Rhoiptelenol and Rhoiptelenone, Two Pentacyclic Triterpenes from *Sideritis macrostachya*

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The pentacyclic triterpenes rhoiptelenol and rhoiptelenone have been isolated from *Sideritis macrostachya.* Rhoiptelenone is a new natural compound, whose structure has been determined as D:B-*friedo*-urs-5-en-3-one. The ^{1}H and ^{13}C NMR spectra of rhoiptelenol, rhoiptelenol acetate and glutinol have been reassigned. The natural occurrence of the D:B-*friedo*-ursene and D:B-*friedo*-oleanene derivatives has been examined.

Key words: Sideritis macrostachya, Triterpenes, Rhoiptelenone

Introduction

In the biosynthesis of the pentacyclic triterpenes, the formation of β-amyrin (**1**) and α-amyrin (**6**) from the lupenyl cation occurs by two and three 1,2-hydride shifts, respectively, and finalizes, in both cases, with the elimination of the H-12 proton to give a 12,13-double bond by neutralization of the 13-oleanyl and the 13-ursanyl cation, respectively (Fig. 1). When the rearrangement of these ions progresses the multiflorenol (**2**) and bauerenol (**5**) frameworks are produced, respectively, by migration of the carbocation to the C-8 position and formation of the 7,8-double bond. It is also known that these types of rearrangement can indeed be more complicated, the C-8 cation migrating to C-5, *via* C-9 and C-10, to finalize giving a 5,6-double bond, as in the case of glutinol (**3**) in the rearrangement of the 13-oleanyl cation (Torssell, 1983). The analogous compound in the corresponding rearrangement of the ursanyl cation had only been isolated from *Ficus thunbergii* by Kitajima *et al.* (1994) and named rhoiptelenol (**4**). Its role in the biosynthesis of the pentacyclic triterpenes, filling a place in the rearrangement of these compounds, has not been highlighted. We have now isolated it, together with a new ketone, rhoiptelenone (**8**), from *Sideritis macrostachya* (Lamiaceae), a plant endemic to Tenerife. Rhoiptelenol (**4**) had previously been obtained from other *Sideritis* species and erroneously identified as glutinol (**3**) (González *et al.*, 1979). The corresponding 28-acid, rhoiptelic acid, has been isolated from *Rhoiptelea chiliantha* (Jiang *et al.*, 1995).

Results and Discussion

The high resolution MS of **4** was in accordance with the molecular formula $C_{30}H_{50}O$ (*m/z* 426.3867) and the resonance values of the ^{1}H and ^{13}C NMR spectra were identical with those reported for rhoiptelenol (Kitajima *et al.*, 1994). However, our 2D NMR studies of this compound, and of its acetate **4a**, were not in accordance with several assignments of these spectra. Therefore, we have reassigned the resonances of 26-H and 27-H in the ^{1}H NMR spectrum (see Experimental) and those of C-1, C-2, C-7, C-26 and C-27 in the ^{13}C NMR spectrum in **4** and **7** (Table I). On the other hand, since the ^{13}C NMR spectrum of glutinol (**3**) of Kitajima *et al.* (1994) showed some erroneous assignments we have also examined four different studies where the carbon resonances of this triterpene have been assigned. The first and second publications (Mahato *et al.*, 1981; González *et al.*, 1987) contained several mistakes, which were corrected in the third and fourth study (Carvalho and Seita, 1993; Olea *et al.*, 1994). However, in these two last papers the C-13 and C-14 resonances are not unambiguously assigned and the

