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Odoratin 7-*O*-β-D-glucopyranoside from *Bowdichia virgilioides*

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Abstract

An isoflavonoid glucoside, odoratin 7-*O*-β-D-glucopyranoside, was isolated from the roots of *Bowdichia virgilioides* and its structure was established by spectroscopic and chemical methods. In vitro tests showed that this compound inhibited both T-lymphocyte and B-lymphocyte proliferation. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Bowdichia virgilioides*; Leguminosae; Roots; Isoflavonoid glucoside; Odoratin 7-*O*-β-D-glucopyranoside

1. Introduction

Bowdichia virgilioides is traditionally used in Brazil for the treatment of rheumatism, arthritis and diabetes (Friese, 1934; LeCointe, 1947). Chemical study of this plant has revealed the presence of anthocyanin (Mell, 1929), alkaloids (Torrenegra, Escarria, Bauereiss & Achenbach, 1985,1989), triterpenes (Marinho, Cunha, Thomas & Barbosa-Filho, 1994), resins (Machado, 1936; Machado & Peixoto, 1939) and essential oils (Arriaga, Machado, Gomes & Craveiro, 1998). As part of our ongoing investigation for biologically active isoflavonoid glycosides (da Silva, Bernardo & Parente, 1998a, 1998b, 1988c; Tostes, Silva & Parente, 1997, 1999), we report in this paper the isolation and structural elucidation of a new isoflavonoid glucoside from *B. virgilioides*.

2. Results and discussion

Fractionation of a MeOH extract from the dried roots of *B. virgilioides* by adsorption chromatography on silica gel yielded the isoflavone glucoside (**1**).

The molecular formula of **1** calculated as $C_{23}H_{24}O_{11}$ by combination of its LSIMS (negative ion mode) m/z 475 $(M\text{-}H)^-$ and ^{13}C-NMR spectral data (Table 1). The UV spectrum of **1** exhibited λ_{max} (log ε) at 219 (4.14), 262 (4.05) and 319 nm (3.67). The chromatographic behavior of **1**, UV, IR 3484 cm^{-1} (OH) and 1624 cm^{-1} (>C=O), ^{1}H-NMR δ 8.39 (1H, *s*, H-2), ^{13}C-NMR δ 153.40 (CH, C-2) and 124.91 (C, C-3) (Jha, Zilliken & Breitmaier, 1980; Murthy, Rao & Ward, 1986; Tostes et al., 1997, 1999) spectra established that **1** is an isoflavone glucoside. In the NOE difference experiments, separate saturation of the methoxyl signal at δ 3.68 and 3.59 resulted in the enhancements of H-5 (δ 7.48, *s*) and H-5′ (δ 6.98, *s*), respectively, indicating that these methoxyl groups were at C-6 and C-4′, respectively. The correlation peaks between each methoxyl proton signal, and its corresponding quaternary carbon resonance in the COLOC spectrum, allowed the carbon resonances at δ 147.68 and 147.73 to be assigned to C-6 and C-4′, respectively. Furthermore, the correlation peaks between H-2′ and C-3′, H-2 and C-4, H-5 and C-4, and H-8 and C-7 confirmed these assignments. The ^{1}H-NMR spectrum displayed, in addition to a single signal for H-2, two singlets at δ 7.48 and 7.29, integrating for single protons, which were assigned to H-5 and H-8, respectively. It is interesting that the aromatic protons H-2′, H-6′ and H-5′ appeared as single signals at δ 7.11, 7.00 and 6.98, respectively. This fact can prob-

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Table 1
^{1}H- and ^{13}C- NMR spectral data for compounds **1**, **1a**, **1b** and **1c** in DMSO-d_6[a,b]

