Indian Journal of Chemistry
Vol. 51B, April 2012, pp 635-638

Note

Antiviral activity of a new flavone glycoside from *Emilia sonchifolia* DC.

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Received 7 December 2010; accepted (revised) 15 February 2012

A new flavone glycoside **1** molecular formula $C_{32}H_{38}O_{19}$, m.p. 246-48°C, $[M]^+$ 726 (FABMS), has been isolated from seeds of *Emilia sonchifolia* DC. It has been characterized as 3,7,3′,4′-tetrahydroxy-flavone-3-O-β-D-xylopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-O-α-L-rhamnopyranoside by various chemical degradations and spectral analysis alongwith two known compounds Luteolin-7-O-β-D-glucoside **2** and Isoetin 5′-methyl ether **3**.

Keywords: *Emilia sonchifolia* DC, compositae, seeds, flavone glycoside

Emilia sonchifolia DC.[1-2] belongs to family Compositae which is commonly known as "Hiranakhuri" in Hindi. It is found almost throughout India. It seeds have been collected from Sagar region. It is a glabrous scabrid or puberulous slender herb, 30-40 cm high. It is edible and used as a salad plant before flowering. The stem-leaves are cooked and eaten as vegetable. The plant is sudorific. A decoction of it is used as febrifuge in infantile tympanites and in bowel complaints. Its root is used for controlling diarrhea. The juice of fresh leaves is used for sore ears, sore eyes and night-blindness.

Earlier workers[3-5] have reported the isolation of various constituents from this plant. In the present paper is reported the isolation and structural elucidation of a new flavone glycoside- 3,7,3′,4′-tetrahydroxy-flavone-3-O-β-D-xylopyranosyl-(1→3)-O-β-D-galactopyrano-syl-(1→4)-O-α-L-rhamnopyranoside by several colour reactions, chemical degradations and spectral analysis along with two known compounds Luteolin-7- O- β- D-glucoside **2** and Isoetin 5′-methyl ether **3**.

Results and Discussion

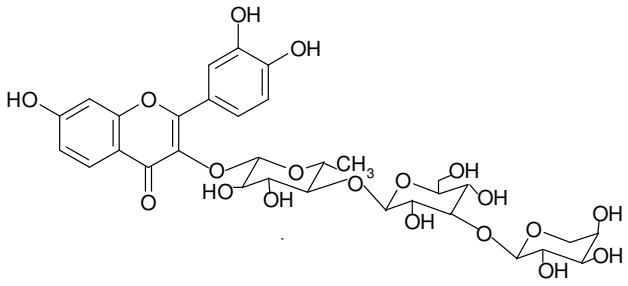
The ethanol soluble fraction of the seeds of the plant afforded a new compound **1**, m.p. 246-48°C, molecular formula $C_{32}H_{38}O_{19}$ $[M]^+$ 726 (FABMS). It responded to Molisch[6] and Shinoda tests[7] showing its flavonoidal glycosidic nature. Its IR spectrum showed absorption bands at 3340, 1635, 1605, 1582, 1530, 1207, and 845 cm^{-1}. In ^1H NMR spectrum, a double doublet at δ 6.94 showed both *ortho* (J = 2.2 Hz) and *meta* (J = 8.3 Hz) coupling for H-6′ doublet of one proton intensity at δ 6.96 (J = 1.8 Hz) for H-2′, proton and doublet of one proton intensity at δ 7.08 (J = 7.4 Hz) for H-5′, proton. A double doublet at δ 7.58 and doublet at δ 7.81, were assigned to H-6 and H-8 proton, respectively and doublet at δ 5.20 (J = 1.2 Hz), 5.71 (J = 7.9 Hz) and 5.11 (J = 7.3 Hz), each of one proton intensity, were assigned for the anomeric proton of L-rhamnose, D-galactose and D-xylose. In ^{13}C NMR spectrum, the chemical shifts at δ 159.2 for C-2, δ 139.1 for C-3 were indicative of 3-hydroxy etherification and a shift at 182.5 revealed the presence of carbonyl group at C-4 position.

Acid hydrolysis of compound **1** with l0% ethanolic H_2SO_4 gave aglycone **1a** m.p. 324-28°C, molecular formula $C_{15}H_{10}O_6$, $[M]^+$ 286 (FABMS). It was identified as 3,7,3′,4′-tetrahydroxy flavone by comparison of its spectral data with reported literature values[8].