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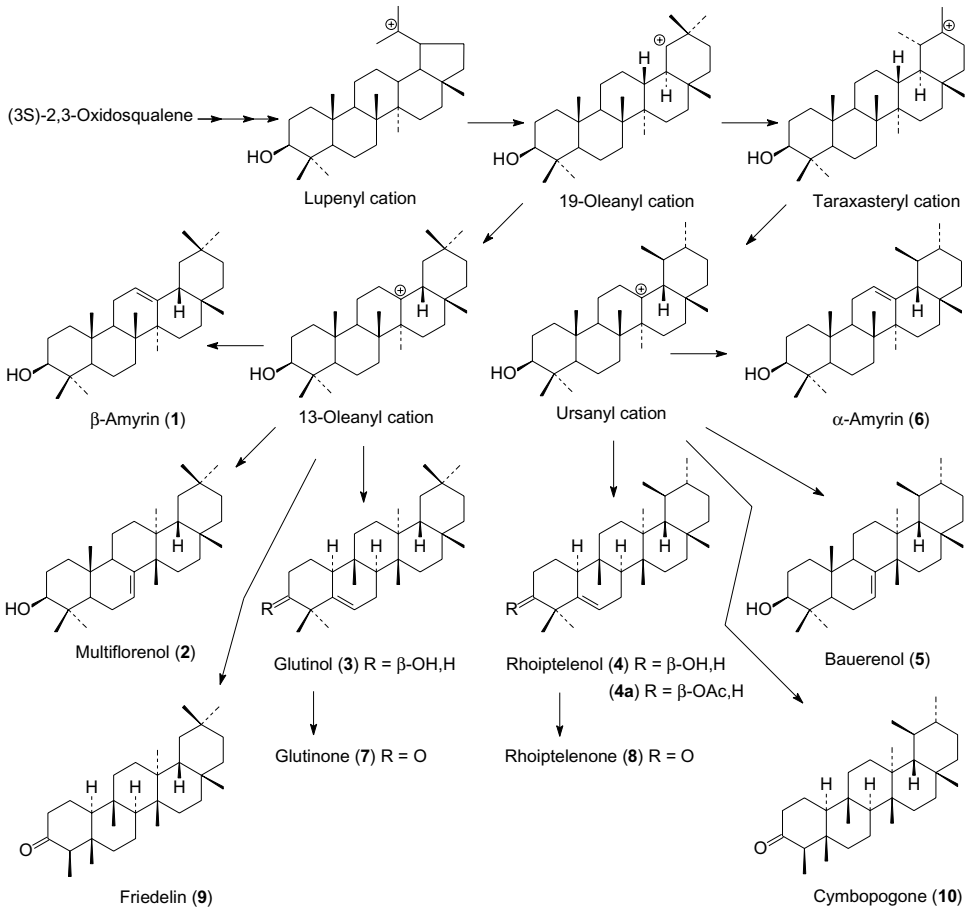

Fig. 1. Biosynthesis of pentacyclic triterpenes.

C-29 and C-30 values are interchanged. Thus, we have reassigned the ^{13}C NMR spectrum of glutinol (**3**) (Table I) using 2D NMR data (COSY, NOESY, HSQC and HMBC). Jiang *et al.* (1995) have assigned the signals of this spectrum in pyridine-d_5. A single crystal X-ray diffraction analysis of its acetate **4a** confirmed the structure of rhoiptelenol. Later a previous X-ray study of this compound was brought to our attention (Kiyotani *et al.*, 1996).

The structure of rhoiptelenone was determined as D:B-*friedo*-urs-5-en-3-one (**8**) on the basis of the following considerations: Its high resolution MS showed the molecular ion at 424.3754 ($C_{30}H_{48}O$) and the base peak at *m/z* 274.2622 ($C_{20}H_{34}$), which is a characteristic fragment due to the retro-Diels-Alder cleavage of ring B. The oxygen atom of the molecule was in the form of a carbonyl group (ν_{max} 1715 cm^{-1}, δ_C 215.4 ppm). Its ^1H NMR spectrum showed the resonances of the vinylic hydrogen at C-6 as a triplet at δ 5.66, of H-29 and H-30 at the two secondary methyl groups at 0.98 and 0.88 (each 1H, d, J = 6 Hz), and of six angular methyl groups. Other resonances observed in this spectrum were the two H-2 at δ 2.41 (m), H-10 at 2.26 (m) and H-18 at 1.33 (d, J = 2.6 Hz). The ^{13}C NMR spectrum (Table I) showed the presence, besides the carbonyl group, of eight methyl, nine methylene and six methine groups and six quaternary carbon atoms. Of special relevance was the high value of the resonance of the C-28 methyl group at δ 38.6, in comparison with that of glutinol at 32.1, which was due to the presence in **4** of the C-29 β-methyl group at C-19. This deshielded resonance of C-28 has also been observed in the spectra of bauerenol (**5**) (δ 38.0)

Table I. ^{13}C NMR data of compouds **3**, **4**, **4a** and **8**.

