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DITERPENOIDS FROM *SIDERITIS VAROI* SUBSPECIES *CUATRECASASII*: ^{13}C NMR OF ENT-13-EPI-MANOYL OXIDES FUNCTIONALIZED AT C-12*

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Key Word Index—*Sideritis varoi*; Labiatae; ent-13(16),14-labdadiene derivative; ent-13-epi-manoyl oxides; ^{13}C NMR data.

Abstract—Several ent-13-epi-manoyl oxides and a new natural product, ent-8α-hydroxylabda-13(16),14-dien-19-oic acid, have been isolated from *Sideritis varoi* subsp. *cuatrecasasii*. In addition, a study has been made of some natural and semisynthetic ent-13-epi-manoyl oxides functionalized at C-12.

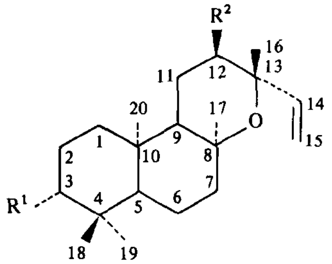
INTRODUCTION

The *Sideritis* genus presents many classification problems. Botanically, *Sideritis varoi* Soc. is a recently described [1] species, which grows in extensive areas of Andaluzia (southern Spain). At present, several of its subspecies are being submitted to phytochemical studies as an aid in their botanical classification.

Genuine *S. varoi* contained seven known ent-kaurenoids (ent-3β,7α-dihydroxy-18-acetoxykaur-16-ene, ent-7α,18-dihydroxy-3β-acetoxykaur-16-ene, ent-3β,7α-dihydroxy-18-acetoxykaur-15-ene, ent-7α,15β,18-trihydroxy-3β-acetoxykaur-16-ene, ent-3β,7α,15β-trihydroxy-18-acetoxykaur-16-ene and ent-18-hydroxy-7α-acetoxykaur-15-ene), three ent-labda-13(16),14-dienes [ent-6α,8α,18-trihydroxylabda-13(16),14-diene, ent-8α,18-dihydroxylabda-13(16),14-diene and ent-8α-hydroxy-13(16),14-labdadien-18-al] and six ent-13-epi-manoyl oxides [ent-3β-hydroxy-13-epi-manoyl oxide (ribenol, **1**), ent-12α-hydroxy-13-epi-manoyl oxide (varol, **2**), ent-3β,12α-dihydroxy-13-epi-manoyl oxide (varodiol, **3**), ent-3β-acetoxy-12α-hydroxy-13-epi-manoyl oxide (3-acetylvarodiol, **4**), ent-3β-hydroxy-12α-acetoxy-13-epi-manoyl oxide (12-acetylvarodiol, **5**) and ent-3β,12α-diacetoxy-13-epi-manoyl oxide (diacetylvarodiol, **6**) [2].

Sesquiterpenes from *S. varoi* subsp. *varoi* and *S. varoi* subsp. *cuatrecasasii* have been studied previously [3]. The present paper deals with the diterpenic content of *S. varoi* subsp. *cuatrecasasii*.

RESULTS AND DISCUSSION



	R¹	R²
1	OH	H
2	H	OH
3	OH	OH
4	OAc	OH
5	OH	OAc
6	OAc	OAc
8	H	H

Sideritis varoi subsp. *cuatrecasasii* contains eudesmanic sesquiterpenes as the main components [3] and several diterpenes as minor products. Nevertheless, ent-kaurenic and ent-isokaurenic diterpenes have not been isolated. The ent-13-epi-manoyl oxides now isolated are similar to those obtained from *S. varoi* (*varoi*) [2], namely ribenol (**1**), varol (**2**), varodiol (**3**), 3-acetylvarodiol (**4**), 12-acetylvarodiol (**5**) and 3,12-diacetylvarodiol (**6**).

Another diterpenoid, **7**, isolated from this subspecies of *S. varoi* was found to have the molecular formula $C_{20}H_{32}O_3$. Its UV spectrum showed a maximum at 227 nm and its IR spectrum showed carboxyl (3000–2500 and 1720 cm^{-1}) absorptions. The ^{1}H NMR spectrum (see Experimental) revealed a vinylic A_2B_2X system similar to that shown by labda-13(16),14-diene derivatives [4] and three methyl singlet signals at δ1.10 (6H) and 0.85 (3H). These data suggest the structure to be 8-hydroxylabda-13(16),14-diene (**7**). As its chemical behaviour indicated an acidic character, biogenetic considerations suggest the presence of an equatorial COOH-4 group and the ent-configuration. On the other hand, ^{13}C NMR experiments with **7** and related compounds, lead us to propose the structure as ent-8α-hydroxylabda-13(16),14-dien-18-oic acid (see Table 1). To confirm this hypothesis, **7** was reduced with lithium aluminum hydride to yield ent-8α,18-dihydroxylabda-13(16),14-diene (6-deoxyandalusol, **11**).

