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FLAVONOID GLYCOSIDES AND AN ANTHRAQUINONE FROM *RUMEX CHALEPENSIS*

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Key Word Index—*Rumex chalepensis*; Polygonaceae; flavonol glycosides; quercetin 3-glucosyl(1 → 4) galactoside; quercetin 3-rhamnosyl(1 → 6) glucoside; kaempferol 3-robinobioside; quercetin 3-rhamnoside; emodin.

Abstract—Besides rutin, quercetin 3-rhamnoside and kaempferol 3-rhamnosyl(1 → 6) galactoside, a new flavonol glycoside, quercetin 3-glucosyl(1 → 4)galactoside, and 1,6,8-trihydroxy-3-methyl anthraquinone (emodin) have been characterized from leaves of *Rumex chalepensis*. The structures were established on the basis of R_f values, acid hydrolysis to aglycone and sugar and UV, EI and FAB-mass spectra, ^{1}H NMR, ^{13}C DEPT NMR, NOE difference measurement, ^{1}H–H COSY and ^{1}H–^{13}C COSY spectral data.

INTRODUCTION

Rumex chalepensis Mill, is a perennial herb widely distributed in Pakistan. Plants belonging to the Polygonaceae are known to produce a large number of biologically important secondary metabolites, such as flavonoid glycosides [1], anthraquinones [2], steroids [3], leucoanthocyanidins and phenolic acids [4]. The genus *Rumex* has attracted the attention of many investigators because of its medicinal properties [5]. Some species of this genus have been reported to contain *C*-glycosylflavonoids and anthraquinones. For example *R. acetosa* [6, 7] and *R. cyprius* [8] contain acetyl derivatives of iso-orientin, and orientin and cyanidin 3-glucoside have been found in *R. crispus* [9] and *R. thrysiforus* [10]. In the present investigation of *R. chalepensis* we report one new flavonol glycoside (**1**), three known flavonol glycosides (**2**–**4**) and the anthraquinone emodin (**5**).

RESULTS AND DISCUSSION

Chromatographic separation of the EtOAc extracts of a concentrated aqueous ethanolic extract of air-dried leaves of *R. chalepensis* afforded three flavonol diglycosides (**1**–**3**) one flavonol monoglycoside (**4**) and an anthraquinone (**5**). Compounds **2**, **4** and **5** were characterized as quercetin 3-rutinoside, quercetin 3-rhamnoside and emodin, respectively, by standard procedures.

The flavonol glycosides **1** and **3** were obtained as yellow crystalline solids, which appeared violet on TLC under UV light, 366 nm, and turned yellow in NH_3. Acid hydrolysis with 2N HCl afforded quercetin and kaempferol, respectively (co-TLC, UV and EI-mass spectrometry), and glucose and galactose from **1** and rhamnose and galactose from **3**. The sugars were identified by co-chromatography in five solvent systems as well as by GC after silylation [11]. The position of attachment of the sugar moieties was determined by UV spectral analysis with the usual shift reagents [11, 12]. The glycosylation site in each case was found to be at C-3 (12–17 nm hypsochromic shift of band I in methanol with respect to the corresponding aglycone). On alkaline hydrolysis both compounds remained unchanged and no organic acid was detected, showing that they were not acylated. The nature of the sugar moieties was confirmed and their linkage determined by mass and NMR analysis.

An EI-mass spectral base peak $[M]^+$ was observed for **1** at *m/z* 302 (100%) along with other diagnostic fragments $[M - H]^+$ 301 (25.4%), $[M - CO]^+$ 274 (8.9%), $[A_1 + H]^+$ 153 (14.2%), $[B_2]^+$ 137 (17.6%) and $[B_2 - CO]^+$ 109 (9.5%), which confirmed quercetin as the aglycone. The sugar sequence was deduced by FAB-mass spectra recorded in positive mode in lactic acid. A molecular ion peak was observed at *m/z* 627 $[M + H]^+$. The fragment ions at 465 $[M + H - 162]^+$ and 303 $[M + H - 324]^+$ showed sequential loss of hexose.

In the ^{1}H NMR, spectrum of **1** two anomeric protons appeared at δ5.12 (1H, *d*, J = 6.0 Hz) and 5.20 (1H, *d*, J = 2.0 Hz) which indicated the presence of two sugars having a β-configuration. Other chemical shifts at δ6.15 (1H, *d*, J = 2.0 Hz), 6.31 (1H, *d*, J = 2.0 Hz), 7.60 (1H, *d*, J = 2.0 Hz) could be assigned to H-6, H-8 and H-2′, of the aglycone part (see Table 1). Chemical shifts in the ^{13}C NMR spectrum of **1** were similar to the reported values for quercetin [13]. Anomeric carbons of the sugar moieties appeared at δ104.8 and 101.3 (Table 2) Carbon-13 chemical shifts for the sugars, i.e glucose and galactose,

