New anti-inflammatory active flavanone glycoside from the Echinops echinatus Roxb.

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A new anti-inflammatory active flavanone glycoside 5,7-di-4′-dimethoxyflavanone-5-O-α-L-rhamnopyranosyl-7-O-β-D-arabinopyranosyl-(1→4)-O-β-D-glucopyranoside A along with a known compound dihydroquercetin-4′-methyl ether B have been isolated from the leaves of Echinops echinatus.

Keywords: Anti-inflammatory activity, flavanone glycoside, Echinops echinatus Roxb, arabinopyranosyl glucopyranoside

IPC: Int. Cl.2 A 61 K

Echinops echinatus Roxb.1,2 (N.O. Compositae) is commonly known as “Gokru or Utakanta” in Hindi. It is distributed throughout India and Afghanistan ascending to 5000 ft on the hills. It is used in hourse cough, hysteria, dyspepsia and ophthalmia. The powdered root of this plant is applied to wounds on cattle to destroy maggots. Mixed with acacia gum, it is used for hair to destroy lice. Earlier workers3,4 have reported various compounds from this plant.

The present paper deals with the isolation and structure elucidation of a new anti-inflammatory active flavanone glycoside, 5, 7-dihydroxy-8, 4′-dimethoxyflavanone-5-O-α-L-rhamnopyranosyl-7-O-β-D-arabinopyranosyl-(1→4)-O-β-D-glucopyranoside A along with a known compound dihydroquercetin-4′-methyl ether B from methanolic extract of the leaves of this plant.

Results and Discussion

Compound A had molecular formula C₃₄H₄₄O₁₉, m.p. 245-48°C, (FABMS): (M⁺) 756. Its IR spectrum showed a strong absorption bands at 3482 (OH group), 2902 (C-H), 2872 (OME), 1648 (aromatic C=C), 1620 (C=O), 1587, 1065 (O-gly), 923, 852 cm⁻¹. In ¹H NMR spectrum of A, two doublets at δ 6.92 (2H, d, J = 8.9 Hz) and 7.36 (2H, d, J = 8.9 Hz) were assigned to H-3′, H-5′ and H-2′, H-6′, respectively. A multiplet at δ 2.92 (2H, m) and a singlet at δ 6.12 (1H, s) were assigned to H-3 and H-6 protons, respectively.

The bathochromic shift of 24 nm with AlCl₃ and 34 nm with NaOAc in band I relative to methanol revealed the presence of –OH group at C-5 and C-7 position, respectively in aglycone C (ref. 5, 6). Two singlets at δ 3.92 (3H, s) and at δ 3.84 (3H, s) suggested the presence of –OMe group at C-8 and C-4′ position. Chemical shifts at δ 94.2 and 157.2 in ¹³C NMR spectrum of compound A also confirmed the presence of –OMe group at C-8 and C-4′ position (see ¹³C NMR in Experimental).

The anomic proton signals at δ 5.06 (1H, d, J = 7.2 Hz), 4.43 (1H, d, J = 7.8 Hz) and 5.10 (1H, d, J = 7.2 Hz) were assigned to H-1″, H-1′″, H-1′″ of L-rhamnose, D-glucose and D-arabinose, respectively.

The position of sugars moieties in compound A was determined by permethylation7 followed by acid hydrolysis which yielded methylated sugars identified as 2, 3, 4-tri-O-methyl-L-rhamnose, 2, 3, 4-tri-O-methyl-D-arabinose and 2, 3, 6-tri-O-methyl-D-glucose (by CO-PC and CO-TLC) according to Petek8 indicating that the C-1″ of L-rhamnose was linked to C-5 of aglycone and C-1′″ of D-arabinose.
was attached with the C-4‴ of the d-glucose and C-1‴ of d-glucose was attached to the C-7 of aglycone. The interlinkages (1→4) between d-arabinose and d-glucose were further confirmed by their $^{13}$C NMR spectra.

Acid hydrolysis of compound A with 10% H$_2$SO$_4$ yielded aglycone C, molecular formula C$_{17}$H$_{16}$O$_6$, m.p. 173-75°C, EIMS: (M$^+$) 316 and identified as 5,7-dihydroxy-8,4′-dimethoxyflavanone by comparison of its spectral data with reported literature values$^9$.

