

TERPENOIDS FROM *SIDERITIS VAROI*

EDUARDO CABRERA, ANDRES GARCIA-GRANADOS and M. ARANZAZU QUECUTY

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Granada, Granada 18071, Spain

(Revised received 18 May 1987)

Key Word Index—*Sideritis varoi*; Labiate; *ent*-manoyl-oxides; eudesmanes.

Abstract—Several germacrenes, eudesmanes, eudesmenes and *ent*-13-*epi*-manoyl oxides and the new natural products 1*α*,4*α*-oxi-6*β*-acetoxyeudesmane, 1*β*-hydroxyeudesm-3,5-diene, 1*β*,5*α*-dihydroxy-6*β*-acetoxyeudesm-4(15)-ene and 6*β*-hydroxyeudesm-1,4-dien-3-one have been isolated from *Sideritis varoi* subsp. *nijarensis*.

INTRODUCTION

Sideritis varoi Soc. is of widespread occurrence in the south of Spain. In order to aid its botanical classification, we are making an extensive and systematic phytochemical study of its subspecies [1-4]. On this occasion, we report the study of the subspecies *nijarensis*, which grows in restricted areas near Níjar (Almería).

RESULTS AND DISCUSSION

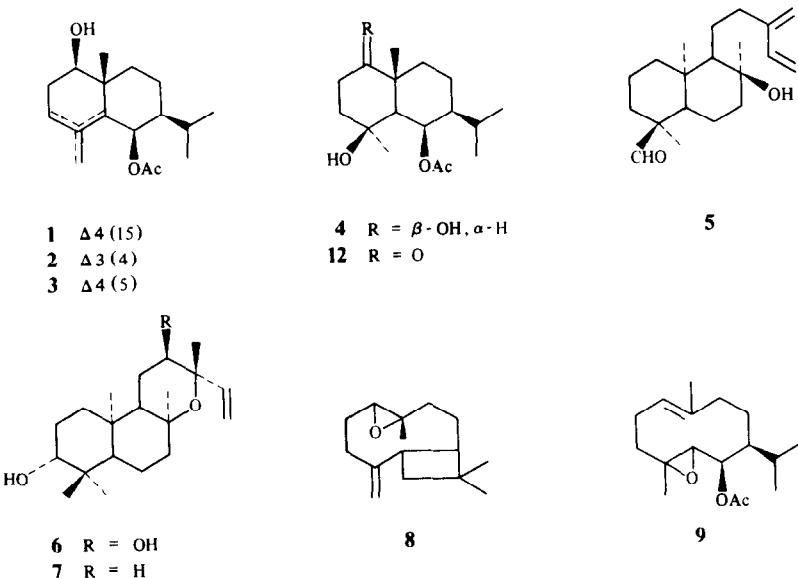
S. varoi subsp. *nijarensis* contained several sesquiterpenes and diterpenes previously isolated from *S. varoi* [1, 2], *S. varoi* subsp. *cuatrecasasii* [3] and *S. varoi* subsp. *oriensis* [4] i.e. 1*β*-hydroxy-6*β*-acetoxyeudesm-4 (15)-ene (1), 1*β*-hydroxy-6*β*-acetoxyeudesm-3-ene (2), 1*β*-hydroxy-6*β*-acetoxyeudesm-4-ene (3), 1*β*,4*β*-dihydroxy-6*β*-acetoxyeudesmane (4), *ent*-8*α*-hydroxylabda-13(16), 14-dien-19-al (5), *ent*-3*β*,12*α*-dihydroxy-13-*epi*-manoyl oxide (6), ribenol (7), caryophyllene oxide (8) and shiromool acetate (9) [5].

Compound 10 was a sesquiterpene which contained an acetoxy group but no hydroxyl group. Its ¹H NMR

spectrum showed signals from two methyl singlets and an isopropyl group, as well as a 1H singlet at δ 5.65. This spectral behaviour was indicative of an eudesmanoid sesquiterpene, similar to those isolated from other subspecies of *S. varoi* [2, 3]. A 1H signal (δ 3.90, *dd*, *J*₁ = 5, *J*₂ = 2 Hz), geminal to an oxygenated function, was also observed. The ¹³C NMR spectrum of 10 showed three oxygenated carbons at δ 84.88 (methine), 82.56 (quaternary) and 69.58 (methine). These data suggested that C-1 and C-4 were bonded by an ether linkage. A compound (11) with this functionalization had been isolated from *Verbesina glabrata* [6]. Table 1 shows the ¹³C NMR assignments for both products 10 and 11. The similar chemical shifts show that the configurations at C-1 and C-4 are the same for both products.

