Pergamon

PII: S0031-9422(98)00051-X

Phytochemistry, Vol. 48, No. 4, pp. 725–728, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

THREE XANTHONES FROM *POLYGALA CYPARISSIAS*

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(*Received* 1 *May* 1997; *accepted in revised form* 17 *November* 1997)

Key Word Index—*Polygala cyparissias*; Polygalaceae; xanthones; 1,7-dihydroxy-2,3-methylenedioxyxanthone; 1,3,6,8-tetrahydroxy-2,7-dimethoxyxanthone; 1,3,7-trihydroxy-2-methoxyxanthone.

Abstract—The ethyl acetate extract of *Polygala cyparissias* afforded three unknown and three known simple xanthones. The structures of the former were established by spectroscopic methods as 1,7-dihydroxy-2,3-methylenedioxy-,1,3,6,8-tetrahydroxy-2,7-dimethoxy- and 1,3,7-trihydroxy-2-methoxyxanthone. Moreover, the hexane extract gave an uncommon sterol (α-spinasterol) and high yields of methyl salicylate. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Polygala cyparissias (Polygalaceae), popularly known as "pinheirinho-da-praia", "avenca-da-praia" or "timutu", is a small herb growing on the southern coast of Brazil as a typical underbrush of dunes [1, 2]. This species is used in folk medicine as an anaesthetic of topical use due to the high concentration of methyl salicylate, which is also responsible for the characteristic smell of the essential oil from the roots [3]. Pharmacological studies using a hydroalcoholic extract of this plant showed a potent analgesic activity inhibiting the abdominal constriction response caused by acetic acid (0.6%) in mice [4]. Preliminary experiments showed that some xanthones are responsible, at least partially, for this activity [4]. Previous phytochemical investigations on the genus have shown the presence of coumarins, saponins, lignans, flavonoids and mainly xanthones [5]. The present paper deals with the isolation and structure elucidation of the constituents of *P. cyparissias*.

RESULTS AND DISCUSSION

Extensive column and thin-layer chromatography of the ethyl acetate extract afforded six xanthones. On the basis of the spectral data and by comparison with those of literature, three were identified as 1,3-dihydroxy-7-methoxyxanthone (**1**), 1,7-dihydroxy-2,3-dimethoxyxanthone (**3**) and 1,3,6-trihydroxy-2,7-dimethoxyxanthone (**6**), previously reported from related species [5–7]. The other three are new xanthones and their structures (**2**, **4**, **5**) were established as follows.

Compound **2** was a dihydroxy-methylenedioxyxanthone, as inferred from EIMS ($[M]^+$ at m/z 272), ^1H NMR (chelated hydroxyl at δ 13.4; methylenedioxy at δ 6.14) and ^{13}C NMR (methylenedioxy at δ 103.5). Accordingly, by acetylation a diacetyl derivative was obtained. In addition, its ^1H NMR spectrum exhibited an isolated aromatic proton singlet at δ 6.66, and signals at δ 8.0 (d, J = 2.8 Hz), δ 7.56 (dd) and δ 7.44 (d, J = 9.0 Hz) characteristic for 7-hydroxyxanthones. The oxygenation 1,2,3, rather than 1,2,4 or 1,3,4, of the other ring was deduced by the low value for C-2 in the ^{13}C NMR spectrum (Table 1). Therefore, compound **2** is 1,7-dihydroxy-2,3-methylenedioxyxanthone.