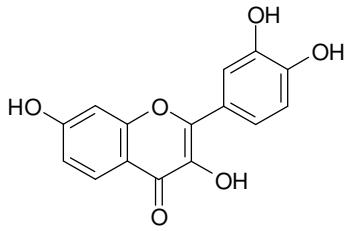
The aqueous hydrolysate, after the removal of the aglycone, was neutralised with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was then concentrated and subjected to paper chromatography examination showing the presence L-rhamnose (R_f 0.36), D-galactose (R_f 0.17), and D-xylose (R_f 0.27). Periodate oxidation[9] of compound **1** confirmed that all the sugars were present in the pyranose form.

The position of sugar moieties in compound **1** was determined by methylation[10] followed by acid hydrolysis which yielded methylated aglycone identified as 3-hydroxy-7,3′,4′-trimethoxy flavone which confirmed that glycosidation was involved at C-3 position of -OH group. The methylated sugars were identified as 2,3-di-O-methyl-L-rhamnose (R_G 1.09), 2,4,6-tri-O-methyl-D-galactose (R_G 0.68) and 2,3,4-tri-O-methyl-D-xylose (R_G 0.96) by (Co-Pc)[11].

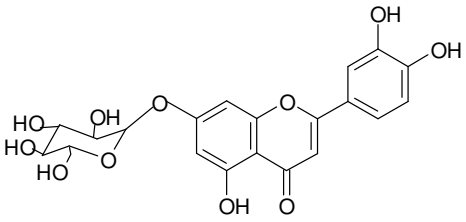
Enzymatic hydrolysis of **1** with almond emulsin at first liberated D-xylose which was followed by D-galactose and proaglycone confirming the presence of β-linkage between D-xylose and D-galactose as well



1



1a



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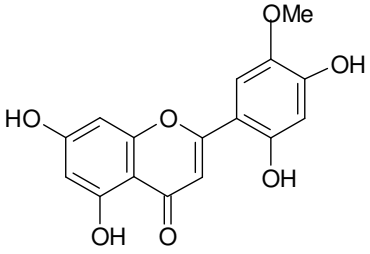

3

as between D-galactose and proaglycone. Proaglycone on further hydrolysis with takadiastase liberated L-rhamnose and aglycone, confirming the presence of α-linkage between L-rhamnose and aglycone.

Thus, on the basis of the above evidence, the structure of compound **1** was characterized as 3,7,3′,4′-tetra hydroxy-flavone-3-O-β-D-xylopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-O-α-L-rhamnopyranoside. Compound **1** was tested against Japanese Encephalitis Virus and showed 50% antiviral activity.

Compound **2** was analysed for molecular formula $C_{21}H_{20}O_{11}$, m.p. 256-58°C, $[M]^+$ 449, It was identified as Luteolin-7-O-β-D-glucoside by comparison of its spectral data with reported literature values[12].

Compound **3** was analysed for molecular formula $C_{16}H_{12}O_7$, m.p. 297-99°C, $[M]^+$ 317, It was identified as Isoetin-5′-methyl ether by comparison of its spectral data with reported literature values[13].

Experimental Section

All the melting points were determined on a thermoelectric m.p. apparatus and are uncorrected. The IR spectra were recorded in KBr disc, ^{1}H NMR spectra 300 MHz in $CDCl_3$ using TMS as internal standard, ^{13}C NMR spectra were recorded at 75 MHz using $CDCl_3$ as solvent. Mass spectra were recorded on a Jeol SX-102 (FAB) mass spectrometer.

Plant material

The seeds of *Emilia sonchifolia* DC. were collected around Sagar region and were taxonomically authenticated by the Department of Botany, Dr. H. S. Gour Central University, Sagar (M.P.) India. A voucher specimen has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H. S. Gour Central University, Sagar (M.P.).

Extraction and isolation

Air dried and powdered seeds (4 kg) of the plant were extracted with ethanol in a Soxhlet apparatus for seven days. The ethanolic extract of the seeds of the plant was successively extracted with $CHCl_3$, CH_3COOCH_3, CH_3COCH_3 and MeOH. The methanolic fraction of the plant was concentrated. On being subjected to TLC examination it showed three spots indicating it to be a mixture of **1**, **2**, and **3**. These compounds were separated by TLC and purified by column chromatography over silica-gel and studied separately.