Table 1. ^{1}H NMR chemical shifts (δ from TMS) of compounds **1** and **3** in CD_3OD

H	1	3
H-6	6.15 *d*, J = 2.0 Hz	6.10 *d*, J = 2.0 Hz
H-8	6.31 *d*, J = 2.0 Hz	6.25 *d*, J = 2.0 Hz
H-2′	7.60 *d*, J = 2.0 Hz	8.09 *d*, J = 9.0 Hz
H-3′		6.90 *d*, J = 9.0 Hz
H-5′	6.90 *d*, J = 8.0 Hz	6.90 *d*, J = 9.0 Hz
H-6′	7.32 *dd*, J = 2 and 9 Hz	8.09 *d*, J = 9.0 Hz
H-1″	5.12 *d*, J = 6.0 Hz	5.03 *d*, J = 6.0 Hz
H-1‴	5.20 *d*, J = 2.0 Hz	5.38 *d*, J = 2.0 Hz
Me		0.90 *d*, J = 6.0 Hz

Table 2. ^{13}C NMR chemical shifts (δ from TMS) of compound **1** (in pyridine-d_5) and compound **3** (in CD_3OD)

C	1	3
C-2	158.2	157.7
C-3	135.8	135.2
C-4	178.9	178.5
C-5	162.7	162.1
C-6	101.3	102.5
C-7	166.3	166.0
C-8	95.7	96.0
C-9	158.9	159.1
C-10	104.5	105.1
C-1′	123.1	122.6
C-2′	116.6	132.0
C-3′	145.8	116.4
C-4′	150.0	162.0
C-5′	116.7	116.4
C-6′	122.9	132.0
C-1″	101.3	102.5
C-2″	71.2	71.9
C-3″	73.2	73.2
C-4″	78.3	69.0
C-5″	75.1	75.7
C-6″	61.9	66.9
C-1‴	104.8	101.7
C-2‴	75.2	72.1
C-3‴	77.1	71.9
C-4‴	70.0	72.3
C-5‴	78.1	69.0
C-6‴	62.5	17.7

were also similar to their reported resonance values [14] except for an 8.5 ppm downfield shift of C-4″ of hexose. This downfield shift shows that either glucose or galactose is a terminal sugar and that they are linked to each other at C-4″. Partial acid hydrolysis afforded quercetin 3-galactoside and glucose, which proved the structure of **1** to be quercetin 3-*O*-β-D-glucopyranosyl(1 → 4)-β-D-galactoside. The (1 → 2) and (1 → 6) isomers have been described but this is the first report of a (1 → 4)-linked disaccharide attached to quercetin.

A 100% base peak $[M]^+$ for **3** was observed at m/z 286 in the EI-mass spectrum along with other diagnostic fragments for kaempferol. The positive FAB-mass spectrum gave a molecular ion peak at m/z 595 $[M + H]^+$, and fragment ions at 449 $[M + H - 146]^+$ and 287 $[M + H - 308]^+$, which indicated sequential loss of rhamnose and galactose moieties. Anomeric carbons of the sugar moieties appeared at 102.5 and 101.7 indicating *O*-linked sugars. The downfield shift of C-6″ of galactose, which appeared 5.0 ppm downfield, showed (6 → 1) linkage of galactose and rhamnose which established the structure as kaempferol 3-*O*-α-L-rhamnopyranosyl (1 → 6)-β-D-galactopyranoside. This diglycoside, also known as kaempferol 3-robinobioside, was first isolated from the leaves of *Strychnos variablilis* [15]. The structure of **3** was further confirmed by partial acid hydrolysis with 0.1 N H_2SO_4, giving kaempferol 3-galactoside and rhamnose.

EXPERIMENTAL

General. UV shift reagents were prepared according to ref. [11]. TLC was carried out on pre-coated silica gel 60 F_{254}, cellulose and polyamide 11 F_{254} (20 × 20 cm) glass plates from Merck. The following solvent systems were employed. $CHCl_3$–MeOH–H_2O (12:4:1) and (36:22:5) for silica gel, 15% HOAc, 60% HOAc, BAW: *n*-BuOH–HOAc–H_2O (4:1:5), BEW: *n*-BuOH–EtOH–H_2O (4:1:2.2), EPAW: EtOAc–pyridine–HOAc–H_2O (36:36:7:21) and BBPW: *n*-BuOH–C_6H_6–pyridine–H_2O (5:1:3:3) for cellulose and H_2O–MeOH–*n*-BuOH–HOAc (75:15:10:2) for polyamide. Compounds were revealed in UV light at 254 and 366 nm. For CC, Polyamide SC6 (0.07 mm) Macherey Nagel (Germany) and Sephadex LH-20 were used. Purity of compounds was checked by HPLC on a Zorbax ODS C_{18} (25 cm × 4.6 mm i.d.) column at a flow rate of 1.7 ml min^{-1}, UV detection at 280 nm, using an isocratic solvent system MeOH–H_2O–HOAc (4:20:1). Sugar analysis was carried out by GC on SE-54 (25 m × 0.25 mm i.d.) at a flow rate of 4 ml min^{-1} using N_2 as carrier gas. FAB-MS were recorded in the positive ion mode using lactic acid as solvent. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz, respectively. TMS was used as int. standard.

