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LUTEOLIN 3′-XYLOSYL(1 → 2) GLUCOSIDE FROM *VIBURNUM GRANDIFOLIUM*

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Key Word Index—Viburnum grandifolium; Caprifoliaceae; luteolin 3′-*O*-β-D-xylosyl(1 → 2) glucoside; apigenin 7-*O*-β-D-xylosyl(1 → 2) glucoside.

Abstract—A novel flavone glycoside, luteolin 3′-*O*-β-xylosyl(1 → 2) glucoside, together with apigenin 7-xylosyl(1 → 2) glucoside has been isolated from *V. grandifolium*. The structure were elucidated by means of ^{1}HNMR, ^{13}CNMR, IR, UV and NOE spectral data. © 1998 Published by Elsevier Science Ltd. All rights reserved

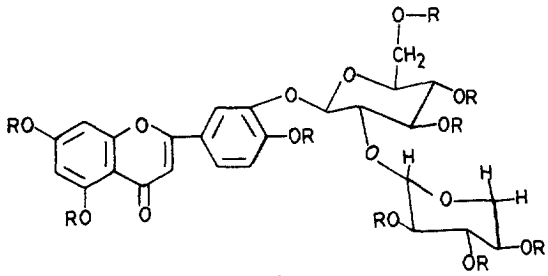
INTRODUCTION

Members of the genus *Viburnum* are known for their medicinal properties e.g. antioxidant [1] antibacterial [2], astringent, sedative and emmengagogue [3] and some seventeen have been tested for their toxicity [4]. This led us to make a comprehensive phytochemical investigation of *Viburnum grandifolium* D.C. we now report the isolation and characterization of a novel flavone glycoside (**1**) and the known glycoside (**2**) from this plant.

RESULTS AND DISCUSSION

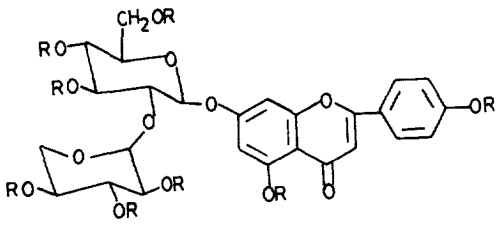
Compound **1** which analysed for $C_{26}H_{28}O_{15}$, responded positively to the Shinoda's [5] and ferric chloride test. The Molish test showed it to be a glycoside and this was also indicated by its IR spectrum which showed strong absorptions at 3340(OH), 1660(C=O) and a broad band at 1100–1000 cm^{-1}. The UV spectrum gave λ_{max} at 270 and 340 nm. UV spectral analysis with diagnostic reagents [6] gave bathochromic shifts of 30 nm with $AlCl_3$, 10 nm with NaOAc and 43 nm with NaOMe, showing the presence of free hydroxyls at the C-5, 7 and 4′-positions. The negative shift with $NaOAc/H_3BO_3$ indicated the absence of an *ortho*-dihydroxyl group [6].

Total hydrolysis of the glycoside (**1**) with 7% HCl gave an equimolar quantity of xylose and glucose (PC and GLC) and an aglycone (**1a**). The UV spectra of the aglycone and the glycoside were similar except that with $AlCl_3$-HCl, band I of **1a** showed an hypsochromic shift of 40 nm, and a



(1)

a) R=H
b) R=Ac
c) R=Me



(2)

a) R=H
b) R=Ac
c) R=Me

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Table 1. ^{1}H NMR ASSIGNMENTS for acetylated **1** and **2** (**1b** and **2b**)

PROTONS	COMPOUNDS **1b**	**2b**
Aglycone		
H-3	6.59(1H, s) (1 H, s)	6.60 (1 H, s)
H-6	6.71 (1 H, d, J = 2.5 Hz)	6.70 (1 H, d, J = 2.5 Hz)
H-8	7.01 (1 H, d, J = 2.5 Hz)	7.01 (1 H, d, J = 2.5 Hz)
H-2′, 6′	7.30–7.69 (2 H, dd, J = 9 and 2.5 Hz)	7.90 (2 H, d, J = 9 Hz)
H-5′	7.35 (1 H, d, J = 9 Hz)	–
H-3′, 5′	–	7.28 (2 H, d, J = 9 Hz)
Aromatic Acetoxyls		
5-OAc	2.45 (3 H, s)	2.47 (3 H, s)
7-OAc	2.33 (3 H, s)	
4′-OAc	2.35 (3 H, s)	2.38 (3H, s)
Sugars		
H-1″ (glc)	5.33 (1 H, d, J = 9 Hz)	5.34 (1 H, d, J = 9 Hz)
H-1‴(xyl)	5.24 (1 H, d, J = 9 Hz)	5.25 (1 H, d, J = 9 Hz)
All Sugar Protons (H-1″, 2″, 3″, 4″, 5″, 6″, H-1‴, 2‴, 3‴, 4‴, 5‴	3.92–5.33 (13 H,m)	3.92–5.34 (13 H,m)
6 × aliphatic acetoxyls	2.05–2.07 (18 H, m)	2.07–2.1 (18 H, m)

