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MINOR CLEISTHANTANE AND TETRANORFRIEDOLABDANE FROM *VELLOZIA FLAVICANS**

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Key Word Index—*Vellozia flavicans*; Velloziaceae; cleistanthane and tetranorfriedolabdane diterpenes.

Abstract—Six diterpenes with the cleistanthane skeleton have been identified from *Vellozia flavicans*, three of which; 8,11,13-cleistanthatrien-7-one-17-oic acid, 8,11,13-cleistanthatrien-7-one-19-oic acid and 8,11,13-cleistanthatrien-17,19-dioic acid are reported herein for the first time as natural products. Two tetranorditerpenes with a friedolabdane skeleton have also been isolated from this same plant.

INTRODUCTION

After veadeirol and veadeiroic acid were isolated from *Vellozia flavicans* Martius & Schultz [1], several of these cleistanthane diterpenes were identified in other species of the Velloziaceae [2]. In a previous paper [3], we have dealt with the chemical study of the hexane extract of *Vellozia flavicans*, which led to the isolation of its major constituents, the cleistanthanes functionalized at C-17 or C-19: 8,11,13-cleistanthatrien-17-ol, 8,11,13-cleistanthatrien-19-ol, 8,11,13-cleistanthatrien-17-al, 8,11,13-cleistanthatrien-17-oic acid and 8,11,13-cleistanthatrien-19-oic acid.

After the isolation of these main components by silica gel column chromatography, the remaining fractions were analysed by HRGC-mass spectrometry. Additionally, two tetranorfriedolabdanes; 5(6)-tetranorfriedolabden-12-oic acid and 5(6)-tetranorfriedolabden-12-ol were identified. The cleistanthanes; 8,11,13-cleistanthatriene, 8,11,13-cleistanthatrien-7-one and 8,11,13-cleistanthatrien-7β-ol, previously described from other *Vellozia* species [4], and 8,11,13-cleistanthatrien-7-one-17-oic acid and 8,11,13-cleistanthatrien-7-one-19-oic acid as methyl esters were also identified. The latter two compounds are constituents of the acidic fraction. The structures of these diterpenes were confirmed by comparison of their mass spectra and co-elution in GC of authentic samples, obtained from chemical transformations of cleistanthanes of known absolute stereochemistry isolated from other *Vellozia* species. Finally, the structural elucidation of 8,11,13-cleistantha-17,19-dioic acid is also described in this paper.

*This paper is dedicated to Prof. Raimundo Braz Filho on the occasion of his sixtieth birthday.

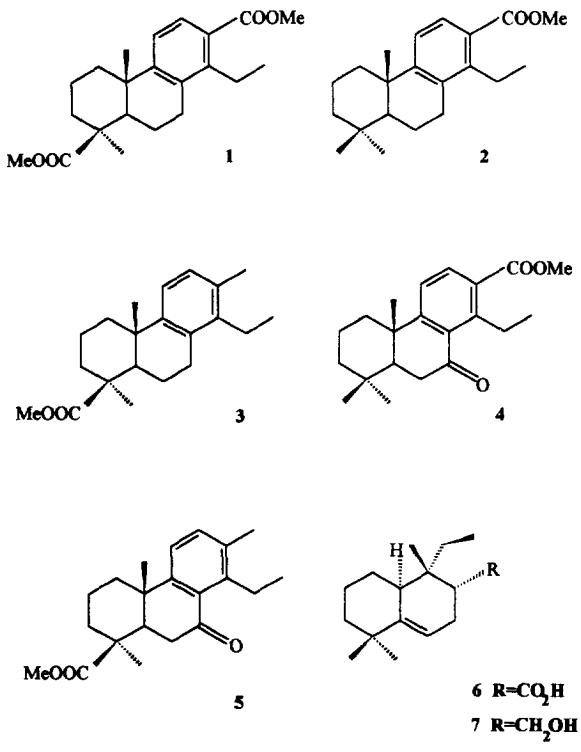
RESULTS AND DISCUSSION

The molecular formula of **1** ($C_{22}H_{30}O_4$) was established by mass and ^{13}C NMR spectrometry, after methylation with diazomethane. The UV spectrum showed an intense absorption at 244 nm ($\log\varepsilon = 4.10$). The IR, ^1H and ^{13}C NMR spectra indicated that **1** contains a 1,2,3,4-tetrasubstituted aromatic ring. The increase of 28 amu after esterification with diazomethane and one intense absorption at 1719 cm^{-1} in the IR spectrum showed that **1** is a diester. The analysis of the ^1H NMR spectrum demonstrated that the two aromatic protons are *ortho* to each other. The downfield shifts observed for these two protons and also those observed for the methylene protons of the ethyl group defined the substitution pattern of the aromatic ring. The ^1H NMR spectrum also showed three methyl groups, two of which were singlets and one was a triplet signal.

