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ISOFREGENEDADIOL: A NOVEL DITERPENIC DIOL FROM *HALIMIUM VISCOSUM*

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Key Word Index—*Halimium viscosum*, diterpenes, isofregenedane.

Abstract—One of the chemotypes of *Halimium viscosum*—La Fregeneda—provided a new natural product with a rearranged skeleton. The new bicyclic diol possesses an aromatic ring B and its structure has been determined on the basis of ^{1}H, ^{13}C and 2D NMR (^{1}H–^{13}C HETCOR and ^{1}H–^{13}C long range inverse detected HMBC) studies.

INTRODUCTION

During the last ten years, the systematic study on the chemical components of *Halimium viscosum* chemotypes, collected near the Spanish–Portuguese border, allowed the separation of several natural products with different skeleta. Among them, from *H. viscosum* La Fregeneda, a bicyclic diol named fregenedadiol (**1**) was isolated and its structure clearly and unambiguously determined as a rearranged labdane skeleton with an aromatic ring B (fregenedane) [1]. A few years later its structure was confirmed by synthesis [2].

A reinvestigation of the unsaponifiable part of the neutral fraction was planned for isolating its major component 7-labden-3β,15-diol (**2**) needed as starting material in homochiral syntheses. Unexpectedly, this work allowed the isolation of a new diterpenic diol, **3**, that posseses a bicycle[4.4.0]decane system with an aromatic ring B. This new compound has been named isofregenedadiol because it differs from **1** only in the substitution pattern on the aromatic ring.

Only two other natural compounds are known so far to possess the same carbon skeleton: chrysolic acid (**4**) [3], reported by Timmermann *et al.* in 1982 and the alcohol **5**, reported more recently [4].

RESULTS AND DISCUSSION

The unsaponifiable neutral fraction obtained from the hexane extract of *H. viscosum* La Fregeneda [5] afforded by crystallization 7-labden-3β,15-diol (**2**). The mother liquor was chromatographed using column chromatography to give six fractions. Fraction IV after acetylation and column chromatography afforded **6**, **7** and **8** (isofregenedadiol diacetate).

Compound **8** showed in the IR spectrum absorption bands at 1740 and 1240 cm^{-1}, due to the acetoxyl groups, and at 1620 and 1580 cm^{-1}, due to an aromatic ring.

The broadband ^{1}H-decoupled ^{13}C NMR spectrum showed the presence of 24 carbon atom resonances (Table 1), the multiplicities of which were determined by DEPT pulse sequence. Five of them (C-5, C-7, C-8, C-9 and C-10) were assigned to a pentasubstituted benzenoid ring on the basis of their chemical shift values, while the remaining signals were assigned to seven methyl (two of them acetate esters at δ20.9 and 21.2), six methylene (one of them oxygen-bearing at δ63.0), three methines (one of them sp^2 hybridized at δ124.8 and one oxygen-bearing at δ77.4) and three fully substituted (two of them ester carbonyls at δ170.8 and 171.0) carbon atoms.

The ^{1}H NMR spectrum showed an aromatic proton (δ7.0, 1H, *s*), geminal hydrogens to a secondary (δ4.96, 1H, *dd*, J = 6.8 and 4.3 Hz) and a primary (δ4.15, 2H, *t*, J = 6.8 Hz) acetoxyl function, respectively. Seven methyl groups were identified, with signals at δ2.21 (3H, *s*) and 2.16 (3H, *s*) assigned to two aromatic methyl groups; at δ2.05 and 2.06, assigned to two acetates; at δ1.31 (6H, *s*) assigned to two quaternary methyl groups and a doublet at δ1.02 (3H, *d*, J = 6.4 Hz).

Alkaline hydrolysis of **8** afforded either the diol **3** [(NaOH/MeOH), isofregenedadiol, IR: 3340 cm^{-1})] or the monoacetyl derivative **9** [(K_2CO_3/MeOH), IR: 3500, 1740 and 1240 cm^{-1})].

Compound **3** had the molecular formula $C_{20}H_{32}O_2$ (*m/z* 304) and compounds **8** and **9** had the molecular formulae $C_{24}H_{36}O_4$ (*m/z* 388) and $C_{22}H_{34}O_3$ (*m/z* 346), respectively. These data are in agreement with a diterpenoid (bicyclic) diol, with an aromatic ring, and **8** and **9** being its diacetyl and monoacetyl derivatives. A distinct peak at *m/z* 185 (100%) corresponds to the loss of water or acetic acid and the typical sidechain of these

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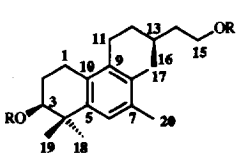
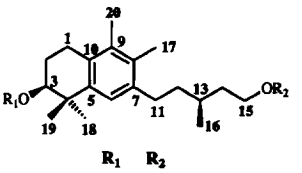
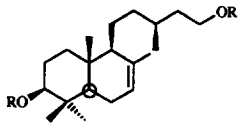


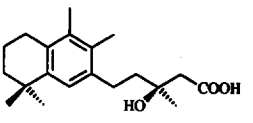
R
1 H
7 Ac



R1 R2
3 H H
8 Ac Ac
9 Ac H



R
2 H
6 Ac



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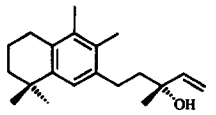

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diterpenes (with a hydroxyl or acetoxyl function at C-15): Compound **3** [304 − (H_2O + $C_6H_{13}O$)]; Compound **8** [304 − (HOAc + $C_8H_{15}O_2$)]; Compound **9**: [346 − (HOAc + $C_6H_{13}O$)].

