Flavonoids from the Leaves of *Impatiens bicolor*

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Three new flavanone glycosides, naringenin 4′-*O*-β-D-glucuronopyranoside, naringenin 4′-*O*-α-L rhamnopyranoside and naringenin 4′-*O*-β-D-xylopyranoside, were characterized from the leaves of *Impatiens bicolor*, together with 6 known glycosides: naringenin 4′-*O*-β-D-glucopyranoside, kaempferol 7-*O*-β-D-glucuronopyranoside, quercetin 3-*O*-β-D-glucopyranoside, kaempferol 5-*O*-β-D-xylopyranoside, kaempferol 3-*O*-β-D-galactopyranoside and kaempferol 7-*O*-β-D-xylopyranoside. The structures were determined by means of co-chromatography, UV, HPLC, EI-MS, FAB-MS and ^{1}H NMR.

Key Words: *Impatiens bicolor,* Balsaminaceae, Naringenin 4′-*O*-β-D-glycosides.

Introduction

Impatiens bicolor 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. The genus *Impatiens* is rich in organic acids, anthraquinones and flavonoids. Charles and Hagen have reported the isolation of 3 monoglucosides of kaempferol, quercetin and pelargonidin from the stem of *I. balsamina*[1]. Pelargonidin and peonidin as glucosides have been characterized from *I. holstii*[2]. Bhom and Towers extracted and purified salicylic acid, sinnapic acid, caffeic acid, scopletin, 2-hydroxy, 1,4-naphthoquinone and 2-methoxy 1,4-naphthoquinone from the stem of I. *balsamina*[3]. Panichayupakaranaut isolated a new biscoumarin, 4,4′-biisofraxidin, from the roots of *I. balsamina*[4]. *I. balsamina* extracts show a long lasting skin moisturizing effect and prevent dryness, rough skin chap, dandruff and split hair ends and are used to prepare lotions, creams, hair tonics, cosmetics, bath preparations and detergents[5]. Thomas et al. extracted antibacterial and antifungal substances from *I. biflora*[6]. In the present investigations performed on *I. bicolor* we now report 3 new flavanone glycosides (**1-3**) and 6 known flavonoid glycosides (**4-9**) from the leaves of *I. bicolor.*

Experimental

Equipment

UV spectra were taken with a Shimadzu (Japan) 1601 UV-vis spectrophotometer. Shift reagents were prepared[7]. Thin layer chromatography (TLC) was performed on polyamide (Riedel-de-Haën 6DF, Ger-

many) using solvent systems A (toluene-methyl ethyl ketone-methanol 4:3:3) and B (water-methanol-methyl ethyl ketone-acetylacetone 13:3:3:1). TLC plates were visualized under UV light (366 nm). For column chromatography (CC) polyamide (Riedel-de-Haën 6S, Germany) and sephadex LH-20 (bead size 25-100 μ Sigma, Switzerland) were used. Purity of compounds was checked by Shimadzu LC-6A HPLC using a Zorbex ODS C_{18} (25 cm x 4.6 mm i.d.) column at a flow rate of a 1 mL/min, UV (SPD-6AV) detection at 320 nm and a gradient system, H_2O-AcOH (1000:20) and CH_3CN-H_2O-AcOH (800:200:20) using Shimadzu LC 6A pumps. Sugar analysis was carried out on a Whatman No. 1 PC along with authentic sugar samples (supplied by Fluka Switzerland) in 3 solvent systems: C (*n*-BuOH-AcOH-H_2O 4:1:5 upper layer), D (*n*-BuOH-EtOH-H_2O 4:1:2.2) and E (*n*-BuOH-AcOH-Et_2O-H_2O 9:6:3:1) as well as by Shimadzu GC 9A on a 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 was recorded on a Shimadzu QP1000A machine. FAB-MS were recorded on a Double Focusing Varian MAT-312 Spectrometer connected to a MAT-188 computer system in the positive mode using lactic acid as solvent. ^{1}H NMR spectra were recorded in CD_3OD on a 300 MHz Brucker WM400. TMS was used as internal standard.

