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TWO MONOISOPRENYLATED FLAVONOIDS FROM *VELLOZIA GRAMINIFOLIA*

ALEXSANDRO BRANCO, RAIMUNDO BRAZ-FILHO,*† CARLOS R. KAISER‡ and ÂNGELO C. PINTO‡

Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, 23851-970 Seropédica, Rio de Janeiro, Brasil; † Setor de Química de Produtos Naturais, Universidade Estadual do Norte Fluminense, 28015-620 Campos, Rio de Janeiro, Brasil; ‡ Departamento de Química Orgânica, Universidade Federal do Rio de Janeiro, 21949-970 Rio de Janeiro, RJ, Brasil

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Key Word Index—*Vellozia graminifolia*; Velloziaceae; monoisoprenylated flavonols; spectral data.

Abstract—A mixture of two monoisoprenylated flavonols velloquercetin 3′,4′-dimethyl ether and velloquercetin 3,3′,4′-trimethyl ether have been isolated and characterized from the hexane whole plant extract of *Vellozia graminifolia*. The structures were determined from spectral data, including 2D-NMR experiments.

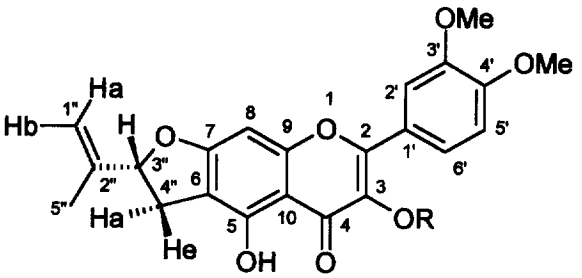
INTRODUCTION

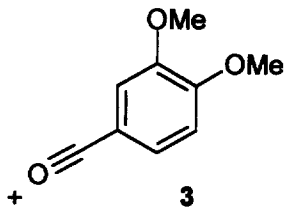
Velloziaceae is a monocotyledonous family containing about 270 species distributed throughout Central Africa and South America, mainly in Brazil [1]. Previous studies on the constituents of *Vellozia* species have resulted in the isolation of significant numbers of different diterpenoids [e.g. 2–4]. The separation and identification of the lipophilic flavonols in leaf tissues of Velloziaceae by a combination of chromatographic and spectral techniques have been described [5]. In this paper, we describe the isolation of a mixture containing a new derivative (**1**) of velloquercetin (2″-isopropenyldihydrofuranol (4″,5″: 6,7) quercetin) and its known 3-*O*-methyl ether (**2**) from *Vellozia graminifolia*. Structural elucidation of these compounds as components of a mixture was based on spectral analysis, including NMR techniques such as ^{1}H-detected Heteronuclear Multiple Quantum Coherence (HMQC), ^{1}H-detected Heteronuclear Multiple Bond Connectivity (HMBC) and Nuclear Overhauser Effect Spectroscopy (NOESY) [6, 7], together with chemical shift correlations.

RESULTS AND DISCUSSION

The mixture of velloquercetin 3′,4′-dimethyl ether (**1**) and velloquercetin 3,3′,4′-trimethyl ether (**2**) showed IR absorption bands for a conjugated carbonyl group (1667 cm^{-1}) and for aromatic ring (1608 and 1508 cm^{-1}). The multiplicity for each carbon signal was deduced by comparative analysis of the PND- and DEPT-^{13}C NMR spectra (Table 1). This analysis in combination with the low-resolution mass spectrum {*m/z* 396 $[M]^{+\cdot}$, $C_{22}H_{20}O_7$ (**1**), and 410 $[M]^{+\cdot}$, $C_{23}H_{22}O_7$ (**2**)} and ^{1}H NMR spectra (Table 1) allowed the deduction of the molecular formula $(C)_{11}$ (C = O) (H-C-O) $(CH)_4$ $(CH_2)_2$ (CH_3) $(OCH_3)_2$ $(OH)_2$ for **1** and $(C)_{11}$ (C = O) (H-C-O) $(CH)_4$ $(CH_2)_2$ (CH_3) $(OCH_3)_3$ (OH) for **2**. The presence of chelatogenic hydroxyl groups at C-3 and C-5 was deduced by singlet signals at δ 11.90 and 12.85, the latter with major relative intensity revealed by integrated curve. The molecular formulas $C_{22}H_{20}O_7$ (**1**) and $C_{23}H_{22}O_7$ (**2**) require 13 degrees of unsaturation, compatible with a flavone skeleton ($C_{15}H_{10}O_2$, 11 degrees of unsaturation), an additional ring and a double bond. In fact, the presence of an isoprenoid moiety involved in a furone ring was evidenced by the chemical shifts for the 2H-1″ [**1**/**2**: δ_H 5.10 (*br s*)/5.10 (*br s*) and 4.95 (*br s*)/4.95 (*br s*)], H-3″ [**1**/**2**: δ_H 5.35 (*m*)/5.35 (*m*)], 2H-4″ [**1**/**2**: δ_H 3.39 (*dd*, *J* = 9.6 and 15.7 Hz)/3.38 (*dd*, *J* = 9.5 and 15.6 Hz) and 3.03 (*dd*, *J* = 7.4 and 15.7 Hz)/3.02 (*dd*, *J* = 7.3 and 15.6 Hz] and 3H-5″ (**1**/**2**: δ_H 1.78 (*s*)/1.78 (*s*)] and for carbon atoms C-1″ to C-5″ [**1**/**2**: δ_C 112.94/112.92 (CH_2-1″), 143.33/143.33 (C-2″), 88.36/88.36 (CH-3″), 30.67/30.67 (CH_2-4″) and 17.13/17.13 (CH_3-5″)]. The location of this isoprenoid moiety in the A ring involving the carbon atom C-6 was defined by heteronuclear coupling via three bonds between this carbon [**1**/**2**: δ_C 108.20/108.39, $^3J_{CH}$] and the hydrogen of the hydroxyl group [**1** and **2**: δ_H 12.89 (*s*)] at C-5 observed in the HMBC spectrum (Table 1).

