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Stigmasterol-3-O-β-D-arabinopyranosyl(1→4)-O-β-D-glucopyranoside from the Roots of *Limonia crenulata* (Roxb.)

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ABSTRACT

The methanol soluble part of the concentrated ethanolic extract of defatted roots of Limonia crenulata (Roxb) when worked up phytochemically yielded a saponin which on various chemical reactions and spectral analysis was identified as; Stigmasterol-3-O-β- D-arabinopyranosyl (1→4)-O-β-D-glucopyranoside.

Key words: Stigmasterol -3 - O - β - D - arabinopyranosyl (1→ **4**) - O - β - D -glucopyranoside from the roots of Limonia crenulata (Roxb).

INTRODUCTION

The plant Limonia crenulata (Roxb)[1] is commonly know as Beli in hindi and belongs to Natural-order Rutaceae It occurs in Western and Southern India, Punjab, N.W. Himalay, Shimla, Kumaon, Bihar, Bengal and Assam. Its roots are purgative, and are reported to be useful for curing colic and cardialagia. The dried fruit of this plant work as antidote to various poisons They function as tonic and diminish intestinal fermentation. They also resist the contagion of small pox along with malignant and pestilent fevers. It's leave are reported to be useful for curing EPILEPSY.

MATERIALS AND METHODS

2.5kg of air dried powdered and defatted roots of Limonia crenulata (Roxb) were extracted with hot rectified spirit in a round bottomed flask fitted with a reflux condenser on an electric water bath. The ethanolic extract was filtered while hot under concentrated reduced pressure to get brown viscous mass, which was partitioned with n-hexane, benzene, chloroform, acetone ethylacetate and methanol.

The study of the methanol soluble part

The methanol soluble part was concentrated under reduced pressure to get a

brown viscous mass which on addition of excess of solvent ether gave a precipitate which on TLC examination over silica gel G, using solvent system n-butanol acetic acid : water (4:1:5) showed two spots indicating it to be a mixture of two compounds. Therefore the precipitate was subjected to coloumn chromatography[2] over silica gel 'G' and eluted with acetone : methanol in different proportions.

Eluates from acetone : methanol (1:1) were of the same R_f value and so combined and subsequently removal of the solvent yielded a homogenous mass (confirmed by the TLC). Therefore it was crystallized from pyridine. It analysed for molecular formula $C_{40}H_{66}O_{10}$, m.p. 190-192°C and $[M^+]$ = 706 (CIMS). It was soluble in methanol, and absolute alcohol but insoluble in petroleum ether and benzene and responded to characteristics tests of steroidal saponin[3-7].

RESULTS AND DISCUSSION

The presence of OH group(s) in steroidal saponin

Characteristic bands at ν_{max}^{KBr} 3755 and 3333 cm^{-1} of the IR spectrum of the saponin indicated the presence of - OH group(s) in it. The number of hydroxyl (-OH) group(s) were estimated by acetylation of saponin with Ac_2O/pyridine when it gave an acetylated product. The percentage of the acetyl group in the acetylated product was estimated by the procedure of Weisenberger[8] as described by Belcher and Godbert[9] (36.54%), which showed the presence of six - OH groups in it.

The presence of double bond in the steroidal saponin

The presence of double bond in the steroidal saponin was indicated by the fact that a solution of saponin in CCl_4 produced yellow colour with tetra nitro-methane[10], thus indicating the presence of double bond in the steroidal saponin. This was further confirmed by a band at ν_{max}^{KBr} 1597 cm^{-1} in the IR spectrum of the steroidal saponin.

The presence of methyl groups in steroidal saponin

Significant band at ν_{max}^{KBr} 1404 cm^{-1} in the IR spectrum of the steroidal saponin indicated the presence of —CH_3 group(s) in it. The number of —CH_3 groups were estimated by Ziesel method (13.00%), which showed the presence of six —CH_3 (methyl) group(s) in it.

Acid hydrolysis of saponin

The saponin was hydrolysed with 6% H_2SO_4 when the sapogenin precipitated out. It was filtered and washed with water.

The sugar moiety(ies) remained in the hydrolysate and studied chromatographically.

