www.saber.ula.ve/avancesenquimica
Avances en Química, 8(3), 131-138 (2013)
Artículo científico

Naphthopyranones from Rhizomes of *Paepalanthus diffissus*

José Gregorio Alvarado*, José Andrés Abad-Reyes, Roger Montealegre, Juan Manuel Amaro-Luis

Laboratorio de Productos Naturales. Departamento de Química. Facultad de Ciencias.
Universidad de Los Andes. Mérida 5101, Venezuela

(*) josealvarado@ula.ve

Recibido: 04/11/2013 **Revisado**: 06/12/2013 **Aceptado**: 08/12/2013

Resumen

Del extracto en diclorometano obtenido de los rizomas de *Paepalanthus diffissus* Moldenke (Eriocaulaceae) fueron aisladas las naftopiranonas bioactivas (+)-semi-vioxantina **[3]** y vioxantina **[8]**. Estos compuestos fueron caracterizados en base a estudios espectroscópicos, incluyendo experimentos de RMN uni- y bi-dimensionales. La revisión de la literatura indica que estos metabolitos son encontrados frecuentemente en hongos y líquenes, pero son compuestos raros en plantas superiores. La presencia de (+)-semi-vioxantina en plantas con flores, es descrita aquí por primera vez.

Palabras clave: Eriocaulaceae; *Paepalanthus*; naftopiranonas; (+)-semi-vioxantina; vioxantina

Abstract

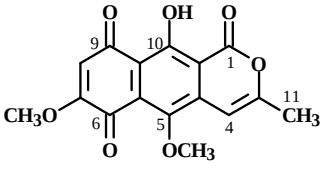
From a dichloromethane extract obtained of rhizomes of *Paepalanthus diffissus* Moldenke (Eriocaulaceae) were isolated the bioactive naphthopyranones (+)-semi-vioxanthin **[3]** and vioxanthin **[8]**. These compounds were characterized on the basis of spectroscopic studies, including 1D- and 2D-NMR experiments. The literature review indicated that these metabolites are frequently found in fungi and lichens, but they are rare compounds in higher plants. Presence of (+)-semi-vioxanthin in flowering plants is here described by first time.

Keywords: Eriocaulaceae; *Paepalanthus*; naphthopyranones; (+)-semi-vioxanthin; vioxanthin

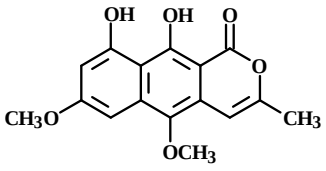
Introduction

Eriocaulaceae is a small family of flowering plants, which contains about 1400 species grouped in 11 genera[1], although the most recent molecular studies tends to reduce the number of genera to ten[2]. Most of the species included in this family are distributed in mountainous regions of South America, especially in the rocky savannas of Brazil and the ñtepuisò (table mountains) of Guyana and Venezuela; only a few species assembled in five genera, extend their habitat into temperate regions of North America and Europe, tropical Africa and eastern Asia[3]. *Paepalanthus* represents the largest genus of the family, with over 450 species distributed disjunctly in neotropical South America, occidental Africa and Madagascar[4].

The genus *Paepalanthus* is well documented as a good source of secondary metabolites such as flavonoids[5-10], naphthopyranones[9-14], naphthoquinones[15] and caffeic acid derivatives[6]. Currently, some of these compounds possess a notable pharmacological interest due to their proven biological activity; for example, it has been reported that the naphthoquinone 5-methoxy-3,4-dehydroxanthomegnin **[1]**, isolated from *Paepalanthus latipes*, possess immune modulatory effects on nitric oxide production on LPS-stimulated macrophages[16], in addition to cytotoxic, antitumoral, antioxidant and anti-*Helicobacter pylori* activity[15-18]. In the same way, the isocoumarin paepalantine **[2]**, a metabolite present in different extracts of *Paepalanthus bromelioides* and *P. vellozioides*, exhibits a wide range of biological activities, including antioxidant[19,20], anti-inflammatory[21], antimicrobial[22-23] cytotoxic[12,24], genotoxic[25] and mutagenic[12,26] activity. Also several flavonoids identified in *Paepalanthus ssp*. have shown to possess biological properties such as antioxidant potential[8], mutagenicity[9] or antimycobacterial activity[10].

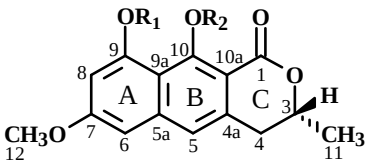


5-Methoxy-3,4-dehydroxanthomegnin [1]

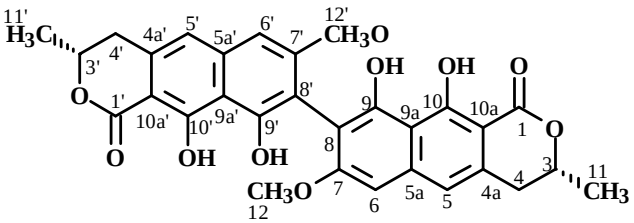


Paepalantine [2]

In the light of the foregoing, as part of our continuing phytochemical studies on medicinal plants of Venezuelaôs Andean, in this paper we describe the isolation of two naphthopyranones from rhizomes of *Paepalanthus diffissus* Moldenke, a species found commonly in Andean moors. These naphthopyranones were identified as (+)-semi-vioxanthin **[3]** and vioxanthin **[8]**, known antifungal antibiotics often isolated from fungi[27-29] and lichens[30]; presence of vioxanthin in flowering plants is up to now limited to a few species of the genus *Paepalanthus* [20,31], but to the best of our knowledge, (+)-semi-vioxanthin has so far not been found in higher plants.



