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VERBACOSIDE: A NEW LUTEOLIN GLYCOSIDE FROM *VERBASCUM THAPSUS*[1]

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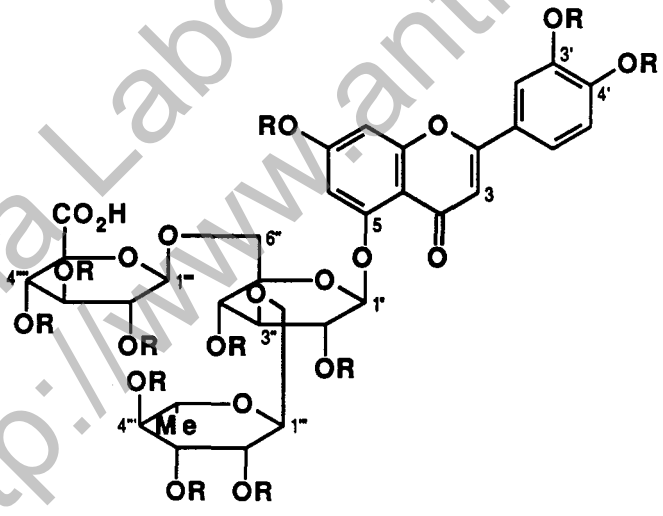
ABSTRACT.—A new triglycoside of luteolin, verbacoside, isolated from the whole plant of *Verbascum thapsus* has been characterized as luteolin 5-*O*-α-L-rhamnopyranosyl(1↦3)-[β-D-glucuronopyranosyl(1↦6)]-β-D-glucopyranoside [**1**] through spectral and chemical studies.

Verbascum thapsus L. (Scrophulariaceae) is used in the indigenous system of Indian medicine for the treatment of inflammatory disease, asthma, spasmodic coughs, and migraine (1–3) and has also been reported to possess antiviral activity against influenza in chicken embryos (4). Veratric acid, α-spinasterol, 5-(ethoxymethyl)furfural, siakogenins, and oligosaccharides have been isolated from the plant (5,6). We report further isolation of a new triglycoside of luteolin from this plant species. Compound **1**, named verbacoside, has been characterized as luteolin 5-*O*-α-L-rhamnopyranosyl(1↦3)-[β-D-glucuronopyranosyl(1↦6)]-β-D-glucopyranoside. The aglycone luteolin was also isolated from the alcoholic extract of the plant.

Compound **1** analyzed for $C_{33}H_{38}O_{21}$ ($[M]^+$ at *m/z* 770 in fdms) and gave positive Shinoda and Molisch tests for flavone glycosides. The ir spectrum exhibited absorptions at 3300 (OH), 1700 (α,β-unsaturated carbonyl), 1600, and 1520 cm^{-1} (aromatic system possessing phenolic moiety). The uv spectrum (λ max (MeOH) 250, 286, 333) showed a bathochromic shift of 48 nm for band I in NaOMe, showing the presence of the 4′-OH group. Furthermore, a 20-nm bathochromic shift for band II in NaOAc showed the presence of a 7-hydroxyl group. A hypsochromic shift of 27 nm observed in band I of the $AlCl_3$ spectrum on the addition of HCl suggested the presence of ortho di-hydroxyl groups in the compound, which



1 R=H
2 R=Ac

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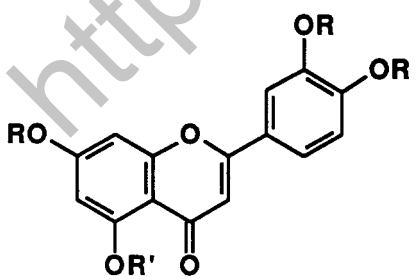
were attributed to 3′- and 4′-positions by the evidence of bathochromic shift of 18 nm in the $NaOAc/H_3BO_3$-induced spectrum (7). The ^1H nmr spectrum of **1** exhibited signals at 1.1 (3H, d, $J=6$ Hz, rhamnosyl-Me), 3.30–4.50 (14H, m, sugar protons), 4.80 (1H, d, $J=8$ Hz, H-1‴), 4.95 (1H, d, $J=8$ Hz, H-1″), 5.30 (1H, d, $J=2$ Hz, H-1‴), 6.05 (1H, d, $J=2$ Hz, H-6), 6.28 (1H, d, $J=2$ Hz, H-8), 6.38 (1H, s, H-3), 6.75 (1H, d, $J=8$ Hz, H-5′), 7.20 (2H, dd, $J=2$, 8 Hz, H-2′ and H-6′), indicating **1** to be a triglycoside. On acid hydrolysis with 5% HCl, **1** afforded an aglycone which was characterized as luteolin by its spectral and analytical data (7,8). The aglycone **3** showed a bathochromic shift of 26 nm in band I in the $AlCl_3/HCl$ spectrum with respect to its uv in MeOH, a characteristic feature of 5-OH. This fact supported the conclusion that the 5-OH of luteolin in **1** was involved in glycosidation. Compound **1** was methylated with Me_2SO_4 which on subsequent hydrolysis and usual work-up furnished aglycone **5**. The uv spectrum of **5** showed a bathochromic shift in band I with respect to the uv spectrum in MeOH, indicating a free hydroxyl at the 5 position. No shifts were observed on the addition of NaOAc, NaOMe, and $NaOAc/H_3BO_3$, showing an absence of free hydroxyls at the 7-, 3′-, and 4′-positions of luteolin. The ^1H-nmr spectrum showed signals at 3.8 (9H, s, 3 × OMe). Compound **5** was thus characterized as 7,3′,4′-trimethoxy-5-hydroxyflavone (7, 3′,4′-trimethylluteolin) which clearly supported the presence of the glycosidic linkage at the 5 position of **1** (7).