C	**3**	**4**	**4a**	**8**
1	18.2	18.0	18.7	21.4
2	27.8	27.7	25.3	38.0
3	76.3	76.3	78.5	215.4
4	40.8	40.7	39.0	49.9
5	141.6	141.7	142.0	142.5
6	122.0	122.1	120.0	121.3
7	23.6	23.9	23.7	23.8
8	47.4	45.2	45.1	44.8
9	34.8	34.7	34.6	34.8
10	49.6	49.7	49.8	50.7
11	34.6	34.2	34.2	33.6
12	30.3	28.3	28.3	28.3
13	39.3	39.8	39.8	38.8
14	37.8	39.8	39.8	39.6
15	32.1	28.3	28.2	28.2
16	36.0	34.6	34.8	34.7
17	30.1	31.3	31.2	31.3
18	43.0	52.3	52.3	52.3
19	34.8	35.6	35.6	35.6
20	20.2	31.8	31.9	31.8
21	33.1	29.6	29.7	28.1
22	38.9	37.5	37.6	37.6
23	28.9	28.4	28.9	29.0
24	25.5	24.4	25.4	25.1
25	16.2	16.5	17.1	22.4
26	19.6	25.1	25.2	24.9
27	18.4	14.9	15.0	14.9
28	32.1	38.6	38.7	38.6
29	34.5	15.0	15.3	15.2
30	32.1	22.4	22.4	16.9

and its derivatives, which possess similar D and E rings to rhoiptelenol (**4**) (Cerda-García-Rojas *et al.*, 1996). The structure of rhoiptelenone (**8**) was confirmed by oxidation of **4** with Cornforth's reagent, which led to a compound identical with the natural product. The corresponding 3-oxo-derivative in the glutinol series, named glutinone or alnusenone (**7**), had been isolated in 1953 from *Alnus glutinosa* (Chapon and David, 1953).

Recently, Kushiro *et al.* (2000) have carried out the functional expression in yeast of a cDNA clone from *Arabidopsis thaliana*, which codes for a multifunctional triterpene synthase catalysing the formation of nine different triterpenes, including **1**, **2**, **5** and **6**. Products such as rhoiptelenol (**4**) should help to identify the function of remaining homologous genomic clones and triterpene constituents in this type of studies.

From the data on the isolation of this type of pentacyclic triterpenes it may be deduced that the rearrangement of the 13-oleanyl cation occurs more easily in plants than that of the 13-ursanyl cation. Thus, glutinol (**3**) occurs more frequently in nature than rhoiptelenol (**4**), and indeed, whilst friedelin (**9**), which is formed by a more complicated rearrangement of the 13-oleanyl cation, had been isolated until 1979 from almost three hundred different plant species (Chandler and Hooper, 1979), the corresponding 3-oxo derivative in the rearranged ursane series, cymbopogone (**10**), has only been isolated once (Crawford *et al.*, 1975).

Experimental

General experimental procedures

Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. IR spectra were recorded in a Perkin-Elmer 1600. ^1H NMR spectra were recorded in $CDCl_3$ solutions 500.13 MHz with a Bruker AMX2-500 spectrometer. ^{13}C NMR spectra were run in $CDCl_3$ at 50.32 and 125.13 MHz with a Bruker AC-200 or a Bruker AMX2-500, respectively. Chemical shifts are given in ppm (δ). Mass spectra and HRMS were taken at 70 eV in a Micromass Autospec spectrometer. Dry column chromatographies were made on Si gel Merck 0.2–0.065 mm.

Plant material, extraction and isolation of compounds

The air-dried aerial part of the plant (4 kg), collected at "El Bailadero" in the Anaga region of Tenerife (Canary Islands) was chopped and extracted several times with EtOH in a Soxhlet. The cold extract was filtered and concentrated *in vacuo*. The part of the syrup soluble in $CHCl_3$ was chromatographed on a silica gel column eluting with hexane, hexane-EtOAc and EtOAc. In this way several mixtures of compounds were obtained, which were rechromatographed in silica gel to afford in order of elution rhoiptelenone (**8**) (40 mg), rhoiptelenol (**4**) (28 mg) and the previously reported β-amyrin, β-sitosterol, erythrodiol and a mixture of ursolic and oleanolic acids (González *et al.*, 1979).