(+)-Semi-Vioxanthin [3]: $R_1 = R_2 = H$
(+)-Semi-Vioxanthin 9-Acetate [4]: $R_1 = Ac$; $R_2 = H$
(+)-Semi-Vioxanthin, 9,10-Diacetate [5]: $R_1 = R_2 = Ac$
(+)-Semi-Vioxanthin 9-Methyl Ether [6]: $R_1 = CH_3$; $R_2 = H$
(+)-Semi-Vioxanthin 10-Methyl Ether [7]: $R_1 = H$; $R_2 = CH_3$



Vioxanthin [8]

Materials and methods

General

Melting points were determined using a Fisher-Johns apparatus and they are uncorrected. Optical rotations were measured on a 60Hz-Steeg & Reuter G.m.b.H. polarimeter using $CHCl_3$ as solvent. UV spectra were obtained in a Perkin-Elmer spectrophotometer, Lambda 3B, using quartz cells with 1cm thick and methanol (Merck-Uvasol) as solvent. IR measurements were obtained on a Perkin-Elmer FT-1725X spectrophotometer as KBr pellets. 1D and 2D NMR spectra in $CDCl_3$ were acquired using a Bruker-Avance DRX-400 instrument, operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Mass spectra were recorded on a Hewlett-Packard Mass Spectrometer, model 5890 (70 *eV*). TLC were developed on 0.25 mm layers of silica gel PF 254 (Merck); spots were visualized using UV light (254 and 365 nm) and subsequently by spraying with a mixture *v/v* CH_3COOH-H_2O-H_2SO_4 (20:4:1) and then heating with air flow at 100°C for few minutes. VCC was performated with silica gel Merck 60 (63-200 ε m, 70-230 mesh). Size-exclusion chromatography columns were packed with Sigma Sephadex LH-20.

Plant material

Plant material (rhizomes) was collected at ñPáramo de San José de Acequias, Municipio Campo Elías, Estado Mérida, Venezuelaò. Species was identified as *Paepalanthus diffissus* Moldeke by Eng. Juan Antonio Carmona Arzola, Department of Pharmacognosy and Organic Medicaments, Faculty of Pharmacy and Bioanalysis, University of Los Andes (ULA); a *voucher specimen* (Amaro-Luis *et al*., N° 2342) was deposited at the Herbario MERF of this faculty.

Extraction

Rhizomes of *Paepalanthus diffissus* (*ca* 1.80 Kg) were air-dried, ground and exhaustively extracted with hexane and then with dichloromethane in a soxhlet. The solutions obtained were filtered and concentrated *in vacuum* on a rotary evaporator, to afford respectively, 54.8 g and 60.4 g of crude extracts.

Isolation and identification of the constituents

The dichloromethane extract was preadsorbed on silica gel and chromatographed (VLC) over silica gel 60, eluting with hexane, dichloromethane and EtOAc in mixtures of increasing polarity. Fifty-four (54) fractions of 500 mL were collected, concentrated *in vacuum*, and combined according to the TLC characteristics to afford twelve major fractions (A-L).

(+)-Semi-vioxanthin **[3]**: From combined fraction E [22-26, eluted with hexane-CH_2Cl_2 (7:3)] precipitated an apple green solid residue (≅ 5.8 g), which was partially purified by flash chromatography (hexane-EtOAc 4:1); crystallization from mixtures EtOAc/hexane provided

pure yellow needles (≅ 353 mg) detected in TLC plates as a homogeneous green-yellow spot, m.p. = 192-193°C, $[\alpha]_D$: +7.3º ($CHCl_3$). UV, λ_{max} (nm).: 263, 379 (CH_3OH); 228, 243, 404 (CH_3OH + $AlCl_3$). IR (KBr), $\nu_{max.}$ (cm^{-1}): 3384 (-OH), 2978-2850 (C-H), 1650 (C=O), 1582 (C=C), 1158 and 1124 (C-O), 844 and 598 (=C-H). ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EI-MS: *m/z (%)* 275 (60.12) [M^+ +1], 274 (42.31) [M^+], 256 (47.31), 229 (27.72), 200 (15.81), 186 (23.18), 158 (16.03), 141 (22.20), 129 (41.63), 115 (49.82), 102 (33.64), 77 (32.95), 62 (22.31), 43 (37.75).

Vioxanthin **[8]**: Combined fraction J [42-46, eluted with CH_2Cl_2-EtOAc (7:3)] was chromatographed on a Sephadex-LH20 column using as eluent a mixture of hexane-CH_2Cl_2-CH_3OH (0.5:4:0.5) which allowed to obtain twenty six fractions. From fraction N° 6 precipitated a crystalline solid as pale yellow needles (10.2 mg.); m.p. = 196-198°C (descomposition); $[\alpha]_D$: + 4.6º ($CHCl_3$). UV, λ_{max} (nm).: 270, 387 (CH_3OH); 232, 276, 418 (CH_3OH + $AlCl_3$). IR (KBr), $\nu_{max.}$ (cm^{-1}): 3398 (-OH), 2976-2848 (C-H), 1632 (C=O), 1584 (C=C), 1128 and 1092 (C-O), 856 and 568 (=C-H). ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EI-MS: *m/z (%)* 548 (4.25) [M^+ +2], 546 (18.42) [M^+], 531 (57.30), 517 (62.25).

