# Planifolin, a New Naphthopyranone Dimer and Flavonoids from *Paepalanthus planifolius*

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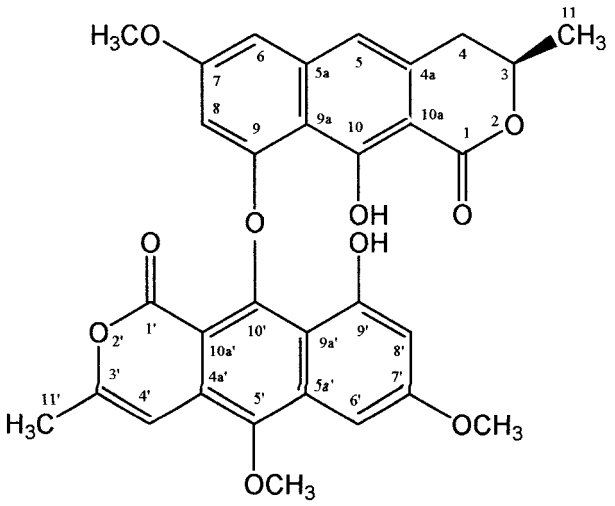
A new naphthopyranone dimer (**1**) named planifolin was isolated from a methylene chloride extract of the capitula of *Paepalanthus planifolius*. The structure of **1** has been determined by chemical and spectroscopic means. In addition, a known dihydronaphthopyranone glycoside and seven known flavonoids were isolated from an ethanolic extract of the leaves of *P. planifolius*.

The Eriocaulaceae is an unusual family of monocotyledonous plants which shares with the Asteraceae the presence of capitula in the inflorescence, a fact that accounts for the phrase "the Compositae of the monocotyledons", which has been employed to emphasize this morphological convergence.[1]

Paepalantine, a naphthopyran-1-one showing potent antibiotic, cytotoxic, and mutagenic activities,[2,3] was isolated from several *Paepalanthus* species.[4] Our interest in naphthopyranones has now led us to investigate a methylene chloride extract of the capitula and an ethanolic extract of the leaves of *Paepalanthus planifolius* Koern. (Eriocaulaceae). *P. planifolius*, known as "sempre-viva" ("everlasting plant"), belongs to the subgenus *Platycaulon*, and it is found commonly in parts of Minas Gerais and Bahia States in Brazil, as well as outside of these regions.[5] We report here the isolation and characterization of a new naphthopyranone dimer (**1**) from a methylene chloride extract of the capitula together with a known dihydronaphthopyranone glycoside and seven known flavonoids from an ethanolic extract of the leaves of *P. planifolius*.



**1**

**Table 1.** $^1$H and $^{13}$C NMR (600 MHz) Data for Compound **1** ($CDCl_3$)[a]

| position | $^1$H (*J* in Hz) | $^{13}$C | position | $^1$H (*J* in Hz) | $^{13}$C |
|---|---|---|---|---|---|
| 1 | | 171.5 | 1′ | | 168.1 |
| 3 | 4.72 m | 77.5 | 3′ | | 152.4 |
| 4 | | 34.7 | 4′ | 6.49 s | 99.4 |
| 4a | 2.94 overlapped | 133.1 | 4a′ | | 122.3 |
| 4b | 2.94 overlapped | | | | |
| 5 | 6.83 s | 116.0 | 5′ | | 140.5 |
| 5a | | 141.5 | 5a′ | | 141.1 |
| 6 | 6.53 d (1.6) | 98.6 | 6′ | 6.94 d (1.6) | 93.6 |
| 7 | | 162.7 | 7′ | | 162.8 |
| 8 | 6.49 d (1.6) | 101.5 | 8′ | 6.56 d (1.6) | 101.9 |
| 9 | | 158.9 | 9′ | | 158.6 |
| 9a | | 108.6 | 9a′ | | 108.3 |
| 10 | | 163.0 | 10′ | | 158.2 |
| 10a | | 99.3 | 10a′ | | 96.1 |
| Me-11 | 1.51 d (6.1) | 20.7 | Me-11′ | 2.27 d (1.0) | 19.4 |
| | | | OMe-5′ | 3.83 s | 61.8 |
| OMe-7 | 3.95 s | 55.4 | OMe-7′ | 3.97 s | 55.4 |
| OH-10 | 13.20 s | | OH-9′ | 9.47 s | |

[a]Assignments were made by DQF-COSY, HSQC, and HMBC data.

