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CAROTANE SESQUITERPENES FROM *FERULA LINKII*

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Key Word Index—*Ferula linkii*; Umbelliferae; sesquiterpenes; carotane; isolancerotriol; isolancerotetrol; epoxyisolancerotetrol.

Abstract—Four new carotane sesquiterpenes have been isolated from *Ferula linkii*. They have been identified as the 5-isovalerate of isolancerotriol, the 5-isovalerate and 5-angelate of isolancerotetrol, and the 5-isovalerate of epoxyisolancerotetrol.

INTRODUCTION

Ferula linkii Webb is a species endemic in the Canary Islands, from which we have isolated several carotane sesquiterpenes [1–3] and two dienic triterpenes [4]. Continuing our studies on this species we describe the structural determination of four new sesquiterpenes with a carotane skeleton.

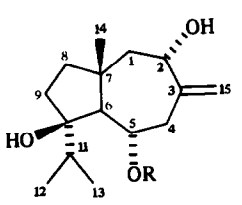
RESULTS AND DISCUSSION

The least polar new compound isolated from this species was the 5-isovalerate of lancerotriol (**1**). In the mass spectrum the molecular ion was not observed, but an ion was produced by the loss of C_3H_7 from the molecular ion. In the literature [5] this is characteristic of a hydroxyl function at C-10 in a carotane skeleton, but our experience indicates that it is true when there is also an acid esterifying an alcoholic group at C-5. In **1** this acid was identified with isovaleric acid, on the basis of the ^{1}H NMR and ^{13}C NMR spectra. The proton geminal to this esterified hydroxyl group at C-5 is equatorial, according to the magnitude of the coupling pattern observed in the ^{1}H NMR spectrum of **1** in benzene-d_6. Other signals observed in this spectrum were those due to a hydrogen geminal to an alcoholic group, an exocyclic double bond, an angular methyl and an isopropyl group.

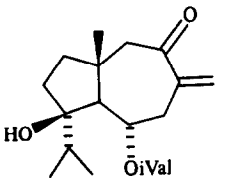
The secondary hydroxyl was located at C-2, allylic to the double bond, because by oxidation of **1** with pyridinium dichromate [6] the compounds **3**, **4** and **5** were obtained. The structures of these products were assigned on the basis of their ^{1}H NMR spectra, and their formation can be explained in accordance with the results obtained by other authors on the oxidation of allylic alcohols [7]. The stereochemistry at C-2 in **1** was given as α. The ^{1}H NMR spectrum suggested that this hydroxyl was equatorial, but this molecule may adopt two conformations in which the equatorial hydroxyl can be α or β. We have chosen the α-stereochemistry on the basis of a biosynthetic hypothesis. Thus, this compound must be derived from epoxyjaeschkeanadiol isovalerate (**6**), also isolated from this plant [2], by enzymatic cleavage of the oxirane ring with formation of a carbocation at C-3, and neutralization of this with the loss of a hydrogen from C-15 and formation of a double bond between C-3 and C-15 (Scheme 1). Thus, the configuration of the hydroxyl group formed must be α, as in the original oxirane ring in **6**. Moreover, the ^{1}H NMR spectrum of **1** was very similar to that of **2** [8] especially for the chemical shift and the coupling pattern of the geminal hydrogen to the alcoholic group at C-2. Compound **2** was obtained by acid treatment of the epoxide **7** [8].

