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Identification of polyalcohols from periodate oxidized polysaccharides of *Strychnos nux vomica* Linn. seeds by Smith degradation method

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ABSTRACT

A water soluble polysaccharide was extracted from *Strychnos nux vomica* Linn. seeds on acid hydrolysis with sulphuric acid and obtained hydrolysate on paper chromatography led to the D galactose and D mannose in the molar ratio of 1 : 4 moles. The periodate oxidized see!ds polysaccharide on reduction with sodium borohydride by Smith degradation method followed by acid hydrolysis (H_2SO_4), which yielded polyalcohols as glycerol, erythritol and thritol in the molar ratio of 1.10 : 4.85 : 0.008 moles on paper chromatogram. The derivatives of polyalcohols were produced from seeds polysaccharide as glycerol tri O *p* nitrobenzoate and tetra O tosyl erythriotol. The absorbance of polyalcohols were recorded in photoelectrocolorimeter on 540 mμ for glycerol and erythritol.

Key words: Polyalcohols, glycerol, erythritol, thritol, *Strychnos nux vomica* seeds.

INTRODUCTION

Strychnos nux vomica Linn.[1,2] (Loganiaceae) plant is commonly known as Kuchla. It occurs in Northern India, Garhwal region particularly in F.R.I. Dehradun and other places like Gorakhpur forests, Orissa, U.P., M.P., A.P., Western Peninsula, Coromandel coast, Western coast, Mysore, Myanmar, Sri Lanka upto an altitude of 360 m in height. It is a middle sized tree upto 13 height and 0.9 1.8 m in girth, bark smooth, and seeds are grey, flat, shining nearly circular. The *nux vomica* alkaloids consists from seeds is a powerful poison in large doses, producing titanic convulsions and eventually death. In comparatively lesser doses it may result in mental derangement. In indiginous medicine, it is used as a tonic stimulant and febrifuge and its preparation are prescribed for nervous disorders. In Konkan, the small doses of seeds are given with aromatic in colic and in Cambodia seeds are used as an emetic. They are also used in the preparation of medicated products for hair and scalp. *Nux vomica* is an effective animal poison and used more as a poison then as a drug. It is also useful as an insecticide to kill vermie in fields. In South East Asian countries, tribal peoples are use the seeds in the preparation of arrow and dart poisons. Strychnine and brucine alkaloids are also occurs in seeds, roots, wood, bark, leaves, fruit pulp and hard fruit shells. There alkaloids are used in paralysis, fever and bites of venomous snakes. Leaves are employed for externally in paralysis and rheumatic swelling of joints and bark in the treatments of dysentery, diarrhoea, fevers, piles, leprosy, skin, spleen disease, leucodermatic. The flowers and fruits extracts are the rich sources of blue dyes. Juice of fresh wood is to be a popular remedy for dysentery, fevers, cholera and dyspepsia. In our earlier communications the nature of sugar are obtained from the water soluble seeds polysaccharides, methylation studies to obtained the methyl sugars for the determination of proposed polysaccharide structure and peridoate oxidation studies of seeds polysaccharide for the confirmation

of proposed polysaccharide structure of *Strychnos nux vomica* Linn. seeds. The present study mainly deals with the identification of polyalcohols by Smith degradation[3] studies of the peridoate oxidized seeds polysaccharide for the confirmation of proposed polysaccharide structure of *Strychnos nux vomica* Linn. seeds.

MATERIALS AND METHODS

Separation of products

The polyalcohol sugars were separated from periodate oxidized hydrolysed compounds by descending technique of paper chromatographic analysis[4] on Whatman No. 3 mm filter paper sheet. The following solvent mixture (v/v) were used as: (A) *n*-butanol, ethanol, water (4 : 1 : 5, upper phase)[5], and (B) ethyl acetate, pyridine, water (2 : 1 : 2 upper phase)[6] and used spray reagent as (R) acetonical silver nitrate, alcoholic sodium hydroxide[7] was applied for the detection of polyalcohols. All evaporations were carried out under reduced pressure at 45 50^{0}C.

