first time from nature.



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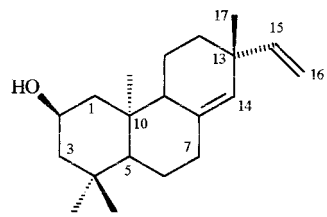

2

TABLE I ^{13}C-NMR data of Compounds **1** and **2**

	1	**2**		**1**	**2**
C-1	40.01	48.44	C-11	17.87	19.27
C-2	17.65	65.39	C-12	27.43	35.67
C-3	35.19	51.11	C-13	39.29	39.10
C-4	37.07	35.05	C-14	31.21	128.60
C-5	34.80	54.07	C-15	62.29	147.29
C-6	26.32	22.21	C-16	78.05	112.81
C-7	74.89	35.67	C-17	17.40	29.45
C-8	48.15	137.60	C-18	18.45	33.76
C-9	45.96	51.08	C-19	17.52	23.06
C-10	38.91	43.07	C-20	14.43	15.70
			OCO-CH_3	21.40	
			OCO-CH_3	178.60	

The second new compound has a pimarane skeleton. The EIMS spectrum of compound **2** gave a molecular ion peak at m/z 288.2. The molecular formula $C_{20}H_{32}O$ derived from its HRMS indicates five degrees of unsaturation, three of which are accounted for by a tricyclic ring system, and two by double bonds. The IR spectrum of **2** shows a hydroxyl absorption at 3440 cm^{-1} and unsaturation absorptions at 1660, 980 and 780 cm^{-1}. The ^{13}C-NMR spectrum (by APT technique) revealed 20 carbon signals consisting of four methyl, seven methylene, five methine and four quaternary carbon atoms. In the ^{1}H-NMR spectrum, the three signals at δ 5.72 dd ($J = 17.5$ and 10.5 Hz), at δ 4.97 dd ($J = 10.5$ and 2 Hz) and at δ 4.92 dd ($J = 17.5$ and 2 Hz) [13,17] are characteristic of an ethylenic side chain (C-15 and C-16 protons). These olefinic signals together with four methyl signals at δ 0.78, 0.90, 0.96, and 1.01 are assigned to a pimarane diterpene skeleton. The presence of the ethylenic group at C-13 is supported by carbon signals observed at δ 147.29 (C-15) and δ 112.82 (C-16). There is an additional double bond observed at δ_H 5.18 (br d, $J = 1$ Hz, H-14), δ_c 128.60 (C-14) with δ_c 137.60 (C-8) indicating a $\Delta^{8(14)}$ placement in the pimarane structure. A 2α-methine proton signal was observed at δ 3.83 as dddd ($J = 4,4,12,12$) [17–21]. The multiplicity indicates it should be placed between two methylene carbon atoms, therefore, it must be located at C-2. Due to the β effect of the hydroxyl group in the ^{13}C-NMR spectrum, the two neighboring carbon atoms appear at δ 48.44 and δ 51.11 which can only be attributed to the C-1 and C-3 signals [22,23], supported the location of the hydroxyl group at C-2. While direct ^{1}H–^{13}C correlations followed by HETCOR experiments, COLOC experiments showed two bond correlations between the δ 3.83 (H-2) proton signal and the 48.44 (C-1) and 51.11 (C-3) resonances, and three bond correlations with 35.05 (C-4) and 43.07 (C-10). The stereochemistry at C-2 was deduced by a NOE experiment. When H-2α was irradiated, the enhancement of the C-20α methyl protons (δ 0.78) is observed supporting the presence of β hydroxyl group at C-2.

The above spectroscopic data verified the assignment of structure **2** as *ent*-2α-hydroxy-8(14),15-pimaradiene.

MATERIALS AND METHODS

General

The spectra were recorded with the following instruments; IR: Perkin-Elmer 980 in $CHCl_3$; NMR: Bruker AC-200 L, 200 MHz and 50.32 MHz for ^{1}H- and ^{13}C-NMR, respectively, in $CDCl_3$; MS: VG ZabSpec high resolution Mass Spectrometer; Silicagel 60 was used for column chromatography and Kieselgel 60F_{254} (E. Merck) for prep. TLC as precoated plates.

