FITOTERAPIA
Rivista di studi ed applicazioni delle piante medicinali

Milano, 19th January 1993

AB/gd

Prof. V.K. SAXENA
Dept. of Chemistry
Dr Harisingh Gour University
(University at Sagar)
Sagar-470 003 (M.P.) INDIA

Dear Sir,

This is to thank you for the forwarding of your paper "TRICHOSANTHES DIOICA (ROXB) LEAF OIL; A POTENTIAL BACTERICIDAL AGENT" and to inform you that in order to simplify and put into better evidence the results obtained in researches on the antibacterial, fungicidal, antiamoebic, molluscicidal, antiviral activities of medicinal plant derivatives, it has been decided to adopt a common outline for all these papers.

We are therefore returning you, herewith attached, your paper along with copy of this outline asking you, should you agree, to rewrite it as per what proposed.

Looking forward to hearing from you, we remain with our best regards.

Yours faithfully,

Dr A. Bonati

enc.

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TRICHOSANTHES DIOICA (Roxb) LEAF; OIL; A POTENTIAL BACTERICIDAL AGENT

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Trichosanthes dioica, Roxb., belongs to the natural order Cucurbitaceae and is a climber, with a perennial root-stock occurring in the plains of north India is commonly known as "parwal". The leaves of the plant are used as a cardiotonic antipyretic, laxative, stomachic and as an antidiabetic agent.

In view of earlier reports about the presence of bitter principle, amorphous saponin and essential oil its leaf oil was subjected to antimicrobial activity which showed that the oil had strong bactericidal activity against various bacteria. The results show the maximum bactericidal activity against Staphylococcus albus and Bacillus subtilis and least against Bacillus pumilus.

Experimental

The plant was supplied by United Chemical and Allied Products Calcutta. 500 gm of air-dried powdered leaves were extracted with petroleum ether (60-80 C) in a Soxhlet extractor for 80 hrs. Excess of hexane was added to the extract and the insoluble matters were removed by filtration. The filtrate extract, concentrated under reduced pressure and kept in a refrigerator overnight, gave 46% Yellowish-green deposite at the bottom of the flask (Table-1).
<table>
<thead>
<tr>
<th>Constant</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity at 33°C</td>
<td>0.8648</td>
</tr>
<tr>
<td>Refractive index at 30°C</td>
<td>1.3007</td>
</tr>
<tr>
<td>Acid Value</td>
<td>0.53</td>
</tr>
<tr>
<td>Saponification value</td>
<td>18.5%</td>
</tr>
<tr>
<td>Iodine value (Wij's 30 min)</td>
<td>117.2</td>
</tr>
<tr>
<td>Unsaponifiable matter</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

Table 1 - Physico-chemical constants of the oil.

After saponification (KOH and alcohol), the mixed fatty acids were esterified with BF₃-methanol (14%) and analysed by GLC (Various Aerograph 180 °C, 6ftx 1/8 in stainless steel column packed with 15% diethylene glycol succinate on chromosorb W 60-80 mesh). Fatty acid composition (% by wt.) is shown in Table - 2.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Retention time (tR) (Min)</th>
<th>Composition (% by wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 : 0</td>
<td>8.2</td>
<td>15.5</td>
</tr>
<tr>
<td>16 : 2</td>
<td>6.2</td>
<td>20.3</td>
</tr>
<tr>
<td>18 : 0</td>
<td>10.5</td>
<td>11.7</td>
</tr>
<tr>
<td>18 : 2</td>
<td>13.6</td>
<td>7.6</td>
</tr>
<tr>
<td>18 : 3</td>
<td>19.4</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Table 2 - Fatty acid composition of oil.

Bactericidal activity of the leaf oil:

The solutions of four different dilution (1:100, 1:250, 1:500 and 1:1000) of the oil in ethylene glycol were prepared and tested against various bacteria. griseofulvin (100 ppm) was used as control. Bactonutrient agar along with Saboraud's dextrose agar was employed as medium. Sterilized paper discs (10 mm diam) of whatman No. 1 filter paper were thoroughly soaked in pure oil and in different dilutions and placed over the seeded agar plates.
The bactericidal activity was measured as zones of inhibition around the discs after incubating them at 33°C and observation is recorded in Table 3.

\[
\begin{array}{cccccc}
\text{Organism} & \text{Pure Oil} & 1:100 & 1:250 & 1:500 & 1:1000 \\
\hline
\text{Staphylococcus albus} & 20 & 19 & 18 & 17 & 08 & 17 \\
\text{Staphylococcus aureus} & 18 & 16 & 15 & 14 & 07 & 13 \\
\text{Bacillus subtilis} & 20 & 19 & 18 & 17 & 06 & 17 \\
\text{Bacillus anthracis} & 15 & 13 & 12 & 11 & 05 & 14 \\
\text{Bacillus pumilus} & 10 & 09 & 08 & 06 & 05 & 09 \\
\text{Vibrocholerae} & 12 & 10 & 09 & 08 & 06 & 11 \\
\end{array}
\]

** 1000ppm griseofulvin.

Table 3 - Bactericidal activity of the oil.

In this view the results show that the oil possesses maximum bactericidal activity against Staphylococcus albus, Bacillus subtilis, Staphylococcus aureus, Bacillus anthracis and vibrocholerae and least against Bacillus pumilus.

