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CAROTDIOL ESTERS FROM *FERULA LINKII*

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Key Word Index—*Ferula linkii*; Umbelliferae; sesquiterpenes; carotdiol; phenylpropanoids.

Abstract—The new carotane sesquiterpenes carotdiol acetate and carotdiol veratrate have been isolated from *Ferula linkii*. The known sesquiterpene daucol and the phenylpropanoids laserine, laserine oxide and helmanticine, have also been obtained from this plant.

INTRODUCTION

The genus *Ferula* (Umbelliferae) is phytochemically characterized by containing mainly coumarins and sesquiterpenes [1]. *Ferula linkii* is a species endemic to the Canary Islands, from which we have isolated two dienic triterpenes [2] and several carotane sesquiterpenes [3–6]. In this work we now describe the structural determination of two new carotane sesquiterpenes, and the identification of several phenylpropanoids.

RESULTS AND DISCUSSION

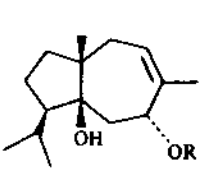
The two new sesquiterpenes isolated were named carotdiol acetate and carotdiol veratrate, and their structures were determined as **1** and **2**, respectively, on the basis of the following considerations. The high resolution mass spectrum of **1** is in accordance with the formula $C_{17}H_{28}O_3$. Its IR spectrum shows absorbances characteristic of hydroxyl and ester groups. The ^{1}H NMR spectrum indicates that this ester is an acetate and that the hydroxyl group is of the tertiary type. This spectrum also shows other signals typical of a vinylic proton, an isopropyl and two methyl groups. One of these methyl functions is located over a double bond. These spectroscopic data and previous phytochemical data of this species [3–6] indicated that this compound had a carotane skeleton. The hydroxyl group was assigned to C-6, because in its mass spectrum the loss of an isopropyl group, characteristic of a carotane sesquiterpene with an alcoholic group at C-10, is not observed [7].

By hydrolysis of **1** the alcohol **3** was obtained, and named carotdiol. Oxidation of **3** with pyridinium dichromate afforded the α,β-unsaturated ketone **4**. Its ^{1}H NMR spectrum showed the resonances of the methyl group at C-3 and the vinylic proton at δ1.82 and 6.55, respectively, indicating that the methyl and the hydrogen are in the α and β-position of the carbonyl group, respectively. Thus the structure **3** for carotdiol is in accordance with these spectroscopic data. The alcohol **3**, obtained by hydrolysis of **1**, was esterified with 3,4-dimethoxybenzoyl chloride in pyridine to give a sesquiterpene ester identical with the second sesquiterpene isolated, carotdiol veratrate (**2**).

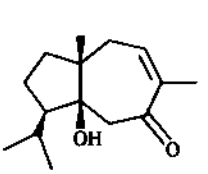
The distinction between structure **3**, and the alternative structure **5** for carotdiol was resolved in the following way. Treatment of carotdiol veratrate (**2**) with *m*-chloroperbenzoic acid gave compound **6**, formed by the opening of the oxirane ring of the intermediate epoxide **11**. The ^{1}H NMR spectrum of **6** showed the geminal proton to the veratrate group as a double doublet at δ5.30 and another hydrogen geminal to a new hydroxyl group. The form of resonance, a double doublet, and the chemical shift, δ3.82, of this last proton are similar to those of the hydrogen at C-2 in daucol (**9**), a substance obtained in the same way by epoxidation of carotol (**13**) [8–10]. Therefore we assigned the C-2, C-3 position to the double bond of the two carotdiol esters **1** and **2**, the same position as in carotol. The formation of the ether **6** also confirmed that the tertiary hydroxyl group is at C-6. When the alcohol **3** was epoxidated the diol **8** was obtained, identical with the compound formed by hydrolysis of **6**.