Plant Material

S. trojana was collected from Marmara region of Turkey (Bayramiç-Çanakkale), June 1995. The plant was identified by Prof Dr. K.H.C. Başer (Eskişehir), a voucher specimen was deposited in the Herbarium of Faculty of Pharmacy, Anadolu University (ESSE 11669).

Extraction and Isolations

The powdered whole plant (1.0 kg) was extracted successively with hexane and methanol to give extracts 25 g and 30 g, respectively. Each extract was fractionated on the silica gel column. The elution of the hexane extract (25 g) was started with hexane and continued with gradients chloroform, and acetone, then methanol. From the hexane extract, except compound *ent*-7α-acetoxy-15β,16β-epoxykaurane **1** (15 mg) all the compounds, *ent*-2α-hydroxy-8(14),15-pimaradiene **2** (23 mg) siderol **3** (2 g), sideridiol **4** (1.7 g), 7-epicandicandiol **5** (10 mg), isocandol B **6** (25 mg), candol A acetate **7** (18 mg) and *ent*-7α-acetoxykaur-15-ene **8** (21 mg) were isolated.

The methanol extract (30 g) was first eluted with $CHCl_3$ and gradients $(CH_3)_2CO$ and CH_3OH were used, and a new compound *ent*-7α-acetoxy-15β,16β-epoxykaurane **1** isolated along with compounds **6**, **7**, **8**. Purification of the new compounds was carried out on prep. TLC. The solvent systems ($CHCl_3$–Hexane) (6 : 4) and ($CHCl_3$–Hexane) (2 : 8) were used in purification for compounds **1** and **2**, respectively.

Ent-7α-acetoxy-15β,16β-epoxykaurane **1** IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 and 1270 (C=O), 1085 (C–O). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 4.85 (1H, t, $J = 2$, H-7), 2.97 (1H, s, H-15), 2.09 (3H, s, OAc), 1.42 (3H, s, Me-17), 1.02 (3H, s, Me-20), 0.78 (6H, s, Me-18 and Me-19), EIMS (rel. int.) *m/z*: 346.2 $[M]^+$ (16), 286.2 $[M - COOCH_3]^+$ (19), 268.2 (81), 253.1 (80), 243.1 (32), 225.1 (36), 201.1 (49), 149.0 (77), 131.0 (62), 119.0 (58), 108.9 (79), 95.1 (66), 81.0 (76), 69.0 (96). HRMS: 346.2469 (calcd. 346.2507 for $C_{22}H_{32}O_2$). ^{13}C-NMR: (see Table I).

Ent-2α-hydroxy-8(14),15-pimaradiene **2** IR $\nu_{max}^{CHCl_3}$$cm^{-1}$: 3440 (OH), 1660, 980, 780 (C=C); ^{1}H-NMR (200 MHz, $CDC1_3$) δ: 5.72 (1H, dd, $J = 17.5$ and 10.5 Hz H-15), 5.18 (1H, br d, J = 1 Hz, H-14), 4.92 (lH, dd, $J = 17.5$ and 2.0 Hz, H-l6a) and 4.97 (1H, dd, $J = 10.5$ and 2.0 Hz, H-16b), 3.83 (1H, dddd, $J = 4.0$, 4.0, 12.0, 12.0, H-2α) 1.01 (3H, s, Me-17), 0.96 (3H, s, Me-l8), 0.90 (3H, s, Me-19), 0.78 (3H, s, Me-20). EIMS (rel. int.) *m/z*: 288 $[M]^+$ (52), 273 $[M - CH_3]^+$(43), 270 $[M–H_2O]^+$ (24), 255 $[M - H_2O - CH_3]^+$ (46), 227 (8), 201 (17), 187 (44), 175 (12), 159 (12), 153 (8), 148 (14), 135 (100), 121 (39), 107 (37), 93 (44), 79 (24), 57 (17); HRMS: 288.2459 (calcd. 288.2453 for $C_{20}H_{32}O$). ^{13}C-NMR: (see Table I).

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