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Flavonol Glycosides from *Davilla flexuosa*

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Este trabalho relata o estudo fitoquímico das folhas de um espécime de *Davilla flexuosa* St. Hill. Do extrato hexânico foi isolado o α-tocoferol. Do extrato de acetato de etila foram isolados dois flavonóis: miricetina e quercetina, bem como dois derivados glicosilados de flavonóis: miricetina 3-ramnosídeo e um novo glicosídeo miricetina 3'-ramnosídeo. Todas as estruturas foram elucidadas através de dados espectrométricos obtidos no UV, IV, RMN e Massas.

This work describes a chemical study of the leaves of a specimen of *Davilla flexuosa* St. Hill. α-Tocopherol was isolated from the hexane extract, and the ethyl acetate extract furnished the flavonols myricetin and quercetin, and two flavonol glycosides: myricetin 3-rhamnoside and a new compound identified as myricetin 3'-rhamnoside. All the structures were determined by NMR, UV, MS and IR spectral data.

Keywords: Davilla flexuosa, *Dilleniaceae, myricetin 3-rhamnoside, myricetin 3'-rhamnoside; myricetin, quercetin, α-tocopherol*

Introduction

Dilleniaceae is a plant family found in tropical and subtropical regions, especially in Australia. It is composed of trees, woody vines, shrubs and occasionally herbs[1]. The Asiatic species have been well studied because most of them present medicinal and nutritional properties. In the Indian *Dillenia* and *Tetracera* species triterpenoids, especially betulinic acid[2] and flavonoids[3], are commonly found. The native Brazilian representatives are predominantly species of the *Davilla*, *Tetracera*, *Curatella* and *Dolicarpus* genera. Some species belonging to these genera are used by local populations as medicinal plants. In spite of this, their chemical composition is almost completely unknown.

Gurn *et al.*[4] previously reported the detection of myricetin 3-rhamnosidesulphate from herbarium samples of *D. macrocarpa* and *D. flexuosa*. This communication presents a chemical study of leaves from *Davilla flexuosa* St. Hill, a climber plant very common in Brazilian restinga on sandy soil. In addition to α-tocopherol two flanovoids and two flanovonoid glycosides were isolated from this species. The structures of these compounds were determined by spectrometric data analyses, and are discussed in this paper.

Experimental

The NMR spectra were recorded on Brucker AC-200 and Varian Gemini-300 equipment, using a mixture of Me_2CO-d_6/D_2O as the solvent and TMS as the internal standard. The ^1H spectra were registered utilizing water peak suppression by the presaturation technique[5]. The Mass spectra were obtained by direct injection on a Hewlett-Packard spectrometer 5888A, and the UV spectra on a Hewlett-Packard 8451A Diode Array spectrophotometer.

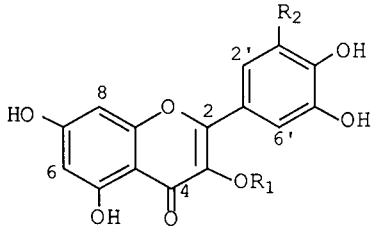
The botanical material was collected at restinga on sandy soil in the Reserva do Parque Metropolitano do Abaeté, Salvador, BA. A voucher is deposited at the Herbarium Alexandre Leal Costa, Biology Institute of the Federal University of Bahia, under the number 026962.

The isolation of the constituents

The dried leaves of *D. flexuosa* (620 g) were extracted with MeOH (2 x 3 L). The crude extract was successively partitioned with hexane/EtOH and AcOEt/H_2O. From the hexane partition (0.62 g), α-tocopherol (152 mg) was isolated by PTLC on silica gel utilizing benzene/acetone

(49:1) as the eluent. The EtOAc fraction (4.3 g) was chromatographed on a Poliamide 6 column using MeOH/H_2O mixtures. The presence of phenols in the fractions was detected by color reactions employing ferric chloride. The mixture of MeOH/H_2O (3:2) furnished a fraction which was submitted to a Sephadex LH-20 column employing MeOH/$CHCl_3$ (4:1) as the eluent, and which yielded **1** (137.4 mg) and **2** (35.3 mg). The fraction obtained by elution with MeOH/H_2O (4:1) yielded **3** (31.5 mg) and **4** (15.0 mg).

Compound **1**. Myricetin 3-rhamnoside. White amorphous powder. Mp 190-191° (uncorr.). UV $\lambda_{max.}^{MeOH}$ nm(ε): 350(10230); 308(sh); 261(13023). UV data with shift reagents: see Table 3. EIMS m/z (rel.int.) 464[$M^{+\cdot}$] (1); 318 (100); 302 (30); 152 (47); 135 (31); 125(43); 108 (37). ^{1}H- and ^{13}C-NMR data: Tables 1 and 2.



1 R_1 = Rha; R_2 = OH
2 R_1 = H; R_2 = ORha
3 R_1 = H; R_2 = OH
4 R_1 = H; R_2 = H

Rhamnosil group =

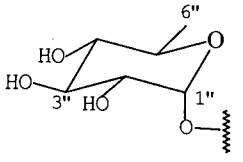


Figure 1.