* Author to whom correspondence should be addressed.





3

m/z 165

	R
1	H
2	Me

The difference of 14 daltons observed in the mass spectrum of **1** and **2**, which was confirmed through HRGC mass spectral analysis, was attributed to the presence of a hydroxyl group at C-3 [δ_H 11.94 (*s*)] of **1** and of a methoxyl function at C-3 [δ_H 3.86 (*s*) and δ_C 60.36 (MeO-3)] of **2**. In fact, selective methylation

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

	1				**2**			
	HMQC		HMBC		HMQC		HMBC	
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
2	145.39	—		H-2′,H-6′	155.73	—		H-2′,H-6′
3	135.82	—			139.04	—		OMe-3
4	175.31	—			179.00	—		
5	156.65	—	HO-5		156.65	—	HO-5	
6	108.20	—	2H-4″	HO-5,H-8	108.39	—	2H-4″	HO-5,H-8
7	166.80	—	H-8	2H-4″	166.41	—	H-8	2H-4″
9	157.35	—			157.25	—	H-8	
10	104.26	—		HO-5,H-8	106.44	—		HO-5,H-8
1′	122.59	—		H-5′	123.14	—		H-5′
3′	148.80	—	H-2′	H-5′	148.91	—	H-2′	H-5′
4′	151.45	—		H-2′,H-6′	151.45	—		H-2′,H-6′
2″	143.33	—	3H-5″	2H-4″	143.33	—	3H-5″	2H-4″
CH								
8	89.30	6.48(*s*)			89.00	6.43(*s*)		
2′	110.62	7.78(*d*, 2.0)		H-6′	111.33	7.69(*d*, 2.0)		H-6′
5′	111.05	7.01(*d*, 8.6)			110.00	7.00(*d*, 8.6)		
6′	121.43	7.83(*dd*; 2.0,8.6)		H-2′	122.26	7.73(*dd*; 2.0,8.6)		H-2′
3″	88.36	5.35(*m*)	H-4″b	3H-5′,2H-1″	88.36	5.35(*m*)	H-4″b	3H-5″,2H-1″
CH_2								
1″	112.94	5.10(*br s*)		3H-5″	112.92	5.10(*br s*)		3H-5″
		4.95(*br s*)				4.95(*br s*)		
4″	30.67	3.39(*dd*; 9.6,15.7)			30.67	3.38(*dd*; 9.5,15.6)		
		3.03(*dd*; 7.4,15.7)				3.02(*dd*; 7.3,15.6)		
CH_3								
5″	17.13	1.78(*s*)			17.13	1.78(*s*)		2H-1″
HO-3	—	11.94(*s*)			—	—		
HO-5	—	12.89(*s*)			—	12.89(*s*)		
MeO-3	—	—			60.36	3.86(*s*)		MeO-3
MeO-3′	56.18	3.98(*s*)			56.18	3.96(*s*)		MeO-3′
MeO-4′	56.18	3.97(*s*)			56.18	3.97(*s*)		MeO-4′

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

Table 2. ^{1}H-^{1}H-NOESY (dipolar couplings) spectral data for flavonoids **1** and **2** (300 MHz, $CDCl_3$, δ)

1				2			
H	δ_H	H	δ_H	H	δ_H	H	δ_H
2′	7.78	MeO-3′	3.98	2′	7.69	MeO-3′	3.96
5′	7.01	MeO-4′	3.97	5′	7.00	MeO-4′	3.97
		6′	7.83			6′	7.73
3″	5.35	1″a	5.10	3″	5.35	1″a	5.10
		4″eq	3.39			4″eq	3.38
		4″ax	3.03			4″ax	3.02
		5″	1.78			5″	1.78
1″b	4.92	1″a	5.10	1″b	4.92	1″a	5.10
		5″	1.78			5″	1.78
4″ax	3.03	4″eq	3.39	4″ax	3.02	4″eq	3.38
		5″	1.78			5″	1.78

of the mixture with diazomethane furnished only one product retaining a hydroxyl groups at C-5 [δ_H 12.89 (*s*)].

