PERGAMON

Phytochemistry 55 (2000) 819–822

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Styryl- and dihydrostyryl-2-pyrones derivatives from *Polygala sabulosa*

Moacir Geraldo Pizzolatti [a,*], Cristiany Luciano [a], Franco Delle Monache [b]

[a] *Department of Chemistry, Universidade Federal de Santa Catarina, 88040-900 Florianopolis, SC, Brazil*
[b] *Centro Chimica Recettori, C.N.R., Largo Francesco Vito 1, 00168 Rome, Italy*

Received 3 January 2000; received in revised form 17 April 2000

Dedicated to Professor Otto Richard Gottlieb on the occasion of his 80th birthday

Abstract

Two dihydrostyrylpyrones and a styrylpyrone were isolated from *Polygala sabulosa*, together with five known styrylpyrones. Their structures were established on the basis of spectral evidence as 4-methoxy-6-(11,12-methylenedioxy-14-methoxydihydrostyryl)-2-pyrone, 4-methoxy-6-(11,12-methylenedioxy-10,14-dimethoxydihydrostyryl)-2-pyrone, and 4-methoxy-6-(11,12-methylenedioxy-14-methoxy-styryl)-2-pyrone.

Keywords: *Polygala sabulosa*; Polygalaceae; Styrylpyrones; Dihydrostyrylpyrones; 6-methoxy-7-prenyloxycoumarin; Protohypericin

1. Introduction

In a previous paper, we reported the occurrence in *Polygala cyparissias* (Polygalaceae) of xanthones with antinociceptive properties, in addition to methyl salicylate in high concentration, justifying the use of this plant as a topical anaesthetic in folk medicine (Pinheiro et al., 1998; Campos et al., 1997). *Polygala sabulosa*, commonly called "timutu-pinheirinho", is used for the same purpose and is morphologically similar, but has a different habitat, growing in the Southern Meridional Highlands of Brazil (Wurdack and Smith, 1971; Schultz, 1990). The present paper deals with the isolation and structural determination of one styrylpyrones and two dihydro-styrylpyrones from this species.

2. Results and discussion

The EtOAc-soluble fraction from the aqueous EtOH (1:4) extract of the whole herb was subjected to silica gel chromatography, followed by repeated flash chromatography or crystallization to afford the dihydrostyrylpyrones **1**–**3**, the styrylpyrones **4**–**6**, 6-methoxy-7-prenyloxycoumarin (**7**) and protohypericin (**8**).

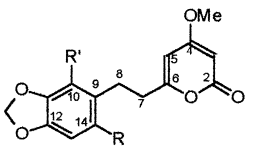
Of these, dihydro-styrylpyrone **1** (Schlemmer et al., 1972), styrylpyrones **5**,**6** (Rossi et al., 1997; Barbosa Filho et al., 1987), compound **7** (Jackson et al., 1990; Cardona et al., 1992) and protohypericin **8** (Banks et al., 1976) are known compounds, and were identified by comparison of their NMR spectral data with literature values. In particular, coumarin **7** was distinguished from the isomeric 6-prenyloxy-7-methoxy coumarin by the $\Delta\delta$ of the ^{13}C NMR signals for C-5 and C-8 ($\Delta\delta$ 9.8 and 6.8, respectively) (Cardona et al., 1992). The ^{13}C NMR spectral data for dihydro-styrylpyrone **1** and protohypericin **8** are reported here for the first time.

On the basis of MS, elemental analysis and NMR spectroscopic evidence, the molecular formulas $C_{15}H_{14}O_5$, $C_{16}H_{16}O_6$, $C_{17}H_{18}O_7$ and $C_{16}H_{14}O_6$ were attributed to compounds **1**, **2**, **3** and **4**, respectively. The mass spectra of compounds **1**–**3** exhibited base peaks at 135, 165 and 195 a.m.u. respectively, attributed to the tropylium ion arising from the aromatic ring. The ^1H and ^{13}C NMR spectra data for **1**–**3** are reported in Tables 1 and 2. In the ^1H NMR spectrum, each compound showed doublets ($J = 2.2$ Hz) at δ 5.41 and δ 5.72 which are typical of di-substituted 2-pyrones, and two triplets ($J = 7.6$ Hz) between δ 2.90 and δ 2.58, which further characterized them as dihydrostyrylpyrone derivatives. With respect to the aromatic ring, the first compound exhibited signals for a methylenedioxy substituent and three protons assignable to a 1,3,4-substituted aromatic

* Corresponding author.
E-mail address: moacir@qmc.ufsc.br (M. G. Pizzolatti).

