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Two *Sideritis* species afforded eleven kaurene and one beyerene diterpenes. Structures of the compounds from *Sideritis sipylea* were elucidated as linearol **(1)**, 7-epicandicandiol **(2)**, sideridiol **(3)**, siderol **(4)**, isolinearol **(5)**, isosidol **(6)** and epoxyisolinearol **(7)**. Linearol was treated with *m*-chloroperbenzoic to afford its analogues *ent*-3β,7α,17-trihydroxy-18-acetoxykaur-15-ene **(1a)** and *ent*-7α,17,18-trihydroxy-3β-acetoxykaur-15-ene **(1b)** as new compounds. From the second species, *Sideritis dichotoma,* the kaurenes sideridiol **(3)** siderol **(4)**, *ent*-7α,18-dihydroxy-15β,16β-epoxykaurane **(8)**, *ent*-7α-acetoxy,18-hydroxy-15β,16β-epoxykaurane **(9)**, *ent*-7α-acetoxy-15,18-dihydroxy-kaur-16-ene **(10)**, *ent*-7α,15,18-trihydroxykaur-16-ene **(11)** and the beyerene *ent*-7α,18-dihydroxybeyer-15-ene **(12)** were isolated. Structural elucidation is based on NMR techniques and mass spectrometer analyses.

Key Words: Labiatae, *Sideritis sipylea*, *Sideritis dichotoma,* diterpenoids, kaurane, kaurene, beyerene.

Introduction

Among the 45 *Sideritis* species growing in Turkey, 34 are endemic[1]. *Sideritis* species have been used in folk medicine for their antiinflammatory, antirheumatic, digestive and antimicrobial activites in Turkey[2] as well as in Europe[3]. They are also widely used as herbal teas in Turkey. In our previous studies, we investigated two *Sideritis* species, *S. athoa*[4] and *S. argyrea*[5], which mainly afforded kaurene diterpenes. We report here diterpenic constituents of *Sideritis sipylea* and *S. dichotoma.* The latter showed diuretic activity[6]. The synthesis of analogues **1a** and **1b** of linearol **(1)** is reported for the first time.

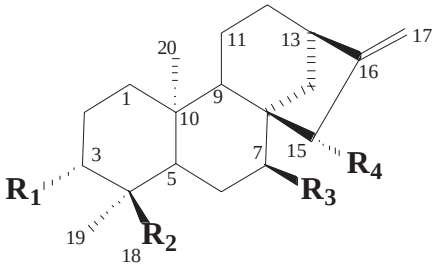
Experimental

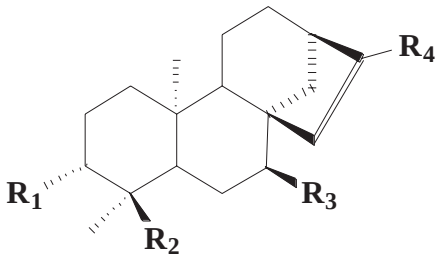
General

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 $CDCl_3$; MS: ZabSpec high resolution mass spectrometer; CC: Si-gel 60 was used for column chromatography and Kieselgel $60F_{254}$ (E. Merck) for prep., TLC as precoated plates.

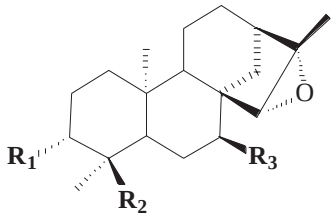
Plant Material

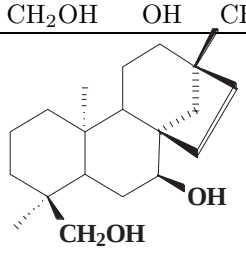
The aerial parts of *Sideritis sipylea* were collected from Sipil mountain (Manisa) in Turkey, in June 1995, while the aerial parts of *Sideritis dichotoma* Huter were collected from the Marmara region (Kazdağı, Balıkesir) in June 1995. The plants were identified by Prof. Dr. K.H.C. Başer (Eskişehir), and voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Anadolu University (ESSE 10141) for *S. sipylea* and (ESSE 11658) *S. dichotoma*.





