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New Constituent from *Argemone mexicana* Linn.

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The present paper reports the isolation and structure elucidation of a new compound **A** identified as 5,7,4'-trihydroxy-8-methoxy flavanone-4'-α-L-rhamno-pyranosyl-7-O-β-D-xylopyranosyl-(1→3)-O-β-D-galactopyranoside along with two known compounds **B** and **C** identified as chrysoeriol and tricin, respectively from the methanolic extract of the defatted seeds of the plant by various colour reactions, chemical degradation and spectral analysis.

Keywords: *Argemone mexicana* Linn. Papaveraceae, Seeds, Chrysoeriol, Tricin.

INTRODUCTION

Argemone mexicana Linn.[1-4] belongs to Papaveraceae family. It is commonly known as 'Bharbhand' or 'Shialkanta' in Hindi. It is a native of Mexico which has run wild in India and is now a troublesome weed. It grows in wasteland and is abundantly found in Asia and Africa over a much extended area. Its medicinal value includes usage of root and stems for chronic skin diseases. Its juice or latex is recommended for treatment of abnormal accumulation of liquid in cellular tissue. Its seeds are also used as a laxative and for removal of mucous secretions from bronchial tubes. Its seeds resemble black mustard seeds and are therefore sometimes found mixed with it and this mixing of argemone oil in mustard oil is probably responsible for outbreaks of epidemic dropsy. Previous workers[5,6] have reported various constituents from this plant. This paper deals with the isolation and structural elucidation of compound **A**, identified as 5,7,4'-trihydroxy-8-methoxy flavanone-4'-α-L-rhamnopyranosyl-7-O-β-D-xylopyranosyl-(1→3)-O-β-D-galactopyranoside along with two known compounds, **B** identified as chrysoeriol and compound **C** identified as tricin from the methanolic extract of the defatted seeds of the plant.

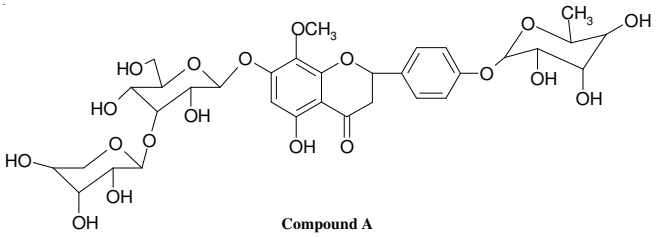
EXPERIMENTAL

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

The seeds of *Argemone mexicana* Linn. were procured from Sagar region and were taxonomically authenticated by the Department of Botany, Dr. H.S. Gour University, Sagar. A voucher specimen has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar, India.

Extraction and isolation: Air-dried and powdered seeds (4 kg) of the plant were extracted with petroleum ether (40-60 °C) in a Soxhlet apparatus for 4 days. The seeds were then successively extracted with $CHCl_3$, $CH_3COOC_2H_5$, CH_3COCH_3 and CH_3OH. The methanol soluble fraction of the plant was concentrated under reduced pressure to give brown viscous mass. It gave three spots on TLC examination using chloroform: methanol:water (6:4:2) as solvent and I_2 vapours as visualizing agent indicating it to be mixture of three compounds **A**, **B** and **C**. These were separated by column chromatography over a silica gel column using $CHCl_3$: MeOH (3:6) as eluent and studied separately.

Compound A (Fig. 1): It was obtained as light brown amorphous powder, m.p. 261-262 °C, m.f., $C_{33}H_{42}O_{19}$, $[M]^+$ *m/z* 742 (FABMS). Found C, 53.81 %; H, 5.62 %; Calcd. for molecular formula $C_{33}H_{42}O_{19}$: C, 53.37 %; H, 5.66 %. UV (MeOH) λ_{max} nm; 286, 328; IR (KBr, ν_{max}, cm^{-1}) 3276, 1630, 1596, 1312, 1249, 1182, 1158, 1078, 828; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.23 (1H, dd, *J* = 2.3, 12.5 Hz, H-2), 2.81 (1H, dd, *J* = 3.7, 16.8 Hz, H-3α), 3.18 (1H, dd, *J* = 11.8, 17.7 Hz, H-3β), 5.69 (2H, s, H-6), 3.77 (3H, s, OMe-8), 7.24 (2H, d, *J* = 8.1 Hz, H-2', H-6'), 7.11 (2H, d, *J* = 8.2 Hz, H-3', H-5'), 12.23

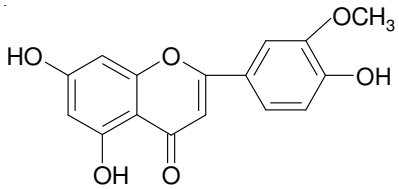


Compound A

Fig. 1.