The structural study of sapogein

The sapogenin was subjected to TLC, examination when it was found to be homogenous. The sapogenin analyzed for molecular formula. $C_{29}H_{48}O$, m.p. 221 — 222°C, and $[M^+]$ = 412 (CIMS). It responded positively to all the characteristics colour reactions of steroids[11].

Presence of hydroxyl group(s) in the sapogenin

Characteristic bands at ν_{max}^{KBr} 3486 and 3310 cm^{-1} in the IR spectrum of sapogenin indicated the presence of —OH group(s) in it. The acetylation of sapogenin with Ac_2O / NaOAc in glacial acetic acid yielded monoacetate of the sapogenin. This mono acetate analysed for molecular formula $C_{31}H_{52}O_2$, m.p 194 — 195°C and $[M^+]$ = 454 (CIMS). The presence of acetyl groups(9.56%) were estimated by Weisenberger[12] as described by Belcher and Godbert[13], which showed the presence of only one acetylable hydroxyl group in the sapogenin.

The position of hydroxyl group(s) in the sapoenin

The sapogenin on Cr_2O_3/ pyridine oxidation, yielded a ketone, m.f. $C_{29}H_{46}O$, m.p. 227 — 228°C and $[M^+]$ = 410 (CIMS), which responded to positive Zimmerman test[14] for C-3 keto group, thereby confirming the presence of hydroxyl group at C-3 and further indicated its nature as secondary in the sapogenin.

The presence and position of double bond(s) in the sapogenin

The characteristics band at ν_{max}^{KBr} 1609 cm^{-1}

in the IR spectrum of the sapogenin showed the presence double bond(s) in it. On catalytic hydrogenation with Pd/C, the sapogenin gave a tetra hydro derivative m.f. $C_{29}H_{50}O$ m.p. 224 — 225°C and $[M^+]$ = 414 (CIMS), which indicated the presence of two double bond in it.

The presence and position of methyl group(s) in the sapogenin

The IR spectrum of sapogenin showed band(s) at ν_{max}^{KBr} 1350 and 1380 cm^{-1}, which indicated the presence of angular methyl group(s) in it. Quantitative estimation of methyl group(s) (22.0%) was done by Ziesels[15] methods, which showed the presence of six angular methyl group(s) in the sapogenin. The position of methyl group(s) was established by the study of ^{1}HNMR spectrum. The ^{1}HNMR spectrum of sapogenin showed three proton intensity singlet each at b 0.70, 0.82 doublets each at b 0.75 J-6.5, b 0.68 J-6.7 and triplet b 0.88 J-5.34, assigned for the position of methyl group at C-18, C-19, C-25, C-26, C-28 and C-29 respectively in the sapogenin.

The study of the sugar hydrolysate of the saponin

The aqueous hydrolysate obtained after separating the sapogenin was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate which was found to reduce Fehling's solution was concentrated to get a syrupy mass.

The concentrated hydrolysate was, subjected to paper chromatography with authentic sugar samples on Whatmann No.1 filter paper using aniline hydrogen phthalate as spraying reagent. The analysis revealed the presence of D-arbinose and D-glucose as sugar moieties (confirmed by CoPC and CoTLC with authentic sugar samples).

