Two Xanthones from *Polygala paniculata* and Confirmation of the 1-Hydroxy-2,3,5-trimethoxy-xanthone at Trace Level by HRGC-MS

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Polygala paniculata L. yielded the xanthones 1-hydroxy-5-methoxy-2,3-methylenedioxy-xanthone (**1**) and 1,5-dihydroxy-2,3-dimethoxyxanthone (**2**), together with coumarin murragatin and flavonol rutin. Their structures were established by chemical and spectroscopic methods (EIMS, IR, ^{1}H and ^{13}C NMR, NOE difference spectroscopy). By posterior analysis of an apolar crude extract using high resolution gas chromatography coupled to mass spectrometry (HRGC-MS) it was possible to characterize two sterol (spinasterol and Δ^{25}-spinasterol) and the minor 1-hydroxy-2,3,5-trimethoxyxanthone (**3**). Thus, the xanthone **3** was confirmed through of co-injection HRGC-MS of the respective extract with a certified standard obtained by methylation of **2** with diazomethane.

Key words: Polygala paniculata, Xanthones, HRGC-MS

Introduction

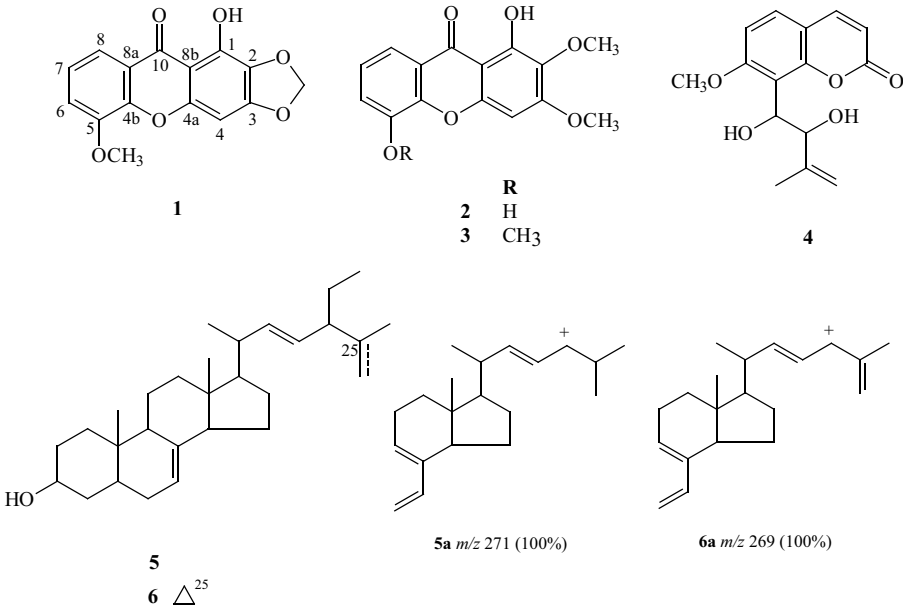
Xanthones are biologically active natural compounds widely distributed in plants of the Polygalaceae family (Peres and Nagem, 1997). Their chemical structures and biological activities have already been described (Li *et al.*, 1999; Ikeya *et al.*, 1991; Bashir *et al.*, 1992a). Previous studies on the apolar extracts of *Polygala paniculata* L., possessing both molluscicidal and antifungal properties, led to the isolation of one pyranocoumarin diester (Hamburger *et al.*, 1984) and four prenylated coumarins (Hamburger *et al.*, 1985), but there has been no report on the presence of xanthones so far.

The analysis of crude extract or prefractioned apolar or medium-polar extracts by high resolution gas chromatography (HRGC) and HRGC coupled to mass spectrometry (HRGC-MS) has been an extremely valuable tool for the quick screening of several natural products (Branco *et al.*, 2001; Patitucci *et al.*, 1995). In continuation of our search on Brazilian *Polygala* species (Pizzolatti *et al.*, 2000), we report the isolation and structural elucidation of 1-hydroxy-5-methoxy-2,3-methylenedioxyxanthone (**1**) and 1,5-dihydroxy-2,3-dimethoxyxanthone (**2**). The coumarin murrangatin and flavonol rutin were also characterized. Their structures were established by chemical and spectroscopic methods (EIMS, IR, ^{1}H and ^{13}C NMR, NOE difference spectroscopy). In addition, the minor 1-hydroxy-2,3,5-trimethoxyxanthone (**3**) was identified through of co-injection HRGC-MS of the respective extract with a certified standard obtained by methylation of **2** with diazomethane.

