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A New Steroidal Saponin from *Trichosanthes dioica* (Roxb.)

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A new steroidal saponin 24-α-ethyl-20-ene-7-hydro-stigmast-8β:14β-di-3-O-β-D-xylofuranoside **1** has been isolated from MeOH soluble fraction of 95% EtOH extract of the leaves of *Trichosanthes dioica.*

INTRODUCTION

Trichosanthes dioica (Roxb.)[1] (Cucurbitaceae) occurs in the plains of North India and the plant has been reported to be used as cardiotonic, laxative, stomachic, antipyretic, antitumor and as antidiabetic agent[2, 3]. Its various parts have been phytochemically investigated by earlier workers who reported the presence of bitter principles along with amorphous saponins and essential oils. Its antidiabetic and antitumor activities have already been evaluated with significant success[4,5].

RESULTS AND DISCUSSION

The MeOH soluble part of the 95% EtOH extract of the leaves of *Trichosanthes dioica* (Roxb.) when worked up gave compound **1** (0.0728), m.p. 205–6°C, molecular formula $C_{34}H_{58}O_6$, $[M]^+$ 562 and $[\alpha]_D^{27} = +19.5$ (in $CHCl_3$). It gave positive foam test[6], honey comb and haemolytic test[7, 8] indicating its nature as saponin.

1 gave the maximum absorption at 218 and 295 nm and responded to positive Molisch's test. On acid hydrolysis **1** yielded sapogenin **2**, m.p. 189–90°C molecular formula $C_{29}H_{50}O_3$, $[M]^+$ 446, $[\alpha]_D^{27} = 9.3$ in $CHCl_3$ and sugar moieties. Compound **2** responded to the positive colour reactions of the steroids[9, 10].

ν_{max}(KBr) 3560 cm^{-1} of **2** indicated the presence of OH group(s), which was further confirmed by ^1H NMR of the monoacetate derivative **3** m.p. 167–68°C, molecular formula $C_{31}H_{52}O_4$, $[M]^+$ 488, showed singlets at δ 2.15 for —OAc and δ 3.15 for OH. Compound **2** on treatment with HCl forms a trianhydrosapogenin **4** m.p. 181–82°C, molecular formula $C_{29}H_{44}$ confirming the presence of three OH group(s), out of which one —OH group was either primary or secondary and remaining two —OH group(s) must be tertiary.

CrO_3/pyridine oxidation of the compound **2** yielded a ketone **5** m.p. 170–71°C, molecular formula $C_{29}H_{49}O_3$, $[M]^+$ 445, which gave a positive Zimmermann test for C-3 keto group thereby confirming the presence of one —OH group at C-3 and further indicating its secondary nature. The position of double bond was confirmed by the KOH hydrolysis of compound **2** giving an isosapogenin **6**, m.p. 254–56°C, molecular formula $C_{29}H_{50}O_3$, $[M]^+$ 445, which showed a quartet at δ 3.8, C-22 H and multiplet at δ 2.3, C-20 H indicating cleavage of double bond in between C-20 and C-22 and forming an epoxy-linkage, which further explains the fixing of tertiary OH group[11] at C-14. The

formation of 8:14 diketone **7** with lead tetraacetate of compound **2** suggested the adjacent position of the tertiary OH group(s)[12].

The monoacetate of compound **2** on oxidation with $KMnO_4$ in acetone yielded a compound **9**, molecular formula $C_{20}H_{32}O_5$, which showed absorption for carbonyl group in IR and was found to be 3β:8β:14β-trihydroxy-etianic acid[13] (confirmed by mmp, Co-TLC and superimposable spectra), in fact establishing the location of a side chain at C-17. The peak in the IR spectrum at ν_{max}(KBr) 1650 cm^{-1} indicated the presence of unsaturation in it which is further supported by the fact that **2** on catalytic hydrogenation gave a dihydrosapogenin **8** m.p. 182–83°C, molecular formula $C_{29}H_{52}O_3$, $[M]^+$ 448. The ^1H NMR in upfield chemical shift at δ 4.22 ppm, C-22 H also confirmed the presence of unsaturation in it (Scheme I).

Attachement of sugar in the steroidal saponin **1** was fixed at C-3 as the **1** itself did not give a positive Zimmermann test, whereas the sapogenin **2** did this, thereby confirming that C_3-OH group was free in the sapogenin but was involved in the glycosylation in **1**.

