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DITERPENOIDS FROM *GALEOPSIS ANGUSTIFOLIA*

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Key Word Index *Galeopsis angustifolia*; Labiatae; new furanic and prefuranic labdane diterpenes; retroaldol reaction.

Abstract—The structures of two new diterpenoids, galeopsin and pregaleopsin, isolated from aerial parts of *Galeopsis angustifolia* (Labiatae) have been shown to be 8β-acetoxy-15,16-epoxy-9α-hydroxylabda-13(16),14-dien-7-one and 8β-acetoxy-9α,13*R*; 15,16-diepoxylabd-14-en-7-one, respectively, by chemical and spectroscopic studies. The previously known diterpenoid hispanolone (15,16-epoxy-9α-hydroxy-8α-labda-13(16),14-dien-7-one) has been also obtained from the same source. A thermal retroaldol reaction in the absence of solvent experienced by these 9-hydroxy-7-keto-labdanes is also described.

INTRODUCTION

In our search for new natural diterpenoids in the Labiatae plants endemic in the Mediterranean area [1–4], we have examined the aerial parts of *Galeopsis angustifolia* Hoffm., a species which grows all over Europe. From this plant three diterpenic compounds have been isolated, one of which is the previously known hispanolone (**1**) [5] and the other two are new substances, whose structures are established as 8β-acetoxy hispanolone (**2**, galeopsin) and 8β-acetoxy-13*R*-prehispanolone (**3**, pregaleopsin).

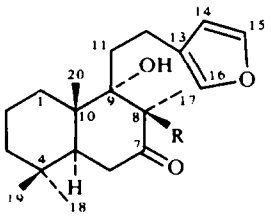
RESULTS AND DISCUSSION

Galeopsin (**2**) had a molecular formula of $C_{22}H_{32}O_5$ and its IR spectrum showed typical absorptions for alcoholic (3550 cm^{-1}), furan (3140, 1500, 880 cm^{-1}), acetoxyl group (2.12, 3 H, *s*) and four methyl singlets, one of groups. The ^1H NMR spectrum of **2** showed characteristic signals for a β-substituted furan ring (two α-furan protons at δ 7.49 and 7.37, and one β-furan proton at 6.40), an acetoxyl group (2.12, 3 H, *s*) and four methyl singlets, one of which (1.51) may be geminal to the acetoxyl group or to a tertiary hydroxyl group. The prominent peaks at *m/e* 81 and 95 in the MS of galeopsin (**2**) were also indicative of the presence of the β-substituted furan ring in the molecule [6].

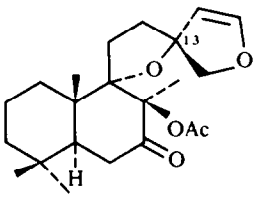
IR absorption at 3550 cm^{-1} and the absence of signals between δ 2.5 and 6.3 in the ^1H NMR spectrum of **2** pointed toward the presence of a tertiary hydroxyl group in this new diterpenoid. In fact, the ^1H NMR spectrum of compound **2** was almost identical to the spectrum of hispanolone (**1**) [5], the only differences being the substitution of the C-17 methyl doublet of **1** by the singlet at 1.51 and the presence of a tertiary acetoxyl function (a 3 H singlet at 2.12). These data were in agreement with structure **2** for galeopsin.

On the other hand, comparison between the ^{13}C NMR spectra of hispanolone (**1**) and galeopsin (**2**) (Table 1), and the reported data on marrubiin and its 8β-hydroxyl derivative [6], were in very good agreement with an 8β-acetoxy-hispanolone structure for compound **2**. The β configuration (axial) of the acetoxyl group was also confirmed by comparing the frequencies of the carbonyl absorption band in the UV spectra of hispanolone (**1**, λ 282 nm, ε 34) and galeopsin (**2**, λ 288.5 nm, ε 81) [7]. However, this small bathochromic shift (typical value for an axial–OAc ≃ +10 nm) [7] suggested an almost axial configuration for the acetoxyl group of galeopsin. In fact, galeopsin (**2**) may possess the B ring in a boat conformation in which the 1,3-diaxial interactions between the 8β-OAc and the C-20 methyl group are minimized. This assumption was also supported by the similar chemical shift of the C-20 methyl group in hispanolone (**1**, 1.17) [5] and galeopsin (**2**, 1.22), because a 1,3-diaxial relationship causes a larger deshielding effect (Δδ ≃ +0.25) [8]. In agreement with all the above data, galeopsin (**2**) showed a small positive Cotton effect ($\Delta\varepsilon_{290} = +0.40$) whereas hispanolone (**1**, with ring B in the chair conformation) [5] possessed a small negative value ($\Delta\varepsilon_{287} = -0.98$) [9].