Material and Methods

General

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were measured at 300 and 75 MHz respectively, on a Varian Gemini 300 spectrometer. IR spectra were recorded using a FT Perkin-Elmer 16 PC with KBr pellets. HRGC analyses were recorded on a HP 5790 A gas chromatography. Mass spectra employed a HP 5973 GC-MS at 70 eV. Column chromatography was performed using silica gel (0.063–0.2 mm). TLC, used to analyze fractions, employed layers of silica gel (Kieselgel 60; Merck, Darmstadt, Germany), spots were visualized by UV at 254 and 360 nm.



1

R
2 H
3 CH_3

4

5

6 $\triangle^{25}$

5a *m/z* 271 (100%)

6a *m/z* 269 (100%)

HRGC and HRGC-MS

A glass capillary column (11 m × 0.25 mm × 0.25 μm) coated with SE-54 was used, hydrogen as carrier gas at a flow rate of 2 ml/min; injector port was set at 280 °C and flame ionization detector (FID) at 290 °C. The temperature program for the analysis of the mixture ranged from 100 to 280 °C (10 °C/min). The data were collected on a HP 3396-II integrator. HRGC-MS analyses were performed using electron impact ionization (70 eV). MS scan range was 40 to 700 Da. The GC-MS interface was held at 150 °C and ion source temperature at 200 °C. Column temperature program and injection mode were as for chromatographic analyses.

Plant material

Polygala paniculata (Polygalaceae) was collected in Massiambú (Santa Catarina State, Brazil) and identified by Professor Dr. Olavo de Araújo Guimarães. A voucher specimen (UPCNB 26027) was deposited in the Herbarium of the Botany Department of the Universidade Federal do Paraná, Curitiba, PR.

Extraction and isolation

Air-dried whole plant (277 g) was cut in small pieces and extracted at room temperature with ethanol-water (4:1 v/v). The ethanol-water residue (28 g) was filtered over 60 g of silica gel with hexane, chloroform and butanol, successively. After concentration of the solvents, under vacuum, the respective extracts were obtained. An aliquot of this hexane extract was analyzed by HRGC and HRGC-MS (Fig. 1).

The chloroform extract (3.5 g) was chromatographed on a silica gel column (21.2 g) using mixtures of hexane/ethyl acetate and ethyl acetate/methanol of increasing polarity. A total of 36 fractions of *ca.* 125 ml each were collected. Fractions 4–10 were combined on the basis of TLC analysis in toluene: ethyl acetate (3:1 v/v) to give **1** (20 mg) by crystallization with acetone. From fraction 32, eluted with ethyl acetate:methanol (4:6 v/v), murrangatin (**4**) precipitated as an amorphous powder (40 mg). Fractions 21–25 were combined (192 mg) on the basis of TLC analysis in toluene:ethyl acetate (1:1 v/v) and re-chromatographed on a silica gel column using mixtures of ethyl acetate and methanol of increasing polarity. Fractions 6 and 7 were combined to give **2** (23 mg). The BuOH extract (7.6 g) was chromatographed on a silica gel column (90 g) in the above manner. Fractions 18–27 afforded rutin (140 mg) as a yellow amorphous powder, with ^{13}C NMR data identical to literature (Markham and Ternai, 1976).

1-Hydroxy-5-methoxy-2,3-methylenedioxyxanthone (**1**)

Yellow needles; melting point 205–207 °C; IR ν_{max}^{KBr} cm^{-1}: 3422 (OH), 1680 (C=O), 1582, 1496 (aromatic ring); EIMS 70 eV *m/z* (relative intensity): 286 ([M^+] $C_{15}H_{10}O_6$, 100%), 271([$M^{+\cdot}$–Me]$^+$, 10), 258 ([M^+ – CO]$^+$, 70), 229 ([$M^{+\cdot}$–CO-CHO]$^+$, 68); ^{1}H NMR ($CDCl_3$), δ (ppm): 4.03 (OMe, s), 6.12 (OCH_2O, s), 6.66 (H-4, s), 7.23 (H-6, dd, *J* = 8.0 and 1.6 Hz), 7.32 (H-7, t, *J* = 8.0 Hz), 7.89 (H-8, dd, *J* = 8.0 and 1.6 Hz), 12.74 (OH, s). ^{13}C NMR data in Table I. N. O. E. between OMe and H-6.

