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NEW DITERPENES FROM *SIDERITIS SICULA*

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Key Word Index—*Sideritis sicula*; Labiatae; tetracyclic diterpenes; new *ent*-kaur-15-ene derivatives.

Abstract—Two new diterpenes have been isolated from *Sideritis sicula*: sideripol, *ent*-18-acetoxy-7α-hydroxykaur-15-ene and epoxysideritriol, *ent*-15β,16β-epoxykauran-7α,17,18-triol. The previously known diterpene eubol, *ent*-7α-acetoxykaur-16-en-15β,18 diol has also been obtained from the same source.

Previous investigations of the genus *Sideritis* have shown that the plants in this genus are rich in diterpenoid constituents. The first species to be examined was *S. sicula* which grows in the mountains of Southern Italy and Sicily [1, 2]. From the corollas of this plant five tetracyclic isokaurene diterpenes have been isolated: sideridiol (*ent*-7α,18-dihydroxykaur-15-ene) [2], siderol (*ent*-7α-acetoxykaur-15-en-18-ol) [2], sideroxol (*ent*-15β,16β-epoxykaurane-7α,18-diol) [3], sideritriol (*ent*-kaur-15-ene-7α,17,18-triol) [4] and epoxysiderol (*ent*-15β,16β-epoxykauran-18-ol) [5]. More recently, eubol (*ent*-7α-acetoxykaur-16-ene-15β,18-diol), was identified in the Greek species *S. euboea* [6].

The present work reports the isolation of three further diterpenes from the inflorescence of the same plant: eubol and two new compounds, sideripol (**1**) and epoxysideritriol (**2**).

The first new diterpene, sideripol (**1**) has the molecular formula $C_{22}H_{34}O_3$ (MS, M^+346). The IR spectrum shows the occurrence of a hydroxyl group (3448 cm^{-1}), an ester group (1709 and 1266 cm^{-1}) and a trisubstituted double bond (3030 and 827 cm^{-1}). Its NMR spectrum shows signals for two tertiary methyl groups, one methyl on a double bond, one acetyl, one C<u>H</u>OH, one —C<u>H</u>$_2$OAc and one vinyl proton. Comparison of the NMR spectra of sideridiol (**3**) and siderol (**4**) with that of the new product indicates that the primary hydroxy group is acetylated (Table 1).

On alkaline hydrolysis, sideripol (**1**) affords (**3**); by acetylation it gives the known diacetylsideridiol [2] (IR spectra superimposable).

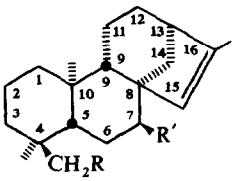
Therefore sideripol is *ent*-18-acetoxy-7-hydroxykaur-15-ene (**1**). This was confirmed by partial acetylation of sideridiol [2] (**3**).

The second diterpene epoxysideritriol (**2**) has the molecular formula $C_{20}H_{32}O_4$ as determined by MS; the IR spectrum lacked carbonyl and double bond absorption, showing the presence of hydroxyl function (3390–3279 cm^{-1}). The NMR spectrum shows signals for two tertiary methyl groups, a CHOH and a CH_2OH equatorial on C-4. Moreover a singlet at 3.81 δ and a quartet AB at 4.5 and 4.0 δ respectively, is characteristic for one proton and a C<u>H</u>$_2$OH on an epoxide ring.