	R_1	R_2	R_3	R_4		R_1	R_2	R_3	R_4
1	OH	CH_2OAc	OH	H	**3**	H	CH_2OH	OH	CH_3
2	H	CH_2OH	OH	H	**4**	H	CH_2OH	OAc	CH_3
10	H	CH_2OH	OAc	OH	**5**	OH	CH_2OAc	OH	CH_3
11	H	CH_2OH	OH	OH	**6**	OAc	CH_2OH	OH	CH_3
					1a	OH	CH_2OAc	OH	CH_2OH
					1b	OAc	CH_2OH	OH	CH_2OH



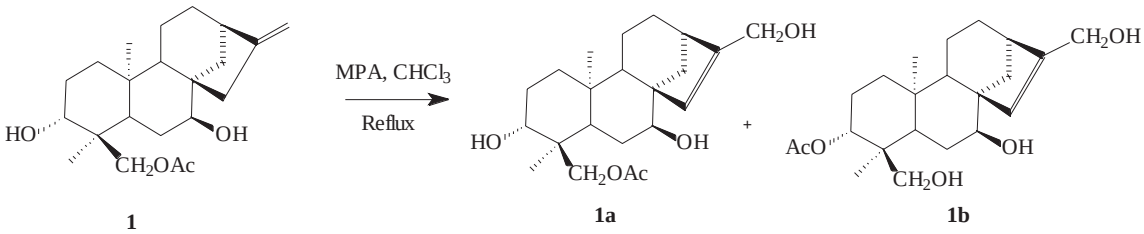


12

	R_1	R_2	R_3
7	OH	CH_2OAc	OH
8	H	CH_2OH	OH
9	H	CH_2OH	OAc

Extraction and Isolation. The air dried plant material from *Sideritis sipylea* (1.5 kg) was extracted successively with hexane and methanol. The hexane extract (30 g) was fractionated on a Si-gel column. The elution of the hexane extract was started with hexane and continued with the gradients chloroform, acetone

and then methanol. Compounds **(1)** and **(2)** were obtained as a mixture from the hexane extract during the elution chloroform-acetone (9:1) and purified on prep. TLC ($CHCl_3$: acetone, 7:3) as 520 mg and 1 g, respectively. Sideridiol **(3)** (1.5 g) siderol **(4)** (200 mg,) and isolinearol **(5)** (40 mg) were isolated from (CH_2Cl_2: acetone, 1:1), isosidol **(6)** (23 mg) from (CH_2Cl_2:acetone, 4:6) and epoxyisaineorol **(7)** (15 mg) from (CH_2Cl_2:acetone, 4:6).



1 **1a** **1b**

Scheme 1. Reaction of linearol with MPA in $CHCl_3$

The aerial parts of *S. dichotoma* (800 g) were extracted successively with hexane and acetone to give the extracts of 17 g and 13 g, respectively. Each extract was fractionated with Si-gel column chromatography. Elution of the hexane extract (17 g) was carried out as for the above plant. Compounds *ent*-7α,18-dihydroxy-15β,16β-epoxykaurane **(8)** (siderexol) (250 mg), *ent*-7α-acetoxy,18-hydroxy-15β,16β-epoxykaurane **(9)** (epoxysiderol) (105 mg), sideridiol **(3)** (23 mg), siderol **(4)** (47 mg), *ent*-7α-acetoxy,15β,18-dihydroxykaur-16-ene **(10)** (35 mg), ent-7α,15β,18-dihydroxykaur-16-ene **(11)** (28 mg) and 7α,18-dihydroxy-beyer-15-ene **(12)** (14 mg) were isolated from both hexane and acetone extracts. The acetone extract on a Si-gel column (13 g) was first eluted with chloroform and then with gradients of acetone and methanol. Compound **9** was isolated from a Si-gel column using (acetone: CH_2Cl_2) (8:2). Purification of compounds **(3)**, **(4)**, and **(9)** was carried out on prep. TLC using the solvent system (CH_2Cl_2:Hexane, 7:3) while compounds **(10)** and **(11)** were purified from (Hexane:CH_2Cl_2, 6:4) and compound **12** was purified from (CH_2Cl_2:Hexane, 8:2).

