A New Flavanone Glycoside from Leaves of *Impatiens balsamina*

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Summary A new flavanone glycoside, naringenin-4′-O-β-D-glucuronopyranoside (**1**) was isolated from leaves of *Impatiens balsamina* together with a known but rare flavanone monoglycoside: naringenin 4′-O-β-D-glucopyranoside (**2**), four rare flavonol diglycosides: quercetin 3-O-α-L-rhamnopyranosyl (1→6)-β-D-galactopyranoside (**3**), kaempferol-3-O-α-L-rhamnopyranosyl-7-O-α-L-arabinopyranoside (**4**), quercetin-3-O-β-D-xylopyranosyl (1→6)-β-D-glucopyranoside (**5**), quercetin-3-O-α-L-arabinopyranosyl (1→2)-β-D-glucopyranoside (**6**) and three flavonol monoglycosides: kaempferol-7-O-α-L-rhamnopyranoside (**7**), kaempferol-7-O-β-D-allopyranoside (**8**) and quercetin-3-O-β-D-glucopyranoside (**9**) Their structures were determined by means of co-chromatography, UV, EI-MS, FAB-MS and ^{1}HNMR spectroscopy

Introduction

Impatiens balsamina Linn. (Balsaminaceae) is an annual herb, 45-60 cm tall. It is distributed in northern areas of Pakistan. This plant species is widely distributed in Murree, Nathia gali and Miran Jani hills. The genus *Impatiens* is reported to be rich in organic acids, anthraquinones and flavonoids. Three monoglucosides of kaempferol, quercetin and pelargonidin have been reported from the stem of *I. balsamina* [1] Pelargonidin and peonidin as glucosides have been characterized from *I. holstii* [2]. Bhom et al. [3] extracted and purified salicylic acid, sinnapic acid, caffeic acid, scopoletin, 2-hydroxy-1,4-napthaquinone and 2-methoxy 1,4-nepthaqui-none from the stem of I. *balsamina.* A new biscou-marin, 4,4′- bi isofraxidin has been reported from the roots of *I. balsamina* [4]. *I. balsamina* extracts have shown a long lasting skin moisturizing effect and prevent dryness, rough skin chap, antidandruff, split hair and are used to prepare lotions, creams, hair tonics, cosmetics, bath preparations and detergents.[5]. Thomas *et al.* [6] reported the extraction of anti-bacterial and anti-fungal substances from leaves of *I. biflora.* Fukomoto *et al.* [7] have reported the isolation of some flavonols and naphthaquinones from the white petals of *I. balsamina* and also studied the antianaphylatic effect of the principal compounds. In the present investigations we report one new flavanone glycosides (1), one known but rare flavanone glycoside (2), four rare flavonol diglycosides (3-6) and three known flovonol monoglycosides (7-9) from *Impatiens balsamina* leaf tissue.

Results and Discussion

Crude *n*-BuOH extract of *Impatiens balsamina* leaves was subjected to column chromatography (CC) on polyamide and sephadex LH-20 to yield a new flavanone glycoside (**1**) as well as eight known flavonoid glycosides (**2-9**). Compounds (**2-9**) were characterized as naringenin 4′-*O*-β-D glucopyranoside, quercetin 3-*O*-α-L-rhamnopyranosyl (1→6)- β-D-galactopyranoside, kaempferol 3-*O*-α-L-rhamnopyranosyl-7-*O*-α-L-arabinopyranoside, quercetin 3-*O*-β-D-xylopyranosyl (1→6)-β-D-glucopyranoside, quercetin 3-*O*-α-L-arabinopyranosyl (1→2)-β-D-glucopyranoside, kaempferol 7-*O*-α-L-rhamnopyranoside, kaempferol 7-*O*-β-D-allopyranoside and quercetin 3-*O*-β-D-glucopyranoside by standard procedures [8-10]. Structures of all isolated compounds are presented in figures 1 & 2.

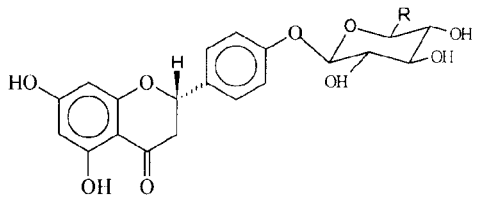

Fig. 1: Structures of compounds **1-2**

R = COOH = *Naringenin 4′-O-β-D-glucuronopyranoside* (**1**)
R = CH_2OH = *Naringenin 4′-O-β-D-glucopyranoside* (**2**).

