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Spinoside, New Coumaroyl Flavone Glycoside from *Amaranthus spinosus*

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Spinoside, new coumaroyl flavone glycoside was isolated from the *n*-butanol fraction of the mathanolic extract of the whole plant of *Amaranthus spinosus* and assigned the structure 7-*p*-coumaroyl apigenin 4-*O*-β-D-glucopyranoside (**1**) on the basis of spectroscopic techniques including 1D and 2D NMR spectroscopy. In addition α- xylofuranosyl uracil (**2**), β-D-ribofuranosyl adenine (**3**) and β-sitosterol glucoside (**4**) have also been isolated for the first time from this species.

Key words: *Amaranthus spinosus*, Amaranthaceae, Flavone glycoside

INTRODUCTION

The roots of *Amaranthus spinosus* Linn. (Amaranthaceae) are used in menorrhagia, gonorrhea, eczema, and colic, and used as lactagogue (Banerji, 1978). Leaves and roots are laxative, emollient, used on boils and as poultice on abscess (Baquar, 1989). Isolation of α-spinasterol, α-spinasterol octacosanoate, and saponins from this plant has previously been reported (Banerji, 1980). Phytochemical investigation on this plant has led to the isolation of spinoside, a rare new *p*-coumaroyl flavone glycoside (**1**), its structure being assigned on the basis of spectroscopic techniques including 1D and 2D NMR. In addition, α-xylofuranosyl uracil (**2**), β-D-ribofuranosyl adenine (**3**) and β-sitosterol glucoside (**4**) have also been isolated for the first time from this species.

MATERIALS AND METHODS

General

Optical rotations were measured on a JASCO DIP-360 polarimeter. Melting points were determined on Buchi melting point apparatus and are uncorrected. UV spectrum was recorded on Hitachi U-3200 spectrophotometer. IR spectra were recorded on FTIR-8900 Shimadzu spectrometer. The ^{1}H-, ^{13}C-NMR, HMQC, and HMBC spectra were recorded on Bruker spectrometers operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively. The chemical shift values are reported in ppm (δ) units and the coupling constants (*J*) are in Hz. MS and HR-MS were obtained on a JMS-HX-110 with a data system on JMS-DA 500 mass spectrometers. Aluminum sheets precoated with silica gel 60 F_{254} (20×20 cm, 0.2 mm thick; E-Merck) were used for TLC and flash silica gel (230–400 mesh) was used for column chromatography. Visualization of the TLC plates was carried out under UV at 254 and 366 nm and also by spraying ceric sulphate reagent with heating.

Plant material

The whole plant of *Amaranthus spinosus* Linn. was collected from Sawat district and identified by Mr. Habib Ahmed (Plant Taxonomist), Department of Botany, Govt. Post Graduate College Sawat, Pakistan. A voucher specimen has been deposited at the herbarium of the Botany Department of Post Graduate College, Sawat, Pakistan.

Extraction and isolation

The shade-dried whole plant (16 kg) of *Amaranthus spinosus* was extracted three times, seven days each, with methanol. The combined methanolic extract was evaporated *in vacuo*. The resulting residue (0.65 kg) was suspended in water and extracted successively with *n*-

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hexane, chloroform, and *n*-butanol. The *n*-butanol soluble fraction (150 g) was subjected to column chromatography (CC) over flash silica gel eluting with $CHCl_3$/MeOH (95:5, 90:10, 85:15, 75:25, 60:40) in increasing order of polarity. The fraction which eluted with $CHCl_3$/MeOH (90:10) was subjected to repeated column chromatography (CC) eluting with $CHCl_3$/MeOH (93:7) to afford compounds **2** (11 mg), **3** (9 mg), and **4** (60 mg). The fraction which was obtained from $CHCl_3$/MeOH (95:5) was then subjected to preparative TLC over silica gel ($CHCl_3$/MeOH; 87:13) to afford compound **1** (22 mg).

The compounds **2**, **3**, and **4** were identified as α-xylofuranosyl uracil, β-D-ribofuranosyl adenine and β-sitosterol glucoside, by comparison of their physical and spectral data with those reported in literature (Kwon *et al.*, 2003), (Collin, 1987), (Iribarren, *et al.*, 1983).