The above deliberations clerly transpire that there is enough scope of the utility of oil as antibacterial agent and may potentially be used for making pharmaceutical preparation for treating bactericidal diseases.
REFERENCES


Prof. V.K. Saxena
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India

Dear Prof. Saxena:

I have received your manuscript entitled "Novel Prenylated Triterpenoidal Saponin from Trichosanths Dioica (Roxb)" to be considered for publication in the Journal of Natural Products. It is being referred to Associate Editor, Richard G. Powell, Northern Regional Research Center, ARS, USDA, 1815 N. University Street, Peoria, Illinois 61604, who will notify you as soon as possible as to its acceptability for publication in the Journal. In all correspondence concerning your paper, please refer to manuscript No. 92307 and address your correspondence to Associate Editor Powell.

Sincerely yours,

James E. Robbers
Professor of Pharmacognosy
Editor

JER/1af
cc: Richard G. Powell
NOVEL PRENYLATED TRITERPENOIDAL SAPONIN;
FROM TRICHOSANTHS DIOICA (Roxb)

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Department of Chemistry
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Sagar - 470 003 (M.P.) INDIA

ABSTRACT

A novel prenylated triterpenoidal saponin 28-O-β-D-glucopyranosyl (1→5)-O-β-D-xylopyranoside 20-(3-methylbut-2-en) 3β,21-dihydroxy olean-12-en-28 oic acid ester 1 has been isolated from MeOH-soluble fraction of 95% EtOH extract of stem part of the Trichosanthes dioica Roxb.

INTRODUCTION

Trichosanthes dioica Roxb (1) (N.O. Cucurbitaceae) occurring in the plains of north India and the plant is reported to be used as cardiotonic, laxative, stomachic, antipyretic antitumour and as an antidiabetic agent (3). Its various parts have been phytochemically investigated by earlier workers who reported the presence of bitter principles alongwith amorphous saponins and essential oils (4). Its antidiabetic and antitumour activities have already been evaluated with the significant success (5,6).
RESULTS AND DISCUSSION

The MeOH soluble part of the 95% EtOH extract of stem of T. dioica Roxb. when worked up by C.C. gave compound 1 (0.03%) m.p. 251-250, C_{45}H_{72}O_{13}, [M]^+ 820, [\alpha]_D^{27} = 16.8 in pyridine. It gave positive foam test (7) honey-comb test (8) Haemolytic test (9), indicating its nature as saponins.

1 did not show any absorption beyond 210 nm and on acid hydrolysis with 7% ethanolic H$_2$SO$_4$ yielded compound 2 (sapogenin) m.p. 308-100, C$_{34}$H$_{54}$O$_4$, [M]$^+$ 526, [\alpha]_D^{27} = 11.2 in pyridine; and sugar moieties. 2 responded to the positive characteristic colour reactions of triterpenes (10-12). (SCHEME-I).

$\nu_{\text{KBr}}^{\text{max}}$ 3590 cm$^{-1}$ of 2 indicated the presence of -OH group(s) in it. Acetylation of 2 with Ac$_2$O in pyridine yielded a diacetyl derivative 3 m.p. 271-73, C$_{38}$H$_{58}$O$_6$, [M]$^+$ 610, acetoxyl grp(s) (5.0%), which was further supported by $^1$H NMR signals at $\delta$ 2.06 (-OAc) and $\delta$ 2.16 (-OAc) and clearly indicated the presence of two acetylated -OH group(s).

$\nu_{\text{KBr}}^{\text{max}}$ 1716 cm$^{-1}$ in 2 indicated -COOH group whose methyl ester derivative 4 m.p. 198-1$^\circ$, C$_{35}$H$_{56}$O$_4$, [M]$^+$ 540, confirmed the presence of only one -COOH group in 2.
CrO₃/pyridine oxidation of compound 4 yielded diketone 5 m.p. 168-70⁰, C₃₅H₅₄O₄, [M]+ 538, gave positive Ziemermond test there by confirming the presence of secondary -OH group(s).

νKBrmax at 1248 cm⁻¹ indicated the presence of olininic double bond which was further supported by the fact, that of 2 on catalytic hydrogenation found a dihydrosapogenin 6, m.p. 289⁰, C₃₄H₅₈O₄ [M]+ 542, thereby confirming the presence of one double bond. The position of this double bond was fixed on the triterpenoidal nucleus, since the compd. 2 gave positive test with tetranitromethane. It indicate high terminal UV absorption, of 12-13 double bond of triterpene of β-amyrin series (13). Signals in ¹HNMR at δ 5.32 (Vinylic proton) of 2 was further confirmed the presence of double bond.

Signals in ¹HNMR δ 3.68 (m, 2H, J = 6.7 Hz, Ar-CH₂), δ 5.24 (t, 1H, CH=C-) and δ 1.63 (S, 6H, Me₂-32) integrated for prenylation (14,15). The presence of prenyl group at C-20 was supported by cyclization of 2 resulting in the formation of a chromane derivative 7, m.p. 312-13⁰, C₃₄H₅₄O₄, [M]+ 526.

Attachment of sugar in the prenylated triterpeniodal glycoside 1 was fixed at C-28 as the glycoside itself did not give a positive acid test where as the
sapogenin 2 gave the positive response, indicated that the
-COOH is bonded in the glycoside but was free in 2, it is
thus suggested that 2 was 20-(methylbut-2-en) 3,21 di-
hydroxy olean-12-en-28-oic acid.

