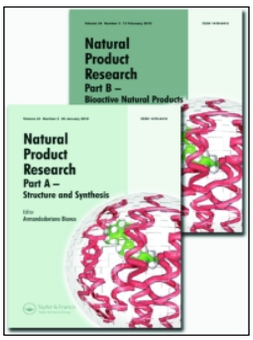

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ENT-KAURENE DITERPENES FROM *SIDERITIS ATHOA*

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Abstract : Two new and six known *ent*-kaurene diterpenes were isolated from the whole plant of *Sideritis athoa* and their structures were elucidated as *ent*-3α,18-dihydroxykaur-16-ene (**1**), athonolone (**2**), *ent*-3β-hydroxykaur-16-ene (**3**) *ent*-3β,7α-dihydroxykaur-16-ene (**4**), 7-*epi*candicandiol (**5**), linearol (**6**), foliol (**7**) and sidol (**8**) based on 1D and 2D NMR techniques and HRMS.

Key Words: *Sideritis athoa*, labiatae, diterpenes, kaurene.

INTRODUCTION

Sideritis species have been used in folk medicine for their anti-inflammatory, antirheumatic, digestive and antimicrobial activities[1] in Turkey as well as in Europe[2] *Sideritis* species are also often used as herbal teas in Turkey. By the identification of seven new species within the last 3 years,[3,4] the number of 38 *Sideritis* species, known in the Flora of Turkey, was raised to 45 with 10 subspecies and 2 varieties. Among them, 34 species, 4 subspecies and 2 varieties are endemic. Since there is only marginal knowledge on the non-volatile components of Turkish *Sideritis* species[5] we began to investigate these compounds and report here on a study of *Sideritis athoa* which is only distributed in the Kazdagı region (Turkey) and at Athoa Mountain in Greece[6]

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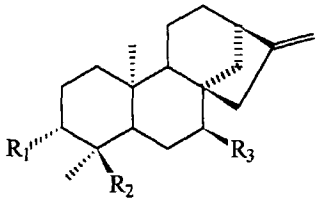
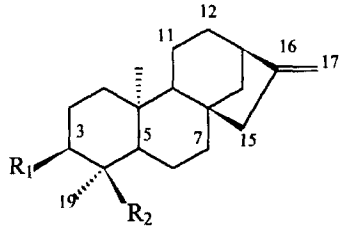
RESULTS AND DISCUSSION

From the whole plant extract of *S. athoa* two new and six known compounds were isolated. The known compounds were identified as linearol **(6)**, 18-deacetyl linearol (foliol) **(7)** , sidol **(8)**, *ent*-3β,7α-dihydroxykaur-16-ene **(4)**[5,7,8] , 7-*epi*candicandiol **(5)**[8,9] and *ent*-3β-hydroxykaur-16-ene **(3)**[8] based on ^{1}H-, ^{13}C-NMR and MS spectral data. Of those linearol, foliol and 7-*epi*candicandiol were isolated in high yield, 0.1, 0.08 and 0.15 %, respectively.



	R_1	R_2	R_3
3	OH	CH_3	H
4	OH	CH_3	OH
5	H	CH_2OH	OH
6	OH	CH_2OAc	OH
6a	OAc	CH_2OAc	OAc
7	OH	CH_2OH	OH
8	OAc	CH_2OH	OH

The molecular ion of the first new compound **(1)** was observed at *m/z* 304.1 in the EI-mass spectrum accounting for a molecular composition $C_{20}H_{32}O_2$.



1 R_1= OH R_2= CH_2OH

1a R_1= OAc R_2= CH_2OAc

The ^{1}H NMR spectrum exhibited exocyclic methylene proton signals at δ 4.62 and 4.72 and two methyl singlets at δ 0.70 and 1.11. An AB system at δ 3.05 and 3.36 (J=12 Hz) was attributed to a hydroxymethylene group located at C-18, as deduced by comparison of the ^{1}H and ^{13}C NMR spectra with