In the epoxidation reaction of **2** a minor compound was obtained, to which the structure **14** was assigned. Its ^{1}H NMR spectrum was similar to that of **6**, but the geminal proton to the secondary hydroxyl group was not observed. Moreover, the signal of a methyl group was substituted by those of a hydroxymethylene group. This product originated by epoxidation of **15**, and then by opening of the oxirane ring of the formed compound **16**. The product **15** may be a natural substance that co-occurred with **2** or it may be formed by isomerization of **2** in the reaction medium.

At this point, only the stereochemistry of the ester group at C-4 in **1** or **2** remains to be resolved. In the corresponding alcohol **3**, using Dreiding stereomodels and assuming a conformation for the 7-membered ring that explains the coupling constants observed between the vinylic hydrogen and the two hydrogens at C-1 (J = 5.5 and 3 Hz), it can be seen that the form of resonance, a broad singlet, for the geminal proton to the alcoholic group at C-4, in its coupling with the two hydrogens at C-5, is in accordance with a β-orientation for this proton. Therefore the hydroxyl group at C-4 in **3** must be α. The conformaton chosen for **3** or their esters, which is similar to that of carotol, also explains the formation of the daucol type hydroxy ether **6** in the epoxidation of **2**, owing to the spatial proximity of the hydroxyl group at



1 R = Ac
2 R = Vr
3 R = H



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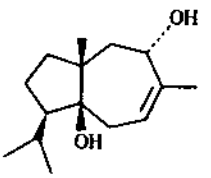

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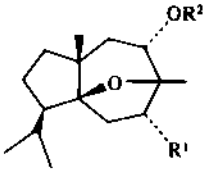
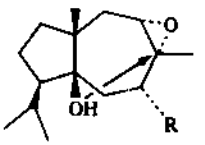
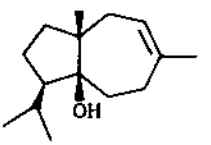


6 R^1 = OVr, R^2 = H
7 R^1 = OVr, R^2 = Ac
8 R^1 = OH, R^2 = H
9 R^1 = R^2 = H
10 R^1 = H, R^2 = Ac



11 R = OVr
12 R = H



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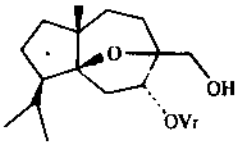

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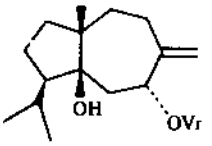

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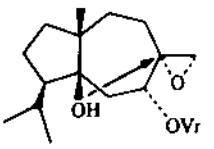

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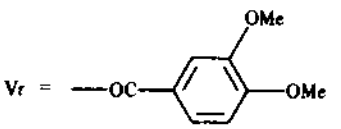

C-6 to the double bond. It is known that an alternative conformation does not form this type of ether [11, 12]. There are other reasons to assign an α-stereochemistry to the alcoholic group at C-4. Thus, the geminal hydrogen at the veratrate group in the compound **14** has been shielded at a lower field (δ5.71) when compared with the chemical shift of this proton in product **6** (δ5.30), indicating a proximity effect between the primary alcohol and this hydrogen. Using Dreiding models it can be seen that there are no interactions between the hydroxymethylene group and an α-hydrogen for an alternative structure with a β-orientated veratrate group. On the other hand, a β-ester group at C-4 in **2** should be associated with the hydroxyl at C-6, which would probably impede the rearrangement in its epoxidation. In the ^{1}H NMR spectrum of **2** (or **1**) no associated hydroxyl has been observed. In Table 1 the ^{13}C NMR spectra of several derivatives of the new sesquiterpenes have been assigned.

The known sesquiterpene daucol (**9**) was also found in this species. Other compounds obtained from this plant were the phenylpropanoids laserine, laserine oxide and helmanticine, whose structures were determined on the basis of their ^{1}H NMR data. Laserine has previously been isolated from *Laser trilobum* [13] and *Ferula loscosii* [14]. Laserine oxide has been obtained from *Guillonea scabra* [15], and helmanticine from *Thapsia villosa* [16].

