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Chemical constituents from *Vellozia graminifolia* (Velloziaceae)

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ABSTRACT

From the hexane and ethyl acetate extracts obtained from stems, roots and leaf sheaths of *Vellozia graminifolia*, a mixture of linear hydrocarbons, a mixture of pentacyclic triterpenes, five monoisoprenylated flavonoids and one labdane diterpene, (-)-ent-3β-hydroxy-8(17)-labden-15-oic acid, were isolated. The linear hydrocarbons and minor triterpenes were identified in their corresponding mixtures by High Resolution Gas Chromatography (HRGC) and HRGC coupled to mass spectrometry. The major pentacyclic triterpenes and the diterpene were characterized by spectral data, including 2D NMR experiments, and chemical transformations.

Key words: *Vellozia graminifolia*, Velloziaceae, triterpenes, diterpene, flavonoids.

INTRODUCTION

Velloziaceae is a family of tropical monocotyledonous plants containing about 270 species, most of which occur in Brazilian tropical scrub on rock outcrops (''campos rupestres'') (Stannard 1995). The *Vellozia* genus presents the largest number of species in the Velloziaceae family. Previous phytochemical studies showed that the main constituents of the *Vellozia* genus are flavonoids (Harborne et al. 1994, Williams et al. 1994), diterpenoids (Pinto et al. 1983, 1991, 1996a) and triterpenoids.

The triterpenoids reported in the literature as constituents of *Vellozia* species are distributed into the following classes: dammarane, lupane, taraxerane, oleanane and euphane (Barnes et al. 1984, Pinto et al. 1996b, Peixoto et al. 1979). The application of High Resolution Gas Chromatography (HRGC) and HRGC coupled to mass spectrometer (MS) can be used to distinguish its triterpenoids (Shiojima et al. 1992, Ogunkoya 1981) by retention time and fragmentation patterns, respectively. Thus, the identification of these compounds can be accomplished by co-injection in the HRGC using authentic samples isolated from other plants (Pinto et al. 1994, Patitucci et al. 1995). On the other hand, HRGC requires special attention in Velloziaceae because the presence of peaks corresponding to sterols (Peixoto et al. 1979) and dimmeric diterpenes (Pinto et al. 1997) can be observed in the same region where the triterpenes appear.

In continuation of our phytochemical studies with *Vellozia graminifolia*, we report in this paper the characterization of its pentacyclic triterpenes (**1-15**), in mixture, by high resolution HRGC and HRGC-MS, together with other spectral data. In addition, we also describe the struc-

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ture of a first labdane-type diterpene (**16**) from the Velloziaceae family, determined by application of NMR techniques, including Heteronuclear Multiple Quantum Coherence (^{1}H-^{13}C-HMQC-$^1J_{CH}$, ^{1}H-detected), Heteronuclear Multiple Bond Connectivity [^{1}H-^{13}C-HMBC-$^nJ_{CH}$ (n = 2 and 3), ^{1}H-detected] and Nuclear Overhauser Effect SpectroscopY (NOESY) (Nakanishi 1990, Sanders and Hunter 1993), and chemical transformations.

MATERIALS AND METHODS

General Experimental Procedures

Mps were uncorr. NMR spectra were recorded in $CDCl_3$ soln at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Bruker AC 300 spectrometer, using TMS as int. standard or by reference to the solvent signals: C<u>H</u>Cl$_3$ at δ_H 7.25 and <u>C</u>HCl$_3$ at δ_C 77.00. HRGC analyses were recorded on a Hewlett Packard model 5790A gas chromatograph. Glass capillary column (11 m $\times$ 0.25μm) coated with SE-54 (df = 0.25μm). Analytical GC conditions: Hydrogen as carrier gas; flow rate of 2 ml/min; injector port was set at 280°C and flame ionization detector (FID) at 290°C. The temperature program for the analysis of mixtures ranged from 260°C to 280°C. The data were collected on an HP 3396-II integrator. HRGC-MS analyses were performed on an HP 5987A spectrometer (Hewlett Packard, Palo Alto, USA), with electron impact ionization (70eV). MS scan range was 40 to 700 Da. The GC-MS interface was at 350°C and ion source temperature at 300°C. Column temperature program and injection mode were as for chromatographic analysis. CC: silica gel (0.063 to 0.2 mm). TLC: silica gel (Merk, Kieselgel 60), spots visualized by UV (254 and 360 nm) and exposure to I_2 vapor. TLC was used to analyze frs collected from CC.

Plant Material

The *Vellozia graminifolia* was collected in Chapada da Diamantina, Minas Gerais State, Brazil and identified by Prof. Nanuza L. de Menezes of the Universidade de São Paulo, São Paulo-SP, Brazil. A voucher of this plant is deposited in the herbarium of the Instituto de Botânica at the same university.

Extraction and Purification

Dried and powdered material (roots, stems and leaf sheaths) was successively extracted with n-hexane and ethyl acetate at room temp. and the solvents removed under vacuum to yield 12.9 and 13.9 g of oily residue, respectively. The n-hexane extract (12.9 g) was chromatographed on a column of silica gel (112 g) and eluted with hexane/EtOAc and EtOAc/MeOH mixtures in increasing polarity. A total of 29 fractions, each containing ca 120 mL, were collected and combined on the basis of TLC comparative analysis. Fraction 1, eluted with hexane, afforded a mixture of alkanes. Fractions 4 and 5, eluted with hexane/EtOAc (3:1), furnished a mixture of pentacyclic triterpenes (**1-15**) (36 mg, as red oil) after removal of aliphatic esters with acetone. Fractions 10 and 11, eluted with hexane/EtOAc (2:1), yielded a mixture of **20** and **21**. Fraction 20 eluted with EtOAc/MeOH (5:1) afforded **16** (48 mg), after recrystallization from acetone. The EtOAc extract (13.90 g) was chromatographed using the same procedures described above, yielding **22-24**.