Identification of polyalcohols by Smith degradation:

The purified *Strychnos nux vomica* Linn. seeds polysaccharide (1.5 gm) was oxidized[8] with sodium metaperiodate (0.125 M, 30 ml) for 72 hrs at 4 8^{0}C in refrigerator. It was further reduced[9] with sodium borohydride (1 gm) for 24 hrs at room temperature and excess periodate was removed by ethylene glycol (5 ml) to decompose the excess of periodate ions and reaction mixture was dialysed against running water for 48 hrs. It was concentrated to syrup and hydrolysed with H_2SO_4 (1 N, 100 ml) at 100^{0}C for 12 hrs. The obtained hydrolysate was neutralized with barium carbonate slurry, filtered and filtrate was deionised by passing through Amberlite Ion exchange resins[10], IR 120 (H^+) and IR 45(OH^-) then concentrated to a thin syrup.

Characterization of polyalcohols

The hydrolysed syrup of periodate oxidized seeds polysaccharide was resolved into its component by paper chromatographic separation method on Whatman No. 3 MM filter paper sheet in solvent mixture (A) and used (R) as spray reagent to revealed the presence of three spots of polyalcohols corresponding to the glycerol, erythritol and thritol. The component sugar strips were cut out with the help of guide spots and eluted with water according to the Dents method[11], which on evaporation gave glycerol, erythritol and thritol were characterized and identified as follows and reactions are shown in Fig. -1.

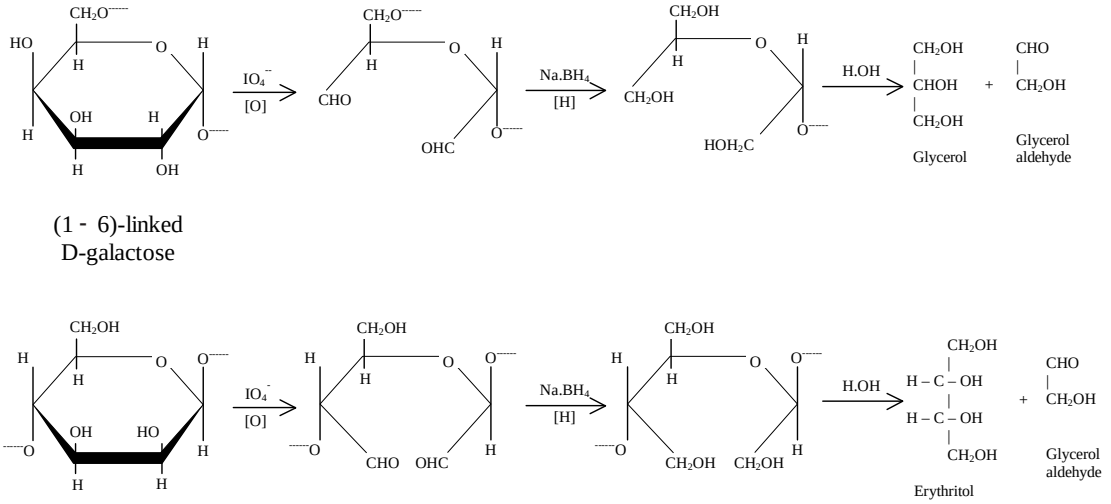

Fig. -1: Smith degradation of polyalcohols from *Strychnos nux-vomica* Linn. seeds polysaccharide

Fraction I: Glycerol

Sugar syrup (350 mg) was dissolved in ethanol (50 ml) and decolourised with aqueous solution of animal charcoal and then it filtered off. The filtrate was concentrated to a syrup and it moved a single spot on paper chromatogram corresponding to the authentic sample of glycerol. The residue (150 mg) was dissolved in pyridine (5 ml) and *p* nitrobenzoyl chloride (2.4 gm) then the content was heated for 1 hr at 70 75^{0}C. The reaction mixture was poured into ice cold solution of sodium bicarbonate to obtain a precipitate which was filtered off. The filtrate gave the crystals of glycerol tri O *p* nitrobenzoate derivative were obtained on cooling the reaction mixture, which were separated by filtration. It on recrystallisation with acetone, had m.p. and mixed m.p. 188 190^{0}C, Lit. m.p. 186 188^{0}C[12].