1,5-dihydroxy-2,3-dimethoxyxanthone (**2**)

Yellow needles; melting point 230–232 °C [lit. 244 °C (Bashir *et al.*, 1992b)]; IR ν_{max}^{KBr} cm^{-1}: 3436 (OH), 1654 (C=O), 1580, 1498 (aromatic ring). EIMS 70 eV *m/z* (relative intensity): 288 ([$M^{+\cdot}$, $C_{15}H_{12}O_6$, 90), 273 ([$M^{+\cdot}$-Me]$^+$, 100), 245 ([$M^{+\cdot}$-Me-CO]$^+$ and/or [M^{+}-CO-Me]$^+$, 70), 229 ([$M^{+\cdot}$-CO-CHO]$^+$, 80). ^{1}H NMR ($CDCl_3$), δ (ppm): 6.57 (H-4, s), 7.28 (H-7, t, *J* = 8.0 Hz), 7.34 (H-6, dd, *J* = 8.0 and 2.0 Hz), 7.80 (H-8, dd, *J* = 8.0 and 2.0 Hz). ^{1}H NMR (pyridine-d_5), δ (ppm): 3.73 (MeO-3, s), 3.98 (MeO-2, s), 5.05 (OH-5, s), 6.32 (H-4, s), 7.30 (H-7, t, *J* = 8.0 Hz), 7.55 (H-6, dd, *J* = 8.0 and 2 Hz), 7.95 (H-8, dd, *J* = 8.0 and 2.0 Hz), 13.43 (OH-1, s). ^{13}C NMR data in Table I. N. O. E. between MeO-3 and H-4, and OH-5 and H-6.

Methylation of **2**

The xanthone **2** was treated with CH_2N_2 in the manner usual to yield 1-hydroxy-2,3,5-trimethoxyxanthone (**3**). GC-EIMS 70 eV *m/z* (relative intensity): 302 ([M^+], $C_{16}H_{14}O_6$, 92), 287 ([$M^{+\cdot}$-Me]$^+$, 100), 259 ([$M^{+\cdot}$-Me-CO]$^+$ and/or [$M^{+\cdot}$-CO-Me]$^+$, 64), 216 (7), 122 (5).

Murrangatin (**4**)

Colorless needles. melting point 95 °C [lit. 116 °C (Ito *et al.*, 1990)]; $[\alpha]_D$ – 10° (*c* 0.29 in $CHCl_3$) IR ν_{max}^{KBr} cm^{-1}: 3386 (OH), 1722 (C=O), 1604, 1566, 1496; EIMS 70 eV *m/z* (relative intensity): 276 ([M^+], absent), 205 (100), 175 (25). ^{1}H NMR (acetone-d_6), δ (ppm): 1.69 (CH_3-5′, s), 3.05 (OH-1′ e OH-2′, s), 3.98 (MeO-7, s), 4.53 e 4.58 (CH_2-4′), 4.73 (H-2′, d, *J* = 8.0 Hz), 5.27 (H-1′, d, *J* = 8.0 Hz), 6.21 (H-3, d, *J* = 10.0 Hz), 7.05 (H-6, d, *J* = 8.0 Hz), 7.57 (H-5, d, *J* = 8.0 Hz), 7.88 (H-4, d, *J* = 10.0 Hz). ^{13}C NMR data identical to Kinoshita *et al.* (1996).

Results and Discussion

Fractionation of the chloroform extract from *P. paniculata* on silica gel followed by extensive TLC analysis afforded compounds **1**, **2** and **4**. Murrangatin (**4**), previously described by Hamburger *et al.* (1985), was identified by comparison with NMR data. From the butanol extract, rutin was isolated and identified by NMR data (Markham *et al.*, 1976).