Mixture of Alkanes

Mp 57 – 58°C. IR ν_{max}^{KBr} cm^{-1}: 2960, 2910, 2820 and 720 (CH_3,CH_2). HRGC-MS 70 eV m/z (rel. int.): 380 ([M]$^{\bullet+}$, 3, $C_{27}H_{56}$); 394 ([M]$^{\bullet+}$, 6, $C_{28}H_{58}$); 408 ([M]$^{\bullet+}$, 3, $C_{29}H_{60}$); 422 ([M]$^{\bullet+}$, 1, $C_{30}H_{62}$).

Mixture of Pentacyclic Triterpenes

IR ν_{max}^{KBr} cm^{-1}: 1730 (C=O). HRGC chromatogram: Figure 1A. HRGC-MS 70 eV m/z (rel. int.): Table I. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): data for **2** and **8** in accordance with the literature data (Olea and Roque 1990, Mahato and Kundu 1994).

(-)-*Ent*-3β-Hydroxylabd-8(17)-en-15-oic Acid (16)

White crystal, Mp 144°C, $[\alpha]_D$ – 52° (*c* 1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3300-2600 (OH), 1700 (C=O), 1640

TABLE I

Principal peaks observed in the mass spectra (*m/z* and relative intensity in parenthesis) of the triterpenes isolated from *V. graminifolia*.

Peak*	R_t**	$[M]^{+\bullet}$	$[M]^{+}$-15	Principal peaks	Compound
a	24.4	422 (100)	407 (15)	See Figure 2A	Olean-9(11),12-dien-3-one (**1**)
b	25.7	424 (22)	409 (14)	See Figure 2B	Taraxer-14-en-3-one (**6**)
c	26.1	424 (11)	409 (10)	311 (5); 205 (100, **8a**); 189 (26); 109 (58); 95 (44)	neo-Lup-20(29)-en-3-one (**7**)
d	26.3	424 (5)	409 (4)	391 (2); 218 (100, **2a**); 203 (56, **2b**); 135 (10); 55 (24)	Olean-12-en-3-one (**2**)
e	26.4	424 (19)	409 (28)	See Figure 2C	Olean-18-en-3-one (**3**)
f	26.9	426 (3)	411 (3)	281 (11); 218 (100, **2a**); 203 (52); 107 (26); 55 (44)	Olean-12-en-3-ol (**5**)
g	27.4	424 (36)	409 (25)	381 (6); 313 (35); 245 (25); 205 (100, **8a**); 109 (74)	Lup-20(29)-en-3-one (**8**)
h	27.8	426 (24)	411 (12)	393 (10); 315 (14); 207 (90, **9a**);189 (91, **9b**); 107 (68)	Lup-20(29)-en-3β-ol (**9**)
i	28.0	424 (15)	411 (11)	313 (18); 274 (100, **10a**); 259 (91, **10b**); 205 (36); 109 (86)	Glutin-5-en-3-one (**10**)
j	28.6	438 (30)	423 (12)	340 (10); 313 (44); 300 (17); 175 (36); 95 (100)	24-methyl-cycloartan-25-en-3-one(**12**)
l	29.0	438 (18)	423 (10)	409 (38); 395 (9); 313 (12); 257 (100); 245 (28)	–
m	29.4	438 (12)	–	424 (69); 408 (11); 325 (38); 205 (100); 189 (82)	–
n	29.6	438 (8)	423 (6)	424 (34); 355 (28); 313 (24); 205 (100); 189 (37)	–
o	29.9	454 (4)	439 (6)	259 (8); 218 (100, **2a**); 203 (59, **2b**);189 (37); 95 (40)	Olean-12-en-3-one-23-oic acid (**4**)
p	30.1	454 (5)	–	281 (11); 274 (100, **10a**); 259 (92, **10b**); 231 (31); 137 (46)	glutin-5-en-3-one-23-oic acid (**11**)
q	30.7	440 (10)	425 (15)	409 (73); 393 (13); 271 (22); 257 (100); 189 (45)	–
r	31.1	454 (5)	439 (4)	385 (5); 344 (10); 218 (100); 203 (68); 189 (22); 107 (27)	–
s	32.1	438 (25)	423 (19)	See Figure 2D	Olean-12-en-3,11-dione (**13**)
t	33.3	438 (28)	–	407 (30); 313 (23); 273 (100, **14a**); 232 (72, **14b**); 135 (80, **14c**)	Ursan-12-en-3,11-dione (**14**)
u	33.7	440 (13)	–	422 (25); 407 (14); 302 (18); 217 (35); 147 (50); 95 (100)	11-hydroxy-olean-12-en-3-one (**15**)

*Peak: see Figure 1B. **Retention Time in minute.