Periodate oxidation studies, with HIO₄ of 1
indicated that the D-glucose was present in pyranose form
while D-xylose in furanose form (16). On further
hydrolysis by the enzyme almond emulsin liberated
D-glucose and D-xylose and indicated the linkage between
sapogenin and D-xylose as well as D-xylose and D-glucose
was β (17).

On partial hydrolysis with 2% H₂SO₄, showed
D-glucose followed by D-xylose, confirming the D-glucose
was the terminal sugar and that D-xylose was attached to
the sapogenin and is to be prosapogenin (18). Which on
hydrolysis with 7% H₂SO₄ yielded 20-(methyl but-2-en) 3,21
dihydroxy olean-12-en-28-oic acid and D-xylose. On
quantitative estimation it was found to be 76.00% thereby
confirming the presence of one mole. of acid and one mole.
of D-xylose.

This was further supported by acid hydrolysis of
permethylate 1 which yielded 2:3 di-o-methyl-D-xylose
(confirmed by Co-PC and TLC) and 2:3:4:6 tetra-o-methyl-D-
glucose (confirmed by Co-PC and TLC), thus the identity of
compound 1 is novel prenylated sapogenin 28-O-β-D-glucopyranosyl (1→5)-O-β-D-xylofuranoside.

EXPERIMENTAL

PLANT MATERIAL: T. dioica (Roxb). was procured from M/s. United Chemical and Allied Products, Calcutta. The plant was authenticated by the Department of Botany, courtesy of Dr. Harisingh Gour University, Sagar, M.P. A herbarium specimen (No. IV-XVII) has been deposited in the Department of Chemistry of the University.

ISOLATION: Air dried, powdered stem (4.0 kg) of T. dioica Roxb. were extracted with 95% EtOH. The extract was concentrated to a green viscous mass under reduced pressure and successively segregated in to pet. ether, \( C_6H_6 \), CHCl\(_3\), EtOAc, Me\(_2\)CO and MeOH soluble fractions.

The MeOH-soluble part was concentrated. It showed three spots on tlc [Si-gel, G plates, acetic acid : Water : Chloroform 30:20:50, visualized by I\(_2\) vapours]. The fraction was subjected to column chromatographed over Si-gel and eluted with acetone : methanol : ethylacetate (3 : 1 : 2). Elvents from fractions 1-7 and 13-20 had same Rf values. The fractions 8-12 provided compound 1 (1.20 gm), colourless needled sharped crystaline solid; m.p. 251-2\(^\circ\)C, \( C_{45}H_{72}O_{13} \) (found C 65.82, H 8.75, calcd. C 65.85, H 8.79), 252-2\(^\circ\)C eims \([M]^+ 820; UV \lambda_{max}^{EtOH} 210 \text{ nm;}\)
$\nu_{\text{max}}^{\text{KBr}}$ 3600, 3035, 2900, 2990, 2848, 1660, 1264, 1360, 1392-1381, 1246, 810 cm$^{-1}$. $^1$H-nmr (60 MHz, CDCl$_3$) of Octaacetyl derivate of compound 1, 0.88 (S, 3H, Me-25); 0.83 (S, 3H, Me-26), 1.01 (S, 3H, Me-29), 2.04 (S, 3H, 3-OAc), 2.20 (S, 2H, 21-OAc), 0.95 (S, 6H, 2x CH$_3$-tertiary methyl), 1.26-2.10 (m, 20H, polymethelene CH$_2$ and CH), 1.60 (S, 6H, Me$_2$-32), 3.64 (m, 2H, J=6.6Hz, Ar-CH$_2$), 5.26 (t, 1H, CH=C=), 2.07 (dd, J=4, H$_2$-11), 4.3 (dd, J=6, 1H methenic proton), 5.37 (dd, J=6.2, H-12 vinylic proton), 4.26 (d, J=4, 1 anomeric proton, l'H), 4.38 (d, J=7.2, 1 anomeric proton, l"H), 3.6-4.28 (m, 11 sugar protons), 2.03 (S, 3H, 2'-OAc), 3.01 (S, 3H, 3'-OAc), 2.8 (S, 6H, 2" and 3'-OAc), 2.06 (S, 3H, 4"-OAc), 2.08 (S, 3H, 6"-OAc); Fabms m/z 820, 657, 641, 545, 509, 481, 426, 409, 233, 248, 207, 190, 189, 175 and 133; $^{13}$C nmr (20 MHz, DMSO, ppm), 38.6 (C-1), 27.4 (C-2), 78.3 (C-3), 39.2 (C-4), 56.4 (C-5), 17.8 (C-6), 30.9 (C-7), 39.5 (C-8), 46.8 (C-9), 37.3 (C-10), 23.2 (C-11), 121.3 (C-12), 144.2 (C-13), 41.5 (C-14), 29.9 (C-15), 22.8 (C-16), 48.2 (C-17), 40.8 (C-18), 47.6 (C-19), 28.3 (C-20), 73.6 (C-21), 32.6 (C-22), 28.0 (C-23), 16.2 (C-24), 148 (C-25), 17.6 (C-26), 25.8 (C-27), 180.9 (C-28), 34.7 (C-29), 23.4 (C-30), 128.1 (C-31), 57.8 (C-32), 32.1 (C-33), 20.8 (C-34), 104.2 (C-1), 78.6 (C-2"), 77.3 (C-3"), 74.8 (C-4"), 75.9 (C-5"), 62.2 (C-6"), 103.8 (C-1"), 73.3 (C-2"), 77.5 (C-3"), 69.2 (C-4"), 66.3 (C-5").
ACID HYDROLYSIS: Compd 1 (500 mg) was hydrolysed (0.02N of 7% sulphuric acid) by refluxing three hours on a water bath and then poured into 250 ml ice cold water. A light yellow crystalline compound 2 yielded (430 mg).