A very polar new compound, isolancerotetrol 5-isovalerate was isolated from this species and the structure **8** assigned on the basis of the following considerations. Its IR spectrum showed bands of an alcoholic group, of an ester and of an exocyclic double bond. In its mass spectrum the molecular ion was not observed, but a fragment ion at m/z 311 was formed from it by loss of C_3H_7. Thus, its molecular formula is $C_{20}H_{24}O_5$. Two of the oxygens of the molecule are part of an ester group and another is in a tertiary hydroxyl group. The other oxygens must form part of two secondary alcohol groups, because in the ^{1}H NMR spectrum of **8**, two protons geminal to hydroxylic groups can be observed at δ3.68 and 4.31. These two hydrogens are coupled one to the other (J = 3 Hz) and they must be placed at C-1 and C-2. This assumption was chemically confirmed because **8** formed an acetonide (**11**) by treatment with acetone and copper sulphate. Other signals observed in the spectrum of **8** are a pair of singlets at δ5.08 and 5.27 of an exocyclic double bond. The hydrogen geminal to the ester appears as a doublet of double-doublets at δ5.32, with coupling values of J = 10, 4 and 2 Hz, thus establishing that the esterified secondary hydroxyl is equatorial and located between a methylene and a methine group. Thus, this function must have the α-configuration at C-5. All the carotane sesquiterpenes isolated from the genus *Ferula* and hydroxylated at C-6 have an α-stereochemistry at this carbon.

Using Dreiding stereomodels and assuming a conformation with an equatorial α-stereochemistry for the esterified hydroxyl group at C-6, it can be seen that the coupling observed for the geminal protons to the alcoholic group at C-1 and C-2 (J = 3 Hz) is in accordance with the fact that these hydroxylic functions must be α-placed.



1 R = iVal
2 R = CO—C_6H_4OMe



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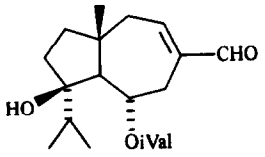

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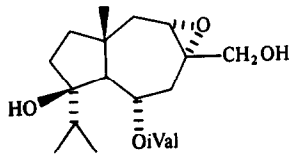

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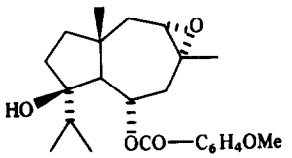

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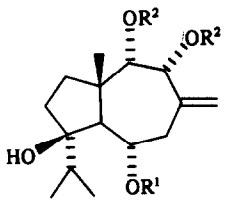
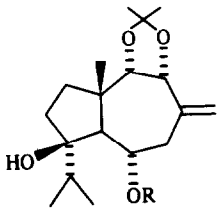
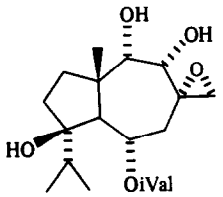


8 R^1 = iVal, R^2 = H
9 R^1 = iVal, R^2 = Ac
10 R^1 = Ang, R^2 = H



11 R = iVal
12 R = Ang
13 R = H



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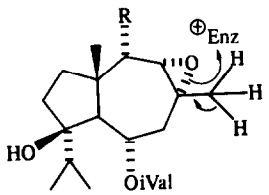
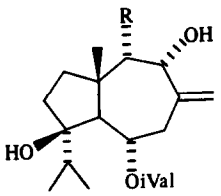


6 R = H
14 R = OH



1 R = H
8 R = OH

Scheme 1.

When the acetonide **11** was formed from a crude sample of **8**, it was observed that another acetonide **12** was present as an impurity. This compound was very similar to the acetonide **11**, differing only in the acid that esterified the alcoholic function at C-6. This acid, in the minor substance, was identified as angelic acid. Thus the structure of this new compound was determined as the 5-angelate of isolancerotetrol (**10**).

Biogenetically, **8** can be derived from **1** by hydroxylation, or from the 5-isovalerate of lapiferol (**14**) by enzymatic cleavage of the oxirane ring with elimination of one of the hydrogens of the C-15 methyl group.

Another compound isolated from this plant was epoxyisolancerotetrol 5-isovalerate (**15**). This new substance was identical with a product obtained by epoxidation of **8** with *m*-chloroperbenzoic acid. The stereochemistry of the oxirane ring was given as α, because it is known that epoxidation occurs on this face [9]. Also in the epoxidation of allylic alcohols the oxiranes formed normally have the same stereochemistry as the hydroxylic function. The ^{13}C NMR spectra of **1**, **8** and **15** (Table 1) are in agreement with the structures assigned to these products.