Compound **4** was a symmetrically substituted tetrahydroxy-dimethoxyxanthone ($[M]^+$ at m/z 320), as deduced by the ^{13}C NMR spectrum which showed only eight signals (Table 1). Analogously, the ^1H NMR spectrum (see Experimental) exhibited only three singlets, one of which at δ 12.61 was attributable to chelated hydroxyl protons. These findings, coupled to the presence in the ^{13}C NMR spectrum of a signal at

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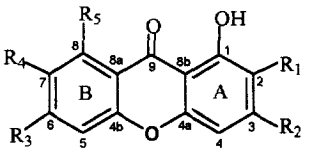
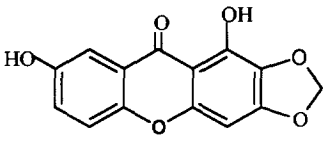


	R_1	R_2	R_3	R_4	R_5
1	H	OH	H	OMe	H
3	OMe	OMe	H	OH	H
4	OMe	OH	OH	OMe	OH
5	OMe	OH	H	OH	H
6	OMe	OH	OH	OMe	H



Compound **2**

Table 1. ^{13}C NMR spectral data of xanthones **2**, **4** and **5** in pyridine-d_5

C	2	4	5
1	154.6*	153.8*	154.1*
2	129.6	131.7	131.8
3	160.3	160.5	160.6
4	89.7	95.0	95.1
4a	155.7*	154.6*	155.4*
4b	†	154.6*	†
5	119.5	95.0	119.4
6	125.5	160.5	125.3
7	155.8*	131.7	155.5*
8	108.8	153.8*	109.3
8a	121.2	101.6	121.4
8b	105.6	101.6	103.5
9	181.7	183.9	181.4
OCH_3-2	—	60.5	60.5
OCH_3-7	—	60.5	—
OCH_2O-2	103.5	—	—

* Assignments interchangeable.
† Overlapped with signals from pyridine-d_5.

δ 60.5 was indicative of *ortho*-disubstituted methoxy groups, led to the structure of 1,3,6,8-tetrahydroxy-2,7-dimethoxyxanthone for compound **4**. To the best of our knowledge, hexa-oxygenated xanthones are rare; 1,2,3,4,6,7-hexamethoxyxanthone has been reported from *P. macradenia* [8] and methyl derivatives of 1,2,3,4,6,8-hexahydroxyxanthone from Gentianaceae [9].

The NMR spectral features of compound **5** were comparable with those of **2**, with a methoxyl (δ 3.87) instead of the methylenedioxy group. The methoxyl group was located on C-2, for the signal at δ 60.5 in the carbon spectrum (Table 1), and compound **5** was assigned the structure of 1,3,7-trihydroxy-2-methoxyxanthone. Other methyl derivatives of 1,2,3,7-tetrahydroxyxanthone were reported from related species [6, 7]. The hexane extract of the plant yielded a sterol identified as α-spinasterol [10] not previously reported from the genus.

EXPERIMENTAL

General experimental procedures

Mps. uncorr. ^1H and ^{13}C NMR spectra were measured at 300 and 75 MHz, on a Varian Gemini VXR 300F spectrometer, respectively; IR spectra were recorded using a FT Perkin-Elmer 16 PC (KBr pellets); UV spectra were obtained in MeOH using a

Hitachi U-2000 (UV-Vis) spectrometer, whereas mass spectra employs a Shimadzu QP 2000 at 70 eV. TLC: Merck Kieselgel 60 F_{254} CC: Merck Kieselgel 60.

Plant material

Polygala cyparissias was collected in April 1993 at the Santa Catarina coast, Brazil, and identified by Prof. Leila da Graça Amaral. A voucher specimen is deposited at the Herbarium FLOR (UFSC) under number 22.744.

Extraction and isolation

Aerial parts and roots (1 kg) of *P. cyparissias* were dried and cut in small pieces and successively extracted at room temperature with hexane (3 × 2 l), ethyl acetate (3 × 2 l) and methanol (3 × 2 l). The EtOAc residue (29 g) was chromatographed on silica gel and eluted with a gradient of EtOAc in hexane to give six main fractions. Each fraction was purified by flash chromatography on silica gel eluted with C_6H_6–EtOAc (4:1) to give xanthones **1** (19 mg), **2** (28 mg), **3** (37 mg), **4** (33 mg), **5** (31 mg) and **6** (3.8 mg), respectively. The hexane extract (17 g) was chromatographed on silica gel with hexane–EtOAc (98:2) to give α-spinasterol (37 mg).