Attribution	**1**		**1a**		**1b**		**1c**	
	δ ^{13}C	δ ^{1}H (J = Hz)	δ ^{13}C	δ ^{1}H (J = Hz)	δ ^{13}C	δ ^{1}H (J = Hz)	δ ^{13}C	δ ^{1}H (J = Hz)
2	153.40	8.39 *s*	152.65	8.29 *s*	153.91	8.50 *s*	154.26	8.55 *s*
3	123.26		122.89		121.91		122.16	
4	174.59		174.23		174.04		174.61	
4[a]	118.02		116.32		119.05		121.95	
5	105.03	7.48 *s*	104.80	7.44 *s*	105.63	7.50 *s*	105.87	7.62 *s*
6	147.68		146.98		150.61		149.30	
OMe	55.88	3.68 *s*	55.83	3.93 *s*	55.84	3.86 *s*	56.26	3.92 *s*
7	151.38		152.86		147.81		144.40	
OH				10.61 *s*				
OCOMe[c]							20.30	2.38 *s*
OCOMe[c]							168.41	
8	103.66	7.29 *s*	102.85	7.15 *s*	105.12	8.32 *s*	113.26	7.64 *s*
8[a]	151.73		151.69		150.66		150.61	
1′	124.91		125.02		124.38		124.16	
2′	116.61	7.11 *s*	116.59	6.96 *s*	123.09	7.38 *d* (1.8)	123.26	7.40 *d* (2.1)
3′	146.19		146.01		138.92		138.92	
OH		9.08 *s*		9.05 *s*				
OCOMe[c]					20.30	2.26 *s*	20.31	2.29 *s*
OCOMe[c]					168.49		167.98	
4′	147.73		147.55		150.51		149.87	
OMe	56.07	3.59 *s*	56.68	3.81 *s*	55.19	3.80 *s*	55.98	3.84 *s*
5′	112.16	6.98 *s*	111.92	6.95 *s*	112.49	7.20 *d* (8.8)	112.54	7.23 *d* (8.6)
6′	120.05	7.00 *s*	119.75	6.99 *s*	127.15	7.52 *dd* (2.1, 8.6)	127.17	7.52 *dd* (2.1, 8.4)
1″	99.96	5.24 *d* (7.7)			97.50	5.72 *d* (7.7)		
2″	73.28				70.43			
3″	77.37				71.85			
4″	69.85				67.96			
5″	69.85				71.22			
6″	6.91				61.61			
OCOMe[c]					20.18	1.97 *s*		
					20.21	1.99 *s*		
					20.32	20.1 *s*		
					20.43	2.03 *s*		
OCOMe[c]					168.89			
					169.31			
					169.52			
					169.89			

[a] Chemical shift (δ) and coupling (J) in Hz in parentheses.
[b] Assignments from DEPT, 2D-COSY, ^{1}H–^{13}C HETCOR and ^{1}H–^{13}C COLOC experiments.
[c] Assignments interchangeable.

ably be explained by assuming that the hydroxyl group at C-3′ forms an intermolecular bond to DMSO. A singlet at δ 9.08 and its D_2O-exchange indicated the presence of a 3′-hydroxyl group. A doublet at δ 5.24 (J = 7.7 Hz) integrating for a single proton was attributed to H-1 of a glucose, indicating a β-linkage (Table 1).

The ^{13}C-NMR spectrum showed two quartets which resonated at δ 55.88 and 56.07, and were assigned to the carbons of the two methoxyl-substituents at C-6 and C-4′, respectively. The signal at δ 174.59 was attributed to the carbonyl carbon. The resonance of the aromatic moiety was assigned by DEPT, ^{1}H–^{13}C COSY and ^{1}H–^{13}C COLOC and by comparison with data from the literature (Jha et al., 1980; Murthy et al., 1986; Tostes et al., 1997). The proposed structure **1** was fully supported by its ^{13}C-NMR spectrum, which exhibited peaks for 23 carbon atoms (Table 1).

On acid hydrolysis, compound **1** yielded odoratin, 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-6-methoxy-4*H*-1-benzopyran-4-one, (**1a**) (Galina & Gottlieb, 1974; Hayashi & Thomson, 1974) and glucose. Mp and UV, IR and ^{1}H-NMR spectral data of **1a** were in accordance with those reported in the literature (Galina & Gottlieb, 1974; Hayashi & Thomson, 1974). The ^{13}C-NMR spectrum of **1a** was consistent with the UV, IR and ^{1}H-NMR data. The resonance of the compound **1a** was assigned by DEPT, ^{1}H–^{13}C HETCOR and ^{1}H–

