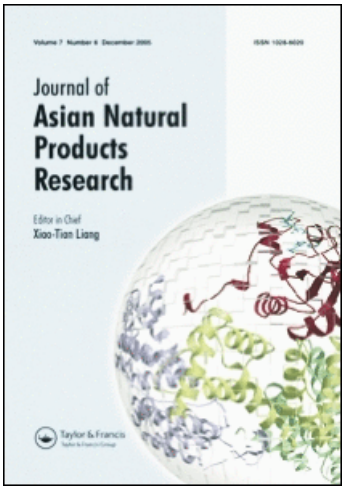

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Flavonoids from *Echinops echinatus*

S. Singh[a]; R. K. Upadhyay[a]; M. B. Pandey[a]; J. P. Singh[a]; V. B. Pandey[a]
[a] Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

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Note

Flavonoids from *Echinops echinatus*

S. SINGH, R. K. UPADHYAY, M. B. PANDEY, J. P. SINGH and V. B. PANDEY*

Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India

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A new isoflavone glycoside, echinoside (**7**), together with 7-hydroxyisoflavone, kaempferol-4′-methylether, kaempferol-7-methylether, myrecetin-3-*O*-α-L-rhamnoside, kaempferol and kaempferol-3-*O*-α-L-rhamnoside, has been isolated from the whole plant of *Echinops echinatus*. The structure of echinoside has been established by chemical and spectral data. This is the first report of the occurrence of these flavonoids in *E. echinatus.*

Keywords: *Echinops echinatus* Roxb; Asteraceae; Isoflavonoid; Echinoside

1. Introduction

Echinops echinatus Roxb. (Family Asteraceae), which is distributed throughout India, is used in the Indian System of Medicine for the treatment of chronic fever, inflammations and various other diseases [1]. A number of terpenoids, flavonoids and other constituents have earlier been reported to be derived from this plant [2–7]. The present study deals with the isolation of a new isoflavone glycoside designated echinoside (**7**) from the MeOH fraction of the whole plant of *E. echinatus* together with six known flavonoids.

2. Results and discussion

Chromatographic resolution of the methanolic extract of the whole plant of *E. Echinatus* yielded a glycoside, echinoside (**7**), mp 268–270°C, $C_{27}H_{30}O_{12}$ ($M^+ + H$, 547, FAB-MS) which was recognised to be an isoflavone from its positive Shinoda test, UV absorption maxima at 255, 292, 312 (sh) nm and a singlet at δ 8.35 (H-2) in its ^{1}H NMR spectrum.

*Corresponding author. Tel.: +91-542-2318178/2307547. Fax: +91-542-2367568.
E-mail: pandeyvb04@reddifmail.com

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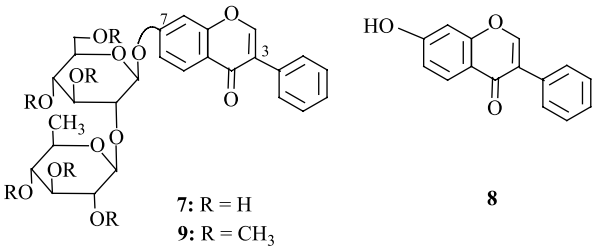
IR spectrum indicated the presence of hydroxyl (3300–3650 cm^{-1}) and chelated carbonyl (1650 cm^{-1}) functions in the molecule. On acid hydrolysis, it gave glucose, rhamnose and an aglycone (**8**).

The aglycone (**8**) showed in its ^1H NMR a characteristic singlet at δ 8.36 (H-2) for isoflavonoid, eight aromatic protons and broad singlet at δ 10.80 for the OH group identical to 7-hydroxyisoflavone [8]. This was confirmed by ^{13}C NMR and MS data and direct comparison with authentic sample.



7: R = H
9: R = CH_3

8

The presence of two sugar units in glycoside **7** was evidenced from the appearance of two anomeric proton signals as doublets at δ 4.80 and 4.90, respectively, for rhamnosyl and glucosyl units in ^1H NMR, which was further supported by two anomeric carbon signals at δ 98.2 and 100.4 ppm in its ^{13}C NMR spectrum. ^1H NMR of **7** exhibited a doublet at δ 1.12 (3H, d, $J = 6.5$ Hz) for rhamnosyl CH_3 group together with other protons of one mole of glucose and one mole of rhamnose.

