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Flavonol Glycosides from Leaves of *Bergenia himalaica*

AURANGZEB HASAN*, AJAZ HUSSAIN and MIR AJAB KHAN†
Department of Chemistry
Quaid-i-Azam University, Islamabad-45320, Pakistan
Fax: (92)(51)2873869; Tel: (92)(51)9219811
E-mail: flavonoids@hotmail.com

A new flavonol triglycosides, quercetin 3-O-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→6)-β-D-glucopyranoside (1) was isolated from the leaves of *Bergenia himalaica* together with three known flavonol diglycosides: kaempferol-3-O-α-L-rhamnopyranosyl-7-O-α-L-arabinopyranoside (2), quercetin-3-O-β-D-xylopyranosyl (1→6)-β-D-glucopyranoside (3), quercetin-3-O-α-L-arabinopyranosyl (1→2)-β-D-glucopyranoside (4) and five known flavonol monoglycosides: kaempferol-7-O-α-L-rhamnopyranoside (5), kaempferol-7-*O*-β-D-allopyranoside (6), quercetin-3-O-β-D-xylopyranoside (7), quercetin-3-O-β-D-gluco-pyranoside (8) and quercetin-3-O-α-L-rhamnopyranoside (9). Their structures were determined on the basis of R_f values, acid hydrolysis, UV-Visible EIMS, FAB^+ MS, ^1H and ^{13}CNMR spectral data.

Key Words: Flavonol glycosides, *Bergenia himalaica* leaves.

INTRODUCTION

Bergenia himalaica Borris (Saxifragaceae) is a medicinal herb commonly known as Zakham-e-hayat or Zakhmi boti. It is distributed in the temperate Himalayas and in Central and East Asia. In Pakistan, this plant species is widely distributed in the Murree Hills and Nathia Gali at an altitude of about 8000 feet. The genus *Bergenia* has attracted the attention of many investigators because species of this genus have been reported to contain a number of secondary metabolites, such as flavonoid glycosides[1], anthraquinones[2], tanins[3], arbutin and phenolic compounds[4, 7], bergenin[5] and lactone[6]. For example, *B. crassifolia* contain 3-O-monoglycoside and 3-O-diglycoside of kaempferol and quercetin and bergenin[1, 5]. A large number of anthraquinones such as aloe emodin, physcion, aloe emodin 8-O-β-glucosides, chrysophanein and emodin 1-O-β- glucopyranoside have been isolated from air-dried roots of *Bergenia hissarica*[2]. Four new polyphenolic compounds were isolated from rhizome of *Bergenia ciliata*[7]. Although locally this plant species is widely used in a number of ailments, but no chemical constituents have been studied. In the present paper, the isolation

†Department of Biology, Quaid-i-Azam University, Islamabad 45320, Pakistan.

and characterization of nine flavonoid glycosides from the leaves of *B. himalaica* have been reported first time.

EXPERIMENTAL

UV shift reagents were prepared as reported and UV-Visible spectral analysis was carried out on a Shimadzu model 1601 spectrophotometer. TLC was carried out on polyamide (Riedel-de-Haën 6DF, Germany). The following solvent systems were employed: (System 1) $C_6H_5CH_3$-MEK-MeOH (4 : 3 : 3) and (System 2) H_2O-MeOH-MEK-acetylacetone (13 : 3 : 3 : 1). Compounds were revealed in UV light at 366 nm. For CC, polyamide (Riedel-de-Haën 6S, Germany) and lipophilic sephadex LH-20 (Sigma, Switzerland) were used. Purity of compounds was checked by Shimadzu LC-6A HPLC on ODS nucleosil C_{18} (25 cm × 4.6 mm i.d.) column at a flow rate of 0.8 mL/min using a gradient system, H_2O—AcOH (1000–20) and CH_3CN—H_2O—AcOH (800–200–20). Detection was carried out at 280 nm on a UV detector. Sugar analysis was carried out according to standard procedure[9] on Whatman paper No.1 along with authentic standard sugars in three different solvent systems *n*-BuOH-AcOH-H_2O (4 : 1 : 5), *n*-BuOH-EtOH-HO (4 : 1 : 2.2) and *n*-BuOH-AcOH-Et_2O-H_2O (9 : 6 : 3 : 1) as well as by Shimadzu GC-9A on SE-54 column (25 m × 0.25 mm i.d.) at a flow rate of 4 mL/min using N_2 as carrier gas. FAB^+ MS was recorded on a Double Focusing Varian MAT-312 spectrometer connected to an MAT-188 computer system in the positive ion mode using lactic acid as solvent. EIMS were recorded on Shimadzu QP1000A. ^1H and ^{13}C NMR spectra were recorded at 400 MHz on Brucker WM300. TMS was used as an internal standard.