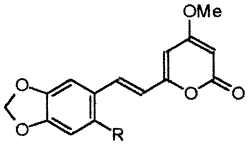
0031-9422/00/$ - see front matter
PII: S0031-9422(00)00301-0

ring, thus confirming the known structure **1** (Schlemmer et al., 1972). Structure **2** was attributed to the second compound, whose ^{1}H NMR spectrum showed an additional OMe signal and whose aromatic protons appeared as two singlets. This structure was supported by data obtained from selective INEPT experiments: the irradiation of H-10 (δ 6.60) showed correlations to C-11 (^2J), C-12 (^3J) and C-14 (^3J), the irradiation of H-13 (δ 6.50) gave correlations to C-12 and C-14 (^2J) and C-9 and C-11 (^3J). In turn, the irradiation at δ 5.72 (H-5) showed correlations to C-4 and C-6 (^2J) and C-2 and C-3 (^3J) and irradiation at δ 2.66 (2H-7) gave correlations to C-6 (^2J) and C-9 (^3J). The third compound was assigned the structure **3**, since it showed ^{1}H signals for a methylenedioxy, two OMe groups and a single aromatic proton.



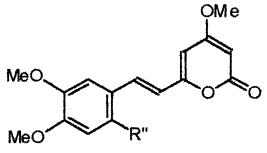
R R'

1 H H
2 OMe H
3 OMe OMe

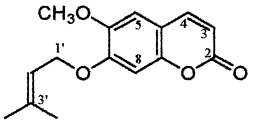


R

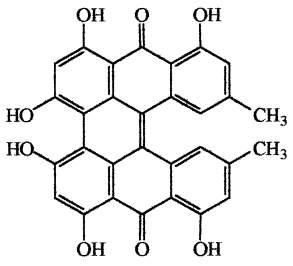
4 OMe
5 H



6



7



8

Some possible locations of the substituents on the aromatic ring were ruled out from a comparison of the ^{13}C NMR spectroscopic data for the C-8 methylenes in compounds **1**–**3** (Table 2), which were found at δ 20.9 for **3**, at δ 27.2 for **2**, and at δ 32.6 for **1**. This required the presence on the adjacent aromatic ring of two, one and no OMe groups, respectively. In accordance with the presence of the doublets (J = 16.0 Hz) at δ 7.76 and δ 6.45 (Table 1), compound **4** is a styrylpyrone derivative, like **5** and **6**. The substitution on the aromatic ring as depicted in **4** followed from comparison of NMR spectral data with those of compound **2**, and was further supported by selective INEPT experiments. In these experiments, H-10 showed a ^3J correlation with C-14 and C-12, and a ^2J correlation with C-11; in turn, selective irradiation at δ 7.76 (H-8) and at δ 6.53 (H-13) gave correlations to C-14 and C-12, respectively. This is the first report of the occurrence of styrylpyrone derivatives among members of the Polygalaceae, which are generally characterized by the accumulation of xanthones.

Table 1
^{1}H NMR spectral data ($CDCl_3$) for compounds **1–4**

H	1	2	3	4
3	5.41 *d* (2.2)[a]	5.41 *d* (2.2)	5.41 *d* (2.2)	5.46 *d* (2.2)
5	5.72 *d* (2.2)	5.72 *d* (2.2)	5.72 *d* (2.2)	5.89 *d* (2.2)
7	2.71 *t* (7.6)	2.66 *t* (7.6)	2.58 *t* (7.6)	6.45 *d* (16.1)
8	2.90 *t* (7.6)	2.86 *t* (7.6)	2.90 *t* (7.6)	7.76 *d* (16.1)
10	6.83 *d* (2.0)	6.60 *s*		6.98 *s*
13	6.75 *d* (8.0)	6.50 *s*	6.22 *s*	6.53 *s*
14 6	68 *dd* (8.0; 2.0)			
4-Ome	3.85 *s*	3.78 *s*	3.78 *s*	3.83 *s*
10-Ome			3.96 *s*	
14-Ome		3.75 *s*	3.71 *s*	3.84 *s*
OCH_2O	5.95 *s*	5.89 *s*	5.86 *s*	5.96 *s*

[a] J (Hz) in parentheses.

