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NEW FLAVONE GLYCOSIDE FROM *CASSIA TORA* LINN.

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ABSTRACT

A new flavone glycoside **A**, m.p. 208-210°C, m.f. $C_{33}H_{40}O_{19}$ $[M]^+$ 740 (FABMS), has been isolated from ethanolic extract of the seeds of *Cassia tora* Linn. It was characterized as 5,7,3'-trihydroxy-4'-methoxyflavone-7-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→4)–O-β-D-xylopyranoside by various chemical degradations and spectral analysis.

Key words: *Cassia tora*, Leguminosae, Flavonol glycoside.

INTRODUCTION

Cassia tora Linn.[1,2] belongs to family Leguminosae. It is commonly known as 'Chakunda' or 'Pamad' in Hindi. It is distributed throughout in India and most tropical countries. Its leaves and seeds are used in skin diseases chiefly in ringworm and itch. Its leaves are used as laxative in the form of decoction. Pods are used in dysentery and in diseases of eye.

Earlier workers[3-6] have reported various constituents from this plant. In the present paper, we report the isolation and structural elucidation of a new flavoneglycoside 5,7,3'-trihydroxy-4'-methoxyflavone-7-O-α-L-rhamnopyranosyl (1→3)-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranoside (**A**) from ethanolic extract of the seeds of this plant.

EXPERIMENTAL

General experimental procedure

All the m.p. were determined on a thermoelectrical melting point apparatus and are uncorrected. The IR spectra were recorded in KBr disc; ^{1}H-NMR spectra at 300 MHz in $CDCl_3$ using TMS as internal standard; ^{13}C-NMR spectra were recorded at 90 MHz using

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$CDCl_3$ as solvent; UV spectra were determined in MeOH and mass spectra on a Jeol D-300 mass spectrometer.

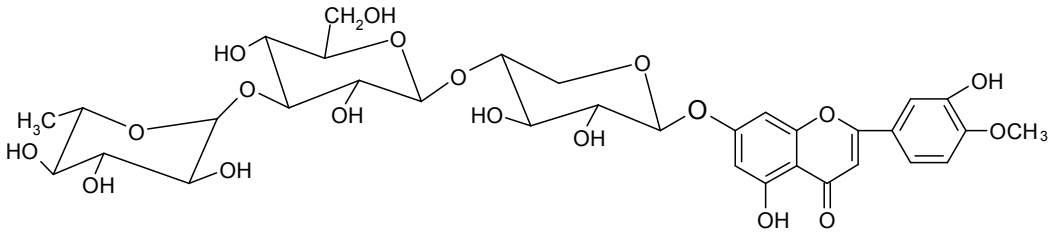
Plant material

The seeds of *Cassia tora* were procured from Sagar region and were taxonomically authenticated by the Department of Botany, Dr. H.S. Gour University, Sagar (M.P.), India. A voucher specimen has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar (M P.), India.

Extraction and isolation

Air dried powdered seeds (2 kg) of the plant were extracted with 95% ethanol in a Soxhlet apparatus for one week. The ethanol extract of the seeds of this plant was further exhaustively partitioned with pet. ether, chloroform, ethyl acetate, acetone and methanol. The methanol soluble fraction of ethanolic extract was concentrated under reduced pressure to yield brown viscous mass (1.85) g. It gave two spots on PC examination using nBAW (4 : 1 : 5) indicating it to be mixture of two compounds. These compounds were separated by TLC and purified by column chromatography over silica gel. The compound **B** was found in small quantity and hence, rejected.

Study of compound A



Compound A

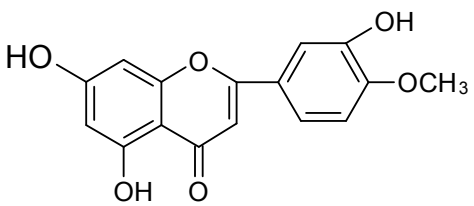
It has m.f. $C_{33}H_{40}O_{19}$, m.p. 208-210°C, $[M]^+$ 740 (FABMS); found (%); C 54.25, H 5.31, calcd. (%) m.f. $C_{33}H_{40}O_{19}$, C 54.5, H 5.39; UV (MeOH) λ_{max} (nm) 250, 342 (+$AlCl_3$) 252, 262; (+$AlCl_3$-HCl) 252, 360; (+NaOAc) 271, 340; IR ν_{max}^{KBr} (cm^{-1}); 3392 (-OH), 3015 (C-H aromatic), 2952 (-C-H saturated), 2865 (-OMe), 1647 (>C=O), 1620 (aromatic ring system), 1490, 1252, 1120, 837cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm); 6.93 (1H, s, H-3), 6.20 (1H, d, J 2.2 Hz, H-6), 6.47 (1H, d, *J* 2.2 Hz, H-8), 7.43 (1H, d, *J* 2.2 Hz, H-2'), 7.09 (1H, d, J 8.1 Hz, H-5'), 7.73 (1H, dd, *J* 8.8, 2.2 Hz, H-6'), 12.93 (s, 5-OH), 3.87 (s, OMe); 5.50 (1H, d, *J* 7.8 Hz, H-1"), 4.58 (1H, dd, *J* 3.8, 10.2 Hz, H-2"), 4.13 (1H, dd, *J*