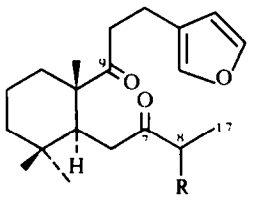
A less probable alternative structure for galeopsin, with the acetoxyl group at C-9 and the tertiary alcohol at C-8, was firmly discarded as follows. When hispanolone (**1**) was heated at 190° for 10 min under N_2 and without solvent, a new compound (**4**, $C_{20}H_{30}O_3$, isomeric with **1**) was obtained in good yield (70%) together with unchanged starting material (30%). The ^1H NMR spectrum of compound **4** showed a typical pattern for a $-CO-CH_2-CH_3$ group (a 2 H quartet at δ 2.38, $J = 7.5$ Hz and a 3 H triplet, $J = 7.5$ Hz at 1.01) whereas its ^{13}C NMR spectrum (Table 1) showed two carbonyl atoms at 214.6 and 209.9 ppm. Thus, a retroaldol reaction occurred and structure **4** must be assigned to the product (see also Experimental). Similarly, treatment of galeopsin (**2**) by the same conditions described for hispanolone, yielded a mixture of two C-8 isomeric compounds (**5**) which could not be separated, the ^1H NMR spectrum of



1 R = H
2 R = OAc



3



4 R = H
5 R = OAc (*R* and *S*)

which showed a typical pattern for the -CO.CHOAc.Me grouping (δ 5.20, 1 H, *q*, J = 7 Hz; 1.39, 3 H, *d*, J = 7 Hz; 2.17, 3 H, *s*, -OAc) in accordance with the mechanism of this retroaldol reaction [10].

The other new diterpenoid isolated from *Galeopsis angustifolia*, pregaleopsin (**3**), also had a molecular formula $C_{22}H_{32}O_5$ which lacked the hydroxyl group (no IR-OH absorption) found in galeopsin (**2**). The ^{1}H NMR spectrum of compound **3** was very similar to that of galeopsin (**2**), the differences being consistent with the occurrence of a β,β-disubstituted dihydrofuran [δ 6.41 and 5.11 (1 H each, *d*, J = 2.5 Hz, H-15 and H-14, respectively), 4.48 and 4.02 (AB system, J = 10.5 Hz, 2 H-16)] instead of the β-monosubstituted furan ring of galeopsin (**2**). The fragments at *m/e* 96 and 82 in the MS of compound **3** further supported this assignment, which was also in accord with its ^{13}C NMR spectrum (Table 1). All the data may be accommodated by structure **3** for pregaleopsin. This assignment was confirmed by the ready conversion of pregaleopsin (**3**) to galeopsin (**2**) by mild acidic reagents [11].

Finally, the 13*R* configuration assigned to pregaloepsin (**3**) was supported by NOE experiments. When the C-17 methyl signal (δ 1.34) was irradiated it gave a clear Overhauser effect (7.5%) on the AB system of the C-16 protons, whereas no NOE was observed on the C-14 proton. Since pregaleopsin showed a small positive Cotton effect ($\Delta\varepsilon_{289.5}$ = +0.37) as galeopsin (**2**), an identical ring B conformation must be expected for both compounds (*vide supra*), and the distance between the C-17 and C-16 protons in the molecule of pregaleopsin (**3**) is *ca* 1.6 Å.

It is possible that pregaleopsin (**3**) is the naturally occurring diterpenoid in *Galeopsis angustifolia* and that galeopsin (**2**) arises as an artefact from the extraction and isolation procedures. This has been previously noted in the case of other prefuranic and furanic diterpenes [11].