Plant material, extraction and isolation

I. bicolor leaves were collected from Murree Hills, Pakistan in August 2002 and authenticated by Dr. Mir Ajab Khan, Associate Professor, Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan. A voucher specimen (no. 3571) has been deposited in the Herbarium of the above department.

Powdered air-dried leaves (500 g) of *I. bicolor* were repeatedly extracted with methanol and methanol-water (8:2), by stirring at room temperature, for 24 h in each solvent. The combined extracts were concentrated to H_2O 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 (4.3 g) was subjected to CC on polyamide. The column was eluted in the gradient mode from H_2O to MeOH. Fifty-six fractions were collected. Fractions 1-10, 11-16, 17-20, 21-24, 25-32, 33-36, 37-39, 40-45, 46-49 and 50-56 were combined. Compounds present in these fractions were purified by a combination of TLC, prep-TLC and 2DTLC on polyamide, in solvent systems A and B. Final purification was carried out by CC on polyamide and sephadex LH-20. As a result, 9 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** (11 mg), **2** (13 mg), **3** (17 mg), **4** (20 mg), **5** (14 mg), **6** (12 mg), **7** (9 mg), **8** (21 mg) and **9** (15 mg).

General method for acid hydrolysis

Each flavonoid glycoside (1-9) (3.0 mg) was refluxed in 2N HCl (5 mL) for 1 h. The aglycones were extracted with EtOAc and identified by co-TLC with authentic standards and UV spectral analysis. The sugars were isolated from the aqueous layer in the usual way and identified by co-paper chromatography (co-PC) with authentic markers on Whatman No. 1 sheets in solvent systems C, D and E.

Results and Discussion

Crude *n*-BuOH extract of *I. bicolor* leaves was subjected to CC on polyamide and sephadex LH-20 to yield 3 new flavanone glycosides (**1-3**) as well as 6 known flavonoid glycosides (**4-9**). Compounds (**4-9**) were characterized as naringenin 4'-*O*-β-D glucopyranoside (**4**), kaempferol 7-*O*-β-D-glucuronopyranoside

(**5**), quercetin 3-*O*-β-D-glucopyranoside (**6**), kaempferol 5-*O*-β-D-xylopyranoside (**7**), kaempferol 3-*O*-β-D-galactopyranoside (**8**) and kaempferol 7-*O*-β-D-xylopyranoside (**9**) by standard procedures[7−9] and are also reported [10].

The 3 flavanone glycosides (**1-3**) were obtained as yellow crystalline solids, which appeared violet on TLC under UV light of 366 nm. Acid hydrolysis of compounds (**1-3**) with 2N HCl followed by the usual workup yielded naringenin as the only aglycone in each case (co-TLC, m.p., UV and EI-MS) and glucuronic acid, rhamnose and xylose respectively The sugars were identified by co-PC in 3 solvents[11] as well as by GC after silylation[7]. The position of attachment of the sugar moieties to naringenin was determined by UV spectral analysis with the usual shift reagents[7−8]. The glycosylation site in each case was found to be at C-4′ since free OH groups were detected at position 5 and 7 of ring A by a bathochromic shift in band II of 23 nm with $AlCl_3$ and a 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 ring B.