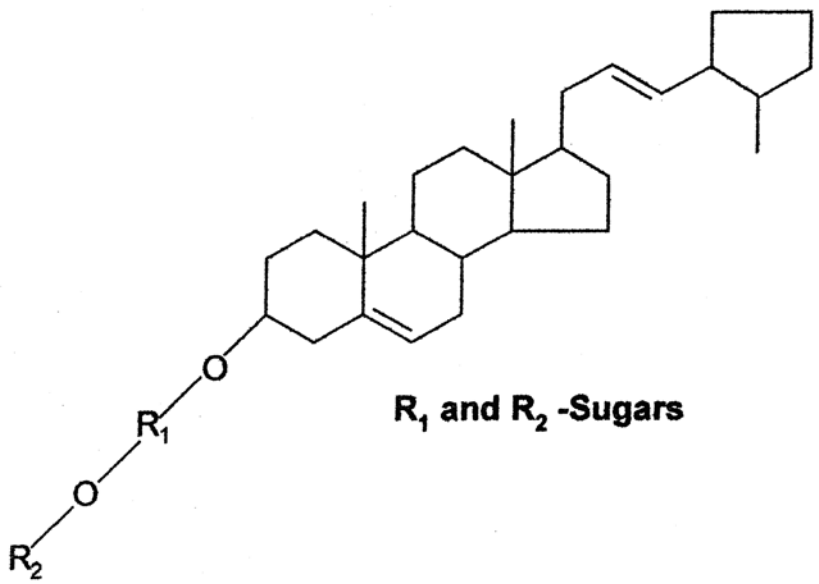
The quantitative estimation of sugars

Quantitative estimation of sugars present in the saponin was done by procedure of Mishra and Rao[16]. It was observed that the two sugars were present in the equimolecular ratio.

Thus, it was concluded that one molecule of the steroidal saponin was made up of one molecule of sapogenin and one molecule each of D-arabinose and D-glucose.

The position of attachment of sugars to the sapogenin

Since there is only one hydroxyl group available for glycoside formation in the sapogenin naturally the sugars must be attached on it, thereby concluding that sugars must be attached at C-3. Thus a tentative structure to the saponin was assigned as below:



The sequence of sugars in the steroidal saponin

On graded hydrolysis with 0.04 N H_2SO_4 for one hour at room temperature the steroidal saponin liberated first D-arabinose followed by D-glucose, thus confirming D-arabinose as terminal sugar.

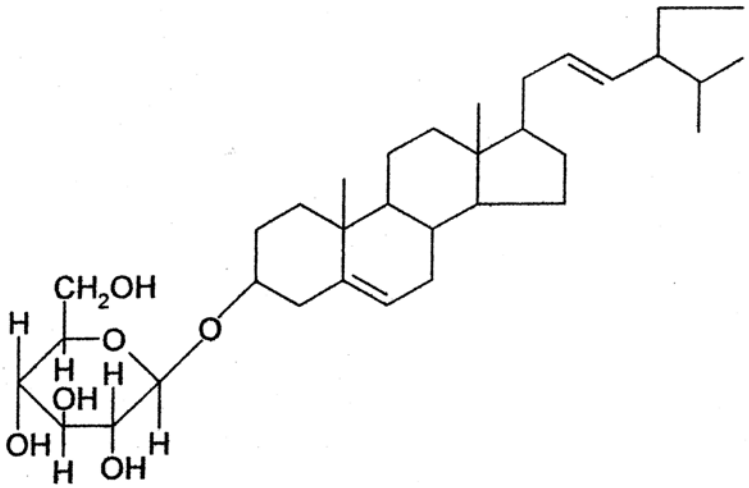
Thereafter the contents of the flask were subjected to column chromatography over silica gel 'G' using chloroform : methanol (1:1) as eluant when a mixture of two prosapogenins were isolated analysed for m.f. $C_{35}H_{58}O_6$, m.p. 219-220 and m.f. $C_{40}H_{66}O_{10}$, m.p. 239-240 respectively.

The study of the prosapogenin

The prosapogenin analysed for m.f. $C_{35}H_{58}O_6$ which on hydrolysis with 0.04 N H_2SO_4 gave a sapogenin and D-glucose which was identified Co-PC and Co-TLC, with authentic sample. The sapogenin was identified as stigmasterol by mixed m.p.

The permethylation and hydrolysis

The prosapogenin was subjected to permethylation which was done by the procedure of Khun[17], The permetylated prospagenin on hydrolysis yielded 2, 3, 4, 6 tetra — O — methyl — D — glucose (The identity was established by Co-PC and Co-TLC, with authentic sample). This showed that C_1 of D-glucose was involved in the glycosidic linkage and also suggested that D-glucose was present in the pyranose form. Thus the prosapogenin was assigned the structure as : Stigmasterol — 3 — O — β— D — glucopyranoside.