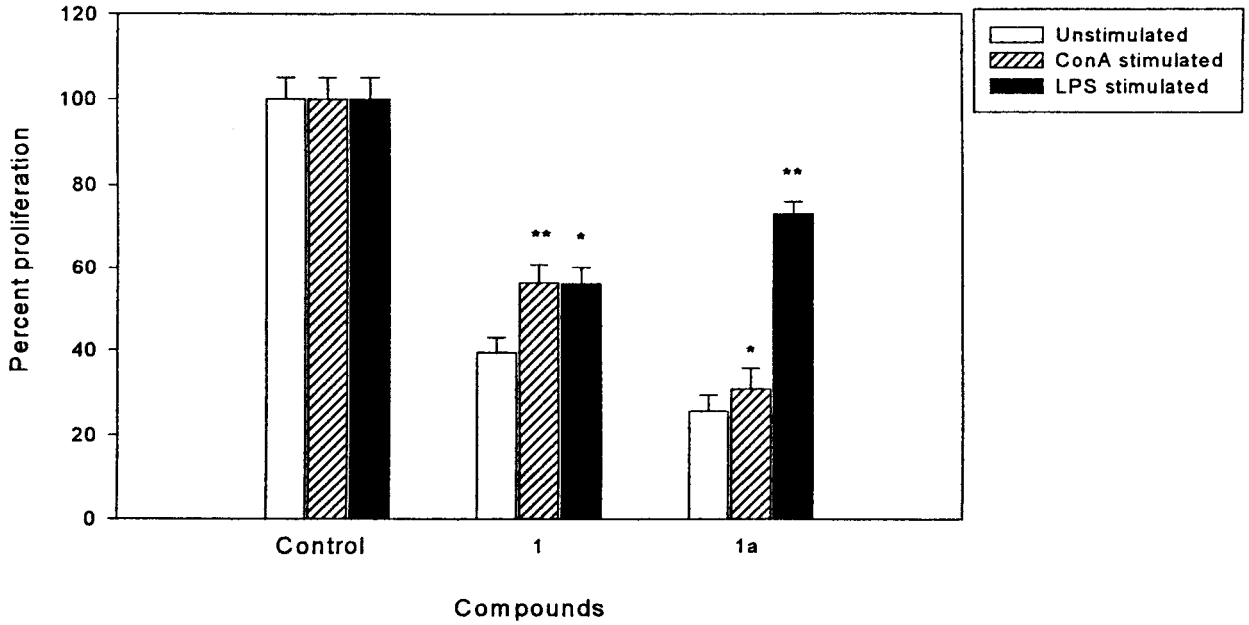


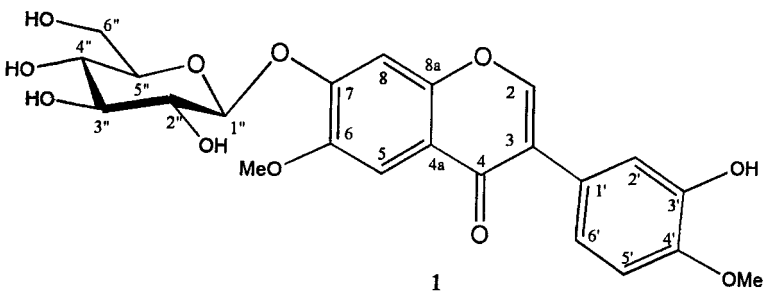
Fig. 1. Inhibition of lymphocyte proliferation by **1** and **1a** (10 μM). Spleen cells were treated with the compounds and mitogens (ConA and LPS) as described in Section 3. Data are mean values ± s.d. ($n = 5$). Significantly different from unstimulated treatment; *: $p < 0.05$, **: $p < 0.01$.

^{13}C COLOC experiments (Table 1) and by comparison with data from the literature (Jha et al., 1980; Murthy et al., 1986). Compound **1a** revealed $[M]^+$ at m/z 314.2970, $C_{17}H_{14}O_6$. The absolute configuration of the glucose was determined by GC of its TMSi (−)-2-butylglucosides. D-glucose was identified by GC-EIMS of the pertrimethylsilylated methylglucosides.

The acetylation reaction of **1** and **1a** yielded **1b** and **1c**, respectively. The ^{1}H-NMR spectrum of **1b** displayed signals at δ 7.38 (1H, *d*, $J = 1.8$ Hz, H-2′), 7.20 (1H, *d*, $J = 8.8$ Hz, H-5′) and 7.52 (1H, *dd*, $J = 8.6$, 2.1 Hz, H-6′) in the B ring, supporting that the B ring had a 3′,4′-disubstituted pattern. The ^{1}H-NMR spectral data of **1c** were consistent with the values observed for **1b** (Table 1).

