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TRITERPENES FROM *FERULA LINKII*

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Abstract—Two new natural triterpenes, 3β-acetoxyolean-9,12-diene and 3β-acetoxy-6β-hydroxyolean-9,12-diene, were isolated from *Ferula linkii*

INTRODUCTION

Ferula linkii Webb (Umbelliferae) is a species endemic to the Canary Islands [1], commonly known as 'cañaheja' or 'julan' Chemically, the *Ferula* genus is characterized by its content in sesquiterpenes and coumarins [2] In a previous work [3], we reported the isolation from this species of a new sesquiterpene, linkiol, with a carotane skeleton, as a major product

This paper describes the characterization of two triterpenoids also isolated from *Ferula linkii* The less polar of these compounds had a mass spectrum in accordance with the formula $C_{32}H_{52}O_2$ (M^+ at m/z 466 3801) The UV spectrum showed an absorption at 283 nm assignable to a homoannular diene The ^{1}H NMR spectrum had a one proton triplet at δ4 53 typical of the geminal proton to an equatorial acetate at C-3 in a triterpenoid and a double doublet at 5 49 and 5 57 corresponding to the two dienic homoannular hydrogens

These data permitted the assignment to this substance of the oleane structure **1**, or its equivalent in the ursane series The non-identity of the new compound with the ursane product previously isolated from *Salvia broussonetii* [4], and the presence in the ^{1}H NMR spectrum of methyl group resonances in the form of singlets pointed to an oleane skeleton

In the mass spectrum, the base peak and molecular ion coincided at m/z 466, due to the stability afforded the molecule by the diene system Another important fragment was observed at m/z 255 assignable to a cleavage in ring B with rearrangement of a hydrogen A further fragmentation of this type with cleavage in ring D formed a fragment at m/z 313, which through loss of acetic acid gave an ion at m/z 253 Assuming that the rearranged hydrogen is derived from the methyl groups at C-8 and C-14, respectively, these fragments may be represented as shown in Scheme 1

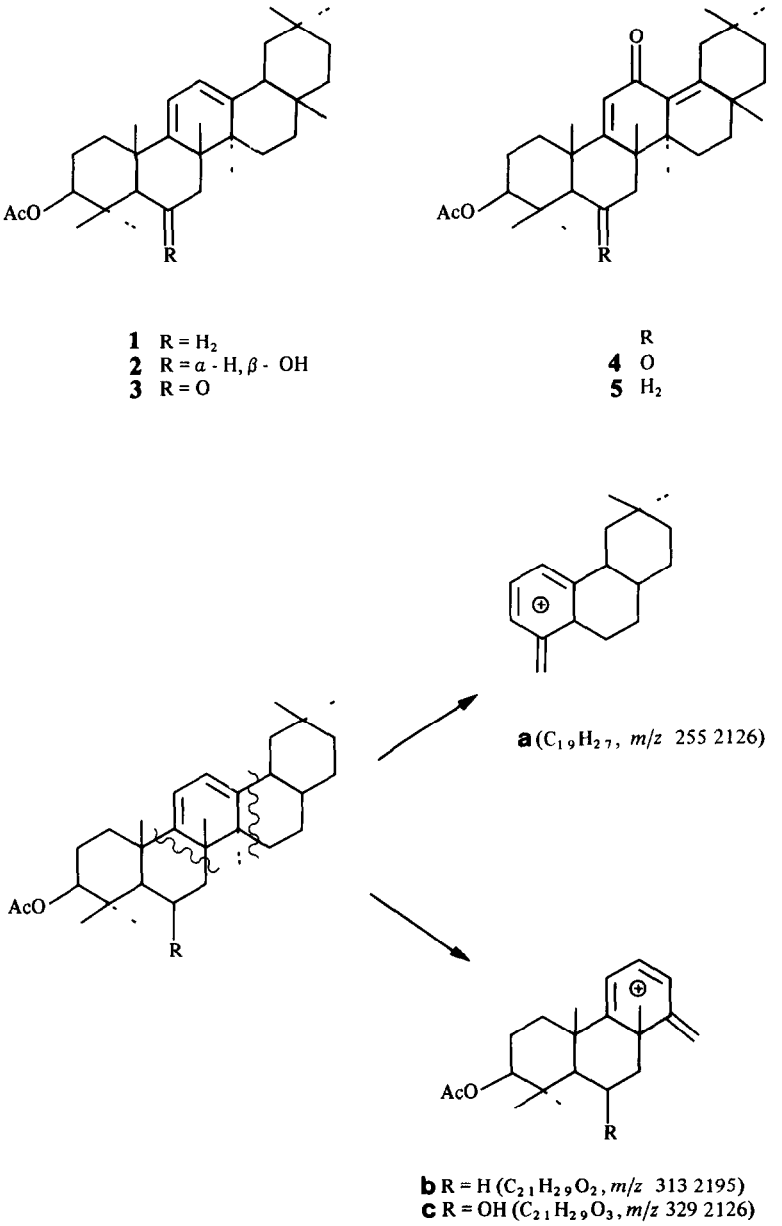
The confirmation of the 3β-acetoxy-olean-9,12-diene structure for **1** was achieved by study of its ^{13}C NMR spectrum (Table 1) and by comparison of its physical properties with those given for a synthetic sample [5, 6] This is the first time that this compound has been isolated from nature