Periodate oxidation[14] of **1** with HIO_4 indicated that D-xylose was present in furanose form and its hydrosate showed the presence of 2:3 di-*o*-methyl-D-xylose (confirmed by Co-Pc and Co-TLC) which also suggested that C-1 of D-xylose was involved in glycosidic linkage, whereas hydrolysis with almond emulsion of **1** yielded D-xylose indicating that the D-xylose was linked *via* β-linkage to the sapogenin. Thus the identity of novel compound **1** is assigned as 24-α-ethyl-20-ene-7-hydro-stigmast-8β:14β-di–3-O-β-D-xylofuranoside.

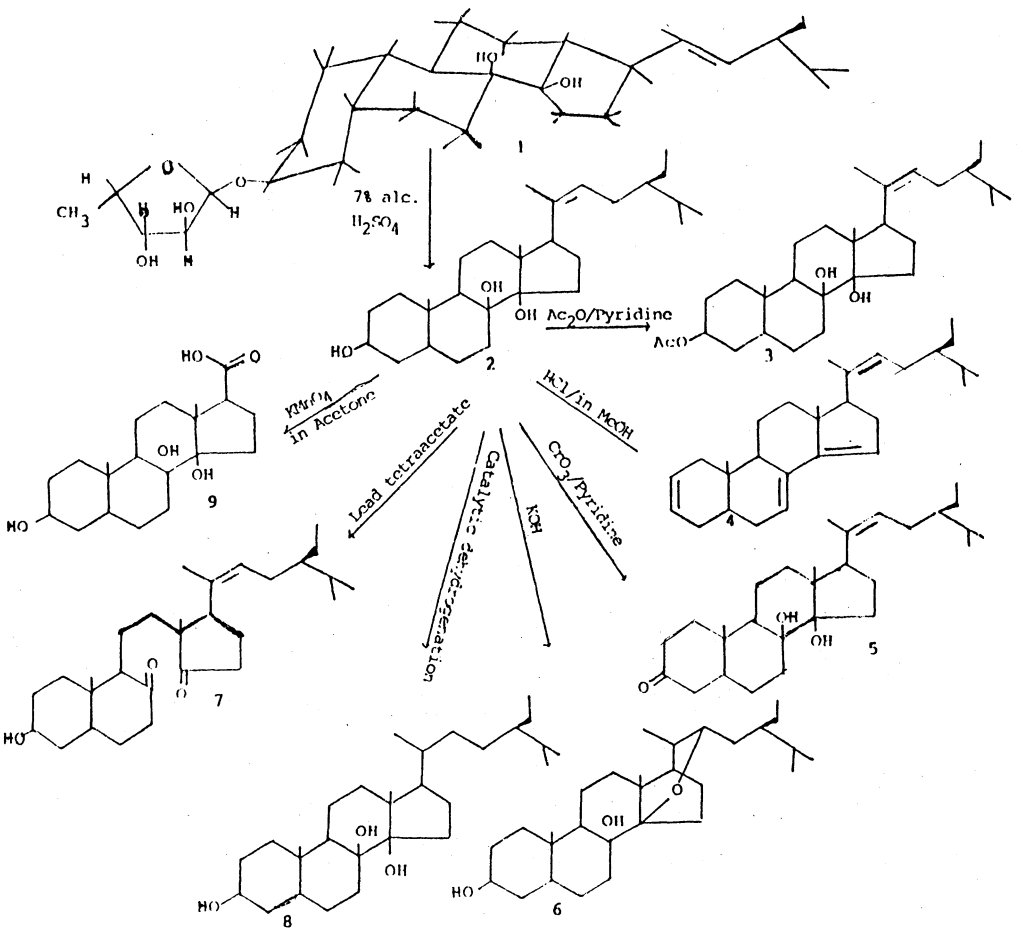
EXPERIMENTAL

Plant Material: *Trichosanthes dioica* (Roxb.) was procured from M/s. United Chemical and Allied Products, Calcutta. The plant was authenticated by the Department of Botany (courtesy Dr. Harisingh Gour University, Sagar, India). A herbarium specimem (No. IV–XVII) has been deposited in the Department of Chemistry of the University.