3.8, 10.2 Hz, H-3''), 4.19 (1H, m, H-4''), 4.38 (2H, d, *J* 6.8 Hz, H-5''), 5.07 (1H, d, *J* 7.8 Hz, H-1'''), 3.82 (1H, dd, *J* 3.8, 10.2 Hz, H-2'''), 3.70 (1H, dd, *J* 3.8, 10.2 Hz, H-3'''), 3.51 (1H, dd, *J* 3.7, 10.2 Hz, H-4'''), 3.67 (1H, m, H-5'''), 3.20 (2H, d, *J* 6.4, Hz, H-6'''), 5.35 (1H, d, *J* 7.0 Hz, H-1''''), 4.59 (1H, dd, *J* 3.2, 9.6 Hz, H-2''''), 4.58 (1H, dd, *J* 3.6, 9.7 Hz, H-3''''), 4.55 (1H, dd, *J* 3.2, 9.8 Hz, H-4''''), 4.18 (1H, m, H-5''''), 1.18 (3H, m, -Ome 4''''), ^{13}C-NMR (90 MHz, $CDCl_3$) δ (ppm); 163.4 (C-2), 103.4 (C-3), 181.6 (C-4), 103.6 (C-4a), 161.4 (C-5), 98.6 (C-6), 164.1 (C-7), 157.2 (C-8a), 122.9 (C-1'), 112.8 (C-2'), 146.7 (C-3'), 151.0 (C-4'), 112.1 (C-5'), 118.6 (C-6'), 55.7 (C-4' OMe); 105.4 (C-1''), 80.2 (C-2''), 175.1 (C-3''), 78.6 (C-4''), 67.2 (C-5''), 100.8 (C-6'', C-6'''), 84.1 (C-1'''), 77.7 (C-2'''), 71.0 (C-3'''), 77.2 (C-4'''), 69.0 (C-5'''), 100.21 (C-1''''), 70.0 (C-2''''), 68.53 (C-3''''), 60.24 (C-4''''), 68.15 (C-5''''), 17.63 (C-6''''); MS: (FABMS) *m/z*, 740 $[M]^+$, 594 [M^+ -rhamnose], 446 [M^+-rhamnose-glucose], 300 $[aglycone]^+$.

Acid hydrolysis of compound A

100 mg of compound A was dissolved in ethanol (25 mL) and refluxed with 15 mL of 10% H_2SO_4 on water bath for 8-10 hrs. The contents were 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 (7 : 3) to give compound **B**, which was identified as 5,7,3' trihydroxy-4'-methoxyflavone 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) and aniline hydrogen phthalate as detecting agent. Sugars were identified as D-glucose, D-xylose and L-rhamnose.

Study of compound B



Compound B

It had m.f. $C_{16}H_{12}O_6$, m.p. 253-254°C, $[M]^+$ 300 (FABMS); found (%); C 64.30, H 4.25, calcd. (%)m.f. $C_{16}H_{12}O_6$, C 64.0, H 4.20; UV (MeOH) λ_{max} (nm) 250, 342, (+$AlCl_3$) 252, 262; (+$AlCl_3$-HCl) 252, 360; (+NaOAc) 271, 340; IR ν_{max}^{KBr} (cm^{-1}); 3458 (-OH), 3010 (-CH aromatic), 2949 (-CH saturated), 1612 (-CH aromatic ring system), 1485, 1255, 870,

830 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm); 6.93(1H, s, H-3), 6.20 (1H, d, J 2.2 Hz, H-6), 6.47 (1H, d, *J* 2.2 Hz, H-8), 7.43 (1H, d, *J* 2.2 Hz, H-2'), 7.09 (1H, d, J 8.1 Hz, H-5'), 7.73 (1H, dd, *J* 8.8, 2.2 Hz, H-6'), 2.65 (s, 5-OH), 3.87 (s, OMe); ^{13}C-NMR (90 MHz, $CDCl_3$) δ (ppm); 163.4 (C-2), 103.4 (C-3), 141.6 (C-4), 103.6 (C-4a), 161.4 (C-5), 98.6 (C-6), 164.1 (C-7), 157.2 (C-8a), 122.9 (C-1'), 112.8 (C-2'), 146.7 (C-3'), 151.0 (C-4'), 112.1 (C-5'), 118.6 (C-6'), 55.7 (C-4' OMe); MS: (FABMS) *m/z*, 300 $[M]^+$,

Permethylation of compound A

Compound A (50 mg) was refluxed with MeI (5 mL) and Ag_2O (20 mL) in DMF (35 mg) for two days and then filtered. The filtrate was hydrolysed with 10% ethanolic H_2SO_4 for 7-8 hrs. to give methylated aglycone, identified as 5,7,3',4' - tetramethoxy flavone and methylated sugars, which were identified as 2, 3, di-O-methyl-D-xylose, 2,4,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose.

