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RESEARCH ARTICLE

STEROIDAL SAPONIN FROM THE ROOTS OF *MOMORDICA CHARANTIA*

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ABSTRACT:

The *Momordica charantia* is known as ' karela' in Hindi and is found through-out in India and also cultivated in the country as a vegetable crop.The roots were extracted and extract were concentrared to a viscous mass and tested to respond the positive tests for saponin(s), than subjected to TLC, when two spots were seen. It was therefore, subjected to the column chromatography and both the compounds were separated. Eluates from chloroform:methanol (3:2) of same Rf values were combined.removal of the solvent yielded a pure compound in white yellow needles shape with the molecular formula $C_{46}H_{76}O_{14}$, m.p.146-148°, and M^+ = 852.It was responded to all the tests of saponin. The saponin gave positive Molisch's test for glycoside and was hydrolysed by sulphuric acid , sapogenin were precipitated out. The structure of saponin as well as structure of sapogenin and sugar moieties were identified by using physicochemical methods like UV, IR, NMR and MASS spectrum.The roots of *Momordica charantia* were found to contain a new steroidal saponin identified as ; stigmast-5,17(20)-dien-3-0-β-D-arabinofuranosyl(1→4)-O-α-L-rhamnipyranosyl(1→4)-O-α-D-glucopyranoside.

Keywords: Saponin, sapogenin, glycoside, moieties, enzyme, eluates, monosaccharide, Molisch'test.

Introduction:

Momordica charantia Linn. (cucurbitaceae)[1], is credited to reputed therapeutic values [2] . The presence of charantin and steroidal saponins have been reported in it by earlier workers [3], but roots remain yet to be worked up and this encouraged us to investigate them further.

Material:

Roots were collected locally and authenticated by the Department of Botany, Govt. M.L.B. Girls College Bhopal.

Isolation of the saponin: The roots (3kg) were air dried ,powdered and defatted with petroleum ether (40 - 60˚) and then extracted with 95% ethanol and the extract concentrated to a brown viscous mass and the mass treated with chloroform:ethanol (2:1) and filtered . The filtrate was concentrated to a brown viscous mass and tested for presence of saponin, responded positive tests for saponin (s).

The viscous mass was then subjected to TLC solvent system, chloroform:acetone: methanol:water with the ratio 60:20:18:2 respectively and then sprryed by 10% H_2SO_4 , then two spots were seen.

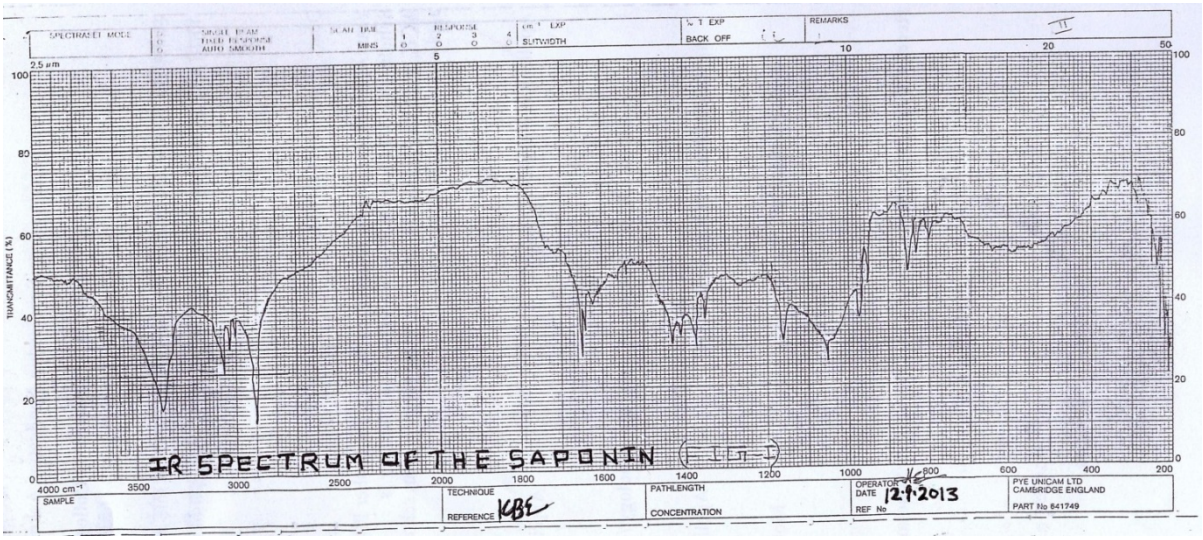
It was therefore, subjected to the column chromatography on activated silica gel using eluates (I) chloroform (II) acetone (III)chloroform:methanol(I:I) and (IV) chloroform:methanol (3:2).