The study of prosapogenin

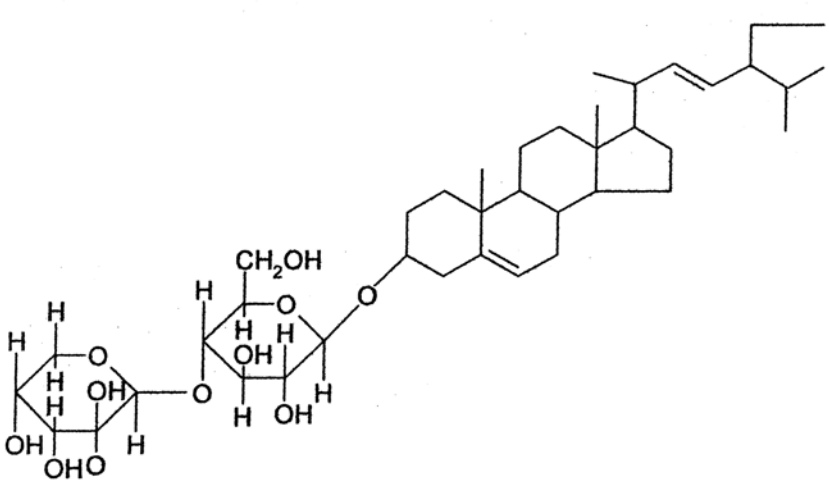
The prosapogenin on hydrolysis with 6% H_2SO_4 yielded a sapogenin which was identified as, stigmasterol. The sugars were identified as D-glucose and D-arabinose (confirmed by Co-PC and Co-TLC with authentic sample).

The permethylation and hydrolysis of prosapogenin

This prosapogenin on permethylation followed by hydrolysis and chromatographic examination of hydrolyrate established the presence of 2, 3, 4, 6 tetra — O — methyl — D — glucose and 2, 3, 4 tri—O—methyl — D — arabinose (by CoPC and Co-TLC with authentic sample) thus indicating that D-arabinose and D-glucose both were present in pyranoside form in it.

The nature of the glycosidic linkage in the saponin

The steroidal saponin[18] when subjected to hydrolysis by enzyme almond emulsion liberated the sugars, The examination of sugars by paper chromatography (confirmed by Co-PC and Co-TLC with authentic sample) indicated the presence of D-arabinose and D-glucose thereby confirming the linkage between D-arabinose and the sapogenin as well as between D-glucose and D-arabinose as b in the saponin.



CONCLUSION

As such structure to the steroidal saponin was assigned as; stigmasterol — 3 — O —β— D — arabinopyranosyl (1→4) —O — β— D — glucopyranoside.

REFERENCES

1. Chopra, R.N., Nayer, S.L. and Chopra, I.C. "Glossary of Indian Medicinal Plans", CSIR Publication, New Delhi, p. 119, (1956).
2. Peachand Tracey, "*Modern Methods of Plant Analysis*", **2**: 49 (1962).
3. Noller, C.R., *J. Amer. Chem. Soc.*, **64**: 3047 (1942).
4. Tsohugajew, *Che. Zig.*, **24**: 542 (1900).
5. Mohan Jag, "Organic Spectroscopy Principles and Applications" Narosa Publishing House New Delhi (2000).
6. Liebermann, C. "Ber. Deash. Chem. Gas", p. 1804 (1885).
7. Salkwski, E., Hoppeseyler Z., **57**: 52 (1908).
8. Weisenberger, *Microchemic,* **33**: 51 (1947).
9. Belcher, R. and Godbert, A.L., *Semi Micro quantitative Organic analysis,* 164 (1954),
10. Rechard, R.E. and Thomas, N.A., *J. Chem. Soc.,* 368-74 (1974).
11. Zeisel, *et al, J. Chem. Soc.,* 2478-82 (1930).
12. Weisenberger, *Microchemic,* **33**: 52 (1947).
13. Belcher, R. and Godbert, A.L. *Semi Micro Quantitative Organic analysis,* 165 (1954).
14. Zimmermann, J. *Helv. Chem. Acad.*, **26**: 642-647 (1943).
15. Zeisel, *et al, J. Chem. Soc.,* 2478 (1930).
16. Mishra, S.B., and Rao M.V.K., *J. Sci. Ind. Res.* 19: 170 (1960).
17. Khun, R. Low, I and Trichmann, H., *Angew. Chem*. 67: **32** (1955).
18. Pakrashi, S.P., *Indian J. Chem.* 21: 468 (1964).