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Flavonoid glycosides from *Crotalaria podocarpa*

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Abstract

The structures of two flavonoid glycosides isolated from the aerial parts of *Crotalaria podocarpa* have been established as apigenin 7-*O*-β-D-apiofuranosyl (1 → 6)-β-glucopyranoside and a new compound, acacetin 7-*O*-β-D-apiofuranosyl (1 → 6)-β-D-glucopyranoside. The structures of these flavonoid glycosides were determined using spectroscopic methods.

Keywords: *Crotalaria podocarpa*; Leguminosae; Flavonoid glycosides; Acacetin 7-apiosyl(1 → 6)glucoside

1. Introduction

The genus *Crotalaria* has 300 species world-wide with only about 60 species reported in southern Africa (Dyer, 1975). The genus produces mainly pyrolizidine alkaloids (Abegaz, Atnafu, Duddeck, & Snartzke, 1987; Toppel, Witte, & Hartmann, 1988; Mattocks & Nwude, 1988; Roeder, Sarg, El-Dahmy, & Ghani, 1993) but some flavonoid glycosides have been reported (Yadava, 1993). The aerial parts of *C. podocarpa* L. are known in homeopathy for their antirheumatic, antiphlogistic and expectorant activities (Kokwaro, 1976; Foster, Niklas, & Lutz, 1980; Charrtte, 1987; Herdberg & Staugard, 1989). *Crotalaria podocarpa* (Leguminosae) is an evergreen shrub that blooms in wet seasons. Earlier phytochemical investigation of *C. podocarpa* has revealed the presence of an alkaloid, 7-hydroxy-1-methylene pyrrolidine (Benn, Mathenge, Munavu, & Were, 1995), which was isolated from the aerial parts.

In the present study, we have isolated two flavonoid glycosides, apigenin 7-apiosyl (1 → 6)glucoside (**1**) and acacetin 7-apiosyl (1 → 6)glucoside (**2**). Compound **1** has also been isolated recently from the leaves of *Gonocaryum calleryanum* (Icacinaceae) (Kaneko et al., 1995).

2. Results and discussion

The methanolic extract from the aerial parts of *C. podocarpa* was worked-up as described in Section 3 to give **1** and **2**. The identification of **1** is based on spectral data as well as by comparison of our data with those reported in the literature for the same compound (Kaneko et al., 1995). Compound **2** was identified using spectroscopic methods, UV, IR, 1D NMR, 2D NMR (COSY, HMQC, HMBC), ESI-MS and by comparison with spectral data for **1**. The signals attributable to the sugar moiety in **1** and **2** corresponded closely to those reported for a lignan glycoside, vomifoliol 3′-*O*-β-D-apiofuranosyl (1 → 6)-β-D-glucopyranoside (Higuchi, Fukui, Kinjo, & Nohara, 1992).

The ESI-MS of **1** gave a pseudomolecular ion at m/z 565.5 (M + H) consistent with a molecular formula of $C_{26}H_{28}O_{14}$ and was identified as apigenin 7-*O*-β-D-apiofuranosyl (1 → 6)-β-D-glucopyranoside (Kaneko et al., 1995) on the basis that the NMR data (Table 1) agreed well with reported values. Compound **2** showed very similar spectral data to those of **1** Table 1 except that one hydroxy group in **1** was replaced by a methoxy group in **2**. The ESI-MS of **2** gave a pseudomole-

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Table 1
^{1}H (300 MHz) and ^{13}C (75.4 MHz) NMR chemical shifts for compounds **1** and **2** in DMSO-d_6