The aqueous hydrolysate was neutralised with BaCO₃, BaSO₄ was filtered off. The residue was concentrated to a golden yellow mass and examined by tlc [n-BuOH : HOAc : H₂O (40 : 10 : 50) and showed the presence of D-glucose & D-xylose.

IDENTIFICATION OF THE SAPOGENIN 2: Light yellow crystalline solid, m.p. 308-10°C, C₃₄H₅₄O₄, [M]+ 526; [ ]D²⁷ 11.2 in pyridine; ir νmax KBr 3035, 3590, 3935, 1716, 1665, 1385-1378, 1248, 836 cm⁻¹; ¹H-nmr (60 MHz, CDCl₃) of diacetyl derivative of compd 20.90 (S, 3H, Me-25), 0.81 (S, 3H, Me-26), 1.01 (S, 3H, Me-29), 2.06 (S, 3H, 3-OAc), 2.16 (S, 3H, 21-OAc), 0.96 (S, 6H, 2x CH₃-tertiary methyl), 1.3-2.13 (m, 20H, polymethylene CH₂ and CH), 1.63 (S, 6H, Me₂-32), 3.68 (m, 2H, J = 6.7 Hz, Ar-CH₂), 5.24 (t, 1H, CH=C-), 2.08 (dd, J = 4.2, H₂-11), 4.7 (dd, J = 6.3, 1H, methenic proton), 5.32 (dd, J = 6.1, H-12, vinylic proton); fabsms m/z 526, 302, 257, 247, 133, 207, 190, 189 and 176; ¹³C nmr (20 MHz, DMSO, ppm); 38.4 (C-1), 27.8 (C-2), 77.9 (C-3), 39.6 (C-4), 56.7 (C-5), 17.5 (C-6), 31.2 (C-7), 39.8 (C-8), 46.4 (C-9), 37.4 (C-10), 23.3 (C-11), 121.8 (C-12), 144.1 (C-13), 41.4 (C-14), 30.1 (C-15), 22.6
(C-16), 48.3 (C-17), 40.6 (C-18), 47.3 (C-19), 28.4 (C-20),
73.2 (C-21), 32.7 (C-22), 28.2 (C-23), 16.5 (C-24), 14.8
(C-25), 17.4 (C-26), 25.6 (C-27), 181.2 (C-28), 34.5 (C-29),
23.2 (C-30), 127.9 (C-31), 58.1 (C-32), 32.6 (C-33), 20.6
(C-34).

COLOUR REACTIONS: (a) The sapogenin was dissolved in
chloroform and concentrated sulphuric acid added, a red
colour is produced in the chloroform layer. (b) produced a
red colour with conc. sulphuric acid and acetic anhydride.
(c) when boiled with acetyl chloride and zinc chloride in
chloroform produced a violet red colour.

ACETYLATION OF COMPOUND 2: (200 mg) of compound 2 was
mixed with 4 ml pyridine and 30 ml of acetic anhydride in
R.B. flask, refluxed on a water bath for about 4 hrs. The
mix. after cooling was poured in ice cold water to get a
ppt. which was washed with water and dried over anhyd.
Na₂SO₄. Then it was recrystallized from acetone to yield
diacetyl derivative 3 m.p. 271-72°C, C₃₈ H₅₈ O₆ (found C 75.73,

METHYL ESTER OF SAPOGANIN 2: (100 mg) of sapogenin was
taken in solvent ether and treated with ethereal solution of
diazomethane with constant cooling till a yellow colour was
produced. Acetic acid was added to destory the action
diazomethane. The mix was washed with water and Na₂CO₃ and
dried over anhyd. Na₂SO₄. The residue was column
chromatographed over si-gel (eluted with methanol : acetic acid : acetone (3:2:1)) and the homogenous residue was crystallized from methanol yielding its methyl ester 4 m.p. 198-190°C, C_{35}H_{56}O_{4} (found C 77.77, H 10.37 calcd C 77.75, H 10.36), eims [M]^+ 540.

CHROMIC ACID OXIDATION OF THE METHYL ESTER 4: (70 mg) of the methyl ester in 5 ml 90% acetic acid in a 100 ml conical flask and solution 200 mg of CrO_3 in 10 ml of acetic acid was added to the cooling mix. A permanent orange brown colour was obtained. Filtered and the ppt. was dried. A crystalline product 5 m.p. 168-70°C C_{35}H_{54}O_{4} (found C 77.54, H 10.24 calcd. C 77.56, H 10.26), eims [M]^+ 538.

CYCLISATION OF COMPOUND 2: (60 mg) of compound 2 on refluxing with 98% fumaric acid produced a chromano derivative 7 m.p. 312-13°C, C_{34}H_{54}O_{4}, [M]^+ 526.