The aqueous hydrolysate, obtained after acid hydrolysis of compound A, was neutralized with BaCO$_3$ and BaSO$_4$ filtered off. The filtrate was concentrated and subjected to PC and sugars were identified as L-rhamnose (0.38), d-arabinose (0.21) and d-glucose (0.19) (co-PC and co-TLC). Periodate oxidation of compound A confirmed that three sugars were present in pyranose form.

Enzymatic hydrolysis of compound A, with takadiastase liberated L-rhamnose first and 5,7-dihydroxy-8,4′-dimethoxyflavanone-7-O-β-d-arabinopyranosyl-(1→4)-O-β-d-glucopyranoside as proaglycone confirming the presence of α-linkage between L-rhamnose and proaglycone. Proaglycone on further hydrolysis with almond emulsion liberated d-arabinose first, then d-glucose and aglycone suggesting the presence of β-linkage between d-arabinose and d-glucose as well as between d-glucose and aglycone.

On the basis of above evidences, the structure of compound A was characterized as 5,7-dihydroxy-8,4′-dimethoxyflavanone-5-O-α-L-rhamnopyranosyl-7-O-β-d-arabinopyranosyl-(1→4)-O-β-d-glucopyranoside (Figure 1). The anti-inflammatory activity of compound A was carried out by non-immunological carrageenin induced hind paw Oedema method$^{10}$. The results are recorded in Table I which show that compound A may be potentially used as anti-inflammatory active drug.

Compound B was analysed for molecular formula C$_{16}$H$_{14}$O$_7$, m.p. 173-74°C; MS: m/z 318 [M$^+$], 289, 166, 165, 164, 153 and 137. It was identified as dihydroquercetin-4′-methyl ether by comparison of its spectral data with reported literature values$^{11}$.

![Figure 1](image-url)
Experimental Section

**General.** All the melting points were determined on a thermoelectrical melting point apparatus and are uncorrected. The IR spectra were recorded in KBr disc; $^1$H NMR spectra in CDCl$_3$ at 300 MHz using TMS as internal standard; $^{13}$C NMR spectra at 90 MHz using DMSO-$d_6$ as solvent; and mass spectra on a Jeol SX-102 mass spectrometer.

**Plant material.** The leaves of *E. echinatus* Roxb. were collected around Sagar region and was taxonomically identified by Taxonomist of Botany Department of this University. The voucher specimen was deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H S Gour University, Sagar (M.P.).

**Extraction and Isolation**

Air-dried and powdered leaves of plant (3 kg) were extracted with pet.ether (40-60°C) in Soxhlet extractor and the defatted leaves extract was extracted with 95% methanol. The methanolic extract was concentrated under reduced pressure to yield a light brown viscous mass that showed two spots on TLC examination using solvent system CHCl$_3$:MeOH (10:6) indicating it to be mixture of two compounds A and B. These compounds were further separated by TLC and purified by column chromatography and studied separately. Compound B was found to be a known compound and identified as dihydroquercetin-4′-methyl ether by various chemical and spectroscopic methods (UV, IR, $^1$H NMR, $^{13}$C NMR and co-PC and co-TLC with authentic sample).

