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Short Communication

Isolation and Biological Activity of New and Known Diterpenoids From *Sideritis stricta* Boiss. & Heldr.

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Abstract: Nine known and one new *ent*-kaurene diterpenoid were isolated from the acetone extract of *Sideritis stricta* Boiss & Heldr. The new compound, identified as *ent*-1β-hydroxy-7α-acetyl-15β,16β-epoxykaurane (**1**) by IR, 1D and 2D NMR techniques and mass spectra, was isolated along with sideroxol (**2**), 7-acetyl sideroxol (**3**), 7-epicandicandiol (**4**), linearol (**5**), *ent*-7α,15β,18-trihydroxy-kaur-16-ene **(6)**, *ent*-7α-acetyl,15,18-dihydroxy-kaur-16-ene (**7**), foliol (**8**), sideridiol (**9**) and siderol (**10**). The antibacterial and antifungal activities of these compounds and the whole crude acetone extract were evaluated against *E. coli, S. aureus, K. pneumeonia* and *C. albicans*.

Keywords: Kaurene, Diterpenoids, *Sideritis stricta*, Lamiaceae, Antibacterial Activity

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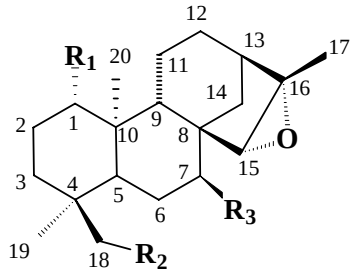
Introduction

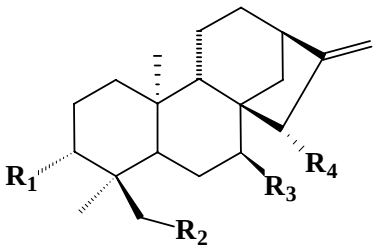
There are 46 *Sideritis* flora species in Turkey, of which 36 species and 10 subspecies are endemic [1]. *Sideritis* species have been used in folk medicine in Turkey and Europe for their antinflammatory, antirheumatic, digestive and antimicrobial properties [2,3]. *Sideritis* species contain mainly kaurene diterpenoids, but they rarely have labdane, pimarane or atisene diterpenoids. In this study, one new and nine known *ent*-kaurene diterpenoids were isolated from *Sideritis stricta* and the antibacterial and antifungal activities of these compounds against *E. coli, S. aureus, K. pneumeonia* and *C. albicans* was evaluated.

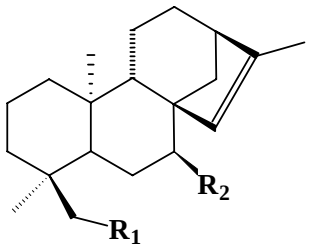
Results and Discussion

A new *ent*-kaurane, identified as *ent*-1β-hydroxy-7α-acetyl-15β,16β-epoxykaurane (**1**) was isolated, along with nine known *ent*-kaurenes, from the whole plant extract of *S. stricta.* The known kaurene diterpenes were identified as sideroxol (**2**) [4,5]**,** 7-acetyl sideroxol (**3**) [4] 7-epicandicandiol **(4**), [6-9] linearol (**5**), [6-10] *ent*-7α,15β,18-trihydroxy-kaur-16-ene (**6**) [10-11], *ent*-7α-acetyl,15,18-dihydroxy-kaur-16-ene (**7**) [10-11], foliol (**8**) [12], sideridiol (**9**) [13-14] and siderol (**10**) [15], respectively (Figure 1). All the compounds were identified based on IR, ^{1}H- and ^{13}C-NMR and mass spectroscopic techniques. The structures of known compounds were confirmed by comparison to literature data.

Figure 1.







