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ANALYTICAL ASPECTS OF THE SEED POLYSACCHARIDE OF WRIGHTIA TINCTORIA R.Br (Roxb.)

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ABSTRACT

Structural elucidation of polysaccharide is one of the most complicated & time consuming operation in carbohydrate chemistry. The structure of Polysaccharide of this species has not been reported. Wrightia Tinctoria R.Br (Roxb) plant belongs to a family Apocynaceae and is a small deciduous tree growing in several part of India. The structure of Polysaccharide has not been reported in this species. The seed yield a deep red semi drying oil(yield-30.5% d^{24} 0.995) with fatty acid composition linoleic -38.8%,oleic -34.0%;myristic—0.1%,palmetic-8.7%,stearic -18.2% and arachidic-5.81%.The unsaponifiable matter(1.42%) consist mostly of sitosterol.The pods without seed contained beta sitosterol;alfa amyrins, ursolic and oleonelic acids. Paper chromatography analysis of hydrolysate on whatmann no. 3 filter paper sheets revealed the presence of glycerol ,erythritol & thritol.

It also showed the presence of D-galactose, D-mannose & 3 disaccharide & one trisaccharide .The mixture of monosaccharide and & oligosaccharide were separated on charcoal celite column chromatography employing the gradient elution method. The oligosaccharide were present not a single component but found a mixture of 4 oligosaccharide .Each Oligosaccharide fraction was separated on whatmann no. 3mm filter paper sheets and then purified , it obtained 5 oligosaccharide .out of them 3 disaccharide & one trisaccharide.

Keywords: WRIGHTIA TINCTORIA R.BR(ROXB.), APOCYNACEAE, PERIODATE OXIDATION, SMITH DEGRADATION, PARTIAL ACID HYDROLYSIS, ERYTHRITOL

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1.0 INTRODUCTION

The seed of Wrightia Tinctoria R.Br (Roxb.) belongs to family Apocyanceae .It occurs in particularly in Dehradun, Coastal forest of Coramandel, Mysore, konkan A.P, U.P, Bihar & many parts of India. It is known as Indrajau in Hindi. Plants are generally up to 1.8m tall & often under 60 cm in grits, sometimes up to 7.5 high. The bark is light grey, scaly, smooth leaves elliptic ovate or obviate –oblong, 7.5-12.5 cm long flower white fragrant terminal cymes, follicles in pair. Flowers are used as vegetables, they are slightly bitter. Leaves are a source of blue indigo called Mysore Pala indigo-0.33-0.50% and have total nitrogen -2% & calcium oxide 3.8% .Seeds are used as an adjuvant to dying material .The seeds , leaves & roots have been shown to contain an indigo-yielding glycosides .

2.0 MATERIAL & METHOD

2.1 PLANT MATERIAL

The seed of Wrightia Tinctoria R.Br (Roxb.) belongs to the family –Apocynaceae were collected in the month of September & February from Forest Research Institute Dehradun (Uttrakhand).

2.2 ISOLATION & PURIFICATION OF SEED POLYSACCHARIDE

The Polysaccharide was isolated from seed by extraction with cold distill water & precipitated with C_2H_5OH . The crude polysaccharide was obtained as grayish amorphous powder had sulphated ash 1.60%, optical rotation $[\alpha]_D^{25}$ $+31.2^\circ$ C (H_2O).The crude seed polysaccharide was purified by redissolving in H_2O & fractionation with ethanol to different concentration (20-60%) . The fractions obtained from the 40% & 60 % C_2H_5OH. Concentration were then titrated with absolute alcohol, acetone & ether (3 to 4 times) & then dried over calcium chloride under vacuum at 60°c.These two fractions (40 &60%) of polysaccharide showed the identical homogeneous spectrogram in IR spectrum (KBr),$[\alpha]^{25}_{D.+30^\circ C}$ (H_2O).sulphated ash 0.82%.

2.3 IDENTIFICATION OF SUGAR BY PAPER CHROMATOGRAPHY

The following columns were used in the column chromatography

(i) Cellulose Column

(ii) Charcoal –celite column

The following solvents (v/v) were used as eluants.

(i) N-Butanol –half saturated with water

(ii) Benzene –ethanol-water (167:17:15, upper layer)

(iii) 2.5%, 5.0%, 7.5%, 10.0%, 12.5% and15% aqueous alcoholic solution.

The sugar mixture has been identified by Paper Column Chromatography carried by descending technique on whatmann No. 1 filter paper sheet. It revealed the presence of D-galactose (RI-08) & D-mannose (Rf-0.24) and Identification was made on the basis of comparison with the corresponding data of authentic compound available in the literature