The availability of a number of C-12 functionalized 13-epi-manoyl oxides, with a relatively scarce skeleton, induced us to perform a ^{13}C NMR study of these compounds as well as determining the spectra of some of the derivatives obtained from the natural products mentioned. The assignments of the chemical shifts are based on previous data [2, 5], on the comparison of pairs of compounds, considerations of substituent effects.

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*Part 19 in the series "Terpenic Components of Spanish Labiatae". For Part 18, see E. Cabrera, A. García-Granados and A Molina. *An. Quim.* (in press).

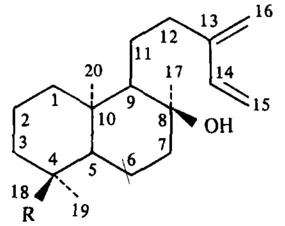
Table 1 ^{13}C NMR chemical shifts of **7**, **11**, **12** and the reference compound **13**

C No.	7	12*	11	13†
1	38.54	38.89	39.28	39.76
2	17.58	16.76	17.80	18.50
3	37.03	32.40	35.29	42.03
4	47.38	48.43	49.66	33.29
5	50.40	49.70	49.19	56.19
6	23.38	23.08	20.28	20.60
7	44.07	44.11	44.25	44.62
8	74.81	74.16	74.12	74.28
9	61.79	61.63	61.81	61.28
10	39.00	38.00	39.02	39.17
11	24.63	24.54	24.60	24.75
12	35.12	35.04	35.10	35.12
13	147.53	147.08	147.49	147.47
14	138.96	138.88	138.85	138.82
15	115.67	115.70	115.43	115.60
16	113.58	113.56	113.42	113.51
17	24.03	24.24	23.94	24.05
18	184.23	206.23	71.82	33.43
19	16.25	14.05	17.38	21.52
20	15.92	15.63	15.81	15.50

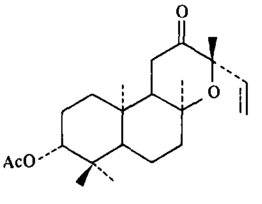
The ^{13}C chemical shifts are given in δ-values relative to TMS. Assignments were made with the aid of DEPT experiments

*Isolated from *S varoi*. See ref [2].

†See ref. [4].



	R
7	COOH
11	CH_2OH
12	CHO
13	Me



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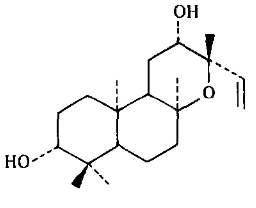

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SFORD experiments and on general chemical shift arguments.

Table 2 shows the estimated chemical shifts for ent-13-epi-manoyl oxides. The equatorial group at C-3 causes the expected shifts on nearby carbons, in accordance to those observed with similarly functionalized triterpenic compounds [6]. In this way, the OAc-3 group produces α-, β- and γ-effects as expected for an equatorial acetoxy group [6, 7].

Table 3 shows some of the substituent effects due to an axial OH-12 group obtained by comparing the spectra of compounds **2** and **8**. Thus, the observed α-effect is in agreement with an axial conformation for the OH-12 group. On the other hand, the pronounced γ-effect observed for C-9 is indicative of the existence of a syn-diaxial effect between the OH-9 group and H-9 and, hence, of a chair conformation for ring C, which is in agreement with the assumed chair conformation for ring C in a series of 14-functionalized 13-epi-manoyl oxide derivatives [8]. Thus, an antiperiplanar γ-effect on C-14 is observed. There is also a remarkable δ-effect (Δδ + 1.18) on C-15 which agrees with the observations made in connection with acyclic olefins and ent-beyer-15-ene systems [9, 10], in that when the transmission of a δ-effect takes place through a π-bond there is a reversal of the sign of the effect. The γ-gauche effect observed on the Me-16 group is also considerable.

