

Diterpenes from *Sideritis sipylea* and *S. dichotoma*

Gülaçtı TOPÇU*, Ahmet C. GÖREN

*University of İstanbul, Faculty of Pharmacy,
34452, Beyazıt, İstanbul-TURKEY

TÜBİTAK, Marmara Research Center, Department of Chemistry,
P.O. Box 21, 41470, Gebze, Kocaeli-TURKEY

Turgut KILIÇ, Y. Kemal YILDIZ

Balikesir University, Necatibey Education Faculty,
Department of Chemistry, 10100 Balikesir-TURKEY

Gülendam TÜMEN

Balikesir University, Art and Science Faculty, Department of Biology,
10100 Balikesir, TURKEY

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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*-3 β ,7 α ,17-trihydroxy-18-acetoxykaur-15-ene (**1a**) and *ent*-7 α ,17,18-trihydroxy-3 β -acetoxykaur-15-ene (**1b**) as new compounds. From the second species, *Sideritis dichotoma*, the kaurenes sideridiol (**3**), siderol (**4**), *ent*-7 α ,18-dihydroxy-15 β ,16 β -epoxykaurane (**8**), *ent*-7 α -acetoxy,18-hydroxy-15 β ,16 β -epoxykaurane (**9**), *ent*-7 α -acetoxy-15,18-dihydroxy-kaur-16-ene (**10**), *ent*-7 α ,15,18-trihydroxykaur-16-ene (**11**) and the beyerene *ent*-7 α ,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 endemic¹. *Sideritis* species have been used in folk medicine for their antiinflammatory, antirheumatic, digestive and antimicrobial activities in Turkey² as well as in Europe³. 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 activity⁶. The synthesis of analogues **1a** and **1b** of linearol (**1**) is reported for the first time.

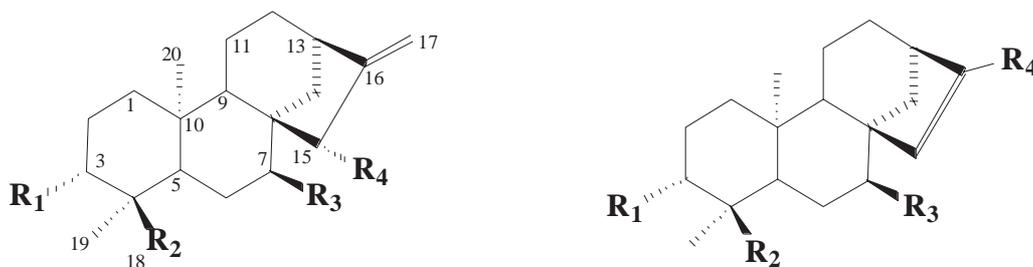
Experimental

General

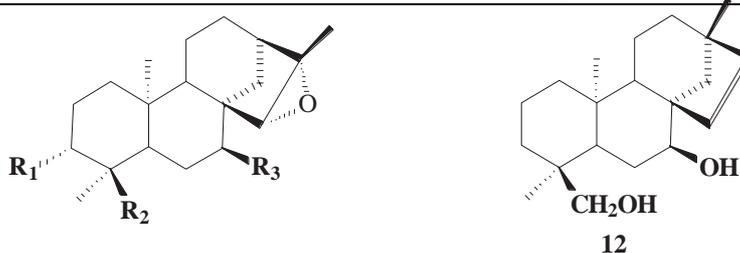
The spectra were recorded with the following instruments: IR: Perkin-Elmer 980 in CHCl₃; NMR: Bruker AC-200 L, 200 MHz and 50.32 MHz for ¹H- and ¹³C- NMR, respectively, in CDCl₃; MS: ZabSpec high resolution mass spectrometer; CC: Si-gel 60 was used for column chromatography and Kieselgel 60F₂₅₄ (E. Merck) 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*.