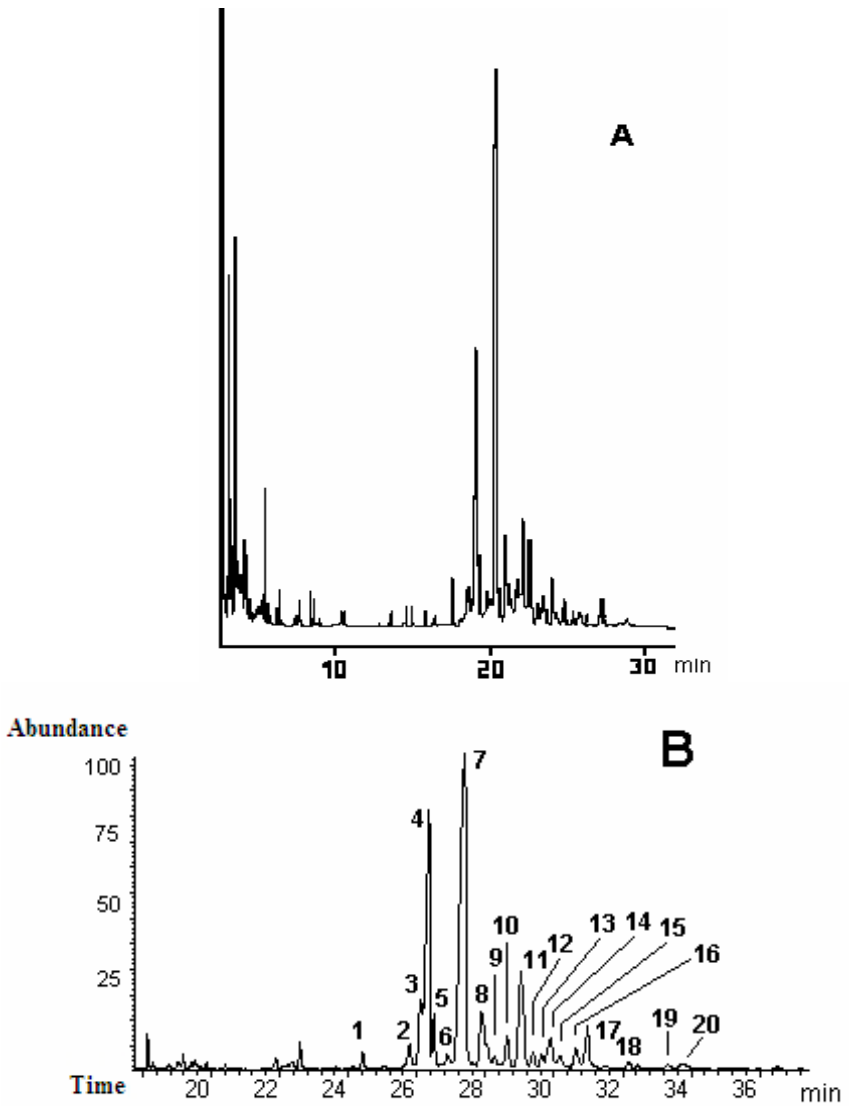


Fig. 1 – **A)** HRGC chromatogram of fraction containing pentacyclic triterpenes from *V. graminifolia* and **B)** Partial Total Ions Chromatogram (TIC) after recrystallization of the fraction. Mass spectra: see Table I.

and 850 (C=CH_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 0.66 (*s*, 3H-20), 0.75 (*s*, 3H-19), 0.95 (*d*, *J*= 6.0, 3H-16), 0.97 (*s*, 3H-18), 1.70 and 1.55 (*m*, 2H-2), 2.30 and 1.90 (*m*, 2H-14), 3.23 (*dd*, *J*= 10.8 and 4.1, H-3), 4.80 (*s*, 1H-17) and 4.47 (*s*, 1H-17). ^{13}C NMR (75 MHz, $CDCl_3$): 35.6 (CH_2-1), 27.8 (CH_2-2), 78.8 (CH-3), 39.0 (C-4), 51.5 (CH-5), 23.9 (CH_2-6), 38.1 (CH_2-7), 148.0 (C-8), 56.5 (CH-9), 39.3 (C-10), 21.0 (CH_2-11), 37.0 (CH_2-12), 30.6 (CH-13), 41.7 (CH_2-14), 178.6 (C-15), 19.5 (CH_3-16), 106.6 (CH_2-17), 28.2 (CH_3-18), 15.3 (CH_3-19), 14.4 (CH_3-20). EIMS 70 eV *m/z* (rel. int.): 322 ($[M]^{\bullet+}$, 3), 307 (M-Me$^\bullet$, 2), 304 (M-H_2O, 21), 289 (*m/z* 304 - Me$^\bullet$ and/or 307 - H_2O, 14), 245 (*m/z* 304 - $^\bullet CH_2COOH$, 4), 193 (**16a**, 5), 190 (**16b**, 5), 175 (**16c**, 21), 135 (**16d**, 100).

METHYL ***Ent***-3β-HYDROXYLABD-8(17)-EN-15-OATE (17)

The crystal of **16** (11.3 mg) was treated with CH_2N_2 in the usual manner to yield **17** (10.4 mg). ^1H (300 MHz) and ^{13}C NMR (75 MHz, $CDCl_3$): Table II. EIMS 70 eV *m/z* (rel. int.): 336 ($[M]^{\bullet+}$, 1), 321 (M-Me$^\bullet$, 2), 318 (M-H_2O, 9), 303 (*m/z* 318 - Me$^\bullet$, 2), 193 (4), 190 (5), 175 (*m/z* 193 - H_2O and/or *m/z* 190 - Me$^\bullet$, 21), 135 (**16d**, 100).

METHYL ***ent***-3-OXO-LABD-8(17)-EN-15-OATE (18)

A soln of **16** (10.7 mg) in Me_2CO (10.0 mL) was allowed to stand with slight excess of Jones' reagent at reflux for 2 hs and usual work-up gave a colorless oil **18** (7.2 mg) after methylation with CH_2N_2. ^1H NMR (300 MHz, $CDCl_3$): δ_H 0.79 (*s*, 3H-20), 0.95 (*s*, 3H-19), 0.88 (*d*, *J*=6.6, 3H-16), 1.02 (*s*, 3H-18), 4.82 (*s*, 1H-17) and 4.49 (*s*, 1H-17). EIMS 70eV *m/z* (rel. int.): 334 ($[M]^{\bullet+}$, 45), 319 (M - Me$^\bullet$, 41), 303 (M - MeO$^\bullet$, 24), 291 (*m/z* 319 - CO, 15), 236 (**18a**, 11), 139 (**18b**,31), 123 (**18c**, 94).