Multiplicity and coupling constants are shown in parenthesis recorded in $CDCl_3$.

bathochromic shift of 22 nm with $NaOAc/H_3BO_3$ (not observed in the glycoside) suggesting the presence of a free *ortho*-dihydroxy group in the B-ring. This was further supported by a positive borate shift and an intense red brown colour with ammonium molybdate and acetic acid [7]. These combined data indicated that the sugars are present as a disaccharide linked at the 3′-position of the aglycone. The aglycone was characterized as luteolin by spectral and chromatographic comparison with an authentic sample [8]. The pyranose structure of the sugars was confirmed by periodate oxidation [9] of the glycoside (**1**). The quantitative estimation of the sugars by Somogyi's copper micro method [10] indicated the presence of 2 moles of sugar per mole of aglycone. Partial hydrolysis of the glycoside with 1% H_2SO_4 yielded D-xylose and a glycoside, which on further hydrolysis with 6% HCl yielded D-glucose and luteolin. This confirmed that xylose is present as the terminal sugar. The sugars were identified by paper chromatography and GLC of their trimethyl silyl ethers.

Acetylation of the glycoside afforded the nonaacetate (**1b**). The ^{1}HNMR spectrum of **1b** (Table 1) indicated it was a xylosylglucoside. Three aromatic acetoxyls at the 7,4′ and 5-positions were seen as singlets at δ 2.33, 2.35 and 2.45 and six sugar acetoxyls appeared in the range of δ 2.05–2.07 as a multiplet integrating for 18 protons. The flavone nucleus was evidenced by the presence of a sharp one proton singlet at δ 6.59 for the H-3 proton. A pair of *meta*-coupled doublets (J = 2.5 Hz) at δ 6.71 and 7.01 assigned the to 6 and 8 protons supported the 5,7-disubstitution pattern. An *ortho*-coupled doublet at δ 7.35 (J = 9 Hz) was ascribed to H-5. A double doublet centered at δ 7.33–7.69 (J = 2.5 & 9 Hz) was attributed to the H-2′ & 6′ protons. The anomeric proton H-1″ (glucose) appeared as a doublet at δ 5.33 (J = 9 Hz) and H-1‴ (xylose) also as a doublet at δ 5.24 (J = 9 Hz), the coupling constant of glucose and xylose indicated a β-configuration. Sugar protons appeared in the range δ 3.92–5.33. The 1 → 2 intersugar linkage was indicated by the absence of the characteristic

Table 2. ^{13}C NMR ASSIGNMENTS for **1** and **2**

Carbon Numbers	AROMATIC REGION **1**	**2**	CARBON NUMBERS	SUGAR REGION **1**	**2**
C-8	94.9	94.8	C-6″	61.4	61.5
C-6	99.5	99.6	C-5‴	65.5	65.4
C-3	103.1	102.8	C-4‴	69.5	69.5
C-10	104.8	105.5	C-4″	70.3	70.4
C-2′	113.5	-	C-1″	99.0	99.1
C-5′	116.3	-	C-2‴	73.7	73.8
C-6′	118.9	-	C-3‴	76.1	76.2
C-1′	121.7	120.9	C-5″	81.5	81.4
C-3′	146.2	-	C-2″	82.1	82.1
C-3′, 5′	-	116.1	C-1‴	103.5	103.6
C-2′, 6′	-	128.3			
C-4′	154.0	161.0			
C-9	157.4	157.0			
C-5	161.4	161.6			
C-7	163.5	162.7			
C-2	164.3	164.4			
C-4	181.7	181.9			

high field signal for a 2″ acetoxyl at δ 1.78 and was further supported by a difference in the NOE. An intense NOE enhancement of the doublet H-2′ caused by irradiation of H-1″ (Glc) indicated, glucose to be attached to the 3′-hydroxyl of luteolin. A strong NOE correlation was observed between H-1‴ of xylose and H-2″ of glucose, thus confirming the intersugar linkage as 1 → 2. Hydrolysis of the methylated glycoside (**1c**) with 0.3 N HCl afforded 2,3,4-tri-*O*-methyl xylose and 3,4,6-tri-*O*-methyl glucose [11]. The ^{13}C NMR spectrum (Table 2) of **1** was in full agreement with the assigned structure. A downfield shift of C-2″ (Glc) at 82.1 ppm was observed due to the 1 → 2 intersugar linkage.