The xanthone **1** presented EI-mass spectra with intense molecular ion (M^+, *m/z* 286), characteristic of the xanthone nucleus. The ^{1}H NMR spectroscopy showed an isolated aromatic hydrogen singlet at δ_H 6.66, attributed to H-4, one chelated hydroxyl (δ_H 12.74), one methylenedioxy (δ_H 6.12) and one methoxyl (δ_H 4.03) groups and a three-proton spin system [δ_H 7.23 (dd), δ_H 7.32 (t) and δ_H 7.89 (dd)], suggesting a C-5 substituted ring-B. In NOE difference experiments, mutual enhancement of the signals at δ_H 7.23 and δ_H 4.03 reinforced the methoxyl group at C-5. The ^{13}C NMR data is shown in the Table I.

Table I. ^{13}C NMR (75 MHz) data for compounds **1** and **2** ($CDCl_3$, δ in ppm).

C	**1** ($CDCl_3$)	**2** (Pyridine-d_5)
1	143.0	153.3*
2	128.4	132.2
3	153.4	160.7
4	89.7	90.9
4a	155.2	154.5*
4b	148.3*	145.2
5	146.3*	147.8
6	115.4	121.2
7	123.7	124.0
8	116.5	115.1
8a	120.9	[a]
8b	103.8	104.3
9	181.1	181.7
OCH_2O-2	102.7	–
CH_3O-2	–	60.4
CH_3O-3	–	56.1
CH_3O-5	56.4	–

[a] Not observed.
* Assignments interchangeable.

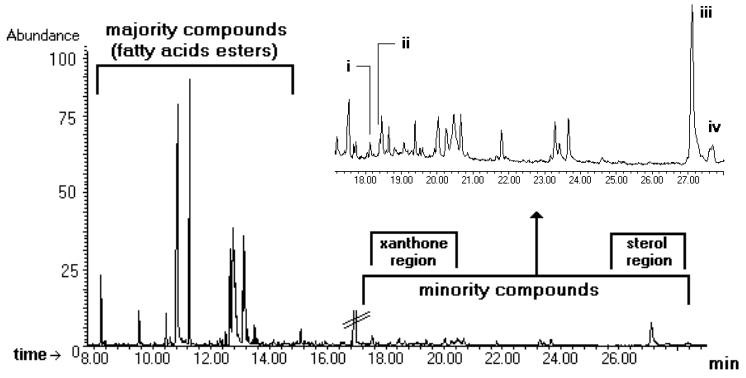


Fig. 1. Total ion chromatograms (TIC) of the apolar crude extract from *Polygala paniculata*. The inserts shows the sub-region of elution of the xanthones and sterol. Majority compounds (t_R min): palmitic acid (10.8), ethyl palmitic acid (11.2), linoleic acid (12.7), ethyl linoleic acid (13.1), ethyl α-linolenic acid (13.2), ethyl oleic acid (13.3), ethyl stearic acid (13.5), ethyl behenic acid (17.5) and ethyl lignoceric acid (19.4) (Dewick, 1997).

The molecular ion at *m/z* 288 ($C_{15}H_{12}O_6$) observed in the EI-MS and NMR data indicated a dihydroxydimethoxylated xanthone for **2**. The ^1H NMR (pyridine-d_5) spectrum showed two methoxyl groups at δ_H 3.73 and δ_H 3.92, two hydroxyl groups at δ_H 13.43 (chelated, OH-1) and δ_H 5.05 and an isolated hydrogen singlet at δ_H 6.32. The signals at δ_H 7.55 (dd), 7.30 (t) and 7.95 (dd) have been attributed to H-6, H-7 and H-8, respectively. NOE difference showed mutual enhancement between the hydroxyl group at δ 5.05 and the aromatic hydrogen at δ_H 7.55 (H-6). This fact confirmed the position of the hydroxyl group at C-5. The ^1H NMR in $CDCl_3$ showed the hydrogen singlet at δ_H 6.57 which was attributed to H-4 in accordance with value attributed to 1,2,3-trisubstituted xanthones (Fujita *et al.*, 1992). The signals at δ_H 7.34, 7.28 and 7.80 have been attributed the hydrogen H-6, H-7 and H-8 (Bashir *et al.*, 1992b). The ^{13}C NMR spectrum data is shown in the Table I. Thus, the xanthones **1** and **2** have been identified as 1-hydroxy-5-methoxy-2,3-methylenedioxyxanthone and 1,5-dihydroxy-2,3-dimethoxy xanthone, respectively.