Eluates from chloroform;methanol (3:2) was worked up by column chromatography and eluates of same Rf value were combined . Removal of the solvent under reduced pressure yielded (0.06%) pure compound (further subjected for TLC ,resulting single spot . Which crystallized in white yellow needles from a mixture of chloroform:methanol (3:2), m.p. 146-148˚, molecular formula $C_{46}H_{76}O_{14}$ $(\alpha)^{22}_{D}$ – 30.0˚ ($CHCl_3$), and $M^+ = 852$.

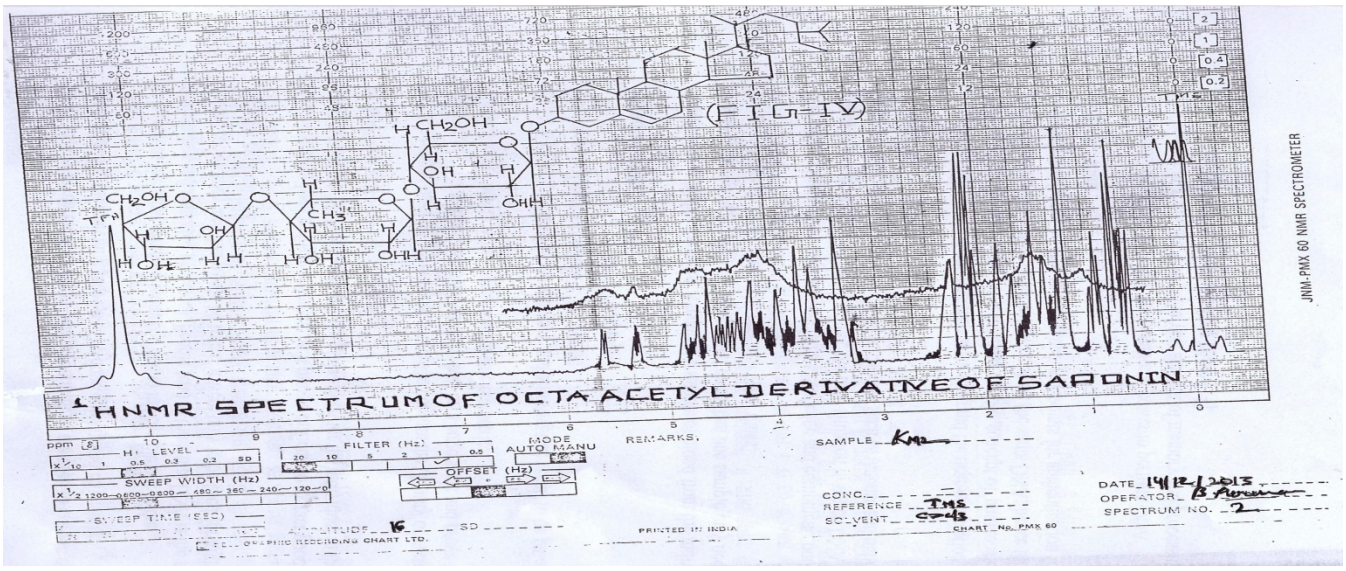
It was insoluble in water and ether and sparingly soluble in chloroform, ethyl acetate and soluble in alcohol, acetone and readily soluble in pyridine gave a brown coloured viscous solution . It responded to all the tests for saponin. which was identified as: stigmast-5, 17(20)-dien -3-O-β-D-arabinofuranosyl(1→4)-O-α-L-rhamnopyranosyl (1→4)-O- β-D-glucopyranoside by spectral studies, eluates from (II) chloroform-methanol (5/7) were of similar Rf value (0.79) when mixed and worked up yielded another compound which could