The fragment observed in the mass spectrum of **1** at m/z 343 $[M - 15]^+$ (20%) corresponds to the loss of the angular methyl group at C-10, and the fragment at m/z 203 $[M - 155]^+$ (30%) results from the cleavage of rings A and B, thus confirming that one of the two carbon methyl groups is localized on the C-aromatic ring. Taken together those data revealed **1** as a cleistanthane type diterpene with a carboxymethyl group located on C-4 (C-18 or C-19).

The position of the two carboxymethyl groups in C-17 and C-19 were confirmed by the ^{13}C NMR spectrum, when compared with model compounds **2** and **3**, which showed a perfect correlation (Table 1). Thus, **1** was identified as the 8,11,13-cleistanthatrien-17,19-dioic acid.

The presence of many cleistanthane acids in this species, associated with the previous knowledge of vigorous oxidative metabolism in the Velloziaceae, led us to investigate the minor constituents in *Vellozia flavicans*. So, the



crude hexane extract was chromatographed on a KOH silica gel column, eluted with dichloromethane and a 5% HCl acidified methanol solution, respectively. After extraction and diazomethane methylation, two interesting compounds were isolated and analysed by HRGC-mass spectrometry. Fragmentation analysis indicated them as **4** and **5**. As we have already isolated the diterpenes **2** and **3** from *Vellozia flavicans*, we oxidized these diterpenes with tert-butyl chromate [5]. Comparison of the EI-mass spectra of the natural diterpenes with those of the products obtained from oxidation, together with coelution in GC, confirmed the structures **4** and **5**. The molecular formulae of the tetranorditerpenes **6** ($C_{16}H_{26}O_2$) and **7** ($C_{16}H_{28}O$) were established by mass and ^{13}C NMR spectra.

The ^1H NMR spectra of **6** and **7** showed three methyl groups on quaternary carbons and one on a tertiary carbon, together with one trisubstituted double bond. These, associated with ^{13}C NMR data (Table 2) and biogenetic considerations suggested the presence of a tetranorfriedolabdane skeleton for both compounds. The presence of a fragment at *m/z* 136 related to a retro-Diels–Alder rearrangement in the B-ring located the double bond between C-5 and C-6.

The oxygen-containing functional group of **6** was shown to be a carboxyl group by its IR spectrum (3449 and 1708 cm^{-1}). The ^1H NMR spectrum of this compound showed two doublets for two geminal protons at δ2.38 and 2.45 (J = 12.0 Hz), suggested the presence of a CH_2CO_2H(Me) residue. In **7** this AB pattern is replaced by a distorted triplet at δ3.70 (J = 8.0 Hz) and the ^1H–^1H COSY spectrum showed that this signal corresponded to a hydroxymethylene group, coupled to a methylene group (δ1.62 and 1.70) which, in turn, is attached to a quaternary carbon. Both compounds also revealed in the mass spectra the fragment at *m/z* 191 as base peak, resulting from the loss of CH_2CO_2H(Me) and CH_2CH_2OH moieties.

The orientation of the C-17 methyl group was deduced from the ^{13}C NMR spectra of **6** and **7**. The C-10 in both compounds showed the upfield shifts (δ41.0 and 40.8, respectively) owing to a strong γ-gauche effect suffered by this carbon atom when in a 1,3-diaxial interaction between H-10 and C-17 methyl group. The relative stereo-