The ^{1}H NMR spectra (1D and 2D ^{1}H-^{1}H-COSY) revealed a 1,3,4-trisubstituted aromatic ring (B ring 3′,4′-dimethoxylated in flavonoid) by analysis of the chemical shifts for the H-2′ [**1**/**2**: δ_H 7.78 (*d*, J = 2.0 Hz)/7.69 (*d*, J = 2.0 Hz)], H-5′ [**1**/**2**: δ_H 7.01 (*d*, J = 8.6 Hz)/7.00 (*d*, J = 8.6 Hz)] and H-6′ [**1**/**2**: δ_H 7.83 (*dd*, J = 2.0 and 8.6 Hz)/7.73 (*dd*, J = 2.0 and 8.6 Hz)], which was confirmed by signals for C-1′ to C-6′ observed in the ^{13}C NMR spectra [**1**/**2**: δ_C 122.59/123.14 (C-1′), 110.62/111.33 (CH-2′), 148.80/148.91 (C-3′), 151.45/151.45 (C-4′), 111.05/110.00 (CH-5′) and 121.43/122.26 (CH-6′)]. The peak at *m*/*z* 165 [5% (**1**); 30% (**2**)] observed in the mass spectra, attributed to ionic fragment **3**, further corroborates this deduction.

Thus, the two monoisoprenylated flavonoids isolated as a mixture from *Vellozia graminifolia* were characterized as velloquercetin 3′,4′-dimethyl ether (**1**) and as 3,3′,4′-trimethyl ether (**2**). To the best of our knowledge, **1** is hitherto unreported as a natural product, whereas its 3-*O*-methyl derivative (**2**) has been isolated previously from the leaf surface of *V. stipitata* [8]. On the basis of intensity of the signals corresponding to H-8 [**1**/**2**: δ_H 6.48 (*s*)/6.43 (*s*)] in the ^{1}H NMR spectrum the percentages of **1** (30%) and **2** (70%) in the mixture were estimated.

The complete assignment of **1** and **2** was accomplished by multi-nuclear NMR techniques of the mixture (Tables 1 and 2). The comparison of the chemical shifts of the quaternary carbons C-2 to C-4 of **1** and **2** also showed downfield shifts by 10.34 [$\Delta\delta$ = 155.73 (**2**) − 145.39 (**1**)], 3.22 [$\Delta\delta_C$ = 139.04 (**2**) − 135.82 (**1**)] and 3.96 ppm [$\Delta\delta_C$ = 179.00 (**2**) − 175.31 (**1**)], respectively (Table 1). These data and the corresponding modifications can be used to characterize 3-hydroxyflavones (flavonols and their 3-*O*-methyl derivatives (3-methoxyflavones).

Homonuclear 2D ^{1}H-^{1}H NOESY (Table 2) was also used to assign unambiguously the chemical shifts of each of the methylidene and methylene hydrogens of carbon C-1″ and C-4″ of **1** and **2**. The cross-peaks indicated dipolar–dipolar interactions between H-3″ [**1**/**2**: δ_H 5.35 (*m*)/5.35 (*m*)] and H-1″a [(**1**/**2**: δ_H 5.10 (*br s*)/5.10 (*br s*)]; 3H-5″ [(**1**/**2**: δ_H 1.78 (*s*)/1.78 (*s*)] and pseudoaxial H-4″ax [**1**/**2**: δ_H 3.03 (*dd*, J = 7.4 and J = 15.7 Hz)/3.02 (*dd*, J = 7.3 and 15.6 Hz): *vicinal* couplings (*trans*-H-3″/H-4″ax) = J_v = 7.4 (**1**) and 7.3 Hz (**2**), *geminal* couplings (H-4″ax/H-4″eq) = J_g = 15.7 (**1**) and 15.6 Hz (**2**)]. Consequently, the chemical shifts for H-1″b [**1**/**2**: δ_H 4.92 (*br s*)/4.92 (*br s*)] and pseudoequatorial H-4″eq [**1**/**2**: δ_H 3.39 (*dd*, J_v = 9.6 and J_g = 15.7 Hz)/ 3.38 (*dd*, J_V = 9.5 and J_g = 15.6 Hz)] were also defined (Tables 1 and 2). Other dipolar coupling data are summarized in Table 2.