3 R=R′=H
4 R=R′=Ac
5 R=Me, R′=H

Mild hydrolysis of **1** with 2% HCl and subsequent co-pc of the hydrolysed product after intervals of 5 min for 2 h indicated the removal first of the rhamnose and glucuronic acid simultaneously and then of glucose afterwards, confirming that the glucose was linked at the 5 position of luteolin. Partial hydrolysis of **1** with 1% HCl in DMSO afforded a mixture containing luteolin 5-*O*-glucoside, luteolin, glucose, rhamnose, and glucuronic acid. Acetylation of **1** afforded **2**, a peracetylated product, which gave no color with $FeCl_3$ and no absorption at 3300 in the ir. The ^1H nmr of **2** exhibited signals at 1.1 (3H, d, $J=8$ Hz, rhamnosyl-Me), 1.70 (3H, s, 2″-Ac), 1.9–2.1 (21H, ms, 7 × Ac of sugars), 2.3 (9H, s, 3 × Ac of aglycone), 2.9, 3.6–4.45, 5.0 (14H, m, sugar carbinolic protons), 4.90 (1H, d, $J=8$ Hz, glucuronyl H-1), 5.05 (1H, d, $J=2$ Hz, rhamnosyl H-1), and 5.20 (1H, d, $J=8$ Hz, glucosyl H-1) along with the usual aromatic protons of aglycone. A singlet at δ 1.70 for -Ac indicated a free -OH at C-2″ in the glucose moiety of **1** (7,8). The methyl and anomeric protons of rhamnose were observed downfield at 1.1 and 5.05, respectively, indicating that the rhamnose unit is linked either at C-3″ or at C-4″ of the glucose unit. Had these protons appeared at high field, the linkage would have been at C-6″ (7,8). The ^1H-nmr spectra of **1** and its acetyl derivative **2** showed coupling constants for the anomeric protons of glucose, glucuronic acid, and rhamnose at 8, 8, and 2 Hz, respectively, which indicated that the rhamnose was linked via an α linkage, whereas glucuronic acid and glucose were linked with β linkages (7). Conclusive proof about the attachment of rhamnose to the glucose unit was obtained by ^{13}C-nmr spectral data. The ^{13}C nmr spectrum of **1** showed three anomeric carbons at 100.0, 101.5, and 102.0 ppm due to C-1″, C-1‴, and C-

1''''. A detailed account of the ^{13}C nmr of **1** was made by taking the glycosidation shift (9,10) into consideration; the signal at 80.5 was assigned to the C-3'' of the glucose where the rhamnose unit is attached by a (1↦3) pattern. As discussed earlier, the ^{1}H-nmr spectrum showed a possibility of attachment of rhamnose either at C-3'' or C-4''; the possibility at C-4'' was excluded by the ^{13}C-nmr chemical shift of C-3''. The ^{13}C nmr of both **1** and **2** showed a signal at 62 indicating the glucuronic acid attachment at C-6'' of the glucose via β linkage.

Based on all these physico-chemical data the structure of **1** was thus assigned as luteolin 5-*O*-α-L-rhamnopyranosyl(1↦3)-[β-D-glucuronopyranosyl(1↦6)]-β-D-glucopyranoside.

EXPERIMENTAL

General experimental procedures.—Melting points are uncorrected. Uv spectra were recorded on a SP8-100 Pye-Unicam Spectrometer using MeOH as solvent; ir spectra were recorded on a 399B Perkin-Elmer spectrometer. ^{1}H-nmr and ^{13}C-nmr spectra were obtained on a Varian FT-80 instrument at 80 MHz and 20 MHz, respectively, with TMS as internal standard. The mass spectra were determined on a Finnigan MAT at 70 eV and a Hitachi RMU 6L mass spectrometer. The plant material was collected from Almora, India in the month of October. Voucher specimens are kept in the Botany Division of CIMAP, Lucknow.