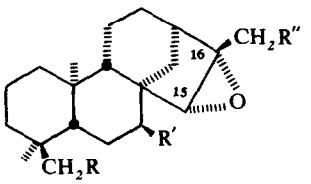
Table 1. NMR spectral signals of *Sideritis* diterpenes

	C<u>H</u>$_2$OR	15-H	C<u>H</u>OR	4-Me
Sideridiol (3)	2.90 and 3.49, *q*	5.24, br	3.60, *t*	0.68, *s*
Siderol (4)	3.03 and 3.35, *q*	5.28, *br*	4.71, *t*	0.71, *s*
Sideripol (1)	3.43 and 4.03, *q*	5.49, *br*	3.68, *br*	0.82, *s*

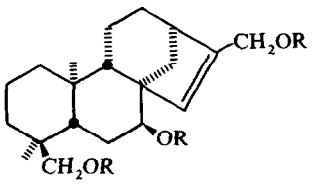
Acetylation of (**2**) with Ac_2O–Py gave triacetylepoxysideritriol (**5**), whose IR spectrum does not show OH absorption. The above data suggest for (**2**) a structure comparable to sideritriol (**6**) in which the 15,16 double bond is epoxidized; hence the new diterpenoid has structure (**2**), *ent*-15β,16β-epoxykauran-7α,17,18-triol.



(**1**) R = OAc, R′ = OH
(**3**) R = OH, R′ = OH
(**4**) R = OH, R′ = OAc



(**2**) R = OH; R′ = OH, R″ = OH
(**5**) R = OAc, R′ = OAc, R″ = OAc



(**6**) R = H
(**7**) R = Ac

The structures of (**2**) and (**5**) were fully confirmed by partial synthesis. Sideritriol (**6**) [4] and triacetylsideritriol (**7**) when oxidized with *p*-nitroperbenzoic acid yielded, respectively, (**2**) and (**5**), identical with the natural products. Thus confirming the αα configuration of the epoxy-ring, as it is known [3, 7] that epoxidation of a *ent*-kaur-15-ene double bond takes place from the less hindered α side (*ent*-β).

EXPERIMENTAL

Mps are uncorr. IR: Nujol mull. NMR: 60 MHz, $CDCl_3$ soln with TMS as int. ref., except where otherwise indicated. MS

were recorded on an Jeol-IMS-OLSG-2 spectrometer, ionisation potential 75 eV. CC was performed on Si gel (0.05–0.20 mm). All the new products here reported gave satisfactory elemental analyses.

Plant material. Sideritis sicula was collected on the high summits of Madonie Mounts (Sicily). A specimen is deposited in the Herbarium of the 'Orto Botanico, University-Palermo'.

Extraction of the diterpenes. The inflorescence were ground and extracted (Soxhlet) with petrol for 48 hr. The solvent was removed under red. pres. and the residue chromatographed on a column. The fraction eluted with cyclohexane–Et_2O (9:1) yielded sideripol (**1**) (100 mg); elution with cyclohexane–Et_2O (1:1) gave eubol (300 mg), mp 194–195° (from EtOAc), mp, IR and NMR identical with reported data [6]: mmp did not depress. Elution with Et_2O–EtOAc (3:7) gave epoxysideritriol (15 mg).

Sideripol (**1**). Mp 121–122° (from petrol); positive TNM test: IR: 3448 cm^{-1} (OH), 1709 and 1266 cm^{-1} (AcO), 3030 and 827 cm^{-1} (trisubstituted C=C); MS: 346 (M^+), 328 (M-H_2O), 313 (M-H_2O—Me), 287 (M-OAc), 255 (M-CH_2OAc—H_2O), 109 (ring A, $C_6H_7Me_2$); NMR: δ 0.82 (3H, s, 4α-Me), 1.08 (3H, *s*, 10α-Me), 1.72 (3H, *d*, J = 1.5 Hz, 16-Me), 2.06 (3H, *s*, OAc), 2.30 (1H, *br*, 13-H), 3.43 and 4.03 (2H, q_{AB} 10.5 Hz, 4β-C$\underline{H}_2$OAc), 3.58 (1H, *br*, 7α-H), 5.49 (1H, *br*, $W_{\frac{1}{2}}$ = 4.5 Hz, 15-H).

By alkaline hydrolysis with 5% KOH–EtOH (at room temp. for 24 hr) it yielded sideridiol (**3**) [2], mp 195–196°; by acetylation diacetoxysideridiol, mp 128–129° [2], (IR spectra superimposable).