EXPERIMENTAL

Mps: uncorr.; IR: $CHCl_3$; NMR: $CDCl_3$; MS: 70 eV (probe). Column chromatography was performed on silica gel

Table 1. ^{13}C NMR data ($CHCl_3$, 50.32 MHz) for compounds **1**, **8**, **11** and **15**

C	1	8	11*	15
1	51.1	74.1	80.9†	75.7
2	72.3†	77.1	81.8†	71.1
3	148.6	143.6	142.5	56.2
4	42.9	40.4	38.5	40.4
5	72.9†	71.6	76.5	69.3
6	55.6	45.0	48.6	45.3
7	42.2	46.3	45.9	47.4
8	32.0	31.7	31.6	31.8
9	39.5	36.8	38.0	36.8
10	85.9	86.4	86.4	86.7
11	36.9	37.0	37.4	37.0
12	17.4	17.6	17.4	17.7
13	18.5	18.3	18.5	18.4
14	20.6	21.2	19.6	21.8
15	113.8	115.1	120.7	48.2
1′	174.4	173.3	173.3	173.1
2′	44.2	44.1	44.2	44.0
3′	25.8	25.7	24.2	25.7
4′	22.6	22.5	22.6	22.6
5′	22.6	22.5	22.6	22.6

*Acetonide: 24.6, 25.8 and 107.5.
†These values can be interchanged.

0.063–0.2 mm. The substances were crystallized from petrol–EtOAc except where otherwise indicated.

Isolation of the sesquiterpenes. The substances were obtained in accordance with the experimental data reported in ref. [4] and by several dry-column chromatographies of a complex mixture of sesquiterpenes. The substances isolated were: **1** (30 mg), **8** (320 mg), **10** (obtained as its acetonide **12**, 20 mg) and **15** (60 mg).

5-Isovalerate of isolancerotriol (**1**). Mp 84–86° (from petrol) $[M-C_3H_7]^+$ at 295.1941, $C_{17}H_{27}O_4$ requires 295.1909. IR ν_{max} cm^{-1} : 3580, 3450, 3000, 2940, 2920, 2860, 1700, 1460, 1380, 1290, 1230, 1045, 970 and 910; ^1H NMR (200 MHz): δ0.80–1.03 (12H, complex signal), 1.12 (3H, *s*, H-14), 1.91 (1H, *d*, J = 10 Hz, H-6), 2.48 and 2.55 (each 1H, *dd*, J = 14 and 5 Hz), 4.20 (1H, *dd*, J = 11 and 6 Hz, H-2), 5.00 and 5.23 (each 1H, *br s*, H-15), 5.23 (1H, *m*, H-5); ^1H NMR (200 MHz, C_6D_6): δ0.76–0.94 (12H, complex signal), 1.04 (3H, *s*, H-14), 2.43 and 2.55 (each 1H, *dd*, J = 14 and 5 Hz, H-4), 3.86 (1H, *dd*, J = 11 and 6 Hz, H-2), 5.01 and 5.22 (each 1H, *s*, H-15), 5.52 (1H, *dt*, J = 10 and 5 Hz, H-5); EIMS *m/z* (rel. int.): 295 $[M-C_3H_7]^+$ (2), 284 (2), 236 (6), 218 (5), 203 (5), 201 (3), 193 (39), 175 (100).