Isolation: Air-dried, powdered leaves of *Trichosanthes dioica* (Roxb.), 4 kg, were extracted with 95% EtOH. The extract was concentrated to a greenish brown viscous mass under reduced pressure and successively segregated in to pet. ether, C_6H_6, $CHCl_3$, EtOAc, Me_2CO and MeOH soluble fractions.

The MeOH-soluble fraction was concentrated to a dark greenish yellow viscous mass which showed two spots on TLC examination [Si-gel, G plates, acetone : chloroform : methanol : water (30:20:45:5) visualized by Kedde's reagent]. The fraction was subjected to column chromatography over Si-gel, eluted with acetone : methanol (3:5). Eluents from 16 to 26 were of the same R_f value and provided compound **1** (2.16 gm), white cream-coloured crystalline solid, m.p. 205–6°C, $C_{34}H_{58}O_6$ (found (calcd.) % C = 72.56 (72.59), H = 10.31 (10.32), eims $[M]^+$ 562; UV λ_{max}(MeOH) 218 and 295 nm; IR ν_{max}(KBr) 3598, 3036, 2976, 2930, 1650, 1460, 1370, 1195, 1248, 1670, 967, 885 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) of the diacetyl derivative of compound **1**, 0.85 (1H, s, C_{19}-3H), 0.92 (3H, s, C_{18}-3H), 1.4–2.00 (polymethylene —CH_2 and —CH), 4.53 (1H, m, C_3-H), 4.72 (3H, s, C_{21}-3H), 2.15 (3H, s, C_3-OAc), 3.54 (1H, s, C_{14}-OH), 2.67 (1H, m, C_{17}-H), 5.56

SCHEME-I



(1H, dd, C_{21}-H vinylic proton), 4.28 (1H, d, 1′-anomeric proton), 3.6–4.3 (9H, m, protons of sugar residue), 2.06 (3H, s, 2′-OAc), 2.04 (3H, s, 3′-OAc); Fabms M/Z 562 (1), 446 (21), 428 (24), 417 (20), 410 (32), 392 (42), 376 (46), 358 (33), 340 (58), 296 (65), 254 (71), 236 (59), 218 (78), 216 (82), 202 (89), 162 (91), 148 (87) and 134 (100), ^{13}C NMR (20 MHz, DMSO, ppm), 26.96 (C-1), 27.88 (C-2), 75.53 (C-3), 32.26 (C-4), 40.00 (C-5), 28.21 (C-6), 26.48 (C-7), 31.23 (C-8), 50.16 (C-9), 57.88 (C-10), 20.43 (C-11), 31.42 (C-12), 48.43 (C-13), 83.36 (C-14), 28.16 (C-15), 27.90 (C-16), 51.29 (C-17), 12.23 (C-18), 214.56 (C-19), 139.52 (C-20), 13.14 (C-21), 118.42 (C-22), 26.20 (C-23), 44.31 (C-24), 28.94 (C-25), 19.18 (C-26), 18.21 (C-27), 24.26 (C-28), 11.21 (C-29), 101.68 (C-1′), 70.86 (C-2′), 75.31 (C-3′), 71.53 (C-4′), 62.98 (C-5′).

Acid Hydrolysis: Compound **1** (500 mg) was hydrolysed with 7% of sulphuric acid (10 mL) by refluxing for 2 h on water bath and then the acidic solution was cooled to give a solid white crystalline compound **2** (420 mg). The aqueous hydrolysated was neturalise with $BaCO_3$ and the resulting $BaSO_4$ was removed.

The filtrate was concentrated to give a light yellow golden mass showing the presence of D-xylose (R_f value = 0.28).

