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Novel Biologically Active Triterpenoid Saponin from the Leaves of *Xanthium strumarium* Linn.

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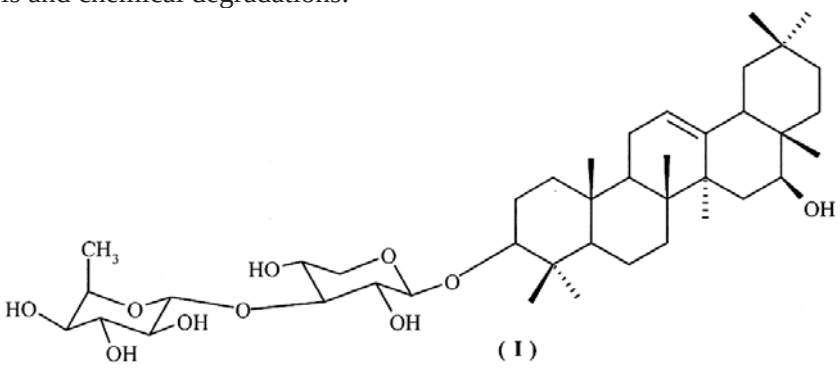
A novel triterpenoid saponin, 3-O[α-L-rhamnopyranosyl-(1→3)-O-β-D-xylopyranosyl]maniladiol has been isolated from the leaves of *Xanthium strumarium*. Its structure was determined by various spectral analysis and chemical degradations. This compound shows antiinflammatory activity.

Key Words: *Xanthium strumarium* Linn., Compositae, Leaves, Triterpenoid saponin, Antiinflammatory activity.

INTRODUCTION

Xanthium strumarium Linn.[1-3] belongs to family compositae. It is commonly known as "Chotagokhru" or "Banokra" in Hindi. It is found almost throughout India and Ceylon. The plant is considered to have diaphoretic and sedative properties. Its leaves are reported to possess astringent, alterative, antisyphilitic and diuretic properties. Its root is bitter tonic and useful in cancer and strumous diseases. Its fruits are useful in small-pox. Earlier workers[4-8] have reported the presence of various constituents from this plant.

The present paper deals with the isolation and structural elucidation of a novel bioactive triterpenoid saponion, 3-O[α-L-rhamnopyranosyl-(1→3)-O-β-D-xylopyranosyl]maniladiol (**I**), alongwith known compound ursolic acid (**II**) from the leaves of this plant on the basis of various spectral analysis and chemical degradations.



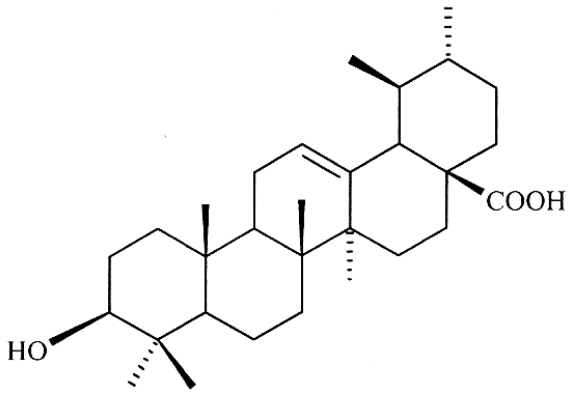
(I)

EXPERIMENTAL

Melting points are uncorrected. The infrared spectra were recorded (KBr discs) on a FT-IR spectrophotometer. ^{1}H NMR spectra were recorded at 300 MHz, in $CDCl_3$, using TMS as an internal standard solvent, ^{13}C NMR spectra at 90 MHz, in $CDCl_3$ as solvent. Mass spectra were determined on Jeol D-300-mass spectrometer.

The leaves of *Xanthium strumarium* were collected locally around Sagar region and were taxonomically identified by the Taxonomist, Department of Botany, Dr. H.S. Gour University, Sagar, India. The voucher specimen was deposited in Chemistry Department of this University.

