

Note

A new flavonol glycoside from the seeds of *Zea Mays*

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The flavonol glycoside, together with known compound quercetin has been isolated from the ethyl acetate extract of methanolic extract of the seeds of locally grown *Zea Mays*. The two isolated flavonoid compounds been have identified on the basis of UV, IR, ¹H NMR, ¹³C-NMR, COSY, DEPT 45, DEPT 90, DEPT 135 and mass spectral data.

Keywords: flavonol glycoside, quercetin, *Zea Mays*

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Zea Mays is a small tree, which grows in the tropical and sub tropical Asian countries, America and currently cultivated in sub-tropical countries of the world and in warm climates. It is locally known as 'Bhutta'. Maize is a potential allergen source from early infancy as it is an ingredient in infant's diets in many parts of the world. A relatively low degree of relation is known to exist between maize and the other cereals² although maize and rice antigens show similarities³. Recently, it is used as a diuretic in acute and chronic cystitis in the bladder irritation of uric acid and phosphatic gravel, gonorrhoea and hepatobiliary disease. The recent surge of interest in chemistry of this plant has led to the isolation of more than 20 components including flavonoids, terpenoids and caffeic acid derivatives with different biological activities^{4,5}. During the course of our investigation on the biologically active constituents of Labiatae plants, we examined the constituents of the seeds of *Zea Mays* widely cultivated in Bangladesh and isolated one flavonol glycoside **1**, together with one known flavonoid compound quercetin **2**. In this paper, we describe the isolation and structure elucidation of the new flavonol glycoside.

Experimental Section

IR spectra were recorded (KBr discs) on a FT-IR spectrophotometer, validation (ν_{\max} in cm^{-1}). ¹H NMR

spectra were recorded on a Bruker (400 MHz) instrument in DMSO-*d*₆ with TMS as an internal standard (chemical shifts in δ , ppm). UV spectra were recorded on a Hitachi, U-2000 spectrophotometer Ultraspeck in methanol (λ_{\max} in nm). TLC was performed with silica gel GF₂₅₄. All solvents were of analytical reagent grade.

Plant Material

The ripe seeds of *Zea Mays* were collected from the district Kushtia of Bangladesh in June 2004. The plant was identified and voucher specimen number was deposited at Bilik Herba of School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia with No. 10132.

Extraction

The dried and milled seeds (500 gm) were extracted with methanol (1L) in a soxhlet extractor for 72 hr. The extract was evaporated in a rotary evaporator and dried under vacuum. The methanolic extract (5 g) was suspended in water and extracted successively with petrol, hexane, chloroform and ethyl acetate to yield petrol (0.320 g), hexane (1.23 g), chloroform (1.50 g) and ethyl acetate-soluble (0.63 g) fractions, respectively. The ethyl acetate soluble fraction (1.26 g) was subjected to chromatography on silica gel (60-120 mesh, Merck) and eluted with hexane-acetone (7:2) solvent system. The major fractions were obtained from the column on further chromatography namely Fraction 1, 0.412 g; Fraction 2, 0.217 g and Fraction 3, 0.099 g.

Chromatographic separation

Fraction 1: The fraction (0.250 g) was chromatographed on LH-20 (160 × 5 cm; methanol) to yield fractions A-D. Fraction B (0.105 g) was rechromatographed on sephadex LH-20 (methanol) to yield compound **1** (4.3 mg).

Fraction 2: It was purified and crystallized from dil. methanol to give compound **2** as yellow crystals (5.9 mg).

Compound 1: Flavonol glycoside 1

It was crystallized from methanol-water as a yellow powder; m.p. 218°C; (M^+ , 664). UV: 255, 261, 278, 343 nm; IR: 3424, 3282, 2991, 2936, 1660, 1594, 1567,