not be studied for want of material.(the yield of the compound was very less or was not sufficient for the degradative and spectral analysis , so unable to identified the compound.).

The saponin $C_{46}H_{76}O_{14}$, had m.p. 146-148˚C (M^+ 852), $(\alpha)_D^{22}$ +22.5($CHCl_3$).IR(KBr): 3375,3015,3032,3050,2900,1650,1645,1425,1410,1370,1040,1030,970-955,860-800;[5]

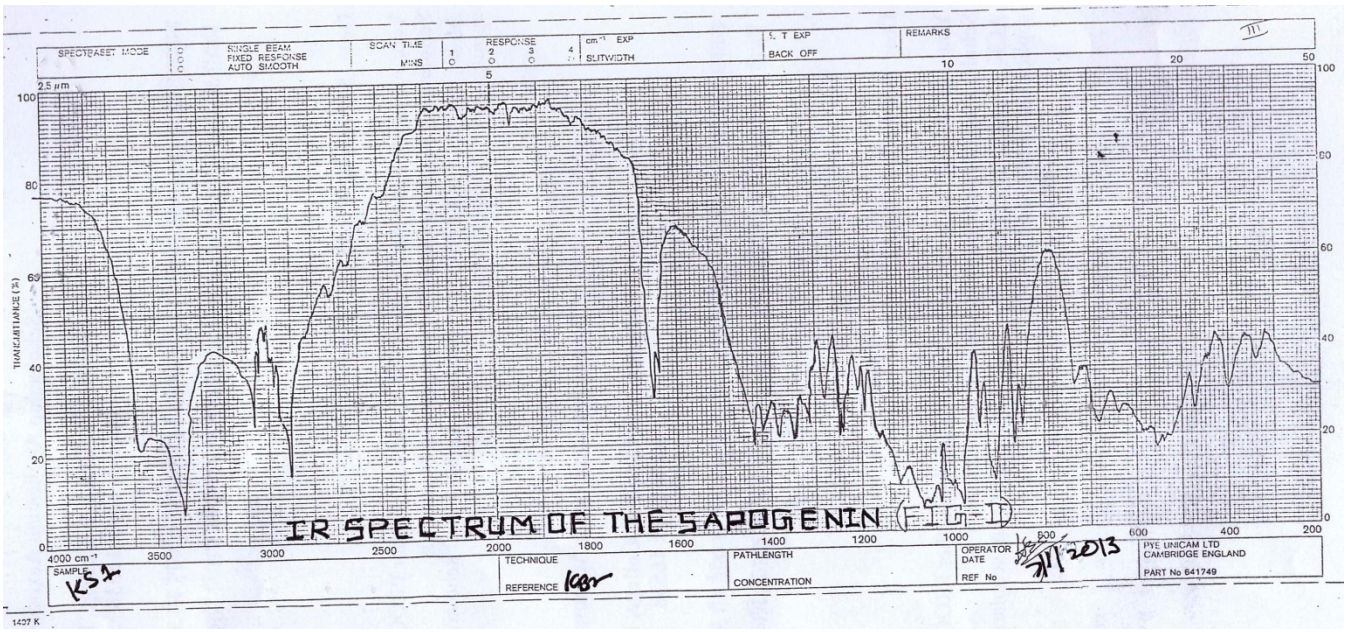


PMR($CDCl_3$) of acetyl derivative :1.80(S, 3H, sec CH_3), 0.63 (S, 3H, tert CH_3), 0.74 (S, 3H, tert CH_3), 0.90(t,3H,J= $6H_Z$ primary CH_3),0.85(d,6H, J = 6.5 Hz sec 2 x CH_3), 3.40(m, 1H carbinolic proton), 4.45 (m, 1H, hydroxyl proton), 5.30(m, 1H, olefinic