A second new triterpene (**2**) isolated from this species showed a similar absorption to **1** in the UV spectrum Its ^{1}H NMR spectrum was also very similar to that of **1**, except for the chemical shift of the methyl groups and the appearance of a new broad singlet at δ4 65, assignable to a proton geminal to an axial hydroxyl group The presence in its mass spectrum of a peak at m/z 255, corresponding to fragment **a** (Scheme 1), and the absence of one for **b**, showed that the hydroxyl group was on ring A or B Moreover, a peak at m/z 329 corresponding to a new fragment **c**, corresponding to a hydroxyl substituted form of **b**, was observed

The axial hydroxyl group was assumed to be located at the 6β- or 7α-position, because under normal acetylation conditions the acetate was not formed The ^{1}H NMR chemical shift of the methyl group in ring A and B pointed to 6β Moreover, the physical and spectroscopic properties of **2**, were also different from those reported for the monoacetate of castanopsin (3β-acetoxy-7α-hydroxyolean-9,12-diene) [7] Therefore the second triterpene was assigned the structure 3β-acetoxy-6β-hydroxyolean-9,12-diene (**2**)

The location of the hydroxyl group at C-6 in **2** was confirmed by oxidation with Jones reagent In this way two compounds were obtained, the less polar was the normal oxidation product with structure **3** In its ^{1}H NMR spectrum a pair of doublets at δ2 72 and 2 05, and a singlet at 2 35, were assigned to the hydrogens at C-7 and C-5 The ^{13}C NMR spectrum of this compound was characteristic of a triterpene with a carbonyl group at C-6 [8] Thus, by comparison of the spectra of **1** and **3** (Table 1), it is seen that in the latter compound the signal corresponding to C-6 disappeared and that those of C-5 and C-7 were displaced downfield These effects produced by the influence of the ketone at C-6 are similar to those given for steroids [9] and for the lupane triterpenes [8] with this substituent

The structure **4** was assigned to the more polar compound obtained from the oxidation The high resolution mass spectrum indicated the introduction into the molecule of a new oxygen atom, according to the formula $C_{32}H_{46}O_4$ Its UV spectrum showed an increase in conjugation compared with the ketone **3** In the IR



Scheme 1 Mass spectral fragmentation of compound **1** (R = H) and compound **2** (R = OH)

spectrum three carbonyl absorptions were observed, one was typical of the acetate, a second was a six membered ring ketone and a third a conjugated ketone The ^{1}H NMR spectrum of this last product showed the proton at C-11 as a singlet at δ 5 92 The appearance in this spectrum of a pair of doublets at 3 39 and 2 72, assignable to the hydrogens at C-19, is also characteristic

The acetate **1** was also subjected to the oxidation conditions mentioned above for **2** In this way the conjugated ketone **5** was obtained In its ^{1}H NMR spectrum there appeared, as in **4**, the proton at C-11 and the pair of doublets typical of the hydrogen at C-19 This compound (**5**) was identical (mp, UV) with one described in the literature [10]