EXPERIMENTAL

General. Mps were uncorr. NMR spectra 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 $C\underline{H}Cl_3$ at δ_H 7.25 and $\underline{C}HCl_3$ at δ_C 77.00. GC/EIMS: the samples were previously analysed on a Hewlett-Packard model 5790 A gas chromatograph and subsequently on a Hewlett-Packard computerized gas chromatograph–mass spectrometer model 5987 A with a quadrupole analyser (electron impact ionization, 70 eV). GC carrier gas H_2, flow rate 2 ml min^{-1}, injection on-column, flame ionization detector temp. 370°, glass capillary columns (10m × 0.3 mm) coated with PS-090 (df = 0.1 μm), temp. programme 40–350° at 12° min^{-1}, Me_2CO as solvent. CC: silica gel (0.063 a 0.2 mm). TLC: silica gel (Merck, Kieselgel 60) spots visualized by UV (254 and 360 nm), exposure to I_2 vapour. TLC was used to analyse frs collected from CC.

Plant material. Vellozia graminifolia Nanuza L. de Menezes was collected in Chapada da Diamantina, Minas Gerais State, Brazil and identified by Professora Nanuza L. de Menezes of the Universidade de São Paulo (USP), São Paulo-SP, Brazil. A voucher specimen has been lodged in the herbarium of the Instituto de Botânica of USP.

Extraction and isolation of flavonoids. Dried and powdered roots, stems and pods were extracted with *n*-hexane at room temp. and the solvent removed under vacuum to yield 12.96 g of an oily residue. This residue was chromatographed on a column of silica gel (112.06 g) using hexane–EtOAc mixts of increasing polarity. A total of 29 frs of *ca* 120 ml each one were collected and combined on the basis of TLC comparison. Frs 10 and 11, eluted with hexane–EtOAc (2:1), furnished a mixt. **1** and **2** (23.07 mg) as yellow needles, mp 128–129°.

Mixture of velloquercetin 3′,4′-dimethyl ether (**1**) *and velloquercetin 3,3′,4′-trimethyl ether* (**2**). Mp. 128–129°. IR ν_{max}^{KBr} cm^{-1}: 3300(OH), 1667(C = O), 1608, 1508 (aromatic ring). ^1H and ^{13}C NMR: Table 1. GCAR/EI-MS 70 eV *m/z* (rel. int.): **2** [R_t 20.81 min], 410 ($[M]^+$, 100), 395 ($[M-Me]^+$, 100), 367 ($[M-Me-CO]^+$ and/or $[M-CO-Me]^+$, 21), 165 (**3**, 30); **1** [R_t 20.95 min], 396 ($[M]^+$, 70), 381 ($[M-Me]^+$, 100), 355 ($[M-CH_2=C-CH_3]^+$, 5), 165 (**3**, 5).

Methylation of the mixture of **1** *and* **2**. The mixt. (10.29 mg) was treated with CH_2N_2 in the usual manner to yield **2** (10.67 mg), mp 134°. IR ν_{max}^{KBr} cm^{-1}: 1673 (C = O), 1620, 1550, 1530 (aromatic ring). ^1H NMR (300 MHz, $CDCl_3$): δ_H 12.90 (*s*, HO-5), 7.74 (*dd*, *J* = 2.0, 8.3 Hz, H-6′), 7.60 (*d*, *J* = 2.0, H-2′), 6.99 (*d*, *J* = 8.3 Hz, H-5′), 6.40 (*s*, H-8), 5.32 (*dd*, *J* = 9.6, 7.1 Hz, H-3″), 5.07 (*br s*, H-1″a), 4.92 (*br s*, H-1″b), 3.94 (*s*, MeO-3′ and MeO-4′), 3.83 (*s*, MeO-3), 3.36 (*dd*, *J* = 15.6, 9.6 Hz, H-4″eq), 2.99 (*dd*, *J* = 15.6, 7.1 Hz, H-4″ax), 1.78 (*s*, 3H-5″). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 154.25(C-2), 138.43(C-3), 178.84(C-4)l, 156.50(C-5), 108.22(C-6), 166.25(C-7), 88.82(C-8), 157.10(C-9), 107.08(C-10), 122.99(C-1′), 111.20(C-2′), 148.76(C-3′), 151.30(C-4′), 110.85(C-5′), 122.07(C-6′), 112.73(C-1″), 143.18(C-2″), 88.19(C-3″), 30.51(C-4″), 16.96(C-5″), 60.19(MeO-3), 56.01(MeO-3′ and MeO-4′).

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