Table 1. ^{13}C NMR spectral data of diterpenes **1**–**5**

C	1	2	3	4	5
1	39.1	39.0	39.8	38.6	38.9
2	20.0	19.0	21.1	18.8	21.6
3	37.4	41.3	37.5	41.4	37.5
4	43.9	33.3	43.9	32.2	44.0
5	51.9	49.3	52.3	47.4	49.5
6	20.9	19.3	20.1	38.5	39.6
7	28.8	27.3	29.0	201.4	201.1
8	134.5	133.1	133.1	132.2	130.2
9	152.3	154.1	146.2	159.5	153.3
10	39.6	38.4	38.5	38.4	39.1
11	123.3	121.9	123.2	120.7	122.0
12	127.5	127.4	127.9	133.4	134.7
13	130.2	128.6	132.8	130.7	130.2
14	143.4	143.2	140.2	146.3	144.6
15	22.1	22.7	22.2	22.8	20.5
16	14.4	14.0	13.0	16.4	14.2
17	169.0	168.7	19.3	168.6	19.2
18	28.4	21.6	28.5	21.3	27.8
19	177.8	33.1	177.9	33.4	177.1
20	22.8	24.6	22.9	23.7	23.8
21	51.8	51.8	51.2	52.1	—
22	51.9		—	—	—

Table 2. ^{13}C NMR spectral data of the tetranorditerpenes **6** and **7**

C	6	7
1	27.8	27.8
2	22.1	22.2
3	40.9	40.9
4	36.1	36.9
5	145.7	145.9
6	115.9	116.1
7	31.6	31.5
8	34.3	34.3
9	38.7	36.1
10	41.0	40.8
11	41.4	39.7
12	178.4	59.0
17	15.6	16.0
18	29.7	29.7
19	28.8	28.9
20	15.6	15.3

chemistry of C-9 is the same found for other tetranorditerpenes isolated from Velloziaceae [6].

Lithium aluminium hydride reduction of **6** afforded **7**, which was identical in all respects to the tetranorditerpene isolated from the same plant.

EXPERIMENTAL

Details of plant material and fractionation of the extract are described in a previous paper [3]. The already known natural compounds were identified by comparison of their spectral data with those of authentic samples.

After the isolation of the major compounds, the remaining fractions analysed by HRGC-MS were monitored by HPLC on a C-18 reversed-phase semiprep. column, from which the compounds were purified. Co-injection of the standards was performed taking care that the addition of the standards enhanced the height of the target peak by 30%.

8,11,13-*Cleistanthatrien*-17,19-*dioic acid dimethyl ester* (**1**). Crystals from hexane, mp 148–150°. UV λ_{max}^{EtOH} nm (logε): 244 (4.10). UV ν_{max} cm^{-1}: 2943, 1719, 1447, 1425, 1260, 1196, 1147 and 875; ^{1}H NMR (300 MHz, $CDCl_3$): δ1.04 (3H, *s*), 1.17 (3H, *t*), 1.27 (3H, *s*), 3.65 (3H, *s*), 3.85 (3H, *s*), 7.22 (1H, *d*, J = 9.0 Hz), 7.55 (1H, *d*, J = 9.0 Hz). ^{13}C NMR: Table 1. EI-MS 70 eV *m/z* (rel. int.): 358 $[M]^+$ (12), 343 $[M-15]^+$ (20), 327 $[M-31]^+$ (15), 284 $[M-74]^+$ (20), 283 $[M-75]^+$ (100), 217 $[M-141]^+$ (17), 203 $[M-155]^+$ (30), 183 $[M-175]^+$ (10), 155 $[M-203]^+$ (17), 141 $[M-217]^+$ (30).

8,11,13-*Cleistanthatrien*-7-*one*-17-*oic acid methyl ester* (**4**). HRGC-EI-MS of the synthetic standard, 70 eV *m/z* (rel. int.): 328 $[M]^+$ (34), 313 $[M-15]^+$ (100), 297 $[M-31]^+$ (10), 231 $[M-97]^+$ (10), 225 $[M-103]^+$ (10), 128 $[M-200]^+$ (10). The synthetic standard was prepd in the following manner. To a soln of the acid **2** (48 mg) in CH_2Cl_2 (0.3 ml) was added *t*-butyl chromate (0.2 ml, 0.04 M) and TBHP (0.2 ml, 7.3 M). The reaction mixt. was left for 24 hr at room temp. Usual work-up ($FeSO_4 \cdot 7H_2O$) and purification by CC afforded **4** (30 mg, 62%). IV ν_{max} cm^{-1}: 2951, 2932, 2871, 1729, 1683, 1588, 1460, 1434, 1392, 1261, 1245, 1224, 1203, 1165, 1096, 841, 803, 754, 636. ^{1}H NMR (300 MHz, $CDCl_3$): δ0.92 (3H, *s*), 1.04 (3H, *s*), 1.15 (3H, *s*), 1.22 (3H, *t*, J = 7.5 Hz), 1.78 (1H, *dd*, J = 7.9 and 10.9 Hz), 2.25 (1H, *d*), 2.69 (1H, *d*, J = 10.9 Hz), 2.70 (1H, *d*, J = 7.9 Hz), 2.95 (1H, *q*, J = 7.5 Hz), 3.40 (1H, *m*), 3.90 (3H, *s*), 7.25 (1H, *d*, J = 8.5 Hz), 7.75 (1H, *d*, J = 8.5 Hz). ^{13}C NMR: Table 1.