Table 1. ^{13}C NMR chemical shifts in ppm relative to TMS

Carbon No.	1	2	3	4
1	34.8 *t*	36.1 *t*	35.9 *t*	35.3 *t*
2	18.5 *t*	18.2 *t*	18.2 *t*	18.3 *t*
3	41.3 *t*	41.2 *t*	41.4 *t*	40.8 *t**
4	33.5 *s*	34.4 *s*	33.1 *s*	35.6 *s*
5	50.8 *d*	49.7 *d*	50.4 *d*	42.6 *d*
6	39.2 *t*	38.4 *t*	38.5 *t*	40.5 *t**
7	211.6 *s*	206.7 *s*	205.6 *s*	209.9 *s*
8	46.3 *d*	88.5 *s**	87.6 *s*	37.6 *t*†
9	81.6 *s*	81.9 *s**	97.0 *s**	214.6 *s*
10	43.3 *s*	44.7 *s*	43.2 *s*	52.1 *s*
11	31.9 *t*	30.8 *t*	33.8 *t*	33.8 *t*†
12	21.5 *t*	21.6 *t*	28.7 *t*	19.2 *t*
13	124.7 *s*	124.7 *s*	94.5 *s**	124.1 *s*
14	110.5 *d*	110.7 *d*	106.9 *d*	110.9 *d*
15	142.7 *d*	142.9 *d*	148.1 *d*	142.3 *d*
16	138.3 *d*	138.6 *d*	80.3 *t*	138.8 *d*
17	8.2 *q*	15.2 *q*	15.9 *q*	7.9 *q*
18	33.0 *q*	32.7 *q*	32.2 *q*	32.7 *q*
19	21.4 *q*	21.4 *q*†	21.3 *q*†	22.8 *q*
20	16.3 *q*	16.6 *q*	17.7 *q*	17.3 *q*
$OCO\underline{C}H_3$	--	21.6 *q*†	21.6 *q*†	—
$O\underline{C}OMe$	—	168.9 *s*	168.9 *s*	--

*† Values in any vertical column may be interchanged, but those given here are considered to be most likely.

EXPERIMENTAL

Mps were determined in a Kofler apparatus and are uncorr. ^{1}H NMR and ^{13}C NMR spectra were measured at 90 and 25.2 MHz, respectively, in $CDCl_3$ soln with TMS as int. standard. Assignments of ^{13}C NMR chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Elemental analyses were carried out in this laboratory (Madrid) with the help of an automatic analyser. Plant materials were collected in July 1979 near Molina de Aragón (Guadalajara, Spain) and voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy (Madrid 'Complutense' University).

Isolation of the diterpenoids. Dried and finely powdered *G. angustifolia* plants (470 g) were extracted 3 × with Me_2CO (2 l.) at room temp. for 2 days. The extracts were evapd to dryness under red. pres. and low temp. (30°), dissolved in EtOAc and washed with H_2O. After evapn of the solvent, the residue (20 g) was chromatographed on Si gel (Merck, 7734, deactivated with 15% H_2O) column (350 g). Elution with petrol and petrol–EtOAc (9:1) gave, in order of elution, hispanolone (**1**, 811 mg), galeopsin (**2**, 400 mg) and pregaloepsin (**3**, 495 mg).

Hispanolone (**1**) was identified by physical and spectroscopic data (mp, $[\alpha]_D$, IR, MS, ^{1}H NMR) and by comparison with an authentic sample.

Galeopsin (**2**): mp 154–156° (EtOAc–*n*-hexane); $[\alpha]_D^{25}$ + 25.7° (*c* 0.42, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3550, 3140, 3025, 3010, 2980, 2950, 1737, 1720, 1500, 1470, 1370, 1250, 1120, 1065, 1030, 990, 950, 880, 780, 740, 645. ^{1}H NMR: see Discussion, other C–Me singlets at δ 1.22, 0.91 and 0.87. ^{13}C NMR: see Table 1. MS (12 eV, direct inlet) *m/e* (rel. int.): 376 (M^+, 18), 358 (3), 334 (5), 316 (7), 289 (18), 281 (11), 253 (23), 210 (16), 193 (50), 175 (9), 137 (12), 123 (100), 115 (12), 109 (50), 95 (16), 87 (9), 81 (23), 69 (18). UV λ_{max}^{EtOH} nm (ε): 288.5 (81). CD: $\Delta\varepsilon_{327} = 0$, $\Delta\varepsilon_{290} = +0.40$, $\Delta\varepsilon_{247} = 0$ (C 0.28, EtOH). [Found: C, 70.31; H, 8.54. $C_{22}H_{32}O_5$ requires: C, 70.18; H, 8.57%].