Plant material. Rumex chalepensis was collected in April 1993 from Islamabad, Pakistan and authenticated by Dr Mir Ajab Khan of the Department of Biology, Quaid-i-Azam University, Islamabad, where a voucher

specimen (no. 66845) has been deposited in the Herbarium.

Extraction. Soluble compounds were exhaustively extracted from 1 kg air-dried leaves of the plant with 80% EtOH. Extracts were concd in H_2O under vacuum, defatted with $CHCl_3$, and repeatedly extracted with EtOAc. The EtOAc extract was evapd to dryness and the dry residue (4.72 g) dissolved in MeOH. The methanolic soln was subjected to CC on polyamide. Gradient elution was made with H_2O with increasing concentrations of MeOH. Thirty frs were collected. Frs 7–12, 13–17, 18–25 and 26–30 were combined. The compounds present in these frs were purified by a combination of prep. TLC on silica gel in $CHCl_3$–MeOH–H_2O (12:4:1) and CC on Sephadex LH-20 to afford **1** (16 mg), **2** (30 mg), **3** (9 mg), **4** (80 mg) and **5** (15 mg).

Acid hydrolysis. Each flavonoid glycoside (**1**–**4**) (3 mg) was refluxed in 2 N HCl (5 ml) for 1 hr. The aglycones were extracted with EtOAc and identified by co-TLC with authentic samples of quercetin and kaempferol, and by UV spectral analysis. The sugars were isolated from the aq. layer in the usual way and identified by co-TLC with authentic markers on cellulose, BAW, BEW, EPAW and BBPW. The nature of sugars was confirmed by GC, of their TMSi derivatives [11].

Partial hydrolysis. Mild acid hydrolysis with 0.1 N H_2SO_4, was used for partial hydrolysis of diglycosides.

Identification of flavonoids. Quercetin 3-O-*β*-D-*glucopyranosyl* (1 → 4)-*β*-D-*galactoside* (**1**). TLC cellulose, BAW (system 1), 15% HOAc (System 2), 60% HOAc (system 3): R_f (× 100) 51, 29 and 68, respectively; silica gel, $CHCl_3$–MeOH–H_2O (system 4): R_f (× 100) 54; polyamide, C_6H_6–MEK–MeOH (system 5): R_f (× 100) 05. UV λ_{max}^{MeOH} nm: 257, 269(sh), 327(sh), 355; + NaOMe 273, 327, 408; + $AlCl_3$ 274, 304(sh), 431; + $AlCl_3$–HCl 268, 303 362(sh), 402; + NaOAc 274, 327, 382; + NaOAc–H_3BO_3 260, 297, 377. EIMS *m/z* (rel. int.): 302 (100), 301 (25.4), 274 (8.9), 153 (14.2), 137 (17.6) 109 (9.5). FAB-MS: (lactic acid, positive ion mode) *m/z* 627 $[M + H]^+$, 465 $[M + H - 162]^+$, 303 $[M + H - 324]^+$. ^1H and ^{13}C NMR: see Tables 1 and 2.

Kaempferol 3-*O*-*α*-L-*rhamnopyranosyl* (1 → 6)-*β*-D-*galactopyranoside* (**3**). TLC (systems 1-5 R_f (× 100) 77, 30, 76, 68 and 14, respectively. UV λ_{max}^{MeOH} nm: 265, 315(sh), 351; + NaOMe 274, 301(sh), 397; + $AlCl_3$ 274, 301, 354, 400; + $AlCl_3$–HCl 275, 299, 348, 400; + NaOAc 274, 318(sh) 384; + NaOAc–H_3BO_3 268, 319(sh) 354. EIMS *m/z* (rel. int.): 286 (100), 285 (32.1), 258 (12), 153 (80), 152 (13), 121 (19.1) 93 (4.9). FAB-MS: *m/z* 595 $[M + H]^+$, 449 $[M + H - 146]^+$, 287 $[M + H - 308]^+$. ^1H and ^{13}C NMR: see Tables 1 and 2.

1,6,8-*Trihydroxy*-3-*methyl anthraquinone* (*emodin*) (**5**). UV, EIMS, FAB-MS, ^1H and ^{13}C NMR values were identical to those reported in the literature.

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