(1H, s, 5-OH); 5.41 (1H, d, *J* = 7.2 Hz, H-1''), 4.58 (1H, dd, *J* = 2.9, 9.4 Hz, H-2''), 4.61 (1H, dd, *J* = 3.6, 9.3 Hz, H-3''), 4.66 (1H, d, *J* = 5.7 Hz, H-6'', CH_3); 5.24 (1H, d, *J* = 2.1 Hz, H-1'''), 4.69 (1H, dd, *J* = 3.1, 9.8 Hz, H-2'''), 4.73 (1H, dd, *J* = 2.8, 9.7 Hz, H-3'''), 4.67 (1H, dd, *J* = 2.9, 10.7 Hz, H-4'''), 4.34 (1H, m, H-5'''); 1.18 (3H, d, *J* = 6.1 Hz, H-6'''); 5.14 (1H, d, *J* = 6.4 Hz, H-1''''), 4.38 (1H, dd, *J* = 3.3, 10.7 Hz, H-2''''), 4.13 (1H, dd, *J* = 3.5, 10.4 Hz, H-3''''), 4.27 (1H, m, H-4''''), 4.68 (2H, d, *J* = 6.1 Hz, H-5''''); ^{13}C NMR (300 MHz, $CDCl_3$) δ 76.87 (C-2), 41.8 (C-3), 196.4 (C-4), 163.2 (C-5), 94.6 (C-6), 164.2 (C-7), 165.3 (C-8), 160.7 (C-9), 101.2 (C-10), 127.9 (C-1'), 129.3 (C-2'), 115.1 (C-3'), 157.6 (C-4'), 115.2 (C-5'), 129.1 (C-6'); 104.9 (C-1''), 84.82 (C-2''), 76.94 (C-3''), 71.03 (C-4''), 67.11 (C-5''), 17.71 (C-6', CH_3); 105.2 (C-1'''), 74.54 (C-2'''), 74.87 (C-3'''), 70.12 (C-4'''), 75.88 (C-5'''), 60.36 (C-6'''); 102.56 (C-1''''), 70.89 (C-2''''), 80.66 (C-3''''), 71.87 (C-4''''), 68.86 (C-5'''').

Compound B (Fig. 2): It was crystallized from MeOH to give light yellow powder. m.p. 325-327 °C, m.f., $C_{16}H_{12}O_6$ [M^+] 300 (FABMS); Found C, 63.97 %; H, 3.96 %; Calcd. for Molecular Formula $C_{16}H_{12}O_6$, C, 64 %; H, 4 %, UV (MeOH) λ_{max} nm; 266, 338; IR (KBr, ν_{max}, cm^{-1}) 3348, 1654, 1626, 1567, 1512, 1436, 1346, 1304, 1211, 1174, 1030, 996, 865, 838, 792, 760. ^{1}H NMR (300 MHz $CDCl_3$) δ 6.31 (1H, d, *J* = 2.3 Hz, H-6), 6.48 (1H, d, *J* = 2.4 Hz, H-8), 6,62 (1H, s, H-3), 7.12 (1H, d, *J* = 8.5 Hz, H-5), 7.54 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 7.71 (1H, d, *J* = 1.8 Hz, H-2''), 4.06 (1H, s, 4' OMe), 12.94 (1H, s, 5-OH).

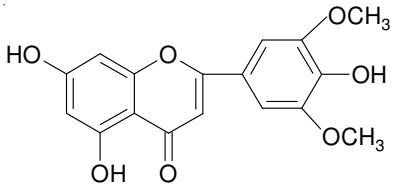


Compound B

Fig. 2.

Compound C (Fig. 3): It was obtained as light brownish yellow powder crystallized from MeOH. m.p. 275-276 °C, m.f., $C_{17}H_{14}O_7$; [M^+] 330 (FABMS); Found C, 61.79 %; H, 4.24 %; Calcd. for Molecular Formula $C_{17}H_{14}O_7$, C, 61.81 %; H 4.24 % ; UV (MeOH) λ_{max} nm 272, 352, IR (KBr, ν_{max}, cm^{-1}) 3452, 1656, 1612, 1504, 1098, 1072, 1030; ^{1}H NMR (300 MHz $CDCl_3$) δ 7.42 (1H, s, H-2', H-6'), 6.76 (1H, s, H-3), 6.38 (1H, d, *J* = 1.8 Hz, H-8), 6.24 (1H, d, *J* = 2.2 Hz, H-6), 3.91 (3H, s, -OMe-3'), 3.90 (3H, s, OMe-5'), 12.83 (1H, s, 5-OH).