Table 2
^{13}C NMR spectral data ($CDCl_3$) for compounds **1–5**

C	1	2	3	4	5
2	171.2	171.2	171.3	171.3	171.2
3	87.7	87.5	87.4	88.1	88.5
4	164.9	165.8	165.8	164.4	164.1
5	100.3	99.9	99.6	100.2	100.7
6	164.2	165.1	165.3	159.6	158.9
7	35.7	34.1	33.6	116.6	116.8
8	32.6	27.2	20.9	130.7	135.5
9	133.6	120.2	112.0	116.8	129.7
10	108.6	109.7	141.9	105.8	108.7
11	147.7	146.6	130.1	149.8	148.9
12	146.1	140.7	147.7	141.6	148.4
13	108.3	94.5	88.5	94.5	105.9
14	121.2	152.3	152.9	154.4	123.5
4-OMe	55.8	55.7	55.7	55.8	55.9
10-OMe			59.5		
14-OMe		56.2	56.3	56.3	
OCH_2O	100.9	100.9	100.6	101.6	101.5

3. Experimental

3.1. General experimental procedures

Mps. are uncorr. IR spectra were recorded with Perkin–Elmer FT 16PC Spectrometer (KBr pellets). ^{1}H and ^{13}C NMR spectra were obtained using a Varian Gemini 300 or a Bruker AC-200 F spectrometer with TMS as the internal standard. GC and MS were recorded with a Shimadzu CG–MS-QP-2000 A spectrometer in the

electron impact mode (EIMS) at 70 eV. Elemental analysis was performed on a Perkin–Elmer 2400 Elemental Analyzer.

3.2. Plant material

Polygala sabulosa A. W. Bennett was collected in Rancho Queimado (Santa Catarina State, Brazil), in November 1997 and identified by Prof. Dr Olavo de Araujo Guimarães. A voucher specimen was deposited at the Herbarium of the Botany Department, Universidade Federal do Paraná, Curitiba, PR, under the number 19640.

3.3. Extraction and isolation

Air-dried and powdered herb (623 g) was extracted with EtOH–water (4:1) at room temperature. The crude extract (169 g) was applied to silica gel (300 g) and eluted successively with hexane, CH_2Cl_2, EtOAc and EtOH. After solvent evaporation of each extract, the hexane, CH_2Cl_2, EtOAc and EtOH extracts were individually obtained.

The CH_2Cl_2 extract (6.2 g) was applied to a silica gel column eluted with increasing amounts of EtOAc in hexane. The combined frs. 28–40 were further fractionated by CC to yield the coumarin **7** (480 mg).

The EtOAc extract (13.9 g) was subjected to silica gel chromotography using mixtures of hexane–EtOAc. The combined frs. 46–48 were submitted to flash chromatography (silica gel; *n*-hexane–EtOAc, 9:1), yielding the dihydrostyrylpyrones **1** (26 mg), **2** (58 mg) and **3** (43 mg), and the styrylpyrone **5** (51 mg). Flash chromatography of frs. 49–56 afforded three subfractions, two of which contained **5** (35 mg) and protohypericin **8** (8 mg). By crystallization (acetone), the third subfraction from frs. 49–56 gave the styrylpyrone **4** (50 mg). Finally, frs. 63–69 yielded the styryl-2-pyrone **6** (78 mg) by crystallization from acetone.

3.4. 4-Methoxy-6-(11,12-methylenedioxydihydrostyryl)-2-pyrone (*1*)

Mp 138–140°C (Me_2CO); IR ν_{max} cm^{-1}: 2902, 1724, 1640, 1618, 1552; ^1H NMR spectral data (200 MHz): Table 1; ^{13}C NMR spectral data (50 MHz): Table 2; EIMS (probe) 70 eV, *m*/*z* (rel. int.): 274 [M^+] (5.5), 135 (100).