Ent-3β-HYDROXYLABD-8(17)-EN-15-OL (19)

The crystal of **16** (11.3 mg) was treated with $LiAlH_4$ in the usual manner to yield **19** (10.4 mg). ^1H NMR (300 MHz, $CDCl_3$): δ_H 0.68 (*s*, 3H-20), 0.77 (*s*, 3H-19), 0.90 (*d*, *J* = 6.4, 3H-16), 0.99 (*s*, 3H-18), 4.83 (*s*, 1H-17) and 4.51 (*s*, 1H-17), 3.62 (m, 2H-15), 3.25 (*dd*, *J* = 11.6 and 4.5, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): 37.2 (CH_2-1), 28.0 (CH_2-2), 79.0 (CH-3), 39.2 (C-4), 54.7 (CH-5), 24.1 (CH_2-6), 38.3 (CH_2-7), 148.4 (C-8), 56.9 (CH-9), 39.5 (C-10), 21.2 (CH_2-11), 36.3 (CH_2-12), 31.1 (CH-13), 40.3 (CH_2-14), 61.3 (CH_2-15), 16.6 (CH_3-16), 106.8 (CH_2-17), 28.4 (CH_3-18), 15.5 (CH_3-19), 14.6 (CH_3-20). EIMS 70 eV *m/z* (rel. int.): 308 ($[M]^{\bullet+}$, 6), 293 (M - Me$^\bullet$, 4), 290 (14), 275 (*m/z* 290 - Me$^\bullet$, 6) 175 (15), 152 (16), 135 (**16d**, 100).

MONOISOPRENYLATED FLAVONOLS (20-24)

Spectral data have been described in previous papers (Branco et al. 1998, 2001, Branco 2001).

RESULTS AND DISCUSSION

The crude hexane extract from *V. graminifolia* was initially analyzed by HRGC and HRGC-MS. The previous knowledge of the heat stable chemical constituents present has been established for retention time (R_t) and comparison of the mass spectral data with those of standards available in our laboratory. The complexity observed in the terpenoid eluting regions in the HRGC chromatogram led to fractionating on open silica gel column in the usual manner (see Experimental), yielding initially two fractions.

The presence of the *n*-alkanes ($C_{27}H_{56}$ to $C_{30}H_{62}$) in the more apolar fraction (eluted with hexane) of the hexane extract have been determined on the basis of mass fragmentograms (*m/z* 57 and 85) (Silverstein et al. 1995) obtained by HRGC-MS.

The HRGC chromatogram of the second fraction showed the presence of several compounds, containing two principal peaks with retention time 19.0 and 21.9 min (Figure 1A). This mixture was submitted to ^1H and ^{13}C NMR analysis involving comparison with literature data (Olea and Roque 1990, Mahato and Kundu 1994), allowing identification of the peaks as olean-12-en-3-one (**2**) and lup-20(29)-en-3-one (**8**), respectively. The 2D ^1H-^{13}C-HMQC-$^1J_{CH}$ spectrum of the mixture revealed cross peaks consistent with the structures of these pentacyclic triterpenoids by heteronuclear coupling via one bond between the olefinic hydrogens H-12 [**2**: δ_H 5.25 (*m*)] and 2H-29 [**8**: 4.56 (*br s*) and 4.68 (*br s*)] and the corresponding carbons CH-12 of **2** (δ_C 121.6) and CH_2-29 of **8** (δ_C 109.5).

MINOR PENTACYCLIC TRITERPENES BY HRGC-MS

The fraction containing **2** and **8** was submitted to recrystallization to remove components in major percentage to improve the analysis of the minor components by HRGC-MS.

HRGC-MS analysis (Figure 1B) of the fraction obtained by the above procedure revealed the presence of the peaks in the mass spectra compatible with the fragmentation patterns of pentacyclic triterpenoids (Shiojima et al. 1992, Ogunkoya 1981).

TABLE II

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data for derivative 17 ($CDCl_3$), including results of heteronuclear 2D experiments ^{1}H-^{13}C-COSY-$^{n}J_{CH}$ (n=1, HMQC; n= 2 and 3, HMBC).*

	HMQC		HMBC	
C	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$
4	39.1	–	H-3,3H-18,3H-19	
8	148.1	–	H-7,H-9	H-11
10	39.4	–	3H-20,H-5,H-1	H-11
15	173.7	–	2H-14	MeO-15
CH				
3	79.2	3.25 (*dd*, *J* = 11.7 and 4.1)	H-2	3H-18,3H-19,H-1
5	54.9	1.08 (*dd*, *J* = 12.5 and 2.0)		H-1,3H-18,3H-19,3H-20
9	57.0	1.54	H-11	2H-17,3H-20
13	31.2	1.92	2H-14,3H-16	
CH_2				
1	37.4	1.79		3H-20
		1.17 (*dt*, *J* = 13.2 and 3.8)		
2	28.3	1.68 / 1.57	H-1	
6	24.3	1.74 / 1.36	H-7a,H-5	
7	38.5	2.39 (*br d*, J = 12.5)		2H-17
		1.96 (*dt*, *J* = 12.5 and 5.1)		
11	21.3	1.42		
12	36.0	1.36 / 1.12	H-11	2H-14,3H-16
14	42.2	2.28 (*dd*, *J* = 6.2 and 14.6)	H-13	
		2.12 (*dd*, *J* = 8.0 and 14.6)		3H-16
17	107.0	4.82 (*br s*) / 4.49 (*br s*)		H-7
CH_3				
16	19.8	0.94 (*d*, *J* = 6.6)		2H-14
18	28.6	0.99 (*s*)		H-3,3H-19
19	15.7	0.77 (*s*)		H-3,3H-18
20	14.8	0.68 (*s*)		H-1,H-9
MeO-15	51.7	3.65 (*s*)		

*Chemical shifts (δ) and coupling constants (J in Hz, in parenthesis) obtained in the one-dimensional ^{1}H NMR spectrum. Number of hydrogens bound to carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C. ^{1}H-^{1}H-COSY 2D NMR also used for these assignments.