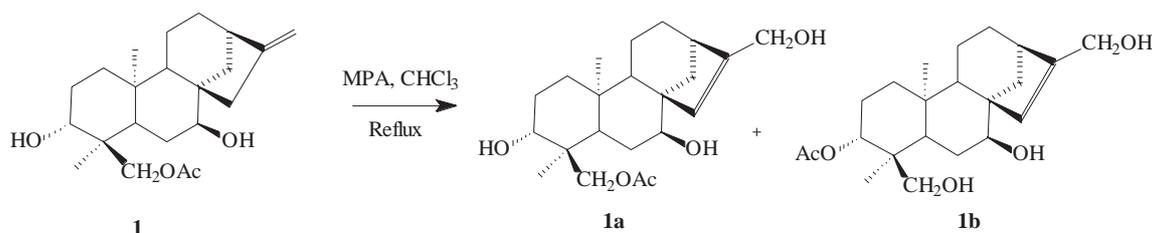
	R ₁	R ₂	R ₃	R ₄		R ₁	R ₂	R ₃	R ₄
1	OH	CH ₂ OAc	OH	H	3	H	CH ₂ OH	OH	CH ₃
2	H	CH ₂ OH	OH	H	4	H	CH ₂ OH	OAc	CH ₃
10	H	CH ₂ OH	OAc	OH	5	OH	CH ₂ OAc	OH	CH ₃
11	H	CH ₂ OH	OH	OH	6	OAc	CH ₂ OH	OH	CH ₃
					1a	OH	CH ₂ OAc	OH	CH ₂ OH
					1b	OAc	CH ₂ OH	OH	CH ₂ OH



	R ₁	R ₂	R ₃
7	OH	CH ₂ OAc	OH
8	H	CH ₂ OH	OH
9	H	CH ₂ OH	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 (CHCl₃: 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 (CH₂Cl₂: acetone, 1:1), isosidol **(6)** (23 mg) from (CH₂Cl₂:acetone, 4:6) and epoxyisaineorol **(7)** (15 mg) from (CH₂Cl₂:acetone, 4:6).



Scheme 1. Reaction of linearol with MPA in CHCl₃

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*-7 α ,18-dihydroxy-15 β ,16 β -epoxykaurane **(8)** (siderexol) (250 mg), *ent*-7 α -acetoxy,18-hydroxy-15 β ,16 β -epoxykaurane **(9)** (epoxysiderol) (105 mg), sideridiol **(3)** (23 mg), siderol **(4)** (47 mg), *ent*-7 α -acetoxy,15 β ,18-dihydroxykaur-16-ene **(10)** (35 mg), *ent*-7 α ,15 β ,18-dihydroxykaur-16-ene **(11)** (28 mg) and 7 α ,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: CH₂Cl₂) (8:2). Purification of compounds **(3)**, **(4)**, and **(9)** was carried out on prep. TLC using the solvent system (CH₂Cl₂:Hexane, 7:3) while compounds **(10)** and **(11)** were purified from (Hexane:CH₂Cl₂, 6:4) and compound **12** was purified from (CH₂Cl₂:Hexane, 8:2).

Linearol (1). IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3445 (OH), 1715 (C=O), 1655 and 875 (C=C). ¹H-NMR (200 MHz, CDCl₃) δ : 4.79 and 4.82 (2H, each br s, H₂-17), 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). ¹³C NMR (50.32 MHz, CDCl₃): 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) (C₂₂H₃₄O₄), 344.2 [M-H₂O]⁺(40), 326.2 [M-2H₂O]⁺ 302.2 [M-HOAc]⁺(35), 284.2 (51), 246.1 (100).

Ent-3 β ,7 α ,17-trihydroxy-18-acetoxykaur-15-ene (1a). IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3450 (OH), 3060, 1720 and 1270 (acetyl), 1055 (C-O), 1660 and 890 (C=C). ¹H-NMR (200 MHz, CDCl₃) δ : 5.81 (1H, s, H-15), 4.20 (2H, br s, H₂-17), 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]⁺ (C₂₂H₃₄O₅). EIMS (rel. int) at *m/z*: 378.2 [M]⁺(12), 360.2 [M-H₂O]⁺(22), 342.2 [M-2H₂O]⁺(60), 300.2 [M-H₂O -HOAc]⁺(44).

Ent-7 α ,17,18-trihydroxy-3 β -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). ¹H-NMR (200 MHz, CDCl₃) 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, H₂-17), 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]⁺ (C₂₂H₃₄O₅). EIMS (rel. int.) m/z : 378.2 [M]⁺(4), 360.2 [M-H₂O]⁺(6), 342.2 [M-2H₂O]⁺ (6), 300.2 [M-H₂O -HOAc]⁺(24).