proton), 1.24-2.00(complex m, 26H, polymethylene protons)4.32(d,1H, J =7.8Hz, anomeric 1'-H), 5.47 (d, 1H , J =2Hz, anomeric 1'' - H) 4.29(d, 1H, J=7 Hz, anomeric 1'''-H), 0.78 (d, 3H, J = 6Hz, 6^{II} = CH_3 of rhamnose), 4.66=4.82(m, 4H, proton of rhamnosyl unit), 3.42 = 4.26 (m, 11H, protons of sugar residue)2.08 (S, 6H,2' OAc and 6' OAc),2.15 (S, 3H, 3' OAc), 2.06(S, 6H, 2" OAc and 3" OAc), 2.04 (S, 6H, 2''' OAc),2.02(S, 3H, 6^{III} OAc), MS: 852 (M^+), 720,574, 412,397, 394,379,300,299,281,271,230, 175 and 159.[6]

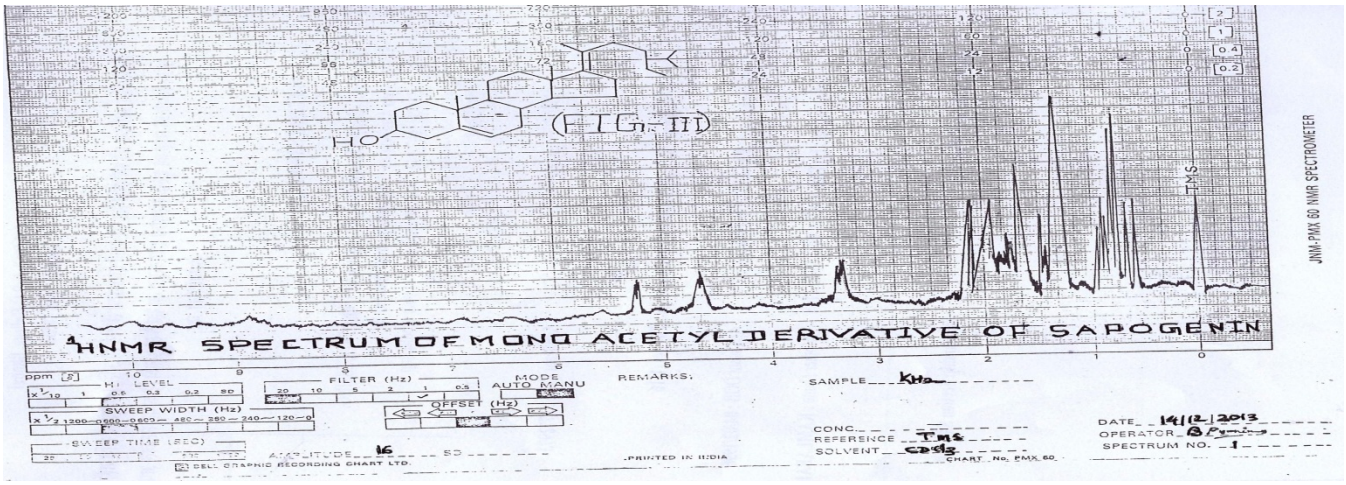


On hydrolysis it afforded a genin and sugars (as; D-arabinose, L-rhamnose and D- glucose, by co-PC and TLC).