Acetylation of (+)-semi-vioxanthin **[3]**

Compound **[3]** (210 mg) was dissolved in pyridine (14 mL) and treated with Ac_2O (35 mL) at room temperature overnight. Cold water was added to the reaction mixture and immediately it was extracted with CH_2Cl_2. The organic layer was washed in successive stages with aqueous solutions of HCl (10% *v/v*), $NaHCO_3$ (2%) and water, dried on $MgSO_4$ and evaporated to yield a solid residue (142 mg) that showed in TLC two spots. Separation was carried out on a silica gel column using as eluent hexane-CH_2Cl_2 (1:4) to furnish compounds **[4]** (36 mg) and **[5]** (22 mg).

(+)-Semi-vioxantin-9-monoacetate **[4]**: pale yellow flakes**;** m.p. = 186-188°C. IR (KBr), $\nu_{max.}$ (cm^{-1}): 3424 (-OH), 1770 (C=O), 1648 (C=O), 1623 (C=C), 1210 (C-O), 858 (=C-H). ^{1}H NMR (Table 1). ^{13}C NMR (Table 2).

(+)-Semi-vioxantin-9,10-diacetate **[5]**: pale yellow needles**;** m.p. > 200°C. IR (KBr), $\nu_{max.}$ (cm^{-1}): 1770 (C=O), 1716 (C=O), 1632 (C=O), 1580 (C=C), 1212 (C-O), 860 (=C-H). ^{1}H NMR (Table 1). ^{13}C NMR (Table 2).

Methylation of (+)-semi-vioxanthin **[3]**

Compound **[3]** (124 mg) was treated with excess ethereal CH_2N_2 and solution was left standing overnight in a refrigerator at 4°C. Evaporation of ether yielded a colorless oil that was chromatographed on a silica gel column eluted with hexane-CH_2Cl_2 (1:4), to obtain pure TLC compounds **[6]** (4 mg) and **[7]** (23 mg).

(+)-Semi-vioxanthin-9- methyl ether **[6]**: yellow solid; m.p. = 142-144°C. ^{1}H NMR (Table 1).

(+)-Semivioxanthin-10-methyl ether **[7]**: colorless oil. IR (KBr), $\nu_{max.}$ (cm^{-1}): 3302 (-OH), 2848 (C-H), 1712 (C=O), 1642 and 1576 (C=C), 1123 (C-O). ^{1}H NMR (Table 1). ^{13}C NMR (Table 2).

Results and Discussion

(+)-Semi-vioxanthin **[3]** was obtained as pale yellow needles [m.p. = 192-193 °C, $[\alpha]_D$: +7.3º ($CHCl_3$)]. The presence in its EIMS of an ion molecular peak at *m/z*: 274 in conjunction with NMR data, allowed to establish the molecular formula $C_{15}H_{14}O_5$. Its IR spectrum showed absorption bands of hydroxyl groups (3384 cm^{-1}), a carbonyl group (1650 cm^{-1}) and C-O (1158 and 1124 cm^{-1}) and aromatic C-H bonds (1582, 844 and 598 cm^{-1}). Its ultraviolet spectrum exhibited maxima at 263 and 379 nm. The ^{1}H NMR spectrum of **[3]** (Table 1) indicated the presence in the molecule of three aromatic protons, a methoxyl group, dos hydroxyl protons and six aliphatic hydrogens that constitute a methylene, an oxymethine and a secondary methyl group. At the same time its ^{13}C NMR (Table 2) shows, apart of ten signals typical of aromatic carbons, a peak assignable to a carbony group and four sp^3 aliphatic carbons signals.

Comparing and contrasting the above information with the data derived from the analysis of the 2D-NMR spectra it was possible to conclude that **[3]** is a naphthopyranone with a lactone moiety, similar to paepalantine **[2]**. In effect, the naphthalene unit was identified by the presence of two aromatic ***ñmetaò***-coupled protons [doublets at δ_H 6.54 and δ_H 6.51; *J* = 2.35 Hz (H-6 and H-8); HMQC: H-6 ↔ C-6 (δ_C 99.5, =CH); H-8 ↔ C-8 (δ_C 101.6; =CH); HMBC: C-7 ↔ H-6 ↔ C-8 and C-6 ↔ H-8], which characterize a 1,2,3,5-tetrasubstituted benzene ring [A] with two substituents identified as a hydroxyl on C-9 (acute singlet at δ_H 9.45, -OH) and a methoxy group on C-7 [δ_H 3.87, *s*, (H-12)]; correlations in HMBC spectra (Fig 1) confirmed the ubication of these substituents [HMBC: C-8 ↔ OH ↔ C-9 (δ_C 158.6; =CH-O-)/C-9a ↔ H-8 and H-12 ↔ C-7 (δ_C 162.7; =CH-O-)]. The other two A-ring substitute carbons [δ_C 140.5; =C< (C-5a) and δ_C 108.4; =C< (C-9a)] conform the link between the second condensed pentasubstituted bencene ring [B], which also possesses two quaternary carbons bridge [δ_C 133.2; =C< (C-4a) and δ_C 99.2; =C< (C-10a)] that configure the fusion with a third aliphatic cycle [C]. The fifth substituent is a chelated hydroxyl group [δ_H 13.74, (-OH)] located at C-10

