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A TETRANORFRIEDOLABDANE DITERPENE FROM *VELLOZIA STIPITATA*

ÂNGELO C. PINTO,* OCTAVIO A. C. ANTUNES, MOACIR G. PIZZOLATTI and VICTOR M. RUMJANEK†

Instituto de Química, Universidade Federal do Rio de Janeiro, CT Bloco A, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ 21945-970, Brazil; †Departamento de Química, Instituto de Ciências Exatas Universidade Federal Rural do Rio de Janeiro, km 47 da Antiga Rio-SP, Seropédica, RJ 23851-970, Brazil

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Key Word Index—*Vellozia stipitata*; Velloziaceae; tetranorfriedolabdane; chemical transformations.

Abstract—Treatment of the methanolic extract of *Vellozia stipitata* with base gave a new norditerpene with a friedolabdane skeleton. The structure was established on the basis of its spectral data and chemical transformations.

INTRODUCTION

In previous papers [1–3], we described the isolation of lupeol, lupenone, vellozone, oleanolic acid, 7,16-epoxy-20-nor-5,7,9,11,13-cleistanthapentaen-3-one and 7,16-epoxy - 20 - nor - 1,5,7,9,11,13 - cleistanthahexaen-3-one from the hexane extract of roots, stem and leaf sheaths of *Vellozia stipitata* L. B. Smith & Ayensu. We now report the isolation of other di- and triterpenes already known from other Velloziaceae species, together with a new tetranorditerpene with a friedolabdane skeleton from the methanolic extract of this species.

RESULTS AND DISCUSSION

Chromatographic fractionation of the methanol extract produced 20-hydroxylupan-3-one [4], betulinic acid [5], a mixture of β-sitosterol, stigmasterol, campesterol and compactone [6]. All these terpenoids were identified from their spectral data and also by comparison with authentic samples.

Fractions that were only partially purified were shown by HRGC–mass spectrometry to contain, as minor components, two diterpenes and three triterpenes: cleistantha-6,8,11,13-tetraene (A. M. R. Mercê, F. R. Aquino Neto and A. C. Pinto, unpublished data) cleistantha-8,11,13-trien-7-one [7], olean-9(11),12-dien-3-one [8], β-amirone [9] and Δ^{18}-friedelen-3-one [10]. All these compounds, with the exception of the last one, were identified by HRGC–mass spectrometry, and selective ion monitoring, and co-elution with standard compounds previously isolated from other Velloziaceae species or their derivatives. The co-elution experiments were carried out using two chromatographic columns of different selectivities. The triterpene Δ^{18}-friedelen-3-one was assigned the proposed structure based on comparison with mass spectral data published in the literature [10].

The methanol extract was dewaxed with hexane, redissolved in diethyl ether and partitioned with 5% NaOH solution and the acidic fractions were worked up separately. From one of these fractions a new tetranorditerpene, **1**, with a friedolabdane skeleton was isolated.

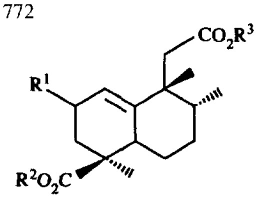
The molecular formula of the diacid **1**, $C_{16}H_{24}O_4$, was determined by HR-mass spectrometry. Its IR spectrum displayed two strong broad carbonyl absorptions at 1700 and 1690 cm^{-1}, a trisubstituted double bond at 1640 and 940 cm^{-1} and a broad band for acidic hydroxyl groups at 3300–2600 cm^{-1}, which was absent in the IR spectrum of its dimethyl ester (**2**).

The ^{1}H NMR spectrum of the dimethyl ester (**2**) showed signals for three methyl groups at δ 0.78 (3H, *d*, $J = 6.9$ Hz), 1.06 (3H, *s*) and 1.16 (3H, *s*), two carboxymethoxyl groups at δ 3.57 and δ 3.66 (3H, *s*) and two doublets for two geminal protons at δ 2.25 and δ 2.95 (1H, *d*, $J = 13.5$ Hz). These two doublets, also present in the ^{1}H NMR spectrum of **1** together with the fragmentation pattern with losses of 59 and 73 amu in the MS of **1** and **2**, respectively, suggested the presence of a $-CH_2CO_2H(Me)$ residue.