In order to confirm these configurations, 10 was oxidized with Jones' reagent to give 12 which was identical to a ketone recently described [4]. Thus, the new natural product 10 has the 6*β*-acetoxy-1*α*,4*α*-epoxyeudesmane structure.

The IR spectrum of compound 13 showed absorptions of hydroxyl and, probably, conjugated double bonds, the presence of which were confirmed by UV spectroscopy. Its



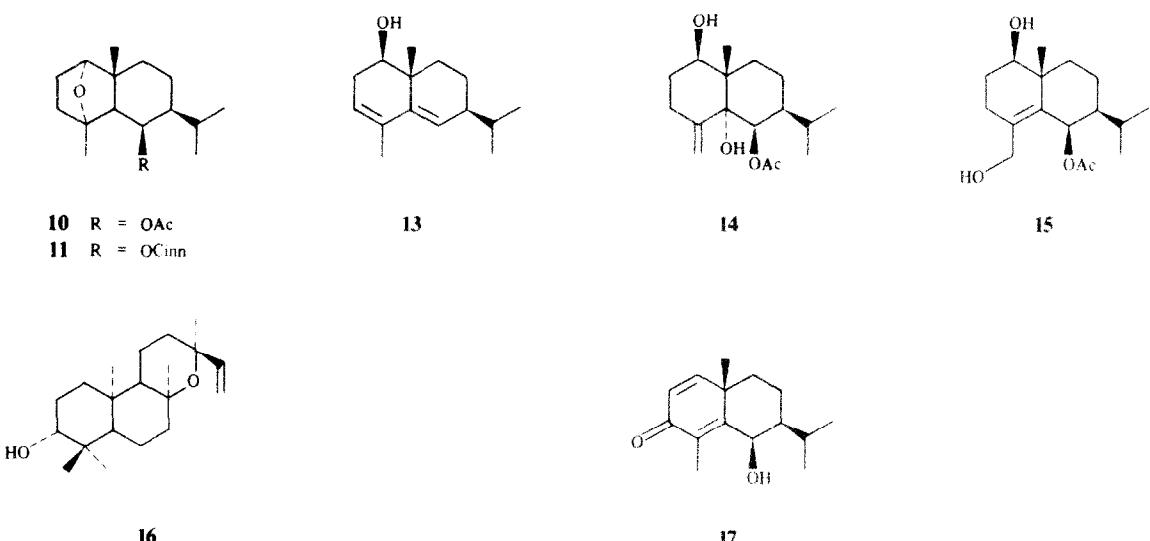


Table 1. ^{13}C NMR data for compounds **10**, **11** and **17**

C	10	11	17
1	84.88	84.50	158.20
2	23.49	23.40	125.17
3	42.84	42.70	187.69
4	82.56	82.40	131.26
5	57.13	57.20	157.79
6	69.58	69.50	69.12
7	49.89	49.90	49.96
8	23.22	23.20	19.43
9	33.06	33.00	37.90
10	48.44	48.30	40.27
11	29.39	29.30	28.87
12	21.51	21.60	21.32
13	20.76	20.60	20.77
14	17.50	17.30	10.50
15	22.28	22.50	25.61

^1H NMR spectrum showed the presence of two vinylic protons at δ 5.40 and 5.52 and one proton geminal to an equatorial hydroxyl group (δ 3.50, dd $J_1 = 10$, $J_2 = 7$ Hz). This product, which is a new natural compound, was identical to another one previously obtained by chemical procedures [2].

Compound **14** was very unstable and only its ^1H NMR spectrum was performed before its decomposition. It showed signals of an acetoxyl group with a geminal proton at δ 5.88 (*br s*), two broad singlets at δ 5.15 and 5.0, attributable to an exocyclic double bond, a methyl singlet at δ 1.02 and an isopropyl group with methyl doublets at δ 0.95 and 0.90 ($J = 6$ Hz). As in the other C-1 hydroxylated eudesmanes and eudesmenes isolated from *Sideritis* [2, 3], an equatorial hydroxyl group was present, with the same coupling constant but considerably deshielded (δ 3.92, dd , $J_1 = 10$, $J_2 = 6$ Hz). This deshielding could be explained if a 5 α -hydroxyl group was present in **14**, which would also explain the instability of **14**, due to its allylic character. Compound **14** gave **15** spontaneously. Compound **15** had a ^1H NMR spectrum with a deshielded

H-6 (δ 6.05, *d*, $J = 2$ Hz) and a Q_{AB} system with doublets centred at δ 5.02 and 4.42 ($J = 12$ Hz). The proton at C-1 was now shielded with respect to the corresponding one at **14** (now δ 3.45, $J_1 = J_2 = 6$ Hz). It was obvious that product **15** was formed as the result of the allylic rearrangement of **14**, which confirms the structure of 1 β ,5 α -dihydroxy-6 β -acetoxyl-eudesm-4(15)-ene for this natural product.