Fraction II: Erythritol

Syrup (850 mg) was treated with aqueous solution of animal charcoal, filtered and filtrate concentrated to a syrup. It moved a single spot on paper chromatogram corresponding to the erythritol. It was again dissolved in ethanol (5 ml), on cooling the crystals of erythritol was obtained after recrystallisation with ethanol and then filtered. It had m.p. and mixed m.p. 118 120^{0}C, Lit. m.p. 117 118^{0}C[12] and 120 122^{0}C[13].

The erythritol syrup (290 mg) was dissolved in the anhydrous pyridine (5 ml), *p* toluene sulphonyl chloride (1.5 gm), then the reaction mixture was left for 24 hrs at room temperature. The content was poured into ice cold water (50 ml) to crystallized out the needle shaped derivatives of erythritol. The erythritol crystals were washed with water followed by ethanol were dried in air. On recrytallisation with acetone and ethanol mixture gave tetra-O-tosyl-erythritol, had m.p. and mixed m.p. 144 -166^{0}C, Lit. m.p. 166-168°C[13].

Fraction III: Thritol

The sugar syrup (50 mg) moved as a single spot on paper chromatogram parallel to thritol. It was obtained in traces, which have *Rf* values more than the D galactose and D mannose was identified as thritol. The spot of thritol is visible only in ultraviolet light.

Quantitative estimation of polyalcohols:

The polyalcohols from *Strychnos nux vomica* Linn. seeds polysaccharide was quantitatively estimated by Chromotropic acid method[14]. The respective polyalcohols were separated by descending technique of paper chromatographic examination on Whatman No. 3 MM filter paper sheets in upper phase of the solvent mixture (B) and used (R) as spray reagent. The polyalcohol components were cut out with the help of guide spots and eluted with water according to the Dent's method[11], producing glycerol, erythritol and thritol in the molar ratio of 1.10 : 4.85 : 0.008. The colour intensity and absorbance were read at 540 mμ in photoelectrocolorimeter and results are given in Table -1 and plotted standard curves are shown in Fig. -2.

RESULTS AND DISCUSSION

Strychnos nux vomica Linn. seeds yielded a water soluble polysaccharide by usual manner as D galactose and D mannose in the molar ratio of 1 : 4. The purified oxidized polysaccharide was

Table - 1: Absorbance of polyalcohols from *Strychnos nux vomica* Linn. seeds polysaccharide at different concentrations

S.No.	Amount in micrograms		Klett reading (absorbance) at 540 mμ	
	Glycerol	Erythritol	Glycerol	Erythritol
1.	2.0	2.0	26	17
2.	4.0	4.0	51	35
3.	6.0	6.0	74	53
4.	8.0	8.0	98	70
5.	10.0	10.0	123	86

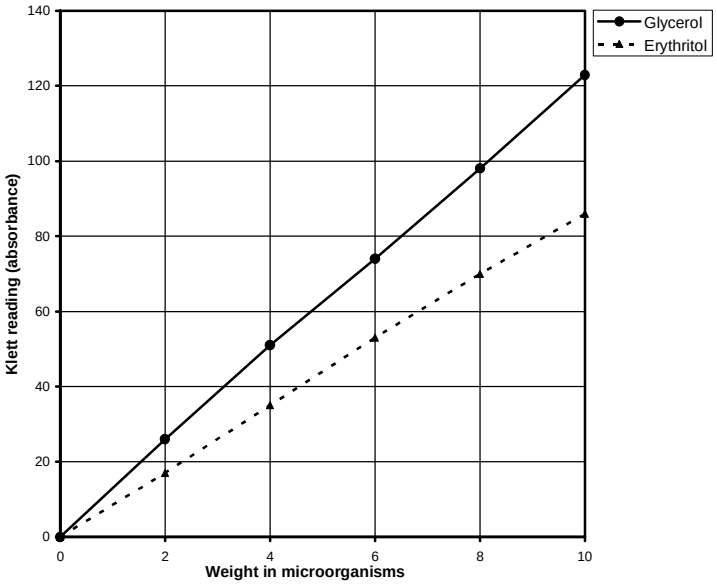

Fig. - 2: Standard curves for glycerol and erythritol of *Strychnos nux-vomica* Linn. seeds polysaccharide