PERIODATE OXIDATION: Compound 1 (30 mg) dissolve in MeOH and treated with NaIO_4 (20 ml of 0.1N) 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 an equal volume of Taka-Diastase and almond emulsion solution and left at room temperature for 2 days. Examination of the hydrolysate on the showed the presence of D-glucose and D-xylose.

ACKNOWLEDGEMENTS

We are thankful to Director, CDRI, Lucknow, for recording various spectra, and to Head of Chemistry Dept. of this University for providing laboratory facilities.
REFERENCES:


Dear Dr. Dave,

We acknowledge the receipt of your research paper entitled, "A new sterculial saponin.............." (Index No. 1049/94) for publication in Asian Journal of Chemistry. In future correspondence, please quote the index number.

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Managing Editor.

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A NEW STEROIDAL SAPONIN FROM TRICHOSANTHES DIOICA 'Roxb'

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Key Word Index: Trichosanthes dioica. Cucurbitaceae.
Steroidal saponin 24 α-ethyl-20-ene-7-hydro-stigmaster—8β:14β-diol-3-O-β-D-xylofuranoside (1).

Abstract: A new steroidal saponin 24-α-ethyl-20-ene-7-hydro-stigmaster—8β:14β-diol-3-O-β-D-xylo furanoside 1 has been isolated from MeOH soluble fraction of 95% EtOH extract of the leaves of Trichosanthes dioica.

INTRODUCTION

Trichosanthes dioica Roxb [1] (Cucurbitaceae) occurring in the plains of north India and 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 the 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 (0.072%) m.p. 205-6°, 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 molischs test. On acid hydrolysis 1 yielded sapogenin 2, m.p. 189-90 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].

\( \nu_{KBr}^{\text{max}} 3560 \text{ cm}^{-1} \) 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° molecular formula \( C_{31}H_{52}O_4 \), \([M]^+ 488\), showed singlets at \( \delta 2.15 \) for -OAc and \( \delta 3.15 \) for OH. Compound 2 on treatment with HCl form a trianhydrosapogenin 4 m.p. 181-82°, 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.

\( \text{C}_8\text{O}_3/\text{pyridine} \) oxidation of the compound 2 yielded a ketone 5 m.p. 170-71° 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 indicated its secondary
nature. The position of double bond was confirmed by the KOH hydrolysis of compound 2 gave a isosapogenin 6 m.p. 254-56\(^\circ\), molecular formula \(C_{29}H_{50}O_3\), [M]\(^+\) 446, which showed a qartert at \(\delta\) 3.8, C-22\(H\) and multipale at \(\delta\) 2.3, C-20 \(H\) indicating cleavage of double bond in between C-20 and C-22 and form a epoxi-linkage, was further explain the fixing of tertiary OH group at C-14[11]. 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 3\(\beta\):8\(\beta\):14\(\beta\) trihydroxy etianic acid [13] (confirmed by mmp, Co-TLC and superimimensible spectra) infact established the location of a side chain at C-17. The peak in the ir spectrum at \(\nu_{KBr}^{max}\) 1650 cm\(^{-1}\) indicated the presence of unsaturation in it which further supported by the fact that 2 on catalytic hydrogenation gave a dihydrosapogenin 8, m.p. 182-83\(^\circ\), molecular formula \(C_{29}H_{52}O_3\) [M]\(^+\) 448. The \(^1\)HNMR in upfield chemical shift at \(\delta\) 4.22 ppm, C-22\(H\) also confirmed the presence of unsaturation in it(Scheme I).

Attachment of sugar in the steroidal saponin 1 was fixed at C-3 as the 1 it self did not give a positive zimmermann test whereas the sapogenin 2 did this, thereby
confirming that C<sub>3</sub>-OH group was free in the sapogenin but was involved in the glycosylation in 1.

Periodate oxidation [14] of 1 with HIO<sub>4</sub> indicated that D-xylose was present in furanose form and it hydrolysate 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 on hydrolysis with almond emulsion of 1 yielded D-xylose indicated 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-en-7-hydro-stigmast-8β:14β-di ol-3-O-β-D-xylofuranoside.

**EXPERIMENTAL**

**PLANT MATERIAL:** T.dioica (Roxb.) was procured from M/s. United Chemical and Allied Products, Calcutta. The plant was authenticated by the Department of Botany; courtesy of Dr.Harisingh Gour University, Sagar, M.P. A herbarium specimen (No. IV-XVII) has been deposited in the Department of Chemistry of the University.

**ISOLATION:** Airdried, 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<sub>6</sub>H<sub>6</sub>, CHCl<sub>3</sub>, EtOAc, Me<sub>2</sub>CO 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 chromatographed over Si-gel eluted with acetone: methanol (3:5). Elvents from (16-26) were of the same Rf value and provided compound 1 (2.16 gm), white cream coloured crystalline solid, m.p. 205-6°, C_{34}H_{58}O_6 (found C 72.56, H 10.31, Calcd. C 72.59, H 10.32); eims[M]^{+} 562; UV \lambda_{\text{max}}^{\text{MeOH}} 218 & 295 nm; ir \nu_{\text{max}}^{\text{KBr}} 3598, 3036, 2976, 2930, 1650, 1460, 1370, 1195, 1248, 1670, 967, 885 cm^{-1}; \text{HNM}

1 (90 MHz, CDCl₃) 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-CH₂ and -CH), 4.53 (1H, m, C₃-H), 4.72 (3H, s, C_{21} 3H), 2.15 (3H, s, C₃-OAc), 3.54 (1H, s, C_{14} -OH), 2.67 (1H, m, C_{17} -H), 5.56 (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) & 134(100), \text{CNMR}(20MHz, DMSO, ppm), 26.96 (C-1), 27.38(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**: Compd 1 (500 mg) was hydrolysed with 7% of sulphuric acid (10 ml) by refluxing two hours on water bath and then the acidic solution was cooled to give a solid white crystalline compd 2 (420 mg). The aqueous hydrolysate was neutralised with BaCO₃ and the resulting BaSO₄ was removed. The filtrate was concentrated to give a light yellow golden mass showed the presence of D-xylose (Rf value = 0.28).

