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Novel Prenylated Isoflavonidal Phytoestrogen from Trichosanthes Dioica (Roxb)

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Abstract **– A novel prenylated isoflavonidal 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 leaves of Trichosanthes Dioica (Roxb).**

Keywords **– Novel Prenylated Isoflavonidal Phytoestrogen Glycoside, Trichosanthes Dioica (Roxb), EtOH.**

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 ,anti-pyretic, anti-tumor and as anti-diabetic 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 anti-diabetic and anti-tumor activities have already been evaluated with the significant success (4, 5).

RESULT AND DISCUSSION

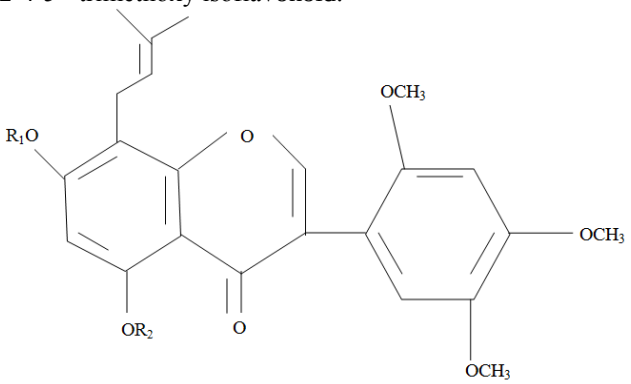
The ethyl acetate soluble extract of air dried and powered leaves of T. Dioica (Roxb), on its column chromatography on Si-gel & elution with chloroform/ ethylacetate furnished a light yellow coloured amorphous substance 1 m. p. 205-6°, $C_{29}H_{34}O_{12}$, M^+ 574 . It gave an olive green colour with $FeCl_3$, a positive test with Na – Hg /HCl and Molish's reagent, suggesting it to be an isoflavonidal 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 moities. The sugar was identified as D-galactose. The aglycone and D-galactose were present in a equimolecular ratio (6), Aglycone 2 m.p. 247-48°, $C_{23}H_{24}O_{17}$, M^+ 412 (EIMS), gave a dark green colour with $FeCl_3$,

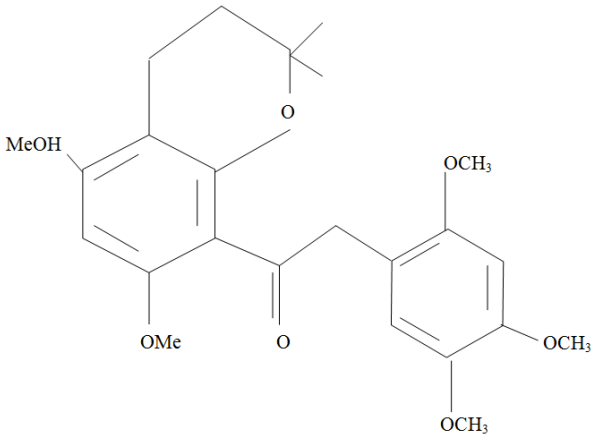
Preparation of its di-acetate indicated the presence of two free OH groups. A bathchromic shift of 14 & 10 nm in band II were observed on addition of $AlCl_3$ + HCl & NaOAc indicating 5, 7 di-hydroxy system in 2 (7), ^{1}HNMR of its di acetate exhibited six proton singlet , two proton doublet & one proton triplet at δ 1.73, 3.30 (J = 7 Hz) & 5.32 respectively for prenyl unit. The downfield shift of singlet at δ 6.89 & δ 7.22 represented magnetically non – equivalent proton at C_3 & C_6 [8] where as the sharp singlet at δ 3.50 & 2.41 were attributed for acetyl groups. These chemical shifts suggested absence of A_2B_2 system in B ring of 2. The EIMS of 2 gave fragment ions at m/z 357 (base peak) & 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 bathchromic shift 55 nm on addition of $AlCl_3$ (13). On mild alkaline hydrolysis followed by cyclization of dimethyl ether of 3 yielded 2: 2, dimethyl chromant identified by PMR (14) also conforming prenylation of C_8. Further $KMnO_4$ oxidation of dimethyl ether of 2 formed 2, 4, 5 tri-methoxy 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, R_1= D-galactose
2, R_1R_2 = H
3, , R_1R_2 = 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 bathchromic shift on addition of NaOMe suggesting the glycosylation of the OH group in ring A. The permethylation (16) confirmed

that C_1-OH of sugar was attached with C_7 – 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 albinorats 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 the University.

ISOLATION: Air-dried powered leaves (4.0 kg) of T. Dioica were extracted exhaustively with hot 95% ethanol. The extract was concentrated under vaccum & segregated into petroleum ether, C_6H_6, EtOAc, $(Me)_2CO$ soluble fractions. The ethyl-acetate 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 / ethyl-acetate in various propotions.

