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Diterpenoids from *Sideritis tmolea* P. H. Davis

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Abstract: Four known *ent*-kaurene diterpenoids **1-4** were isolated from the acetone extract of *Sideritis tmolea* P. H. Davis by using different chromatographic methods. The structures of the isolated compounds were determined as siderol (*ent*-7α-acetoxy-18-hydroxy-kaur-15-ene) **(1)**, 7-acetoxysideroxol (*ent*-7α-acetoxy-18-hydroxy-15β,16β-epoxykaurane) **(2)**, *ent*-7α,15β,18-trihydroxykaur-16-ene **(3)** and *ent*-7α-acetoxy-15β,18-dihydroxykaur-16-ene **(4)** by using IR, NMR (^{1}H,^{13}C, APT and DEPT) techniques and mass spectroscopy. The antimicrobial and antituberculous activities of the acetone and methanol extracts and compound **1** have been reported.

Key words: Lamiaceae, *Sideritis tmolea*, Kaurene, Diterpenoids, Antimicrobial activity

1. Introduction

Sideritis (Labiatae=Lamiaceae) species, collected under two sections, 46 species, 12 subspecies and two varieties in Turkey, are found widely in Southern and Central Anatolia, especially in Western Anatolia [1,2]. 36 species, 10 subspecies and two varieties of them are endemic. Moreover *Sideritis* is one of the genera with high endemism ratio (77%) among all the plants growing in Turkey.

Sideritis (Labiatae Fam.) species are distributed mainly in the Mediterranean area from Caucasus to the Canary Islands with 120 species and have been used in folk medicine for their anti-inflammatory, antirheumatic, digestive and antimicrobial activites in Turkey and Europe [3]. *Sideritis* species have been widely consumed as a herbal tea in Anatolia due to above mentioned effects, especially in Aegean and Mediterranean regions [4]. Although *Sideritis* species are known with names like mountain tea, plateau tea, etc., the tea served under the name of sage in rural coffee houses are mostly prepared from *Sideritis* species rather than *Salvia*.

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In our country, some morphological, anatomic, and palinological studies were made on *Sideritis* species [4] besides some biological activity studies [3, 5]. Even though the researches on *Sideritis* species' volatile oils for most of the species grown in Turkey are completed [6,7], the number of researches on non-volatile secondary metabolites, diterpenoids [8,9], flavonoids and other phenolic compounds [10,11] have increased in the last years. In the last 10 years, our group has been continuing systematical researches on the various species of this plant (*S. argyrea, S. athoa, S. caesarea, S. dichotoma, S. leptoclada, S. lycia, S. sipylea, S. trojana, S.stricta)* for their diterpenoids [12-18]. The aim of this study is to isolate and enlighten the structures of diterpenoids from *Sideritis tmolea,* growing in Turkey. Also, the biological activities of crude extracts and pure substances obtained have been examined.

2. Materials and Methods

2.1. Plant Materials

Aerial parts of *Sideritis tmolea* P.H. Davis was collected in July 2005 from Ödemiş - Bozdağ (İzmir Province, Turkey). The species was identified by Dr. Tuncay Dirmenci, in Balıkesir University. A voucher specimen was deposited at the Herbarium, Faculty of Arts and Science, Department of Biology, Balıkesir University, Balıkesir, Turkey (TD 2811).

2.2. General

^{1}H and ^{13}C spectra were obtained in $CDCl_3$ by using Varian 500 MHz NMR. Mass spectra were recorded on a Thermo LCQ Deca LC/MS. Silica gel 60 was used for column chromatography and Kieselgel $60F_{254}$ precoated plates (E. Merck) for prep. TLC. All the solvents were purchased from Merck.

2.3. Extraction and Isolation

The plant material which dried in shade was cutted in small pieces. The whole plant (1.5 kg) was extracted with acetone to give a crude extract (35 g). This extract was fractionated on a silica gel column. Elution was started with hexane and continued with gradients of chloroform, acetone and then methanol. From the acetone extract siderol (*ent*-7α-acetoxy,18-hydroxy-kaur-15-ene) **(1)** and 7-acetoxysideroxol (*ent*-7α-acetoxy-18-hydroxy-15β,16β-epoxykaurane) **(2)** were isolated from CH_2Cl_2: Acetone (9:1) system, while *ent*-7α,15β -18-trihydroxykaur-16-ene **(3)** and *ent*-7α-acetoxy-15β,18-dihydroxykaur-16-ene **(4)** were isolated from CH_2Cl_2: Acetone (8:2) system. For purification of the isolated compounds preparative TLC was applied using by pre-coated silicagel F_{254} aluminium plates (0,2 mm, Merck) and they developed by the solvent system CH_2Cl_2: Acetone (9:1) or (8:2). All compounds characterized by using spectral methods.