Table 1: ^{1}H-NMR ($CDCl_3$, 400 MHz) Chemical Shifts (δ_H)

Compound	[3]	[4]	[5]	[6]	[7]	[8]*
H-3	4.73 (*m*)	4.71 (*m*)	4.74 (*m*)	4.70 (*m*)	4.62 (*m*)	4.75 (*m*)
H-4	2.96(*m*)	2.95 (*m*)	2.96 (*m*)	2.97 (*m*)	2.97 (*m*)	3.01 (*m*)
H-5	6.85 (*s*)	6.93 (*s*)	7.44 (*s*)	6.85 (*s*)	7.20 (*s*)	6.95 (*s*)
H-6	6.54 (*d*) J = 2.35	6.87 (*d*) J = 2.40	6.95 (*d*) J = 2.35	6.57 (*d*) J = 2.33	6.60 (*d*) J = 2.33	6.70 (*s*)
H-8	6.51 (*d*) J = 2.35	6.74 (*d*) J = 2.40	6.82 (*d*) J = 2.35	6.46 (*d*) J = 2.33	6.55 (*d*) J = 2.33	-
H-11	1.54 (*d*) J = 6.32	1.53 (*d*) J = 6.32	1.47 (*d*) J = 6.20	1.52 (*d*) J = 6.30	1.51(*d*) J = 6.30	1.56 (*d*) J = 6.04
H-12	3.87 (*s*)	3.90 (*s*)	3.89 (*s*)	3.90 (*s*)	3.87 (*s*)	3.84 (*s*)
C-9 (O<u>H</u>)	9.45 (*s*)	-	-	-	9.85 (*s*)	9.70 (*s*)
C-10 (O<u>H</u>)	13.74 (*s*)	12.90 (*s*)	-	13.20 (*s*)	-	13.79 (*s*)
H-1ô	-	-	-	3.99 (*s*)	4.12 (*s*)	-
H-2ô	-	2.38 (*s*)	2.39 (*s*)	-	-	-
H-2ò	-	-	2.46 (*s*)	-	-	-

* Only shows δ_H of a monomer unit, since δ_H of second monomer unit are identical

Table 2: ^{13}C-NMR ($CDCl_3$, 100 MHz) Chemical Shifts (δ_C)

Compound	[3]	[4]	[5]	[7]	[8]*
C-1	171.6 (*s*)	170.3 (*s*)	169.9 (*s*)	162.8 (*s*)	171,1 (*s*)
C-3	76,5 (*d*)	76.1 (*d*)	74.6 (*d*)	74.5 (*d*)	76,0 (*d*)
C-4	34,7 (*t*)	35.1 (*t*)	35.9 (*t*)	36.3 (*t*)	34,2 (*t*)
C-4a	133,2 (*s*)	134.3 (*s*)	136.1 (*s*)	136.1 (*s*)	132,3 (*s*)
C-5	116,1 (*d*)	115.6 (*d*)	114.4 (*d*)	120.8 (*d*)	115,6 (*d*)
C-5a	140,5 (*s*)	140.8 (*s*)	139.5 (*s*)	139.7 (*s*)	134,5 (*s*)
C-6	99,5 (*d*)	104.9 (*d*)	104.5 (*d*)	98.6 (*d*)	97,5 (*s*)
C-7	162,7 (*s*)	162.2 (*s*)	159.9 (*s*)	162.0 (*s*)	162,3 (*s*)
C-8	101,6 (*d*)	111.8 (*d*)	122,9 (*d*)	102.6 (*d*)	107,7 (*s*)
C-9	158,6 (s)	150.0 (*s*)	148.0 (*s*)	157.4 (*s*)	154,9 (*s*)
C-9a	108,4 (*s*)	112.0 (*s*)	116.5 (*s*)	112.6 (*s*)	108,0 (*s*)
C-10	163,0 (*s*)	161.0 (*s*)	160.1 (*s*)	161.6 (*s*)	160,9 (*s*)
C-10a	99,2 (*s*)	101.5 (*s*)	113.2 (*s*)	109.5 (*s*)	98,8 (*s*)
C-11	20,8 (*q*)	20. 8 (*q*)	20.7 (*q*)	20. 7 (*q*)	20,2 (*q*)
C-12	55,5 (*q*)	55.7 (*q*)	55.8 (*q*)	55.5 (*q*)	55,4 (*q*)
C-1ô	-	171.2 (*s*)	169.2 (*s*)	64.4 (*q*)	-
C-2ô	-	21.3 (*q*)	21.2 (*q*)	-	-
C-1ò	-	-	169.6 (*s*)	-	-
C-2ò	-	-	21.6 (*q*)	-	-

* Only shows δ_C of a monomer unit, since δ_C of second monomer unit are identical