	R_1	R_2	R_3
1	OH	H	OAc
2	H	OH	OH
3	H	OH	OAc

	R_1	R_2	R_3	R_4
4	H	OH	OH	H
5	OH	OAc	OH	H
6	H	OH	OH	OH
7	H	OH	OAc	OH
8	OH	OH	OH	H

	R_1	R_2
9	OH	OH
10	OH	OAc

The IR spectrum of compound **1** showed the presence of an acetyl group, with absorption bands at 1720 and 1280 cm^{-1}. An epoxy group at 1085 cm^{-1} and a hydroxyl group at 3400 cm^{-1} were also observed. In the HRMS spectrum, compound **1** gave a molecular ion peak at *m/z* 362.2560, corresponding to a molecular composition of $C_{22}H_{34}O_4$. In the ^{1}H-NMR spectrum four signals (s, 3H) for four methyl groups were observed at δ 0.78, 0.82, 1.08, and 1.44 ppm. In addition, there was an acetyl methyl signal at δ 2.08, which was corroborated with a signal at δ 4.86 appearing as a narrow triplet (*J* = 2.5 Hz) and attributed to the C-7 α proton. The presence of a hydroxyl group at C-1 was observed as a dd (*J*=10 and 5 Hz) and the corresponding C-1 carbon signal was observed at 80.3 ppm. These chemical shifts and the doublet of doublets are characteristic signals for the α position of C-1 [16]. The presence of a singlet at δ 2.98 was indicative of a characteristic H-15β-epoxy proton, as observed in similar kaurane diterpenes [17]. The APT ^{13}C-NMR spectrum revealed 22 carbon signals, consisting of five methyls, six methylenes, six methines and five quaternary carbon atoms. A methine carbon at δ 74.1 was assigned to C-7, while the one at δ 80.3 was assigned to C-1. Another methine carbon at δ 62.4 was attributed to the epoxy methine carbon (C-15), while the quaternary carbon of this epoxy group was observed at δ 77.9. The assignments of protonated carbon signals were carried out by a HMQC experiment. Thus, the structure of this diterpenoid **1** was elucidated as *ent*-1β-hydroxy-7α-acetyl-15β,16β-epoxykaurane, which has now been isolated for the first time from Nature.

Biological activity

The acetone extract of *S. stricta* and the pure compounds **1**-**10** were tested against standard bacterial and fungal strains (Table 1). The MIC values indicated that they showed very little activity against the bacterial and fungal species tested, compared to gentamycin and flucanozole.

Table 1. Antibacterial and antifungal activity of acetone extract of *S. stricta* and kaurene diterpenoids.

Tested material	*E. coli*	*S. aureus*	*K. pneumonia*	*C. albicans*
S. stricta extract	300	600	300	NA
1	NA	600	NA	NA
2	NA	NA	NA	NA
3	NA	600	600	NA
4	200	200	NA	NA
5	600	600	600	NA
8	600	600	NT	NA
9	300	600	600	NA
10	NA	NT	300	NA
*Gentamycin**	0.97	0.48	0.48	NT
*Flucanozole**	NT	NT	NT	15.6

[a] MIC values are given as mg/L, NA: Non-Active; NT: Not tested

* Gentamycin and Flucanozole were used as positive controls and results were given as μg/mL.

Conclusions

We have reported the isolation from *S. stricta* of several known diterpenoids and a new *ent*-kaurane diterpenoid, identified as e*nt*-1β-hydroxy-7α-acetyl-15β,16β-epoxykaurane (**1**). The antimicrobial activity of the crude acetone extract of the studied plant and the pure compounds is reported. Neither the extract nor any of the individual kaurane diterpenoids showed good activity against *E. coli, S. aureus, K. pneumonia* and *C. albicans.*

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Experimental

General

^{1}H- and ^{13}C-NMR spectra were obtained in $CDCl_3$ at 500 and 125 MHz, respectively, using a Bruker Avance 500 NMR. IR and mass spectra were recorded with a IR: Perkin-Elmer 980 (in $CHCl_3$) and a VG ZabSpec High Resolution Mass Spectrometer. Silicagel 60 was used for column

chromatography and Kieselgel 60F_{254} precoated plates (E. Merck) for prep. TLC. All the solvents were purchased from Merck.