**Compound A:** It has molecular formula C$_{34}$H$_{44}$O$_{19}$, yield 1.14 g, m.p. 245-48°C; Anal. Found: C, 53.91; H, 5.86. Caled for C$_{34}$H$_{44}$O$_{19}$: C, 53.96; H, 5.82%; FAB.MS: [M$^+$] 756; UV (MeOH, nm): 274, 326; (+AlCl$_3$) 263, 350; (+AlCl$_3$-HCl) 267, 317; (+NaOMe) 278, 310; (+NaOAc) 274, 360; IR (KBr): 3482 (OH group), 2902, (C-H), 2872 (OMe), 1648 (aromatic C=C), 1620 (C=O), 1587, 1065 (O-gly), 923, 852 cm$^{-1}$; $^1$H NMR: δ 2.92 (2H, m, H-3), 2.94-3.12 (4H, m, H-2″, H-3″, H-4″, H-5″), 3.50 (2H, m, H-5″), 3.52-3.64 (4H, m, protons of rhamnose), 3.84 (3H, s, OMe-4′), 3.92 (2H, d, J = 2.2, 4.6 Hz, H-6″), 3.94 (3H, s, OMe-8), 4.43 (1H, d, J = 7.8 Hz, H-1″), 4.48-5.52 (3H, m, H-2″, H-3″, H-4″), 5.06 (1H, d, J = 7.2 Hz, H-1″), 5.10 (1H, d, J = 7.2 Hz, H-1″), 5.37 (1H, d, d, J = 5.2, 12.6 Hz, H-2), 6.12 (1H, s, H-6), 6.92 (2H, d, J = 8.9 Hz, H-3′ and H-5′), 7.36 (2H, d, J = 8.9 Hz, H-2′ and H-6′); $^{13}$C NMR: δ 43.3 (C-3), 54.2 (C-9), 62.6 (C-5″), 62.7 (C-6″), 69.3 (C-4″), 9.8 (C-4″), 71.4 (C-4″), 73.2 (C-2″), 74.2 (C-3″), 74.6 (C-2″), 75.1 (C-3′), 76.4 (C-5″), 77.2 (C-3″′), 77.4 (C-2″), 78.2 (C-5″), 79.2 (C-2), 94.2 (C-8), 98.2 (C-1″), 103.3 (C-10), 104.3 (C-1″), 106.1 (C-1″), 114.5 (C-3′ and C-6′), 127.2 (C-2′ and C-6′), 129.6 (C-1″), 130.3 (C-6), 157.2 (C-4″), 158.4 (C-5″), 160.5 (C-7), 196.9 (C-4); MS: m/z 756 [M$^+$], (absent), 610 [M$^+$-rhamnose], 478 [M$^+$-arabinose], 316 [M$^+$-glucose, aglycone], 301, 286, 183, 182, 167, 154, 149, 139, 134, 121, 119, 91, 83, 69.

**Acid hydrolysis of compound A**

Compound A (150 mg) was dissolved in 35 mL of EtOH and refluxed with 25 mL of 10% H$_2$SO$_4$ on a water-bath for 7-8 hr. The contents were concentrated and allowed to cool and residue extracted with Et$_2$O. The ethereal layer was washed with water and residue chromatographed over silica gel using CHCl$_3$:MeOH (10:6) to give compound C (0.75 g) which was identified as 5,7-dihydroxy-8, 4′-dimethoxyflavanone by comparison of its known spectral data. IR (KBr): 3485 (OH), 2900, (C-H), 2873 (OMe), 1650 (aromatic

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**Table I — Anti-inflammatory activity of compound A**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test solutions applied</th>
<th>Dose (mg/kg)</th>
<th>Volume of paw after drug administration</th>
<th>Total increase in paw volume</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>i.p.</td>
<td>0 hr</td>
<td>1 hr</td>
<td>2 hr</td>
</tr>
<tr>
<td>1.</td>
<td>Control group</td>
<td>...</td>
<td>0.51</td>
<td>0.68</td>
<td>0.79</td>
</tr>
<tr>
<td>2.</td>
<td>Treated group</td>
<td>45</td>
<td>0.53</td>
<td>0.61</td>
<td>0.68</td>
</tr>
<tr>
<td>3.</td>
<td>ASA</td>
<td>30</td>
<td>0.57</td>
<td>0.62</td>
<td>0.69</td>
</tr>
</tbody>
</table>
C=O), 1622 (C=O), 1590, 920, 850 cm\(^{-1}\); \(^1\)H NMR: δ 2.90 (2H, m, H-3), 3.82 (3H, s, OMe-4), 3.93 (3H, s, OMe-8), 5.35 (1H, d, J = 5.2, 12.6 Hz, H-2), 6.10 (1H, s, H-6), 7.37 (2H, d, J = 8.9 Hz, H-3' and H-5'), 7.37 (2H, d, J = 8.9 Hz, H-2' and H-6'), 11.75 (1H, s, OH); \(^13\)C NMR: δ 43.2 (C-3), 54.1 (C-9), 79.1 (C-2), 94.6 (C-8), 103.1 (C-10), 114.3 (C-3' and C-6'), 127.6 (C-2' and C-6'), 129.8 (C-1'), 130.6 (C-6), 157.5 (C-4'), 158.7 (C-5), 160.2 (C-7), 196.7 (C-4); MS: m/z 316[M]+, 301, 286, 183, 182, 167, 154, 149, 139, 134, 121, 119, 91, 83, 69.