The mass spectra of these compounds are summarized in Table I. The triterpene with retention time at 27.8 min (peak h, Figure 1B) was recognized as lup-20(29)-en-3β-ol (**9**), which was confirmed by co-injection technique in HRGC using authentic sample (Patitucci et al. 1995)

The chromatographic profile observed by HRGC-MS analysis showed in the chromatogram ion total (Figure 1B) two distinct patterns of elution to pentacyclic triterpenoids: monoxygenated (24 to 28 min) and di- or trioxygenated (28 to 35 min), respectively. This profile is similar to other triterpenes isolated from *Vellozia* species (Pinto et al. 1983, 1996a, 1991). On the basis of mass spectra analy-

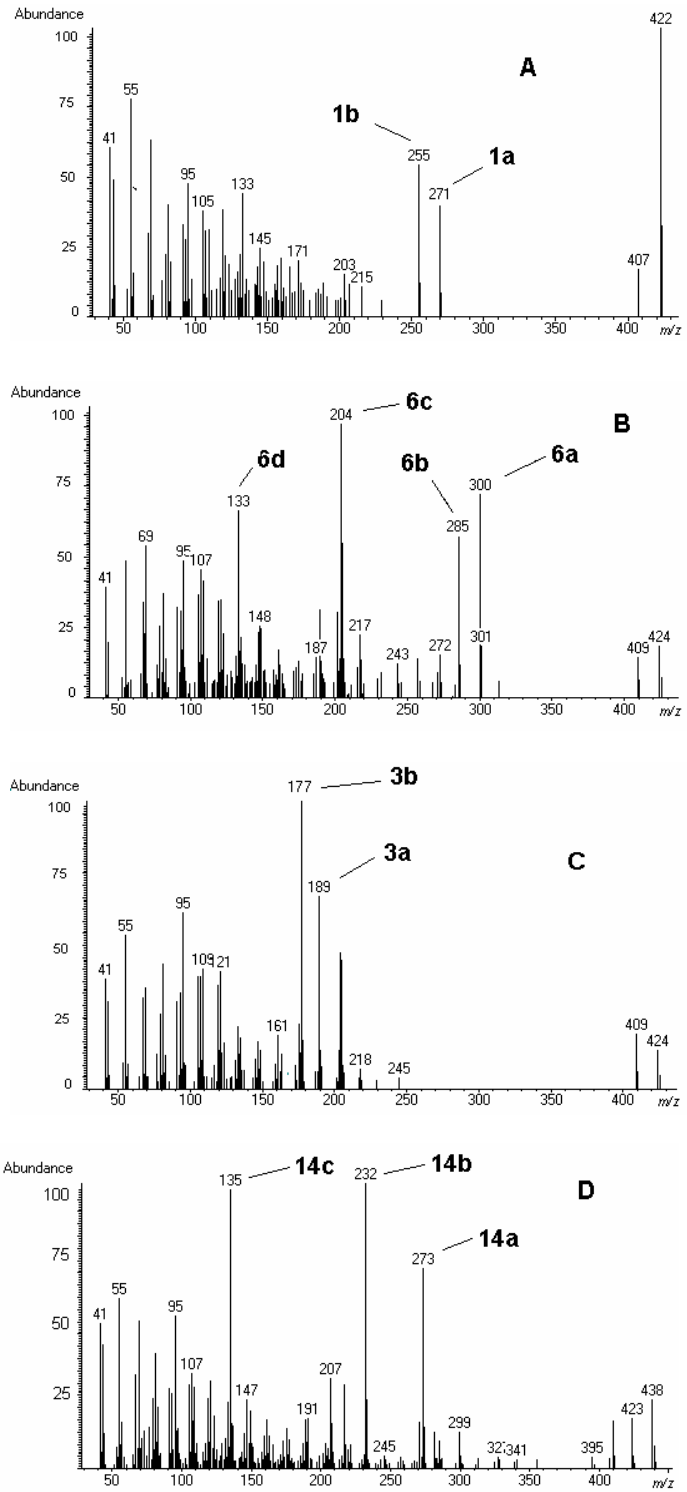


Fig. 2 – Mass spectra of some minor pentacyclic triterpenes: A) **1**, B) **6**, C) **3** and D) **13**. Total Ion Chromatogram: see Figure 1B.

sis, the compounds that appear at 24.4 (peak a), 25.7 (peak b), 26.1 (peak c), 26.4 (peak e), 26.9 (peak f), 28.0 (peak i) and 28.6 (peak j) min (Figure 1B and Table I) have been characterized as olean-9(11),12-dien-3-one (**1**) (Barnes et al. 1984), taraxer-14-en-3-one (**6**) (Sasaki et al. 1965), neo-lup-20(29)-en-3-one (**7**) (Ageta et al. 1981), olean-18-en-3-one (**3**) (Budzikiewich et al. 1963), olean-12-en-3-ol (**5**) and glutin-5-en-3-one (**10**) (Shiojima et al. 1992), 24-methyl-cycloartan-25-en-3-one (**12**) (Kostova et al. 1997, Alves 2000), respectively. The 3-oxo-triterpenes acid eluted at 29.9 (peak o) and 30.1 (peak p) min have been characterized as olean-12-en-3-one-23-oic acid (**4**) and glutin-5-en-3-one-23-oic acid (**11**), respectively.

The compounds eluted at 32.1 (peak s), 33.3 (peak t) and 33.7 (peak u) min, along with biogenetic considerations (Barnes et al. 1984, Torssell 1997), allowed postulating of the **13**, **14** and **15**, respectively, as probable structures. This conclusion was supported by peaks at m/z 273 (68%, **13a** and 100%, **14a**)/ 135 (100%, **13c** and 80%, **14c**) and by peak at m/z 232 (100%, **13b** and 72%, **14b**) in the mass spectra of the diketones, a characteristic and well documented peak due to retro-Diels-Alder fragmentation (Budzikiewich et al. 1963).

Thus, the above triterpenoids **1-15** (Figure 3) were identified by their mass spectra (Table I and Figure 4) and by comparison with standards previously isolated from species of Velloziaceae.

Obviously, pentacyclic triterpenoids of the ursane-type may be included as structural alternatives, since the occurrence of mixtures containing oleanane and ursane isomers is relatively common. Secondary biomodifications of triterpenes may involve the introduction of an additional hydroxyl group and double bond, oxidation of hydroxyl functions to carbonyl groups, reduction of double bond, Wagner-Meerwein rearrangement and alkylation by S-adenosyl methionine (Torssell 1997).