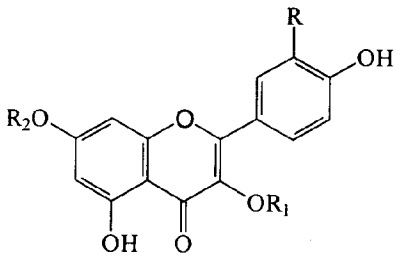

Fig. 2: Structures of compounds **3-9**
R = OH, R_2 = H, R_1 = α-L-rhamnopyranosyl (1→6)-ß-D-glucopyranoside = (**3**).
R = H, R_2 = α-L-rhamnopyranosyl, R_1 = α-L-arabinopyranoside = (**4**).
R = OH, R_2 = H, R_1 = β-D -xylopyranosyl (1→6)-β-D-glucopyranoside = (**5**).
R = OH, R_2 = H, R_1 = α-L-arabinopyranosyl (1→6)-β-D-glucopyranoside = (**6**).
R = H, R_2 = α-L-rhamnopyranoside. R_1 = H.= (**7**)
R = H, R_2 = β-D-allopyranoside, R_1 = H = (**8**).
R = OH, R_2 = H, R_1 = β-D-glucopyranoside = (**9**)

Flavanone glycoside (**1**) was obtained as yellow crystalline solid, which appeared violet on TLC under UV light 366 nm. Acid hydrolysis of compound (**1**) with 2N HCl followed by usual workup yielded naringenin as the aglycone (co-TLC, m.p., UV and EI-MS) and glucuronic acid as the sugar moiety. The sugar was identified by co-paper chromatography (PC) in three solvents [11] as well as by GC after silylation [8]. The position of attachment of the sugar moiety was determined by UV spectral analysis with the usual shift reagents [8-9]. The glycosylation site was found to be at C-4´ since free OH groups were detected at position 5 and 7 by a bathocromic shift of 23 nm with $AlCl_3$ and 34 nm with NaOAc respectively. Oxidation of the aglycone with $KMnO_4$ gave *p*-hydroxy benzoic acid thereby fixing the position of the remaining OH group at position 4´ in the B-ring.

EI-MS spectral analysis of aglycone of **1** exhibited a base peak $[M]^+$ at m/z 272 (44%) along with other diagnostic fragments $[M-H]^+$ 271(30%), $[A_1+H]^+$ 153 (35%), $[A_1^{+\bullet}]$ 152 (100), and $[B_3^{+\bullet}]$ 120 (9%) which confirmed naringenin as the aglycone. The sugar sequence in the glycoside of **1** was deduced by FAB-MS spectrum recorded in positive mode in lactic acid. Molecular ion peak was observed at *m/z* 449, $[M+H+176]^+$ and fragment ion at 273 [M+H-176], thereby confirming the attachment of glucuronic acid to naringenin. ^{1}HNMR spectrum of compound **1** displayed an anomeric proton at δ 5.16 ppm (1H, *d*, $J_{1''-2''}$ = 5.6 Hz), showing β configuration of the sugar. Other sugar protons appeared in the range of δ 3.33- 3.54 ppm. H-6 of ring-A appeared at δ 6.15 ppm (1H, *d*, $J_{6,8}$ = 2.5Hz) showing *meta-coupling* with H-8 which resonated at δ 6.90 ppm (1H, *d*, $J_{8,6}$ = 2.5Hz). The remaining resonance arises from the four hydrogens on the B- ring. The H-2´ and H-6´ pair occurs in identical environment are centred at ca δ 7.72 ppm while the H-3´ and H-5´pair, also in identical environment appears as a resonance centred at ca δ 6.81 ppm. Their observed positions in the spectrum are the result of *ortho-coupling* (*J* = 9.0 Hz) thus showing a 4´- OH subtituion pattern. ^{1}HNMR spectrum showed a diastreotopic effect due to two methylene protons at C-3, which are adjacent to the chiral centre at C-2. These methylene protons H-3a and H-3b appear at δ 3.26 ppm (*dd*, *J* = 17.1 & 12.1Hz) and at δ 2.69 ppm (*dd*, *J* =17.1 & 6.0 Hz) respectively. Proton at chiral centre appeared at δ 5.43 ppm (*dd*, *J* = 12.7 & 6.0 Hz), which is due to the adjacent heteroatom. ^{1}HNMR spectral data of flavanone glycoside **1** is presented in Table-1.