**IDENTIFICATION OF THE SAPOGENIN 2**: White crystalline solid, m.p. 189-90°, C₂₉H₅₀O₅, [M]+ 446; [α]D²⁷ = 9.3 in CHCl₃, iνmax KBr 3560, 2935, 2900, 1440, 1372, 1350, 1315, 1248, 1070, 955, 800 cm⁻¹. ¹H NMR (60 MHz, CDCl₃) of monoacetyl derivative of compd 0.89 (3H, s, C₁₉' -3H'), 0.93 (3H, s, C₁₈' -3H'), 3.00 (3H, s, C₈-OH), 3.54 (1H, s, C₁₄-OH), 2.15 (3H, s, C₃-OAc0, 0.72 (3H, s, C₂₁' -3H'), 1.4-2.00 (2H, m, polymethene CH₂ and CH), 4.55 (1H, p, J=3.4 Hz, C₃-H), 5.55 (1H, dd, J = 4.7, C₂₁' -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)& 134(100). ¹³C NMR (20MHz, 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).

**ACETYULATION OF COMPOUND 2** : (60 mg) of compd 2 was mixed with 5 ml pyridine and 10 ml of acetic anhydride in R.B. flask, refluxed on a water bath for about 4 hrs. The mix after cooling filtrated off and dried over anhyd. Na₂SO₄. Then it was recrystallized from acetone to yield monoacetyl derivative 3, m.p. 167-68°, C₃₁H₅₂O₄ (Found C 76.21, H 10.64, Calcd. C 76.22, H 10.65), eims [M]+ 488.

**PREPARATION OF ANHYDROSAPOGENIN 4** : (80 mg) of compnd 2 in 50% alcohol (10 ml) containing 7N Hydrochloric acid (0.5 ml) was boiled under reflux for 1½ hours. The solution was diluted with 10 ml water and alcohol was remove by evaporation after standing for sometime the semicrystalline deposite (50 mg) was separated and crystallized from acetone : ethylacetate (4:3) to get anhydrosapogenin 4, m.p. 181-82°, molecular formula C₂₉H₄₄ (Found C 88.75. H 11.20, Calcd. C 88.77, H 11.22), eims [M]+ 392.
PREPARATION OF KETONE 5: Solution of compd 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 C 78.18, H 11.00 Calcd C 78.20, H 11.01), eims [M]$^+$ 445.

PREPARATION OF ISOSAPOGENIN 6: (80 mg) of compd 2 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 hours the solution was cooled & concentrated under reduced pressure when crystalline compd 6 was obtained, m.p. 254-56°C, molecular formula C$_{29}$H$_{50}$O$_3$ (Found C 78.00, H 11.18, Calcd. C 78.02, H 11.20), eims [M]$^+$ 446.

PREPARATION OF DIHYDRO SAPOGENIN 7: 60 mg of compd 2 was dissolved in 5 ml of 80% EtOH and the mixture shaken with Pd-black and hydrogen until no more gas was absorbed. After 12 hours the crystalline deposit (80 mg) was separated & recrystallized with ethylacetate, m.p. 182-83°C Molecular Formula C$_{29}$H$_{52}$O$_3$ (found C 77.64, H 10.70 calcd. C 77.67, H 10.71), eims [M]$^+$ 448.

PERIODATE OXIDATION: Compound 1 (30 mg) dissolve in MeOH and treated with NaIO$_4$ (20 ml of 0.1N) for 2 days. The liberated HCOOH & cosumed 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 hours. Examination of the hydrolysate on TLC using n-butanol : acetic acid : water (4:1:5) showed the presence of D-xylose.

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NOVEL PRENYLATED ISOFLAVONOIDAL PHYTOESTROGEN FROM

TRICHOSANTHES DIOICA (Roxb)

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Abstract: A novel prenylated isoflavonoidal phytoestrogen glycoside; 8-prenyl, 2′4′5′-trimethoxy isoflavone-7-O-β-D-glucopyranoside 1 has been isolated from ethyl acetate soluble fraction of 95% EtOH extract of the leaves of Trichosanthes dioica (Roxb.).

INTRODUCTION

Trichosanthes dioica Roxb (1) (Cucurbitaceae) occurring in the plains of north India and plant has been reported to be used as cardiotonic, laxative, stomachic, antipyretic, antitumor and as an 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 the significant success (4,5).

RESULTS AND DISCUSSION

The ethyl acetate soluble extract of air dried and powdered leaves of T. dioica (Roxb.), on its column chromatography on Si-gel and elution with chloroform/
ethylacetate furnished a light yellow colored amorphous substance 1 m.p. 205-6⁰, C₂₉H₃₄O₁₂, M⁺ 574. It gave an olive green colour with FeCl₃, a positive test with Na-Hg/HCl and Molish's reagent, suggesting it to be an isoflavonoidal glycoside, also been supported by IR absorption at 3500, 1650, 1610, 1285, 1145, 885, 825, 766 cm⁻¹.

