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NAPHTHOPYRANONE GLYCOSIDES FROM *PAEPALANTHUS BROMELIOIDES*

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Key Word Index—*Paepalanthus bromelioides*; Eriocaulaceae; naphthopyranone glycosides.

Abstract—Two new naphtho[2,3-C]pyran-1-one glycosides, paepalantine-9-*O*-*β*-D-glucopyranoside and paepalantine-9-*O*-*β*-D-allopyranosyl(1 → 6)glucopyranoside, were isolated from an ethanolic extract of capitula from *Paepalanthus bromelioides* and identified from their spectrometric data.

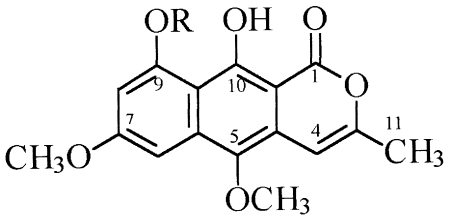
INTRODUCTION

The vegetation pattern found high in the Espinhaço chain in Brazil is classified as "campos rupestres". Plants grow either on stone or in sandy soils [1]. The monocotyledoneous family Ericaulaceae grows wild in this region and has ca 1200 species in 10–14 genera. *Paepalanthus* is the largest genus of this family, comprising ca 500 species [2]. It is primarily concentrated in South America, with few species in Central America and two in Africa [3]. Many plants from this family are called "sempre-vivas" ("everlasting plants"), because they still look alive years after being collected. Only a few studies on the chemical components of these species have been performed [4]. A previous investigation on *P. bromelioides* demonstrated that the naphthopyranone **1,** now named paepalantine, is the main constituent of the $CHCl_3$ extract of the capitula and has promising antibiotic activity against bacteria, yeasts and dermatophytes [5]. More recently, **1** also showed strong cytotoxicity and mutageniticity [6, 7]. Our interest in this type of compound has now led us to investigate the EtOH extract of capitula from *P. bromelioides* in the search for more polar compounds.

RESULTS AND DISCUSSION

Capitula were powdered and extracted successively with hexane, CH_2Cl_2, EtOAc and EtOH. TLC of the EtOH extract revealed two pronounced yellow-green spots, similar to that of paepalantine, but with lower R_f values. The EtOH extract was then partitioned between *n*-BuOH and water. The layers were evaporated and then separately submitted to DCCC fractionation affording **1** [5], **2** and **3**. Characterization of **2** and **3** were relatively straightforward. The spectral data of both substances reflected the similarities of these structures with that of **1** (Tables 1 and 2), the main differences being the sugar moieties, present.

Compound **2** showed a yellowish-green spot on TLC with an R_f value of 0.70 and was obtained subsequently as a yellow amorphous powder. The ^{13}C NMR and DEPT spectra exhibited 22 signals, of which six could be assigned to a glucopyranosyl moiety [8]. The other 16 signals were similar to those of **1** (Table 1). When compared with **1**, the ^1H NMR



	R
1	H
2	-glc
3	-glc$\overset{6}{-}$all

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Table 1. ^{1}H NMR assignments for compounds **1–3**

	H	**1**†	**2**‡	**3**‡
	4	6.49 *q* (1.0)	6.64 *s*	6.54 *s*
	6	6.86 *d* (2.5)	6.86 *d* (2.5)	6.94 *d* (2.5)
	8	6.52 *d* (2.5)	6.96 *d* (2.5)	6.86 *d* (2.5)
	11	2.23 *d* (1.0)	2.25 *s*	2.22 *s*
	OH-9	9.43 *br s*	-	-
	OH-10	13.22 *br s*	12.48 *br s*	12.47 *br s*
	OMe-5	3.93 *s*	3.79 *s*	3.77*s*
OMe-7	3.84 *s*	3.92 *s*	3.90 *s*	
Glucose	H1′		5.10 *d* (7.5)	4.98 *d* (7.9)
	H2′		3.3-3.9*	3.42 *dd* (7.9; 9.0)
	H3′		3.3-3.9*	3.30 *dd* (9.0; 9.0)
	H4′		3.3-3.9*	3.26 *dd* (9.0; 9.0)
	H5′		3.3-3.9*	3.65 *m*
	H6′		3.3-3.9*	4.02 *dd* (2.5; 11.0)
				3.61 *dd* (5.0; 11.0)
Allose	H1″			4.51 *d* (7.4)
	H2″			3.16 *dd* (7.4; 2.5)
	H3″			3.83 *dd* (2.5; 4.0)
	H4″			3.28 *dd* (4.0; 9.2)
	H5″			3.49 *m*
	H6″			3.64 *dd* (3.0; 12.0)
				3.42 *dd* (5.0; 12.0)