EI-MS spectral analysis exhibited identical spectra for the aglycones of **1-3** with 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 in all 3 cases. The sugar sequence in compounds **1-3** was deduced by FAB-MS spectra recorded in positive mode in lactic acid. For compounds **1-3** quasi-molecular ion peaks $[M+H]^+$ were observed at m/z 449, 419, 405 and fragment ions at 273 [M+H-176], 273 [M+H-146] and 273 [M+H-132] thus confirming the attachment of glucuronic acid, rhamnose and xylose respectively to naringenin. The ^{1}H NMR spectrum of compound **1** displayed an anomeric proton at δ 5.16 (1H, *d*, $J_{1''-2''} = 5.6$ Hz), showing the β configuration of the sugar. Other sugar protons appeared in the range of δ (3.33 - 3.54). H-6 of ring A appeared at δ 6.15 ppm (1H, *d*, $J_{6,8}$= 2.5 Hz) showing meta−coupling with H-8, which resonated at δ 6.90 ppm (1H, *d*, $J_{8,6} = 2.5$ Hz). The remaining resonance arises from the 4 hydrogens on the B ring. The H-2′ and H-6′ pair occur in an identical environment and these are centered at ca. δ 7.32 ppm while the H-3′ and H-5'pair, also in an identical environment appear as a resonance centered 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 substitution pattern. ^{1}H NMR spectrum showed a diastreotopic effect due to 2 methylene protons at C-3, which are adjacent to the chiral center at C-2. These methylene protons Ha and Hb appear at δ 3.26 ppm (*dd, J* = 17.1 & 12.8 Hz) and at δ 2.69 ppm (*dd, J* = 17.1 & 6.0 Hz) respectively. The proton at the chiral center gives a peak at δ 5.43 ppm (*dd, J* = 12.8 & 6.0 Hz), which is due to the adjacent heteroatom. ^{1}H NMR spectral data of flavanone glycosides **1-3** are presented in the Table.

On the basis of acid hydrolysis, UV, EI-MS, FAB-MS and ^{1}H NMR spectral data, the structures of the 3 flavanone glycosides (**1-3**) were established as naringenin-4′-*O*-β-D-glucuronopyranoside, naringenin 4′-*O*-α-*L* rhamnopyranoside and naringenin 4′-*O*-β-D-xylopyranoside respectively (Figure). Other naringenin 4′-*O*-substituted glycosides have been reported[12−14], but this is the first report of glucuronic acid, rhamnose and xylose as the sugars attached at position 4′ of naringenin.

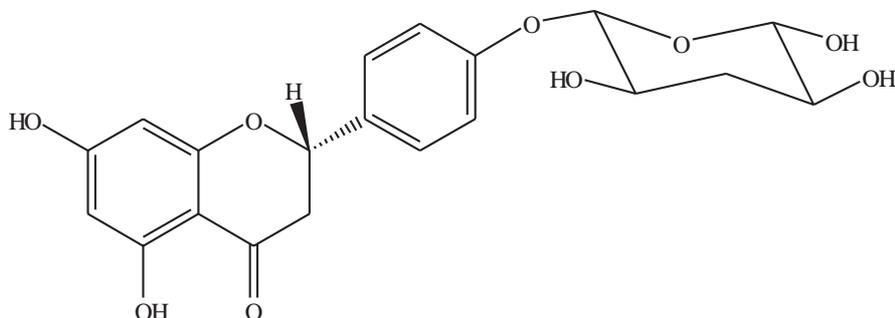### *Naringenin 4′ − O − β-D-glucuronopyranoside (1)*