Another natural product isolated from this plant (**16**, 6 mg) showed IR bands of hydroxyl and double bond groups. Its ^1H NMR spectrum clearly showed the characteristic ABX system of a manoyl oxide, with the signal of part X at δ 5.90 (*dd*, $J_{\text{AX}} + J_{\text{BX}} = 29$ Hz) and that of part AB as a complex signal between δ 5.25 and 4.75. The five methyl groups appeared at δ 1.31, 1.28, 0.98, 0.81 and 0.80, besides a signal at δ 3.25, similar to those in the spectrum of ribenol (**7**) [7]. The only differences were the chemical shifts of methyl groups bonded to an oxygenated carbon, (δ 1.31 and 1.28 in **16** and 1.20 and 1.15 in ribenol).

This spectroscopic behaviour suggested that both products were epimers in C-13 [7, 8], which could be proved by ^{13}C NMR and particularly by the chemical shift of C-9 [9-11]. Fortunately this chemical shift appeared in a zone where only C-5 and C-9 were present. For the 13-*epi* configuration the chemical shift of C-9 would have to be at δ 5.85 whereas for the 13-normal configuration this chemical shift would have to be at δ 5.57 [9-11]. The ^{13}C NMR spectrum was determined with great difficulty but two clear signals at δ 56 and 54 were assigned to C-5 and C-9 respectively. So the 13-normal configuration for this product (**16**) was assigned. As all the diterpenes isolated from *Sideritis*, had *ent*-configuration, product **16** must be *ent*-3 β -hydroxy-manoyl oxide, a new natural compound.

After saponification of a complex mixture, product **17** was isolated. Its ^1H NMR spectrum contained a clear AB system at δ 6.70 and 6.17 (*d*, $J = 11$ Hz), a doublet at δ 5.15 (1H, $J = 2$ Hz) and two singlet methyls at δ 2.00 and 1.42. The characteristic doublets of an isopropyl group at δ 1.09 and 1.02 were also observed. The signal at δ 2.00 was firstly assigned to an acetoxyl group, but was later reassigned to an allylic methyl group because the IR spectrum of **17** contained no acetoxyl group bands. According to the spectroscopic data the structure of 6 β -hydroxy-eudesm-1,4-dien-3-one can be proposed. This was confirmed by its

UV spectrum with λ_{max} at 245 nm (ϵ 12 000). The ^{13}C NMR spectrum (Table 1) of **17** also confirmed the proposed structure. We report product **17** as a new natural product although it is possibly acetylated at C-6 in its natural form.

EXPERIMENTAL

Mps: uncorr; ^1H NMR: 80 MHz, CDCl_3 , TMS as int. stand; ^{13}C NMR: 20.13 MHz, CDCl_3 (which also provided the lock signal), TMS as int. standard. Assignments were made with the aid of distortionless enhancement by polarization transfer (DEPT) using a 'flip angle' of 135°.

Silica gel Merck 7729 (< 0.08 mm) and Scharlau 60 were used for flash chromatography. The eluents used were CH_2Cl_2 , and CH_2Cl_2 containing increasing amounts of Me_2CO . Plant material was collected in June 1983 in 'Los campos de Níjar' (Almeria) and voucher specimens have been deposited at the Herbarium of the Faculty of Pharmacy (University of Granada).

Extraction and isolation of the terpenoids. Dried and finely powdered aerial parts of *S. varoi* subsp. *nijarensis* (1.76 kg) were extracted with hexane (4 l) in a Soxhlet and processed as described in ref. [1] to give 55.5 g of terpenoid fraction. This material in CH_2Cl_2 was washed with aq. NaHCO_3 . The neutral fraction (45 g) was chromatographed on a silica gel column.