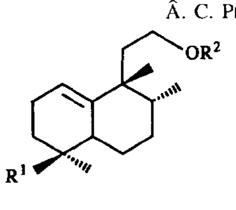
These data, in combination with the analysis of the proton noise decoupled and DEPT ^{13}C NMR spectrum, allowed the further expansion of the molecular formula of the dimethyl ester (**2**), $C_{18}H_{28}O_4$, to (CH_2CO_2Me) (CO_2Me) $(-C{=}CH)$ $(CH{-}CH_3)$ $(-CH_3)_2$ (CH) $(C)_2$ $(CH_2)_4$ and enabled us to consider **1** as the working hypothesis for the new tetranorfriedolabdane.

The moiety $=CHCH_2CH_2-$ was deduced from the homonuclear (^{1}H × ^{1}H-HOMOCOSY) NMR spectrum of **2**, which showed the expected vicinal spin coupling of the olefinic proton at δ 5.35 (1H, *bs*) with the C-2

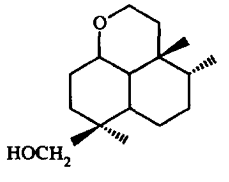
*Author to whom correspondence should be addressed.



	R^1	R^2	R^3
1	H	H	H
2	H	Me	Me
7	=O	Me	Me



	R^1	R^2
3	CH_2OH	H
4	CH_2OAc	Ac
5	CH_2OH	Ac
8	CHO	Ac



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methylene group at δ 2.10, which, in turn, was coupled to two hydrogens at δ 1.50 and δ 1.80 (C-3).

Reduction of **2** with lithium aluminum hydride and subsequent acetylation with acetic anhydride/DMAP produced diacetate **4**. When the acetylation was carried out at 0° for 1 hr it gave both the mono and diacetylated derivatives **5** and **4**.

In order to confirm the position of the double bond, the following chemical reactions were carried out. Treatment of diol **3** with $Hg(OAc)_2$ in THF at reflux, followed by reaction of the crude material with $NaBH_4$, yielded the cyclic ether **6**. Final proof for structure came from the oxidation of dimethyl ester **2** with tert-butyl chromate which yielded an α,β-unsaturated ketone **7**, showing the olefinic proton at δ 5.82 and an AB pattern for a methylene group adjacent to a carbonyl group at δ 2.37 and 2.85 ($J = 15.9$ Hz). Therefore, the double bond position was clearly confirmed at C(1)–C(10), in agreement with structures **1** and **2**. Once the chemical structure of this molecule had been established, the determination of the relative stereochemistry was undertaken. This was done by using ^1H–^1H NOE of the diester **2**.

Irradiation of the methyl signal at δ 1.06 resulted in

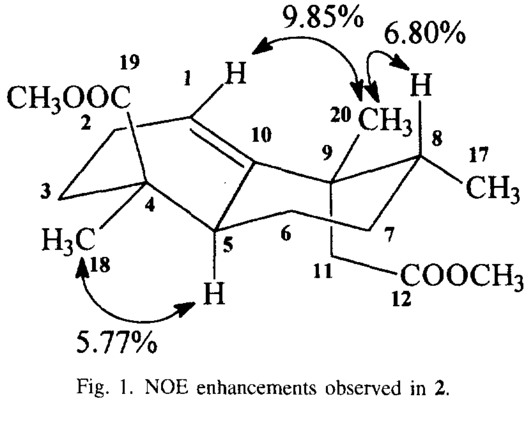

Fig. 1. NOE enhancements observed in **2**.

an enhancement of 9.85% for the vinylic proton at δ 5.35 and of 6.80% for the C(8)-H, thus showing a *cis* relationship between C(9)-Me and C(8)-H. No enhancement was observed for C(5)-H indicating a *trans* relationship between C(9)-Me and C(5)-H. Irradiation of the methyl signal at δ 1.16 (C(4)-Me) resulted in an enhancement of 5.77% for C(5)-H, thus pointing to a *cis* relationship between C(4)-Me and C(5)-H. The relative configuration as well as the NOE enhancements are shown in Fig. 1.

EXPERIMENTAL

General methods. Mps uncorr. ^1H NMR (200 and 300 MHz) and ^{13}C NMR (50.3 and 75.5 MHz): TMS as int. standard; IR: KBr pellets; EIMS: HRGC–MS Hewlett-Packard 5987 A; TLC: Kieselgel 60 HF (Merck) compounds located under UV light and/or by spraying a 0.2% soln of $Ce(SO_4)_2$ in 1M H_2SO_4 followed by heating on a hot plate.