The assigned structure for **1** was further supported by the mass spectrum of the glycoside acetate (**1b**). The molecular ion peak as expected was not observed [12]. The fragment ion at *m/z* 700 was attributed to the partial glycoside. The ion peaks at *m/z* 259, 289 and 286 are due to acetylated xylose, acetylated glucose and the aglycone, respectively. A retro-Diel-Alder fragmentation pattern resulted in the formation of ions at *m/z* 152 [A_1^+] and 134 [B_1^+], which supported the presence of two hydroxyl groups in the B-ring and two hydroxyl groups in the A-ring.

On the basis of the above results **1** was identified as luteolin 3′-*O*-β-D-xylosyl(1 → 2) glucoside, which has not been reported previously.

Compound **2** was characterized as the known glycoside, apigenin 7-*O*-β-D-xylosyl(1 → 2) glucoside by standard procedures (acid hydrolysis, MS and UV spectral analysis)[13] CNMR data for **2** are given in Table 2 and of the acetylated derivative (**2a**) in Table 1.

EXPERIMENTAL

Mps were uncorr. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WM-400 instrument using $CDCl_3$ and DMSO-d_6 as solvent. Chemical shifts are quoted in δ and TMS was used as int. standard.

Plant material

Leaves of *V. grandifolium* D.C. collected from Srinagar Kashmir, were identified by Professor Wazahat Husain, Department of Botany, A.M.U., Aligarh, India. A herbarium specimen has been deposited in the same department.

Extraction and isolation of flavone glycosides

Air dried leaves (1.5 Kg) were defatted with hot light petroleum (40–60°) and then exhaustively extracted with MeOH. The concd. MeOH extract was extracted successively with benzene, EtOAc and MeOH. The benzene and EtOAc extracts, containing mainly resinous matter, were discarded. The MeOH extract was chromatographed over a Si-gel column using EtOAc-MeOH (9:1) as eluant to give a fraction which on TLC examination showed two major bands. The two bands were separated into their individual components (**1** and **2**) by prep. TLC using EtOAc:EtMeCO:HOAc:H_2O (20:3:1:1) as solvent. Compound **1** cryst. from MeOH as pale yellow crystals (150 mg) mp, 315° (Found C 53.6, H 4.7, $C_{26}H_{28}O_{15}$) required C 53.7, H 4.8%). UVλ_{max}^{MeOH} 245 sh, 270, 348; +$AlCl_3$ 274, 300, 345; +$AlCl_3$-HCl 274, 300, 346; +NaOAc 280, 326 sh, 357; +NaOAc-H_3BO_3, 250 sh, 275, 349; +NaOMe 269, 328 sh, 391 nm. ^{13}C NMR in DMSO-d_6 (see Table 2).

Acetylation of 1. Acetylation with Ac_2O and pyridine afforded the nonaacetate **1b** $C_{44}H_{46}O_{24}$, mp. 125–27°. ^{1}H NMR ($CDCl_3$) (Table 1). MS *m/z*, M^+. absent, 700 [9, M-Xyl $(Ac)_3]^+$. 289 [25, Glc $(Ac)_3]^+$.; 286 [15, aglycone]$^+$. 259 [28, Xyl $(Ac)_3]^+$. RDA fragments 152 [12, $A_1]^+$. 135 [10,$B_1]^+$.

Acid hydrolysis of 1. Acid hydrolysis of **1** with 7% HCl gave luteolin (UV and TLC comparison with an authentic marker), glucose and xylose. The sugars were identified by PC in BAW (*n*-butanol: HOAc: H_2O; 4:1:5) compared with authentic markers (Rfs: glucose 0.18, $[\alpha]_D^{20} = +52.4°$ and xylose 0.28, $[\alpha]_D^{20} = +19°$, respectively). The sugars were detected by spraying with anisidine phthalate and *p*-anisidine phosphate. The identities of the sugars were confirmed by comparative GLC of their trimethyl silyl ethers compared with authentic markers (Rts: glucose 1.0 and xylose 0.38).

Methylation of 1 *followed by hydrolysis.* Compound **1** was methylated with NaH and DMSO using standard procedures. The methylated glycoside was hydrolysed with Killiani's mixture (HOAc-HCl-H_2O, 7:3:1) and the methylated sugars were characterized as 2,3,4,-tri-*O*-methyl xylose and 2,4,6-tri-*O*-methyl glucose (PC and Si-gel TLC in toluene: MeOH, 4:1).

Periodate-oxidation of **1**. Compound **1** (25 mg) was oxidized by $NaIO_4$ at room temp. in 90% EtOAc (25 ml). A blank was run simultaneously. After 6 hr. 3.14 mol of periodate was consumed with the liberation of 1.12 mol of formic acid per mole of **1**.

Apigenin 7-xylosyl (1 → 2) glucoside (**2**) was identified by standard procedures (UV, MS, acid hydrolysis, acetylation, methylation followed by hydrolysis). For NMR see Tables 1 and 2.

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