Pregaleopsin (**3**): mp 146–148° and 178–180° (decomp.) (from EtOAc–*n*-hexane); $[\alpha]_D^{26}$ − 43.5° (*c* 1.09, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3135, 3100, 3050, 3000, 2970, 1720 (br), 1612, 1465, 1370, 1250, 1150, 1110, 1070, 1055, 1020, 955, 930, 895, 870, 855, 840, 795, 780, 730. ^{1}H NMR: δ 6.41 and 5.11 (1 H each, *d*, *J* = 2.5 Hz, H-15 and H-14, respectively), 4.48 and 4.02 (AB system, *J* = 10.5 Hz, 2 H-16), 2.05 (3 H, *s*, —OAc), C–Me singlets at 1.34 (3 H-17), 1.15 (3 H-20), 0.87 and 0.83 (3 H-18 and 3 H-19); Overhauser effect, irradiation of the signal at δ 1.34 caused a NOE of 7.5% on the 2 H-16 AB system, no NOE was observed when the signal at δ 1.15 was irradiated. ^{13}C NMR: see Table 1. MS (12 eV, direct inlet) *m/e* (rel. int.): 376 (M^+, 20), 334 (50), 317 (30), 289 (17), 281 (11), 253 (22), 210 (33), 193 (50), 179 (22), 175 (11), 137 (17), 123 (100), 115 (11), 109 (47), 96 (17), 95 (22), 87 (11), 82 (22), 81 (27), 69 (22). UV λ_{max}^{EtOH} nm (ε): 288 (75). CD: $\Delta\varepsilon_{328} = 0$, $\Delta\varepsilon_{289.5} = +0.37$, $\Delta\varepsilon_{247} = 0$ (C 1.04, EtOH). [Found: C, 70.43; H, 8.49. $C_{22}H_{32}O_5$ requires: C, 70.18; H, 8.57%].

Diketone **4** *from hispanolone* (**1**). Hispanolone (**1**, mp 146°, 1.2 g) was heated under N_2 at 190° for 10 min (without solvent). The resulting material was chromatographed on Si gel (Merck, 7734, deactivated with 15% H_2O) column (130 g). Elution with petrol–EtOAc (19:1) gave, in order of elution, compound **4** (760 mg) and starting material (**1**, 320 mg). Compound **4** was a syrup: n_D^{14} 1.5049; $[\alpha]_D^{24}$ − 18.1° (*c* 1.51, $CHCl_3$). IR ν_{max}^{NaCl} cm^{-1}: 3145, 3120, 1710, 1695, 1505, 1030, 880, 790. ^{1}H NMR: δ 7.29 and 7.21 (1 H each, *m*, $W_{1/2}$ = 3 Hz, H-15 and H-16), 6.29 (1 H, *m*, $W_{1/2}$ = 3 Hz, H-14), 2.38 (2 H, *q*, *J* = 7.5 Hz, partially overlapped with other signals, 2 H-8), 1.01 (3 H, *t*, *J* = 7.5 Hz, 3 H-17), C–Me singlets at 1.09, 0.86 and 0.78. On irradiation of the signal at δ 1.01 collapsed to singlet the quartet at δ 2.38, and irradiation at the last field transformed into a singlet the C-17 Me triplet (δ 1.01). ^{13}C NMR: see Table 1. MS (75 eV, direct inlet) *m/e* (rel. int.): 318 (M^+, 9), 223 (3), 195 (18), 177 (9), 166 (3), 152 (6), 123 (93), 109 (57), 95 (24), 81 (69), 69 (36), 57 (100). [Found: C, 75.67; H, 9.36. $C_{20}H_{30}O_3$ requires: C, 75.43; H, 9.50%].

Compounds **5**. Thermal treatment of galeopsin (**2**, 80 mg) as described above for hispanolone, gave a ~3:2 mixture of compounds **5** (43 mg) besides starting material (**2**, 21 mg). **5** was a syrup: IR ν_{max}^{NaCl} cm^{-1}: 3140, 3120, 1730, 1695, 1503, 1240, 1030, 880, 790. ^{1}H NMR major isomer (minor isomer): δ 7.45 and 7.35 (1 H each, *m*, H-15 and H-16), 6.37 (1 H, *m*, $W_{1/2}$ = 3 Hz, H-14), 5.20 (5.15) (1 H, *q*, *J* = 7 Hz, H-8), 2.17 (2.15) (3 H, *s*, —OAc), 1.39 (1.37) (3 H, *d*, *J* = 7 Hz, 3 H-17), C–Me singlets at 1.09 (1.12), 0.90 (0.88) and 0.82 (0.79). MS (75 eV, direct inlet) *m/e* (rel. int.): 376 (M^+, 6), 316 (2), 289 (8), 281 (6), 253 (8), 210 (6), 193 (26), 175 (6), 137 (10), 123 (78), 115 (6), 109 (40), 95 (30), 87 (12), 81 (76), 69 (36), 55 (17), 43 (100). $C_{22}H_{32}O_5$ MW 376.

Galeopsin (**2**) *from pregaleopsin* (**3**). A suspension of pregaloepsin (**3**, 40 mg), EtOH (3 ml) and Amberlite IR-120 (H^+ form, 80 mg) was stirred at room temp. for 2 hr. The soln was filtered and the solvent removed. After PLC purification it yielded galeopsin (**2**, 30 mg), identical with the sample previously isolated (mp, mmp, $[\alpha]_D$, IR, ^{1}H NMR and MS).

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