Compound **2**. Myricetin 3'-rhamnoside. White amorphous powder. Mp 186-188° (uncorr.). UV $\lambda_{max.}^{MeOH}$ nm(ε): 350(13814); 308(sh); 262(16048). UV data with shift reagents: see Table 3. EIMS m/z (rel. int.): 464 [$M^{+\cdot}$] (1); 446 (2); 430 (3); 318 (100); 302 (87); 286 (26); 153 (53); 136 (27); 121 (22) and 108 (33). ^{1}H- and ^{13}C-NMR data: Tables 1 and 2.

Compound **3**. Myricetin. Yellow crystals. Mp. 230-231° (uncorr.). UV $\lambda_{max.}^{MeOH}$ nm(ε): 376(3561); 296(2385). UV data with shift reagents, ^{1}H- and ^{13}C-NMR data: Tables 1, 2 and 3.

Compound **4**. Quercetin. Yellow amorphous powder. Mp. 330° with decomposition. ^{1}H- and ^{13}C- data: Tables 1 and 2.

Hydrolisis of compound **2** with 0.05 M H_2SO_4. Compound **2** (30 mg) was refluxed in 0.05 M H_2SO_4/EtOH (5 ml) for 2 h and then water was added. The solution was submitted to a partition with EtOAc, furnishing the aglicone **3**(19 mg).

α-tocopherol. Crystals. Mp 182-185° (uncorr.). IR $\lambda_{max.}^{KBr}$ cm^{-1}: 3450, 2930, 2860, 1460, 1250, 1080. EIMS m/z (rel. int.): 430 [$M^{+\cdot}$] (20); 205 (15); 203 (6); 165 (100); 164 (37). ^{1}H-NMR [200 MHz, δppm), $CDCl_3$]: 0.75-0.80 (CH_3, 4'a, 7'a, 12'a, 13'a); 1.15 (CH_3, 2a); 2.03 (CH_3, 5a, 7a); 2.08 (CH_3, 8b); 2.51 (t, CH_2-4); 4.02 (s, OH). ^{13}C-NMR [50 MHz, δ(ppm); $CDCl_3$]: 145.5 (C-8a); 144.5 (C-6); 122.6 (C-8); 121.0 (C-7); 118.5 (C-5); 117.3 (C-4a); 74.5 (C-2); 39.7 (C-1'); 39.3 (C-11'); 37.4 and 37.3 (C-3', C-5', C-7' and C-9'); 32.8 and 32.7 (C-4' and C-8'); 31.5 (C-3); 27.9 (C-12'); 24.8 (C-10'); 24.4 (C-6'); 23.8 (C-2a); 22.7 and 22.6 (C-12'a and C-13'); 21.0 (C-2'); 20.7 (C-4); 19.7 and 19.6 (C-4'a and C-8'a); 12.2, 11.8 and 11.3 (C-7a, C-8b and C-5a).

Table 1. ^{1}H-NMR data of flavonoids [200 (**1**, **2** and **4**)and 300 MHz (**3**), δ(ppm), J(Hz), Me_2CO-d_6/D_2O (1:1)].

H	1	2	3	4
6	6.16 (d, J = 2.0)	6.15 (d, J = 1.5)	6.19 (d, J = 2.1)	6.20 (d, J = 2.0)
8	6.37 (d, J = 2.0)	6.35 (d, J = 1.5)	6.49 (d, J = 2.1)	6.46 (d, J = 1.9)
2'	6.97 (s)	7.00 (s)	7.37 (s)	7.59 (dd, J = 8.4; 2.1)
3'	-	-	-	6.92 (d, J = 8.4)
6'	6.97 (s)	6.90 (s)	7.37 (s)	7.70 (d, J = 2.1)
1"	5.23 (d, J = 1.1)	5.21 (d, J = 1.1)		
2"	4.22 (d, J = 2.7)	4.20 (d, J = 2.8)		
3"	3.80-3.71 (m)	3.81-3.73 (m)		
4"	3.80-3.71 (m)	3.81-3.73 (m)		
5"	3.32-3.20 (m)	3.34-3.22 (m)		
6"	0.85 (d, J = 7.4)	0.84 (d, J = 7.2)		

Results and Discussion

The dried leaves were submitted to extraction with methanol and the concentrated extract was partitioned successively with hexane, chloroform and ethyl acetate. From the hexane partition α-tocopherol was isolated by PTLC using a mixture of benzene:acetone (49:1) as the eluent. The identification of this compound was based on spectral data (IR, MS and NMR) and by comparison with data from the literature[6].

