Naphthopyranone Glycosides from *Paepalanthus microphyllus*

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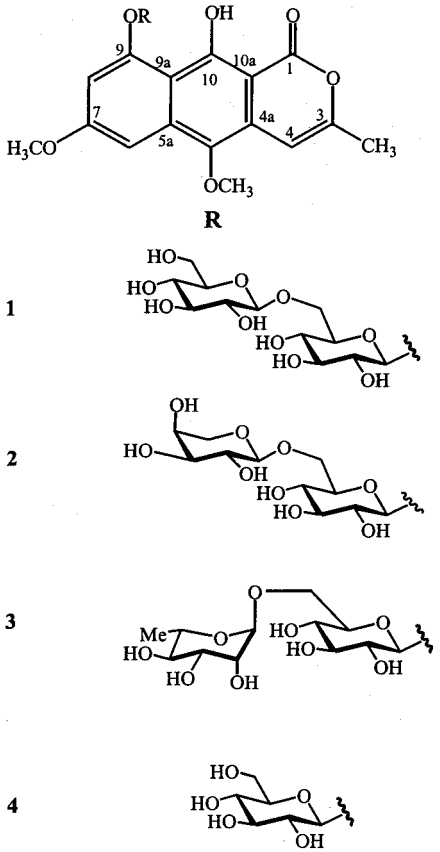
Three new naphthopyranone glycosides, paepalantine-9-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**), paepalantine-9-*O*-α-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside (**2**), and paepalantine-9-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**3**), along with the known paepalantine-9-*O*-*β*-D-glucopyranoside (**4**) were isolated from aerial parts of *Paepalanthus microphyllus*. These compounds were characterized by spectrometric methods, including electrospray mass spectrometry and 1D and 2D NMR experiments. As a part of our program for screening natural compounds for anti-HIV activity, compounds **1**−**4** were tested in C8166 cells infected with HIV-1_{MN}.

The Eriocaulaceae are the dominant herbal population of Cipó hill region (Minas Gerais State, Brazil), together with Poaceae, Cyperaceae, and Xyridaceae.[1] According to Giulietti et al., the monocotyledoneous family Eriocaulaceae has ca. 1200 species, distributed in 10 genera.[2] The taxonomy of Eriocaulaceae is complex and is based mostly on morphological features. Limited chemical data are available in the literature.[1,2] We have described the isolation and structural determination of a naphthopyran-1-one (paepalantine) from *Paepalanthus bromelioides* as well as a number of its glycosylated derivatives in previous reports.[3,4] These compounds showed potent antibiotic, cytotoxic, and mutagenic activities.[5] Other compounds belonging to this class of natural metabolites present antitumor, antileukemic, and even antiviral activities.[6] We have now examined the constituents of the aerials parts of *P. microphyllus* (Guill.) Kunth., belonging to the subgenus *Paepalocephalus*. This investigation led to the isolation of three new paepalantine glycosides (**1**−**3**) together with paepalantine-9-*O*-*β*-D-glucopyranoside (**4**), which was previously isolated from *P. bromelioides*.[4]

The ESMS (100 V, positive ion) spectrum of **1** gave the protonated molecular ion $[M + H]^+$ at *m*/*z* 627, corresponding to the molecular formula $C_{28}H_{34}O_{16}$, as well as the peak at *m*/*z* 649 corresponding to the sodium adduct $[M + Na]^+$. The loss of a hexose moiety (162 u) from the molecular ion led to the fragment at *m*/*z* 465 $[M - 162 + Na]^+$, and subsequent loss of another hexose unit gave the protonated aglycon $[A + H]^+$ at *m*/*z* 303 and a sodium adduct $[A + Na]^+$ at *m*/*z* 325. The complete structure of **1** was elucidated by 1D and 2D NMR experiments at 600 MHz. The ^{13}C NMR and DEPT spectra showed 28 signals, 12 of which were assigned to a disaccharide moiety. The ^1H NMR spectrum displayed two signals of *meta*-coupled protons at *δ* 7.08 and 7.11 and one singlet at *δ* 6.64. Further signals were evident at *δ* 2.32 corresponding to a methyl group and at *δ* 3.88 and 4.00 ascribable to methoxyl groups. The two anomeric signals appearing at *δ* 5.07 and 4.40 (*J* = 7.5 Hz) indicated the *β*-configuration of the sugars. The signal assignments of all ^1H and ^{13}C NMR signals were based on HMBC, HSQC, and TOCSY experiments. The HSQC spectrum,