	1		**2**	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ^{13}C
Aglycone				
2	–	164.5 (s)	–	162.8 (s)
3	6.85 s	103.3 (d)	6.97 s	102.7 (d)
4	–	182.2 (s)	–	181.1 (s)
5	–	161.3 (s)	–	160.1 (s)
6	6.41 d, J=2.2 Hz	99.5 (d)	6.43 d, J=2.2 Hz	98.2 (d)
7	–	163.0 (s)	–	161.7 (s)
8	6.78 d, J=2.2 Hz	94.9 (d)	6.83 d, J=2.2 Hz	93.7 (d)
9	–	157.1 (s)	–	155.9 (s)
10	–	105.5 (s)	–	104.3 (s)
1′	–	121.2 (s)	–	121.6 (s)
2′	7.94 d, J=8.4 Hz	128.9 (d)	8.07 d, J=8.3 Hz	127.4 (d)
3′	6.92 d, J=8.7 Hz	116.2 (d)	7.13 d, J=8.3 Hz	113.5 (d)
4′	–	161.6 (s)	–	161.4 (s)
5′	6.92 d, J=8.7 Hz	116.2 (d)	7.13 d, J=8.3 Hz	113.5 (d)
6′	7.94 d, J=8.4 Hz	128.9 (d)	8.07 d, J=8.3 Hz	127.4 (d)
OCH_3	-	–	3.86 s	54.4 (q)
Glucose				
1	5.10 d, J=7.5 Hz	98.8 (d)	5.17 d, J=7.5 Hz	96.8 (d)
2		73.5 (d)		72.8 (d)
3		75.8 (d)		75.6 (d)
4		68.6 (d)		68.5 (d)
5		73.8 (d)		74.4 (d)
6		64.4 (t)		63.0 (t)
Apiose				
1	5.29 d, J=2.9 Hz	109.0 (d)	5.34 d, J=2.9 Hz	107.34 (d)
2		76.2 (d)		75.82 (d)
3		79.5 (s)		78.11 (s)
4		74.2 (t)		74.79 (t)
5		60.4 (t)		59.23 (t)

cular ion 579.5 (M+H), 14 mass units higher than **1**, and consistent with the molecular formula $C_{27}H_{30}O_{14}$. The ^{1}H and ^{13}C NMR data indicates the presence of glucose, apiose and flavone moieties. The nature and identity of the flavone was evident from the ^{1}H NMR spectrum which showed the presence of a 4-oxyphenyl group (δ_H 7.13, 2H, d, J=8.3 Hz and 8.07, 2H, d, J=8.3 Hz), a 2,4,6-trioxyphenyl (δ_H 6.83, 1H, d, J=2.2 Hz and 6.43, 1H, d, J=2.2 Hz) and a sharp singlet at δ_H 6.97 characteristic of flavone H-3 proton. These data are consistent with the flavone being apigenin and agree well with those reported for apigenin (Kaneko et al., 1995). The nature and stereochemistry of the glycosyl moieties viz- β-D-apiosyl and β-D-glucosyl were determined from anomeric proton resonances at δ_H 5.34 (1H d, J=2.9 Hz, H-1‴) and 5.17 (1H d, J=7.5 Hz, H-1″), respectively. The connectivity of the sugar residues to the apigenin nucleus were deduced from HMBC which showed a correlation between the glucose anomeric proton (δ_H 5.17) and the flavone C-7 (δ_C 161.8) carbon. Furthermore, a methoxy at δ_H 3.86 showed an HMBC correlation with a flavone C-4′ (δ_C 161.4) carbon. UV shift reagents also indicated that C-7 and C-4′ were substituted (Mabry, Markham, & Thomas, 1970). The apiose anomeric proton (δ_H 5.34) showed an HMBC correlation with a quaternary carbon at δ_C 78.1 (apiose C-3), a methylene carbon at 74.8 (apiose C-4) and another methylene carbon at 63.0 (glucose C-6), also indicating 1 → 6 linkage between apiose and glucose. The sequencing of sugar residues is further confirmed by ESI-MS which showed loss of apiose (m/z 447.4 [M-apiose]) before loss of glucose (m/z 285.3 [M-(glucose–apiose)]). Compound **2** was therefore found to be a 4′-methoxy derivative of **1**, namely acacetin 7-*O*-β-D-apiofuranosyl (1 → 6)-β-D-glucopyranoside and is reported for the first time.