Leaves of *Bergenia himalaica* were collected in August 2002, at Nathia Gali, Pakistan and authenticated by one of us (MAK). A voucher specimen (no. 3429) has been deposited in the herbarium, Department of Biological Sciences, Quaid-i-Azam University, Islamabad.

Extraction of flavonoid glycosides: Dried leaves (500g) of *Bergenia himalaica* were repeatedly extracted with MeOH, MeOH-H_2O (8 : 2), MeOH-H_2O (5 : 5) by stirring at room temperature, for 24 h in each solvent. The combined hydro-alcoholic extracts were evaporated to dryness under reduced pressure between 30–35°C. The dried extract was dissolved in hot distilled H_2O defatted with petroleum-ether 40–60 (no non-polar polyphenolic compounds were detected by TLC in the petroleum-ether extract) and repeatedly extracted with *n*-BuOH. The *n*-BuOH extract was evaporated to dryness and the dried residue (4.1 g) dissolved in MeOH. The methanolic solution was subjected to CC on polyamide. The column was run in the gradient mode from H_2O to MeOH. Fifty-four fractions were collected. Identical fractions were combined and compounds present therein were purified by a combination of PTLC in $C_6H_5CH_3$-MEK-MeOH (4 : 3 : 3) and 2D-TLC in $C_6H_5CH_3$-MEK-MeOH (4 : 3 : 3) and H_2O-MeOH-MEK-acetylacetone (13 : 3 : 3 : 1) on polyamide and CC on polyamide and sephadex LH-20. As a result of CC, TLC, PTLC and 2DTLC seven compounds were obtained and their purity was checked by TLC. Purity of each of the seven-flavonoid glycosides was further examined by Shimadzu LC-6A

HPLC coupled to a Shimadzu SPD-6AV UV detector. Glycosides (1–6) were found pure enough to be used for their characterization by spectroscopic techniques; however, flavonoid glycoside (7 appeared to be a mixture of three glycosides. This mixture of flavonoid glycoside was further purified by preparative HPLC using an ODS nucleosil (25 cm × 10.0 mm i.d.) column. Compound 7 was thus resolved into three flavonoid glycosides (7–9). As a result of CC, PTLC, 2D-TLC and HPLC, leaves of *Bergenia himalaica* yielded nine flavonoid glycosides.

Acid hydrolysis: Flavonoid glycoside (3 mg each) was refluxed in 2 N HCl (5 mL) for 1 h. The aglycones were extracted with EtOAc and identified by co-TLC (with authentic samples of kaempferol and quercetin) and UV spectral analysis. The sugars were isolated from the aqueous layer in the usual way and identified by PC and GC.

Alkaline hydrolysis: 1 M NaOH, room temperature, 3 h in the absence of air to avoid oxidation[9].

RESULTS AND DISCUSSION

The crude *n*-BuOH extract of *B. himalaica* leaves was subjected to column chromatography on polyamide, sephadex LH-20 and HPLC to afford **1** (21 mg), **2** (25 mg), **3** (18 mg), **4** (29 mg), **5** (18 mg), **6** (15 mg), **7** (7 mg), **8** (9 mg) and **9** (7 mg). Compounds **2–9** were characterized as kaempferol-3-O-α-L-rhamnopyranosyl-7-O-α-L-arabinopyranoside (**2**), quercetin-3-O-β-D-xylopyranosyl (1→6)-D-glucopyranoside (**3**), quercetin-3-O-α-L-arabinopyranosyl (1→2)-β-D-glucopyranoside (**4**) and five known flavonol monoglycosides: kaempferol-7-*O*-β-L-rhamnopyranoside (**5**), kaempferol-7-O-β-D-allopyranoside (**6**), quercetin-3-O-β-D-xylopyranoside (**7**), quercetin-3-O-β-D-glucopyranoside (**8**) and quercetin-3-O-α-L-rhamnopyranoside (**9**) by standard procedures[8–10] and have been reported collectively in a hand book on natural flavonoids.