reduced with sodium borohydride and sulphuric acid by Smith degradation method. It yielded polyalcohols as glycerol, erythritol and thritol in 1.10 : 4.85 : 0.008 molar ratio by paper chromatographic analysis. The large proportion of erythritol released with acid hydrolysis of polyalcohols produced by sodium borohydride serves as evidences that the main polymer linkages are of (1→4) ² type with D galactopyranose and D mannopyranose units. The ratio of erythriol to the amount of glycerol was obtained due to the presence of D galactose and D mannose at the non reducing end with (1→6)–α– type linkages in the main chain of the polysaccharide structure. It indicated three branching point on the average of 20 hexoses units in the main chain and side chain in polysaccharide structure. The derivative of glycerol was obtained by usual manner as glycerol tri O-*p*-nitrobenzoate while erythritol as tetra-O-tosyl erythritol. The absorbance of polyalcohols was recorded in photoelectrocolorimeter on 540 mm for glycerol and erythritol. It indicated three branching point on the average of 17 hexoses units are in the backbone and 3 hexoses units in the non reducing end for the support of the earlier proposed polysaccharide structure of water soluble *Strychnos nux vomica* Linn. seeds polysaccharide as shown in Fig. 3.

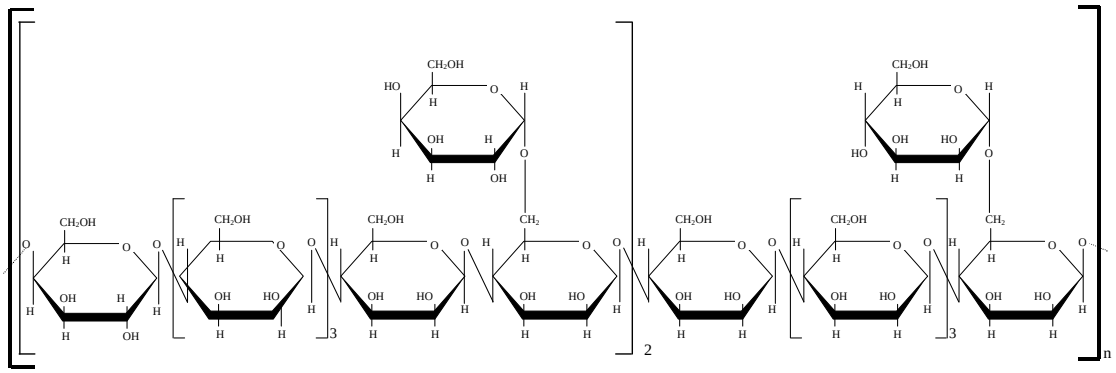

Fig. - 3: Polysaccharide structure of *Strychnos nux-vomica* Linn. seeds polysaccharide

REFERENCES

1. Drury, Colonel Heber. *The Useful Plants of India.* Allied Book Centre, Dehradun, India, 407 408 (1991).
2. Chadha, Y.R. *The Wealth of India, Raw Materials,* Publication and Information Directorate, CSIR, New Delhi, India, **10:** 62 66 (1976).
3. Smith, F. *J. Chem. Soc.,* **47:** 1035 1038 (1940).
4. Partridge, S.M. *Nature (London),* **158 :** 270 274 (1946).
5. Partridge, S.M. and Westall, R.G., *Biochem. J.,* **42 :** 238-241 (1948).
6. Jermyn, M.A. and Isherwood, F.A. *Biochem. J.,* **44:** 402-405 (1949).
7. Trevelyan, W.E., Procter, D.P. and Harrison, J.S. *Nature (London)*, *166* : 444 446 (1950).
8. Abdel, A.M., Hamilton, J.K., Montogomery, R. and Smith, F. *J. Amer. Chem. Soc*., **74 :** 4970 4973 (1952).
9. Morimoto, I.J.Y., Unrau, C.J. and Unrau, A.M. *J. Agri. Food. Chem*., **10 :** 134 136 (1960).
10. Adams, B.A. and Halmes, E.L. *J. Soc. Chem. Ind*., **54 :** 17 20 (1956).
11. Dent, C.E. *Biochem. J., 41* : 240 243 (1947).
12. Unrau, A.M. *J. Org. Chem*., **26 :** 3097 3101 (1961).
13. Hamilton, J.K. and Smith, F. *J. Amer. Chem. Soc.* **75 :** 5907 5910 (1956).
14. Lambert, M. and Neish, A. *Can. J. Res*., **28**B, 83 86 (1950).