The substitution effects due to an OAc-12 group are given in Table 4. In any events there is a parallel with the substituent effects observed for ent-12α-acetoxybeyer-15-enes [10] but, in our case, a chair conformation for ring C is even more obvious.

We have also studied the effects produced by an equatorial OH-12 group and they are given in Table 5. The chemical shift of C-13 could not be determined in the spectrum of **10**. The observed δ-effect for C-15 in **10** is very significant and may be due to a conformational change in the vinyl group of this product.

EXPERIMENTAL

Mps were determined in a Kofler apparatus and are uncorr. ^{1}H NMR spectra were measured at 80 MHz ($CDCl_3$ soln with TMS as int. standard). ^{13}C NMR spectra were determined at 22.5 MHz also in $CDCl_3$ soln (which also provided the lock signal) with TMS added as int. reference Assignments of ^{13}C NMR chemical shifts were made with the aid of broad band proton decoupling and SFORD experiments, setting the decoupler frequency in the middle of the proton range in the former and δ2 to the right of the TMS in the latter On some occasions, ^{13}C NMR spectra were determined at 20.13 MHz, also in $CDCl_3$, in a Bruker WP80S4 spectrometer equipped with a three-clock multipulser. In these cases, assignments were made with the aid of distortionless enhancement by polarization transfer (DEPT), using a 'flip angle' of 135° Silica gel, Merck 7729 (less than 0.08 mm) was used for CC. Plant material was collected in April 1981 near Carboneras (Almería) and voucher specimens are deposited at the herbarium of the Faculty of Pharmacy (University of Granada)

Extraction and isolation of the diterpenoids. Dried and finely powdered plants of *S. varoi* subsp *cuatrecasasii* (2.8 kg) were extracted with hexane (4 l.) in a Soxhlet and processed as in ref [2], to give 84 g of a terpenoid fraction This material (24 g) in CH_2Cl_2, was washed with aq $NaHCO_3$ The neutral fraction (19 g) was chromatographed on a silica gel column and eluted with CH_2Cl_2–Me_2CO mixtures of increasing polarity The homogeneous fractions were repeatedly chromatographed on 10% $AgNO_3$–silica gel columns and eluted with CH_2Cl_2–Me_2CO mixtures of increasing polarity, yielding the following compounds in order of elution: 22 mg diacetylvarodiol (**6**), 150 mg ribenol (**1**), 65 mg varol (**2**), 60 mg 12-acetylvarodiol (**5**), 90 mg 3-acetylvarodiol (**4**) and 200 mg varodiol (**3**). From the acidic fraction, 1.5 g ent-8α-hydroxylabda-13(16),14-dien-18-oic acid (**7**) was isolated as a colourless gum: $[\alpha]_D^{20}$ − 25.03° (EtOH, *c*

Table 2. ^{13}C NMR chemical shifts of ent-13-epi-manoyl oxides

C No.	**8***	**1**	**2**	**3**	**4**	**5**	**6**	**9**	**10**
1	39.4	37.81	39.08	37.47	36.85	38.01	37.10	36.60	37.63
2	18.7	27.42	18.54	27.23	23.73	27.11	23.88	23.37	27.29
3	42.2	78.91	42.19	78.95	80.70	78.64	80.65	80.39	78.86
4	33.4	38.93	33.34	38.90	37.73	38.74	37.86	37.74	38.88
5	56.5	55.39	56.68	55.52	55.42	55.19	55.42	54.38	55.12
6	19.9	19.51	20.03	19.65	19.45	19.51	19.40	19.34	19.62
7	43.1	43.00	42.87	42.78	42.49	42.53	42.44	41.89	42.34
8	76.1	75.92	76.36	76.13	75.93	76.11	76.03	75.28	76.42
9	58.5	58.42	49.92	49.69	49.32	50.01	49.98	55.00	57.68
10	36.9	36.25	36.50	36.17	35.98	37.35	35.88	37.01	36.66
11	15.9	16.01	23.72	23.83	23.44	21.61	21.64	33.77	25.42
12	34.9	34.96	69.22	69.13	68.93	71.37	71.32	221.25	77.63
13	73.3	73.35	75.93	75.74	76.23	74.90	74.96	82.10	—
14	147.8	147.45	147.33	147.19	147.09	146.58	144.61	142.35	140.43
15	109.5	109.80	110.68	110.83	110.64	111.01	111.03	113.22	117.14
16	32.7	32.73	26.96	27.04	26.84	27.99	27.96	28.65	28.03
17	24.0	24.13	24.45	24.32	24.22	23.81	23.19	22.12	25.30
18	33.4	28.02	33.34	28.01	27.91	27.11	27.08	28.09	28.03
19	21.3	15.15	21.29	15.27	16.24	15.22	16.29	16.56	15.19
20	15.9	15.97	15.85	15.95	15.86	15.66	16.81	14.74	16.10
COOMe	—	—	—	—	21.20	21.27	21.30 21.30	21.27	—
COOMe	—	—	—	—	171.01	170.47	170.60 170.90	—	—
CO	—	—	—	—	—	—	—	211.25	—