Extraction and preliminary fractionation. Details of the plant material and the extraction procedures are described in a previous paper [3]. The methanolic extract, after drying, was dissolved in Et_2O and extracted with 5% NaOH. The Et_2O soln was washed with H_2O, dried and concd yielding the neutral fraction. The alkaline solution was acidified with HCl, extracted with Et_2O, the organic phase washed with H_2O, dried and concd, yielding the acidic fr.

Isolation of 13,14,15,16-*tetranorfriedo*-1(10)-*labden*-12,19-*dioic acid* (**1**). This compound was isolated from the acidic fr. by silica gel CC, eluted with hexane–EtOAc (9:1) and purified by recrystallization from Me_2CO: mp 202–203°; IR ν_{max}^{KBr} cm^{-1} 3300–2600(*br*), 1700, 1690, 1640, 1410, 1285, 1140, 940, 850, 765 and 680; MS m/z (rel. int.): 280 $[M]^+$ (15) ($C_{16}H_{24}O_4$), 262 (5), 235 (35), 221 (65), 175 (100), 159 (12), 147 (6), 133 (18), 119 (40), 107 (15), 105 (25) and 91 (23); ^1H NMR (300 MHz, $CDCl_3$): δ 0.83 (3H, *d*, $J = 6.9$ Hz), 1.12 (3H, *s*), 1.19 (3H, *s*), 2.31 (1H, *d*, $J = 13.5$ Hz), 2.89 (1H, *d*, $J = 13.5$ Hz) and 5.39 (1H, *bs*); ^{13}C NMR (25.2 MHz, $CDCl_3$): δ 15.6 (CH_3), 22.3 (CH_3), 22.8 (CH_2), 22.8 (CH), 25.5 (CH_2), 26.6 (CH_2), 29.8 (CH_2), 39.2 (CH), 41.5 (C), 43.2 (C), 44.6 (CH_2), 119.3 (CH), 139.9 (C), 175.2 (C) and 180.9 (C).

Methylation of **1**. The acid **1** (80 mg) was methylated with CH_2N_2 in Et_2O to furnish the diester **2** (88 mg) as crystals (from hexane), mp 93–94°; IR ν_{max}^{KBr} cm^{-1}: 3040, 2920, 1725, 1450, 1420, 1370, 1300, 1200, 1180, 940, 850, 765 and 675; MS m/z (rel. int.): 308 $[M]^+$ (10), 279 (5), 249 (55), 235 (40), 217 (15), 202 (15), 175 (100), 159 (20), 119 (40) and 105 (30); ^1H NMR (300 MHz, $CDCl_3$): δ 0.78 (3H, *d*, $J = 6.9$ Hz), 1.06 (3H, *s*), 1.16 (3H, *s*), 2.25 (1H, *d*, $J = 13.5$ Hz), 2.95 (1H, *d*, 13.5 Hz), 3.57 (3H, *s*), 3.66 (3H, *s*) and 5.35 (1H, *bs*).

Reduction of **2**. The methyl ester **2** (50 mg), dissolved in dry THF (5 ml), was stirred for 3 hr in the presence of $LiAlH_4$ (100 mg) at room temp. Excess reagent was destroyed by successive addition of EtOAc, H_2O and drops of 1M HCl. The reaction mixture was worked up in the usual way to yield, after purification by silica gel CC, pure diol **3** (46.3 mg) as a colourless oil. IR ν_{max}^{NaCl} cm^{-1}: 3458, 3342, 2937, 1654, 1459, 1085, 1035, 865, 811 and 740; MS m/z (rel. int.): 252 $[M]^+$ (3), 221 (100), 177 (75), 149 (25), 119 (34), 105 (44), 91 (44), 79 (26) and 55 (25); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79 (3H, *d*, $J = 7.0$ Hz), 0.94 (3H, *s*), 0.98 (3H, *s*), 1.06 (1H, *m*), 1.10 (2H, *m*), 1.43 (1H, *ddd*, $J = 12.0$, 9.1 and 5.6 Hz), 2.06 (2H, *m*), 2.35 (1H, *ddd*, $J = 12.0$, 9.1 and 5.6 Hz), 3.33 (1H, *d*, $J = 10.6$ Hz), 3.49 (1H, *d*, $J = 10.6$ Hz), 3.51 (1H, *ddd*, $J = 12.0$, 9.1 and 5.5 Hz), 3.58 (1H, *ddd*, $J = 12.0$, 9.1 and 5.5 Hz) and 5.39 (1H, *t*, $J = 3.6$ Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 15.1 (CH_3), 21.6 (CH_3), 22.3 (CH_2), 22.5 (CH_3), 24.0 (CH_2), 26.7 (CH_2) 29.2 (CH_2), 36.1 (C), 39.7 (CH), 40.6 (CH), 41.5 (CH_2), 42.1 (C), 60.0 (CH_2), 69.9 (CH_2), 119.9 (CH) and 141.5 (C).