5-Isovalerate of epoxy-isolancerotetrol (**15**). $[M-C_3H_7]^+$ at 327.1819. $C_{17}H_{27}O_6$ requires 327.1808; IR ν_{max} cm^{-1}: 3590, 3500, 3010, 3000, 2950, 2920, 2860, 1715, 1460, 1365, 1290, 1230, 1160, 1070, 970; ^1H NMR (200 MHz): δ0.87–0.94 (12H, complex signal), 1.17 (3H, *s*, H-14), 2.87 and 3.09 (each 1H, *d*, J = 4 Hz, H-15), 3.75 and 3.97 (each 1H, *d*, J = 3 Hz, H-2 and H-1), 5.40 (1H, *dd*, J = 11 and 6 Hz, H-5); EIMS *m/z* (rel. int.): 327 $[M-C_3H_7]^+$ (2), 279 (2), 268 (1), 225 (33), 207 (23), 189 (35).

5-Isovalerate of isolancerotetrol (**8**). $[M-C_3H_7]^+$ at 311.1865, $C_{17}H_{27}O_5$ requires 311.1858; IR ν_{max} cm^{-1}: 3580, 3460, 3070, 3000, 2950, 2920, 2860, 1705, 1640, 1460, 1380, 1365, 1290, 1230, 1160, 1050, 1000, 975, 910, 830; ^1H NMR (360 MHz): δ0.90 and 0.92 (each 3H, *d*, J = 2.7 Hz), 0.97 and 0.99 (each 3H, *d*, J = 3 Hz), 1.20 (3H, *s*, H-14), 3.68 and 4.31 (each 1H, *d*, J = 3 Hz, H-2 and H-1), 5.08 and 5.27 (each 1H, *s*, H-15), 5.32 (1H, *ddd*, J = 10, 4 and 2 Hz, H-5); EIMS *m/z* (rel. int.): 311 $[M-C_3H_7]^+$ (2), 252 (2), 234 (10), 209 (27), 191 (59), 173 (16), 163 (13). Diacetate **9**: $[M-C_3H_7]^+$ at 395.2072, $C_{21}H_{31}O_7$ requires 395.2070; ^1H NMR (200 MHz): δ0.88 (6H, *d*), 0.96 (6H, complex signal), 1.28 (3H, *s*, H-14), 2.00 and 2.09 (each 3H, *s*), 2.53 (1H, *d*, J = 10 Hz, H-6), 5.03 and 5.14 (each 1H, *s*, H-15), 5.09 and 5.38 (each 1H, *d*, J = 3 Hz, H-2 and H-1), 5.33 (1H, *m*, H-5); EIMS *m/z* (rel. int): 395 $[M-C_3H_7]^+$ (2), 335 (1), 293 (6), 233 (100), 216 (63).

Acetonides **11** *and* **12**. Fractions (150 mg) containing **8** were dissolved in dry Me_2CO and treated with copper sulphate (5 g) under reflux for 10 hr. The soln was filtered and the solvent evaporated off. Chromatography of the residue eluting with petrol–EtOAc (10%) gave the acetonide of isolancerotetrol 5-angelate (**12**) (20 mg): $[M-Me]^+$ at 377.2327, $C_{22}H_{33}O_5$ requires 377.2328; ^1H NMR (90 MHz): δ0.80–1.02 (6H, complex signal), 4.11 and 4.57 (each 1H, *d*, J = 8 Hz, H-2 and H-1), 5.19 (2H, *br s*, H-15), 5.19 (1H, *m*, H-5), 6.07 (1H, *m*, Ang); EIMS *m/z* (rel. int.): 377 $[M-Me]^+$ (6), 349 (6), 292 (4), 274 (4), 249 (20), 234 (20), 217 (13), 191 (97), 173 (2). Further elution afforded a mixture (20 mg) of **12** and **11**, and finally the acetonide of isolancerotetrol 5-isovalerate (**11**) (90 mg), mp 86–88°, $[M-Me]^+$ at 379.2458. $C_{22}H_{35}O_5$ requires 379.2484; ^1H NMR (90 MHz): 0.82–1.02 (12H, complex signal), 1.10 (3H, *s*, H-14), 1.34 and 1.51 (each 3H, *s*), 4.09 and 4.53 (each 1H, *d*, J = 8 Hz, H-2 and H-1), 5.18 (2H, *br s*, H-15), 5.18 (1H, *m*, H-5); EIMS *m/z* (rel. int.): 379 $[M-Me]^+$ (10), 351 (7), 292 (3), 274 (3), 249 (15), 234 (16), 217 (15), 191 (100).