(cross peaks in HMBC among OH and C-10, C-9a and C-10a); the chelation requires that the lactone carbony group in ring [C] is situated on C-1. The gross molecular structure is completed with the presence en ring [C] of a methylene group [δ_H 2.96, *m*, (H-4); HMQC: H-4 ↔ C-4 (δ_C 34.7; $>CH_2$)] adjacent to a oxymethine [δ_H 4.73, *m*, (H-3); HMQC: H-3 ↔ C-3 (δ_C 76.5; >CH-O-)] that supports to the secondary methyl [δ_H 1.54; *d*, *J* = 6.32 Hz (H-11); HMQC: H-11 ↔ C-11 (δ_C 20.8; - CH_3)]. Many other correlations in HMBC spectrum (Fig. 1) ensure the lineal condensation of the rings A/B/C and, unequivocally, confirm the position of substituents in [B] and [C] rings [HMBC: C-6 ↔ H-5 ↔ C-9a ↔ H-6 ↔ C-5 ↔ H-4 ↔ C-3 ↔ H-11 ↔ C-4 ↔ H-5 ↔ C-4a ↔ H-4↔ C-10a ↔ H-5 ↔ C-5a].

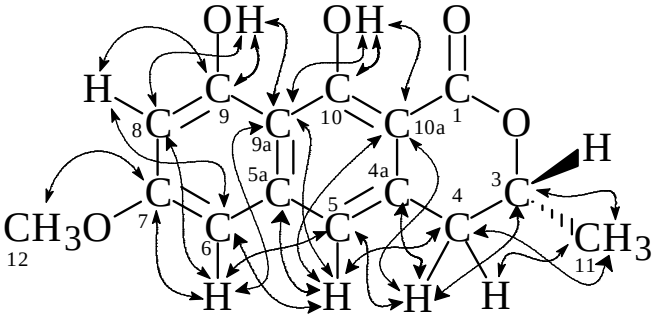
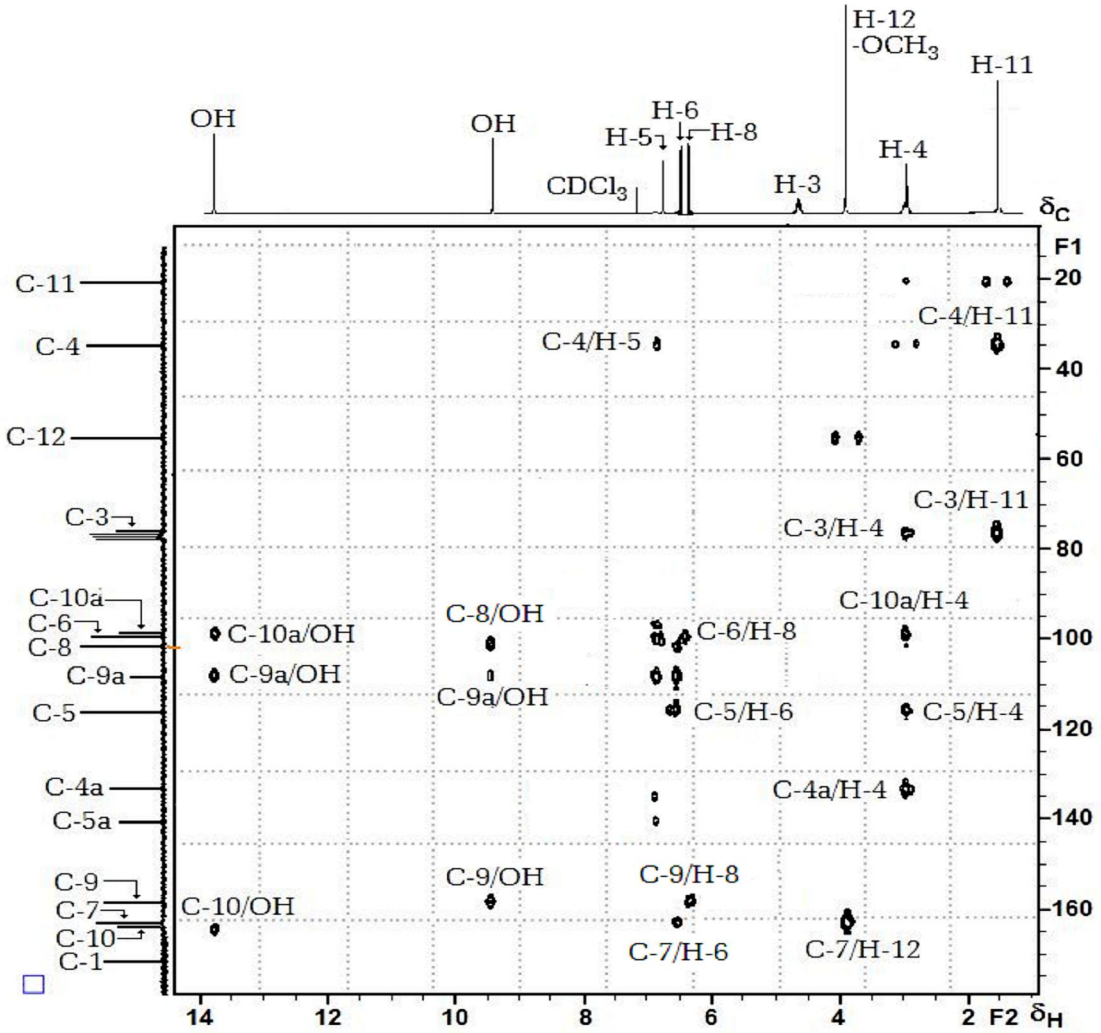