Spinoside (1)

Amorphous white solid, UV λ_{max}^{MeOH} nm: 225, 269, 318; IR ν_{max}^{KBR} cm^{-1}: 3390 (OH), 1685 (ester CO), 1654 (CO); $[\alpha]_D^{25}$ +14.5 (c= 0.025); HR-FABMS (negative) *m/z*: 577.1335 $[M-H]^+$ (calcd. for $C_{30}H_{25}O_{12}$: 577.1346); ^{1}H-NMR (300 MHz, DMSO-d_6) δ; 5.15 (1H, d, J = 7.1 Hz, H-1'''), 6.32 (1H, d, J = 15.8 Hz, H-2''), 6.46 (1H, d, J = 1.9 Hz, H-6), 6.64 (2H, d, J = 8.7 Hz, H-(3), H-(5), 6.79 (1H, d, J = 1.9 Hz, H-8), 6.82 (1H, s, H-3), 6.90 (2H, d, J = 8.7 Hz, H-3', H-5'), 7.35 (2H, d, J = 8.7 Hz, H-(2), H-(6)), 7.45 (1H, d, J = 15.8 Hz, H-3''), 7.91 (2H, d, J= 8.7 Hz, H-2', H-6'), 12.91 (1H, s, 5-OH); ^{13}C-NMR (75 MHz, DMSO-d_6): Table I.

Acid hydrolysis

Compound **1** (15 mg) in 10% HCl was refluxed for 40 minutes. The cooled reaction mixture was neutralized with 10% NaOH. The resulted aqueous mixture was extracted with ethyl acetate. The aqueous phase was concentrated and D-glucose was identified by Co-TLC using solvent system EtOAc:MeOH:H_2O:HOAc; 11:2:2:2, and the sign of its optical rotation ($[\alpha]_D^{20}$ = +52). It was further confirmed by comparing retention time of its TMS ether with standard sample in GC. The residue recovered from the organic phase was a binary mixture of two components which could be separated through preparative TLC using solvent system (1:3; EtOAc:n-hexane and pure EtOAc) and identified as apigenin (yellow needles, m.p. 351; lit. m.p. 352) and *p*-coumaric acid (amorphous solid, m.p. 210; lit. m.p. 210-213).

RESULTS AND DISCUSSION

Compound **1** was isolated from the *n*-butanol soluble fraction of *Amaranthus spinosus*. It was recognized as flavonoid glycoside from its positive reaction with Molish and Shinoda reagents (Shinoda *et al.*, 1928). The molecular

Table I. ^{13}C-NMR data of compound **1** (75 MHz, DMSO-d_6, δ ppm)

Carbon number	1 (δ)
C-1	
C-2	161.4
C-3	102.9
C-4	181.8
C-5	156.8
C-6	99.4
C-7	164.2
C-8	94.4
C-9	161.1
C-10	105.3
C-1′	120.8
C-2′	128.5
C-3′	115.9
C-4′	162.7
C-5′	115.9
C-6′	128.5
(1)	124.8
(2)	130.0
(3)	115.6
(4)	159.7
(5)	115.6
(6)	130.0
C-1″	166.4
C-2″	113.6
C-3″	144.9
C-1‴	99.4
C-2‴	73.0
C-3‴	76.2
C-4‴	70.0
C-5‴	76.9
C-6‴	62.0

formula was deduced from the HR-FABMS (negative mode) as $C_{30}H_{26}O_{12}$. The IR spectrum showed a strong band of a *p*-disubstituted phenyl ring at 830 cm^{-1} and a chelated carbonyl band at 1653 cm^{-1} flanked by a weak band around 1684 cm^{-1} for the cinnamoyl ester group. The nuclear magnetic resonance (^{1}H-NMR) spectrum confirmed the presence of para-substituted ring B showing AA'BB' pattern (doublets, at δ 7.91 and 6.90, J = 8.7 Hz). The olefinic proton α-to the carbonyl group resonated as singlet at δ 6.82 revealing the presence of hydroxyl group at C-5 which was subsequently confirmed by a downfield singlet of chelated hydroxyl at δ 12.91. The presence of doublets of *meta*-coupled protons at δ 6.79 and 6.46 (J = 1.9 Hz) confirmed the presence of 1, 2, 3, 5-substituted