Ent-3β-Hydroxylabd-8(17)-en-15-oic Acid (16)

The IR spectrum of **16** revealed bands corresponding to a carboxylic acid function (ν_{max} 3300-2600 and 1700 cm^{-1}) and terminal methylene (ν_{max} 1640 and 850 cm^{-1}). The signals corresponding to quaternary, methine, methylene and methyl carbon atoms were deduced by comparative analysis involving HBBD- and DEPT- ^{13}C NMR spectra. This analysis in combination with the low-resolution mass spectrum (m/z 322 [M]$^+$) and ^{1}H NMR spectra allowed the deduction of the molecular formula $C_{20}H_{34}O_3$ = $(C)_2$ $(CH_2)_7$ $(CH_3)_4$ $(CH)_4$ $(C{=}CH_2)$ (COOH) (OH) for **16**. The peaks at m/z 193 (6%), 190 (5%), 175 (21%) and 135 (100%) in the mass spectrum of **16**, attributed to ionic fragment **16a**, **16b**, **16c** and **16d**, respectively, are characteristic of a diterpene with labdane skeleton oxygenated at C-3 (Figure 5).

The carboxyl function was confirmed by reaction with diazomethane, which furnished the methyl ester derivative **17**. This derivative was also characterized by comparative analysis of the HBBD- and DEPT- ^{13}C NMR and ^{1}H NMR [1D and 2D ^{1}H-^{1}H-COSY] spectra (Table II). The ^{1}H NMR spectra showed the presence of signals for an axial carbinolic hydrogen [δ_H 3.25 (*dd*, J = 11.7 and 4.1 Hz), H-3], two terminal methylidene hydrogens [δ_H 4.82 (*br s*) and 4.49 (*br s*), 2H-17] and four methyl groups: three tertiary at δ_H 0.68 (*s*), 0.77 (*s*) and 0.99 (*s*), and one secondary at δ_H 0.94 (*d*, J = 6.6 Hz).

2D shift-correlated NMR techniques ^{1}H-^{13}C-HMQC-$^1J_{CH}$ and ^{1}H-^{13}C-HMBC-$^nJ_{CH}$ (Table II) and ^{1}H-^{1}H-NOESY (Table III) of the methyl ester derivative **17** were also used for the correct ^{1}H and ^{13}C NMR chemical shift assignments and stereochemistry deductions of the natural diterpene **16**. The HMBC spectrum revealed the long-range heteronuclear couplings between C-8 (δ_C 148.1) and the hydrogen atoms 2H-11 [δ_H 1.42, $^3J_{CH}$], 2H-7 [2.39 (*br d*, J = 12.5), $^2J_{CH}$] and H-9 (1.54, $^2J_{CH}$).

The oxidation with Jones' reagent of **17** yielded the methyl 3-oxo-labd-8(17)-en-15-oate (**18**), which was characterized on the basis of spectral data (Experimental).

The diol **19** was obtained by reaction of **17** with $LiAlH_4$. The ^{1}H NMR spectrum of **19** showed the