Oxidation of **2** *with tert-butyl chromate.* A soln of **2** (20 mg) in CCl_4 (6 ml) was added to a mixt. of HOAc (1 ml), Ac_2O (0.5 ml) and tert-butyl chromate CCl_4 soln (0.5 ml). This mixt. was heated to reflux temp. for 2 hr. The reaction mixt. was extracted with 5% oxalic acid several times and then the acids were removed from the organic layer by washing with 10% Na_2CO_3. The organic layer was dried, filtered through silica gel, and concd *in vacuo*. Recrystallization from Me_2CO yielded **7** (16.9 mg): mp 95–96°; IR ν_{max}^{KBr} cm^{-1}: 2960, 2880, 1730, 1670, 1610, 1460, 1380, 1245, 1110, 1010, 860 and 770; MS m/z (rel. int.): 322 $[M]^+$ (13), 291 (7), 263 (31), 231 (28), 189 (100), 173 (14), 147 (9) and 121 (26); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80 (3H, *d*, $J = 7.0$ Hz), 1.15 (3H, *s*), 1.28 (3H, *s*), 1.58 (2H, *m*), 1.88 (2H, *m*), 2.36 (1H, *d*, $J = 14.5$ Hz), 2.37 (1H, *d*, $J = 15.9$ Hz), 2.74 (1H, *dd*, $J = 13.2$ and 4.5 Hz), 2.85 (1H, *d*, $J = 15.9$ Hz), 3.16 (1H, *d*, $J = 14.5$ Hz), 3.60 (3H, *s*), 3.70 (3H, *s*) and 5.82 (1H, *s*); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 15.0 (CH_3), 21.8 (CH_3), 23.4 (CH_3), 26.0 (CH_2), 28.6 (CH_2), 41.1 (CH), 41.8 (CH_2), 43.2 (CH), 44.0 (CH_2), 44.8 (C), 46.4 (C), 51.4 (CH_3), 52.0 (CH_3), 124.0 (CH), 165.2 (C), 171.3 (C), 175.5 (C) and 197.7 (C).

Acetylation of diol **3**. To a soln. of diol **3** (35 mg) in EtOAc (0.5 ml) was added Ac_2O (2 ml) and a catalytic amount of DMAP. The mixture was left for 12 hr at 0°. At intervals an aliquot of the reaction was examined by TLC and stopped after 4 hr. Mono- and di-acetylated products were sepd by prep. TLC yielding 13 mg of monoacetyl **5**: IR ν_{max}^{NaCl} cm^{-1}: 3495, 3040, 2960, 1760, 1470, 1380, 1250, 1040 and 830; MS m/z (rel. int.): 294 $[M]^+$ (3), 263 (93), 251 (4), 207 (20), 203 (100), 175 (15), 161 (16), 147 (44), 119 (40), 105 (55), 91 (44) and 81 (25); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80 (3H, *d*, $J = 7.0$ Hz), 0.94 (3H, *s*), 0.96 (3H, *s*), 1.10 (1H, *dt*, $J = 13.0$, 4.4 and 4.4 Hz), 1.50 (1H, *ddd*, $J = 12.0$, 10.6 and 5.9 Hz), 2.00 (3H, *s*), 2.30 (1H, *ddd*, $J = 12.0$, 10.6 and 5.9 Hz), 3.85 (1H, *dt*, $J = 10.6$, 10.6 and 5.25 Hz), 4.00 (1H, *dt*, $J = 10.6$, 10.6 and 5.91 Hz) and 5.36 (1H, *t*, $J = 3.8$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.1, 21.6, 22.3, 22.5, 24.0, 27.1, 28.4, 36.7, 39.5, 47.5, 42.6, 61.1, 61.6, 70.8, 120.4, 124.6 and 197.0; 7 mg of diacetyl **4**: IR ν_{max}^{NaCl} cm^{-1}: 3054, 2930, 1735, 1640, 1242, 1033 and 737; MS m/z (rel. int.): 336 $[M]^+$ (20), 321 (5), 263 (45), 249 (30), 175 (100), 147 (30), 119 (50), 105 (55), 91 (40) and 81 (20); ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.80 (3H, *d*, $J = 7.0$ Hz), 0.94 (3H, *s*), 0.96 (3H, *s*), 2.05 (6H, *s*), 3.33 (1H, *d*, $J = 10.6$ Hz), 3.50 (1H, *d*, $J = 10.6$ Hz), 3.85 (1H, *dt*, $J = 10.4$, 10.4 and 5.3 Hz), 4.00 (1H, *dt*, $J = 10.4$, 10.4 and 5.3 Hz) and 5.36 (1H, *t*, $J = 3.6$ Hz); and 15 mg of recovered **3**.