The homogeneous fractions were repeatedly chromatographed on a 10% AgNO_3 -silica gel column, yielding the following compounds in order of elution: Caryophylene oxide (**8**) (10 mg), $1\alpha,4\alpha$ -oxy- 6β -acetoxyeudesmane (**10**) (14 mg), $4\beta,5\alpha$ -epoxy- 6β -acetoxy-*trans*-germacr-1(10)-ene (shiromool acetate) (**9**) (11 mg) 1β -hydroxy- 6β -acetoxy-eudesm-3,5-diene (**13**) (15 mg), 1β -hydroxy- 6β -acetoxy-eudesm-4(15)-ene (**1**) (600 mg), 1β -hydroxy- 6β -acetoxy-eudesm-3-ene (**2**) (1.8 g), 1β -hydroxy- 6β -acetoxy-eudesm-4-ene (**3**) (1.1 g), *ent*-3 β -hydroxy-13-*epi*-manoyl oxide (**7**) (400 mg), $1\beta,5\alpha$ -dihydroxy- 6β -acetoxy-eudesm-4(15)-ene (**14**) (10 mg), *ent*-8 α -hydroxilabda-13(16), 14-dien-18-al (**5**) (110 mg), *ent*-3 β -hydroxy-manoyl oxide (**16**) (5 mg), $1\beta,4\beta$ -dihydroxy- 6β -acetoxy-eudesmane (**4**) (250 mg), *ent*-3 $\beta,12\alpha$ -dihydroxy-13-*epi*-manoyl oxide (**6**) (300 mg), and 6β -hydroxyeudesma-1,4-dien-3-one (**17**) (14 mg).

1 $\alpha,4\alpha$ -Oxi- 6β -acetoxyeudesmane (10**).** Colourless gum; $[\alpha]_D^{20} = -17.6$ (CHCl_3 ; c1); IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3421, 2928, 2864, 1737, 1454, 1377, 1241, 1022, 982, 925; ^1H NMR: δ 5.65 (1H, br s, H-6), 3.90 (1H, dd, $J_1 = 5$, $J_2 = 2$ Hz, H-1), 2.02 (3H, s, AcO group), 1.36 and 1.30 (3H each, s, C-14 and C-15 Me groups), 0.92 (6H, d, $J = 7$ Hz, C-12 and C-13 Me groups); ^{13}C NMR: See Table 1.

1 β -Hydroxy- 6β -acetoxyeudesm-3,5-diene (13**).** Mp 48–50°; $[\alpha]_D^{20} = -7^\circ$ (CHCl_3 ; c1); IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3400, 1600, 1060, 1050, 860; ^1H NMR: δ 5.52 (1H, d, $J = 2$ Hz, H-6), 5.40 (1H, m, $W_{1/2} = 9$ Hz, H-3), 3.50 (1H, dd, $J_1 = 10$, $J_2 = 7$ Hz, H-1), 1.75 (3H, d, $J = 2$ Hz, C-15 Me group), 0.92 (9H, complex signal, C-12, C-13 and C-14 Me groups); ^{13}C NMR: See Table 1; UV λ_{max} 240 nm (ϵ 6000).

1 $\beta,5\alpha$ -Dihydroxy- 6β -acetoxyeudesm-4(15)-ene (14**).** ^1H NMR: δ 5.88 (1H, br s, H-6), 5.15 and 5.00 (1H each, br s, C-15 protons), 3.92 (1H, dd, $J_1 = 10$, $J_2 = 6$ Hz, H-1), 2.00 (3H, s, AcO group), 1.02 (3H, s, C-14 Me group), 0.95 and 0.90 (3H each, d, $J = 6$ Hz, C-12 and C-13 Me groups).

1 $\beta,15$ -Dihydroxy- 6β -acetoxyeudesm-4-ene (15**).** Colourless gum; $[\alpha]_D^{20} = +32.5^\circ$ (CHCl_3 , c 0.2); IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3392, 2935, 1712, 1371, 1241, 1023; ^1H NMR: δ 6.05 (1H, d, $J = 2$ Hz, H-6), 5.02 and 4.42 (1H each, AB system, d, $J = 12$ Hz, C-15 protons), 3.45 (1H, t, $J = 6$ Hz, H-1), 2.00 (3H, s, AcO group), 1.10 (3H, s, C-