Alkaline hydrolysis of the mixture of **11** *and* **12**. A mixture (20 mg) of the acetonides **11** and **12** (1:1) in MeOH (0.5 ml) was treated with methanolic KOH (3%) (1 ml) at room temp. for 48 hr. TLC showed only one product of hydrolysis. Usual work up afforded **13** (14 mg), mp 152–154°, $[M-Me]^+$ at 295.1907, $C_{17}H_{27}O_4$ requires 295.1909; ^1H NMR (90 MHz): δ0.99 (6H, *t*, J = 8 Hz, H-12 and H-13), 1.25 (3H, *s*, H-14), 1.35 and 1.53 (each 3H, *s*), 3.93 (1H, *m*, H-5), 4.09 and 4.60 (each 1H, *d*, J = 8 Hz, H-2 and H-1), 5.16 (2H, *br s*, H-15); EIMS *m/z* (rel. int.): 295 $[M-Me]^+$ (8), 267 (14), 249 (9), 217 (7), 191 (100).

Epoxidation of **8**. The isolancerotetrol 5-isovalerate (**8**) (160 mg) in $CHCl_3$ (2 ml) was added to a soln of *m*-chloroperbenzoic acid (77 mg) in $CHCl_3$ (2 ml). The mixture was left at room temp. in the dark for 2 hr, and then washed with a saturated soln of $NaHCO_3$. Usual work up and chromatography of the residue with C_6H_6–MeCN (25%) gave **15** (120 mg), identical with the natural compound.

Oxidation of **1**. The 5-isovalerate of isolancerotriol (**1**) (25 mg) in dry CH_2Cl_2 (3 ml) was treated with pyridinium dichromate (18 mg) and stirred under nitrogen for 7 hr. The soln was diluted with dry Et_2O, filtered and evaporated. The residue was chromatographed by elution with petrol–EtOAc (15%) giving **3** (7 mg), IR ν_{max} cm^{-1}: 3450, 3020, 1710, 1685, 1600 and 950; ^1H NMR (200 MHz): 0.98–1.00 (12H, complex signal), 1.10 (3H, *s*, H-14), 2.00 (1H, *d*, J = 11 Hz, H-6), 5.23 (1H, *ddd*, J = 11, 9 and 3 Hz), 5.44 (1H, *br s*, H-15) and 6.16 (1H, *d*, J = 2 Hz, H-15); EIMS *m/z* (rel. int.): 293 $[M-C_3H_7]^+$ (5), 234 (10), 191 (77), 173 (6), 163 (11). Further elution gave the α,β-unsaturated aldehyde **4** (2 mg), ^1H NMR (200 MHz): δ0.90–1.05 (12H, complex signal), 1.25 (3H, *s*, H-14), 1.98 (1H, *d*, J = 10 Hz, H-6), 5.02 (1H, *dt*, J = 10 and 3 Hz, H-5), 6.80 (1H, *m*, H-2), 9.38 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 293 $[M-C_3H_7]^+$ (3), 234 (6), 219 (2), 217 (3), 216 (3), 191 (68), 173 (16), 163 (11). Further elution afforded **5** (3 mg), ^1H NMR (200 MHz): δ0.86–0.96 (12H, complex signal), 1.23 (3H, *s*, H-14), 3.10 (1H, *t*, J = 7 Hz, H-2) 3.63 and 3.83 (each 1H, *d*, J = 12 Hz, H-15), 5.14 (1H, *m*, H-5); EIMS *m/z* (rel. int.): 311 $[M-C_3H_7]^+$ (1), 293 (1), 252 (1), 234 (3), 209 (21), 193 (5), 191 (39), 179 (9), 175 (13).

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