Extraction and isolation: Dried powdered leaves (3 kg) of *Xanthium strumarium* were extracted with 95% EtOH in a Soxhlet extractor. The combined ethanolic extract was concentrated under reduced pressure to yield a brown viscous mass, which was successively extracted with petroleum ether (40-60ºC), benzene, chloroform, ethyl acetate, acetone and methanol. The acetone soluble fraction was concentrated under reduced pressure to give a light brown viscous mass (2.10 g), which yielded a precipitate on addition of excess Et_2O. On TLC examination, it gave three spots indicating it to be mixture of three compounds. These compounds were separated by column chromatography over silica-gel using MeOH-$CHCl_3$ (6:4) to yield compound **I** (0.89 g), compound **II** (0.35 g) and compound **III**. Compound **III** was found in very small quantity therefore it was rejected.



(II)

Compound I: m.f. $C_{41}H_{68}O_{10}$, m.p. 254-256ºC, [M^+]; 720 (FABMS), Calcd. (found) (%): C 68.33 (68.38), H 9.44 (9.40), IR (KBr, ν_{max}, cm^{-1}) 3485, 1375, 1361, 1064, 1025. ^{1}H NMR (300 MHz-$CDCl_3$), δ 0.70 (3H, s, CH_3), δ 0.79 (3H, s, CH_3), δ 0.85 (3H, s, CH_3), δ 0.86 (3H, s, CH_3), δ 0.88 (3H, s, CH_3), δ 0.92 (3H, s, CH_3), δ 0.96 (3H, s, CH_3), δ 1.12 (3H, s, CH_3), δ 3.62 (1H, dd, J 5.43 Hz, 10.4 Hz, H-3), δ 3.82 (1H, m, H-16), δ 5.17 (1H,

t, J 6.5 Hz, H-12), δ 5.04 (1H, d, J 7.5 Hz, H-1'), δ 3.50-4.43 (5H, m, H-2', H-3', H-4', H-5'), δ 5.28 (1H, br, s, H-1''), δ 4.16 (1H, br, d, J 3.6 Hz, H-2''), δ 3.83 (1H, dd, J 8.4, 3.1 Hz, H-3''), δ 3.44 (1H, dd, J 9.2 Hz, H-4''), δ 3.35 (1H, dq, J 9.1, 6.1 Hz, H-5'') and δ 1.14 (3H, d, J 5.6, Rham-Me). ^{13}C NMR (90 MHz-$CDCl_3$), 38.4 (C-1), 27.6 (C-2), 78.8 (C-3), 38.6 (C-4), 55.2 (C-5), 18.3 (C-6), 32.5 (C-7), 39.9 (C-8), 47.6 (C-9), 37.1 (C-10), 23.4 (C-11), 122.8 (C-12), 143.9 (C-13), 41.8 (C-14), 27.9 (C-15), 78.0 (C-16), 47.2 (C-17), 41.4 (C-18), 46.3 (C-19), 30.9 (C-20), 35.7 (C-21), 31.5 (C-22), 28.1 (C-23), 16.0 (C-24), 15.8 (C-25), 17.2 (C-26), 25.6 (C-27), 28.8 (C-28), 32.9 (C-29), 22.2 (C-30), 102.5 (C-1'), 79.6 (C-2'), 78.3 (C-3'), 70.7 (C-4'), 67.0 (C-5'), 111.4 (C-1''), 71.3 (C-2''), 69.6 (C-3''), 73.2 (C-4''), 67.7 (C-5''), 18.6 (C-6''), [M^+]; 720, m/z, 574, 442, 234, 216, 208, 122.

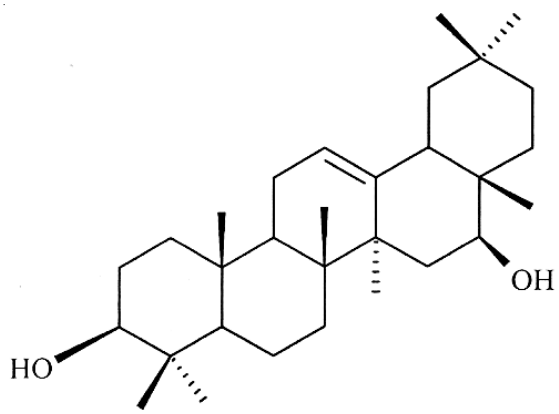
Compound II: It gave a positive test for triterpenes, m.f. $C_{30}H_{48}O_3$, m.p. 283-285ºC, [M^+] 456 (FABMS), Calcd. (found) %: C 78.95 (78.92), H 10.53 (10.55). Compound **II** was identified as ursolic acid by direct comparison with authentic sample[9].