EXPERIMENTAL

Mps: uncorr. IR: $CHCl_3$. NMR: $CDCl_3$. MS: 70 eV (probe). CC was performed on silica gel 0.063–0.2 mm. The substances were crystallized from petrol–EtOAc except where otherwise indicated.

Isolation of the sesquiterpenes. The compounds were obtained in accordance with the experimental data reported in ref. [2], and by chromatography of the complex mixture of products. The order of polarity and the amounts of the natural products isolated were acetate of carotdiol (**1**) (300 mg), veratrate of carotdiol (**2**) (90 mg), daucol (**9**) (200 mg), laserine (30 mg), laserine oxide (7 mg), and helmanticine (25 mg).

Carotdiol acetate (**1**). Oil, $[M]^+ - H_2O$ at *m/z* 262.1952. $C_{17}H_{26}O_2$ requires 262.1932; IR ν_{max} cm^{-1}: 3590, 3000, 2940, 2860, 1720, 1455, 1370, 1245, 1125, 1075, 1030, 1010, 970, 950, 905, 830; ^{1}H NMR (60 MHz): δ0.95 (3H, *s*, H-14), 0.93 and 1.03 (each 3H, *d*, *J* = 4 Hz, H-12 and H-13), 1.67 (3H, *br s*, H-15), 2.08 (3H, *s*), 5.53 (2H, complex signal, H-2 and H-4); ^{1}H NMR (C_6D_6, 60 MHz): δ0.91 (3H, *s*, H-14), 1.02 (6H, *t*, H-12 and H-13), 1.64 (3H, *br s*, H-15), 1.66 (3H, *s*, AcO-), 5.47 (2H, complex signal, H-2 and H-4); EIMS *m/z* (rel. int.): 262 $[M]^+ - H_2O$ (2), 238 (2), 220 (46), 205 (9), 192 (7), 177 (21).

Carotdiol veratrate (**2**). Oil, $[M]^+$ at *m/z* 402.2406. $C_{24}H_{34}O_5$ requires 402.2406; IR ν_{max} cm^{-1}: 3590, 3020, 3000, 2940, 2920, 2860, 1700, 1595, 1510, 1460, 1410, 1265, 1160, 1130, 1020;

Table 1. ^{13}C NMR data of compounds **3–6** and **8–10** (50.32 MHz)

C	3	4	6	8	9	10
1	40.91[a]	41.37	40.87	40.88	41.13	37.92
2	123.08	141.64	70.51	70.50	71.67	73.83
3	139.39	140.15	87.20	87.38	85.47	83.55
4	72.78	200.14	74.25	71.26	29.58	31.18
5	41.76[a]	55.95	50.22	53.13	41.13	40.98
6	85.53	82.45	91.23	90.36	91.76	92.17
7	50.48	50.48	44.80	44.68	45.91	45.64
8	39.45	40.25	33.27	33.35	33.12	33.08
9	25.09	26.37	26.48	26.45	26.46	26.53
10	57.18	57.52	52.42	53.13	52.60	52.78
11	27.57	27.43	31.50	31.70	31.60	31.67
12	21.50[b]	21.22[b]	21.93[b]	21.90[b]	21.92[b]	21.92[b]
13	22.31[b]	22.98[b]	23.36[b]	23.56[b]	23.60[b]	23.57[b]
14	22.00	23.65	21.80	21.90	22.52	22.27
15	24.33	18.26	18.26	18.09	23.08	23.03

[a, b] The assignments for these signals may be reversed.