Methylation of **7** by the Hakomori method [9] yielded a methylated product (**9**) which was methanolysed to give 3,4,6-trimethoxy-D-glucose (**10**) and 2,3,4-trimethoxy-L-rhamnose (**11**) and the aglycone, 7-hydroxyisoflavone (**8**). The formation of compounds **10, 11** and **8** on acid hydrolysis indicated that C-1 and C-2 of the glucose unit are attached respectively with C-7 of 7-hydroxyisoflavone and C-1 of rhamnose. The structure of the glycoside is thus settled as **7**, which is a new glycoside, designated echinoside. ^{13}C NMR data fits structure **7** well.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Toshniwal apparatus and are uncorrected. Infrared spectra were recorded in KBr pellet on a Perkin-Elmer spectrophotometer model 720 and UV on a Carry-14 spectrophotometer using spectral MeOH. ^1H and ^{13}C NMR images were taken at 100, 300 and 400 MHz NMR on a Bruker HX-90 in $CDC1_3$ and DMSO-d_6 with TMS as internal standard. Mass spectrometry was performed on a Kratos MS-30 and MS-50 mass spectrometer operating at 70 eV with evaporation of sample in the ion source at 200°C. CC: silica gel columns (BDH, 60–120 mesh); TLC: silica gel G (Merck); PC: Whatmann No.1 paper; solvents for TLC: $CHCl_3$ (solvent A), $CHCl_3$—MeOH (4:1) (solvent B), $CHCl_3$—MeOH—H_2O (65:35:10) (solvent C); solvent for PC: n-BnOH-HOAc-H_2O (4:1:5) (solvent D); paper chromatogram developed with acetonic $AgNO_3$/5% alcoholic NaOH/$Na_2S_2O_3$/H_2O. Analytical samples were dried routinely over P_2O_5 for 24 h *in vacuo*.

3.2 Plant material

The whole plant of *Echinops echinatus* was collected from Varanasi district, India and identified by Dr N.K. Dube, Department of Botany, Banaras Hindu University. A specimen sample is preserved in the department.

3.3 Extraction and isolation

Dried whole plant (3 kg) was powdered and repeatedly extracted with MeOH by cold percolation at 25°C. A brown gummy mass (125 g) of MeOH extract so obtained was chromatographed over a silica gel column. Fractions eluted from C_6H_6—EtOAc (8:1), (4:1), (1:2); EtOAc; EtOAc—MeOH (9:1), (4:1) and (1:1) furnished, respectively, 7-hydroxyisoflavone (**1**) (15 mg), yellowish granules, Rf 0.52 (solvent A), mp 215°C [8]; kaempferol-4′-methylether (**2**) (21 mg), yellow shining granules, Rf 0.45 (solvent A), mp 223–225°C; kaempferol-7-methylether (**3**) (28 mg), yellow granules, Rf 0.38 (solvent A), mp 218-220°C [10]; kaempferol (**4**) (31 mg), yellow granules, Rf 0.32 (solvent A), 273–275°C; kaempferol-3-*O*-α-L-rhamnoside (**5**) (28 mg), yellow shining granules, Rf 0.20 (solvent A) mp 177–179°C; myrecetin-3-*O*-α-L-rhamnoside (**6**) (24 mg), yellow granules, Rf 0.10 (solvent A), mp 211–213°C [11] and a new flavone glycoside, echinoside (**7**).

3.3.1 Echinoside (7). Compound **7** crystallised from MeOH as yellowish granules, Rf 0.22 (solvent B), Rf 0.72 (solvent C), mp 268–270°C, UV λ_{max} (MeOH, nm): 255 (log ϵ 4.52), 292 (log ϵ 3.32), 312 sh (log ϵ 3.12); IR ν_{max} (KBr, cm^{-1}): 3300–3650 (OH), 1650 (chelated carbonyl), 1550, 1450, 1380; ^{1}H NMR (400 MHz DMSO-d_6) δ: 1.12 (3H, d, $J = 6.5$ Hz, rhamnosyl CH_3), 3.00–4.60 (16 H, overlap, rhamnosyl-glucosyl H), 4.80 (1 H, d, $J = 2$ Hz, rhamnosyl anomeric H), 4.90 (1 H, d, $J = 7.5$ Hz, glucosyl anomeric H), 4.85 (1 H, d, $J = 2$ Hz, H-8), 6.92(1 H, d, $J = 8$ Hz, H-5), 7.40 (3 H, overlap H-3′, H-4′ and H-5′), 7.55 (2 H, overlap H-2′and H-6′), 7.95 (1 H, d, $J = 8$ Hz, H-6), 8.35 (1 H, s, H-2); ^{13}C NMR (100 MHz DMSO-d_6) δ: 153.95 (C-2), 123.71 (C-3),174.63 (C-4), 116.73 (C-4a), 127.91 (C-5), 115.49 (C-6), 162.91 (C-7), 101.90 (C-8), 157.64 (C-8a), 132.25 (C-1′), 128.30 (C-2′), 129.10 (C-3′), 127.48 (C-4′), 129.10 (C-5′), 128.30 (C-6′), 98.2 (C-1″), 72.51 (C-2″), 72.56 (C-3″), 71.70 (C-4″), 73.25 (C-5″), 61.36 (C-6″), 100.4 (C-11‴), 70.70 (C-2‴), 70.58 (C-3‴), 72.11(C-4‴), 67.88 (C-5‴), 18.20 (C-6‴); FAB-MS (*m/z* relative intensity %): 547 ([$M^+ + H$], 30), 238 (50), 210 (30), 121 (35), 120 (45), 108 (20), 102 (35); Elemental analysis: Found: C, 59.32%, H, 5.40%; calcd for $C_{27}H_{30}O_{12}$: C, 59.34%, H, 5.49%.