The flavonol glycoside (**1**) was obtained as a dark yellow crystalline solid, which appeared violet on TLC under 366 nm UV light and turned yellow in ammonia. Acid hydrolysis with 2 N HCl afforded quercetin (co-TLC, UV, EIMS and ^{1}H NMR) as the aglycone part and glucose, galactose and rhamnose as the sugar moieties. The sugars were identified by co-chromatography in five solvent systems as well as by GC after trimethylsilylation.

The UV spectrum of **1** recorded in MeOH showed two absorption maxima at 355 and 257 nm. This range of absorption is typical for 3-O-substituted. Presence of a free hydroxyl group at position 7 of ring A was indicated by the addition of sodium acetate (NaOAc) which exhibited a 17 nm bathochromic shift in band II with respect to the methanol spectra. A bathochromic shift of 22 nm in the presence of sodium acetate/boric acid (NaOAc/H_3BO_3) showed the presence of 3′,4′-orthodihydroxylation. A bathochromic shift of 76 nm with 5% methanolic solution of aluminum chloride and a decrease of 29 nm after the addition of hydrochloric acid indicated the presence of free hydroxyl group at position 5 of ring A and 3′,4′-orthodihydroxylation at ring B. UV spectra recorded in MeOH

and on addition of diagnostic shift reagents, suggested compound 1 to be a 3-O-substituted flavonol with all the three sugars (glucose, galactose and rhamnose) attached at position C-3 of the aglycone.

An EI-mass spectral base peak [M^+] was observed for **1** at m/z 302 (100%) along with other diagnostic fragments $[M\text{-}H]^{2-}$ 301 (26), $[M\text{-}CO]^+$ 274 (9), $[Al_2+H]^+$ 153 (14) and $[Br_2]^+$ 137 (18) which confirmed quercetin as the aglycone. The sugar sequence of **1** was determined by FAB-MS recorded in positive mode in lactic acid, which showed a molecular ion peak at m/z 773 $(M+H)^+$. Other diagnostic fragment ions appeared at m/z 627 $[M+H\text{-}146]^+$, m/z 465 $[M+H\text{-}146\text{-}162]^+$ and m/z 303 $[M+H\text{-}146\text{-}162\text{-}162]^+$ indicating successive loss of a pentose and two hexoses, *i.e.*, rhamnose, galactose and glucose.

The ^{1}H NMR spectrum of 1 showed three anomeric proton signals at δ5.45 (1H, d, J = 7.1 Hz) assignable to 1-H β-glucoside proton, 5.57 (1H, d, J = 8 Hz) assignable to 1-H β-galactosyl proton and 5.05 (1H, d, J = 2.0 Hz) assignable to 1-H rhamnosyl proton. Rest of sugar protons resonated in the range of 3.33-4.50 ppm. CH_3 of rhamnose appeared at 1.25 (3H, d, J = 6.0 Hz) (Table-1). ^{13}C NMR of **1** was similar to the reported values for quercetin[12]. Anomeric carbons of glucose, galactose and rhamnose appeared at δ 101.3, 104.8 and 100.6 respectively. ^{13}C NMR signals were similar to their reported values except for a 5.9 ppm downfield shift of C-6″ of glucose and 6.8 ppm down fieldshift of C-2‴ of galactose. This downfield shift established the 1→6 linkage between galactose and glucose and a 1→2 linkage between rhamnose and galactose (Table-2) On the basis of FAB$^+$MS, ^{1}H NMR, and ^{13}C NMR spectroscopic data, compound **1** was identified as quercetin 3-O-α-L-rhamnopyranosyl(1→2)-β-galactopyranosyl (1→6)-β-D-glucopyranoside (Fig. 1) which is a new linear triglycoside of quercetin. Linear and branched triglycosides of kaempferol and quercetin have been reported in literature[14, 15].