Compound **1**, molecular formula $C_{31}H_{26}O_{10}$, showed in its IR spectrum absorptions for hydroxyl (3384 $cm^{-1}$) and carbonyl groups (1651 and 1640 $cm^{-1}$). The complete structure of **1** was elucidated by 1D and 2D NMR experiments at 600 MHz. The $^{13}$C NMR spectrum showed 31 signals. The $^1$H NMR spectrum displayed signals of aromatic protons at $\delta$ 6.49 (2H), 6.53 (1H), 6.56 (1H), 6.83 (1H), and 6.94 (1H). Further signals appeared at $\delta$ 2.94 (overlapped) and at $\delta$ 4.72 (1H). Also evident were signals at $\delta$ 3.97, 3.95, and 3.83, typical of aromatic methoxyl groups. Assignments of the $^1$H and $^{13}$C NMR data of **1** were based on HMBC, HSQC, and DQF-COSY experiments (Table 1). The DQF-COSY spectrum indicated the sequence $H_2$ ($\delta$ 2.94, overlapped), C*H* ($\delta$ 4.72), and C$H_3$ ($\delta$ 1.51), typical of the A ring of a naphthopyranone.[6] In the HMBC spectrum (Figure 1 and table in Supporting Information) were observed diagnostic long-range correlations between the proton signal at $\delta$ 6.49 (H-8) and the carbon resonances at $\delta$ 98.6 (C-6), 108.6 (C-9a), 158.9 (C-9), and 162.7 (C-7); the proton signal at $\delta$ 6.83 (H-5) and the carbon resonances at $\delta$ 98.6 (C-6), 108.6 (C-9a), 99.3 (C-10a), and 141.5 (C-5a); the proton signal at $\delta$ 6.53 (H-6) and the carbon resonances at $\delta$ 101.5 (C-8), 108.6 (C-9a), 116.0 (C-5), and 162.7 (C-7); the proton signals at $\delta$ 2.94 (overlapped, $H_2$-4) and the carbon resonances at $\delta$ 77.5 (C-3), 133.1 (C-4a), and 99.3 (C-10a); and the methyl doublet at $\delta$ 1.51 ($CH_3$-11) and the

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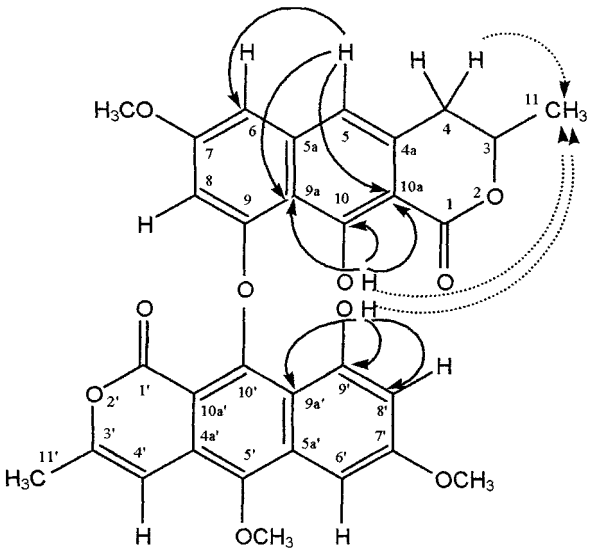

**Figure 1.** Major HMBC (solid arrows) and ROESY (dotted arrows) correlations of compound **1**.

carbon signal at $\delta$ 77.5 (C-3). A further correlation was observed between the proton signal at $\delta$ 3.95 and the carbon signal at $\delta$ 162.7 (C-7). On the basis of the above evidence it was possible to deduce the presence of semi-vioxanthin as one of the monomeric units of the molecule.[6] For the other monomeric unit (paepalantine) diagnostic long-range correlations were observed between the proton signal at $\delta$ 6.56 (H-8′) and the carbon resonances at $\delta$ 93.6 (C-6′) and 108.3 (C-9a′), and the proton signal correlations peak at $\delta$ 6.94 (H-6′) and the carbon resonances at $\delta$ 101.9 (C-8′) and 108.3 (C-9a′). Also seen were the proton signal at $\delta$ 6.49 (H-4′) and the carbon resonances at $\delta$ 122.3 (C-4a′), 140.5 (C-5′), and 152.4 (C-3′); the methyl signal at $\delta$ 2.27 and the carbon signal at $\delta$ 152.4 (C-3′); and the methoxy group at $\delta$ 3.83 ($OCH_3$-5′) and the carbon signal at $\delta$ 140.5 (C-5′).