Relatively few natural triterpenes are homoannular dienes but among those known are saikogenin B [11], echinatic acid [12, 13], α-amiradienyl acetate [4], isomacedonic acid [14], castanopsin [7] and isomeristropic acid [15]

EXPERIMENTAL

Mps are uncorr Optical activities and IR spectra were taken in $CHCl_3$ UV spectra in EtOH and NMR in $CDCl_3$ MS were measured at 70 eV (probe)

Isolation of the triterpenes The dry fruits of *Ferula linkii* Webb (3 1 kg), collected in San Mateo (Gran Canaria, Canary Islands), were extracted with EtOH in a Soxhlet The alcoholic extract was steam distilled and the residue then extracted with petrol, C_6H_6 and $CHCl_3$ This yielded 300 g of petrol extract, 125 g of this

Table 1 ^{13}C NMR spectral data for compounds 1 and 3 (90 MHz)

Carbon	1	3	Carbon	1	3
1	37 1	37 1	16	27 2	27 1
2	24 3	23 8	17	32 1	32 3
3	80 6	80 1	18	45 6	45 9
4	37 9	36 9	19	46 8	46 7
5	51 2	59 9	20	31 1	31 2
6	18 2	211 9	21	34 6	34 6
7	32 1	48 5	22	36 9	36 9
8	38 6*	41 2	23	28 7	28 7
9	153 9	149 6	24	16 8	16 4
10	40 7*	47 6	25	20 0†	19 3‡
11	115 9	116 6	26	21 0†	20 8‡
12	120 7	120 9	27	28 1	27 9
13	147 2	148 9	28	25 3	25 5
14	42 7	42 8	29	33 2	33 2
15	25 6	25 7	30	23 7	23 3

*,†,‡These values may be interchanged

material were chromatographed on silica gel A mixture of waxes and triterpenes was initially obtained from which **1** (200 mg) was isolated by crystallization, leaving a complex mixture of sesquiterpenes including linkiol

The triterpene **2** was isolated as a minor product (90 mg) from the air-dried roots collected in the same place and in accordance with the experimental data reported [3] and by rechromatography of some fractions obtained when linkiol was isolated

3β-Acetoxy-olean-9,12-diene (**1**) Mp 223–226° $[\alpha]_D$ +315° (*c* 1 5) (lit [5] mp 217°, $[\alpha]_D$ +342°), $[M]^+$ at *m/z* 466 3801 (calc for $C_{32}H_{50}O_2$ 466 3810) IR ν_{max} cm^{-1} 2940, 2850, 1720, 1460, 1380, 1365, 1260, 1030, 980, 900, 835, 820 UV λ_{max} nm 283 (lit 281 [6]) ^1H NMR (90 MHz) δ 5 49 and 5 57 (each 1H, *d*, *J* = 6 Hz, H-11 and H-12), 4 53 (1H, *t*, H-3), 2 06 (3H, *s*), 1 28 (6H, *s*), 1 23, 1 16 and 0 99 (each 3H, *s*) and 0 90 (9H, *s*) EIMS *m/z* (rel int) 466 $[M]^+$ (100), 451, 313, 255, 253, 218

3β-Acetoxy-6β-hydroxy-olean-9,12-diene (**2**) Mp 224–229°, $[M]^+$ at 482 3744 (calc for $C_{32}H_{50}O_3$ 482 3760) IR ν_{max} cm^{-1} 3600, 2910, 2820, 1720, 1600, 1460, 1380, 1365, 1260, 1030, 980, 940, 835, 830 UV λ_{max} nm 295 ^1H NMR (60 MHz) δ 5 57 and 5 72 (each 1H, *d*, *J* = 6 Hz, H-11 and H-12), 4 65 (1H, *br s*, H-6), 4 50 (1H, *t*, H-3), 2 07 (3H, *s*), 1 58 and 1 41 (each 3H, *s*), 1 25, 0 95 and 0 86 (each 6H, *s*) ^1H NMR (90 MHz, C_6D_6) δ 5 68 (2H, *s*, H-11) and H-12), 4 70 (1H, *t*, H-3), 4 30 (1H, *br s*, H-6), 1 92 (3H, *s*), 1 83, 1 54, 1 44 and 1 09 (each 3H, *s*), 1 05 and 0 98 (each 6H, *s*) EIMS *m/z* (rel int) 482 $[M]^+$ (100), 329 (6), 255 (7), 123 (34), 109 (12), 95 (13)