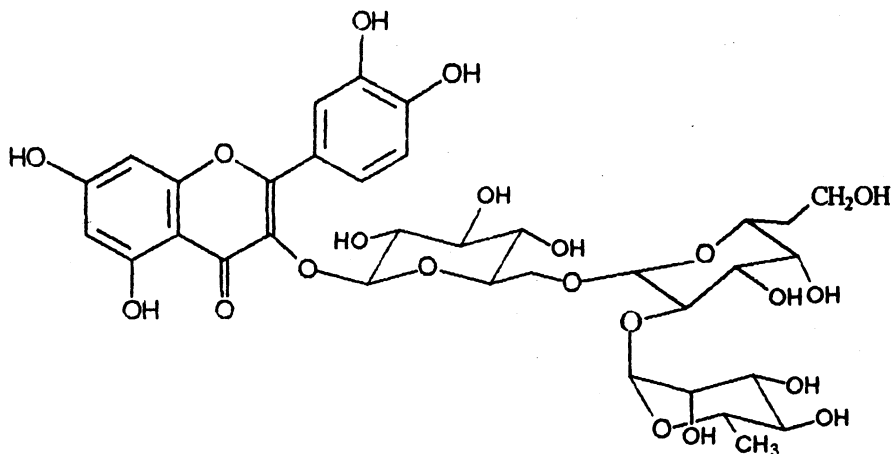

Fig. 1. Structure of quercetin 3-O-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→6)-β-D-glucopyranoside (1)

TABLE-1
^{1}H NMR SPECTRAL DATA OF COMPOUND 1 IN MeOH-d_4 AT 300 MHz

H	1	H	1
H-6	6.17 *d* j = 2.2 Hz	H-1″	5.45 *d* J = 7.1 Hz
H-8	6.37 *d* J = 2.2 Hz	H-1‴	5.57 *d* J = 8.0 Hz
H-2′	7.62 *d* J = 9.0 Hz	H-1⁗	5.05 *d* J = 2.0 Hz
H-5′	6.90 *d* J = 9.1 Hz	CH_3 (rham.)	1.25 *d* J = 6.0 Hz
H-6′	7.32 *dd* J = 2 and 9.1 Hz		

TABLE-2
^{13}C NMR SPECTRAL DATA OF COMPOUND 1 IN MeOH-d_4 AT 75.5 MHz

Aglycone		Sugars	
C	1	C	1
C-2	158.2	C-1″	101.3
C-3	135.8	C-2″	74.3
C-4	178.9	C-3″	76.7
C-5	162.7	C-4″	70.2
C-6	101.3	C-5″	77.2
C-7	166.4	C-6″	67.8
C-8	95.7	C-1‴	104.8
C-9	158.7	C-2‴	78.1
C-10	104.5	C-3‴	73.7
C-1′	123.1	C-4‴	71.2
C-2′	116.6	C-5‴	75.2
C-3′	145.8	C-6‴	61.9
C-4′	150.0	C-1⁗	100.6
C-5′	116.6	C-2⁗	71.8
C-6′	122.9	C-3⁗	72.2
		C-4⁗	73.7
		C-5⁗	68.1
		CH_3	18.1

Similarly, the structures of the remaining 8-flavonoid glycosides isolated from the leaves of *B. himalaica* were characterized. However, the characterization was only based on acid hydrolysis to aglycone and sugar(s) UV-Visible and EIMS, because the NMR spectral data of these flavonoids is already reported in literature[16–22]

Quercetin 3-O-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→6)-β-D-glucopyranoside (1): TLC polyamide (system 1): R_f (× 100) 55, (system 2): R_f (× 100) 31. UVλ_{max} 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. EI-MS m/z (rel. int.): 302 (100%), 301 (26), 274 (9), 153 (15), 137 (18), 109 (10). FAB-MS (lactic acid positive ion mode) m/z 773 $(M+H)^+$, 627 $[M+H-146]^+$, m/z 465 $[M+H-308]^+$, 303 $[M+H-470]^+$. ^1H and ^{13}C NMR: (Tables 1 and 2).

Kaempferol-3-O-α-L-rhamnopyranosyl-7-O-α-L-arabinopyranoside (2): TLC polyamide, (system 1): R_f (× 100) 49, (system 2): (× 100) 22. UV λ_{max} nm: 265, 315 (sh), 351; +NaOMe 274, 301 (sh), 397; +$AlCl_3$-HCl 274, 301, 354, 400; +$AlCl_3$-HCl 275, 299, 348, 400; +NaOAc 265, 318 (sh), 358, 406 (sh); +NaOAc-H_3BO_3 268, 319 (sh), 354. EI-MS m/z (rel. int.): 286 (100), 285 (32.1), 258 (12), 153 (80), 121 (19.1) and 93 (4.9).