Thus, the molecule **1** appeared to be made up by two monomeric portions, semi-vioxanthin and paepalantine,[2] linked together via an ether bond. To define the sites of linkage, several complementary observations were made. In the $^1$H NMR spectrum, the signal at $\delta$ 9.47 suggested the occurrence of a free hydroxyl group at position 9 or 9′ and the signal at $\delta$ 13.20 clearly indicated a hydroxyl group at position 10 or 10′ chelated to the carbonyl group. The proton signal at $\delta$ 13.20 correlated in the HMBC spectrum to the carbon signals at $\delta$ 99.3 (C-10a), 108.6 (C-9a), and $\delta$ 163.0 (C-10). The signal at $\delta$ 9.47 showed in the HMBC spectrum correlations with the carbon resonances at $\delta$ 101.9 (C-8′), 108.3 (C-9a′), and $\delta$ 158.6 (C-9′). On the basis of these findings the signal at $\delta$ 13.20 was attributed to the hydroxyl group at C-10 of the semi-vioxanthin unit and the signal at $\delta$ 9.47 to the hydroxyl group at C-9′ of the paepalantine moiety. Thus, the linkage between the two monomeric units was deduced to be between C-9 and C-10′ and the structure of planifolin was established as **1**. Additional evidence for this was afforded by the ROESY spectrum (Figure 1), which showed correlations between the methyl group at $\delta$ 1.51 ($CH_3$-11) and the signals at $\delta$ 13.20 (OH-10), at $\delta$ 9.47 (OH-9′), and at $\delta$ 4.72 (H-3), and the signal at $\delta$ 13.20 (OH-10) and the signal at $\delta$ 9.47 (OH-9′). Despite the unusual structure of dimer **1**, the presented data explain the observed correlations very well. Naphthopyranone glycosides with semi-vioxantin as aglycon were previously found in *P. vellozioides* and in *P. latipes*, and the stereochemistry at C-3 was determined as *R*.[6]

From the ethanolic extract of the leaves of *P. planifolius*, 3,4-dihydro-10-hydroxy-7-methoxy-3-methyl-1*H*-3,4-dihydronaphtho[2,3*c*]pyran-1-one-9-*O*-$\beta$-D-allopyranosyl-(1→6)-$\beta$-D-glucopyranoside was isolated. This compound has been found in *P. latipes* and *P. vellozioides*.[6] In addition, from the same extract, the flavonoids 6-hydroxyluteolin-7-*O*-$\beta$-D-glucopyranoside, 6-hydroxyquercetin-3-*O*-$\beta$-D-glucopyranoside, 5,6,7,8,3′,4′-hexahydroxyflavone, luteolin-6,8-di-*C*-$\beta$-D-glucopyranoside, 6-hydroxyapigenin-7-*O*-$\beta$-D-glucopyranoside, 6-hydroxy-7-methoxyapigenin, and 6-hydroxyluteolin-7,4′-di-*O*-$\beta$-D-glucopyranoside were purified and identified on the basis of NMR data and comparison with literature values.[7,8] The occurrence of a flavone in the leaves of *P. planifolius* represents the first report for a plant in the subgenus *Platycaulon*. Common to all the *Paepalanthus* species belonging to the subgenus *Platycaulon* is the occurrence of naphthopyranone derivatives. Thus, the presence of naphthopyranones in *P. planifolius* reinforces the fact that such compounds can be considered taxonomic markers of *Paepalanthus* species belonging to the subgenus *Platycaulon*. Naphthopyranone dimers are typical of fungi of the genera *Aspergillus* and *Penicillium*,[9] but these are symmetric and characterized by C−C linkages. From the capitula of *Paepalanthus bromelioides* the symmetric 8−8′ C−C dimer of paepalantine was isolated and characterized.[10]

## Experimental Section

**General Experimental Procedures.** Melting points were determined using a Bausch & Lamb apparatus. UV spectra were obtained on a Beckman DU 670 spectrometer. IR measurements were performed on a Bruker IFS-48 spectrophotometer. NMR spectra in $CDCl_3$ (compound **1**) and $CD_3OD$ (dihydronaphthopyranone glycoside and flavonoid glycosides) were obtained using a Bruker DRX-600 spectrometer, operating at 599.19 MHz for $^1$H and 150.85 MHz for $^{13}$C. 2D experiments: $^1$H−$^1$H DQF-COSY[11] (double filtered direct chemical shift correlation spectroscopy), inverse detect $^1$H−$^{13}$C HSQC[12] (heteronuclear single quantum coherence), and HMBC[13] (heteronuclear multiple bond connectivity) were obtained using UXNMR software. EIMS were performed by using a TRIO 2000 apparatus (70 eV); the mass range (50−650 amu) was analyzed using a scan time of 0.6 s. HREIMS were performed by using a VG-70 250S apparatus (VG Analytical, Ltd., Manchester, UK) (70 eV). Elemental analyses 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 with a Waters $\mu$-Bondapak $RP_{18}$ column and UK6 injector. TLC were performed on silica gel SiF254 (Merck). The plates were visualized using UV light (254 and 365 nm).