Plant material

Sideritis stricta Boiss. & Heldr. was collected in July 2004 from Termesos National Park (Antalya Province, Turkey). The plant was identified by Prof Dr. G. Tümen (Balıkesir University), and a voucher specimen (TD 1485) was deposited at the Herbarium of the Faculty of Pharmacy, Anadolu University.

Extraction and isolation

The powdered whole plant (1.5 kg) was extracted with acetone to give a crude extract (54 g). A portion of this extract (25 g) was fractionated on a silica gel column. Elution was started with hexane and continued with gradients of chloroform, acetone and then methanol to give *ent*-1β-hydroxy-7α-acetyl-15β,16β-epoxykaurane (23 mg, **1**), sideroxol (54 mg, **2**), 7-acetylsideroxol (102 mg, **3**), 7-epi-candicandiol (178 mg, **4**), linearol (210 mg, **5**), *ent*-7α, 15β, 18-trihydroxy-kaur-16-ene (32 mg, **6**), *ent*-7α-acetyl-15,18-dihydroxy-kaur-16-ene (17 mg, **7**), foliol (48 mg, **8**), sideridiol (205 mg, **9**) and siderol (183 mg, **10**). Purification of the new compound **1** was carried out by preparative TLC, using $CHCl_3$-acetone (9:1) as eluent. IR $\nu_{max}^{CHCl_3}$ *ent*-1β-hydroxy-7α-acetyl-15β,16β-epoxykaurane **(1)** IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3400, 1725 and 1270 (C=O), 1050 (C-O); ^{1}H-NMR δ: 4.86 (1H, t, *J*=2.5, H-7), 3.32 (1H, dd, *J*=10 and 5 Hz, H-1), 2.97 (1H, s, H-15), 2.09 (3H,s, OAc), 2.98 (1H, s, H-15), 2.08 (3H, s, OAc),1.44 (3H, s, Me-17), 1.08 (3H, s, Me-20), 0.78 (3H,s, Me-18) 0.82 (3H,s, Me-19); ^{13}C-NMR δ: 80.3 (C-1), 29.2 (C-2), 34.8 (C-3), 37.6 (C-4), 34.8 (C-5), 26.3 (C-6), 74.1 (C-7), 48.3 (C-8), 45.9 (C-9), 38.9 (C-10), 17.3 (C-11), 27.4 (C-12), 39.3 (C-13), 31.2 (C-14), 62.4 (C-15), 77.9 (C-16), 17.9 (C-17), 18.6 (C-18), 17.5 (C-19), 15.3 (C-20), 21.1 (OCO<u>C</u>H$_3$), 178.6 (O<u>C</u>O-CH$_3$); EIMS (rel.int.) *m/z*: 362.2 $[M]^+$ (10), 344 $[M\text{-}OH]^+$ (28) 302.2 $[M\text{-}COOCH_3]^+$(23), 288.2 (45), 254.1 (85), 225.1 (30), 201.1 (50), 131.0 (60), 120.0 (50), 108.9 (82), 95.1 (65), 80.0 (23), 69.0 (97); HRMS: 362.2560 (calcd for $C_{22}H_{34}O_4$ 362.2457).

Antibacterial and antifungal activity

The acetone extract of *S. stricta* and the individual compounds **1**, **2**, **3**, **4**, **5**, **8**, **9**, and **10** were tested against standard bacterial strains such as *E. coli* ATCC 29995, *S. aureus* ATCC 6538P, *K. pneumonia* CCM 2318, and the yeast *C. albicans* ATCC 10239. The agar diffusion method was used to determine the inhibition zones of the tested compounds and acetone extract of *S. stricta* against these standard bacterial strains. The acetone extract of the species and the pure compounds with inhibition zones larger than 7 mm were selected for determination of quantitative antimicrobial activity expressed as minimum inhibition concentrations (MIC) [18]. The broth microdilution method was applied for this purpose [18-20].

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Sample Availability: Available from the author.