^{1}H NMR (60 MHz): δ1.02 (3H, *s*, H-14), 1.02 (6H, *t*, H-12 and H-13); 1.73 (3H, *br s*, H-15), 3.91 (6H, *s*), 5.5–6.00 (2H, complex signal, H-2 and H-4), 6.90 (1H, *d*, J = 9 Hz, H-5'), 7.61 (1H, *d*, J = 2 Hz, H-2'), 7.74 (1H, *c*, J = 2 and 9 Hz, H-6'); EIMS *m/z* (rel. int.): 402 $[M]^+$ (1), 220 (6), 202 (11), 177 (10), 159 (12).

Hydrolysis of **1**. A soln of carotdiol acetate (**1**) (100 mg) in MeOH (1 ml) was treated with 3% methanolic KOH (10 ml) leaving the mixture at room temp. for 3 hr. Usual work-up and subsequent dry CC, eluting with petrol–EtOAc (9:1), gave the alcohol **3** (92 mg), mp 76–78°, $[M]^+$ at *m/z* 238.1942. $C_{15}H_{26}O_2$ requires 238.1933; IR ν_{max} cm^{-1}: 3600, 3470, 2950, 2920, 2860, 1460, 1455, 1450, 1380, 1370, 1240, 1120, 1080, 1030, 970, 910, 840; ^{1}H NMR (200 MHz): δ0.90 and 1.01 (each 3H, *d*, J = 6 Hz, H-12 and H-13), 1.03 (3H, *s*, H-14), 1.76 (3H, *s*, H-15), 2.03 (1H, *dd*, J = 3 and 15 Hz, H-1α), 2.25 (1H, *dd*, J = 5.5 and 15 Hz, H-1β), 4.21 (1H, *br s*, H-4), 5.38 (1H, *m*, H-2); EIMS *m/z*: 238 $[M]^+$, 220, 205, 192, 177.

Oxidation of **3**. The diol **3** (90 mg) in CH_2Cl_2 (10 ml) was treated with pyridinium dichromate (215 mg) at room temp. for 4 hr. The soln was diluted with Et_2O, filtered and evapd. The residue was chromatographed, eluting with petrol–EtOAc (9:1), to give **4** (70 mg), $[M]^+$ at *m/z* 236.1796. $C_{15}H_{24}O_2$ requires 236.1777; UV λ_{max} nm^{-1}: 238; IR ν_{max} cm^{-1}: 3590, 3000, 2950, 2920, 2860, 1650, 1460, 1450, 1370, 1085, 1000, 970, 855; ^{1}H NMR (90 MHz): δ0.90 and 1.02 (each 3H, *d*, J = 6 Hz, H-12 and H-13), 1.82 (3H, *br s*, H-15), 2.90 (2H, *s*, H-5), 6.55 (1H, *m*, H-2); EIMS *m/z* (rel. int.): 236 $[M]^+$ (3), 221 (3), 218 (3), 193 (7), 175 (4), 151 (8).