2.4 Biological Assessment

The acetone and methanol extracts and compound (**1**) were tested against standard bacterial strains such as *Escherichia coli* ATCC 29995*, Staphylococcus aureus ATCC 6538P, Mycobacterium smegmatis, Mycobacterium tuberculosis* H37Ra (ATCC 25177) and the yeast *Candida albicans* ATCC 10239 for the determination of their antimicrobial activity. The disc-diffusion method was used to determine the inhibition zones of the tested compound against the standard bacterial strains [19-20].

Acetone and methanol extracts and compound **1** were first dissolved in a very little amount of chloroform and diluted to 1:10 (alcohol:water). For more dilutions sterile pure water was used. When the microorganisms in culture have reached nearly 10^6 c.f.u/mL concentration, these microorganisms

were planted in Salubris brand cultures, sold in the market in petri's dish. After making them waited in 37 ^{0}C for one night, the radius of the areas where microorganisms have died was measured [21]. Ethanol and ampicillin were used for control.

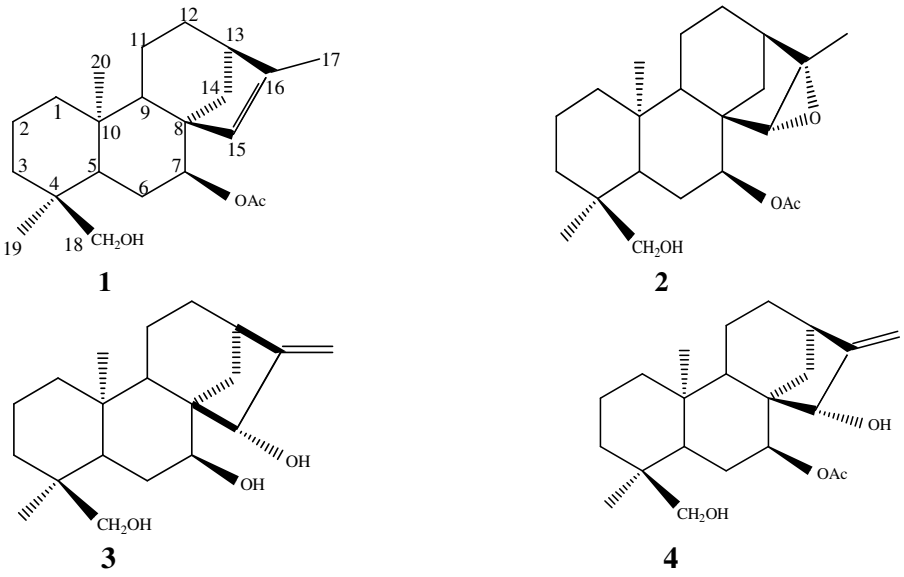

Figure 1. Isolated *ent*-kaurane diterpenoids from *Sideritis tmolea* P.H.Davis

3. Results and Discussion

Generally, *Sideritis* species are rich in kaurene diterpenoids, however they differ in the substituents attached to this skeleton. [22-26]. In this study, diterpenoids of *Sideritis tmolea* plant, an endemic species only grown in Turkey, were studied, and four known diterpenoids were isolated having *ent*-kaurene skeleton and alike each other.

The plant was collected during flowering period, which was dried on shadow and choped into small pieces and then extracted at room temperature for two weeks. Crude extracts were concentrated until dryness under the vacuum.

The crude extracts were separated into fractions by open column chromatography, packed with silica gel. The collected fractions were applied onto silica gel coated glass plates and were developed on using proper solvent systems. The plates were examined by UV fluorescence and sprayed cerium (IV) sulphate reagent prepared in H_2SO_4, followed by heating at 105° C for 1-2 min. and similar fractions were combined. The combined fractions were subsequently purified by applying either the colon chromatography and/or preparative thin layer chromatography. The compounds were compared in thin layer chromatography with those of standard compounds which were obtained previously from other *Sideritis* species by our group.

For determination of the structures of pure compounds, spectral methods were used consisting of IR, 1D- and 2D-NMR (^{1}H, ^{13}C, COSY, HMQC, APT or, DEPT techniques) and mass spectra.

The structures of the isolated diterpenoids were identified as siderol (ent-7α-acetoxy,18-hydroxy-kaur-15-ene), 7-acetoxysideroxol (*ent*-7α-acetoxy-18-hydroxy-15β,16β-epoxykaurane), *ent*-7α,15β-18-trihydroxykaur-16-ene, *ent*-7α-acetoxy-15β,18-dihydroxykaur-16-ene (Figure 1).