The genin crystallized from pyridine as light yellow crystals m.p. 165-170˚C $(\alpha)_D^{22}$ - 51.0˚($CHCl_3$), molecular formula $C_{29}H_{48}O$, M^+412. It gave colour reactions characteristic of steroids[7], and showed IR peaks ν_{max}^{KBr} at 3375 cm^{-1} (OH). 1645 (for unsaturated)The C-OH stretching band at 1040 cm^{-1} indicated the presence of equatorial hydroxyl group at C-3 of an A/B transteroid[8]



Sapogenin Showed Signals in ^{1}HNMR at δ = 0.63 and 0.74 each of (3H,S)were assigned to two tertiary methyl groups. While a doublet at δ =0.85 (6H, J = 6.5 Hz) was assigned to isopropyl group of the side chain[9] .Further signals at δ = 0.90(3H,t, J = 6Hz) indicated the presence of primary methyl group at C-28 and δ = 1.80 (3H,s) for the methyl group at C - 21. This indicated the presence of a double bond either between C-17 and C-20 or C-20 and C-22. Moreover the NMR spectrum demonstrated the signals at δ =3.40(1H,m) 4.45 (1H,m), 5.30(1H,m) corresponding to carbinolic protons respectively.

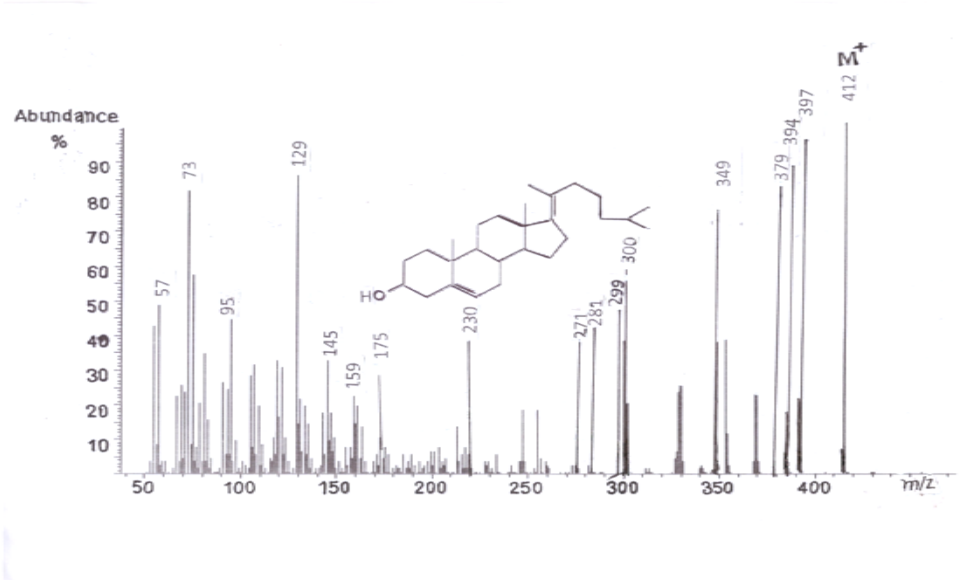


Results and discussion:

The above data concluded that the compound under examination was a steroidal saponin having a hydroxyl group at C-3, and one trisubstituted double bond and another double bond which was located on the side chain.

Peaks in the EI mass spectrum were at ; m/z = 412 and 397 (M-Me)$^{+}$, 394 (M – H_2O)$^{+\cdot}$, 349 (M-Me-H_2O)$^{+}$, 299 (base peak) and 271. The peak at m/z 271 were assigned to the fragment formed due to the lose of the side chain from the molecular ion along with one hydrogen atom from the charge retaining fragment. The fragment at (m/z 271 is characteristic feature in mass spectrum fragmentation of steroids(^{5} - 3β- ol- moity) having double bond in the side chain[13] , which was fixed at ^{5} and was confirmed by the absence of a characteristic peak in the mass spectrum at m/z 289 which is formed with 5-α- steroids by fragmentation of the ring B, involving migration of the 5α-hydrogen [11] Absence of a peak at m/z 246, 232 , 231 , 180 . 166 , 95 and 81 produced due to the cleavage of ring D , excluded possibility of the location of the double bond between C-20 and C-22 [10,12,13], thereby confirming the position of another double bond between C-17 and C-20. The peaks at 397 (

M-Me)$^+$ and at 394 (M-H_2O)$^+$ were due to loss of CH_3 group and water molecule repectively. These facts and available literature identified the genin as; stigmast – 5, 17 (20)-diene-3β- ol.