Flavonoids (**1-4**) were obtained from the ethyl acetate partition, and the identification of these compounds was carried out by analysis of their spectral data. The molecular formulae of **1** and **2** ($C_{21}H_{20}O_{12}$) were determined by low resolution mass spectroscopy, combined with proton and carbon counts by ^{1}H- and ^{13}C-NMR (proton-noise-decoupled and DEPT) spectra. Detailed analyses of the ^{13}C-NMR spectra of these compounds indicated the presence of one methyl group (δ 17.1), four oxymethine carbons with chemical shifts between δ 71 and 76, and one CH signal at δ 102.4 due to an anomeric carbon of a glycoside. This evidence clearly suggests that the glycoside is formed by a rhamnosyl group. The ^{1}H-NMR data are in agreement with this conclusion, and the H-1" coupling constant (J = 1.1 Hz) confirms that **1** and **2** are α-rhamnosides. The other NMR spectra signals reveal the presence of a flavonol skeleton for these compounds. The similarity of the data with the literature[7] led us to conclude that **1** and **2** are 3,5,7,3',4',5'-*O*-substituted flavonols. The unique difference between **1** and **2** derives from the H and C signals of the B-ring. While H-2' and H-6' of **1** appear at δ 6.97 as a singlet (Table 1), in **2** they are displayed as two singlets at δ 6.90 and 7.00. The ^{13}C-NMR data from the B-ring indicate the presence of only four signals for **1**, while this part of the compound **2** spectrum shows six signals (Table 2). The assignment of carbon shifts was carried out by the analysis of ^{13}C-^{1}H Heteronuclear Long-range Correlation. This evidence shows that **1** and **2** differ only in the position of the sugar moiety on the aglycone. Band I in the aluminium chloride UV spectra (Table 3) appears at 430 nm (+80 nm relative to Band I in methanol), and in aluminium chloride-hydrochloric acid at 410 nm. These large shifts are in agreement with 5 or 3-, 4', 5'-hydroxyflavonols. The large bathochromic shift of Band I with sodium methoxide confirms that 4'-OH is free. A 14 nm bathochromic shift of Band II in the presence of sodium acetate indicates 7-hydroxyl groups. These results indicate that **1** is myricetin 3-rham-

Table 2. ^{13}C-NMR spectra data of flavonoids [50 (**1** and **2**)and 75 MHz (**3** and **4**); Me_2CO-d_6/D_2O (1:1); δ(ppm)].

C	1	2	3	4
2	158.5	158.6	147.7	147.5*
3	135.1*	136.8*	136.9*	136.3
4	178.5	178.5	177.0	176.3
5	105.0	105.0	104.4	103.6
6	157.4	157.3	158.2	157.3
7	99.2	99.3	99.7	98.8
8	164.6	164.5	165.7	164.7
9	94.4	94.5	95.0	94.2
10	161.7	161.5	162.5	161.2
1'	121.0	121.0	123.2	123.1
2'	109.1	109.2$^+$	108.9	115.4$^+$
3'	145.7	145.6	146.9	145.5
4'	135.3*	135.2*	137.2*	148.0*
5'	145.7	145.6	146.9	115.9$^+$
6'	109.1	109.8$^+$	108.9	121.2
1"	102.4	102.4		
2"	70.7	70.6		
3"	71.0	70.8		
4"	72.0	71.9		
5"	71.2	71.2		
6"	17.1	17.1		

*,$^+$ - signals can be changed.

Table 3. UV spectral data for flavonoids **1-3**.

	$\lambda_{max.}^{MeOH}$ (nm)Band I/Band II		
Compound	1	2	3
MeOH	350, 308(sh)/261	350, 308(sh)/262	376
$AlCl_3$	430, 308(sh)/275	430, 310/275	450, 368, 312
$AlCl_3$/HCl	410, 352(sh) 308(sh)/ 275	410, 352(sh), 308(sh)/ 275	430, 362, 312
NaOMe	396, 322(sh)/275	376, 322(sh)/275	decomp.
NaOAc	380, 320(sh)/275	388, 322(sh)/275	decomp.
NaOAc/HBO_3	370, 320(sh)/268	370, 322(sh)/268	decomp.

decomp. = decomposition

noside (myricitrin) while **2** is the isomeric compound myricetin 3'-rhamnoside. This assumption was confirmed by the acid hydrolisis of **2,** which yielded myricetin (**3**), a compound also present in the methanol extract. The ^{1}H- (Table 1), ^{13}C- (Table 2) NMR and UV (Table 3) data for **3** are compatible with those obtained from the literature for myricetin[7,8]. In spite of **1** being a well-known glycoside, isolated for the first time from *Myrica rubra*[9], the 3'-*O*-rhamnosil(**2**) derivative is a new compound.

In addition to **1** and **2**, compounds **3** and **4** were respectively identified as myricetin and quercetin, by the comparison of their physical and spectral data with those obtained from the hydrolitic derivative of **2** and with the literature[7]. The isolation of **3** and **4** in *D. flexuosa* is in agreement with previous studies[10] which demonstrate that species of the genera *Davilla*, *Curatella* and *Doliocarpus* contain either myricetin or quercetin or both.

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