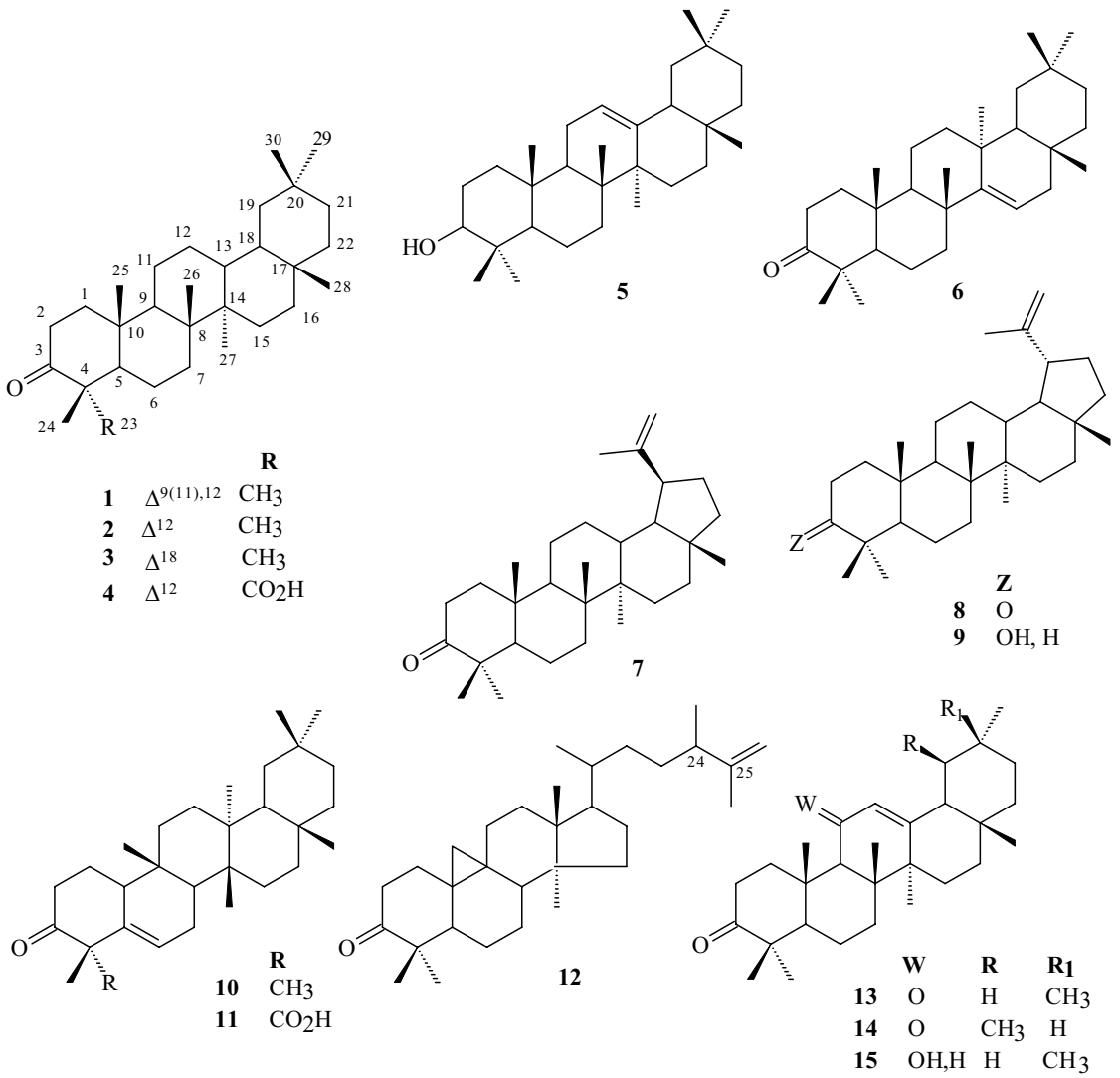


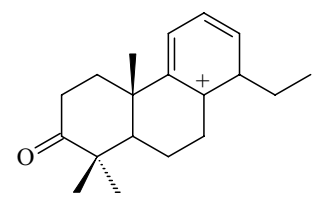
Fig. 3 – Structures of the Triterpenoids characterized in the *V. graminifolia*.

presence of the signal corresponding to hydrogens of a hydroxymethylene group at δ_H 3.62 (*m*). The molecular ion at *m*/*z* 308 observed in the mass spectrum was also used to characterize the structure of this diol. The negative optical rotation compared with the recently synthesized (+)-3β-hydroxylabd-8(17)-ene-15-ol (Pemp and Seifert 1997) allowed determination of **19** as an *ent*-labdane.

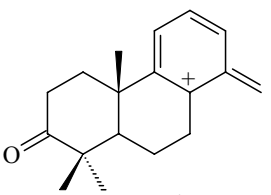
Thus, the natural diterpene isolated from *Vellozia graminifolia* was characterized as (-)-3β-hydroxylabd-8(17)-en-15-oic acid (**16**). To the best of our knowledge **16**, is the first diterpene with a labdane skeleton from the Velloziaceae family; its enantiomer was isolated from *Araucaria imbricata* (Chandra et al. 1964) and from *Moldenhawera nutans* (David et al. 1998).

Monoisoprenylated Flavonols

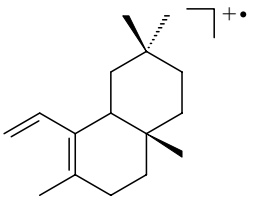
The structures of the new prenylated flavonols (**20-22**) (Figure 6) have been identified on the basis of spectral analysis, including 2D NMR techniques



