



THREE ISOMERIC DITERPENES FROM *VELLOZIA FLAVICANS*

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Key Word Index—*Vellozia flavicans*; Velloziaceae; isopimarane; abietane and strobane diterpenes.

Abstract—Three new diterpenes have been isolated from the roots, stem and leaf sheaths of *Vellozia flavicans*. Their structures were elucidated by spectroscopic methods as 19-hydroxy-8(9),15-abietadiene, 19-hydroxy-8(9),13(16)-14S,17-cyclolabdadiene and 17-hydroxy-8(9),15-isopimaradiene.

INTRODUCTION

In previous papers [1-4], we described several cleistanthane, one totarane and two tetranorfriedolabdane diterpenes from *Vellozia flavicans* Martius and Schultze. The present paper describes the structural elucidation of three new diterpenoids, isolated from the hexane extract of roots, stem and leaf sheaths from the same species.

RESULTS AND DISCUSSION

Analysis by GC-mass spectrometry of one of the apolar fractions of *V. flavicans* showed the presence of three tricyclic isomeric diterpenes with a parent molecular ion of m/z 288, corresponding to the molecular formulae $C_{20}H_{32}O$, in the proportion of 24:30:43. This fraction was separated by HPLC [C-18, H_2O -MeOH (2:3)]. Each of the three diterpenes exhibited in its IR spectra the presence of a hydroxyl group. The 1H NMR spectra showed the presence of a hydroxymethylene group attached to quaternary carbons, and the ^{13}C NMR spectra suggested the presence of a tetrasubstituted double bond. The moieties involving the A-ring of these three isomeric diterpenes were determined based on the chemical shifts of the carbon atoms by spectral comparison with reported data in the literature [5]. The diterpenes **1** and **2** were shown to have the hydroxymethylene group attached to C-4, while in **3** this group was found to be linked to C-13 (Table 1). The tetrasubstituted double bond in each of these diterpenes was located at C(8)/C(9). The presence of the double bond at $\Delta^{8(9)}$ in **1** and **2** results in a fragmentation pattern similar to that of the C-ring

monoaromatic hydrocarbon diterpenes [6-9], leading to the formation of three indanyl ions at m/z 187, 175 and 161. In **3** this fragmentation pattern was not observed owing to the presence of the hydroxymethylene group in the C-ring, which drives the fragmentation pattern.

The 1H NMR spectrum of **1** contains signals due to three methyl groups, two of which are on the quaternary carbons (δ 0.94 and 1.00) and one is part of an isopropylidene moiety at δ 1.72, which is coupled with the methylenic group at δ 4.69 (*br s*). The only tertiary hydrogen attached to the C-ring appeared at δ 2.07, as a complex multiplet. From the 1H - 1H COSY

Table 1. ^{13}C NMR chemical shifts of compounds **1**, **2** and **3** ($CDCl_3$)

C	1	2	3
1	35.5	35.4	36.4
2	18.6	18.7	18.9
3	36.3	36.4	41.7
4	38.6	38.7	32.8
5	52.6	52.8	51.8
6	19.1	19.8	19.0
7	32.6	35.7	32.5
8	125.8	130.0	123.6
9	137.7	143.2	137.6
10	37.5	38.7	36.8
11	24.6	27.2	20.3
12	28.6	34.0	29.3
13	41.7	157.1	37.6
14	36.2	38.8	36.6
15	150.5	18.6	142.0
16	108.1	107.2	115.2
17	20.6	40.7	70.8
18	26.7	26.7	21.7
19	65.2	65.6	33.3
20	19.6	19.9	19.4

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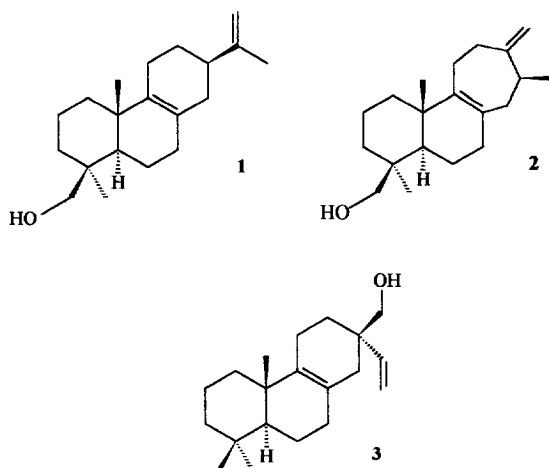
NMR, it was seen that this tertiary hydrogen coupled with four different protons at δ 1.27 and 1.80 (one CH_2), and δ 1.80 and δ 1.85 (the second CH_2). The two vicinal hydrogens at δ 1.27 and 1.80 coupled with two hydrogens at δ 1.95 and 2.04 (the third CH_2); however, the third methylene group only revealed coupling with the first methylene group. By a detailed analysis of the ^1H - ^1H COSY and ^1H - ^{13}C HETCOR spectra, the C-ring was completely assigned. Thus, the isopropylidene group was located on C-13, with a pseudoequatorial orientation. Compound **1** was therefore identified as 19-hydroxy-8(9),15-abietadiene.

In the diterpene **2**, the structure of the C-ring was determined by its ^1H NMR spectrum, which showed a methyl group at δ 0.97 (*d*, $J = 7.1$ Hz) and an exocyclic methylene group at δ 4.51 (*d*, $J = 2.4$ Hz) and 4.57 (*d*, $J = 2.4$ Hz). The ^1H - ^1H COSY and ^1H - ^{13}C HETCOR spectra exhibited the presence of two isolated moieties CH_2CH_2 and $\text{CH}_2\text{CH}(\text{CH}_3)$. The tertiary and the three methylene hydrogens appeared deshielded (δ 1.82–2.40), suggesting that all these hydrogens are allylic. The tertiary hydrogen (H-14) appeared as a double quintet at δ 2.39 ($J = 2.4$, 7.1 and 7.1 Hz), coupling to the methyl group at δ 0.97 and to an allylic methylene at δ 1.82 and 2.15. Compound **2** was then identified as 19-hydroxy-8(9),13(16)-14S,17-cyclolabdadiene.