† $CDCl_3$; ‡ DMSO-d_6; * signal pattern unclear due to overlapping.

spectrum of **2** (Table 2) shows only the signal of a chelated OH at δ12.48 but not that at δ9.43, as occurs in **1**, thus suggesting that the glucose unity is linked to position 9. The anomeric signal in the ^{1}H NMR spectra of **2** appeared at δ5.07 (*d*, $J = 7.6$ Hz), which showed the β-configuration of the sugar moiety. The other signals were partly overlapped by the signals of OMe-5, OMe-7 and DMSO. ES mass spectrometry of **2** exhibited a strong $[M+Na]^+$ ion at m/z 487, the ion $[M+H]^+$ at m/z 465, the aglycone ion $[A+H]^+$ ion at m/z 303, the adduct $[A+Na]^+$ at m/z 325 and a dimer $[2M+Na]^+$ at m/z 951. Thus, **2** could be characterized as paepalantine-9-*O*-β-D-glucopyranoside.

Compound **3** also showed a yellowish-green spot on TLC and on R_f of 0.40, and was obtained as yellow needles. The ^{1}NMR spectrum (Table 2) showed the broad singlet of a chelated OH at δ 12.47, thus indicating that the 10-OH is free and the sugar unity is bonded to OH-9, as in **2**. Two anomeric proton signals were also observed. The two doublets appeared at δ 4.98 ($J = 7.9$ Hz, H1′, glc) and δ 4.51 ($J = 7.4$ Hz, H1″, all), which showed the β configuration in both sugars. The ^{13}C NMR spectrum (Table 1) showed 28 signals, 16 of which were similar to those of **1**. The remaining signals confirmed the two anomeric carbons at δ 101.0 and δ 101.6. The lack of signals in the region of δ 79-80 and the CH_2 signal a δ 68.8 clearly indicate that the two sugar units are $1 \rightarrow 6$ bonded. Assignments of the remaining ^{1}H and ^{13}C NMR signals were based on 2D DFQ-COSY, 1D HOHAHA and HSQC experiments. The ES mass spectrum of **3** showed an intense $[M+Na]^+$ ion at m/z 649 and a smaller $[M+H]$ ion at m/z 627. The loss of the terminal allose moiety was observed as the $[M-all+H]^+$ ion at m/z 465 and the base peak resulted from the protonated aglycone $[A+H]^+$ ion at m/z 303. Thus, the structure of **3** was deduced as paepalantine-9-O-β-D-allopyranosyl($1 \rightarrow 6$)-β-D-glycopyranoside.

The identity of **2** and **3** was further confirmed by acid hydrolysis followed by spectroscopic and TLC comparison of the products. As expected, both substances afforded **1** as the aglycone; **1** furnished glucose and **2** furnished glucose and allose.

Naphthopyranones and their glycosides have hitherto been isolated from the capitula of *P. bromelioides*. Preliminary investigation showed that they occur also in other species of this genus, as well as in the scapes, but not in the leaves. The details of these investigations will be published later.

EXPERIMENTAL

General

NMR spectra of **2** were recorded at 200 MHz for ^{1}H and 50 MHz for ^{13}C in DMSO-d_6. NMR spectra

Table 2. ^{13}C NMR assignments for compounds **2** and **3**.