3. Experimental

3.1. General

^{1}H (300 MHz) and ^{13}C (75.4 MHz) NMR in DMSO-d_6. All spectra were referenced to residual solvent signal. MS: Finnagen MAT SSQ 700 single Quadrupole instrument, ESI source used; CC: polyamide-11 (Merck); VLC: silica gel HF_{254} 5–15 μm mesh (Merck): Sephadex LH-20 (Sigma); HPLC: Shimadzu LC-6AD, Waters RI 401 detector, column-Phase Sep. S5-ODS 5, 250 × 21 mm.

3.2. Plant material

Flowering plants of *C. podocarpa* were collected from central district at Palapye-Bobonong, identified by Dr. L. M. Turton and a voucher specimen (Cp 003) was deposited at the University of Botswana Herbarium.

3.3. Extraction and isolation

Air-dried and pulverized plant material (210 g) was extracted sequentially with CH_2Cl_2/MeOH (1:1), MeOH and MeOH/H_2O (1:1). Removal of solvent from the combined extracts gave a brown residue (23 g), which was subjected to VLC and eluted with hexane, CH_2Cl_2 and MeOH in increasing polarity. The flavonoid glycosides were obtained from the 1:1 CH_2Cl_2/MeOH and MeOH fractions. These fractions were combined and applied to a Sephadex LH-20 column (eluted with 1:1 $CHCl_3$/MeOH) to give fractions A, B and C. Fraction C was concentrated and applied to polyamide-11 CC and eluted sequentially with H_2O, H_2O/MeOH (1:1), MeOH, MeOH/Me_2CO and Me_2CO. Fractions eluted with MeOH were evaporated to dryness, dissolved in MeOH/H_2O (17:8) or (68:32)

mixture and injected into HPLC (running conditions for HPLC: mobile phase-MeOH/H_2O 68:32, injection volume. 1 ml, flow rate 5 ml/min, RI detector) to yield **1** (100 mg) and **2** (56 mg). The extracts and the pure compounds were monitored using TLC-silica gel 60-F_{254} precoated Al sheets, using BAW (*n*-BuOH–HOAc–H_2O, 4:1:5) as developing solvent, visualized using UV (254 and 366 nm) and vanillin–sulphuric acid spray.

3.4. Compound *1*

Yellow solid, mp 245–248°C, $R_f = 0.67$ BAW (4:1:5), $R_t = 11$ min, $[\alpha]_D - 24.3$ (MeOH, *c* 1.27). UV λ_{max} MeOH nm: 268, 335; +NaOAc 266, 388; +NaOAc/H_3BO_3 267, 340; + NaOMe 254, 264, 390; +$AlCl_3$ 276, 297, 348, 384; +$AlCl_3$/HCl 276, 298, 344, 383. ESI-MS *m*/*z* (rel. int) 587.5 (10) $[M+Na]^+$, 565.5 (80) $[M+H]^+$, 433.4 (5) [M-apio]$^+$, 271.3 (100) [M-(gluc–apio)]$^+$. ^1H and ^{13}C NMR (see Table 1).

3.5. Compound *2*

Yellow powder, mp 240–244°C, $R_f = 0.67$ (BAW 4:1:5); $R_t = 15$ min, $[\alpha]_D - 23.0$ (MeOH, *c* 1.00). UV λ_{max} MeOH nm: 268, 325; +NaOAc 266, 325; +NaOAc/H_3BO_3 268, 325; +NaOMe 271, 295; +$AlCl_3$ 277, 300, 344, 381; +$AlCl_3$/HCl 277, 299, 339, 382. ESI-MS *m*/*z* 601.6 (22) $[M+Na]^+$, 579.5 (58) $[M+H]^+$, 447.4 (9) [M-apio]$^+$, 285.3 (100) [M-(gluc–apio)]$^+$. ^1H and ^{13}C NMR (see Table 1).

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