The ^1H NMR of **3** showed three olefinic protons corresponding to a vinyl group at δ 5.03 (1H, *dd*, $J = 1.5$ and 17.5 Hz), 5.18 (1H, *dd*, $J = 1.5$ and 11.0 Hz) and 5.63 (1H, *dd*, $J = 11.0$ and 17.5 Hz) and also three methyl groups on quaternary carbons (δ 0.84, 0.88 and 0.96).

The localization of the hydroxyl group at C-17 was deduced from the chemical shifts of its vicinity. In comparison with 8(9),15-isopimaradienes [10], C-12 showed a 4 ppm upfield shift due to a γ -*gauche* effect relative to 1,3-interactions and C-13 was deshielded due to a β -effect of the hydroxyl group. The position of the hydroxyl group was determined to be C-17 by comparison with known models [5]. Compound **3** was thus identified as 17-hydroxy-8(9),15-isopimaradiene.

This is the first time that the strobane and abietane skeletons have been found in the Velloziaceae family.



They are, however, commonly found in the *Pinus* genus [11].

EXPERIMENTAL

General. The hexane extract (90 g) of roots, stem and leaf sheaths (3.5 kg) of *V. flavicans*, collected on the Chapada dos Veadeiros, Goiás, Brazil, was subjected to silica gel CC (hexane–EtOAc gradient). Fr. 28 (35% EtOAc–hexane, 7:13 (70 mg) on semiprep. C-18 RP HPLC [H_2O –MeOH (2:3), 2.4 ml min $^{-1}$, RI], gave compounds **1** (10 mg), **2** (35 mg) and **3** (9 mg).

19-hydroxy-8(9),15-abietadiene (1). Viscous oil. IR ν_{max} cm $^{-1}$: 3382, 2962, 2926, 2852, 1695, 1645, 1451, 1376, 1343, 1331, 1262, 1099, 886, 803; ^1H NMR (300 MHz, CDCl_3): δ 0.94 (3H, *s*), 1.00 (3H, *s*), 1.27 (H-12, *m*), 1.80 (H-1, H-12 and H-14, *m*), 1.85 (H-14, *m*), 1.95 (H-11, *m*), 2.04 (H-11, *m*), 2.07 (H-13, *m*), 3.48 (H-19, *d*, $J = 11.1$ Hz), 3.79 (H-19, *d*, $J = 11.1$ Hz) and 4.69 (2H-16, *br s*); ^{13}C NMR: Table 1; HRGC–EIMS, 70 eV m/z (rel. int.): 288 [$\text{M}]^+$ (42), 273 (30), 257 (78), 245 (20), 229 (20), 187 (24), 175 (32), 161 (46), 155 (50), 131 (100), 105 (94) and 91 (98).

19-hydroxy-8(9),13(16)-14S,17-cyclolabdadiene (2). Crystals from hexane, mp 115–117°. IR ν_{max} cm $^{-1}$: 3377, 3084, 2963, 2924, 1635, 1457, 1425, 1405, 1377, 1025 and 887; ^1H NMR (300 MHz, CDCl_3): δ 0.90 (3H, *s*), 0.97 (3H, *d*, $J = 7.1$ Hz), 1.00 (3H, *s*), 1.82 (H-17, *m*), 2.07 (2H-12, *m*), 2.15 (H-14, *m*), 2.39 (H-14, *dq*, $J = 2.4$ and 7.1 Hz), 3.47 (H-19, *d*, $J = 10.9$ Hz), 3.75 (H-19, *d*, $J = 10.9$ Hz), 4.51 (H-16, *d*, $J = 2.4$ Hz) and 4.57 (H-16, *d*, $J = 2.4$ Hz); ^{13}C NMR: (Table 1); HRGC–EIMS, 70 eV m/z (rel. int.): 288 [$\text{M}]^+$ (50), 273 (23), 257 (70), 187 (35), 175 (78), 161 (55), 147 (50), 134 (96), 119 (65), 105 (80), 91 (100), 79 (65) and 67 (50).

17-Hydroxy-8(9),15-isopimaradiene (3). Viscous oil. IR ν_{max} cm $^{-1}$: 3382, 2923, 2867, 1693, 1640, 1458, 1440, 1387, 1375, 1261, 1093, 1029, 973 and 911; ^1H NMR (300 MHz, CDCl_3): δ 0.84 (3H, *s*), 0.88 (3H, *s*), 0.96 (3H, *s*), 3.41 (H-17, *d*, $J = 10.3$ Hz), 3.83 (H-17, *d*, $J = 10.3$ Hz), 5.03 (H-15, *dd*, $J = 1.5$ and 17.5 Hz), 5.18 (H-16, *dd*, $J = 1.5$ and 11.0 Hz), 5.63 (H-16, *dd*, $J = 11.0$ and 17.5 Hz). ^{13}C NMR: Table 1; HRGC–EIMS, 70 eV m/z (rel. int.): 288 [$\text{M}]^+$ (40), 273 (100), 257 (30), 229 (8), 203 (18), 185 (20), 177 (20), 159 (50), 131 (50), 117 (52), 105 (80) and 91 (80).

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