Acid hydrolysis of 1 yielded an aglycone 2 and sugar moieties. The sugar was identified as D-galactose. The aglycone and D-galactose were present in a equimolecular ratio (6), Aglycone 2 m.p. 247-48⁰, C₂₃H₂₄O₇, M⁺ 412 (EIMS), gave a dark green colour with FeCl₃.

Preparation of its diacetate indicated the presence of two free OH groups. A bathochromic shift of 14 & 10 nm in band II were observed on addition of AlCl₃ + HCl and NaOAc indicating 5, 7 dihydroxy system in 2 (7), ¹HNMR of its diacetate exhibited six proton singlet, two proton doublet and one protons triplet at δ 1.73, 3.30 (J = 7 Hz) and 5.32 respectively for prenyl unit. The downfield shift of singlet at δ 6.89 and δ 7.22 represented magnetically nonequivalent proton at C₃', and C₆ [8] where as the sharp singlet at δ 3.50 and 2.41 were attributed for acetyl groups. These chemical shifts suggested absence of A₂B₂ system in B ring of 2. The EIMS of 2 gave fragment ions at m/z 357 (base peak) and 356 (40%) by the loss of M-55 & M-56 suggesting prenylation adjacent to OH group (9,10).
Appearance of pronounced peak at M/z 381 due to M-31 indicated 2' methoxy substitution (11,12). A peak at m/z 192 (14%) concluded trimethoxy substitution in ring B.

Location of prenyl unit at C-8 position in ring A was established by the significant bathochromic shift 55nm on addition of AlCl₃ (13). On mild alkaline hydrolysis followed by cyclization of dimethyl ether of 3 yielded 2,2, dimethyl chromanant identified by PMR (14) also conforming prenylation of C₈. Further KMnO₄ oxidation of dimethyl ether of 2 formed 2,4,5 trimethoxy benzoic acid thereby conforming methoxyl position in ring B, thus the aglycone 2 was identified as 5,7 dihydroxy 8 prenyl 2'4'5' trimethoxy isoflavonoid.

1, R₁ = D-glucose
2, R₁,R₂ = H
3, R₁,R₂ = Me
Periodic oxidation and enzymatic hydrolysis (15) revealed that the glycoside 1 consists of one mole of the aglycone and one mole of D-galactose and linkage was β. The glycoside did not show the bathochromic shift on addition of NaOMe suggesting the glycosylation of the OH group in ring A. The permethylation (16) confirmed that C₁-OH of sugar was attached with C₇-OH of aglycone, thereby confirming that the 1 as 8-prenyl 2',4',5'-trimethoxy isoflavone 7-O-β-D-galactopyranoside.

The isolated compound 1 was found to be estrogenically active when administrated (17) on albino rats because of the fact that it led to the increase in the weight of uterine.

EXPERIMENTAL

PLANT MATERIAL: T.dioica (Roxb) was procured from M/s United Chemical and Allied Products, Calcutta. The plant was authenticated by the Department of Botany, courtesy of Dr.Harisingh Gour University, Sagar, M.P. A herbarium specimen (No. IV-XVII) has been deposited in the Department of Chemistry of University.

ISOLATION: Air dried, powdered leaves (4.0 kg) of T.dioica were extracted exhaustively with hot 95% ethanol. The extract was concentrated under vaccum and segregated into petroleum ether, C₆H₆, EtOAc and (Me)₂CO soluble fractions. The ethylacetate soluble fraction showed
the presence of two spots on TLC. These were separated by column chromatography on Si gel (60-120 mesh) column and eluted with chloroform/ethylacetate mixture in various proportions.

**Compound 1**: Eluate with CHCl₃/MeOH (5:6), light yellow amorphous compound m.p. 205-206, m.f. C₂₉H₃₄O₁₂ (Found C 60.59, H 5.91, Calcd. C 60.62, H 5.92). M⁺ 574 (EIMS), intense green color with FeCl₃, pink color with Na-Hg/HCl and positive molish's test. UV λ max MeOH 262, 291 (sh), 300 (sh), λ AlCl₃ 275, 310, λ AlCl₃/HCl 276, 309; IR ν max KBr 3500, 2930, 2820, 1650, 1625, 1557, 1570, 1385, 1368, 1285, 1215, 1145, 885, 825, 766, 710 and 690 cm⁻¹; fambms m/z 574(1), 412(23), 384(42), 381(56), 357(31), 356(74), 192(93), 166(100).