8,11,13-*Cleistanthantrien*-7-*one*-19-*oic acid* (**3**). HRGC-EI-MS of the synthetic standard, 70 eV *m/z* (rel. int.): 328 $[M]^+$ (76), 310 $[M-18]^+$ (12), 268 $[M-60]^+$ (10), 253 $[M-75]^+$ (90), 236 $[M-92]^+$ (20), 235 $[M-93]^+$ (100), 199 $[M-129]^+$ (20), 187 $[M-141]^+$ (27). The synthetic standard was prepd as described for **4**, in a yield of 70%. IR ν_{max} cm^{-1}: 2952, 2937, 2873, 1728, 1677, 1591, 1567, 1470, 1380, 1366, 1310, 1274, 1245, 1231, 1208, 984, 957, 937, 820. ^{1}H NMR (300 MHz, $CDCl_3$): δ1.06 (3H, *s*), 1.12 (1H, *d*, J = 4 Hz), 1.17 (1H, *d*, J = 4 Hz), 1.20 (3H, *t*, J = 7 Hz), 1.25 (3H, *s*), 2.00 (1H, *m*), 2.05 (1H, *dd*, J = 4.5 and 14.0 Hz), 2.73 (3H, *s*), 2.88 (1H, *dd*, J = 4.5 and 18.0 Hz), 2.95 (2H, *m*), 3.30 (1H, *dd*, J = 14.0 and 18.0 Hz), 7.18 (1H, *d*, J = 8.5 Hz), 7.29 (1H, *d*, J = 8.5 Hz). ^{13}C NMR: Table 1.

5(6)-*Tetranorfriedolabden*-12-*oic acid* (**6**). Crystals from MeOH–H_2O (4:1), mp 64–66°. IR ν_{max} cm^{-1}: 3499, 2931, 1708, 1665, 1639, 1630, 1459, 1407, 1383, 1236 and 818. ^{1}H NMR (300 MHz, $CDCl_3$): δ0.75 (3H, *s*), 0.93 (3H, *d*, J = 6.0 Hz), 1.02 (3H, *s*), 1.09 (3H, *s*), 2.35 (1H *d*, J = 12.0 Hz), 2.48 (1H, *d*, J = 12.0 Hz), 5.41 (1H, *m*). ^{13}C NMR: Table 2. EI-MS 70 eV *m/z* (rel. int.) of Me ester of **6**: 264 $[M]^+$ (10), 249 $[M-15]^+$ (10), 232 $[M-32]^+$ (5), 191 $[M-73]^+$ (100), 190 $[M-74]^+$ (80), 175 $[M-89]^+$ (72), 136 $[M-128]^+$ (10), 119 $[M-145]^+$ (45), 105 $[M-159]^+$ (65).

5(6)-*Tetranorfriedolabden*-12-*ol* (**7**). Crystals from hexane, mp 58–62°. IR ν_{max} cm^{-1}: 3361, 3048, 2934, 1456, 1382, 1046, 820. ^{1}H NMR (300 MHz, $CDCl_3$): δ0.64 (3H, *s*), 0.85 (3H, *d*, J = 6.7 Hz), 0.88 (3H, *s*), 1.04 (3H, *s*), 2.14 (1H, *d*), 3.70 (1H, *dt*, J = 8.0 Hz), 5.43 (1H, *m*). ^{13}C NMR: Table 2. EI-MS 70 eV *m/z* (rel. int.): 236 $[M]^+$ (5), 221 $[M-15]^+$ (5), 191 $[M-45]^+$ (100), 136 $[M-100]^+$ (30), 121 $[M-115]^+$ (35), 80 $[M-156]^+$ (70). Compound **7** was prepd from **6** by reduction in the following manner. To the Me ester of **6** (9 mg) in dry THF (0.5 ml) under Ar was added $LiAlH_4$ (3 mg) suspended in THF (1 ml). After 2 hr, usual work-up afforded **7** (6.4 mg, 80%), identical in all respects to the natural product.

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