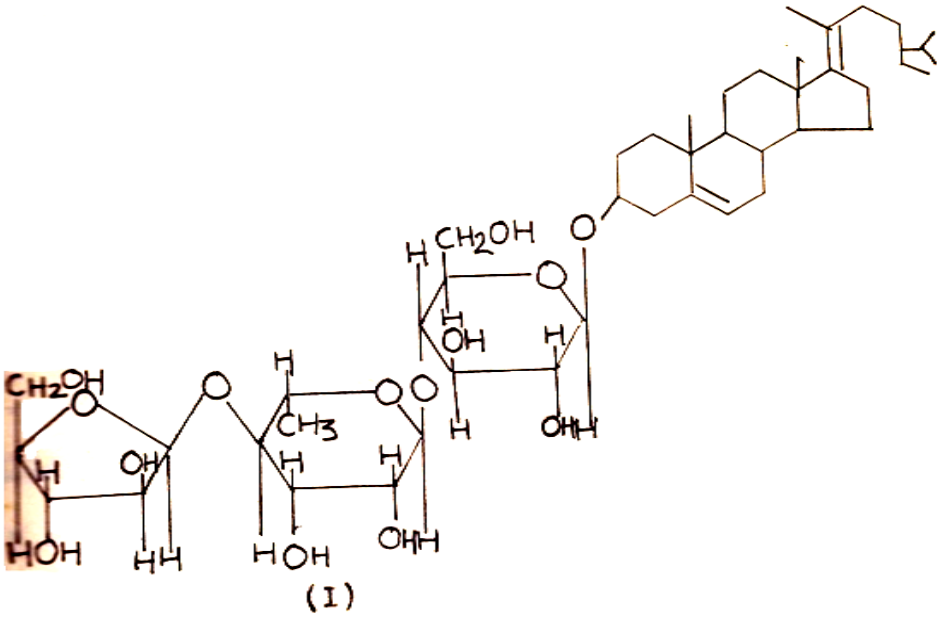
Mass Spectrum of the Sapogenin

The position of attachment of sugar moieties to the genin must be at C-3 in it because of the presence of only –OH group at C-3.

The saponin on hydrolysis with enzyme " emulsion " [14]liberated monosaccharide arabinose and some other carbohydrates which on hydrolysis with enzyme "diastease" [15],liberated the monosaccharide rhamnose and glucose (Co-Pc and TLC). These along with partial hydrolysis (2% H_2SO_4) and periodate oxidation (consumption of three moles of periodate to produce three moles of formic acid per mole of the saponin), indicated that one mole of saponin was made up of one mole of sapogenin and one each of D-arabinose, L-rhamnose and D-glucose and that D-arabinose was the terminal sugar and D-glucose was attached to the genin . The above facts also concluded that the linkage between D-arabinose and L-rhamnose was β and D-glucose was linked to the genin by β –linkage.

Acid hydrolysis of permethylated saponin (DMS/dry K_2CO_3), yielded 2,3,6 tri-O-methyl-D-glucose, 2,3-di-O-methyl- L-rhamnose and 2,3,5 tri-O-methyl D-arabinose (co-PC and TLC), thereby showing that arabinose was linked to the rhamnose via (1→4) linkage and

rhamnose was attached to the glucose via (1→4) linkage and glucose was linked to the genin by anomeric C_1.



STRUCTURE OF THE SAPONIN

Conclusion :

Thus the saponin was identified as: stigmast – 5 , 17(20)-dien-3-o-β-D-arabinofuranosyl (1→4) –o-α-L-rhamnopyranosyl (1→4)-O-β-D-glucopyranoside (I).On the basis of the above facts.

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