Synthesis of compounds 1a and 1b. 167 mg of m-chloroperbenzoic acid dissolved in CHCl₃(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 NaHCO₃ 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, ¹H- and ¹³C-NMR and MS spectral data. Linearol was reacted with m-chloroprebenzoic acid in CHCl₃ to afford compounds **1a** and **1b**.

From *S. dichotoma* were isolated the compounds sideridiol (**3**), siderol (**4**), ent-7 α -18-dihydroxy-15 β ,16 β -epoxykaurane (**8**)^{11,13,14}, ent-7acetoxy-18-hydroxy-15 β ,16 β -epoxy-kaurane (**9**)⁹, ent-7 α -acetoxy,15,18-dihydroxykaur-16-ene (**10**)^{15,16}, ent-7 α ,15,18-trihydroxykaur-16-ene (**11**)^{15,16} and the beyerene ent-7 α ,18-dihydroxybeyer-15-ene (**12**)¹⁷ All known structures were identified by comparison with literature data (IR, ¹H-, ¹³C- 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 C₂₂H₃₄O₅. In the ¹H- 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 sinferol¹⁸. 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-3 α ,7 β ,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 $\text{C}_{22}\text{H}_{34}\text{O}_5$. The ^1H -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*-3 α -acetoxy,7 β ,17,18-trihydroxy kaur-15-ene.

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References

1. P.H. Davis, **Flora of Turkey and The East Aegan Islands**, Univ. Press, Edinburgh, (1988).
2. N. Kırmıer, M. Kürkçüoğlu, T. Özek, K.H.C. Başer, G. Tümen, **Flavour and Fragrance Journal**, **11**, 315-1317 (1996).
3. F.P. Quer, **Planta Medicinales**, Ed. Labor, Barcelona, (1962)
4. G. Topcu, A.C. Gören, Y.K. Yıldız, G. Tümen, **Natural Product Letters**, **14**, 123-129 (1999).
5. G. Topcu, A.C. Gören, T. Kılıç, Y.K. Yıldız and G. Tümen, **Fitoterapia**, **72**, 1-4 (2001).
6. A. Başaran, G. Timuralp, S. Şimşek, K.H.C. Başer, **FABAD, J. Pharm. Sci.**, **22**,101-103 (1996).
7. K.H.C. Başer, M.L. Bondi, N. Kırmıer, F. Piozzi, G. Tümen, N. Vasollo, **Phytochemistry**, **43**, 1293-1296 (1996).
8. I. Aljancic, S. Macura, S. Juranic, N. Andjelkovic, N. Randjelovic, S. Milosavljevic, **Phytochemistry**, **43**, 169-172 (1996).
9. E. Cabbera, A. Garcia-Granados, A.S. De Buruaga, J.M.S. De Buruaga, **Phytochemistry**, **22**, 2779-2781 (1983).
10. J. Algarra, A. Garcia-Granados, A.S. De Buruaga, J.M.S. De Buruaga, **Phytochemistry**, **22**, 1779-1782 (1983).
11. P. Venturella, A. Bellino, F. Piozzi, **Phytochemistry**, **14**, 1451-1452 (1975).
12. T.G. De Queseda, B. Rodriguez, S. Valverde, S. Huneck, **Tetrahedron Letters**, 2187 (1972)
13. C.A. Henrick, P.R. Jefferies, **Aust. J. Chem**, **17**, 915-933 (1964).
14. D.E.U. Ekong, A.U. Ogan, **J. Chem. Society, Perkins** **1**, 311-314 (1967).
15. P. Venturella, A. Bellino, M.L. Marino, **Phytochemistry**, **22**, 2537-2538 (1983).
16. A.G. Gonzalez, B.M. Fraga, G. Hernandez, J.G. Luis, **Phytochemistry**, **12**, 1113-1116 (1973).

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17. A. Garcia-Granados, A. Martinez, M.E. Onorato and O. Socorro, **Phytochemistry**, **23**, 607-610 (1984).
18. C. Fernandez, B.M. Fraga, M.G. Hernandez, **Phytochemistry**, **25**, 2573-2576 (1986).
19. B.M.R. Bandara, W.R. Wimalasiri, J.K. Macleod, **Phytochemistry**, **27**, 869-871 (1988).
20. S. Yahara, M.Ishida, K.Yamasaki, O.Tanaka, S. Mihashi, **Chem. Pharm. Bull.**, **22**, 1629-1631 (1974).