**Acetylation of compound 1**: Compd (1) heated with fused sod. acetate and acetic anhydride at 130° in oil bath for 4 hrs and workup as usual and acetyl derivative crystallised with methanol white needles, m.p. 185-86° analysed for C₃₉H₄₄O₁₇ (Found C 59.68, H 5.59, Calcd. C 59.69, H 5.61). IR KBr max 1720, 1645 cm⁻¹. PMR (CDCl₃ 90 MHz, TMS ppm) 8.02 (s, H, C₂), 7.23 (s, H, C₆), 6.82 (s, H, C₃), 5.32 (t, H, C₂n), 3.92 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃), 3.30 (d, J=7 Hz, H, C₆n), 1.72 (s, 6H, C₄n, C₅n), 5.16 (d, J = 7 Hz, H, C₁n), 4.3-4.8 (m, 6H of galactose), 2.52 (s, 3H, OAc C₆n), 2.14 (s, 3H, OAc C₄n), 2.05 (s, 3H, OAc, C₂n).
Acid hydrolysis of 1: 1 was refluxed with 7% alc H₂SO₄ for 7 hrs. The alcohol was removed and a cream colored substance 2 was separated. The concentrated hydrosate after neutralization, was examined on FC (BAW 4:1:5) and found to contain galactose. The quantitative estimation of sugar showed that the aglycone & galactose present in a equimolecular ratio.

**Compound 2**: Cream coloured substance recrystallized with MeOH : acetone, m.p. 247-48°, analysed for C₂₃H₂₄O₇ (Found C 66.98, H 5.81, Calcd. C 66.99, H 5.82), M⁺ 412 (EIMS), gave dark green colour with FeCl₃. Pink colour with Na-Hg/HCl. UV \( \lambda_{max} \) (MeOH) 254, 290 (sh), 307 (sh), \( \lambda_{max} \) (AlCl₃) 271, 309, \( \lambda_{max} \) (AlCl₃ +HCl) 273, 306; IR \( \nu_{max} \) (KBr) 3510, 2915, 2830, 1640, 1560, 1500, 1380, 1367, 1240, 1190, 805 cm⁻¹. Famsb m/z (r.int) 412(24), 384(40), 381(57), 357(29), 356(70), 192(91) & 166(100). \(^{13}\)C NMR (DMSO-d₆, 22.6 MHz), 153.6 (C-2), 120.1 (C-3), 186.6 (C-4), 158.4 (C-5), 98.4 (C-6), 161.1 (C-7), 154.3 (C-9), 104.8 (C-10), 108.8 (C-1′), 155.6 (C-2′), 102.4 (C-3′), 159.1 (C-4′), 105.6 (C-5′), 132.1 (C-6′), 22.4 (C-1″), 123.3 (C-2″), 129.6 (C-3″), 25.1 (C-4″), 17.3 (C-5″), 60.2 (20 CH₃), 55.1 (4′ OCH₃), 55.9 (5′ OCH₃).

**Acetylation of 2**: The acetyl derivative of 2 was prepared by sodium acetate and acetic anhydride and obtained as silk needles, m.p. 226-27° C₂₇H₂₈O₉, (Found C 65.31, H 5.62, Calcd. C 65.32, H 5.64), IR \( \nu_{max} \) (KBr) 1750, 1645 cm⁻¹; PMR (90 MHz, CDCl₃ ppm), 8.02 (s, H, C₂), 7.23 (s, H, C₆′),
6.87 (s, H, C₃), 5.32 (t, H, C₂), 3.94 (s, 3H, OCH₃), 6.87 (s, H, C₃), 5.32 (t, H, C₂), 3.94 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃), 3.30 (d, J = 7 Hz, 2H, C₁), 2.50 (s, 3H, OAc, C₅), 2.41 (s, 3H, OAc.C₇), 1.73 (s, 6H, C₄-C₅).

**Methylation of 2 to 3:** Compd 2 (400 mg) + DMSO₄ + anhydrous K₂CO₃ + dry acetone were refluxed for 8 hrs. The inorganic salts were filtered off, acetone was evaporated to a syrupy mass and it was charged over small silica gel column & eluted with ether. The removal of ether & on crystallization it yielded white needles (260 mg) m.p. 216-17° analysed for C₂₅H₂₈O₇ (found C 68.17, H 6.34 calcd. C 68.18, H 6.36).

**Alkaline hydrolysis and cyclization:** The dimethyl ether 3 (200 mg) in 20% alco. NaOH (10 ml) refluxed for 20 minutes. The reaction mixture after cooling acidified and water was added and extracted with ether. The ether soluble part on evaporation yielded a residue which was mixed with HCOOH (5 ml) and kept for an hour, followed by addition of H₂O and extracted with ether. Removal of ether from ether soluble part yielded a gummy substance, analysed for C₂₄H₃₀O₇ and was identified as 2,2 dimethyl chroman.

**KMnO₄ oxidation of 3:** Potassium permanganate was added in small quantities to 3 in acetone. The mixture was refluxed and more KMnO₄ was added until slight excess was present. The mixture was refluxed for further 15 minutes. The
precipitated manganese dioxide was filtered off and washed with $H_2O$. The combined filtrate and washing were acidified with $H_2SO_4$, saturated $(NH_4)_2SO_4$ and extracted with ether. Removal of ether and crystallization of product yielded 2,4,5 trimethoxy benzoic acid.

**Peridate oxidation**: Compound 1 was dissolved in methanol (40 ml) and kept with sodium metaperiodate (15 ml) in a conical flask for 2 day. The liberated formic acid and consumed periodate was estimated by Jone's method.

**Permethylation and Acid hydrolysis**: Compound 1 was treated with $CH_3I$ and $Ag_2O$ in dimethyl formamide at room temperature. After two days the reaction mixture was filtered and the residue was washed with dimethyl formamide. The filtrate was concentrated in a vaccum and hydrolysed by alc. $H_2SO_4$. After usual workup methylated sugar was identified on $PC$ as 2,3,4,6 tetra methyl galactose.

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