The ^{13}C chemical shifts are given in δ-values relative to TMS, Assignments were made with the aid of CW experiments.

*See ref. [5].

Table 3. Substituent effects of an axial OH-12 group

C No.	$\Delta\delta$*
9	−8.58
11	7.82
12	34.32
13	2.63
14	−0.47
15	1.18
16	−5.74

*The numbers given are the chemical shift differences δ(**2**) − δ(**8**) for corresponding carbon atoms. A positive sign means a downfield shift.

Table 4. Substituent effects of an axial OAc-12 group

C No.	$\Delta\delta$*
9	−8.41
11	5.60
12	36.47
13	1.55
14	−0.87
15	1.21
16	−4.74

*See footnote to Table 3.
$\Delta\delta = \delta$(**5**) − δ(**1**).

1); IR ν_{max}^{neat} cm^{-1}: 3000–2500, 1720, 1610, 1465, 1390, 1250, 1160, 1120, 1090, 1080, 1030, 1000, 970, 935, 905. UV λ_{max}^{hexane} nm (ε): 227 (12000). ^{1}H NMR (80 MHz): δ6.37 (1H, *dd*, part X of an A_2B_2X system, $J_{AX}+J_{BX}$ = 29 Hz, H-14), 4.90–5.50 (4H, part A_2B_2 of an A_2B_2 X system, 2H-15, 2H-16), 1.10 (6H), 0.85 (3H), (*s*, Me-17, Me-19 and Me-20); ^{13}C NMR: see Table 1. (Found: C, 74.58; H, 10.43. Requires: C, 74.96; H, 10.06%)

LiAlH$_4$ reduction of compound 7. Compound 7 (200 mg) was dissolved in 10 ml dry Et_2O and refluxed with 200 mg $LiAlH_4$ for 72 hr The usual work-up procedure yielded 52 mg 6-deoxyandalusol (**8**) [11]. ^{13}C NMR: see Table 1.

ent-3β,12β-Dihydroxy-13-epi-manoyl oxide (**10**). Jones oxidation of 90 mg 3-acetyl varodiol (**4**) gave 86 mg ent-3β-acetoxy-12-oxo-13-epi-manoyl oxide (**9**) [2]. $LiAlH_4$ reduction of this ketone (**9**) gave ent-3β,12β-dihydroxy-13-epi-manoyl oxide (**10**) (72 mg): mp 157–158°; $[\alpha]_D^{20}$ −42.02° ($CHCl_3$; *c* 1); IR ν_{max}^{KBr} cm^{-1}: 3470, 3080, 3000, 2980, 2940, 1640, 1450, 1410, 1380, 1080, 1050, 1015, 990, 960, 910, 840; ^{1}H NMR (80 MHz): δ6.30 (1H, *dd*, part X of an ABX system, $J_{AX}+J_{BX}$ = 29 Hz, H-14), 5.1–5.6 (2H, part AB of an ABX system, 2H-15), 3.50 (1H, *dd*, J_1 = 5 Hz, J_2 = 9 Hz, H-12), 3.20 (1H, *dd*, J_1 = 5 Hz, J_2 = 10 Hz, H-3), 1.35 (3H), 1.25 (3H), 1.00 (3H), 0.81 (3H), 0.79 (3H), (*s*, Me-16–Me-20);

Table 5. Substituent effects of an equatorial OH-12 group

C No.	Δδ*
9	−0.74
11	9.41
12	42.67
14	−7.02
15	7.34
16	−4.70

*See footnote to Table 3.
Δδ = δ (**10**) − δ (**1**).

^{13}C NMR: see Table 2. (Found: C, 74.40; H, 10.70. Requires: C, 74.49; H, 10.63%.)

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