Linearol (1). IR$\nu_{\max}^{CHCl_3}$cm^{-1}: 3445 (OH), 1715 (C=O), 1655 and 875 (C=C). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 4.79 and 4.82 (2H, each br s, H_2-17), 4. 07 (1H, d, J=11.5 Hz, H-18), 3.99 (1H, d, J=11.5 Hz, H-18′), 3.61 (1H, t, J= 2.5 Hz, H-7), 3.53 (1H, dd, J= 7.5 and 9 Hz), 2.72 (1H, m, H-13), 2.09 (3H, s, OAc), 0.77 (3H, s, Me-19), 1.05 (3H, s, Me-20). ^{13}C NMR (50.32 MHz, $CDCl_3$): 38.2 (C-1), 26.1 (C-2), 72.1 (C-3), 41.3 (C-4), 37.8 (C-5), 26.7 (C-6), 76.5 (C-7), 47.6 (C-8), 49.9 (C-9), 38.4 (C-10), 17.5 (C-11), 33.2 (C-12), 43.1 (C-13), 38.0 (C-14), 44.7 (C-15), 154.9 (C-16), 103.0 (C-17), 65.8 (C-18), 11.6 (C-19), 17.7 (C-20), 172.1 (C-21), 20.4 (C-22). EIMS (rel. int.) m/z: 362.2 [M]$^+$(14) ($C_{22}H_{34}O_4$), 344.2 [M-H_2O]$^+$(40), 326.2 [M-2H_2O]$^+$ 302.2 [M-HOAc]$^+$(35), 284.2 (51), 246.1 (100).

***Ent*-3β,7α,17-trihydroxy-18-acetoxykaur-15-ene (1a).** IR$\nu_{\max}^{CHCl_3}$cm^{-1}: 3450 (OH), 3060, 1720 and 1270 (acetyl), 1055 (C-O), 1660 and 890 (C=C). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.81 (1H, s, H-15), 4.20 (2H, br s, H_2-17), 4.05 (1H, d, J=11.5 Hz, H-18), 3.99 (1H, d, J=11.5 Hz, H-18′), 3.67 (1H, t, J= 2.5 Hz, H-7), 3.52 (1H, dd, J= 7.5 and 9 Hz), 2.65 (1H, m, H-13), 2.09 (3H, s, OAc), 0.79 (3H, s, Me-19), 1.08 (3H, s, Me-20). HRMS m/z: 378.2411 [M]$^+$ ($C_{22}H_{34}O_5$). EIMS (rel. int) at m/z: 378.2 [M]$^+$(12), 360.2 [M-H_2O]$^+$(22), 342.2 [M-2H_2O]$^+$(60), 300.2 [M-H_2O -HOAc]$^+$(44).

***Ent*-7α,17,18-trihydroxy-3β-acetoxykaur-15-ene (1b).** IR$\nu_{max}^{CHCl_3}$cm^{-1} : 3450 (OH), 3000, 2900, 1710 (C=O), 1055 (C-O), 1660 and 875 (C=C). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.81 (1H, s, H-15), 4.90 (1H, dd, J=5.1 and 11.3 Hz, H-3), 4.20 (2H, br s, H_2-17), 3.67 (1H, t, J= 2.5 Hz, H-7,) 3.0 (1H, d, J=12 Hz, H-18), 3.33 (1H, d, J=12 Hz, H-18′) 2.60 (1H, m, H-13), 2.08 (3H, s, OAc), 0.68 (3H, s, Me-19), 1.09 (3H, s, Me-20). HRMS m/z: 378.2409 $[M]^+$ ($C_{22}H_{34}O_5$). EIMS (rel. int.) m/z: 378.2 $[M]^+$(4), 360.2 $[M\text{-}H_2O]^+$(6), 342.2 $[M\text{-}2H_2O]^+$ (6), 300.2 $[M\text{-}H_2O$ -HOAc$]^+$(24).

Synthesis of compounds 1a and 1b. 167 mg of m-chloroperbenzoic acid dissolved in $CHCl_3$(50 mL) was added to linearol **(1)** (196 mg). The mixture was refluxed for 4 h, and then it was washed with a satd. soln. of $NaHCO_3$ and purified by prep TLC to afford **(1a)** (47 mg) and **(1b)** (38 mg).