Oxidation of **2** The monoacetate **2** (70 mg) in Me_2CO was treated with a slight excess of the 8 N CrO_3 reagent at room temp for 20 min MeOH was then added, the mixture was poured into H_2O, the products recovered in EtOAc and subjected to dry column chromatography on silica gel Elution with petrol–EtOAc (20 1) gave 3β-acetoxy-6-oxo-olean-9,12-diene (**3**) (42 mg), mp 189–193°, $[M]^+$ 480 3614 (calc for $C_{32}H_{48}O_3$ 480 3603) IR ν_{max} cm^{-1} 2910, 2840, 1720, 1710, 1460, 1380, 1260, 1040, 990, 970, 840, 820 UV λ_{max} nm 295 ^1H NMR (60 MHz) δ 5 66 (2H, *br s*, H-12 and H-13), 4 44 (1H, *t*, H-3), 2 72 and 2 05 (each 1H, *d*, *J* = 16 Hz, H-7), 2 35 (1H, *s*, H-5), 2 06 (3H, *s*), 1 29 (3H, *s*), 1 23 (6H, *s*), 1 06 (3H, *s*), 0 90 (6H, *s*) and 0 86 (3H, *s*) EIMS *m/z* (rel int) 480 $[M]^+$ (100), 465 (6), 405 (5), 327 (7), 297 (7), 283 (13), 255 (34), 203 (13), 159 (10), 149 (17), 135 (13) Further elution gave *3β-acetoxy-6,12-dioxo-olean-9,13-diene* (**4**) (16 mg), mp 224–228°, $[M]^+$ 494 3414 (calc for $C_{32}H_{46}O_4$, 494 3396) IR ν_{max} cm^{-1} 2960, 2920, 2860, 1720, 1710, 1640, 1590, 1460, 1380, 1370, 1360, 1280, 1260, 1180, 1140, 1100, 1040, 990, 970, 910, 890 UV λ_{max} nm 225, 252 and 301 ^1H NMR (90 MHz) δ 5 92 (1H, *s*, H-11), 4 46 (1H, *t*, H-3), 3 39 and 2 72 (each 1H, *d*, *J* = 15 Hz, H-19), 2 40 (1H, *s*, H-5), 2 08 (3H, *s*), 1 36, 1 34, 1 30, 1 27 and 1 13 (each 3H, *s*), 1 02 (6H, *s*), 0 95 (3H, *s*) EIMS *m/z* (rel int) 494 $[M]^+$ (100), 479 (36), 343 (17), 269 (89), 217 (22), 173 (14), 83 (22)

Treatment of **1** *with Jones reagent* Compound **1** (80 mg) was treated as described above for **2** with 8 N CrO_3 for 30 min In this way starting material (14 mg) and *3β-acetoxy-12-oxo-olean-9,13-diene* (**5**) (56 mg) were obtained after chromatography on silica gel, using petrol–EtOAc (20 1) Compound **5**, mp 186–188°, IR ν_{max} cm^{-1} 2960, 2880, 1725, 1630, 1585, 1460, 1380, 1360, 1310, 1260, 1170, 1030, 980, 905, 890 UV λ_{max} nm 260 and 298 ^1H NMR (90 MHz) δ 5 80 (1H, *s*, H-11), 4 52 (1H, *t*, H-3), 2 37 and 3 35 (each 1H, *d*, *J* = 15 Hz, H-19), 2 04 (3H, *s*), 1 26 and 1 12 (each 6H, *s*), 0 97 (3H, *s*), 0 92 (9H, *s*) EIMS *m/z* (rel int) 480 $[M]^+$ (53), 465 (34), 405 (3), 329 (6), 269 (100), 231 (14), 217 (10), 189 (10), 173 (3), 149 (6)

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