The units described, i.e. six carbon atoms side-chain substituted with a primary hydroxyl function and a methyl doublet, a secondary hydroxyl function, four methyl singlets (two aromatic) and one aromatic proton, as being present in compound **3**, are found also in fregenedadiol, a compound which contains a tetrahydronaphthalenic (THN) structure. The difference observed is in the aromatic ring substitution pattern.

Comparison between the ^{13}CNMR data of **1** and **3** (Table 1) indicated that the differences are in the chemical shift values of a methyl (C-20) and a methylene (C-11) group, supporting that **3** is a positional isomer of **1**.

The substitution pattern on the aromatic ring was determined by 2D (^1H–^{13}C) NMR correlation experiments at 200 MHz for **8** and at 500 MHz for **9**. The H-6 proton showed long range heteronuclear correlations to C-4 (quaternary bearing the gem-dimethyl group) and to C-11 (benzylic methylene on the side-chain), indicating that both are *ortho* to the aromatic hydrogen. These data support the position of the side-chain and the substitution pattern depicted in **8**. The gem-dimethyl protons showed long range heteronuclear correlations to a quaternary aromatic carbon atom (C-5), indicating that the gem-dimethyl group is attached to a quaternary benzylic carbon atom. The same protons also showed correlations with a methine oxygen-bearing carbon, indicating the secondary acetoxyl function position at C-3.

Table 1. NMR data for compounds **1**, **3**, **8** and **9**

			8			9		
Position	1 ^{13}C*	3 ^{13}C*	^{13}C*	^1H*	HCCORR*	^{13}C*	^1H†	HMBC†
1	24.3	25.4	24.9	2.75		24.9	2.7	2. 3, 5, 9, 10
2	27.1	27.0	23.9	2.0–2.1		23.9	1.98	
3	76.4	75.3	77.4	4.96		77.4	4.93	
4	39.1	39.1	37.7			37.8		
5	141.6	141.6	141.1			141.1		
6	126.2	125.2	124.8	7.00	4, 8, 10, 11	124.7	6.98	4, 8, 10, 11
7	134.7	138.8	138.6			138.8		
8	132.1	131.8	131.8			131.9		
9	138.7	134.7	134.6			134.6		
10	129.9	130.6	130.6			130.6		
11	27.2	32.2	32.1	2.63		32.1	2.64/2.56	6, 7, 8, 12, 13
12	36.5	38.6	38.3			38.5	1.54/1.36	
13	30.6	30.0	30.3	1.10		30.0	1.36	
14	39.9	39.9	35.6	1.35/1.90		40.0	1.64/1.43	
15	61.2	61.2	63.0	4.15		61.2	3.69	13, 14
16	19.5	19.7	19.5	1.02		19.7	0.99	12, 13, 14
17	15.2	15.2	15.4	2.21	7, 8, 9	15.4	2.18	7, 8, 9
18	29.4	29.4	29.9	1.31	3, 4, 5, 19	29.9	1.28	3, 4, 5, 19
19	25.3	25.3	26.0	1.31	3, 4, 5, 18	26.0	1.27	3, 4, 5, 18
20	20.9	15.7	15.7	2.16	8, 9, 10	15.8	2.14	8, 9, 10
MeCOO			21.2	2.05		21.2	2.04	
MeCOO			170.9			170.9		
MeCOO			20.9	2.06				
MeCOO			170.8					

*200 MHz.
†500 MHz.

The aromatic methyl protons showed long range heteronuclear correlations between Me-17 and C-7, C-8 and C-9 and between Me-20 with C-8, C-9 and C-10, establishing the *ortho* relationship between both methyl groups and that C-8 and C-9 support Me-17 and Me-20, respectively. This substitution pattern is also supported by the H-6/C-8 and H-6/C-10 correlations observed.

Finally, Me-16 (methyl doublet on the side-chain) showed correlations with C-12 and C-14 and a two-bond correlation with C-13 (the methine directly attached to the methyl group) and the C-1 methylene protons correlated with the quaternary aromatic C-10, allowing the complete and unambiguous assignment of the ^{13}C NMR spectrum of compound **8** and its structure.

The HMBC correlations observed for compound **9** (the C-1 benzylic methylene protons with C-5, C-9 and C-10 and the C-11 benzylic protons with C-6, C-7 and C-8) as well as the nOe interactions observed between H-6 and the gem-dimethyl protons confirmed the THN system and the proposed substitution pattern on ring B.

