International Journal of Innovative Research in Engineering & Management (IJIREM)
ISSN: 2350-0557, Volume-10, Special Issue-1, February-2023
National Conference on Digital transformation through Intelligent Computing Systems and Methods (NCDICM 2023) on 13th January, 2023
Organized by Lloyd Institute of Engineering & Technology, Greater Noida, India

Degradation Studies of Rouwolfia Serpentina Benth Water Soluble Seed Polysaccharide

Dhiresh Kumar Pathak[1], and Ashish Parmar[2]

[1,2]Lloyd Institute of Engineering and Technology, Greater Noida, India

Correspondence should be addressed to Dhiresh Kumar Pathak; abc@gmail.com

ABSTRACT- Rauwolfia Serpentina Benth Seed polysaccharide was degraded by degradation method with the reduction of sodium borohydride and then hydrolysed. The paper chromatographic analysis of the hydrolysate revealed the presence of glycerol and erythritol. The mixture was separated on whatman No.3 MM filter paper sheet and fraction were ientified as Glycerol, Erythritol & Thritol.

KEYWORDS- Rouwolfia Serpentina, Polysaccharide, Water Soluble Seed, Polysaccharide

I. INTRODUCTION

Rauwolfia Serpentina Benth. plant belongs to the family-Loganiaceae, occurring in northern India particularly in Gharwal region and other places like Gorakhpur forests, Orissa, Western Peninsula, Myanmar, Sri Lanka etc, up to an altitude of 360m in height. An evergreen tree Rauwolfia Serpentina Benth. usually extends to the height of 13m and girth 0.9- 1.8m, has fairly straight and cylindrical bole. The strychnine and brucine alkaloids occurs in seeds, roots, wood, bark, leaves, fruit pulp and hard fruit shells. Rauwolfia Serpentina Benth. contains the alkaloids together with traces of a glucoside, loganin. Loganin, a glucoside, is inert and the alkaloids in union with igasuric acid They also contain fatty matter (about 3 per cent.), caffeotannic acid, and a trace of copper. The total alkaloid present varies from 1.8 to 5.3 per cent.; seeds of good quality usually yield from 2.5 to 3.0 per cent. Rauwolfia Serpentina Benth. is an extremely bitter and poisonous alkaloid and one of the narcotico-acrid class of poisons and seems to act directly upon the spinal cord.

Seeds of Rauwolfia Serpentina Benth. Contains a water soluble sugar extract as D-galactose and D-mannose in 1:4 molar ratio. The reaction of periodate oxidation was first discovered by Malaprade (1928). Fluery and Lange (1993) have given a better method for the extensive use of periodate acid for oxidation of glycol. Perlin (1959) has given two important reagents are periodic acid and lead tetra acetate, showed that the glycol group undergo cyclic ester formation with oxidation and reaction considered to be dialdehyde type of oxidation. The control atom of the oxidation reagent must be able to co-ordinate at least two hydroxy group.

II. MATERIAL & MEHTODS

A. Isolation of Polysaccharide

(250gm) of Rauwolfia Serpentina Benth. Plant belongs to the family-Loganiaceae , occurring in northern India particularly in Gharwal region and other places like Gorakhpur forests, Orissa, Western Peninsula, Myanmar, Sri Lanka etc, upto an altitude of 360m in height. An evergreen tree Rauwolfia Serpentina Benth. Usually extends to the height of 13m and girth 0.9-1.8m, has fairly straight and cylindrical bole. The alkaloids occurs in seeds, roots, wood, bark, leaves, fruit Pulp and hard fruit shells. Rauwolfia Serpentina Benth. Contains the alkaloids and of a Glucoside, they also contain fatty matter (about 3 percent), caffeotannic acid, and a trace of copper. The total alkaloid present varies from 1.8 to 5.3 per cent seeds of good quality usually Yield from 2.5 to 3.0 per cent. Seeds of Rauwolfia Serpentina Benth. Contains a water soluble sugar extract as D-galactose and D-mannose in 1:4 molar ratio. The reaction of periodate oxidation was first discovered by Malaprade (1928). Fluery and Lange (1993) have given a better method for the extensive use of periodate acid for oxidation of glycol. Perlin (1959) has given two important reagents are periodic acid and lead tetra acetate, showed that the glycol