Epoxidation of **2**. The veratrate of carotdiol (**2**) (90 mg) in $CHCl_3$ (1.5 ml) was added to a soln of *m*-chloroperbenzoic acid (40 mg) in $CHCl_3$ (1.5 ml). The mixture was left at room temp. in the dark for 5 hr and then washed with a saturated soln of $NaHCO_3$. Usual work-up and chromatography of the residue with petrol–EtOAc (17:3%) gave **14** (3 mg), ^{1}H NMR (200 MHz): δ0.95 and 1.01 (each 3H, *d*, J = 6 Hz, H-12 and H-13), 1.08 (3H, *s*, H-14), 2.51 (1H, *t*, J = 12 Hz, H-5), 3.92 and 3.94 (each 3H, *s*), 5.41 and 5.60 (each 1H, *d*, J = 12 Hz, H-15), 5.71 (1H, *dd*, J = 2 and 12 Hz, H-4), 6.88 (1H, *d*, J = 9 Hz, H-5'), 7.55 (1H, *d*, J = 2 Hz, H-2'), 7.71 (1H, *dd*, J = 2 and 9 Hz, H-6'); EIMS *m/z* (rel. int.): 418 $[M]^+$ (1), 306 (3), 298 (1), 279 (1), 203 (8), 182 (52), 175 (8), 165 (100). Further elution afforded **6** (55 mg), ^{1}H NMR (200 MHz): δ0.82 (6H, *t*, J = 6 Hz, H-12 and H-13), 1.08 (3H, *s*, H-14), 1.41 (3H, *s*, H-15), 2.86 (1H, *dd*, J = 7 and 14 Hz, H-5α), 3.82 (1H, *dd*, J = 6 and 11 Hz, H-2), 3.90 and 3.92 (each 3H, *s*), 5.30 (1H, *dd*, J = 2 and 7 Hz, H-4), 6.88 (1H, *d*, J = 9 Hz, H-5'), 7.55 (1H, *d*, J = 2 Hz, H-2'), 7.71 (1H, *dd*, J = 2 and 9 Hz, H-6'); EIMS *m/z* (rel. int.): 418 $[M]^+$ (10), 236 (3), 218 (1), 208 (2), 203 (2), 193 (6), 192 (11), 182 (55), 175 (4), 165 (100). *Acetate* **7**. Gum, $[M]^+$ at *m/z* 4600.2480. $C_{26}H_{36}O_7$ requires 460.2460; ^{1}H NMR (200 MHz): δ0.78 and 1.06 (each 3H, *d*, J = 6 Hz, H-12 and H-13), 1.10 (3H, *s*, H-14), 1.28 (3H, *s*, H-15), 2.01 (3H, *s*), 2.86 (1H, *dd*, J = 7 and 14 Hz, H-5α), 3.87 and 3.89 (each 3H, s), 4.98 (1H, *dd*, J = 6 and 11 Hz, H-2), 5.29 (1H, *dd*, J = 2 and 7 Hz, H-4), 6.88 (1H, *d*, J = 9 Hz, H-5'), 7.55 (1H, *d*, J = 2 Hz, H-2'), 7.71 (1H, *dd*, J = 2 and 9 Hz, H-6'); EIMS *m/z*: 460 $[M]^+$, 418, 331, 278, 236, 219, 192, 182, 165.

Hydrolysis of **6**. A soln of **6** (30 mg) in C_6H_6 (0.15 ml) was treated with 3% methanolic KOH (2 ml) leaving the mixture at room temp. for 3 hr. Usual work-up and subsequent dry CC, eluting with C_6H_6–EtOAc (1:1), gave **8** (25 mg), mp 177–179°, $[M]^+$ at *m/z* 254.1879. $C_{15}H_{26}O_3$ requires 254.1882; IR ν_{max} cm^{-1}: 3600, 2980, 2970, 2860, 1460, 1370, 1040, 1010, 970, 900, 870; ^{1}H NMR (200 MHz): δ0.79 and 1.05 (each 3H, *d*, J = 6 Hz, H-12 and H-13), 1.03 (3H, *s*, H-14), 1.33 (3H, *s*, H-15), 2.72 (1H, *dd*, J = 7 and 14 Hz, H-5α), 3.77 (1H, *dd*, J = 6 and 11 Hz, H-2), 4.05 (1H, *dd*, J = 2 and 7 Hz, H-4); EIMS *m/z*: 254 $[M]^+$, 236, 208, 167, 149, 137, 123.

Esterification of **3**. Compound **3** (10 mg), obtained by hydrolysis of **1**, dissolved in dry pyridine (2 ml) was treated with 3,4-dimethoxybenzoyl chloride (30 mg) under N_2 for 70 hr. Usual work-up and chromatography, eluting with petrol–EtOAc (2:1) afforded **2**, identical with the natural product.

Epoxidation of **3**. The product **3** (50 mg) was epoxidised as above for **2** to give, after chromatography eluting with C_6H_6–EtOAc (3:2), the compound **8** (35 mg), identical with that obtained by hydrolysis of **6**.

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