14 Me group), 0.92 (6H, d, $J = 6.5$ Hz, C-12 and C-13 Me groups). **ent**- 3β -Hydroxy-manoyl oxide (**16**). Colourless gum; $[\alpha]_D^{20} = 17.6^\circ$ (CHCl_3 , c 0.5); IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3410, 2933, 1640, 1377, 1260, 1044; ^1H NMR: δ 5.90 (1H, dd, part X of an ABX system $J_{\text{AX}} + J_{\text{BX}} = 29$ Hz, H-14), between 5.25 and 4.75 (2H, m, part AB of an ABX system, C-15 protons), 3.25 (1H, dd, $J_1 = 11$, $J_2 = 4$ Hz, H-3), 1.31, 1.28, 0.98, 0.81 and 0.80 (3H each, s, C-16, C-17, C-18, C-19 and C-20 Me groups). MS m/z , (rel. int.): 291 [$\text{M} - 15$]⁺ (12), 255(10), 219(27), 203(16), 191(12), 189(14), 175(15), 167(14), 161(17), 149(75), 147(25), 135(19), 121(25), 111(27), 109(27), 97(40), 95(35), 85(44), 83(47), 81(48), 71(55), 69(68), 57(100).

6 β -Hydroxyeudesma-1,4-dien-3-one (17**).** Colourless gum;

$$[\alpha]_D^{20} = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365}{+ 98.42 + 98.34 + 98.06 + 96.11 \quad 0.00}$$

(CHCl_3 , c 1.7); UV $\lambda_{\text{max}}^{\text{MeOH}}$ 245 nm (ϵ 12 000); IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} : 3416, 2927, 2858, 1654, 1618, 1453, 1368, 1148, 1027, 833; ^1H NMR: δ 6.70 and 6.17 (1H each, AB system, $J = 11$ Hz, H-1 and H-2), 5.15 (1H, d, $J = 2$ Hz, H-6), 2.00 and 1.44 (3H each, s, C-15 and C-14 Me groups), 1.09 and 1.02 (3H each, d, $J = 7$ Hz, C-12 and C-13 Me groups); ^{13}C NMR: See Table 1.

Oxidation of compound 10. Compound **10** (3 mg) was dissolved in Me_2CO (2 ml) and oxidized with Jones reagent [13]. CC gave 4α -hydroxy- 6β -acetoxyeudesm-1-one (12.25 mg).

Acknowledgements—We thank Dr O. Socorro, Departamento de Botanica, Universidad de Granada, for the identification of plant material and Isabel J. MacDonald for her assistance in the translation of the text. This work was supported by a grant from Comision Asesora de Investigacion Cientifica y Tecnica.

REFERENCES

1. Algarra, J. L., Garcia-Granados, A., Saenz de Buruaga, A. and Saenz de Buruaga, J. M. (1983) *Phytochemistry* **22**, 1779.
2. Garcia-Granados, A., Molina, A., Saenz de Buruaga, A. and Saenz de Buruaga, J. M. (1985) *Phytochemistry* **24**, 97.
3. Garcia-Granados, A., Martinez, A., Molina, A., Onorato, M. E., Rico, M., Saenz de Buruaga, A. and Saenz de Buruaga, J. M. (1985) *Phytochemistry* **24**, 1789.
4. Garcia-Granados, A., Martinez, A., Molina, A. and Onorato, M. E. (1986) *Phytochemistry* **25**, 2171.
5. Cabrera, E., Garcia-Granados, A. and Molina, A. (1986) *Tetrahedron* **42**, 81.
6. Bohlmann, F., Grenz, M., Gupta, R. K., Dhar, A. K., Ahmed, M., King, R. M. and Robinson, H. (1980) *Phytochemistry* **19**, 2391.
7. Gonzalez, A. G., Fraga, B. M., Hernandez, M. G. and Luis, J. L. (1973) *Phytochemistry* **12**, 1113.
8. Teresa, P., Urones, J. G. and Montes Sanchez, A. (1978) *Anal. Quim.* **74**, 959.
9. Buckwalter, B. L., Burfitt, I. R., Nagel, A. A., Wenkert, E. and Naf, F. (1975) *Helv. Chim. Acta* **58**, 1567.
10. Wenkert, E., Baddeley, G., Burfitt, I. R. and Moreno, L. N. (1978) *Org. Magn. Reson.* **11**, 337.
11. Wherli, F. W. and Nishida, T. (1979) *Prog. Chem. Org. Nat. Prod.* **36**, 56.
12. Barton, D. H. R., Levisalles, J. C. D. and Pinhey, J. T. (1962) *J. Org. Chem.* **3**, 3472.
13. Curtis, R. G., Heilbron, I., Jones, E. H. R. and Woods, Q. F. (1953) *J. Chem. Soc.* 457.