Crude extract analysis by HRGC-MS and confirmation of **3**

HRGC-MS analysis of the hexane crude extract of the *P. paniculata* is shown in Fig. 1. The respective total ion chromatograms (TIC) showed two distinct regions of elution: majority compounds [retention time (R_t) values 10–17 min], characterized as common for naturally occurring fatty acids esters, and minority compounds (R_t values 18–28 min). The investigation by mass fragmentation of the second region and comparison with compounds previously isolated from *Polygala* species (Pinheiro *et al.*, 1998), others two sub-regions of elution can to be postulated to contend: xanthones (R_t values 18–19 min) and sterols (R_t values 27–28 min) (Fig. 1). The peak **i** was recognized as compound **1** ($M^{+\bullet}$, 286, 100%) and a hydroxy-

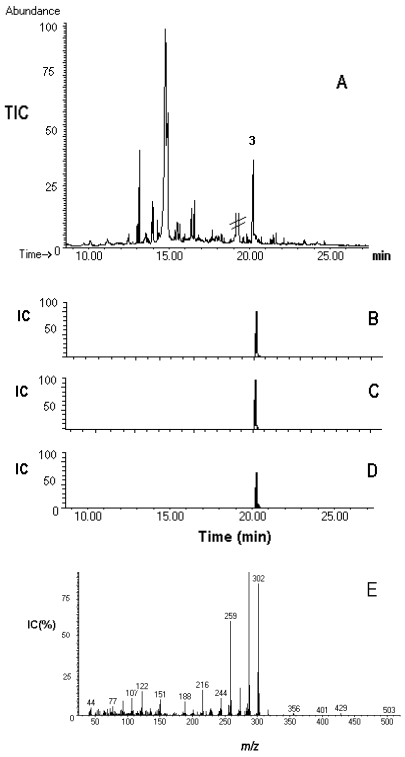


Fig. 2. Total ion chromatogram (TIC) and mass fragmentograms of co-injection on HRGC-MS between hexane extract of *Polygala paniculata* and standard. A) Total ion chromatogram of co-injection; B) Mass fragmentogram *m/z* 302; C) Mass fragmentogram *m/z* 287; D) Mass fragmentogram *m/z* 259; E) Mass spectrum of 1-hydroxy-2,3,5-trimethoxy-xanthone (**3**).

trimethoxylated xanthone was suggested for peak **ii** ($[M]^{+\bullet}$, 302, 84%). Previous isolation of xanthone **2** and fragments at *m/z* 287 ($[M]^{+\bullet}-CH_3$, 100%) and 259 ($[M]^{+\bullet}-C{=}O-CH_3$, 77%) in the EI-mass spectrum of the peak **ii** resulted in the suggestion of 1-hydroxy-2,3,5-trimethoxyxanthone (**3**) as the probable structure. On other hand, the peaks **iii** ($[M]^{+\bullet}$ 412) and **iv** ($[M]^{+\bullet}$ 410) have been characterized as spinasterol (**5**) and Δ^{25}-spinasterol (**6**) due to the presence of intense fragments at *m/z* 271 (100%, **5a**) and 269 (100%, **6a**) in the respective mass spectra. These ionic fragments were attributed to a retro-Diels-Alder fragmentation in ring-B.

The xanthone **2** was submitted to methylation with diazomethane and yielded 1-hydroxy-2,3,5-trimethoxyxanthone (**3**) as the only product. This derivative compound showed identical fragmentation of peak **ii** of the crude extract (Fig. 1) and was used a as certified standard. Thus, the xanthone **3** was confirmed in *P. paniculata* through co-injection on HRGC-MS of the crude extract with certified standard (Fig. 2). Sterol **5** also was confirmed in the above manner with an authentic standard obtained in our laboratories.

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