3.5. 4-Methoxy-6-(11,12-methylenedioxy-14-methoxydihydrostyryl)-2- pyrone (*2*)

Mp 149–151°C (Me_2CO); IR ν_{max} cm^{-1}: 2902, 2850, 1726, 1648, 1568, 1510. ^1H NMR spectral data (300 MHz): Table 1; ^{13}C NMR spectral data (75 MHz): Table 2; EIMS (probe) 70 eV, *m*/*z* (rel. int.): 304 [M^+] (10), 165 (100), 135 (12), *Anal.* calcd. For $C_{16}H_{16}O_6$: C 63.15, H 5.30 found C 63.01, H 5.35.

3.6. 4-Methoxy-6(11,12-methylenedioxy-10,14-dimethoxydihydrostyryl)-2-pyrone (*3*)

Mp 166–168°C (Me_2CO); IR ν_{max} cm^{-1}: 2954, 2850, 1728, 1646, 1570, 1506, 1474; ^1H NMR spectral data (300 MHz): Table 1; ^{13}C NMR spectral data (75 MHz): Table 2; EIMS (probe) 70 eV, *m*/*z* (rel. int.): 334 [M^+] (8), 195 (100), *Anal.* Calcd. For $C^{17}H^{18}O^7$: C 61.07, H 5.43 found C 60.95, H 5.48.

3.7. 4-Methoxy-6-(11,12-methylenedioxy-14-methoxystyryl)-2-pyrone (*4*)

Mp 189–191°C (Me_2CO); IR ν_{max} cm^{-1}: 3090, 2906, 1730, 1634, 1556, 1484, 1416, 1306. ^1H NMR spectral data (300 MHz): Table 1; ^{13}C NMR spectral data (75 MHz): Table 2; EIMS (probe) 70 eV, *m*/*z* (rel. int.): 302 M^+ (100%), 271 (18), 229 (22), 137 (25), 125 (32), 69 (45), *Anal.* Calcd. For $C_{16}H_{14}O_6$: C 63.57, H 4.67 found C 63.45, H 4.75.

3.8. 4-Methoxy-6-(11,12-methylenedioxystyryl)-2-pyrone (*5*)

Mp 200–203°C (EtOAc); IR ν_{max} cm^{-1}: 2924, 2852, 1726, 1646, 1570, 1502, 1476, 1412; ^1H NMR spectral data (200 MHz, $CDCl_3$) δ: 7.42 (1H, *d*, $J=15.8$ Hz, H-8), 7.04 (1H, *d*, $J=1.9$ Hz, H-10), 6.98 (1H, *dd*, $J=8.2$ and 1.9 Hz, H-14), 6.81 (1H, *d*, $J=8.2$ Hz, H-13), 6.40 (1H, *d*, $J=15.8$ Hz, H-7), 6.0 (2H, *s*, OCH_2O), 5.90 (1H, *d*, $J=2.1$ Hz, H-5), 5.48 (1H, *d*, $J=2.1$ Hz, H-3), 3.86 (3H, *s*, OMe); ^{13}C NMR spectral data (50 MHz): Table 2; MS *m*/*z* (rel. int.): 272 [M^+] (100), 201 (85), 115 (38), 89 (64), 69 (90).

3.9. 4-Methoxy-6-(11,12-dimethylstyryl)-2-pyrone (*6*)

Mp 147–149°C (EtOAc); IR ν_{max} cm^{-1}: 2942, 2838, 1702, 1638, 1594, 1552, 1516, 1450; ^1H NMR spectral data (300 MHz, $CDCl_3$) δ: 7.46 (1H, *d*, $J=16$ Hz, H-8), 7.09 (1H, *dd*, H-14), 7.03 (1H, *d*, $J=1.9$ Hz, H-10), 6.87 (1H, *d*, $J=8.3$ Hz, H-13), 6.46 (*d*, $J=16$ Hz, H-7), 5.92 (*d*, $J=2.1$ Hz, H-5), 5.48 (*d*, $J=2.1$ Hz, H-3), 3.93, 3.92, 3.83 (9H, *s*, OMe-4, OMe-11, OMe-12); ^{13}C NMR spectral data (75 MHz): comparable to published values (Rossi et al., 1997); EIMS (probe) 70 eV, *m*/*z* (rel. int.): 288 [M^+] (100), 217 (45), 151 (25), 69 (45).