Fig. 1: HMBC Spectrum of (+)-Semivioxanthin **[3]**



The preceding analysis allows us to conclude that the structure of compound under study corresponds to 9,10-dihydroxy-7-methoxy-3-methyl-1-oxo-1*H*-naphto [2,3-*c*] pyran. This structure possesses a single chiral center (C-3) whose possible configurations (*R* o S) characterizes two enantiomeric molecules. Both molecules have been previously described as natural products: The levorotatory enantiomer, named (-)-semi-vioxanthin ($[\alpha]_D$: -10.6º), possesses configuration ñ*S*ò in C-3 and it was obtained from cultures of *Cryptosporiopsis abietina*[32], a coelomycete endophytic fungus isolated from *Chamaecyparis obtusa*; this enantiomer exhibited absicic activity against Hinoki cypress leaves and, in an antifungal test, inhibited spore germination of *Cladosporum herbarum*. The dextrorotatory isomer, (+)-semi-vioxanthin (configuration *R* in C-3) is a rare natural compound first isolated from the fungus *Penicillium citreo-viride*[28] and subsequently also found in soil[33] and marine-derived fungi[34]. Its properties as an antifungal antibiotic and as a tumor necrosis factor-α regulator have been documented[33-34]. Compound described in this study is dextrorotatory and consequently its configuration in C-3 is *R* with the secondary methyl group α-oriented; it is obvious that the same was clearly identified as **(+)-semi-vioxanthin [3]**.

Identification of (+)-semi-vioxanthin **[3]** was also confirmed by obtaining some derivatives. Thus, on acetylation with Ac_2O/Py, compound **[3]** gave two acetyl derivatives, which were characterized by their spectroscopic data (Table 1) as monoacetate **[4]** [IR, ν_{max}: 1770cm^{-1} (O=C-O-); ^{1}H NMR: substitution of O<u>H</u> singlet at δ_H 9.45, by a new 3H singlet at δ_H 2.38, *s*, O=C-C<u>H</u>$_3$ (H-2ô); ^{13}C NMR: δ_C 171.2; O=<u>C</u>-O- (C-1ô) and δ_C 21.3; O=C-<u>C</u>H$_3$ (C-2ô)] and as diacetate **[5]** [IR, ν_{max}: 1770 and 1716 cm^{-1} (O=C-O-); ^{1}H NMR: substitution of both O<u>H</u> signals by two new singlets at δ_H 2.39 and δ_H 2.46; *s*, O=C-C<u>H</u>$_3$ (H-2ô and H-2ò); ^{13}C NMR: δ_C 169.2 and δ_C 169.6; O=<u>C</u>-O- (C-1ô and C-1ò) and δ_C 21.2 and δ_C 21.6; O=C-<u>C</u>H$_3$ (C-2ô and C2ò)]. Treatment of **3** with CH_2N_2/ether gave two dimethyl ether derivatives: (+)-Semi-vioxanthin-9 methyl ether **[6]** [δ_H 3.99; *s*, -OC<u>H</u>$_3$ (H-1ô)] and (+)-semivioxanthin-10-methyl ether **[7]** [δ_H 4.12; *s*, -OC<u>H</u>$_3$ (H-1ô) and δ_C 64.4; O=C-<u>C</u>H$_3$ (C-1ô].

Vioxanthin **[8]:** yellow needles observed in TLC plates as a orange spot; m.p. = 196-198°C (descomposition); $[\alpha]_D$: + 4.6º ($CHCl_3$). Comparison of its ^{1}H NMR spectrum (Table 1) with that of (+)-semi-vioxanthin **[3]** revealed only two notable changes: The absence of singlet attributed to H-8 and the transformation of H-6 doublet in a sharp singlet [δ_H 6.70; *s*, (H-6/H-6ô)]; these changes indicate that C-8 is a quaternary carbon (=C<) and that ring A is pentasubstituted. In accordance with the foregoing data, the ^{13}C NMR spectrum (Table 2) displays, in the DEPT-90, only two peaks assignable to aromatic methines [δ_C 115.6 and δ_C 97.5; =<u>C</u>H (C-5 and C-6)], and consequently, it is also observed a new peak typical of a quaternary sp^2 carbon [δ_C 107.7; =C< (C-8/8ô)]. The detection in its EI-MS of an ion molecular peak at *m/z*: 546 [M$^+$] allows us to conclude that this compound is a symmetric dimer of (+)-semi-vioxanthin **[3]**, with a bridge C-8/C-8ô between the two monomer units; the symmetry of the structure justifies the not duplicity of the NMR signals. The above analysis led to the structure **[8]**, named in the literature as **vioxanthin**. This naphthopyranone has been previously isolated of several fungi[27-29] and lichens[30] and also it has been found in other *Paepalanthus* species[31]. Its wide range of biological activities is well documented in the literature[14,20,35,36].

Acknowledgments

The authors are grateful to CDCHTA-ULA (C-1808-12-08-A) and to Venezuelan Ministry of Popular Power for Science, Technology and Innovation (MCTI), ñScience Missionò Program (Grant Nº 2008000937), for financial support. Thank are also due to Eng. Juan Carmona Arzola, Department of Pharmacognosy and Organic Medicaments, Faculty of Pharmacy and Bioanalysis, University of Los Andes (ULA) for identification of plant material.