Rhoiptelenol (**4**)

M. p. 210–213 °C (MeOH). – $[\alpha]_D$ + 65° (*c* 1.90, $CHCl_3$) (Lit: 219–221, $[\alpha]_D$ + 63°). – IR ν_{max} 3440, 2890, 1460, 1390, 1040, 980, 830 cm^{-1}. – ^1H NMR

($CDCl_3$, 500 MHz) δ 0.87 (3H, s, H-25), 0.88 (3H, d, J = 7 Hz, H-30), 0.90 (3H, s, H-27), 0.96 (3H, d, J = 6.5 Hz, H-29), 0.97 (3H, s, H-26), 1.03 (3H, s, H-23), 1.05 (3H, s, H-28), 1.12 (3H, s, H-24), 1.33 (1H, d, J = 2.7 Hz, H-18), 1.50 (1H, dd, J = 10.7 and 2.7 Hz, H-19), 2.02 (1H, m, H-10), 3.46 (1H, br s, H-3) and 5.60 (1H, m, H-6). – EIMS *m/z* 426 [M^+] (7), 408 (15), 393 (5), 274 (100), 259 (30), 245 (6), 205 (36), 173 (18), 161 (7), 150 (18), 134 (45), 123 (42), 107 (20). – HREIMS *m/z* 426.3857 (calcd. for $C_{30}H_{50}O$, 426.3861). *Acetate* (**4a**): M. p. 190–192 °C (MeOH). – $[\alpha]_D$ + 72° (*c*, 1.36) (Lit: 205–207, $[\alpha]_D$ + 73). – ^1H NMR ($CDCl_3$, 500 MHz) δ 0.87 (3H, s, H-25), 0.89 (3H, d, J = 7 Hz, H-30), 0.90 (3H, s, H-27), 0.97 (3H, d, J = 6.5 Hz, H-29), 0.98 (3H, s, H-26), 1.02 (3H, s, H-24), 1.04 (3H, s, H-28), 1.05 (3H, s, H-23), 1.32 (1H, d, J = 2.5 Hz, H-18), 1.60 (1H, dd, J = 10.6 and 2.7 Hz, H-19), 1.82 (1H, m, H-2), 1.99 (3H, s, –OAc), 2.01 (1H, m, H-10), 5.52 (1H, t , J = 2.5 Hz, H-6). – EIMS *m/z* 468 [M^+] (7), 408 (13), 393 (5), 274 (100), 259 (25), 205 (27), 187 (5), 173 (15), 159 (6), 150 (11), 134 (23), 123 (29), 109 (26). – HREIMS *m/z* 468.3966 (calcd. for $C_{32}H_{52}O_2$, 468.3967).

Rhoiptelenone (**8**)

M. p. 158–160 °C (acetone), $[\alpha]_D$ + 51° (*c*, 1.48). – IR ν_{max} 2865, 1715, 1452, 1382, 1265, 1000, 815 cm^{-1}. – ^1H NMR ($CDCl_3$, 500 MHz) δ 0.84 (3H, s, H-25), 0.88 (3H, d, J = 6 Hz, H-30), 0.92 (3H, s, H-27), 0.97 (3H, s, H-26), 0.98 (3H, d, J = 6 Hz, H-29), 1.05 (3H, s, H-28), 1.21 (3H, s, H-24), 1.22 (3H, s, H-23), 1.33 (1H, d, J = 2.6 Hz, H-18), 1.85 (1H, m, H-1), 1.93 (1H, m, H-7), 2.26 (1H, m, H-10), 2.41 (2H, m, H-2), 5.66 (1H, t, J = 2.5 Hz, H-6). – EIMS *m/z* 424 [M^+] (11), 274 (100), 259 (22), 245 (7), 205 (29), 189 (8), 188 (5), 163 (6), 150 (13), 149 (11), 137 (18), 123 (40), 109 (25). – HREIMS *m/z* 424.3754 (calcd. for $C_{30}H_{48}O$, 424.3705).

Oxidation of **4**

A mixture of the alcohol **4** (10 mg), pyridine (0.2 ml) and Cornforth's reagent (0.3 ml) was stirred at room temperature for 48 h. The excess of reagent was destroyed with EtOH. The solvent was removed and the residue chromatographed on a short silica gel column eluting with hexane-EtOAc (85:15 v/v) to afford rhoiptelenone (**8**) (6.2 mg, 63 %). Cornforth's reagent was prepared by adding dropwise a solution of chromium oxide (VI) (100 mg) in water (0.1 ml) to pyridine (1 ml) at 0 °C.

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