literature data.[8,9] The presence of a hydroxymethine proton signal at δ 3.28 as a doublet of doublets (*J*=5 and 9 Hz) was assigned to that vicinal to a secondary hydroxy group at C-3. After acetylation of **1** **(1a)**, this signal was shifted to δ 4.55, and the resonances of the primary alcohol methylene protons were shifted to δ 3.57 and 3.85. The high resolution mass spectrum of the diacetate gave a molecular ion at *m/z* 388.261431 (calc. for $C_{24}H_{36}O_4$: 388.261360). The ^{13}C NMR spectrum (APT) supported the structure displaying 24 signals for four methine, four methyl, ten methylene and six quaternary carbon atoms. The primary alcohol carbon signal was observed at δ 72.07 while the hydroxymethine group carbon signal was seen at δ 83.67, and the exocyclic double bond gave resonances at δ 103.14 and 155.48. A literature survey showed that *ent*-3β,19-dihydroxykaur-16-ene[10], *ent*-3β,18-dihydroxykaur-16-ene and *ent*-3α,19-dihydroxykaur-16-ene should be isomers of **(1)**. In fact, the ^1H NMR data of these isomers are very similar to those of compound **(1)**. The H-3 signal had more or less the same multiplicity and *J* values in either an α or β position in accord with Dreiding model studies. The β orientation of the hydroxyl group at C-3 was proven by 2D NOE experiments in which NOE interactions between H-3 and H-19, as well as H-3 and H-20 were observed, indicating that H-3, H-19 and H-20 had the same orientation, viz. α. If an α hydroxyl group was present at C-3, a NOE interaction would be observed between H-3β and H_2-18 as well as between H-5 and H_2-18. The ^{13}C-NMR data of none of the isomers were exactly compatible with those of **(1)**. An comparison of the ^{13}C NMR spectral data of **(1a)** with those of *ent*-3β,19-diacetoxy,12α,15β-dihydroxykaur-16-ene showed on the C-3 signal at δ 78.9 for the later compound, and at δ 83.67 for compound **(1a)**.[10,11] All the spectral data indicated that the structure of **(1)** is ent-3α,18-dihydroxykaur-16-ene.

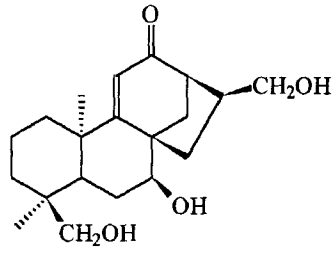
Table 1. The ^1H NMR data of **(1)**, **(1a)** and **(2)** in $CDCl_3$ (200MHz)

	1	**1a**	**2**
H-3	3.28 dd (5 and 9)	4.55 dd (5 and 10)	
H-7			3.65 t (2.5)
H-11			5.80 br s
H-13	2.58 m	2.61 m	2.57 m
H-17	4.62 br s	4.63 br s	4.65 br s
H-17'	4.72 br s	4.72 br s	4.65 br s
H-18	3.05 d (12)	3.57 d (12)	2.98 d (12)
H-18	3.36 d (12)	3.85 d (12)	3.47 d (12)
Me-19	0.70 s	0.90 s	0.69 s
Me-20	1.11 s	1.22 s	1.06 s
OAc		2.09 s	

J values are given in parantheses as Hz.

The HRMS of the second compound **(2)** gave a molecular ion at *m/z* 334.213128 accounting for $C_{20}H_{30}O_4$ (calc. 334.214410). The molecular formula showed six degrees of unsaturation which

correspond to four ring systems, one double bond and one keto group. The IR spectrum showed hydroxyl absorption at 3430 cm^{-1}, and a conjugated carbonyl group (enone) at 1720 and 1660 cm^{-1}. Accordingly, the UV spectrum gave a maximum at 256 nm. Generally, the characteristic exocyclic methylene protons of C-17 appear around 5.00 ppm in the ^1H NMR spectrum of kaurenes, but they were not observed for compound **(2)**. There were two hydroxymethylene groups present, one of which gave two doublets at δ 2.98 and 3.47 (J=12 Hz) attributed to a C-18 hydroxymethylene group. The signal of the second CH_2OH appeared at δ 4.65 (2H) as a broad singlet. Its location was assumed to be at C-16 since there were only two methyl signals at δ 0.69 and 1.06 as singlets which were assigned to H-19 and H-20, respectively.



2

The stereochemistry at C-16 followed from 1D NOE experiments. Irradiation of H-16 (δ 2.32, m) caused an enhancement of H-13 (δ 2.57, m) indicating a β position for the CH_2OH group at C-16. Moreover on irradiation of H-13, an enhancement was observed on H-16. The chemical shift of the H-17 signal was further downfield than expected, but this could be explained by the influence of a neighbouring enone group. The presence of the enone moiety followed from the ^1H NMR spectrum which showed an olefinic proton signal at δ 5.80, the IR (1660 cm^{-1}) and UV (256 nm) spectra supporting this finding[12,13]. The observation of a hydroxymethine proton signal at δ 3.65 as a triplet (J=2.5 Hz) indicated the presence of a secondary hydroxy group at C-7 in a β position, while there was no signal for the presence of a hydroxy group at C-3.

The ^{13}C NMR spectrum taken by the APT technique, revealed 20 carbon signals corresponding to two methyl, four methine (one being olefinic), eight methylene (two of them next to oxygen), and five quaternary carbon atoms. The HETCOR correlations allowed us to determine unambigously protonated carbons of the structure.

Based on the spectral data, compound **(2)** was established as *ent*-7α,17,18-trihydroxykaur-9(11)-ene-12 one, and it was given the trial name athonolone.