The aqueous hydrolysate was neutralised with BaCO\(_3\) and BaSO\(_4\) filtered off. The filtrate was concentrated and extracted by PC [B.A.W (4:1:5)] and sugars were identified as L-rhamnose (R\(_f\) 0.38), D-arabinose (R\(_f\) 0.21) and D-glucose (R\(_f\) 0.19).

**Permethylolation of compound A**

The compound was treated with MeI (5 mL) and Ag\(_2\)O (20 mg) in DMF (50 mL) for 24 hr and then filtered. The filtrate was hydrolysed with 10% ethanolic H\(_2\)SO\(_4\) for 5-6 hr to give aglycone identified as 5,7-dihydroxy-8,4'-dimethoxyflavanone and methylated sugars were identified as 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4-tri-O-methyl-D-arabinose and 2,3,6-tri-O-methyl-D-glucose.

**Enzymatic hydrolysis of compound A**

Compound A (20 mg) was dissolved in MeOH (25 mL) and hydrolysed with equal volume of takadiastase in a 150 mL round bottomed flask fitted with air condenser. The contents were left at room temperature for two days and filtered. The proaglycone and hydrolysate were studied separately.

The hydrolysate was concentrated and subjected to paper chromatography examination using n-BAW (4:1:5) solvent system which showed the presence of L-rhamnose (R\(_f\) 0.38) (CO-PC).

The proaglycone (50 mg) was dissolved in MeOH (35 mL) and hydrolysed with equal volume of almond emulsion. The reaction mixture was allowed to stay at room temperature for 48 hr and filtered. The aglycone was identified as 5,7-dihydroxy-8,4'-dimethoxyflavanone. The hydrolysate was concentrated and studied by paper chromatography examination for sugar moities using n-BAW (4:1:5) solvent system and aniline hydrogen phthalate as spraying reagent. The sugars were identified as D-arabinose (R\(_f\) 0.21) (CO-PC) and D-glucose (R\(_f\) 0.19) (CO-PC).

**Anti-inflammatory activity of compound A**

Anti-inflammatory activity of the methanol soluble fraction of the compound A was carried out by non-immunological carrageen induced hind paw Oedema method.

Adult albino rats of either male or female weighing 160-190 g were taken for present investigation. The volume was recorded by plethysmograph. Original volume of right hind paw of albino rats was measured by plethysmograph without administration of test drug. Acetyl salicylic acid was used as a standard drug. Activity was done by measuring the change in the volume of inflamed foot produced by injection of 0.03 mL of 1% freshly prepared carrageen suspension.

Albino rats were divided into three groups, each group consisting of four rats. First groups of rats were treated intraperitonially (i.p.) with 45 mg/kg body weight of the methanol soluble fraction of the compound.

Second group was administered i.p. with 30 mg/kg body weight of the aqueous suspension of acetylsalicylic acid. Third group was administered with the same volume of distilled water. After one hour of the drug administration, the rats were injected 0.03 mL suspension of carrageen in the right hind paw.

The measurement of the paw volume was carried out by mercury displacement technique with the help of plethysmograph immediately before and after the carrageen injection after 1, 2 and 3 hr (Table 1). The percentage inhibition (I) of inflammation after 3 hr was calculated by the following formula:

\[
I = 100 \left(1 - \frac{a - x}{b - y}\right)
\]

where, ‘x’ is the mean foot volume of rat before the administration of carrageenin injection in the test and standard drug; ‘a’ is the mean foot volume of rats after the administration of carrageenin and the test drug injection in the test and standard group; ‘y’ is the mean foot volume of rats before the administration of carrageenin injection in the control group; and ‘b’ is the mean foot volume of rats after the administration of carrageenin injection in the control group.

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References