References

1. MJ Gomes de Andrade, AM Giulietti, A Rapini, L Paganucci de Queiroz, A de Souza Conceição, PR Machado de Almeida, C van den Berg. A comprehensive phylogenetic analysis of Eriocaulaceae: Evidence from nuclear (ITS) and plastid (*psbA-trnH* and *trnL-F*) DNA sequences. **Taxon, 59**, 379-388 (2010).
2. AM Giulietti, N Hensold, LR Parra, MJ Gomes de Andrade, C Van den Berg, RM Harley. The synonymization of *Philodice* with *Syngonanthus* (Eriocaulaceae). **Phytotaxa, 60**, 50-56 (2012).
3. T Stützel. Eriocaulaceae. In: K Kubitzki (Ed.) ñThe Families and Genera of Vascular Plants-IV: Flowering Plants Monocotyledons Alismatanae and Commelinanae (except Gramineae)ò. Springer-Verlag. Berlin-Heidelberg (GE). p. 197-207 (1998).
4. N Hensold. Morphology and systematics of *Paepalanthus* subgenus *Xeractis* (Eriocaulaceae). **System. Bot. Monogr., 23**, 1-150 (1988).
5. FDP de Andrade, L Campaner dos Santos, AL Dokkedal, W Vilegas. Acyl glucosylated flavonols from *Paepalanthus* species. **Phytochemistry, 51**, 411-415 (1999).

6. L Campaner dos Santos, M Sannomiya, S Piacente, C Pìzza, PT Sano, W Vilegas. Chemical profile of the polar extract of *Paepalanthus microphyllus* (Guill.) Kunth (Eriocaulaceae). **Braz. J. Pharm. Sci., 40**, 433-436 (2004).
7. AL Dokkedal, F Lavarda, L Campaner dos Santos, W Vilegas. Xeractinol, a new flavanonol C-glucoside from *Paepalanthus argenteus* var. *argenteus* (Bongard) Hensold (Eriocaulaceae). **J. Braz. Chem. Soc., 18**, 437-439 (2007).
8. F Pereira do Amaral, A Napolitano, M Masullo, L Campaner dos Santos, M Festa, W Vilegas, C Pizza, S Piacente. HPLC-ESIMSn profiling, isolation, structural elucidation and evaluation of the antioxidant potential of phenolics from *Paepalanthus geniculatus*. **J. Nat. Prod., 75**, 547-556 (2012).
9. FV Zanutto, PK Boldrin, EA Varanda, S Fernandes de Souza, PT Sano, W Vilegas, L Campaner dos Santos. Characterization of flavonoids and naphthopyranones in methanol extracts of *Paepalanthus chiquitensis* Herzog by HPLC-ESI-IT-MS^n and their mutagenic activity. **Molecules, 18**, 244-262 (2013).
10. RRD Moreira, GZ Martins, RCLR Pietro, DN Sato, FR Pavan, SRA Leite, W Vilegas, CQF Leite. *Paepalanthus spp*.: Antimycobacterial activity of extracts, methoxylated flavonoids and naphthopyranone fractions. **Braz. J. Pharmacogn., 23**, 268-272 (2013).
11. W Vilegas, NF Roque, A Salatino, AM. Giesbrecht, S Davino. Isocoumarin from *Paepalanthus bromelioides*. **Phytochemistry, 29**, 2299-2301 (1990).
12. EA Varanda, MS Gonçalves Raddi, F de Luz Dias, MCP Araújo, S Cardoso Alvares Gibran, CS. Takahashi, W Vilegas. Mutagenic and cytotoxic activity of an isocoumarin (paepalantine) isolated from *Paepalanthus vellozioides*. **Teratogen. Carcinogen. Mutagen., 17**, 85-95 (1997).
13. S Piacente, L Campaner dos Santos, N Mahmood, A Zampelli, C Pizza, W Vilegas. Naphthopyranone glycosides from *Paepalanthus microphyllus*. **J. Nat. Prod., 64**, 680-682 (2001).
14. AF Cálgaro-Helena, KF Devienne, T Rodrigues, DJ Dorta, MS Gonçalves Raddi , W Vilegas, SA Uyemura, AC Santos, C Curti. Effects of isocoumarins isolated from *Paepalanthus bromelioides* on mitochondria: Uncoupling, and induction/ inhibition of mitochondrial permeability transition. **Chem.-Biol. Inter., 161**, 155-164(2006).
15. RR Kitagawa, MS Gonçalves Raddi, L Campaner dos Santos, W Vilegas. A new cytotoxic naphthoquinone from *Paepalanthus latipes*. **Chem. Phar. Bull., 52**, 1487-1488 (2004)
16. RR Kitagawa, W Vilegas, IC Zeppone, MS Gonçalves Raddi. Antitumor and immunomodulatory effects of the naphthoquinone 5-methoxy-3,4- dehydroxanthomegnin**. Braz. J. Pharmacogn., 21**, 1084-1088 (2011).
17. RR Kitagawa, LM da Fonseca, VF Ximenes, NM Khalil, W Vilegas, MS Gonçalves Raddi. Ascorbic acid potentiates the cytotoxicity of the naphthoquinone 5-methoxy-3,4-dehydro xanthomegnin. **Phytochemistry, 69**, 2205-2208 (2008).
18. RR Kitagawa, C Bonacorsi, LM da Fonseca, W Vilegas, MS Gonçalves Raddi. Anti-*Helicobacter pylori* activity and oxidative burst inhibition by the naphthoquinone 5-methoxy-3,4-dehydroxanthomegnin from *Paepalanthus latipes*. **Braz. J. Pharmacogn., 22**, 53-59 (2012).
19. RR Kitagawa, MS Gonçalves Raddi, NM Khalil, W Vilegas, LM da Fonseca. Effect of the isocoumarin paepalantine on the luminol and lucigenin amplified chemiluminescence of rat neutrophils. **Biol. Pharm. Bull., 26**, 905-908 (2003).
20. KF Devienne, AF Cálgaro-Helena, DJ Dorta, IMR Prado, MS Gonçalves Raddi, W Vilegas, SA Uyemura, AC Santos, C Curti. Antioxidant activity of isocoumarins isolated from *Paepalanthus bromelioides* on mitochondria. **Phytochemistry, 68**, 1075-1080 (2007).
21. LC Di Saso, D Camuesco, A Nieto, W Vilegas, A Zarzuelo, J Galvez. Intestinal anti-inflammatory activity of paepalantine, an isocoumarin isolated from the capitula of *Paepalanthus bromelioides*, in the trinitrobenzenesulphonic acid model of rat colitis. **Planta Medica, 70**, 315-320 (2004).
22. KF Devienne, MS Gonçalves Raddi. Screening for antimicrobial activity of natural products using a microplate photometer. **Braz. J. Microbiol., 33**, 166-168 (2002).
23. KF Devienne, MS Gonçalves Raddi, R Gomes Coelho, W Vilegas. Structure-antimicrobial activity of some natural isocoumarins and their analogues. **Phytomedicine, 12**, 378-381(2005).
24. KF Devienne, MS Gonçalves Raddi, EA Varanda, W Vilegas. *In vitro* cytotoxicity of some natural and semi-synthetic isocoumarins from *Paepalanthus bromelioides*. **Z. Naturforsch., 57c**, 85-88 (2002).
25. DC Tavares, EA Varanda, FDP Andrade, W Vilegas, CS Takahashi. Evaluation of the genotoxic potential of the isocoumarin paepalantine in *in vivo* and *in vitro* mammalian systems. **J. Ethnopharmacol., 68**, 115-120 (1999).
26. EA Varanda, KF Devienne, MS Gonçalves Raddi, EM Furuya, W Vilegas. Mutagenicity of paepalantine dimer and glycoside derivatives from *Paepalanthus bromelioides*. **Toxicology in Vitro, 18**, 109-114 (2004).
27. AS Ng, G Just, F Blank. Metabolites of pathogenic fungi. VII. Structure and stereochemistry of xanthomegnin, vioxanthin and viopurpurin, pigments from *Trichophyton violaceum*. **Can. J. Chem., 47**, 1223-1227 (1969).
28. A Zeeck, P Russ, H Laatsch, W Loeffler, H Wehrle, H Zaehner, H Holst. Isolation of the antibiotic semi-vioxanthin from *Penicillium citreo-viride* and synthesis of xanthomegnin. **Chem. Ber., 112**, 957-978 (1979).
29. KA Scudamore, JH Clarke, MT Hetmanski. Isolation of *Penicillium* strains producing ochratoxin A, citrinin, xanthomegnin, viomellein and vioxanthin from stored cereal grains. **Lett. Appl. Microbiol.,17**, 82-87 (1993).
30. JA Elix. Vioxanthin from a lichen source. **Austr. Liquenol., 55**, 14-15 (2004).