3.10. 7-Prenyloxy-6-methoxycoumarin (*7*)

Mp 80–82°C (Me_2CO); IR ν_{max} cm^{-1}: 3054, 2974, 2936, 2882, 1714, 1612, 1562, 1514, 1448; ^1H NMR spectral data (200 MHz, $CDCl_3$) and ^{13}C NMR spectral

data (50 MHz, $CDCl_3$) were in good agreement with published data (Cardona et al., 1992).

3.11. Protohypericin (**8**)

IR ν_{max} cm^{-1}: 3536, 3454, 3056, 2920, 2850, 1618, 1558, 1478, 1386, 1258; ^1H NMR spectral data (300 MHz, DMSO-d_6) δ: 11.95 (*br s*, OH), 7.52 (2H, *d*, $J = 1.4$ Hz, H-7), 7.28 (2H, *s*, H-2), 7.19 (2H, *br q*, H-5), 2.42 (6H, *s*, Me-6); ^{13}C NMR spectral data (75.MHz, DMSO-d_6) δ: 189.3 (*s*, C-9), 181.5 (*s*, C-10), 162.5, 161.6, 159.8 (*s*, C-1, C-3, C-8), 148.6 (*s*, C-6), 132.9, 132.3 (*s*, C-5a, C-4a), 124.4 (*s*, C-5), 120.7 (*s*, C-7), 113.7, 108.7, 106.2 (*s*, C-8a, C-1a, C-4), 108.9 (*s*, C-2), 21.7 (*q*, Me-6); EIMS (probe) 70 eV, *m/z* (rel. int.): 304 [M^+] (100).

Acknowledgements

The authors wish to thank Dr O. G. Miguel and O. A. Guimarães (UFP) for identification of the plant.

References

Banks, H.J., Cameron, D.W., Raverty, W.D., 1976. Chemistry of Coccoideaa. II Condensed policyclic pigments from the Australian *Pseudo coccids* (Hemiptera). Australian J. Chem. 29, 1509.

Barbosa Filho, J.M., Yoshida, M., Gottlieb, O.R., Barbosa, R.C.S.B., Giesbrecht, A.M., Young, C.M., 1987. Benzoyl esters, amides, styrylpyrones and neolignans from the fruits of *Aniba riparia*. Phytochemistry 26, 2615.

Campos, R.O.P., Santos, A.R.S., Vaz, Z.R., Pinheiro, T.R., Pizzolatti, M.G., Cechinel, V., Delle Monache, F., Yunes, R.A., Calixto, J.B., 1997. Antinociceptive properties of the hydroalcoholic extract and preliminary study of a xanthone isolated from *Polygala cyparissias*. Life Science 61, 1619.

Cardona, L., Garcia, B., Pedro, J.R., Perez, J., 1992. 3-Prenyloxy-7-methoxycoumarin, a coumarin-hemiterpene from *Carduus tenuiflorum*. Phytochemistry 31, 3989.

Jackson, G.E., Campbell, W.E., Davidowitz, B., 1990. Two dimensional NMR study of some natural coumarins. Spectroscopy Letters 23, 359.

Pinheiro, T.R., Cechinel, V., Santos, A.R.S., Calixto, J.B., Delle, Monache F., Pizzolatti, M.G., Yunes, R.A., 1998. Three xanthones from *Polygala cyparissias*. Phytochemistry 48, 725.

Rossi, M.H., Yoshida, M., Maia, J.G.S., 1997. Neolignans, styrylpyrones and flavonoids from an *Aniba* species. Phytochemistry 45, 1263.

Schlemmer, F., Hugel, H., Petri, H.R., Kuhlmey, S.R., 1972. Pharmakognostische Charakterisierung von *Piper sanctum*. Deutsche Apotheker-Zeitung 112, 591–596.

Schultz, A., 1990. Introdução a Botânica Sistematica vol. 2, Porto Alegre, RGSBrazil.

Wurdack, J.J., Smith, L.B., 1971. Flora Ilustrada Catarinese. Poligolaceae. Itajai, SC, Brazil, p. 28.