which correlated the ^1H resonances with those of the corresponding carbons, and the HMBC spectrum allowed us to deduce the aglycon structure 9,10-dihydroxy-7-methoxy-3-methyl-1*H*-naphtho[2,3*c*]pyran-1-one, previously isolated from *P. bromelioides*.[3] On the basis of 1D TOCSY and 2D NMR experiments it was possible to deduce that the disaccharide chain was made up of two *β*-D-glucopyranosyl units (1→6) linked. HMBC correlation between the anomeric signal of glucose (*δ* 5.07) and the carbon resonance at *δ* 159.9 (C-9) of the aglycon allowed us to establish that the sugar portion was linked to C-9. Correlation between the anomeric proton signal at *δ* 4.40 (H-$1_{glc}''$) and the carbon resonance at *δ* 70.5 (C-$6_{glc}'$) confirmed the interglycosidic linkage. Thus, **1** was char-

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acterized as paepalantine-9-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Similar procedures were used to determine the structures of compounds **2** and **3**, which were found to be the new paepalantine-9-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside and paepalantine-9-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, respectively. Compound **4** was identified as paepalantine-9-*O*-β-D-glucopyranoside by comparison with literature data.[4]

As a part of our program for screening natural compounds for anti-HIV activity,[7,8] pure compounds isolated from *P. microphyllus* were tested in C8166 cells infected with HIV-1_{MN}.

However, all compounds showed disappointing activities. Methods and results of tests are available as Supporting Information.

The classic taxonomy of the Eriocaulaceae is rather complex, and chemical studies on plants belonging to this family are scarce.[2−4,9−11] *Paepalanthus* is the largest genus and the one with the most morphological variation.[12]

Experimental Section

General Experimental Procedures. UV spectra were obtained from a Beckman DU 670 spectrophotometer. IR measurements were performed on a Bruker IFS-48 spectrophotometer. NMR spectra in CD_3OD were obtained using a Bruker DRX-600 spectrometer, operating at 599.19 MHz for ^1H and 150.86 MHz for ^{13}C. 2D experiments: $^1H-^1H$ DQF-COSY (double filtered direct chemical shift correlation spectroscopy), inverse detected $^1H-^{13}C$ HSQC (heteronuclear single quantum coherence), and HMBC (heteronuclear multiple bond connectivity) were obtained using UXNMR software. Selective excitation spectra, 1D TOCSY were acquired using waveform generator-based GAUSS-shaped pulses, mixing time ranging from 100 to 120 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse. ESMS were performed by using a Fisons Platform spectrometer in the positive mode (100 V). Samples were dissolved in MeOH and injected directly. Elemental analysis were made with a Carlo Erba EA 1110 apparatus. HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector and with a Waters μ-Bondapak RP18 column and a U6K injector. TLC were performed on silica gel Si F254 (Merck). The plates were visualized using UV light (254 and 365 nm).

Plant Material. Aerial parts of *P. microphyllus* were collected in February 1997, at Cipó Hill, in the Espinhaço Chain, Minas Gerais State, Brazil, and authenticated by Prof. Paulo Takeo Sano from Instituto de Biociências, USP, São Paulo. A voucher specimen (CFCR 5610) is on file at the Herbarium of the Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Brazil.

Extraction and Isolation. Dried and powdered aerial parts of *P. microphyllus* (300 g) were extracted successively with hexane, CH_2Cl_2, and EtOH (one week each). Solvents were evaporated under vacuum. Two grams of the EtOH extract was chromatographed on a Sephadex LH-20 column (100 × 5 cm), with MeOH as eluent. Fractions (8 mL) were collected and checked by TLC [Si gel plates, *n*-BuOH−AcOH−H_2O (12:3:5)]. Fractions 56−70 contained pure **4** (10 mg). Fractions 32−40 (230 mg) containing the crude glycosidic mixture were further purified by HPLC in a Waters (μ-Bondapak RP-18) column (30 cm × 7.6 mm i.d.) using MeOH−H_2O (1:1) as eluent to afford pure compounds **1** (9 mg, t_R = 18.0 min), **2** (6.5 mg, t_R = 21.0 min), and **3** (9.5 mg, t_R = 21.5 min).