1,3-*dihydroxy*-7-*methoxyxanthone* (**1**). Yellow needles, mp. 244.5–245° (lit. [5] 245–246°). Showed yellow fluorescence under UV light and brown colour by exposure to I_2 vapour. UV and IR spectra were identical to Ref. [5]. EI-MS (probe) *m/z* (rel. int.): 258 $[M]^+$ (100), 243 (25), 228 (23), 215 (2.7), 187 (29), 69 (28); ^{1}H NMR (pyridine-d_5), δ (ppm): 3.71 (OMe, *s*), 6.71 (H-4, *d*, J = 2.1 Hz), 6.75 (H-2, *d*, J = 2.1 Hz), 7.41 (H-5, *d*, J = 9.0 Hz), 7.36 (H-6, *dd*, J = 2.9 and 9.0 Hz), 7.79 (H-8, *d*, J = 2.9 Hz), 13.60 (OH-1, *s*); N.O.E. between OMe and H-6/H-8. ^{13}C NMR (pyridine-d_5), δ (ppm): 55.8 (OMe), 94.9 (C-4), 99.3 (C-2), 103.3 (C-8b), 106.1 (C-8), 119.4 (C-5), 121.4 (C-8a), 124.7 (C-6), 151.0 (C-4b), 156.4 (C-7), 158.6 (C-4a), 164.5 (C-1), 167.5 (C-3), 180.7 (C=O).

1,7-*dihydroxy*-2,3-*methylenedioxyxanthone* (**2**). Yellow needles, mp. 243–245°. Showed yellow fluorescence under UV light and brown colour by exposure to I_2 vapour. UV λ_{max}^{MeOH} nm (log ε): 295 (3.79), 326 (3.69), 377 (3.44); IR ν_{max}^{KBr} cm^{-1}: 3376, 1676, 1616, 1478, 928; EI-MS (probe) *m/z* (rel. int.): 272 [M, $C_{14}H_8O_6]^+$ (100), 243 (8), 214 (30), 186 (14), 158 (5), 136 (24), 121 (15), 77 (30), 53 (28); ^{1}H NMR (pyridine-d_5) δ: 6.16 (OCH_2O, *s*), 6.66 (H-4, *s*), 7.44 (H-5, *d*, J = 9.0 Hz), 7.56 (H-6, *dd*, J = 2.7 and 9.0 Hz), 8.0 (H-8, *d*, J = 2.7 Hz), 13.4 (OH-1, *s*); ^{13}C NMR data in Table 1. On acetylation, **2** gave a diacetyl derivative: ^{1}H NMR (C_6D_6) δ: 2.24 (OAc-7, *s*), 2.55 (OAc-1, *s*), 6.19 (OCH_2O, *s*), 6.95 (H-4, *s*), 7.42 (H-5, *d*, J = 9.0 Hz), 7.56 (H-6, *dd*, J = 9.0 and 2.8 Hz), 8.18 (H-8, *d*, J = 2.8 Hz); ^{13}C NMR (pyridine-d_5) δ: 20.9 and 21.1 (OAc × 2), 96.5 (C-4), 104.4 (OCH_2O), 110.4 (C-8b), 118.5 (C-8), 119.4 (C-5), 122.8 (C-8a), 129.0 (C-6), 138.0 (C-2), 147.5 (C-3), 150.3 (C-4b), 152.9 (C-1), 154.5 (C-4a), 154.7 (C-7), 169.31 and 169.6 (OAc × 2), 174.6 (C=O).

1,7-*dihydroxy*-2,3-*dimethoxyxanthone* (**3**). Yellow needles, mp. 243–245° (lit. [6] 245–246°). Showed yellow fluorescence under UV light and brown colour by exposure to I_2 vapour; IR ν_{max}^{KBr} cm^{-1}: 3412, 1650, 1588, 1478; UV, EI-MS and ^{1}H NMR identical to Ref. [6]. ^{13}C NMR (pyridine-d_5) δ: 56.5 (OMe-3), 60.7 (OMe-2), 91.30 (C-4), 104.4 (C-8b), 109.1 (C-8), 119.5 (C-5), 121.4 (C-8a), 125.6 (C-6), 132.2 (C-2), 150.2 (C-4b), 153.9 (C-1), 154.7 (C-4a), 155.7 (C-7), 160.7 (C-3), 181.6 (C=O).