Isolation.—The shade-dried powdered plant (3 kg) was extracted with EtOH, and the extract was concentrated in vacuo and fractionated into $CHCl_3$-, EtOAc-, and *n*-BuOH-soluble fractions. The *n*-BuOH-soluble fraction concentrate (25 g), on repeated cc on Si gel, yielded **1** after eluting the column with EtOAc-MeOH (4:1), **1** crystallized from MeOH-$CHCl_3$ (4:1) as yellow hygroscopic flakes (1.0 g): mp 203–204°, ir ν max (KBr) 3300 (OH), 1700 (C=O), 1600, 1520, 1440, 1390, 1160, 1120, 1040, 810 cm^{-1}; uv λ max (MeOH) 250, 286, 333; (NaOMe) 260, 300, 382; ($AlCl_3$) 262, 285, 360; ($AlCl_3$/HCl) 250, 288, 333; (NaOAc) 270, 340; (NaOAc/H_3BO_3) 260, 290, 352; ^{1}H nmr (Me_2CO-d_6) is given in the text; ^{13}C nmr (Me_2CO-d_6) 166.0 (C-2), 103 (C-3), 185 (C-4), 154 (C-5), 101 (C-6), 165 (C-7), 94.5 (C-8), 154 (C-9), 110 (C-10), 120 (C-1'), 114.5 (C-2'), 145 (C-3'), 149.5 (C-4'), 115 (C-5'), 118 (C-6'), 100.5 (C-1''), 77 (C-2''), 80 (C-3''), 70 (C-4''), 77 (C-5''), 62 (C-6''), 101.5 (C-1'''), 68.5 (C-2'''), 69 (C-3'''), 71 (C-4'''), 68 (C-5'''), 17.5 (C-6'''), 101.5 (C-1''''), 75 (C-2''''), 74.5 (C-3''''), 73.0 (C-4''''), 73.5 (C-5''''), 181 (C-6'''').

Acid hydrolysis of **1**.—Compound **1** (100 mg) was dissolved in 5% HCl with the addition of few drops of MeOH, and the reaction mixture was refluxed for 2 h. Workup afforded luteolin [**3**]: 25 mg, mp 234–236° (7,8). The aqueous layer of the hydrolyzed product was worked up as usual and co-chromatographed on paper [Whatman No. 1, *n*-BuOH–C_6H_6–C_5H_5N–H_2O (5:1:3:3), 48 h] along with authentic samples which showed the presence of glucose, rhamnose, and glucuronic acid. Compound **3**, characterized as luteolin, was acetylated with Ac_2O-C_5H_5N (1:1) and worked up as usual to get **4** characterized as luteolin-tetraacetate.

Methylation and subsequent hydrolysis of **1**.—A mixture of **1** (100 mg), K_2CO_3 (300 mg), and Me_2SO_4 (1.5 ml) in dry Me_2CO (25 ml) was refluxed (6 h) under an N_2 atmosphere. After completion of the reaction, the mixture was filtered, and the filtrate was concentrated and hydrolyzed by 2% H_2SO_4 to furnish compound **5**: ^{1}H nmr ($CDCl_3$) 3.85 (9H, s, 3 × OMe), 6.10 (1H, d, J = 2 Hz, H-6), 6.30 (1H, d, J = 2 Hz, H-8), 6.40 (1H, s, H-3), 6.80 (1H, d, J = 8 Hz, H-5'), 7.30 (2H, dd, J = 2, 8 Hz, H-2', H-6').

Mild hydrolysis of **1**.—Compound **1** (50 mg) was dissolved in 2% HCl with the addition of 2 drops of MeOH and gently heated on an H_2O bath. The hydrolysate was co-chromatographed with authentic samples of sugars after intervals of 5 min for 2 h, which indicated removal of rhamnose and glucuronic acid simulaneously, followed by glucose.

Partial hydrolysis of **1**.—Compound **1** (50 mg) was added to 1% HCl-DMSO (5 ml) and refluxed for 1 h. The mixture was analyzed to contain luteolin and luteolin-5-*O*-glucoside, glucose, rhamnose, and glucuronic acid (7,8).

Peracetylation of **1**.—Compound **1** (100 mg) was acetylated as usual (Ac_2O/C_5H_5N, room temperature, 24 h), which furnished **2** (80 mg) after purification by chromatography on Si gel. ^{1}H-nmr ($CDCl_3$) data are given in the text.

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