1545, 1506, 1457, 1353, 1287, 1205, 1128, 1096, 1068, 100, 942, 728 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 0.988 (d, 3H, $J = 6.1$ Hz, rhamnosyl $-\text{CH}_3$), 3.33 (m, rhamnosyl glucose protons), 4.38 (t, 1H, rhamnosyl H-1), 5.05 (m, 1H, $J = 7$ Hz, glucosyl H-1), 6.19 (d, 1H, $J = 9$ Hz, H-6), 6.38 (d, 1H, $J = 9$ Hz, H-8), 7.53 (d, 1H, $J = 9$ Hz, H-2'), 7.55 (d, 2H, $J = 9$ Hz, H-5' and H-6'), 9.1, 9.6, 10.8 and 12.75 (4s, 4H, $-\text{OH} \times 4$); ^{13}C NMR (DMSO- d_6): δ 18.5 (C-6'''), 69.17 (C-5'''), 72.68 (C-4'''), 71.38 (C-3'''), 71.20 (C-2'''), 101.58 (C-1'''), 40.99 (C-6''), 74.90 (C-5''), 70.83 (C-4''), 94.40 (C-3''), 99.49 (C-2''), 102.01 (C-1''), 122.00 (C-6'), 117.05 (C-5'), 149.24 (C-4'), 145.00 (C-3'), 116.05 (C-2'), 134.13 (C-1'), 157.48 (C-2), 134.13 (C-3), 178.20 (C-4), 162.06 (C-5), 104.80 (C-6), 164.89 (C-7), 99.49 (C-8), 157.25 (C-9), 101.58 (C-10); MS: 633 $[\text{M}+\text{Na}]^+$, 611.1 $[\text{M}+\text{H}]^+$, 464.9 $[[\text{M}+\text{H}-\text{rhamnose}]^+$, 302.7 $[[\text{M}+\text{H}-\text{rhamnosylglucose}]^+$.

Compound 2: Quercetin 2

It crystallized from methanol-acetone as yellow needles (3 mg), m.p. 308°C (lit.⁶ m.p. 308-309°C); (M^+ , 302). It gave positive ferric chloride test. UV: 253, 278, 261, 370. IR: 3289, 3122, 2991, 2936, 1660, 1594, 1567, 1545, 1506, 1457, 1353, 1287, 1205, 1128, 1096, 1068, 100, 942, 728; ^1H NMR (DMSO- d_6): δ 6.20 (d, 1H, $J = 9$ Hz, H-6), 6.45 (d, 1H, $J = 9$ Hz, H-8), 7.59 (d, 1H, $J = 9$ Hz, H-2'), 7.62 (d, 2H, $J = 9$ Hz, H-5' and H-6'), 9.5, 10.2, 10.8, 12.75 and 13.02 (5s, 5H, $-\text{OH} \times 5$); ^{13}C NMR (DMSO- d_6): δ 146.7 (C-2), 135.7 (C-3), 175.8 (C-4), 160.25 (C-5), 98.01 (C-6), 163.4 (C-7), 93.4 (C-8), 156.34 (C-9), 102.21 (C-10), 121.95 (C-1'), 115.97 (C-2'), 145.32 (C-3'), 147.87 (C-4'), 115.62 (C-5'), 119.87 (C-6'); MS: 302 (M^+), 156, 137, 109. It was identified as quercetin 2.

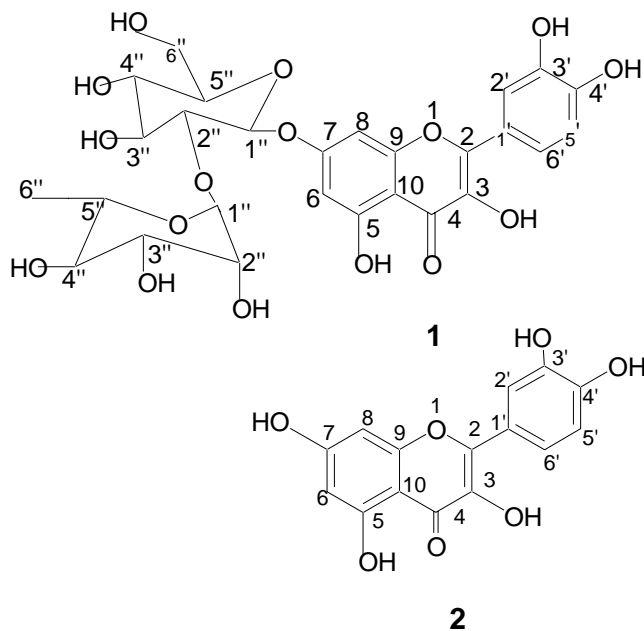
Hydrolysis of flavonol glycoside 1 (Quercetin)