Acid hydrolysis of compound I: Compound **I** (450 mg) was treated with MeOH (30 mL) and 2N H_2SO_4 (25 mL) and refluxed on water bath for 8-10 h. The solution was then concentrated under reduced pressure and allowed to cool. The residue was extracted with Et_2O. The aqueous layer was worked-up separately for identification of sugars. The ethereal layer was washed with water and evaporated to dryness and the residue was chromatographed over a silica-gel using MeOH-$CHCl_3$ (8:6) to yield compound **IV** (0.255 g) (aglycone), which was identified as maniladiol with known reported literature values.

The aqueous hyrolyzate was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filterate was concentrated and subjected to paper chromatography [n-BAW (4:1:5)], showed the presence of L-rhamnose (R_f. 0.36) and D-xylose (R_f : 0.29).

Compound IV: m.f. $C_{30}H_{50}O_2$, m.p. 224-226ºC, [M^+]; 442 (FABMS), Calcd. (found) % : C 81.45 (81.42), H 11.31 (11.34). IR (KBr, cm^{-1}) 3486, 1376, 1363, 1063, 1027. ^{1}H NMR (300 MHz-$CDCl_3$), δ 0.69 (3H, s, CH_3), δ 0.80 (3H, s, CH_3), δ 0.85 (3H, s, CH_3), δ 0.87 (3H, s, CH_3), δ 0.89 (3H, s, CH_3), δ 0.91 (3H, s, CH_3), δ 0.97 (3H, s, CH_3), δ 1.11 (3H, s, CH_3), δ 3.64 (1H, dd, J 5.46 Hz, J 10.6 Hz, H-3), δ 3.82 (1H, m, H-16), δ 5.18 (1H, t, J 6.4 Hz, H-12) and identified as maniladiol as aglycone with known reported literature values.

Permethylation followed by hydrolysis of compound I: Compound **I** (25 mg) in CH_3I (5 mL) and Ag_2O (50 mg) in DMF (10 mL) were treated for 24 h at room temperature. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$ (30 mL) yielded permethylated aglycone and methylated sugars which were identified as 2,4-di-O-methyl-D-xylose and 2,3,4-tri-O-methyl-L-rhamnose according to Petek.



(IV)

Periodate oxidation of compound I: Compound **I** (25 mg) was dissolved in MeOH (35 mL) and treated with 10 mL of 1N sodium metaperiodate. The reaction mixture was left for 24 h at room temperature. The liberated formic acid and consumed metaperiodate were estimated by Jone's method.

Enzymatic hydrolysis of compound I: The compound **I** (25 mg) was dissolved in MeOH (20 mL) and hydrolyzed with enzyme takadiastase yielded L-rhamnose (R_f: 0.36) and proaglycone, suggesting the presence of α-linkage between L-rhamnose and D-xylose. Proaglycone on further hydrolysis with equal volume of almond emulsin at room temperature for 65-68 h, gave D-xylose (R_f : 0.29) and aglycone (Sapogenin) which confirmed the presence of β-linkage between D-glucose and aglycone.

RESULTS AND DISCUSSION

The acetone soluble fraction of the plant afforded a compound **I**, m.f. $C_{41}H_{68}O_{10}$, m.p. 254-256ºC, [M^+]; 720 (FABMS). It gave all the characteristic reactions of saponin[10,11]. Its IR spectrum showed absorption peaks at (KBr, cm^{-1}) 3485, 1375, 1361, 1064 and 1025. Compound **I** did not display any absorption in the UV spectrum above 200 nm (in MeOH).

In ^{1}H-NMR spectrum of compound **I**, a signal at δ 5.17 (t, J 6.5 Hz) was assigned to one vinylic proton at H-12. The double doublet at δ 3.62 (dd, J 5.43 Hz, 10.4 Hz) was assigned to H-3 and resonance signals at δ 0.70 (3H, s, CH_3), δ 0.79 (3H, s, CH_3), δ 0.85 (3H, s, CH_3), δ 0.86 (3H, s, CH_3), δ 0.88 (3H, s, CH_3), δ 0.92 (3H, s, CH_3), δ 0.96 (3H, s, CH_3) and δ 1.12 (3H, s, CH_3) corresponding to eight methyl groups. A multiplet at δ 3.82 was assigned to H-16. The anomeric proton signals at δ 5.04 (d, J 7.5 Hz) and δ 5.28 (br, s) were assigned to H-1' and H-1" of D-xylose and L-rhamnose. Characteristic ions appeared at m/z 573 [M-H-146] and 441 [M-H-146-132] were obtained by subsequent losses from the molecular

ion of one L-rhamnose and one D-xylose units suggesting L-rhamnose as terminal sugar and D-xylose was directly linked to sapogenin.