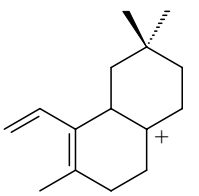
1a *m/z* 271



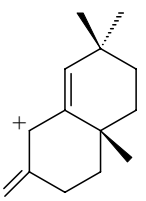
1b *m/z* 255



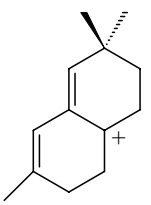
2a *m/z* 218



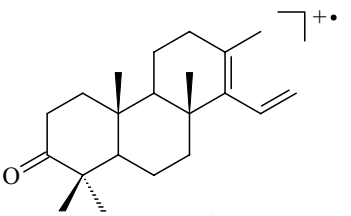
2b *m/z* 203



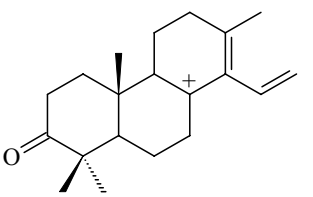
3a *m/z* 189



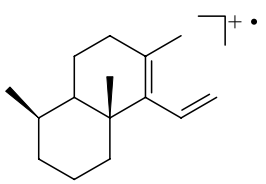
3b *m/z* 177



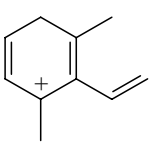
6a *m/z* 300



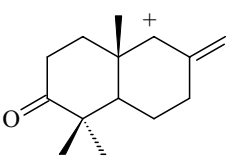
6b *m/z* 285



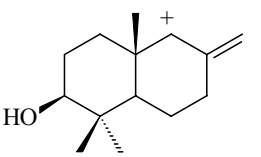
6c *m/z* 204



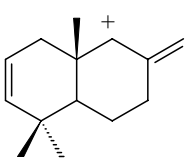
6d *m/z* 133



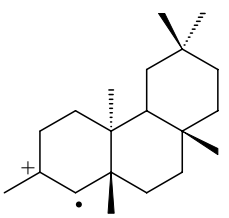
8a *m/z* 205



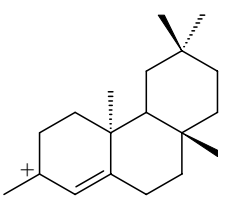
9a *m/z* 207



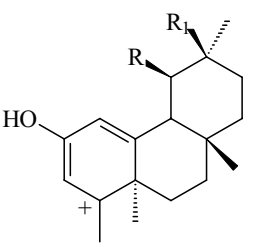
9b *m/z* 189



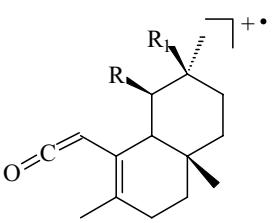
10a *m/z* 274



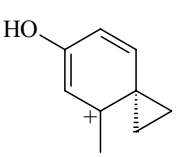
10b *m/z* 259



	R	**R1**	
13a	H	CH_3	*m/z* 273
14a	CH_3	H	*m/z* 273

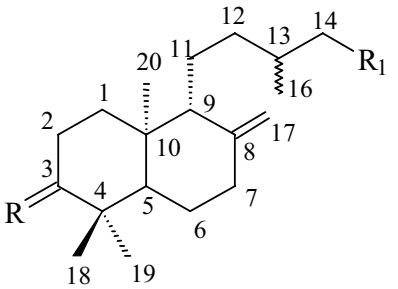


	R	**R1**	
13b	H	CH_3	*m/z* 232
14b	CH_3	H	*m/z* 232

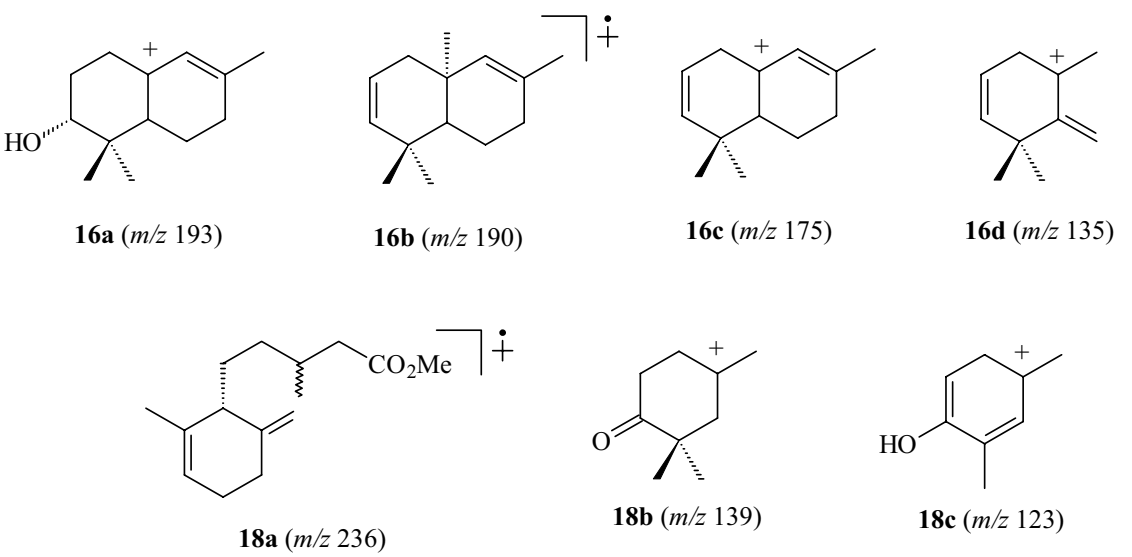


13c/14c *m/z* 135

Fig. 4 – Ionic fragments corresponding to principal peaks observed in the mass spectra. Obviously, the ionic fragments that appear with numbers and letters to indicate the triterpene correspondents can be used for other triterpenoids if compatible.



	R	R_1
16	βOH,H	CO_2H
17	βOH,H	CO_2Me
18	O	CO_2Me
19	βOH,H	CH_2OH



16a (*m/z* 193) **16b** (*m/z* 190) **16c** (*m/z* 175) **16d** (*m/z* 135)

18a (*m/z* 236) **18b** (*m/z* 139) **18c** (*m/z* 123)

Fig. 5 – Structure of the labdane diterpenoid **16**, derivatives **17-19** (upper) and their ionic fragments corresponding to principal peaks observed in the mass spectra (lower).

such as HMQC, HMBC and NOESY, together with chemical shift correlations (Branco et al. 1998, 2001, Branco 2001). The additional new flavonoids **23** and **24** were characterized by HT-HRGC (high temperature high resolution gas chromatography) and HT-HRGC coupled to mass spectrometry experiments (Branco et al. 2001, Branco 2001).

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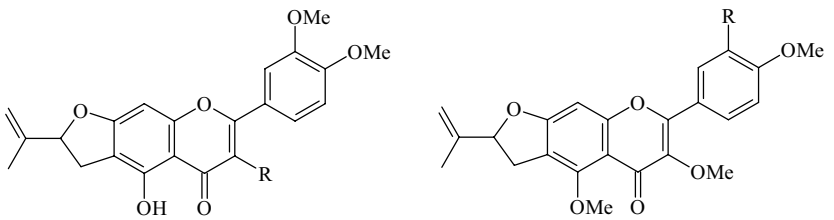

	R
20	H
21	Me

	R
22	OH
23	OMe
24	H

Fig. 6 – Structures of the Monoisoprenylated flavonols isolated from *V. graminifolia*.

TABLE III

^{1}H-^{1}H-NOESY (dipolar couplings) spectral data for 17 (300 MHz,$CDCl_3$,δ).

H	δ_H	H	δ_H
17a	4.82	7eq	2.39
17b	4.49	11	1.42
3	3.25	2eq	1.68
		1ax	1.17
		5	1.08
		18	0.99
9	1.54	5	1.08
18	0.99	6 eq	1.74
		5	1.08
		19	0.77
19	0.77	2ax	1.57
		6ax	1.36
		18	0.99
		20	0.68
20	0.68	2ax	1.57
		6ax	1.36
		11	1.42
		19	0.77

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RESUMO

Dos extratos em hexano e em acetato de etila obtidos a partir de caules, raízes e bainhas foliares de *Vellozia graminifolia* foram identificados hidrocarbonetos lineares, uma mistura de triterpenos pentacíclicos, cinco flavonóides monoisoprenilados e um diterpeno com esqueleto labdano, o ácido (-) -ent-3β-hidroxi-8(17)-labdeno-15-óico. Este é o primeiro diterpeno com esqueleto labdano isolado da família Velloziaceae.

A identificação dos hidrocarbonetos lineares e dos triterpenos minoritários presentes nas duas misturas isoladas foi realizada por Cromatografia Gasosa de Alta Resolução (CGAR) e CGAR acoplada a espectrometria de massas. A determinação estrutural dos triterpenos pentacíclicos majoritários e do diterpeno foi realizada por dados espectrais, incluindo RMN 2D, e transformações químicas. As estruturas dos flavonóides monoisoprenilados são discutidas em artigos anteriores.

Palavras-chave: *Vellozia graminifolia*, Velloziaceae, triterpenos, diterpeno, flavonóides.

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