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CLITORIACETAL 11-*O*-β-D-GLUCOPYRANOSIDE FROM *CLITORIA FAIRCHILDIANA*

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Key Word Index—*Clitoria fairchildiana*; Leguminosae; roots; rotenoid glycoside; clitoriacetal 11-*O*-β-D-glucopyranoside.

Abstract—A new rotenoid glycoside, clitoriacetal 11-*O*-β-D-glucopyranoside, was isolated from the roots of *Clitoria fairchildiana*. Its structure was established by spectroscopic and chemical methods. © 1997 Elsevier Science Ltd

INTRODUCTION

The genus *Clitoria* has 60 reported species [1], some of which are used for their healing properties and, in particular, *C. macrophylla* for the treatment of skin diseases in Thailand. From its roots stemonacetal and clitoriacetal have been isolated [2]. The latter was reported as the major component and possessed remarkable anti-inflammatory and antipyretic activities [3]. Moreover, from the roots of *C. macrophylla* 6-deoxyclitoriacetal was isolated, which *in vitro* tests showed strong cytotoxic activity against cultured P-388 lymphocytic leukaemia cells [4]. *Clitoria fairchildiana*, known in Brazil as sombreiro, is widely grown as a shade tree but no medicinal use is reported [5]. In the present work, we report on the isolation of a new rotenoid glycoside, clitoriacetal 11-*O*-β-D-glucopyranoside (**1**), from the roots of *C. fairchildiana*.

RESULTS AND DISCUSSION

Fractionation of a methanol extract by adsorption chromatography on silica gel afforded compound **1** that was obtained as white amorphous powder, which gave a positive Rogers–Calamari test for rotenoids [6] but did not give any colour with $FeCl_3$. Its molecular formula was calculated as $C_{25}H_{28}O_{14}$ by a combination of LSI mass (negative ion mode) *m/z* 551 $[M-H]^+$ and ^{13}C NMR spectral data (Table 1). The UV spectrum of **1** showed single maxima at 286 nm (4.33) and 320 nm (3.80). The chromatographic behaviour, UV spectrum, IR 3436 (OH), 1670 cm^{-1} (>C = O), 1613, 1573 and 1511 cm^{-1} (aromatic), ^1H NMR δ 5.50 (1H, *bs*, H-6, after D_2O-exchange) and 4.57 (1H, *bs*, H-6a) [2, 7], ^{13}C NMR δ 70.02 (C, C-12a) and 77.48 (CH, C-6a) [4, 8] and 90.85 (CH, C-6) spectral data, established that **1** is a rotenoid glycoside. Two doublets at δ 5.50 (J = 6.3 Hz) and 7.60 (J = 6.3 Hz) integrating for single protons and the D_2O-exchange of the latter indicated the presence of a 6-hydroxy group. The ^1H NMR spectrum displayed, in addition to signals for three methoxyl groups, H-6a, H-6 and 6-OH of a rotenoid nucleus, two doublets at δ 6.10 and 6.34 for H-8 and H-10, respectively. Two singlets at δ 6.78 and 6.41 integrating for single protons were assigned to H-1 and H-4 of the aglycone moiety, respectively. Based on the H-1 chemical shift value (δ 6.78), the B/C ring junction in **1** was determined to be *cis* [9–11]. The appearance of a broad singlet at δ 5.50, attributed to H-6, and a broad singlet at δ 4.57, assigned to H-6a suggested the presence of two hydroxyl groups at C-6 and C-12a and that H-6 exists in **1** as H-6*ax* [11, 12]. The assignments of the chemical shifts of the methoxyl groups (Table 1) were established by 2D-NMR correlations (Table 2) and by comparison of the data with those of 6-deoxyclitoriacetal [4]. A doublet at δ 4.70 (J = 7.2 Hz) integrating for a single proton was assigned to H-1 of a glucose, indicating β-linkage.