Acid hydrolysis of compound A: Compound A (500 mg) was dissolved in ethanol (20 mL) and refluxed with 10 % H_2SO_4 (10 mL) on water bath for 8 h. The contents were concentrated and allowed to cool and residue was extracted with

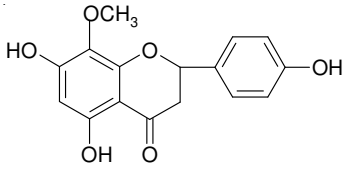


Compound C

Fig. 3

diethyl ether. The ethereal layer was washed with water and the residue was chromatographed over silica gel using $CHCl_3$: MeOH as solvent to give aglycone **A-1** which was identified as 5, 7, 4'-trihydroxy-8-methoxy flavanone by comparison of its spectral data with reported literature values. The aqueous hydrolyzate was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination using *n*-butanol:acetic acid: water *n*-BAW (4:1:5) as solvent system and the sugars so obtained were identified as D-xylose (R_f 0.26), L-rhamnose (R_f 0.36), D-galactose (R_f 0.15) by (Co-PC and Co-TLC).

Compound A-1 (Fig. 4): It is light yellow powder, m.p. 244-246 °C, m.f. $C_{16}H_{14}O_6$; [$M]^+$ 302 (FABMS); Found C, 65.55 %; H, 4.62 %; Calcd. for Molecular Formula $C_{16}H_{14}O_6$, C, 63.57 %; H, 4.64 % ; UV (MeOH) λ_{max} nm; 284, 326; IR (KBr, ν_{max}, cm^{-1}) 3272, 1632, 1394, 1310, 1252, 1186, 1160, 1054, 826; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.32 (1H, dd, *J* = 2.1, 12.3 Hz, H-2), 2.78 (1H, dd, *J* = 3.9, 16.5 Hz, H-3α), 3.21 (1H, dd, *J* = 11.6, 17.4 Hz, H-3β), 5.66 (2H, s, H-6), 3.81 (3H, s, OMe-8), 7.28 (2H, d, *J* = 8.4 Hz, H-2', H-6'), 7.19 (2H, d, *J* = 8.6 Hz, H-3', H-5'), 12.29 (1H, s, 5-OH); ^{13}C NMR (300 MHz, $CDCl_3$) δ 76.7 (C-2), 42.4 (C-3), 197.2 (C-4), 163.6 (C-5), 94.1 (C-6), 165.8 (C-7), 164.8 (C-8), 161.6 (C-9), 100.9 (C-10), 129.3 (C-1'), 128.8 (C-2'), 115.7 (C-3'), 158.1 (C-4'), 115.7 (C-5'), 128.8 (C-6').



Compound A-1

Fig. 4

Permethylation of compound A: Compound A (20 mg) was dissolved in DMF (30 mL) and treated with MeI (5 mL) and Ag_2O (15 mg) in 150 mL round bottomed flask fitted with condenser and refluxed for 2 days. The contents were filtered and washed with DMF. The filtrate was concentrated under reduced pressure and treated with $CHCl_3$ (25 mL) and washed with water. After removal of solvent a syrupy mass was found which was hydrolyzed with 7 % H_2SO_4 (5 mL) to give methylated aglycone, identified as 7,4'-dihydroxy, 5,8-di-methoxy flavanone and methylated sugars. The aqueous hydrolyzate obtained after the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography on Whatmann filter paper No.1 using *n*-butanol:ethanol:water (5:1:4) as solvent system and aniline hydrogen phthalate as spraying agent. The methylated sugars were identified as 2,3,4-

tri-O-methyl L-rhamnose, 2,4,6-tri-O-methyl D-galactose, 2,3,4-tri-O-methyl D-xylose.

Enzymatic hydrolysis of compound A: Compound **A** (40 mg) was dissolved in MeOH (25 mL) and hydrolyzed with equal volume of almond emulsin enzyme. The reaction mixture was allowed to stay undisturbed at room temperature for 2 days and filtered. The hydrolyzate was concentrated and subjected to paper chromatography examination using *n*-BAW (4:1:5) as solvent system and aniline hydrogen phthalate as a spraying reagent which showed the presence of D-galactose (R_f 0.15) and D-xylose (R_f 0.26). The proaglycone was dissolved in MeOH (10 mL) and further hydrolyzed with equal volume of enzyme takadiastase at room temperature which yielded aglycone identified as 5,7,4'-trihydroxy-8-methoxy flavanone and the sugar was identified as L-rhamnose R_f (0.36) by Co-PC and Co-TLC.

