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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 broussonetti* [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.

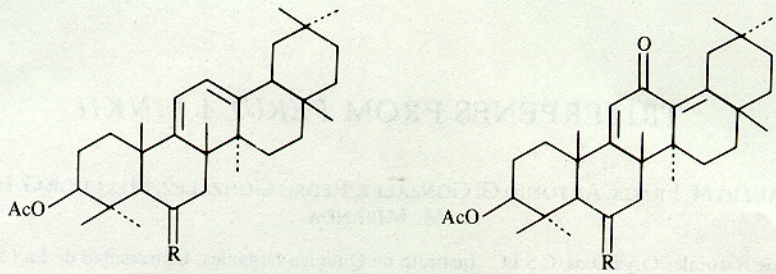
The confirmation of the 3β-acetoxy-olean-9,12-diene structure for **1** was achieved by study of its ^{13}C NMR spectrum (Table 1) and by comparison of its physical properties with those given for a synthetic sample [5, 6]. This is the first time that this compound has been isolated from nature.

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**).

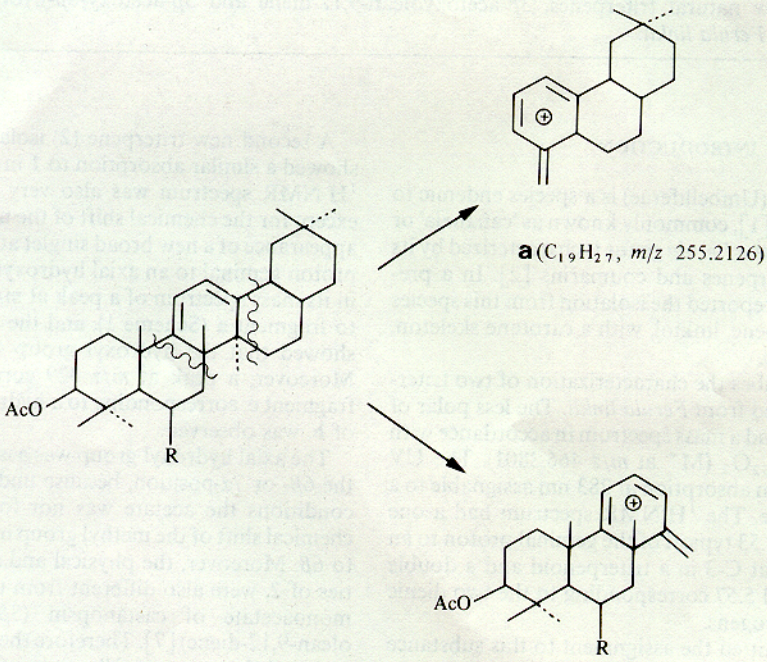
The location of the hydroxyl group at C-6 in **2** was confirmed by oxidation with Jones reagent. In this way two compounds were obtained, the less polar was the normal oxidation product with structure **3**. In its ^{1}H NMR spectrum a pair of doublets at δ 2.72 and 2.05, and a singlet at 2.35, were 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



1 R = H_2
2 R = α - H, β - OH
3 R = O

R
4 O
5 H_2



a ($C_{19}H_{27}$, *m/z* 255.2126)

b R = H ($C_{21}H_{29}O_2$, *m/z* 313.2195)
c R = OH ($C_{21}H_{29}O_3$, *m/z* 329.2126)

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): δ5.49 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): δ5.57 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): δ5.68 (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): δ5.66 (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): δ5.92 (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): δ5.80 (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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REFERENCES

1. Pitart, J. and Proust, L. (1908) *Les Iles Canaries, Flore de l'Archipel*, p. 205. P. Klincksiek, Libraire des Sciences Naturelles, Paris.
2. Saidkhodzhaev, A. I. (1979) *Khim. Prir. Soedin*, 437.
3. González, A. G., Fraga, B. M., Hernández, M. G., Luis, J. G., Estévez, R., Baez, J. L. and Rivero, M. (1977) *Phytochemistry* **16**, 265.
4. González, A. G., Fraga, B. M. and Ravelo, A. G. (1972) *An. Quím.* **68**, 1433.
5. Picard, C. W. and Spring, F. S. (1940) *J. Chem. Soc.* 1198.
6. Agata, I., Corey, E. J., Hortmann, A. G., Klein, J., Proskow, S. and Ursprung, J. J. (1965) *J. Org. Chem.* **30**, 1698.
7. Pant, P. and Rastogui, P. R. (1978) *Phytochemistry* **19**, 575.
8. Dantanarayana, A. P., Kumar, N. S., Muthukuda, P. M. and Wazeer, M. I. M. (1973) *Phytochemistry* **21**, 2065.
9. Eggert, H. and Djerassi, C. (1973) *J. Org. Chem.* **38**, 3788.
10. Beaton, J. M., Johnston, J. D., McKean, L. C. and Spring, F. S. (1953) *J. Chem. Soc.* 3660.
11. Kubota, T. and Tonami, F. (1967) *Tetrahedron* **23**, 3335.
12. Kirlayov, N. P. and Naugolnaya, T. N. (1963) *Zh. Obsch. Khim.* **33**, 700.
13. Kiryalov, N. P. and Bogatkina, V. F. (1969) *Khim. Prir. Soedin* 447.
14. Zorina, A. D., Matyukhina, L. G., Saltykova, I. A. and Shavva, A. G. (1973) *Zh. Org. Chem.* **9**, 1673.
15. Amirov, G. S. (1982) *Khim. Prir. Soedin* 262.