The ^{13}C NMR spectrum of **1** was consistent with

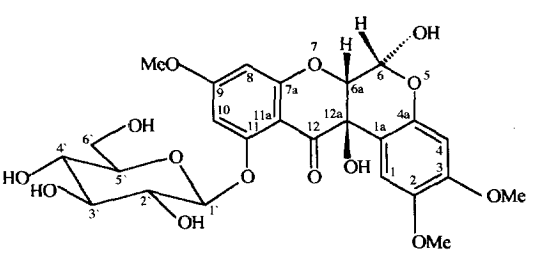

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Table 1. ^1H and ^{13}C NMR spectral data for compound **1** in DMSO-d_6

Attribution	δ ^{13}C	δ ^1H (J = Hz)	DEPT
1	110.9	6.78 *s*	CH
1a	109.8		C
2	143.4		C
2-OMe	55.9	3.83 *s*	CH_3
3	150.4		C
3-OMe	55.8	3.73 *s*	CH_3
4	102.2	6.41 *s*	CH
4a	147.6		C
6	90.9	5.50 *d* (6.3)	CH
6-OH		7.60 *d* (6.3)	
6*	90.9	5.50 *bs*	CH
6a	77.5	4.57 *bs*	CH
7a	160.0		C
8	95.1	6.10 *d* (2.3)	CH
9	165.6		C
9-OMe	55.7	3.65 *s*	CH_3
10	97.1	6.34 *d* (2.3)	CH
11	161.7		C
11a	103.8		C
12	188.5		C
12a	70.0		C
1′	100.8	4.70 *d* (7.2)	CH
2′	73.4		CH
3′	77.5		CH
4′	69.9		CH
5′	76.4		CH
6′	60.9		CH_2

* After D_2O-exchange.

the UV, IR and ^1H NMR data. Three quartets resonated at δ 55.7, 55.8 and 55.9. They were assigned to the carbons of the three methoxy-substituents at C-9, C-3 and C-2, respectively. The signal at δ 188.5 was attributed to the carbonyl carbon. The resonance of the aromatic moiety was assigned by DEPT (Table 1), HETCOR (^{13}C) (Table 2) and by comparison with data from the literature [4, 8, 13, 14]. The proposed structure **1** was fully supported by its ^{13}C NMR spectrum, which exhibited peaks for 25 carbon atoms (Table 1).

On acid hydrolysis, compound **1** yielded clitoriacetal (**2**) [2] as the aglycone and glucose. UV and IR spectra of **2** were in accordance with those reported in the literature [2]. The ^1H NMR spectral data of **2** (Table 3) were identified from direct comparison with

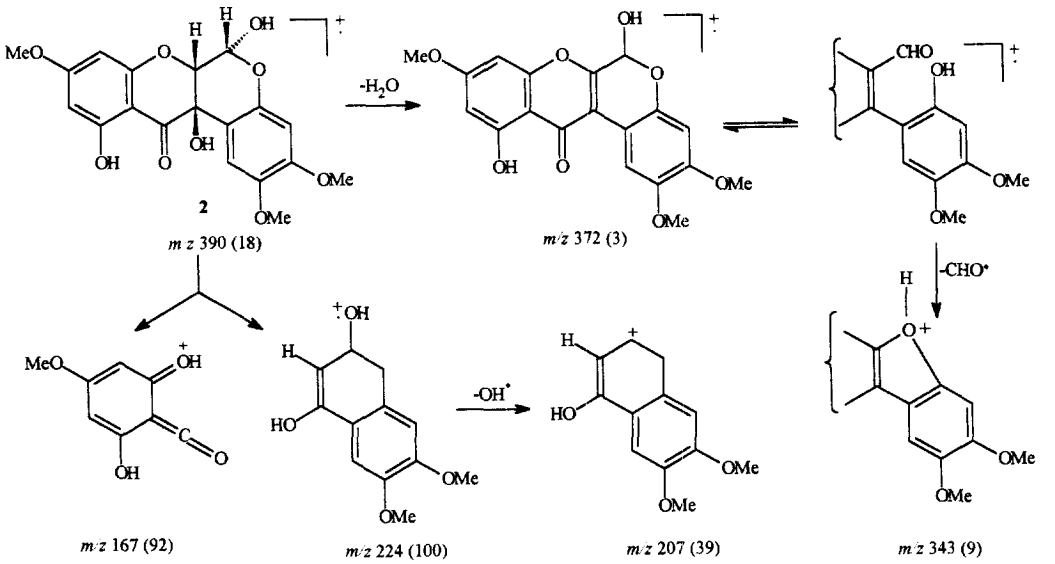
Table 3. ^1H and ^{13}C NMR data of compound **2** in $CDCl_3$