Study of compound 1

It has m.p. 246-48°C, molecular formula $C_{32}H_{38}O_{19}$ $[M]^+$ *m/z* 726 (FABMS). Anal. Found: C, 52.96; H, 5.10. Calcd for $C_{32}H_{38}O_{19}$: C, 53.03; H, 4.97; IR (KBr): 3340, 1635, 1605, 1582, 1530, 1207, 845 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (1H, d, *J* = 8.5 Hz, H-5), 7.58 (1H, dd, *J* = 8.9, 2.7 Hz, H-6), 7.81 (1H, d, *J* = 2.8 Hz, H-8), 6.96 (1H, d, *J* = 1.8 Hz, H-2′), 7.08 (1H, d, *J* = 7.4 Hz, H-5′), 6.94 (1H, dd, *J* =

8.3, 2.2 Hz, H-6′), 5.20 (1H, d, *J* = 1.7 Hz, H-1″), 4.82 (1H, dd, *J* = 3.9, 10.1 Hz, H-2″), 4.81 (1H, dd, *J* = 3.9, 10.1 Hz, H- 3″), 4.59 (1H, dd, *J* = 3.9, 10.1 Hz, H- 4″), 4.23 (1H, m, H-5″), 1.30 (3H, d, *J* = 6.7 Hz, H-6″), 5.72 (1H, d, *J* = 7.9 Hz, H-1‴), 3.91 (1H, dd, *J* = 3.9, 10.1 Hz, H-2‴), 3.98(1H, dd, *J* = 3.9, 10.1 Hz, H-3‴), 3.99 (1H, d, *J* = 3.8 Hz, H-4‴), 4.51(1H, m, H-5‴), 4.43 (2H, d, *J* = 6.3 Hz, H-6″), 5.14 (1H, d, *J* = 7.3 Hz, H-1⁗), 4.44 (1H, dd, *J* = 3.8, 10.1 Hz, H-2⁗) , 4.14 (1H, dd, *J* = 3.9, 10.1 Hz, H-3⁗), 4.23 (1H, m, H-4⁗), 4.43 (2H, d, *J* = 6.5 Hz, H-5⁗); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.2 (C-2), 138.8 (C-3), 182.5 (C-4), 161.1 (C-5), 101.9(C-6), 163.4 (C-7), 94.9 (C-8), 161.5 (C-9), 106.2 (C-10), 121.1 (C-1′), 115.2 (C-2′), 144.9 (C-3′), 149.3 (C-4′), 116.4 (C-5′), 122.9 (C-6′), 107.50 (C-1″), 72.2 (C-2″), 72.11 (C-3″), 73.42 (C-4″), 69.51 (C-5″), 67.32 (C-6″), 105.20 (C-1‴), 72.10 (C-2‴), 75.43 (C-3‴), 68.94 (C-4‴), 60.84 (C-5‴), 66.5 (C-6‴), 102.9 (C-1⁗), 74.2 (C-2⁗), 73.9 (C-3⁗), 71.9 (C-4⁗), 69.8 (C-5⁗).

Acid hydrolysis of compound 1

Compound **1** (90 mg) was dissolved in ethanol (25 mL) and refluxed with 20 mL of 10% H_2SO_4 on water bath for 8-10 hr. The reaction mixture was concentrated and allowed to cool and residue was extracted with diethyl ether. The ethereal layer was washed with water and the residue was chromatographed over silica gel using $CHCl_3$:MeOH (4:6) to give compound **1a** which was identified as 3,5,7,4′-tetrahydroxy flavone by comparison of its known spectral data. The aqueous hydrolysate was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography examination using *n*-BAW (4 : 1 : 5) as solvent and aniline hydrogen phthalate as detecting agent which confirmed the presence of D-xylose (R_f 0.27), D-galactose (R_f 0.19) and L-rhamnose (R_f 0.36) by (Co-Pc).

Study of compound 1a

It has m.p. 324-28°C, molecular formula $C_{15}H_{10}O_6$, $[M]^+$ *m/z* 286; IR (KBr): 3340, 1635, 1605, 1582, 1530, 1207, 845 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (1H, d, *J* = 8.5 Hz, H-5), 7.58 (1H, dd, *J* = 8.9, 2.7 Hz, H-6), 7.81 (1H, d, *J* = 2.8 Hz, H-8), 6.96 (1H, d, *J* = 1.8 Hz, H-2′), 7.08 (1H, d, *J* = 7.4 Hz, H-5′), 6.94 (1H, dd, *J* = 8.3, 2.2 Hz, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1 (C-2), 139.1 (C-3), 182.1(C-4), 161.1 (C-5), 101.9 (C-6), 163.1 (C-7), 94.9 (C-8), 161.1 (C-9), 106.2 (C-10), 121.1 (C-1′), 115.1 (C-2′), 144.9 (C-3′), 149.3 (C-4′), 116.4 (C-5′), 122.9 (C-6′).