Consequently, on the basis of UV, IR, ^{1}H- and ^{13}C-NMR spectroscopy, mass spectrometry and chemical reactions, the structure of compound **1** was established as 7-(β-D-glucopyranoslyoxy)-6-methoxy-3-(3-hydroxy-4-methoxyphenyl)-4H-benzopyran-4-one, named odoratin 7-*O*-β-D-glucopyranoside.

Isoflavonoid glucosides have demonstrated anti-inflammatory (Noreen, El-Seedi, Perera & Bohlin, 1988) and immunosuppressant (Namgooing, Lee & Kim, 1994) activities. In order to investigate the popular use of the plant against autoimmune diseases, compounds **1** and **1a** were evaluated for inhibitory activity against lymphocyte proliferation induced by T-mitogen (ConA) or B-mitogen (LPS) (see Fig. 1). Compound **1a** showed a more pronounced effect upon T-cell than B-cell proliferation. In contrast to this, compound **1** was found to inhibit T-mitogen as well as B-mitogen induced lymphocyte proliferation. These results suggest that compound **1** may be a potential therapeutic agent involved in inflammatory/immunoregulatory disorders, supporting the traditional use of *B. virgilioides* in Brazilian folk medicine (Friese, 1934; LeCointe, 1947).



1

3. Experimental

3.1. General

Mps were determined by an Electrothermal 9200 micro melting point apparatus and are uncorrelated, or were measured on a Perkin Elmer 243B polarimeter. UV and IR spectra were measured on a Shimadzu UV-1601 and on a Perkin Elmer 599B, respectively. ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian Gemini 200 NMR spectrometer operating at 200 MHz for ^{1}H and 50 MHz for ^{13}C in DMSO-d_6, with TMS as internal standard. GC was carried out with FID, using a glass capillary column (0.31 mm × 25 m) SE-30. EIMS was recorded at 70 eV. Negative LSIMS was carried out using HMPA-glycerol as matrix, 35 kV anodic voltage, 8 kV accelerating voltage using Cs ions.

Silica gel column (230–400 mesh ASTM, Merck) was used for CC. TLC was performed on silica gel (Merck) using the following solvent systems: (A) $CHCl_3$–MeOH (85 : 15) for **1**, (B) $CHCl_3$–MeOH (97 : 3) for **1a**, (C) $CHCl_3$–MeOH (98 : 2) for **1b** and **1c** and (D) 1-BuOH–pyridine–H_2O (6 : 4 : 3) for sugar. Compounds **1**, **1a**, **1b** and **1c** were detected under UV of 254 and 366 nm and by spraying with orcinol-H_2SO_4. Sugar was detected by spraying with orcinol-H_2SO_4.

3.2. Plant material

Roots of *B. virgilioides* Kunt were collected at Paço do Lumiar, Maranhão, on March 1997, and identified by T.J.A.S. Rego. A voucher specimen (no. 01137) is deposited at the Ático Seabra Hebarium.

3.3. Extraction and isolation

Dried and powdered roots of *B. virgilioides* (1 kg) were extracted with cold MeOH. Evaporation of solvent gave a residue (20 g), a part of which (10 g) was submitted to CC (90 × 3 cm) on silica gel which was eluted with $CHCl_3$–MeOH mixtures of increasing polarity (up to 10% MeOH). Frs. eluted with $CHCl_3$–MeOH (9 : 1) yielded **1** as a homogeneous compound (495 mg), TLC, R_f = 0.25, dark blue color with orcinol-H_2SO_4 and negative $FeCl_3$ test.

3.4. Odoratin 7-O-β-D-glucopyranoside (1)

Pale yellow amorphous powder from MeOH, mp 185–188°C, $[\alpha]_D^{20}$ − 83.2° (DMSO, *c* 0.001). UV λ_{max}^{MeOH} nm (log ε): 219 (4.14), 262 (4.05), 319 (3.67). IR ν_{max}^{KBr} cm^{-1}: 3484 (OH), 1624 (>C=O), 1494, 1435, 1289, 1262, 1219, 1201, 1085, 1020, 872, 810, 719. ^{1}H- and ^{13}C-NMR spectral data: Table 1. Negative LSIMS, *m/z* (rel. int.): 475 $[M-H]^-$ (58), 313 [M-163] (100).