31. I Provost, M Garcia. First report on the isolation of vioxanthin from a plant source: Occurrence in *Paepalanthus* species. **Planta Medica, 56**, 647 (1990).

32. H Yada, H Sato, H Toshima, M Deura, A Ichihara. (-)-Semivioxanthin, a new abscisic active compound against Hinoki cypress leaves isolated from *Cryptosporiopsis abietina*. **Biosci. Biotechnol. Biochem., 65**, 484-486 (2001).

33. J Yang, XL Qi, G Shao, XS Yao, S Kitanaka. Isolation and structure elucidation of an antifungal antibiotic A73 produced by a soil fungus strain N° 131. **Chem. Res. Chin. Univ., 15**, 83-86 (1999).

34. XY Yang, SX Cai, WJ Zhang, XL Tang, HY Shin, JY Lee, QQ Gu, H Park. Semi-vioxanthin isolated from marine-derived fungus regulates tumor necrosis factor-α, cluster of differentiation (CD) 80, CD86, and major histocompatibility complex class II expression in RAW264.7 cells via nuclear factor-κ B and mitogen-activated protein kinase signaling pathways. **Biol. Pharm. Bull., 31**, 2228-2233 (2008).

35. M Saito, I Matsuura, H Okazaki, Tf-26Vx, An antibiotic produced by a thermophilic fungus. **J. Antibiot., 32**, 1210-1212 (1979).

36. TSP Teixeira, RF Freitas, O Abrahão (Jr.), K F Devienne, L R de Souza, SI Blaber, M Blaber, MY Kondo, MA Juliano, L Juliano, L Puzer. Biological evaluation and docking studies of natural isocoumarins as inhibitors for human kallikrein 5 and 7. **BioOrg. Med. Chem. Lett., 21**, 6112-6115 (2011).