TLC polyamide, (Toluene-MEK-MeOH 4:3:3 system): R_f (x 100) 60, 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
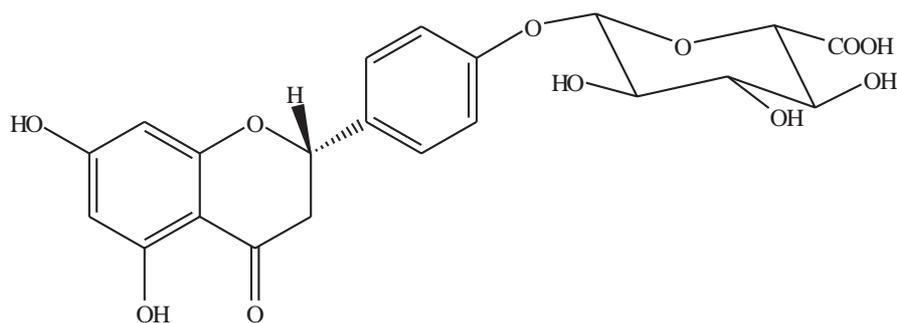
Naringenin 4'-*O*-β–D-glucuronopyranoside (**1**)
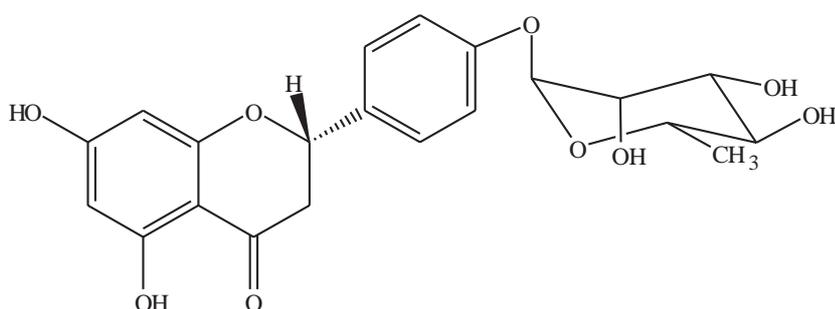
Naringenin-4' *O*-α–L-rhamnopyranoside (**2**)

Naringenin-4'-*O*-β–D-xylopyranoside (**3**)

Figure. Structure of compounds (1-3).

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-mass spectra recorded in positive mode in lactic acid. m/z 449 $[M+H]^+$and a fragment ion at 273 [M+H-176]; ^{1}H NMR data are given in the Table.

Naringenin 4′-O-α − L rhamnopyranoside (2)

TLC polyamide, (Toluene-MEK-MeOH 4:3:3 system): R_f (x 100) 57, 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$_3$BO$_3$ 290, 332; EI-MS *m/z* (rel. int.) 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-mass spectra recorded in positive mode in lactic acid. m/z 419 [M+H]$^+$and a fragment ion at 273 [M+H-146]; ^{1}H NMR data are given in the Table.

Table. The ^{1}H NMR spectroscopic data for compounds **1-3** (300 MHz in CD$_3$OD).

H	1	2	3
6	6.15 *d, J* = 2.5 Hz	6.15 *d, J* = 2.5 Hz	6.15 *d, J* = 2.5 Hz
8	6.90 *d, J* = 2.5 Hz	6.90 *d, J* = 2.5 Hz	6.90 *d, J* = 2.5 Hz
2	5.43 *dd, J*=12.8,6.0 Hz	5.43 *dd, J*=12.8,6.0 Hz	5.43 *dd, J*=12.8,6.0 Hz
3a	3.26 *dd J*= 12.8,17.1 Hz	3.26 *dd J*= 12.8,17.1 Hz	3.26 *dd J*=12.8,17.1 Hz
3b	2.69 *dd J*= 6.0,17.1 Hz	2.69 *dd J*= 6.0,17.1 Hz	2.69 *dd J*= 6.0,17.1 Hz
2′ & 6′	7.32 *d, J* = 9.0 Hz	7.32 *d, J* = 9.0 Hz	7.32 *d, J* = 9.0 Hz
3′ & 5′	6.81 *d, J* = 9.0 Hz	6.81 *d, J* = 9.0 Hz	6.81 *d, J* = 9.0 Hz
1″	5.16 *d, J* = 5.6 Hz	4.85 *d, J* = 1.8 Hz	4.35 *d, J* = 6.0 Hz
6″	———————	0.93 *d, J* = 7.0 Hz	—————
2″-5″	3.33-3.61m	3.33-3.61m	3.33-3.61m

Naringenin 4′ − O − β − D-xylopyranoside (3)

TLC polyamide, (Toluene-MEK-MeOH 4:3:3 system): R_f (x 100) 52, 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$_3$BO$_3$ 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-mass spectra recorded in positive mode in lactic acid. m/z 405 [M+H]$^+$and a fragment ion at 273 [M+H-132]; ^{1}H NMR data are given in the Table.

Acknowledgments

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