Identification of Sapogenin **2**: White crystalline solid, m.p. 189–90°C, $C_{29}H_{50}O_3$, $[M]^+$ 446; $[\alpha]_D^{27} = 9.3$ in $CHCl_3$, IR ν_{max}(KBr) 3560, 2935, 2900, 1440, 1372, 1350, 1315, 1248, 1070, 955, 800 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$) of monoacetyl derivative of compound 0.89 (3H, s, C_{19}-3H), 0.93 (3H, s, C_{18}-3H), 3.00 (3H, s, C_8-OH), 3.54 (1H, s, C_{14} – OH), 2.15 (3H, s, C_3-OAc), 0.72 (3H, s, C_{21}-3H), 1.4–2.00 (2H, m, polymethylene CH_2 and CH), 4.55 (1H, t, J = 3.4 Hz, C_3-H), 5.55 (1H, dd, J = 4.7, C_{21}-H vinylic proton). Fabms m/z 446 (1), 428 (19), 417 (22), 410 (31), 392 (36), 376 (25), 358 (41), 340 (46), 296 (58), 254 (54), 236 (68), 218 (70), 216 (61), 202 (78), 162 (86), 148 (91) and 134 (100). ^{13}C NMR (20 MHz, DMSO, ppm); 26.92 (C-1), 27.36 (C-2), 73.51 (C-3), 32.27 (C-4), 40.10 (C-5), 28.19 (C-6), 26.44 (C-7), 31.21 (C-8), 50.57 (C-9), 57.88 (C-10), 20.41 (C-11), 31.42 (C-12), 48.40 (C-13), 83.33 (C-14), 28.15 (C-15), 27.89 (C-16), 51.29 (C-17), 12.21 (C-18), 210.54 (C-19), 139.50 (C-20), 13.12 (C-21), 118.40 (C-22), 26.18 (C-23), 44.29 (C-24), 28.91 (C-25), 19.16 (C-26), 18.10 (C-27), 24.23 (C-28), 11.21 (C-29).

Acetylation of Compound **2**: 60 mg of compound **2** was mixed with 3 mL of pyridine and 10 mL of acetic anhydride in R.B. flask and refluxed on a water bath for about 4 h. The mixture after cooling was flltered off and dried over anhydrous Na_2SO_4. Then it was recrystallized from acetone to yield monoacetyl derivative **3**, m.p. 167–68°C, $C_{31}H_{52}O_4$ (found (calcd.) % C = 76.21 (76.22); H = 10.64 (10.65)), eims $[M]^+$ 488.

Preparation of Anhydrosapogenin **4**: 80 mg of compound **2** in 50% alcohol (10 mL) containing 7 N hydrochloric acid (0.5 mL) was boiled under reflux for 1.5 h. The solution was diluted with 10 mL water and alcohol was removed by evaporation; after standing for some time the semicrystalline deposit (50 mg) was separated and crystallized from acetone : ethylacetate (4:3) to get anhydrosapogenin **4**, m.p. 181–82°C, molecular formula $C_{29}H_{44}$, found (calcd.) % C = 88.75 (88.77), H = 11.20 (11.22), eims $[M]^+$ 392.

Preparation of Ketone **5**: Solution of compound **2** (90 mg) in pyridine (10 mL) was prepared and mixed with chromium trioxide (40 mg), refluxed on a sand bath at 120°C and cooled when a crystalline ketone **5** was obtained, m.p. 170–71°C, molecular formula $C_{29}H_{49}O_3$, found (calcd.) % C = 78.18 (78.20), H = 11.00 (11.01), eims $[M]^+$ 445.

Preparation of Isosapogenin **6**: 80 mg of compound **2** was dissolved in 10 mL 10% solution of KOH in EtOH (5 mL) and kept for 30 minutes, then the solution was diluted with water (10 mL) and acidified with 10% HCl (5 mL); after standing for 1 h the solution was cooled and concentrated under reduced pressure when crystalline compound **6** was obtained, m.p. 254–56°C, molecular formula $C_{29}H_{50}O_3$, found (calcd.) % C = 78.00 (78.02), H = 11.18 (11.20)), eims $[M]^+$ 446.

Preparation of Dihydro Sapogenin **7**: 60 mg of compound **2** was dissolved in 5 mL of 80% EtOH and the mixture shaken with Pd-black and hydrogen until no more gas was adsorbed. After 12 h the crystalline deposit (80 mg) was separated and recrystallized with ethylacetate, m.p. 182–83°C, molecular formula $C_{29}H_{52}O_3$ found (calcd.) % C 77.64 (77.76), H 10.70 (10.71), eims $[M]^+$ 448.

Periodate Oxidation: Compound **1** (30 mg) was dissolved in MeOH and treated with $NaIO_4$ (20 mL of 0.1 N) for 2 days. The liberated HCOOH and consumed periodate were estimated by the Jones method.

Enzymatic Hydrolysis: Compound **1** (40 mg) in EtOH was treated with almond emulsion solution (35 mL) in conical flask and kept at room temperature for 72 h. Examination of the hydrosate on TLC using *n*-butanol: acetic acid: water (4:1:5) showed the presence of D-xylose.

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