3.5. Acid hydrolysis of 1

The compound (**1**, 100 mg) was hydrolyzed with 1 M HCl in dioxane–H_2O (1 : 1, 10 ml) under reflux for 30 min. After diluting with H_2O (90 ml), the aglycone was extracted with $CHCl_3$–MeOH (4 : 1) and evaporated to dryness in vacuo. The residue was dissolved in MeOH, and on concentration the solution yielded a pale yellow compound, which on further recrystallization gave odoratin (**1a**, 49 mg), mp 214–216°C [lit. (Hayashi & Thomson, 1974; Galina & Gottlieb, 1974) mp 213–215°C]. Odoratin **1a** was analyzed by silica gel-TLC in the above described solvent system. After spraying with orcinol-H_2SO_4 it gave a yellow spot at $R_f = 0.45$. UV λ_{max}^{MeOH} nm (log ε): 223 (4.33), 260 (4.19), 320 (3.90), (NaOAc): 215 (4.35), 255 (4.26), 346 (4.03); (NaOAc + H_3BO_3): 213 (4.36), 259 (4.18), 323 (3.86). IR ν_{max}^{KBr} cm^{-1}: 3513 (OH), 3157, 2994, 1622, 1578, 1566, 1514, 1480, 1455, 1320, 1207, 1183, 1137, 1051, 1015, 859, 762. ^{1}H- and ^{13}C-NMR spectral data: Table 1. EIMS (probe) 70 eV *m/z* (rel. int.): 314 $[M]^+$ (100), 299 (21), 285 (5), 271 (17), 243 (11), 167 (10), 105 (13). HRMS *m/z*: 314.2970 $[M^+]^{1-}$ $C_{17}H_{14}O_6$. Calcd. = 314. The aqueous layer was adjusted to pH 6 by addition of $NaHCO_3$. After lyophilization, sugar was dissolved in pyridine and analyzed by silica gel-TLC in the above described solvent system. After spraying, glucose gave a blue spot at $R_f = 0.70$. The configuration of the sugar was established by capillary GC of its TMSi (−)-2-butyl glycosides (Gerwig, Kamerling & Vliegenthart, 1978).

3.6. Acetylation of 1

Compound **1** (100 mg) was treated overnight with Ac_2O and pyridine at room temperature. Usual warm-up gave (**1b**, 141 mg, TLC: $R_f = 0.55$), mp 103–105°C, $[\alpha]_D^{20}$ − 52.4° (DMSO, *c* 0.001). UV λ_{max}^{MeOH} nm (log ε): 244 (4.73), 322 (4.40), IR ν_{max}^{KBr} cm^{-1}: 2944, 1758, 1641, 1619, 1514, 1495, 1434, 1371, 1272, 1217, 1130, 1068, 1040, 906, 878, 820, 752. ^{1}H- and ^{13}C-NMR spectral data: Table 1. CIMS, *m/z* (rel. int.): 704 $[M + NH_4]^+$ (78), 686 [M] (7).

3.7. Acetylation of 1a

Compound **1a** (100 mg) was treated as **1** to give (**1c**, 113 mg, TLC: $R_f = 0.50$), mp 185–187°C [lit. (Hayashi & Thomson, 1974; Galina & Gottlieb, 1974) mp 185–187°C]. UV λ_{max}^{MeOH} nm (log ε): 208 (4.41), 260 (4.38), 326 (3.90). IR λ_{max}^{KBr} cm^{-1}: 2957, 2849, 1770, 1655, 1618, 1515, 1494, 1436, 1372, 1272, 1198, 1183, 1126, 1015, 905, 821, 601. ^{1}H- and ^{13}C-NMR spectral data: Table

1. EIMS (probe) 70 eV *m/z* (rel. int.): 398 $[M]^+$ (23), 356 (55), 314 (100), 299 (15), 285 (8), 271 (9), 243 (10), 192 (16), 167 (8), 133 (10), 119 (9), 105 (12), 69 (15).

3.8. Lymphocyte proliferation

Male BALB/c mice weighing from 20 to 25 g were used. Spleen cells (1×10^6 cells/ml) were collected, counted and seeded in 96-well microtitre plates and cultured in RPMI 1640 medium supplemented with 5% FBS containing test compounds with or without 1 μg/ml concanavalin A or 50 μg/ml lipopolysaccharide for 24 h at 37°C in a 5% CO_2 incubator. At this time, 10 μl of 10 μg/ml MTT stock solution was added to the wells and incubation was continued for 4 h at 37°C. After this period, 100 μl of 10% SDS was added and the absorbance was determined by usual methods (Mosmann, 1983).

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