Table 2. The ^{13}C NMR data of compounds **(1a)** and **(2)** in $CDCl_3$ (50.32 MHz)

	1a	2		1a	2
C-1	41.35	39.24	C-11	19.74	133.44
C-2	24.59	18.20	C-12	33.30	204.13
C-3	83.67	35.22	C-13	43.79	43.50
C-4	42.27	39.25	C-14	40.26	26.36
C-5	49.50	38.25	C-15	49.21	42.09
C-6	29.71	25.68	C-16	155.48	41.10
C-7	40.26	74.67	C-17	103.14	71.08
C-8	43.79	43.41	C-18	72.07	62.11
C-9	55.40	142.10	C-19	14.32	17.60
C-10	37.90	42.10	C-20	17.58	22.41

Spectral data: *Ent*-3α,18-dihydroxykaur-16-ene (1)- $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH),1650 and 875 (C=C),1480, 1460, 1440, 1385, 1365, 1250, 1225, 1045, 915. ^1H NMR ($CDCl_3$): See Table 1. EI-MS *m/z* (rel.int): 304.1 $[M]^+$(4) ($C_{20}H_{32}O_2$), 286.1 $[M-H_2O]^+$(44), 271.1 $[M-H_2O-CH_3]^+$ (20), 268.1$[M-2H_2O]^+$ (10), 255.1 $[M-2H_2O-CH_3]^+$(100).

***Ent*-3α,18-diacetoxykaure-16-ene (1a)-** ^1H NMR ($CDCl_3$): See Table 1.HREIMS *m/z* 388.261431 $[M]^+$($C_{24}H_{36}O_4$), EI-MS *m/z* (rel.int.): 388.1 $[M]^+$ (14), 328.1 $[M-HOAc]^+$ (32), 268.1 $[M-2HOAc]^+$ (43), 255.1 $[M-133]^+$ (100).

***Ent*-7α,17,18-trihydroxykaur-9(11)-ene-12-one(2)-** UVλ_{max}(MeOH) nm: 256. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430 (OH), 1720 (C = O), 1660 (C = C), 1450, 1380, 1210, 1020, 910. ^1H NMR ($CDCl_3$): See Table 1. HREIMS *m/z* 334.213128 $[M]^+$ ($C_{20}H_{30}O_4$).

EXPERIMENTAL

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 ^1H and ^{13}C NMR, respectively, in $CDCl_3$; HRMS: VG ZabSpec (max mass resolution 10.000). For the isolation and purifications of the compounds TLC: Kieselgel $60F_{254}$ (E.Merck) precoated plates, CC: Silica gel 60 and Sephadex LH-20 (Fluka) were used.

Plant material: *Sideritis athoa* Papanikolau et Kokkini was collected from the Kazdağı region in 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 the Pharmacy, Anadolu University (ESSE 9211).

Extraction and Isolation: The powdered whole plant (2 kg) was extracted successively with hexane and acetone to give extracts 60 g and 70 g, respectively. Each extract was fractionated on a silica gel column. The hexane extract was first eluted with hexane, and gradients chloroform, acetone and methanol, respectively. From the hexane extract, *ent*-3β-hydroxykaur-16-ene **(3)** (10 mg), *ent*-3β,7α-dihydroxykaur-16-ene **(4)** (20 mg), *ent*-3α,18-dihydroxykaur-16-ene **(1)** (22 mg), 7-epicandicandiol *(ent*-7α,18-dihydroxykaur-16-ene) **(5)** (3 g), linearol **(6)** (2 g), foliol **(7)** (1.6 g) and sidol **(8)** (30 mg) were isolated, successively. During elution with chloroform compounds **(1), (3)** and **(4)** were obtained together, and then compound **(1)** was purified on prep. TLC ($CHCl_3$: acetone) (98:2). Compounds **(3)** and **(4)** were separated from each other by prep TLC using ($CHCl_3$: acetone) (95:5) solution systems. The acetone extract was first eluted with $CHCl_3$, as gradient acetone and methanol were used, respectively. From the acetone extract, athonolone (*ent*-7α,17,18-trihydroxy-9(11)-ene-12-one) **(2)** (25 mg) , and compounds **(4)** (10 mg), **(5)** (25 mg), **(6)** (0.3 g) and **(8)** (15 mg) were isolated. Athonolone was obtained during the elution with chloroform: acetone (7:3) and purified on a Sephadex LH-20 column (petrol ether-chloroform-methanol) (7:4:1). Compounds **(5), (7)** and **(8)** were easily crystallized in CH_2Cl_2:acetone (1:1) while linearol **(6)** was crystallized from isopropyl alcohol .

Acetylation of compound 1(1a): Compound **(1)** (22 mg) was dissolved in 1 mL pyridine, 1 mL of acetic anhydrid was added, and the mixture left at room temp. overnight, and then evapd under a vacuum and purified by TLC to yield the acetate (18 mg).

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