Enzymatic hydrolysis of compound A

Compound **A** (10 mg) was dissolved in MeOH (25 mL) and hydrolysed with equal volume of enzyme takadiastase. The reaction mixture was allowed to stay 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) as solvent, which showed the presence of L-rhamnose (R_f 0.35). The proaglycone was dissolved in MeOH (25 mL) and hydrolysed with equal volume of almond emulsin at room temperature as usual procedure yielded aglycone, which was identified as 5,7,3'-tridydroxy-4'-methoxy flavone and sugars were identified as D-xylose (R_f 0.25) and D-glucose (R_f 0.19) (Co-PC).

RESULTS AND DISCUSSION

Compound **A** had a m.f. $C_{33}H_{40}O_{19}$, m.p. 208-210^{o}C, $[M]^+$ 740 (FABMS). It gave Molisch and Shinoda tests[7] showing its flavonoidal glycosidic nature. Its IR spectra showed absorption bands at 3392 (-OH) 3015 (-CH aromatic), 2952 (-CH saturated), 2865 (-OMe), 1647 (<C=O), 1620 (aromatic ring system), 1490, 1252 1120, 870 cm^1. The bands in its UV spectrum at 272 nm and 352 nm showed its flavonoid skeleton. The bathochromic shift of 20 nm with $AlCl_3$ in band revealed the presence of –OH group at C-5 position in compound **A**. In ^{1}H-NMR spectrum of compound **A**, two singlets at δ 6.20 and 6.47 were assigned to H-6 and H-8, respectively. One singlet at 1.18 was assigned to

OMe- group at C-4' position. The signal at δ 55.7 in ^{13}C-NMR spectra confirmed the presence of OMe group at C-4' position. The anomeric proton signals at δ 5.50 (1H, d, *J* 7.0 Hz), 5.07 (1H, d, *J* 7.2 Hz) and 5.35 (1H, d, *J* 7.0 Hz) were assigned H-1", H-1'" and H-1"" of D-xylose, and L-rhamnose, respectively. Characteristic ions appeared at m/z 740 $[M]^+$, 594 [M^+- rhamnose], 446 [M^+- rhamnose-glucose], $[M]^+$ 300, [aglycone] were obtained by subsequent lose from the molecular ion of one L-rhamnose, D-glucose and D-xylose, suggesting L-rhamnose as terminal sugar, D-glucose a middle sugar and D-xylose was linked to aglycone.

Acid hydrolysis of compound **A** with ethanolic 10% H_2SO_4 gave aglycone B, m.p. 253-254°C, m.f. $C_{16}H_{12}O_6$, $[M]^+$ 300 (FABMS). It was identified as 5,7,3'-trihydroxy-4'-methoxyflavone by comparison of its spectral data with reported literature values[8-10].

The aqueous hydrolysate, after the removal of aglycone, was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to PC[11] and sugars were identified as D-glucose (R_f 0.19), D-xylose (R_f 0.25) and L-rhamnose (R_f 0.35) (CO-PC)[11]. Periodate oxidations of compound **A** confirmed that all the sugars were present in the pyranose form[12].

The position of sugar moieties in compound **A** were determined by permethylation[13] followed by acid hydrolysis which yielded methylated sugars identified as 2,3,4-tri-O-methyl-L-rhamnose, 2,4,6-tri-O-methyl-D-glucose and 2,3-di-O-methyl-D-xylose (by Co-PC), which showed that C-1"" of L-rhamnose linked with C-3'" of D-glucose, C-1'" of D-glucose attached with C-4" of D-xylose and C-1" of D-xylose was linked with C-7 position of the aglycone[14]. The interlinkages (1→3) between L-rhamnose and D-glucose as well as (1→4) between D-glucose and D-xylose was further confirmed by ^{13}C-NMR spectra (see experimental section).

Enzymatic hydrolysis of compound **A** with takadiastase enzyme liberated L-rhamnose (R_f 0.35) and proaglycone, identified as 5, 7, 3'-trihydroxy-4'-methoxy flavone 7-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylophyranoside, that confirmed the presence of α-linkage between L-rhamnose and proaglycone. Proaglycone on further hydrolysis with almond emulsion liberated D-glucose (R_f 0.19) first, followed by D-xylose (R_f 0.25) and confirmed the presence of β- linkage between D-glucose and D-xylose as well as between D-xylose and aglycone.

On the basis of above evidences, the structure of compound **A** was characterized as 5,7,3' - trihydroxy - 4' - methoxyflavone - 7 - O - α - L - rhamnopyranosyl (1→3) - β - D -

glycopyranosyl - (1→4) - β - D - xylopyranoside.

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