3.3.2 Methanolysis of echinoside (7). Compound **7** (22 mg) was dissolved in MeOH (8 ml) and refluxed with 6% aqueous HCl for 6 h. The reaction mixture was poured into H_2O (15 ml) and the MeOH removed by evaporation on water bath and the remained aqueous solution was extracted with $CHCl_3$. The chloroform extract yielded 7-hydroxyisoflavone (**8**) (10 mg) as granules, mp 214–215°C, Rf 0.52 (solvent A); UV λ_{max} (MeOH, nm): 260 (log ϵ 4.13), 290 sh (log ϵ 3.40), 315 sh (log ϵ 3.34); λ_{max} (MeOH + NaOAc, nm): 270, 290 sh 316 sh; IR ν_{max} (KBr, cm^{-1}): 3200–3600 (OH), 1630 (chelated carbonyl), 1570, 1450, 1370, 1230, 1260, ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.90 (1 H, d, $J = 2$ Hz, H-8), 6.94 (1 H, d, $J = 8$ Hz, H-5), 7.39 (3 H, overlap H-3′, H-4′, H-5′), 7.56 (2 H, overlap H-2′, H-6′), 7.92

(1 H, d, $J = 8$ Hz, H-6), 8.36 (1 H, s, H-2), 10.80 (1 H, br s, OH-7; ^{13}C NMR (100 MHz DMSO-d_6) δ: 153.4 (C-2), 123.3 (C-3), 174.0 (C-4), 116.4 (C-4a), 127.0 (C-5), 115.0 (C-6), 162.4 (C-7), 101.9 (C-8), 157.1 (C-8a), 131.8 (C-1′), 127.8 (C-2′), 128.6 (C-3′), 127.4 (C-4′), 128.6 (C-5′), 127.8 (C-6′); HR EIMS (relative intensity %) *m/z*: 238.0630 $[M]^+$, calcd for $C_{15}H_{10}O_3$ 238.0629 (48); Elemental analysis: Found: C, 75.59%, H, 4.18%; calcd for $C_{15}H_{10}O_3$: C, 75.62%, H, 4.20%. The hydrolysate showed two spots on PC which corresponded to D-glucose and L-rhamnose (solvent D) (co-PC with authentic sample).

3.3.3 Methylation of echinoside (7) by the Hakomori method. Echinoside (**7**) (30 mg) dissolved in DMSO (10 ml) was treated with NaH (110 mg) and MeI (3 ml) in N_2 stream. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$ in the usual way. The methylated product (**9**) on purification by prep. TLC furnished a crystalline mass, Rf 0.52 (solvent B), IR ν_{max} (KBr, cm^{-1}): 1632, 1570, 1445.

3.3.4 Methanolysis of permethylated product (9). Compound **9** was refluxed with 6% HCl in MeOH for 3 h. The MeOH was removed from the reaction mixture which was then extracted with $CHCl_3$. The $CHCl_3$ extract on crystallisation from MeOH furnished 7-hydroxyisoflavone (**8**), mp 215°C, which was identified by spectral data and comparison with authentic sample (mmp, co-TLC). The sugar residues in the hydrolysate was identified as 3,4,6-trimetthoxy-D-glucose (**10**) and 2,3,4-trimethoxy-L-rhamnose (**11**) by co-PC with authentic samples available in the laboratory.

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