Oxidation of **5** *with PCC.* Compound **5** (10 mg) was treated with PCC (20 mg) in dry CH_2Cl_2 (2 ml) at room temp. After 2.5 hr, dry Et_2O (5 ml) was added and the supernatant decanted from a brown gum. This gum was washed thoroughly with dry Et_2O, the Et_2O solns were combined, filtered through a pad of silica gel and evapd at red. pres. yielding aldehyde **8** (8 mg): IR ν_{max}^{NaCl} cm^{-1}: 2940, 2880, 2720, 1745, 1730, 1460, 1370, 1240 and 1030; MS m/z (rel. int.): 292 $[M]^+$ (2), 263 (10), 233 (2), 219 (10), 103 (10), 175 (100), 159 (26), 145 (36), 131 (24), 119 (44), 105 (70), 91 (48) and 81 (24).

Preparation of compound **6** *through oximercuration–demercuration.* In a 50-ml round-bottomed flask was placed 1.60 g of $Hg(OAc)_2$ and to this was added 5.0 ml of H_2O. The mixture was stirred until the salt dissolved to produce a clear soln. Finally, 5.0 ml of THF was added to produce a yellow prpt. To the vigorously stirred suspension was added compound **3** (30 mg) dissolved in THF (3 ml) and refluxed for 3 hr. A 3.0 M NaOH soln (0.3 ml) was added to the cold mixt. followed by 0.3 ml 0.5 M $NaBH_4$ in 3.0 M NaOH with vigorous stirring. The mixt. was stirred until most of the mercury had coagulated (40 min). The aq. phase was satd with K_2CO_3, extracted with Et_2O (3×5 ml) and the combined extracts were dried over K_2CO_3, then filtered through a Zn powder/silica gel (1:3) layer to remove the Hg–Zn amalgam. The solvent was evapd under red. pres. Recrystallization from Me_2CO yielded crystals of **6** (28 mg) mp 98–99°; IR ν_{max}^{KBr} cm^{-1}: 3315, 2940, 1465, 1376, 1256, 1090 and 1028; MS m/z (rel. int.): 252 $[M]^+$ (5), 222 (100), 104 (8), 177 (65), 121 (18) and 95 (28); ^{1}H NMR (300 MHz, $CDCl_3$): 0.70 (1H, *m*), 0.90 (3H, *d*, $J = 7.0$ Hz), 0.94 (3H, *s*), 0.98 (3H, *s*), 2.31 (1H, *dt*, $J = 13.0$, 13.0 and 6.5 Hz), 3.49 (1H, *d*, $J = 10.6$ Hz), 3.72 (1H, *m*), 377 (1H, *d*, $J = 10.6$ Hz) and 3.76–3.92 (2H, *m*); ^{13}C NMR (75.5 MHz, $CDCl_3$): 14.9 (CH_3), 19.7 (CH_2), 23.4 (CH_3), 25.1 (CH_3), 27.9 (CH_2), 28.9 (CH_2), 29.2 (CH_2), 31.8 (CH_2), 34.3 (C), 37.3 (C), 38.9 (CH), 39.7 (CH), 40.3 (CH), 61.4 (CH_2), 64.4 (CH_2) and 70.5 (CH).

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