Results and Discussion

From *Sideritis sipylea* extract, seven known kaurene diterpenes were isolated. They were identified as linearol **(1)**[7], 7-epicandicandiol **(2)**,[8] sideridiol **(3)**,[9] siderol **(4)**,[10] isolinearol **(5)**[11,12], isosidol **(6)**[11,12] and epoxyisolinearol **(7)**[11,12] based on IR, ^{1}H- and ^{13}C-NMR and MS spectral data. Linearol was reacted with *m*-chloroprebenzoic acid in $CHCl_3$ to afford compounds **1a** and **1b**.

From *S. dichotoma* were isolated the compounds sideridiol **(3)**, siderol **(4)**, ent-7α-18-dihydroxy-15β,16β-epoxykaurane **(8)**[11,13,14], ent-7acetoxy-18-hydroxy-15β,16β-epoxy-kaurane **(9)**[9], *ent*-7α-acetoxy,15,18-dihydroxykaur-16-ene **(10)**[15,16], *ent*-7α,15,18-trihydroxykaur-16-ene **(11)**[15,16]and the beyerene *ent*-7α,18-dihydroxybeyer-15-ene **(12)**[17] All known structures were identified by comparison with literature data (IR, ^{1}H-, ^{13}C- NMR and mass spectra) and with the authentic samples when available.

Sideridiol **(3)** and siderol **(4)** were the only compounds isolated from both species, and sideridiol was found in very high yield, 0.1%, followed by linearol with a yield of 0.035% . Compounds **3** and **4** have also been isolated from two other Turkish *Sideritis* species, *S. argyrea*[5] and *S. Huber Morathi*[7].

Linearol **(1)** is one of the most common compounds found in the studied *Sideritis* species[4,5,7] in the world. However it did not show any remarkable activity, and therefore our aim was to prepare its analogues in order to obtain more active compounds. However, compounds **1a** and **1b** did not show any satisfactory activity against standard bacteria or some tumor cell lines.

The IR spectrum of the first new analogue compound **1a** showed the presence of hydroxyl groups with bands at 3450 cm^{-1} and the acetyl group with bands at 1725 and 1270 cm^{-1}. In the HRMS spectrum, compound **1a** gave a molecular ion peak at m/z 378.2411, accounting for a molecular composition of $C_{22}H_{34}O_5$. In the ^{1}H- NMR spectrum, the exocyclic methylene protons at δ 4.79 and 4.82 of linearol disappeared, and an olefinic methine signal was observed at δ 5.81 (1H,s) like sinfernol[18]. There were two hydroxymethylene groups present, one of which gave two doublets at δ 3.99 and 4.05 (J=11.5 Hz), attributed to the C-18 hydroxymethylene group. The signal of the second hydroxymethylene was observed at δ 4.20 (2H) as a broad singlet. Its location was assumed to be at C-16,[19,20] since there were only two methyl signals at δ 0.79 and 1.08 as singlets which were assigned as Me-19 and Me-20, respectively. Furthermore, an acetyl methyl singlet was observed at δ 2.09. The presence of hydroxymethine proton signals at δ 3.52 as a doublet (J=7.5 and 9 Hz) and at δ 3.67 as a triplet (J=2.5) were assigned to H-3 and H-7 protons, respectively.

All the spectral data indicated that the structure of **1a** is *ent*-3α,7β,17-trihydroxy,18-acetoxy kaur-15-ene.

The second new analogue **(1b)** was found to be similar to compound **(1a)**. The IR absorption bands were observed at 3450 (OH), 1710 (C=O) 1660 (C=C) cm^{-1}. In the HRMS spectrum, compound **1b** gave a molecular ion peak at m/z 378.2409 for the molecular composition $C_{22}H_{34}O_5$. The ^{1}H- NMR spectrum exhibited an olefinic proton signal at δ 5.81 (1H) and three methyl singlets at δ 0.68, 1.09 and 2.08. The signal at δ 4.90 (1H, dd, J=5.1 and 11.3 Hz) was assigned to the acetylated hydroxymethine proton of C-3. Another hydroxymethine proton (C-7) was observed at δ 3.67 (1H, t, J=2.5 Hz). An AB system at δ 3.00 and 3.33 (d, J=12 Hz) was attributed to a hydroxymethylene group which is located at C-18 and the second hydroxymethylene group was observed at δ 4.20 (2H) as a broad singlet for H_2-17.

Based on the spectral data, compound **(1b)** was established as *ent*-3α-acetoxy,7β,17,18-trihydroxy kaur-15-ene.

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