COMPOUND 1: Elaute with $CHCl_3$ / MeOH (5:6), light yellow amorphous compound m.p. 205-206, mf. $C_{29}H_{34}O_{12}$ (found C 60.59, H 5.91, Calcd. C 60.62, H 5.92). M^+ 574 (EIMS), intense green colour with $FeCl_3$ pink colour with Na-Hg / HCl & positive molish's test . UV λ_{max}^{MeOH} 262, 291 (sh), 300 (sh), $\lambda_{max}^{AlCl_3}$ 275, 310, $\lambda_{max}^{AlCl_3/HCl}$ 276, 309, ; IR ν_{max}^{KBr} 3500, 2930, 2820, 1650, 1625, 1557, 1570, 1385, 1368, 1285, 1215, 1145, 885, 825, 766, 710 . & 690 cm^{-1}; fabms 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 sodium acetate and acetic anhydride at 130° in oil bath for 4 hrs & workup as usual & acetyl derivative crystallized with methanol white needles, m.p. 185-86°analysed for $C_{39}H_{44}O_{17}$ (found C 59.68, H 5.59, Calcd. C 59.69, H 5.61). IR ν_{max}^{KBr} 1720, 1645 cm^{-1}. PMR ($CDCl_3$ 90 MHz , TMS ppm) 8.02 (s , H , C_2), 7.23 (s , H , C_6), 6.82 (s , H , C_3) , 5.32 (t , H , $C_{2''}$), 3.92 (s, 3H , OCH_3), 3.82 (s , 3H , OCH_3), 3.79 (s , 3H , OCH_3) 3.30 (d , J = 6 Hz , H , $C_{1''}$) , 1.72 (s , 6H, $C_{4''}$, $C_{5''}$), 5.16 (d , J = 7 Hz , H , $C_{1''}$) , 4.3-4.8 (m , 6H of galactose), 2.52 (s , 3H , OAc $C_{6'''}$) , 2.14 (s , 3H , OAc, $C_{4'''}$) , 2.05 (s , 3H , OAc, $C_{2'''}$)

ACID HYDROLYSIS OF 1: 1 was refluxed with 7 % alc. H_2SO_4 for 7 hrs. The alcohol was removed and a cream coloured substance 2 was separated. The concentrated hydrosate after neutralization, was examined on PC (BAW 4:1:5) & found to contain galactose. The quantitative estimation of sugar showed that the aglycone & galactose are present in a equimolecular ratio.

COMPOUND 2: Cream coloured substance re-crystallised with MeOH : acetone , m.p. 247-48° , analysed for $C_{23}H_{24}O_7$ (found C 66.98 , H 5.81 , Calcd. C 66.99, H 5.82) , M^+ 412 (EIMS), gave dark green colour with $FeCl_3$, Pink colour with Na-Hg / HCl . UV λ_{max}^{MeOH} 254 , 290(sh), 307(sh) , $\lambda_{max}^{AlCl_3}$ 271 , 309 , $\lambda_{max}^{AlCl_3+HCl}$ 273 , 306 ; IR ν_{max}^{KBr} 3510 , 2915 , 2830 , 1640 , 1560 , 1500 , 1380 , 1367 , 1240 , 1190 , 805cm^{-1} . Fambs m/z (r .int) 412 (24) , 384 (40) ,381 (57) , 357 (29) , 356 (70) , 192 (91) , & 166 (100) . ^{13}C NMR (DMSO – d_6 , 22.6 MHz) , 153.6 (C-2) , 120.1(C-3) , 178.6 (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 CH_3), 55.1 (4' OCH_3) , 55.9 (5' OCH_3).

ACETYLATION OF 2: The acetyl derivative of 2 was prepared by sodium acetate and acetic anhydride and obtained as silk needled, m.p. 226-27° $C_{27}H_{28}O_9$ (found C 65.31, H 5.62, Calcd. C 65.32, H 5.64), IR ν_{max}^{KBr} 1750, 1645 $cm^{-1;}$ PMR (90 MHz, $CDCl_3$ ppm) , 8.02 (s, H , C_2), 7.23 (s, H , C_6) , 6.87 (s , H , C_3) , 5.32 (t , H , $C_{2''}$) , 3.94 (s, 3H , OCH_3) , 6.87 (s , 3H, C_3) , 5.32 (t , H , $C_{2''}$) , 3.94 (s , 3H , OCH_3) 3.79(s , 3H , OCH_3) , 3.30 (d , J = 7 Hz , 2H, $C_{1''}$) , 2.50 (s , 3 H , OAc , C_5) , 2.41 (s, 3H , OAc , C_7), 1.73 (s , 6H , $C_{4''}$ $C_{5''}$) .

METHYLATION OF 2 TO 3: Compound 2 (400mg) + $DMSO_4$ + anhydrous K_2CO_3 + dry acetone were refluxed for 8 hours. The inorganic salts were filtered off, acetone was evaporated to a syrupy mass & 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° analyzed for $C_{25}H_{28}O_7$ (found C 66.17 , H 6.34 , Calcd. C 68.18, H 6.36).

ALKALINE HYDROLYSIS AND CYCLIZATION: The di-methyl ether 3 (200 mg) in 20% alco. NaOH (10 ml) refluxed for 20 minutes. The reaction mixture was acidified after cooling and water was added & extracted with ether. The ether soluble part on evaporation yielded a residue which was mixed with HCOOH (5 ml) & kept for an hour, followed by addition of H_2O and was extracted with ether. Removal of ether from ether soluble part yielded a gummy substance, analyzed for $C_{24}H_{30}O_7$ and was identified as 2 , 2 di-methyl chroman.

$KMnO_4$ OXIDATION OF 3: Potassium permanganate was added in small quantities to 3 in acetone. The mixture was refluxed and more $KMnO_4$ was added until slight excess was present. The mixture was refluxed for further 15 minutes. The precipitated magnese dioxide was filtered off and washed with H_2O. The combined filtrate and washing were acidified with H_2SO_4, saturated $(NH_4)_2SO_4$ & extracted with ether. Removal of ether and crystallization of product yielded 2, 4, 5 tri-methoxy benzoic acid.

PERIODATE OXIDATION: Compound 1 was dissolved in methanol (40 ml) and kept with sodium meta -periodate (15 ml) in a conical flask for 2 days. The liberated formic acid and consumed periodate was estimated by Jone's method.

PERMETHYLATION AND ACID HYDROLYSIS: Compound 1was treated with CH_3I & Ag_2O in dimethyl formamide at room temperature. After 2 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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