Table 1. ^{1}HNMR(CD_3OD) 300 MHz Spectral data of flavanone glycoside (**1**)

Protons	δ
H-6	6.15 *d*, *J*= 2.5 Hz
H-8	6.90 *d*, *J*= 2.5 Hz
H-2	5.43 *dd*, *J*=12.8,6.0Hz
H-3a	3.26 *dd J*= 12.8,17.1Hz
H-3b	2.69 *dd J*= 6.0,17.1Hz
H-2´ & H-6´	7.72 *d*, *J*= 9.0 Hz
H-3´ & H-5´	6.81 *d*, *J*= 9.0 Hz
H-1´´	5.16 *d*, *J*= 5.6 Hz
H-2´´- H-6´´	3.33-3.61 *m*

On the basis of acid hydrolysis, UV, EIMS, FAB-MS and ^{1}HNMR spectral data the structure of flavanone glycoside (**1**) was established as naringenin 4´-*O*-β-D-glucuronopyranoside. Other naringenin 4´-*O*-substituted glycosides have been reported [12-14], but this is the first report of glucuronic acid as the sugar moiety at position 4´ of naringenin.

Experimental

UV spectra were taken on a Shimadzu 1601 UV-vis spectrophotometer. Shift reagents were prepd. according to ref. [8]. TL chromatography was performed on polyamide (Riedel-de-Haën 6DF, Germany) using solvent system 1 (Toluene-MEK-MeOH 4:3:3), solvent system 2 (H_2O-MeOH-MEK-Acetyl.acetone 13: 3: 3:1). TLC plates were

visualized under UV light (366 nm). For CC polyamide (Riedel-de-Haën 6S, Germany) and sephadex LH-20 (bead size 25-100μ, Sigma, Switzerland) were used. Purity of compounds were checked by Shimadzu LC-6A HPLC using Zorbex ODS C_{18} (25cm x 4.6mm i.d.) column at a flow rate of 0.8 ml/min, UV (SPD-6AV) detection at 320nm and a gradient system, H_2O-AcOH (1000:20) and CH_3CN-H_2O-AcOH (40:10:1) using Shimadzu LC 6A pumps. Sugar analysis was carried out on Whatman No.1 PC along with authentic sugar samples in three solvent systems: 3 (*n*-BuOH-AcOH-H_2O 4:1:5 top layer), 4 (*n*-BuOH-EtOH-H_2O 4:1:2.2) and 5 (*n*-BuOH-AcOH-Et_2O-H_2O 9:6:3:1) as well as by Shimadzu GC 9A on SE-54 (25 m x 0.25 mm i.d.) column at a flow rate of 4 ml/min using N_2 as carrier gas. EI-MS were recorded on Shimadzu QP1000A. FAB-MS were recorded on Double Focusing Varian MAT-312 Spectrometer connected to a MAT-188 computer system in the positive mode using lactic acid as solvent. ^{1}HNMR spectra were recorded in CD_3OD on a 300 MHz Brucker WM400. TMS was used as an int. standard.

Plant material, extraction and isolation

I. balsamina Linn leaves were collected Miran Jani Hills, Pakistan in February 2003 and authenticated by one of us (MAK) A voucher specimen(no.3572) has been deposited in the Herbarium of Department of Biology, Quaid-i-Azam University, Islamabad.. Powdered air-dried leaves (300g) of *I. balsamina* were repeatedly extracted with methanol, methanol-water (4:1), and methanol-water (1:1) by stirring at room temperature for 24 hours in each solvent. The combined extracts were concentrated under reduced pressure defatted with $CHCl_3$ and repeatedly extracted with n-BuOH. The *n*-BuOH extract was then evaporated to dryness and the dried residue (12.0g) subjected to CC on polyamide. The column was run in the gradient mode from H_2O to MeOH. Fifty-six fractions were collected. Compounds present in these fractions were purified by a combination of TLC, prep-TLC and 2DTLC on polyamide, in solvent systems 1 and 2. Final purification was carried out by CC on polyamide and Sephadex LH-20. As a result, nine flavonoid glycosides (1-9) were obtained and their purity was monitored by TLC on polyamide and RP-HPLC. The yield of each flavonoid glycoside was 1 (11mg), 2 (13mg), 3 (17mg), 4 (20mg), 5 (14mg), 6 (22mg), 7 (16mg), 8 (21mg) and 9 (15mg).