Partial acetylation of sideridiol (**3**). To a soln of (**3**) (200 mg) in Py (5 ml), cold Ac_2O (2.5 ml) was added at 0° and the mixture left at this temp. for 15 min. Dry CC (cyclohexane–EtOAc, 3:1) of the residue gave some diacetate (3 mg), 18-monoacetate (70 mg), identical (mmp, IR, NMR) with natural sideripol (**1**) and the 7-monoacetate (**4**) (8 mg), identified by comparison with an authentic marker [2] (mmp, TLC, IR, NMR) and starting material (75 mg).

Epoxysideritrol (**2**). $C_{20}H_{32}O_4$, mp 234–235° (from EtOAc); TNM reaction: negative; IR: 3390–3279 cm^{-1} (OH); MS: 305 (M-CH_2OH), 287 (M-CH_2OH—H_2O), 270 (M-CH_2OH—H_2O—OH), 256 (M-2CH_2OH—H_2O), 109 *m/e* (ring A, $C_6H_7Me_3$); NMR (60 MHz, pyridine-d_5) δ0.95 (3H, *s*, 4α-Me), 1.08 (3H, *s*, 10α-Me), 2.70 (1H, *br*, 13α-H), 3.45 and 3.66 (2H, $q_{AB}J$ = 11 Hz, 4β-C$\underline{H}_2$OH), 3.81 (1H, *s*, 15-H), 4.12 (1H, *t* J = 2 Hz, 7α-H), 4.45 and 4.0 (2H, q_{AB} J = 12 Hz, 16β-C$\underline{H}_2$OH).

Triacetylepoxysideritriol (**5**). Obtained by reaction with Py–Ac_2O; mp 138–140° (from cyclohexane); NMR δ0.81 (3H, *s*, 4-Me, 1.06 (3H. *s*, 10—Me), 2.02 (3H, *s*, OAc), 2.07 (6H, *s*, 20Ac), 2.75 1H, *br*, 13α-H), 3.13 (1H, *s*, 15-H), 3.69 (2H, *s*, 4β-C$\underline{H}_2$OAc), 4.08 and 4.63 (2H, q_{AB} J = 12 Hz, 16β-C$\underline{H}_2$OAc), 4.78 (1H, *br* $W_{\frac{1}{2}}$ = 6 Hz, 7α-H). The products (**2**) and (**5**) were also prepared by treatment of sideritriol (**6**) and triacetylsideritriol (**7**) [4] with *p*-nitroperbenzoic acid in ether at room temp. for 24 hr as described for similar derivatives [3, 5, 8].

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THE MICROBIOLOGICAL TRANSFORMATION OF EPICANDICANDIOL, *ENT*-7α,18-DIHYDROXYKAUR-16-ENE, BY *GIBBERELLA FUJIKUROI*

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Key Word Index—*Gibberella fujikuroi*; microbiological transformation; kauranoid diterpenes.

Abstract—Incubation of *ent*-7α,18-dihydroxykaur-16-ene with *Gibberella fujikuroi* affords *ent*-7α,18,19-trihydroxykaur-16-ene and *ent*-7α,18-dihydroxykaur-16-en-19-oic acid. There was no transformation into 7,18-dihydroxykaurenolide.

INTRODUCTION

The microbiological transformation of artificial substrates by fungi can be divided into two groups. One which is typified by the hydroxylation of steroids utilizes induced enzyme systems with a definite regiospecificity but of low substrate specificity whilst the other, of which there are relatively few examples, utilizes the natural biosynthetic pathway and substrates related to the normal metabolites. Thus steviol (**1**) which is related to the normal metabolite of *Gibberella fujikuroi*, *ent*-kaur-16-en-19-oic acid, is metabolized [1] to 7,13-dihydroxykaurenolide (**2**), a 13-hydroxy analogue of the normal metabolite This work has subsequently been extended in an elegant