group undergo cyclic ester formation with oxidation and reaction considered to be dialdehyde type of oxidation. While Chatterjee (1970), Kumar (1976) and Sarkar (1976) have been used periodate oxidation to determine the polysaccharide structure. The control atom of the oxidation reagent must be able to co-ordinate at least two hydroxy group were collected from F.R.I. Dehradun, then washed the seeds with water dried, crushed to a greyish powder. Powdered seeds (100gm) were dissolved in distilled water (800ml) for 24 hrs. The contents were stirred thorough with the help of mechanical stirrer then the viscous solution was filtered through muslin cloth then it was again filtered by sharpel's super centrifuge to remove all finely suspended particles. Filtrate was precipitated with ethanol to precipitate out all polysaccharide in light brown form. At the time of precipitation of polysaccharide was well stirred with the help of mechanical stirrer. The precipitate of polysaccharide was filtered through sintered funnel G-3 under suction and dried in vaccum at 60°c after washing with acetone and petroleum ether, obtained as greyish powder (8.67gm) had sulphated ash 1.84% and optical{α}25 D + 31.2°c H2O.

B. Degradation of the Polysaccharide

In degradation studies of the purified polysaccharide (1.5 gm) was oxidized by Smith degradation methods with sodium metaperiodate (0.125M,10 ml) for 36 hrs in refrigerator at 4-8°C. The remaining periodate oxidized compound was treated with ethylene glycol (5 ml) to decomposed the excess of periodate then it was dialysed against running water up to 48 hrs and concentrated to 50 ml volume. The resulting solution was reduced with sodium borohydride (1gm) with mechanical stirring at room temperature for 24 hrs. Excess sodium borohydride was acidified with glacial acetic acid (5ml) and resulting solution was dialysed against running water then evaporated to dryness. Residue was distilled with methyl alcohol to remove the borate ion as methyl borate. The borate free reduction product was dialysed against running water for 48 hrs to remove inorganic ions. It was concentrated to a thin syrup which was hydrolysed with sulphuric acid (0.5N,10 ml) for 12 hrs. Hydrolysed product was neutralized with carbonate slurry with the help of mechanical stirrer then content left for 24 hrs. It was then filtered off and obtained filtrate was deionised by Amberlite ion exchange resin IR-120 (H+) and IR-45 (OH-) then concentrated to syrup.

Hydrolysed product was resolved into its component by paper chromatographic analysis on Whatman No.3MM filter paper sheet using solvent mixture and used a spray reagent for the detection of polyalcohals. It was eluted with water according to the Dent's method and polyalcohals were identified and characterised as glycerol , erythritol , and traces of thritol.

Glycerol- Sugar syrup (280mg) was dissolved in 5 ml ethanol, filtered and filtrate concentrationto a syrup .It moved a single spot parallel to the authentic sample of glycerol on paper chromatogram , Derivative was prepared with sugar residue (240mg) in pyridine (5ml) and p-nitrobenzol chloride (3gm) content was heated over water-bath for 1 hr at 75-80°C , cooled and added saturated solution of sodium bicarbonate then finally filtered. After cooling the filtrate yielded the crystals of glycerol-tri-O-p-nitro benzoate after recrystallisation with acetone having m.p. and mixed m.p. 185-187°C, Lit. m.p. 186- 188°C.

Erythritol- Sugar syrup (560mg) was treated with 20ml water it purified by animal charcoal for 24 hrs, filtered and filtrate concentrated to syrup. It dissolved in 5 ml ethanol, cooling the solution of erythritol was crystallized out from the solution. Crystals of erythritol were filtered and on recrystalysation with ethanol had m.p. and mixed m.p.117 - 119°C, Lit. m.p. 118°C.

Derivative of erythritol was prepared by dissolving the syrup (280g) I 5 ml of anhydrous pyridine solution and 1.5 gm of p-toluene sulphonyl chloride for 24 hrs. content was poured into ice cold water, derivative were crystallized out as crystals, filtered and washed with water followed by ethanol .On recrystallisation with acetone and ethanol gave crystals of tetra-O-tosyl-erythritol having m.p. and mixed m.p. 168 - 170°C, Lit. m.p. 166-168°C.