3.1 Spectral Data

Siderol (Ent-7α-acetoxy,18-hydroxy-kaur-15-ene) **(1)**: IR bands (cm^{-1}): 3450 (OH), 2960 (C-H), 1730 (C=O), 1250 (C-O), 1640 and 780 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$): δ: 5.19 (1H, s, H-15), 4.63 (1H, t, *J* = 2.5 Hz, H-7), 3.26 (1H, d, *J* = 11.5 Hz, H-18a), 2.94 (1H, d, *J* = 11.5 Hz, H-18b), 2.30 (1H, m, H-13), 2.05 (3H, s, OAc), 1.65 (3H, s, Me-17), 1.11 (3H, s, Me-20), 0.64 (3H, s, Me-19). ^{13}C NMR (125 MHz, $CDCl_3$): δ: 42.1 (C-1), 18.4 (C-2), 35.3 (C-3), 37.0 (C-4), 44.6 (C-5), 23.6 (C-6), 78.3 (C-7), 51.8 (C-8) 44.9 (C-9), 39.2 (C-10), 17.9 (C-11), 24.6 (C-12), 39.8 (C-13), 39.8 (C-14), 129.8 (C-15), 144.01 (C-16), 17.9 (C-17), 71.7 (C-18), 17.5 (C-19), 17.5 (C-20), 21.6 (OCO<u>CH$_3$</u>), 170.8 (O<u>CO</u>CH$_3$). EIMS (rel.int.): *m/z* **:** 346.0 $[M]^+$ (33) ($C_{22}H_{34}O_3$), 315.0 $[M-31]^+$(4), 303.9 [M- Ac]$^+$ (71), 287.0 [M-OAc]$^+$ (76).

7-Acetoxysideroxol (Ent-7α-acetoxy–18-hydroxy-15β,16β-epoxykaurane) **(2)**: ^{1}H NMR (500 MHz, $CDCl_3$): δ: 4.71 (1H, t, *J* = 3 Hz, H-7α), 2.93 (1H, d, *J* = 12 Hz H-18a), 3.26 (1H, d, *J* = 12 Hz, H-18b), 2.91 (1H, s, H-15), 1.99 (3H, s, OAc), 1.36 (3H, s, Me-17), 0.97 (3H, s, Me-20), 0.63 (3H, s, Me-19). ^{13}C NMR (125 MHz, $CDCl_3$): δ: 40.1 (C-1), 18.2 (C-2), 35.8 (C-3), 37.1 (C-4), 35.1 (C-5), 26.3 (C-6), 74.8 (C-7), 46.8 (C-8) 46.4 (C-9), 39.2 (C-10), 18.1 (C-11), 27.6 (C-12), 39.3 (C-13), 39.9 (C-14), 63.9 (C-15), 61.8 (C-16), 17.5 (C-17), 71.8 (C-18), 21.4(C-19), 17.6 (C-20), 20.9 (OCO<u>CH$_3$</u>), 170.3 (O<u>CO</u>CH$_3$).

Ent-7α,15β,18-trihydroxykaur-16-ene **(3)**: ^{1}H NMR (500 MHz, $CDCl_3$): δ: 5.23 (1H, s, H-17a), 5.08 (1H, s, H-17b), 4.13 (1H, s, H-15), 3.91 (1H, t, *J* = 2.5 Hz, H-7), 3.52 (1H,d , *J* = 12 Hz, H-18a), 2.97 (1H, d, *J* = 12 Hz, H-18b), 1.03 (3H, s, Me-19), 0.71 (3H, s, Me-20).

Ent-7α-acetoxy-15β,18-dihydroxykaur-16-ene **(4)**: ^{1}H NMR (500 MHz, $CDCl_3$): δ: 5.28 (1H, s, H-17a), 5.12 (1H, s, H-17b), 5.01 (1H, *J* = 2 Hz, t, H-7), 4.22 (1H, s, H-15), 3.25 (1H, d, *J* = 12 Hz, H-18a), 2.97 (1H, d, *J* = 12 Hz, H-18b), 2.78 (1H, m, H-13), 1.02 (3H, s, Me- 20), 0.66 (3H, s, Me-19).

3.2. Biological activity

Acetone and methanol extracts of *Sideritis tmolea* plant and siderol were tested against microorganisms. The obtained results are given in Table 1.

Table 1. Antimicrobial activity tests results (mm)

Substance tested	*E. coli*	*S. aureus*	*M. smegmatis*	*C. albicans*
Acetone extract	13	21	9	16
Methanol extract	16	24	11	17
Siderol	8	6	9	3
Ethanol	0	0	0	0
Ampicilline (10 µg)	11	30	20	0

As the result of activity studies, it is found that S*ideritis* species' crude acetone and methanol extracts have not shown activity against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) microorganism.

In conclusion, we have reported the isolation of four known diterpenoids from *S. tmolea*. The antimicrobial activity of the crude acetone and methanol extracts and the isolated pure compound **1** and antituberculous activity of the crude acetone and methanol extracts of the studied plant are reported. Neither the extract nor any of the isolated compounds showed good antimicrobial or antituberculous activity.

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