	2		
Attribution	δ ^{13}C	δ ^1H (J = Hz)	DEPT
1	109.0	6.59 *s*	CH
1a	107.6		C
2	144.8		C
2-OMe	56.3	3.82 *s*	CH_3
3	151.8		C
3-OMe	55.8	3.73 *s*	CH_3
4	101.8	6.42 *s*	CH
4a	147.9		C
6	91.5	5.52 *d* (6.4)	CH
6-OH		5.40 *d* (6.4)	
6*	91.5	5.52 *bs*	CH
6a	77.0	4.64 *bs*	CH
7a	160.8		C
8	94.6	5.87 *d* (2.3)	CH
9	169.0		C
9-OMe	55.8	3.78 *s*	CH_3
10	95.8	5.95 *d* (2.3)	CH
11	164.2		C
11-OH		11.72 *s*	C
11a	100.0		CH_3
12	194.1		C
12a	69.5		C

* After D_2O-exchange.

Table 2. Summary of 2D-NMR correlations of compounds **1** and **2**

H	**1** and **2** COSY (^1H)	HETCOR (^{13}C)	COSY (^1H) LR
1	4	1	
2-OMe		2-OMe	1
3-OMe		3-OMe	4
4	1	4	
6	6a	6	
6a	6	6a	
8	10	8	
9-OMe		9-OMe	8, 10
10	8	10	
	1		
1′	2′	1′	
2′			
3′			
4′		4′	
5′			
6′		6′	



Scheme 1. Mass spectral fragmentation of clitoriacetal (**2**).

those of clitoriacetal [2], COSY (^{1}H) and COSY (^{1}H) LR experiments (Table 2). The ^{13}C NMR spectrum of **2** was consistent with the UV, IR and ^{1}H NMR data. Two quartets resonated at δ 55.8 (OMe-3 and OMe-9) and 56.3 (OMe-2). The signals at δ 69.5, 7.0 and 91.5 were assigned to C-12a, C-6a and C-6, respectively. The signal at δ 194.13 was attributed to the carbonyl carbon. The resonance of the aromatic moiety of clitoriacetal (**2**) was assigned by DEPT (Table 3), HETCOR (^{13}C) (Table 2) and by comparison with data from the literature [4, 8, 13, 14]. The mass spectrum of **2** exhibited a $[M]^+$ at *m/z* 390, strongly suggesting that **2** is clitoriacetal. Confirmation of this observation was supported by the HR mass spectrum, which indicated a molecular formula $C_{19}H_{18}O_9$. Two prominent fragment ion peaks at *m/z* 167 and 224, due to cleavage between the B/C rings (Scheme 1), revealed that ring D possessed methoxyl and hydroxyl groups (*m/z* 167), and rings A and B had two methoxyl and two hydroxyl groups (*m/z* 224). Clitoriacetal (**2**) obtained from the hydrolysate indicated that **1** has a sugar moiety attached to C-11. The sugar was identified as glucose and its absolute configuration determined by GC of its TMSi (−)-2-butylglycosides [15]; D-glucose was detected. Consequently, on the basis of UV, IR, ^{1}H and ^{13}C NMR spectroscopy, LSI mass spectrometry and chemical reactions, the structure of compound **1** was established as (+)-(6*S*, 6a*R*, 12a*R*)-6,12a-dihydroxy-2,3,9-trimethoxy-rotenoid 11-*O*-*β*-D-glucopyranoside (clitoriacetal 11-*O*-*β*-D-glucopyranoside).