Quercetin-3-O-α-L-xylopyranosyl(1→6)-β-D-glucopyranoside (3): TLC polyamide, (system 1): R_f (× 100) 50, (system 2): R_f (× 100) 29. UV λ_{max}nm: 256, 269 (sh), 329 (sh), 357; +NaOMe 275, 327, 412; +$AlCl_3$-HCl 274, 304 (sh), 435; +$AlCl_3$-HCl 269, 303, 362 (sh) 404; +NaOAc 274, 327, 384; +NaOAc-H_3BO_3 260, 293, 379. EI-MS m/z (rel. int.); 302 (100), 301 (25.2), 274 (9.7), 153 (15.3), 137 (18.9) and 109 (10.5).

Quercetin-3-O-α-L-arabinopyranosyl(1→2)-β-D-glucopyranoside (4): TLC polyamide, (system 1): R_f (× 100) 54, (system 2): R_f (× 100) 28. UV λ_{max}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. EI-MS m/z (rel. int.): 302 (100), 301 (24.8), 274 (9.2), 153 (14.3), 137 (18.4) and 109 (9.5).

Kaempferol-7-O-β-L-rhamnopyranoside (5): TLC polyamide, (system 1): R_f (× 100) 45, (system 2): R_f (× 100) 18. UV λ_{max} nm: 266, 323, 364; +NaOMe 245, 267, 335 (sh), 424; +$AlCl_3$ 259 (sh), 266, 301, 354, 425; +$AlCl_3$-HCl 259 (sh), 266, 350, 422; +NaOAc 268, 325, 384, 419 (sh); +NaOAc-H_3BO_3 265 (sh), 319 (sh), 370. EI-MS m/z (rel. int.): 286 (100), 285 (32.2), 258 (12.3), 153 (65.2), 121 (18.7) and 93 (5.9).

Kaempferol-7-O-β-D-allopyranoside (6): TLC polyamide (system 1): R_f (× 100) 46, (system 2): (× 100) 15. UV λ_{max} nm: 265, 315 (sh), 351; +NaOMe 274, 301 (sh), 397; +$AlCl_3$ 274, 301, 354, 400; +$AlCl_3$-HCl 275, 299, 348, 400; +NaOAc 268, 318 (sh), 384; +NaOAc-H_3BO_3 268, 319 (sh), 354. EI-MS m/z (rel. int.): 286 (100), 285 (32.1), 258 (12), 153 (80), 121 (19.1) and 93 (4.9).

Quercetin-3-O-α-L-xylopyranoside (7): TLC polyamide (system 1): R_f (× 100) 48, (system 2): R_f (× 100) 16. UV λ_{max} nm: 257, 269 (sh), 300 (sh), 361; +NaOMe 273, 327, 408; +$AlCl_3$ 274, 304 (sh), 331 (sh) 436; +$AlCl_3$-HCl 268, 303, 365 (sh), 402; +NaOAc 274, 327, 382; +NaOAc-H_3BO_3 263, 297 (sh), 377. EI-MS m/z (rel. int.): 302 (100), 301 (24.8), 274 (9.2), 153 (14.3), 137 (18.4) and 109 (9.5).

Quercetin-3-O-β-D-glucopyranoside (8): TLC polyamide (system 1): R_f (× 100) 48, (system 2): R_f (× 100) 16. UV λ_{max} nm: 257, 269 (sh), 300 (sh), 361; +NaOMe 273, 327, 408; +$AlCl_3$ 274, 304 (sh): 331(sh) 436; +$AlCl_3$-HCl

268, 303, 365 (sh) 402; +NaOAc 274, 327, 382; +NaOAc-H_3BO_3 263, 297 (sh), 377. EI-MS m/z (rel. int.); 302 (100), 301 (24.6), 274 (10.2), 153 (14.9), 137 (19.2) and 109 (9.7).

Quercetin-3-O-βL-rhamnopyranoside (9): TLC polyamide (system 1): R_f (× 100) 45, (system 2): (× 100) 19. UV λ_{max} nm: 259, 269 (sh), 356; + NaOMe 273, 337, 411; +$AlCl_3$ 278, 310 (sh), 344 (sh) 436; + $AlCl_3$-HCl 268, 303 (sh), 375, 404; +NaOAc 274, 327 (sh), 392; +NaOAc-H_3BO_3 263, 381. EI-MS m/z (rel. int.): 302 (100), 301 (20.5), 274 (11.3), 153 (13.9), 137 (18.4) and 109 (7.9).

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