The isomeric diols, fregenedadiol (**1**) and isofregenedadiol (**3**), should have the same biogenetic origin, i.e. the 3β-hydroxy-ring B-unsaturated labdanes, such as **2**, present in the same extract as **1** and **3**. This type of compound could undergo a rearrangement of either Me-20 and/or the side-chain leading to **1** and/or **3**, retaining the same configuration at C-3 and C-13.

The carbon skeleton shared by **3**, **8** and **9** is named isofregenedane, to indicate its common biogenetic origin with the fregenedane skeleton, and thus retaining the numbering of the labdane precursor.

It can be concluded that the derivatives of both classes: fregenedanes and isofregenedanes, can be easily distinguished by analysis of their ^{13}C NMR chemical shift values (C-20 and C-11). Isofregenedane derivatives will show a 5 ppm shielding for Me-20 (Me-C-9) as in compound **3**, compared to fregenedane derivatives (C-20: Me-C-7), as in compound **1**. The same effect will be observed for C-11, when comparing **1** to **3**, meaning that the carbon attached to C-9 (a methyl group in isofregenedane and methylene group in fregenedane) is always 5 ppm upfield with respect to the carbon (either methyl or methylene group) bonded to C-7, probably due to the fact that C-9 is *ortho* disubstituted and C-7 is *ortho* monosubstituted.

EXPERIMENTAL

Spectral analysis. NMR: 200 MHz for ^{1}H and 50.3 MHz for ^{13}C or 500 MHz for ^{1}H. Chemical shifts are given in δ (ppm) and are referenced to the residual $CHCl_3$, 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C, respectively. EI-MS: VG TS 250 MS, 70 eV.

Extraction and isolation. Aerial parts of *H. viscosum* La Fregeneda were dried (9.6 kg) and extracted with *n*-hexane in a Soxhlet over 24 hr. The extract (584 g) was dewaxed in MeOH and then extracted (422 g) with 10% Na_2CO_3 and 4% NaOH. The neutral fraction (172 g) was saponified with 10% NaOH/MeOH. The unsaponifiable extract (65 g) was crystallized from Et_2O to give **2** (13 g). The mother liquor (52 g) was chromatographed on a silica gel column with *n*-hexane-EtOAc mixtures affording six fractions. Fraction IV (28 g) was acetylated and chromatographed on a silica gel column affording **6** (9.48 g), **7** (0.13 g) and **8** (0.03 g).

Isofregenedadiol (**3**). IR ν_{max} (film) cm^{-1}: 3340, 3040, 1610, 1575, 1380, 1360; ^{1}H NMR δ: 7.05 (1H, *s*, H-6), 3.75 (2H, *m*, H-15), 3.72 (1H, *m*, H-3), 2.21 (3H, *s*, Me-17), 2.16 (3H, *s*, Me-20), 1.34 (3H, *s*, Me-18), 1.30 (3H, *s*, Me-19), 1.02 (3H, *d*, J = 6.4 Hz, Me-16); ^{13}C NMR δ: Table 1; EIMS *m/z* (rel. int.): 304 $[M]^+$ (60), 286 (86), 271 (25), 217 (18), 201 (90), 185 (100), 183 (80), 159 (70), 133 (48), 119 (75), 105 (42), 91 (50), 69 (55), 54 (100).

Isofregenedadiol diacetate (**8**). $[\alpha]_D$ = + 4.1° ($CHCl_3$, 1.23%). IR ν_{max} (film) cm^{-1}: 1740, 1720, 1620, 1580, 1380, 1360, 1240, 885; EIMS *m/z* (rel. int.): 388 $[M]^+$ (15), 328 (80), 316 (35), 253 (18), 200 (38), 185 (100), 157 (35), 131 (58), 119 (57), 83 (44), 13 (60); ^{1}H and ^{13}C NMR: Table 1.

Isofregenedadiol monoacetate (**9**). IR ν_{max} (film) cm^{-1}: 3500 (broad), 1740, 1240, 1500; EIMS *m/z* (rel. int.): 346 $[M]^+$ (20), 286 (98), 200 (58), 185 (100), 183 (90), 157 (42), 119 (70), 83 (56), 69 (60); ^{1}H and ^{13}C NMR: Table 1.

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REFERENCES

1. Urones, J. G., Marcos, I. S., Garrido, N. M. and Moro, R. F. (1990) *Phytochemistry* **29**, 3042.
2. Urones, J. G., Marcos, I. S., Basabe, P., Garrido, N. M., Jorge, A., Moro, R. F. and Lithgow, A. M. (1993) *Tetrahedron* **49**, 6079.
3. Timmermann, B. N., Hoffmann, J. J., Jolad, S. D., Schram, K. H. Y., Klenck, R. E. and Bates, R. B. (1982) *J. Org. Chem.* **47**, 4114.
4. Zdero, C., Bohlmann, F. and Niemeyer, H. M. (1991) *Phytochemistry* **30**, 3689.
5. Urones, J. G., Marcos, I. S., Basabe, P. and Garrido, N. M. (1988) *Phytochemistry* **27**, 501.