EXPERIMENTAL

General. Mps are uncorr. Optical rotations were measured at 20°. IR spectra were recorded in KBr discs. ^{1}H NMR were run at 200 MHz, $CDCl_3$ or DMSO-d_6, TMS as int. standard. ^{13}C NMR-edited DEPT spectra were obtained at 50 MHz from $CDCl_3$ or DMSO-d_6 solns. 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 columns (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 (4:1) for rotenoid glycoside and (B) $CHCl_3$–MeOH (19:1) for rotenoid aglycone and © *n*-BuOH–pyridine–H_2O (6:4:3) for sugars. Compounds **1** and **2** 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.

Plant material. Roots of *C. fairchildiana* Howard were collected at Ilha do Fundão, Rio de Janeiro, on February 1995, and identified by Luci Mendonça de Senna. A voucher specimen (no. R190871) is deposited at the Museu Nacional, Rio de Janeiro, Brazil.

Isolation of constituents. Dried and powdered roots (200 g) were extracted with cold MeOH. Evapn of solvent gave a residue (56 g) a part of which (30 g) was submitted to CC (90 × 3 cm) on silica gel which was eluted with $CHCl_3$–MeOH mixts of increasing polarity (up to 10% MeOH). Frs eluted with $CHCl_3$–MeOH (9:1) yielded **1**, TLC homogeneous compound (1.5 g), $R_f = 0.50$, dark blue colour with orcinol–H_2SO_4, negative $FeCl_3$ test and positive Rogers–Calamari tests.

Clitoriacetal 11-*O*-*β*-D-glucopyranoside (**1**). Amorphous powder (MeOH), mp 165–167° $[\alpha]_D$ + 100° (DMSO; *c* 0.001). UV λ_{max}^{EtOH} nm (log ε): 220 (4.40), 286 (4.33), 320 (sh, 3.80). IR ν_{max}^{KBr} cm^{-1}: 3436 (OH), 2939, 1670 (>C = O), 1613, 1573, 1511, 1445, 1412, 1343, 1268, 1220, 1170, 1148, 1101, 1076, 973, 955, 905, 864, 830, 781, 741. ^{1}H and ^{13}C NMR: Tables

1 and 2. Negative LSIMS, *m/z* (rel. int.): 551 $[M-H]^-$ (53), 389 [M-163] (100).

Acid hydrolysis of **1**. The compound (**1**, 100 mg) was hydrolysed with 1 M HCl in dioxane–H_2O (1:1, 10 ml) at room temp. for 10 min under N_2. After diluting with H_2O (90 ml), the aglycone was extracted with $CHCl_3$–MeOH (4:1) and evapd to dryness *in vacuo*. The residue was dissolved in MeOH and the soln on concn yielded a white compound which on further recrystallization gave clitoriacetal (**2**, 53 mg), mp 130°. $[\alpha]_D$+258° ($CHCl_3$, *c* 0.275), (lit [2] $[\alpha]_D$+259.5° ($CHCl_3$, *c* 0.275). Clitoriacetal was analysed by silica gel-TLC in the above described system. After spraying with orcinol–H_2SO_4 it gave a yellow spot at $R_f = 0.45$. Compound **2** gave a brown colour with $FeCl_3$. UV λ_{max}^{EtOH} nm (log ε): 230 (4.43), 292 (4.30), 320 (sh, 3.88); ($AlCl_3$): 220 (4.95), 295 (4.00), 310 (sh, 4.08). IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 3007, 2937, 2850, 1641 (>C = O), 1575, 1510, 1451, 1410, 1364, 1268, 1195, 1161, 1104, 1039, 975, 957, 907, 867, 830, 753. ^1H and ^{13}C NMR: Table 3. EIMS (probe) 70 eV *m/z* (rel. int.): 390 $[M]^+$ (18), 372 (3), 343 (9), 224 (100), 208 (39), 167 (92); HRMS found: $[M]^+$ 390.3490, $C_{19}H_{18}O_9$ requires 390.3493. The aq. layer was adjusted to pH 6 by addition of $NaHCO_3$. After lyophilization, sugar was extracted with pyridine and analysed by silica gel-TLC in the above described system. After spraying, glucose gave a blue spot at R_f 0.70. For co-chromatography glucose was used. The configuration of the sugar was established by capillary GC of its TMSi (−)-2-butyl glycosides [15].

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