C	1†	2‡	3‡
1	167.8	166.6	166.9
3	152.4	152.8	152.4
4	99.2	98.5	98.3
4a	122.4	123.1	123.3
5	140.5	138.8	139.0
5a	135.9	135.7	135.9
6	93.6	94.3	94.8
7	163.4	161.7	161.3
8	101.9	101.9	102.2
9	158.7	158.4	158.4
9a	108.6	109.7	109.8
10	158.2	159.4	159.6
10a	96.7	97.4	97.5
11	19.6	18.9	19.0
OMe-5	61.7	61.6	61.3
OMe-7	55.5	55.4	55.4
Glucose			
C1′		101.1	101.0
C2′		73.4	73.6
C3′		77.3	76.4
C4′		69.7	69.7
C5′		76.4	75.6
C6′		60.7	68.8
Allose			
C1″			101.6
C2″			70.6
C3″			71.4
C4″			67.5
C5″			74.5
C6″			61.3

† $CDCl_3$; ‡ DMSO-d_6.

of **3** were recorded at 600 MHz for ^1H and 150 MHz for ^{13}C in CD_3OD. Chemical shifts are given in δ from TMS. DCCC was performed on a Tokyo Rikakikai Co. equipment with 300 columns of 2 mm id. ES-MS was performed on a quadrupole instrument and samples were directly injected into the mass spectrometer via a Rheodyne injector. MeCN–H_20 (1:1) was used as the mobile phase. N_2 was used both as a drying gas and for nebulization.

Plant material

Capitula of *P. bromelioides* Silv. were collected at Serra do Cipó, Minas Gerais, Brazil. A voucher specimen was deposited at the herbarium of Departamento de Botânica do Instituto de Biociências-USP (CFSC 13839).

Extraction and isolation

Powdered capitula (189 g) were successively extracted with hexane, CH_2Cl_2, EtOAc and EtOH. The EtOH extract was concd (4.8 g) and partitioned between *n*-BuOH and H_2O. The layers were separated and concd. The *n*-BuOH extract (2 g) was fractionated by DCCC in $CHCl_3$–MeOH–H_2O (13:7:4)(Solvent 1), descending. Frs 1–10 afforded **1** and frs 12–25 afforded pure **2**. The aq. layer was fractionated by DCCC in $CHCl_3$–MeOH–H_2O(7:13:8)(Solvent 2), descending. Frs 5–7 afforded 20 mg of **1** frs 30–35 20 mg of pure **2** and frs 90–100 80 mg of pure **3**. TLC were carried out on silica gel 60 (Merck) plates eluted with the lower phase of solvent 1.

*Paepalantine-9-O-β-D-glucopyranoside (**2**)*. Yellow amorphous powder, mp 191–192° (uncorr.). [α_{546} −200°, [α]$_{578}$ −105° (MeOH). UV λ_{max}^{MeOH} nm (log ε): 253 (sh), 273 (4.65), 284 (4.67), 391 (3.71); +NaOH: 263 (4.49), 283 (4.49), 390 (3.90). IR ν_{max}^{KBr} cm^{-1}: 3379 (br OH), 2920 (CH), 1682 (C═O), 1653 (C═C), 1617 (C═C), 1579 (C═C), 1460 (C═C). ^1H and ^{13}C NMR: Tables 1 and 2.

*Paepalantine-9-O-β-D-allopyranosyl(1 → 6)glucopyranoside (**3**)*. Yellow needles, mp 146-147° (uncorr.). [α]$_{546}$ −114°, [α]$_{578}$ −76° (MeOH + DMSO). UV λ_{max}^{MeOH} nm (log ε): 245 (sh), 273 (4.89), 284 (4.93), 393 (3.97); +NaOH: 262 (4.72), 284 (4.74), 395 (4.14). IR ν_{max}^{KBr} cm^{-1}:3385 (broad OH), 2914 (CH), 1681 (C═O), 1653 (C═C), 1580 (C═C), 1460 (C═C). ^1H and ^{13}C NMR:Tables 1 and 2.

*Acid hydrolysis of **2** and **3***

A soln of **2**–**3** (each 10 mg) in 10% HCl was refluxed for 1 h. The reaction mix. was neutralized with 5% NaOH and extracted with $CHCl_3$. The $CHCl_3$ layer was evapd to give **1**, identified through TLC with an authentic standard and ^1H NMR. The H_2O layer was evapd and tested by TLC with sugar standards, affording glucose and allose.

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