Acid hydrolysis of compound **I** with MeOH and 2N H_2SO_4 gave compound **IV**, m.f. $C_{30}H_{50}O_2$, m.p. 224-226ºC, [M^+] 442 (FABMS). It responsed to all the characteristic colour reaction of triterperoids[12,13] and identified as maniladiol by comparison of its spectral data with known reported literature[14].

The aqueous hydrolyzate obtained after the acid hydrolysis of compound **I** was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to PC [n-BAW (4:1:5)] yielded L-rhamnose (R_f : 0.36) and D-xylose (R_f : 0.29)[15].

Compound **I** was not hydrolyzed with a specific reagent (5N NH_4OH) for the hydrolysis of sugar esters, revealing that sugars were linked to hydroxyl group at C-3 position. The sequence of the sugars residue in compound **I** was confirmed by its partial hydrolysis which gave prosapogenin PS_1 and L-rhamnose PS_1 which on further hydrolysis afforded maniladiol and D-xylose and suggesting that D-xylose was directly linked to sapogenin.

Permethylation of compound **I** follwed by acid hydrolysis yielded permethylated sapgenin and permethylated sugars which were identified as 2,4-di-O-methyl-D-xylose and 2,3,4-tri-O-methyl-L-rhamnose. According to Petek[16] C-1" of L-rhamnose was linked with C-3' of D-xylose. The inter sugar linkage (1→3) was further confirmed by its ^{13}C NMR spectral data (see experimental). Periodate oxidation[17] of compound **I** confirmed that the sugars were presented in pyranose form.

Enzymatic hydrolysis of compound **I** with takadiastase liberated L-rhamnose and proaglycone, suggesting the presence of α-linkage between L-rhamnose and D-xylose. Proaglycone on further hydrolysis with almond emulsin gave D-xylose and aglycone confirming the presence of β-linkage between D-xylose and agylcone (sapogenin).

On the basis of above evidences, the strucutre of compound **I** was assigned as 3-O-[α-L-rhmnopyranosyl(1→3)-O-β-D-xylopyranosyl]-maniladiol.

Antiinflammatory activity of compound I

Antiinflammatory activity of the acetone soluble fraction of compound **I** was carried out by non-immunological carrageenin induced hind paw Oedema method[18].

Adult albino rats of either male or female weighing 120-170 g were taken in the present study. The volume was measured by Plethysmograph. Initial volume of right hind paw of albino rats were 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 inflammed foot produced by injection of 0.06 mL of 1 % freshly prepared carrageenin suspension.

Albino rats were divided in to three groups, each group consists of four rats. First group of rats were treated intraperitonically (i.p.) with 60 mg/kg body weight of the acetone soluble fraction of compound **I**.

Second group was administered (i.p.) with 50 mg/kg body weight of the aqueous suspension of acetyl salicylic acid and the third control group was fed with the same volume of distilled water. After 1 h of the drug administration, the rats were injected 0.06 mL suspension of carrageenin in the right hind paw.

The measurement of the paw volume was taken by mercury displacement technique with the help of Plethysmograph immediately before and after the carrageenin injection after 1, 2 and 3 h. The percentage inhibition of inflammation after 3 h was calculated by the formula given by Newbould[19].

$$I = 100\ [1-(a-x)/(b-y)]$$

where: x = mean foot volume of rats before the administration of carrageenin injection in the test and the standard group, a = mean foot volume of rats after the administration of carrageenin injection in the test and the standard group, y = mean foot volume rats before the administration of carrageenin injection in the test and the control group and b = mean foot volume of rats after the administration of carrageenin injection in the test and the control group.

S. No.	Test solution applied	Dose mg/kg i.p.	Volume of paw after drug administration				Total increase in paw volume	Inhibition (%)
			0 h	1 h	2 h	3 h		
1.	Control group	–	0.60	0.69	0.80	0.94	0.34	–
2.	Treated group	60	0.60	0.58	0.68	0.76	0.16	53
3.	ASA	50	0.60	0.66	0.76	0.81	0.21	38

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