**Plant Material.** *P. planifolius* was collected in February 1995, at Cipó Hill, in the Espinhaço Chain, Minas Gerais State, Brazil, and authenticated by Prof. Paulo Takeo Sano from the Instituto de Biociências, USP, São Paulo. A voucher specimen (CFSC 13848) is on file of the Herbarium of the Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Brazil.

**Extraction and Isolation.** The capitula of *P. planifolius* (91.0 g) were powdered and extracted successively with hexane, methylene chloride, and ethanol. The methylene chloride extract (3.0 g) was chromatographed over a Si gel column using as solvent toluene−EtOAc (9:1). Altogether, 56 fractions (10 mL) were collected and checked by TLC in several eluents [Si gel plates, toluene−EtOAc (5:5, 6:4, 7:3, 8:2, and 9:1)]. Fractions 5−9 (50 mg) contained pure **1**.

The dried leaves of *P. planifolius* (200 g) were powdered and extracted successively with hexane, methylene chloride, and ethanol. The ethanol extract (1.2 g) was chromatographed on a Sephadex LH-20 column (80 × 2 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 30−40 (124 mg) were further purified by HPLC in a Waters ($\mu$-Bondapak RP-18) column (30 cm × 7.6 mm i.d.) using MeOH−$H_2O$ (2:3) as eluent to afford pure 3,4-dihydro-10-hydroxy-7-methoxy-3-methyl-1*H*-3,4-dihydronaphtho[2,3*c*]pyran-1-one-9-*O*-$\beta$-D-allopyranosyl(1→6)-$\beta$-D-glucopyranoside (8.2 mg, $t_R$ = 33.0 min),[6] and luteolin-6,8-di-*C*-$\beta$-D-glucopyranoside (8.5 mg, $t_R$ = 23.0 min).[7,8] Fractions 36−41 (98 mg) were purified using the same conditions indicated for fractions 30−34 and yielded 6-hydroxyquercetin-3-*O*-$\beta$-D-glucopyranoside (6.8 mg, $t_R$ = 20.0 min)[7,8] and 6-hydroxyapigenin-7-*O*-$\beta$-D-glucopyranoside (3.0 mg, $t_R$ =12.0 min).[7,8] Fractions 42−45 contained pure 6-hydroxyluteolin-7-*O*-$\beta$-D-glucopyranoside (10 mg, $t_R$ = 24.0 min).[7,8] Fractions 47−49 (purified by RP-HPLC using MeOH−$H_2O$, 9:11 as eluent) gave 6-hydroxyluteolin-7,4′-di-*O*-$\beta$-D-glucopyranoside (6.5 mg, $t_R$ = 17 min).[7,8] Fractions 52−55 contained pure 6-hydroxy-7-methoxyapigenin (7.5 mg),[7,8] while fractions 57−60 contained pure 5,6,7,8,3′,4′-hexahydroxyflavone (10.5 mg).[8]

**Planifolin (1):** yellow solid; mp 144−145 °C (toluene−EtOAc, 9:1); UV $\lambda_{max}$ (MeOH) (log $\epsilon$) 261 (4.75), 270 (4.70), 371 (4.06) nm; (KOH) 209 (4.78), 263 (4.70), 382 (4.24) nm; ($AlCl_3$) 274 (4.74), 281 (4.75), 409 (4.04) nm; ($AlCl_3$/HCl) 274 (4.80), 281 (4.83), 408 (4.00) nm; NaOAc 263 (4.82), 383 (4.22) nm; NaOAc/$H_3BO_3$ 262 (4.86), 270 (4.80), 370 (4.22) nm; IR (KBr) $\nu_{max}$ 3382, 1651, 1640 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 600 MHz), see Table 1; $^{13}$C NMR ($CDCl_3$, 150 MHz), see Table 1; EIMS (70 eV) *m*/*z* 558 $[M]^+$ (2), 302 $[M - C_{15}H_{12}O_4]^+$ (22), 287 $[M - C_{15}H_{12}O_4 - CH_3]^+$(33), 256 $[M - C_{16}H_{14}O_6]^+$ (15); HREIMS *m*/*z* $[M]^+$ absent, 302.0788 (calcd for $C_{16}H_{14}O_6$ 302.0790), 287.0549 (calcd for $C_{16}H_{15}O_4$ 287.0555), 274.0838 (calcd for $C_{15}H_{14}O_5$ 274.0841); *anal*. C 66.52%, H 4.43%, calcd for $C_{31}H_{26}O_{10}$, C 66.72%, H 4.68%.

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**Supporting Information Available:** Table of HMBC correlations for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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