RESULTS AND DISCUSSION

Compound **A** has m.p. 261-262 °C, m.f. $C_{33}H_{42}O_{19}$ [M^+] 742 (FABMS).

It gave positive Molisch[7] and Shinoda[8] test showing its flavonoidal glycosidic nature. Its UV spectrum showed absorption bands at 286 and 328 nm suggesting it to be a flavonoid. Its IR spectrum showed bands at 3272 cm^{-1} for OH group and at 1632 cm^{-1} for carbonyl group. In its ^1H NMR spectra signals at δ 7.24 (2H, d, J = 8.1 Hz) and at δ 7.11 (2H, d, J = 8.2 Hz) were assigned to H-2', H-6' and H-3', H-5' respectively. Characteristics peak were observed at δ 5.23 (1H, dd, J = 2.3, 12.5 Hz) for H-2 and at δ 2.81 (1H, dd, J = 3.7, 16.8 Hz) and δ 3.18 (1H, dd, J = 11.8, 17.7 Hz) for H-3α and H-3β respectively. Three singlets at δ 5.69 for H-6 at δ 3.77 for OMe-8 and at δ 12.23 for OH group at H-5 were present. The anomeric proton signals at δ 5.41 (1H, d, J = 7.2 Hz), δ 5.24 (1H, d, J = 2.1 Hz) and at δ 5.14 (1H, d, J = 6.4 Hz) were assigned to H-1" of L-rhamnose, H-1"' of D-galactose and H-1"" of D-xylose respectively. In ^1H NMR spectrum coupling constant J = 2.1 Hz and J = 6.4 Hz value of anomeric proton of D-galactose and D-xylose confir-med β configuration between galactose and xylose and coupling constant J = 7.2 Hz value for anomeric proton of L-rhamnose confirmed the α configuration of L-rhamnose.

Acid hydrolysis of compound **A** with 10 % ethanolic H_2SO_4 gave aglycone **A-1** m.p. 244-246 °C, m.f., $C_{16}H_{14}O_6$ [M^+] 302 (FABMS) identified as 5, 7, 4'-trihydroxy-8-methoxy flavanone by comparison of its spectral data with reported literature values[9-11]. The aqueous hydrolysate obtained was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography[12] showing the presence of L-rhamnose, D-xylose and D-galactose. Quantitative estimation[13] of sugars revealed that all the three sugars were present in equimolecular ratio (1:1:1). Periodate oxidation[4] of compound A confirmed that all the three sugars were present in pyranose form.

The position of the sugar moieties in compound **A** were determined by permethylation[14,15] followed by acid hydrolysis, yielding methylated aglycone and methylated sugars. The methylated aglycone was identified as 7,4'-dihydroxy, 5,8-dimethoxy flavanone and the methylated sugars were identified as 2,3,4-tri-O-methyl L-rhamnose 2,4,6-tri-O-methyl D-galactose and 2,3,4-tri-O-methyl-D-xylose, confirming that hydroxyl group at C-7 and C-4' position of aglycone were involved in glycosidation.

Therefore it was concluded that C-1" of L-rhamnose was linked with C-4' position of aglycone and C-1"' of D-galactose was linked to C-7 position of aglycone and also that C-3"' of D-galactose is linked to C-1"" of D-xylose. The inter glycosidic linkage (1→3) was found between D-xylose and D-galactose.

Enzymatic hydrolysis[16] of compound **A** with almond emulsin enzyme liberated D-xylose, D-galactose and proaglycone confirming the presence of β linkage between D-xylose and D-galactose and also between D-galactose and proaglycone. Proaglycone 5, 7, 4'-trihydroxy 8 methoxy flavanone -4'-α-L-rhamnopyranosyl, on further hydrolysis with takadiastase liberated L-rhamnose and aglycone, this confirmed the presence of α-linkage between L-rhamnose and aglycone. Thus on the basis of above evidence the structure of compound A was established as 5,7,4'-trihydroxy-8-methoxy flavanone-4'-α-L-rhamnopyranosyl-7-O-β-D-xylopyranosyl-(1→3)-O-β-D-galactopyranoside.

Compound **B** was analyzed for m.f., $C_{16}H_{12}O_6$, m.p. 325-327 °C [M^+] 300 (FABMS) and was identified as Chrysoeriol by comparison of its spectral data with reported literature values[17].

Compound **C** was analyzed for m.f., $C_{17}H_{14}O_7$, m.p. 275-276 °C [M^+] 330 (FABMS) and was identified as Tricin by comparison of its spectral data with reported literature values[18].

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