To a solution of **1** (20 mg) in methanol (10 mL), 3 N HCl (25 mL) was added and boiled in a water bath for 15 min. It was diluted with water (150 mL) and extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried over anhydrous sodium sulphate and concentrated ten times. TLC examination of the residue showed several spots and the major product was purified by preparative TLC using chloroform-ethyl acetate (6:4) as developing solvent. It was crystallized from methanol-acetone as yellow needles (7.3 mg), m.p. 308°C (lit.⁶ m.p. 308-309°C); (M^+ , 302). It gave positive ferric chloride test. It was identified as quercetin on the basis of Co-UV, Co-IR, Co-NMR, m.m.p.

Results and Discussion

Maize is the only cereal of American origin and it formed the staple diet of the American Indians. The USA produces nearly 50% of the world's maize. Its popularity as a table vegetable is increasing as fresh, frozen and canned. It is widely used in starch manufacture and for whisky distilling. The methanol extract of the seeds of local *Zea Mays* was extracted successively with petroleum spirit, *n*-hexane, chloroform and ethyl acetate. The ethyl extract was subjected to column chromatography on silica gel eluted with a chloroform-methanol mixture. Repeated chromatography, preparative TLC and crystallisation, two flavonoids were isolated from the ethyl acetate fraction. Among these flavonoids, quercetin **2** has already been characterized in this plant⁶ (Scheme I). Previously several authors reported quercetin from different species. But Akowuan *et al.*⁶ reported the isolation and characterization of quercetin from *Gynura procumbens*.

Flavonol glycoside **1** was isolated from this plant for the first time in our laboratory. Flavonol glycoside **1** was obtained as yellow crystals. It had a m.p. 218°C. High-resolution mass spectrometry of compound **1** indicated the molecular formula $\text{C}_{27}\text{H}_{30}\text{O}_{16} \cdot 3\text{H}_2\text{O}$ (M^+ , 664). The compound turned dark green with FeCl_3 and pale red with $\text{Mg} + \text{HCl}$. IR spectra of compound **1** showed frequencies at 3424 and 1650 cm^{-1} indicating the presence of hydroxyl group and keto group in conjugation and the absorption peaks at 1605 and 1599 cm^{-1} indicate the presence of unsymmetric ethylenic



Scheme I

double bond and the aromatic rings, respectively. In ^1H NMR spectrum four singlets at δ 9.10, 9.64, 10.82 and 12.75 indicate the presence of four hydroxyl group at C-4', C-5, C-3' and C-7 on the aromatic rings. Two doublets at δ 6.19 and 6.38 indicate the presence of H-6 and H-8 protons. A broad doublet at δ 0.98 (6.1 Hz) indicating the presence of rhamnosyl methyl protons and a multiplet at δ 3.33 indicates the presence of rhamnosyl glucose protons, respectively. Two doublets at δ 7.53 and 7.55 indicating the presence of H-2', H-5' and H-6' in B-ring. The compound **1** on hydrolysis afford the parent compound querectin **2**. To the best of our knowledge this compound has not been previously reported. The compound **1** and querectin were confirmed by comparing their melting points with that obtained from the natural source⁶. In addition, spectral data and elemental analysis were consistent with the reported values⁶. The compound **2** and querectin were confirmed by comparing their melting points with that obtained from the natural source⁶. In addition, spectral data and elemental analysis were consistent with the reported values⁶.

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References

- 1 World Health Organisation, *Geneva Legal Status of Traditional Medicine and Complementary/Alternative Medicine*, A worldwide Review, **2001**, 129.
- 2 Kalveram K & Forck G, *Int Archs Allergy Appl Immunol*, **57**, **1978**, 549.
- 3 Hoffman D R, *Immunochem*, **12**, **1975**, 53
- 4 Don D D, Ham N N, Khac D H, Lam N T, Son P T, Dau N V, Grab M & Stjernstrom N E, *J Ethnopharmacology*, **36**, **1992**, 225.
- 5 Gruber J W, Slebert D J, Marderoslam A H D & Hock R S, *Phytochemical Analysis*, **10**, **1999**, 22.
- 6 Akowuan G A, Sadikun A & Mariam A, *J Trop Med Plants*, **2**, **2001**, 193.