Thritol- It was obtained in traces (20 mg) on paper chromatogram having Rf values more than D-glucose and D-mannose. It was identified as thritol and spot was visible only in ultraviolet light in UV-spectrum.

Polyalcohol's were quantitatively esteemed by chromatropic acid method and respective polyalcohol's were separated by paper chromatographic analysis on What man No.3MM filter paper sheet in solvent mixture and spray reagent. Polyalcohols were characterized as glycerol and erythritol in 1.42:3.84 molar ratio with traces of thritol.

Seed polysaccharide (780mg) was dissolved in 50 ml water and aliquot were put into six test tubes and each tube adjusted with water (2ml). Phenol solution (5%, 1ml) was added into each solution followed by sulphuric acid (0.5N, 5ml) then tubes were allowed to stand for 20 min. and cooled it in running water. A blank reading for each sugar was also prepared in the same way.

The color intensity and absorbance of polyalcohol's were recorded on 530 mμ color filter in a K let summer son photoelectron colorimeter for each sugar. The analytical data of polyalcohol's

From Rauwolfia Serpentina Benth.Seeds polysaccharide are given in following Table.

Table 1: Benth Seeds polysaccharide

S. No.	Wt. in Microgram		Phenol (Ml.)	H2SO4 (Ml.)	K let. Reading (Absorbance)	
	Glycerol	Erythritol			Glycerol	Erythritol
1	2	2	1	5	25	19
2	4	4	1	5	49	37
3	6	6	1	5	72	58
4	8	8	1	5	95	74
5	10	10	1	5	117	96
6	12	12	1	5	138	118

III. RESULT & DISCUSSION

Oxidized polysaccharide of Rauwolfia Serpentina Benth. Seeds was reduced with sodium borohydride and sulphuric acid yielded glycerol, erythritol in 1.42:3.84 molar ratio with trace of thritol on Whatman No.3 filter paper sheet by chromatography. A large proportion of erythritol released by acid hydrolysis (H2SO4) was obtained by the reduction of borohydride. The evidence of linkages in polysaccharide structure showed that the main polymer linkages are of (1→4)-β- type and branching point with (1→4)-α-type. Ratio of erythritol to the amount if glycerol indicates that the branching point after every two repeating unit in the main polymer chain. It indicates a branching point on the average of 7 hexoses units are in the backbone and 2 hexoses units are in the non-reducing end for the support of the earlier

proposed polysaccharide structure of seed as shown in figure. The molar ratio of D-glucose and D-mannose was found to be 3:6 moles by paper chromatography of the hydrolysed products.

REFERENCES

[1] Fluery, P. and Lange, J.; J.Pharm. Chem., 17: 107 (1933).
[2] Halsal, T.G.; Hirst, E.L. And Jones, J.K.N., J. Chem, Soc., 1427 (1947).
[3] Malaparde, L., Bull. Soc. Chem., 43: 683 (1928).
[4] Perlin., A.S., J. Amer. Chem. Soc. 76: 4101 (1957).
[5] Perlin., A.S., Adv. Carbohydr. Chem. 14-B: (1959).
[6] Sarkar, M., Indian J. Chem., 14-B: 919 (1976).
[7] Abdel, A.M. and Smith, F., (1951) J.Amer. Chem. Soc., 73:994.
[8] Singh, R.B., (2002) Oriental J.Chem., 18 (3): 509-512.
[9] Purdie,T. And Irvine, J.C.;J.Chem Soc., 83:1026 (1903).
[10]Whistler, R.I., (1965) Methods of Carbohy.Chem.Academic Press, London.5: 296-299
[11]Krishnamurthi,A.(1969).Wealth of India Raw Materials.Publication & Identification Directorate,CSIR,New Delhi (India),8(Ph-Re):376.
[12]Kolthoff, J.M. and Blecher, R.; Volumetric Analysis, Intersciences Pub. Inc, New York, 3: 276 (1957).
[13]Kumar, P., J.Int Chem. (India), 48: 192 (1976).
[14]Chadha Y.R. (1976)The Wealth of India, Raw materials, Publication and Information, CSIR, New Delhi, India,
[15] (SP-W): 62
[16] Pathak D.K.(2014)IJHEPS,2(08);106-108