Compound 1: yellow needles, UV λ_{max} (MeOH) (log ε) 245 (sh), 273 (4.89), 284 (4.92), 390 (3.94) nm; (NaOH) 262 (4.72), 284 (4.74), 395 (4.14) nm; IR (KBr) ν_{max} 3385, 2914, 1681, 1653, 1618, 1580, 1460 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 7.11 (1H, d, *J* = 1.5 Hz, H-6), 7.08 (1H, s, H-8), 6.64 (1H, s, H-4), 5.07 (1H, d, *J* = 7.5 Hz, H-$1_{glc}'$), 4.40 (1H, d, *J* = 7.5 Hz, H-$1_{glc}''$), 4.21 (1H, dd, *J* = 2.5, 11.0 Hz, H-$6a_{glc}'$), 4.00 (3H, s, OMe-7), 3.92 (1H, dd, *J* = 5.0, 11.0 Hz, H-$6b_{glc}'$), 3.89 (1H, dd, *J* = 2.5, 11.0 Hz, H-$6a_{glc}''$), 3.88 (3H, s, OMe-5) 3.85 (1H, ddd, *J* = 2.5, 5.0, 9.0 Hz, H-$5_{glc}'$), 3.69 (1H, dd, *J* = 7.5, 9.0, H-$2_{glc}'$), 3.67 (1H, dd, *J* = 5.0, 11.0 Hz, H-$6b_{glc}''$), 3.56 (1H, dd, *J* = 9.0, 9.0 Hz, H-$3_{glc}'$), 3.48 (1H, dd, *J* = 9.0, 9.0 Hz, H-$4_{glc}'$), 3.38 (1H, dd, *J* = 9.0, 9.0 Hz, H-$3_{glc}''$), 3.33 (1H, dd, *J* = 9.0, 9.0 Hz, H-$4_{glc}''$), 3.30 (1H, dd, *J* = 7.5, 9.0 Hz, H-$2_{glc}''$), 3.28 (1H, ddd, *J* = 2.5, 5.0 Hz, 9.0 Hz, H-$5_{glc}''$), 2.32 (3H, *J* = 6.1, Me-11); ^{13}C NMR (CD_3OD, 600 MHz) δ 169.2 (C-1), 163.6 (C-7), 160.8 (C-10), 159.9 (C-9), 154.2 (C-3), 141.1 (C-5), 137.6 (C-5a), 124.7 (C-4a), 111.8 (C-9a), 105.2 (C-8), 105.1 (C-$1_{glc}''$), 103.7 (C-$1_{glc}'$), 99.9 (C-4), 99.2 (C-10a), 96.1 (C-6), 78.0 (C-$3_{glc}''$, C-$5_{glc}''$), 77.6 (C-$3_{glc}'$), 77.5 (C-$5_{glc}'$), 75.2 (C-$2_{glc}'$, C-$2_{glc}''$), 71.7 (C-$4_{glc}''$), 71.6 (C-$4_{glc}'$), 70.5 (C-6′), 62.8 (C-$_{glc}''$), 62.3 (OMe-5), 56.2 (OMe-7), 19.4 (Me-11); ESMS *m/z* 649 $[M + Na]^+$, 627 $[(M + H]^+$, 487 $[M - 162 + Na]^+$, 465 $[M - 162 + H]^+$, 325 $[M - 2glc + Na]^+$, 303 $[M - 2_{glc} + H]^+$; *anal.* C 53.49%, H 5.39%, calcd for $C_{28}H_{34}O_{16}$, C 53.67%, H 5.47%.

Compound 2: yellow needles, UV λ_{max} (MeOH) (log ε) 244 (sh), 271 (4.87), 285 (4.93), 393 (3.97) nm; (NaOH) 260 (4.70), 284 (4.74), 394 (4.13) nm; IR (KBr) ν_{max} 3382, 2910, 1684, 1650, 1619, 1583, 1463 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 7.13 (1H, d, *J* = 1.5 Hz, H-8), 7.03 (1H, d, *J* = 1.5 Hz, H-6), 6.64 (1H, s, H-4), 5.07 (1H, d, *J* = 7.5 Hz, H-1_{glc}), 4.37 (1H, d, *J* = 6.2 Hz, H-1_{ara}), 4.20 (1H, dd, *J* = 2.5, 11.0 Hz, H-$6a_{glc}$), 4.00 (3H, OMe-7), 3.89 (3H, OMe-5), 3.89 (1H, dd, *J* = 2.0, 12.0 Hz, H-$5a_{ara}$), 3.85 (1H, dd, *J* = 5.0, 11.0 Hz, H-$6b_{glc}$), 3.81 (m, H-4_{ara}), 3.76 (1H, ddd, *J* = 2.5, 5.0, 9.0 Hz, H-5_{glc}), 3.70 (1H, dd, *J* = 7.5, 9.0 Hz, H-2_{glc}), 3.64 (1H, dd, *J* = 6.2, 8.5 Hz, H-2_{ara}), 3.57 (1H, dd, *J* = 9.0, 9.0 Hz, H-3_{glc}), 3.55 (1H, dd, *J* = 3.0, 8.5 Hz, H-3_{ara}), 3.53 (1H, dd, *J* = 3.5, 12.0 Hz, H-$5b_{ara}$), 3.51 (1H, dd, *J* = 9.0, 9.0 Hz, H-4_{glc}), 2.32 (3H, d, *J* = 6.1 Hz, Me-11); ^{13}C NMR (CD_3OD, 600 MHz): δ 169.2 (C-1), 163.6 (C-7), 160.8 (C-10), 159.8 (C-9), 153.9 (C-3), 140.9 (C-5), 137.6 (C-5a), 124.7 (C-4a), 111.5 (C-9a), 105.1 (C-1_{ara}), 104.7 (C-8), 103.8 (C-1_{glc}), 99.7 (C-4), 98.5 (C-10a), 77.6 (C-3_{glc}), 77.3 (C-5_{glc}), 75.2 (C-2_{glc}), 74.2 (C-3_{ara}), 72.5 (C-2_{ara}), 71.4 (C-4_{glc}), 69.4 (C-6_{glc}), 69.3 (C-4_{ara}), 66.4 (C-5_{ara}), 62.1 (OMe-5), 56.3 (OMe-7), 19.0 (Me-11); ESMS *m/z* 619 $[M + Na]^+$, 597 $[(M + H]^+$, 487 $[M - 132 + Na]^+$, 303 $[M - 162 + H]^+$; *anal.* C 54.28%, H 5.36%, calcd for $C_{27}H_{32}O_{15}$, C 54.35%, H 5.41%.