Permethylation of compound 1

Compound **1** (45 mg) was refluxed with MeI (5 mL) and Ag_2O (5 mg) in DMF (40 mg) for 1 day and then filtered. The filtrate was hydrolysed with 10% ethanolic H_2SO_4 for 7-8 hr to yield methylated aglycone identified as 3-hydroxy-7,3′,4′-trimethoxy-flavone and methylated sugars, which were identified as 2,3-di-O-methyl-L-rhamnose 2,4,6-tri-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-xylose.

Enzymatic hydrolysis of compound 1

The compound **1** (25 mg) was dissolved in ethanol (25 mL) and hydrolysed with an equal volume of almond emulsin at RT in a 150 mL round bottomed flask fitted with air condenser. The contents were left undisturbed for 2 days and filtered to yield D-xylose (R_f 0.27) first, followed by D-galactose (R_f 0.17) and proaglycone **3** confirming the presence of β-linkage between D-xylose and D-galactose as well as between D-galactose and proaglycone. Proaglycone **3** on further hydrolysis with takadiastase liberated L-rhamnose (R_f 0.36) and aglycone, confirming the presence of α-linkage between L-rhamnose and aglycone.

Study of compound 2

It has m.p. 256-58°C, molecular formula $C_{21}H_{20}O_{11}$, $[M]^+$ *m/z* 449; ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.3 (1H, s, 5-OH), 6.98 (1H, s, H-3), 6.52 (1H, d, *J* = 2.2 Hz, H-6), 6.81 (1H, d, *J* = 2.4 Hz, H-8), 7.36 (1H, d, *J* = 2.5 Hz, H-2′), 6.99 (1H, d, *J* = 8.6 Hz, H-5′), 7.49 (1H, dd, *J* = 8.4, 2.4 Hz, H-6′), 5.09 (1H, d, *J* = 7.4 Hz, H-1″); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4 (C-2), 105.3 (C-3), 182.6 (C-4), 162.4 (C-5), 98.8 (C-6), 165.4 (C-7), 94.7 (C-8), 158.5 (C-9), 104.1 (C-10), 121.6 (C-1′), 116.4 (C-2′), 146.2 (C-3′), 147.4 (C-4′), 116.5 (C-5′), 121.3 (C-6′), 92.7 (C-1″), 71.5 (C-2″), 76.3 (C-3″), 71.3 (C-4″), 75.1 (C-5″), 62.5 (C-6″).

Study of compound 3

It has m.p. 297-99°C, molecular formula $C_{16}H_{12}O_7$, $[M]^+$ *m/z* 317; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (1H, s, H-3), 6.88 (1H, d, *J* = 2.1 Hz, H-6), 5.12 (1H, d, *J* = 2.2 Hz, H-8), 6.63 (1H, s, H-2′), 67.49 (1H, s, H-5′), 3.97 (3H, d, *J* = 6.2 Hz, OCH3); ^{13}C NMR (75

MHz, $CDCl_3$): δ 164.6 (C-2), 112.4 (C-3), 178.7 (C-4), 162.5 (C-5), 98.8 (C-6), 165.5 (C-7), 94.8 (C-8), 158.6 (C-9), 104.2 (C-10), 109.7 (C-1′), 156.7 (C-2′), 106.3 (C-3′), 157.5 (C-4′), 146.6 (C-5′), 119.4 (C-6′), 58.8 (OCH_3 C-5′).

Antiviral activity of compound 1

Compound **1** was tested for antiviral activity against Japanese Encephalitis Virus *in vitro* (Vero cells). The results showed 50% antiviral activity at 62.5 μg/mL concentration of compound **1**. Thus compound **1** may be used as an antiviral agent against diseases caused by these viruses.

Acknowledgement

Authors are thankful to Head, SAIF, Central Drug Research Institute, Lucknow for providing elemental analysis and various spectra and Head, Department of Chemistry, Dr. Hari Singh Gour Central University, Sagar (M.P.), India for providing laboratory facilities.

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