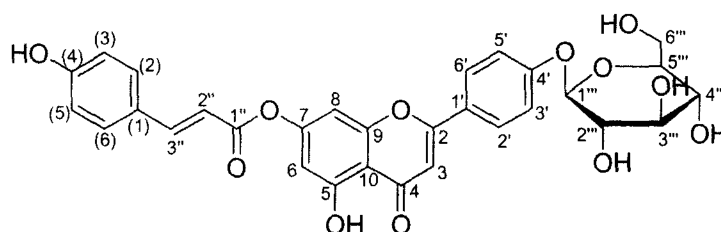

Fig. 1. Structure of Spinoside **1**

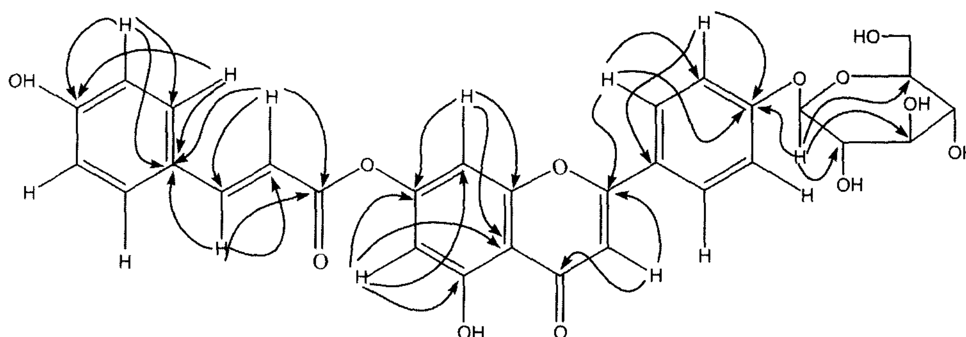

Fig. 2. Important HMBC correlations of **1**

ring A. The presence of coumaroyl moiety could be inferred from *trans* olefinic protons at δ 7.45 and δ 6.32 (d, J = 15.8 Hz) (Karl *et al.*, 1976) and *p*-hydroxyl phenyl ring showing another AA'BB' pattern (δ 7.35 and 6.64, each d, J = 8.7 Hz). The presence of hexose moiety was revealed by the signal of anomeric proton at δ 5.15 (d, J = 7.1 Hz) thereby confirming its β-linkage. Further signals of oxymethine and oxymethylene were observed at δ 3.85 (m, H-2'''), 3.75 (m, H-5'''), 4.15 (m, H-3'''), 4.25 (dd, J = 11.8, 5.0, H-6'''), 4.40 (dd, J = 11.8, 2.3, H-6'''). The ^{13}C-NMR spectra (BB and DEPT) showed the presence of 26 signals including one methylene, 14 methine, and 11 quaternary carbons. The conjugated carbonyls resonated at δ 181.8 and 166.4, besides the olefinic carbons at δ 161.4, 102.9, 144.9 and 113.6. The anomeric carbon was observed at δ 99.4 and further signals of hexose moiety were observed at δ 73.0 (C-2'''), 76.2 (C-3'''), 70.0 (C-4'''), 76.9 (C-5'''), 62.0 (C-6''').

The key to the structure of spinoside **1** was provided by acid hydrolysis which afforded, besides the sugar moiety, the aglycones identified as *p*-coumaric acid and apigenin, respectively. The sugar moiety could be identified as D-glucose through Co-TLC and sign of its optical rotation $[\alpha]_D^{20}$ + 52° (c, 10 in H_2O). The identity was further confirmed through comparison of retention time in gas chromatography of its TMS ether with a standard sample. Thus spinoside **1** is identified as derivative of apeginin. This was also confirmed by CIMS which showed fragment at *m/z* 417 $[M\text{-glu}+1]^+$ for hydroxyl cinnamoyl attached to the apigenin molecule. The fragment at *m/z* 271 $[M\text{-glu-coumaroyl}+1]^+$ is formed by the loss of glucose and coumaroyl moiety corresponding to $[M+1]^+$ of the apigenin. In addition, the presence of fragment at *m/z* 121 $[120+H]^+$ is due to apigenin after the RDA fission. The remaining problem was to locate the points of attachments of the sugar and *p*-coumaroyl moieties. The HMBC experiments showed ^3J correlation of the anomeric proton at δ 5.15 with C-4' (162.7), C-3''' (76.2), C-5''' (76.5), and ^2J correlations with C-2''' (73.0). This allowed us to assign the sugar moiety at C-4' of ring B and hence the *p*-coumaroyl moiety at C-7 of ring A. The important HMBC correlations reflected in Fig. 2 are in complete agreement to the assigned structure.

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