Compound 3: yellow needles, UV λ_{max} (MeOH) (log ε) 247 (sh), 275 (4.89), 283 (4.92), 392 (3.96) nm; (NaOH) 262 (4.71), 282 (4.73), 394 (4.13) nm; IR (KBr) ν_{max} 3382, 2914, 1680, 1653, 1617, 1581, 1463; ^1H NMR (CD_3OD, 600 MHz) δ 7.09 (1H, d, *J* = 1.5 Hz, H-6), 6.98 (1H, d, *J* = 1.5 Hz, H-8), 6.61 (1H, s, H-4), 5.00 (1H, d, *J* = 7.5 Hz, H-1_{glc}), 4.75 (1H, d, *J* = 1.5 Hz, H-1_{rha}), 4.11 (1H, dd, *J* = 2.5, 11.0 Hz, H-$6a_{glc}$), 4.00 (3H, OMe-7), 3.97 (1H, dd, *J* = 1.5, 3.0 Hz, H-2_{rha}), 3.87 (3H, OMe-5), 3.75 (1H, dd, *J* = 3.0, 9.0 Hz, H-3_{rha}), 3.69 (1H, dd, *J* = 7.5, 9.0 Hz, H-2_{glc}), 3.69 (1H, m, H-5_{rha}), 3.68 (1H, ddd, *J* = 2.5, 5.0, 9.0 Hz, H-5_{glc}), 3.65 (1H, dd, *J* = 5.0, 9.0 Hz, H-$6b_{glc}$), 3.56 (1H, dd, *J* = 9.0, 9.0 Hz, H-3_{glc}), 3.47 (1H, dd, *J* = 9.0, 9.0 Hz, H-4_{glc}), 3.39 (1H, dd, *J* = 9.0, 9.0 Hz, H-5_{rha}), 2.31 (3H, Me-11), 1.25 (3H, d, *J* = 6.5 Hz, H-6_{rha}); ^{13}C NMR data (CD_3OD, 600 MHz) δ 169.2 (C-1), 163.9 (C-7), 160.8 (C-10), 160.0 (C-9), 154.7 (C-3), 141.1 (C-5), 137.6 (C-5a), 124.7 (C-4a), 111.7 (C-9a), 105.3 (C-8), 103.8 (C-1_{glc}), 102.2 (C-1_{rha}), 100.0 (C-4), 98.7 (C-10a), 95.8 (C-6), 77.6 (C-3_{glc}), 77.1 (C-5_{glc}), 74.9 (C-2_{glc}), 74.0 (C-4_{rha}), 72.2 (C-3_{rha}), 71.8 (C-2_{rha}), 71.3 (C-4_{glc}), 69.6 (C-5_{rha}), 67.7 (C-6_{glc}), 62.0 (OMe-5), 56.4 (OMe-7), 19.1 (Me-11), 17.7 (C-6_{rha}); ESMS *m/z* 633 $[(M + Na]^+$, 611 $[M + H]^+$, 487 $[M - 146 + Na]^+$, 303 $[M - 162 + H]^+$; *anal.* C 49.98%, H 5.55%, calcd for $C_{28}H_{34}O_{15}$, C 55.07%, H 5.61%.

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Supporting Information Available: Table: Anti-HIV activity of compounds **1**−**4**. Experimental section: Antiviral assays and virus infectivity assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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