General method for acid hydrolysis

Three mg each of flavonoid glycosides (1-9) was refluxed in 2N HCl (5ml) for 1 hr. The aglycones were extracted with EtOAc and identified by co-TLC with authentic samples and UV spectral analysis. The sugars were isolated from the aqueous layer in the usual way and identified by co-PC chromatography with authentic markers on Whatman No. 1 sheets in solvent systems 3, 4 and 5.

Idendification of glycosides

Naringenin 4`-O-β-D-glucuronopyranoside (1)

TLC polyamide, (system 1): R_f (x 100) 60, (system 2): R_f (x 100) 25.UV λ_{max} in MeOH nm 289, 326sh; + NaOMe 245, 323, 345(sh); + $AlCl_3$ 312, 375; + $AlCl_3$-HCl 311, 371; + NaOAc 284sh, 323; + NaOAc-H_3BO_3 290, 332; EI-MS *m/z* (rel. int.) 272 (44%) along with other diagnostic fragments $[M-H]^+$ 271 (30%), $[A_1+H^+]$ 153 (35%), $[A_1^{+\bullet}]$ 152 (100%) and $[B_3^{+\bullet}]$ 120 (9%); FAB-MS spectra recorded in positive mode in lactic acid. *m/z* 449 $[M+H+176]^+$and a fragment ion at 273 $[M+H-176]^+$; ^{1}HNMR data is given in Table 1.

Naringenin 4`-O-β-D-glucopyranoside (2)

TLC polyamide, (system 1): R_f (x 100) 52, (system 2): R_f (x 100) 30. UV λ_{max} in MeOH nm 289, 326sh; + NaOMe 323, 345; + $AlCl_3$ 312, 375; + $AlCl_3$-HCl 311, 371; + NaOAc 284sh, 323; + NaOAc-H_3BO_3 290, 332. Mass and nmr spectral same as reported in reference [15].

Quercetin 3-O-α-L-rhamnopyranosyl (1→6)-β-D-galactopyranoside (3).

TLC polyamide, (system 1) R_f (x 100) 50, (system 2) R_f (x 100) 26. 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. Mass and nmr spectral same as reported in reference [16].

Kaempferol 3-O-α-L-rhamnopyranosyl-7-O-α-L-arabinopyranoside (4).

TLC polyamide, (system 1): R_f (x 100) 49, (system 2) R_f (x 100) 22. 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 265, 318(sh), 358, 406(sh); + NaOAc-H_3BO_3 268,

319(sh), 354. Mass and nmr spectral same as reported in reference [17].

Quercetin 3-O-α-L-xylopyranosyl (1→6)-β-D-glucopyranoside (5).

TLC polyamide, (system 1): R_f (x 100) 54, (system 2) R_f (x 100) 29. UV λ_{max} nm 256, 269(sh), 329(sh), 357; + NaOMe 275, 327, 412; + $AlCl_3$ 274, 304(sh), 435; + $AlCl_3$-HCl 269, 303 362(sh) 404; + NaOAc 274, 327, 384; + NaOAc-H_3BO_3 260, 293, 379. Mass and nmr spectral same as reported in reference [18].

Quercetin 3-O-α-L-arabinopyranosyl (1→2)-β-D-glucopyranoside (6).

TLC polyamide, (system 1): R_f (x 100) 54, (system 2) R_f (x 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. Mass and nmr spectral same as reported in reference [19].

Kaempferol 7-O-α-L-rhamnopyranoside (7).

TLC polyamide, (system 1): R_f (x 100) 45, (system 2) R_f (x 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. Mass and nmr spectral same as reported in reference [20].

Kaempferol 7-O-β-D-allopyranoside (8).

TLC polyamide, (system 1): R_f (x 100) 46, (system 2) R_f (x 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. Mass and nmr spectral same as reported in reference [17].

Quercetin 3-O-β-D-glucopyranoside (9).

TLC polyamide, (system 1): R_f (x 100) 48, (system 2) R_f (x 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. Mass and nmr spectral same as reported in reference [21].

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