1,3,6,8-*tetrahydroxy*-2,7-*dimethoxyxanthone* (**4**). Yellow needles, mp. 235–237°. Showed yellow fluorescence under UV light and brown colour by exposure to I_2 vapour. UV λ_{max}^{MeOH} nm (log ε): 234 (3.36), 254 (3.24), 326 (2.87); IR ν_{max}^{KBr} cm^{-1}: 3386, 1630, 1452; EI-MS (probe) *m/z* (rel. int.): 320 [M, $C_{15}H_{12}O_8]^+$ (100), 305 (83), 277 (81), 262 (13), 234 (58), 206 (19), 167 (18), 131 (14), 98 (14), 77 (11), 69 (58); ^{1}H NMR (pyridine-d_5) δ: 4.00 (OMe-2, OMe-7, *s*), 6.77 (H-4, H-5, *s*), 12.61 (OH-1, OH-8, *s*); ^{13}C NMR in Table 1. On acetylation, **4** gave a tetra-acetyl derivative. ^{1}H NMR [$(CD_3)_2CO$] δ: 2.32 and 2.42 (OAc × 4), 3.85 (OMe × 2), 6.96 (H-4, H-5).

1,3,7-*trihydroxy*-2-*methoxyxanthone* (**5**). Yellow needles, mp. 240–242°. Showed yellow fluorescence under UV light and brown colour by exposure to I_2 vapour. UV λ_{max}^{MeOH} nm (log ε): 257 (4.30), 307 (3.98), 374 (3.80); IR ν_{max}^{KBr} cm^{-1}: 3390, 2924, 1652, 1582; EI-MS (probe) *m/z* (rel. int.): 274 [M, $C_{14}H_{10}O_6]^+$ (61), 259 (54), 231 (100), 202 (10), 174 (4), 147 (8), 137 (11), 93 (13), 77 (9), 65 (16); ^{1}H NMR [$(CD_3)_2CO$] δ: 3.88 (OMe-2, *s*), 6.49 (H-4, *s*), 7.36 (H-6, *dd*, J = 8.8 and 3.0 Hz), 7.46 (H-5, *d*, J = 8.8 Hz), 7.58 (H-8, *d*, J = 3.0 Hz), 13.16 (OH-1, *s*); ^{13}C NMR in Table 1.

1,3,6-*trihydroxy*-2,7-*dimethoxyxanthone* (**6**). Yellow needles, mp. 229–231.5° (lit. [7] 231–233°). Showed yellow fluorescence under UV light and brown colour by exposure to I_2 vapour. UV λ_{max}^{EtOH} nm: 220, 252, 317, 358; IR, EI-MS, ^{1}H and ^{13}C NMR identical to Ref. [7].

α-*spinasterol* (24-*ethyl*-5α-*cholesta*-7,22-*dien*-3β-*ol*). White needles, mp. 166.5–168° (lit. [11] 168–169°). Showed pink colour with anisaldehyde-sulfuric acid (5%) on TLC. IR ν_{max}^{KBr} cm^{-1}: 3446, 2920, 2848, 1706, 1466; GC-MS (fused silica capillary column, OV-1, 25 m × 0.25 mm, i.d., 0.25 μm, column program temp. 40–250°, H_2, flow rate 10° min^{-1}, RR_t 37.987 min.) *m/z* (rel. int.): 412 $[M]^+$ (23), 397 (15), 368 (18), 300 (26), 271 (100), 255 (54), 107 (28), 81 (60), 69 (38); ^{1}H and ^{13}C NMR identical to data reported in Ref. [10].

Acknowledgements—The authors are grateful to Dr Jacir Dal Magro for providing the UV spectral data. This work was supported by grants from CNPq, Brazil.

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