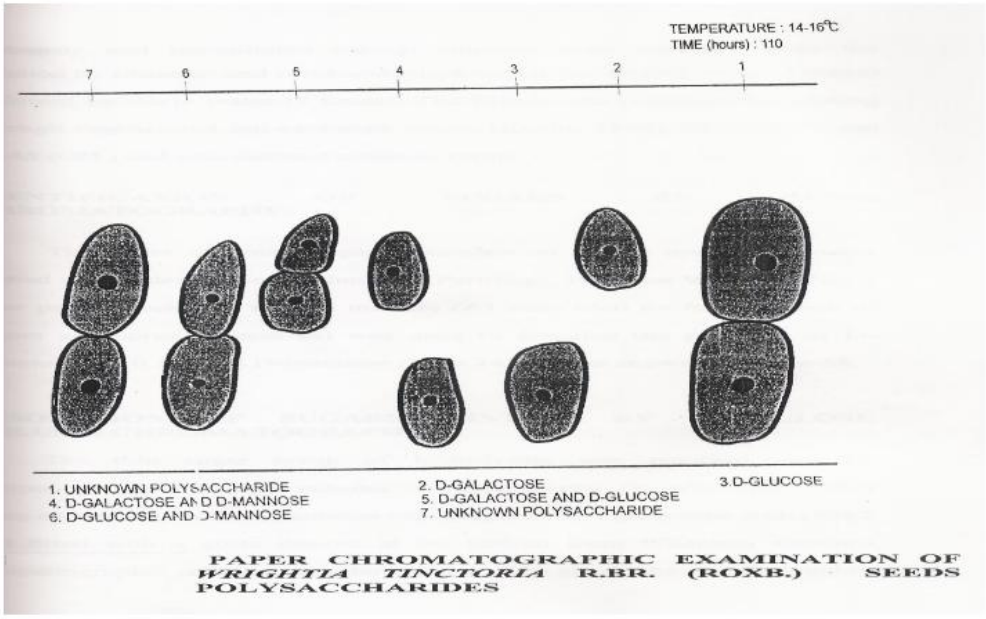


Table 1: Resolution of sugar mixture by cellulose column chromatography

S.no.	Fraction No.	Sugar present
1	01-42	No Sugar
2	43-61	D-Mannose only
3	62-84	Mixture of Dmannose & Dgalactose
4	85-109	D-Galactose only
5	110-onwards	No sugar

Table 2: Identification of sugar from wrightia tinctoria r.br(roxb.) seed polysaccharide

S.No.	Sugars	Melting point & Mixed Melting Point	$[\alpha]^{25}_{D\ H2O}$	Phenyl Hydrazone Derivative
1	D-Galactose	165-167^{0}c	+86.9^{0}c	169-170^{0}c
2	D-Mannose	131-133^{0}c	+12.6^{0}c	194-195^{0}c

2.5 METHYLATION STUDY

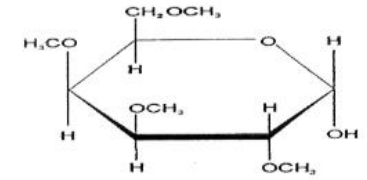
Wrightia Tinctoria R.BR.(Roxb.) seed polysaccharide was subjected to methylation by Haworth's method & Srivastava's method by 3 successive treatment with dimethyl sulphate & NaOH (45%) yielded a glassy yellow brown product. It exhibit slight absorption band of hydroxyl group, at 3500 cm^{-1} .region in IR spectrum. The fully methylated seed polysaccharide was fractionated with petroleum ether (40-60^{0}c) & $CHCl_3$. Mixture containing increasing proportion of latter which obtained seven fractions out of them 3 are oily & 4 are as crispy solids.

Table 2: Fractionation of wrightia tinctoria r.br(roxb.) methylated seed polysaccharide.

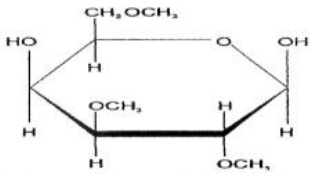
S.no.	State of methyl sugar	Solvent Composition		Yield gms	-OCH_3 (%)	$[\alpha]^{24}_D$ $CHCl_3$
		Pet Ether	$CHCl_3$			
1	Oily Liquid	100	00	0.2258	-	-
2	Oily Liquid	95	05	0.3624	-	-
3	Oily Liquid	90	10	0.4728	-	-
4	Crispy solid	85	15	0.7644	55.6	+73.6^0
5	Crispy solid	80	20	0.8458	40.8	+80.4^0
6	Crispy solid	75	25	0.2436	41.4	+15.6^0
7	Crispy solid	70	30	1.2546	29.6	+65.2°

Table 3: Identification of methylated sugar from wrightia tinctoria r.br(roxb.) seed polysaccharide.

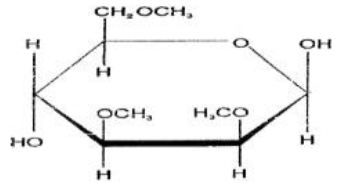
Fraction No.	Methylated sugar	$[\alpha]^{24}_D$ $CHCl_3$	$-OCH_3$ %	Demethylation Analysis	Crystalline derivative	Molar Ratio
1	2,3,4,6-tetra –o-methyl-D-galactose	$+73.6^0$c	54.6	D-Galactose	Anilide 191-193^{0}c	1.00
2	2,3,6-tri-o-methyl-D-galactose	$+80.4^0$c	40.8	D-Galactose	Anilide 175-177^{0}c	1.00
3	2,3,6-tri-o-methyl-D-Mannose	$+15.6^0$c	41.4	D-Mannose	Anilide 130-131^{0}c	5.00
4	2,3-di-o-methyl-D-Mannose	$+65.2^0$c	29.6	D-Mannose	Lactone 106-107^{0}c	1.00



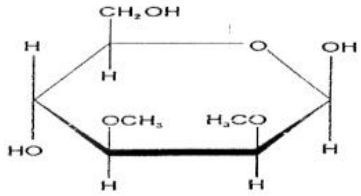
(I) 2,3,4,6-tetra-O-methyl-D-galactose



(II) 2,3,6-tri-O-methyl-D-galactose



(III) 2,3,6-tri-O-methyl-D-mannose

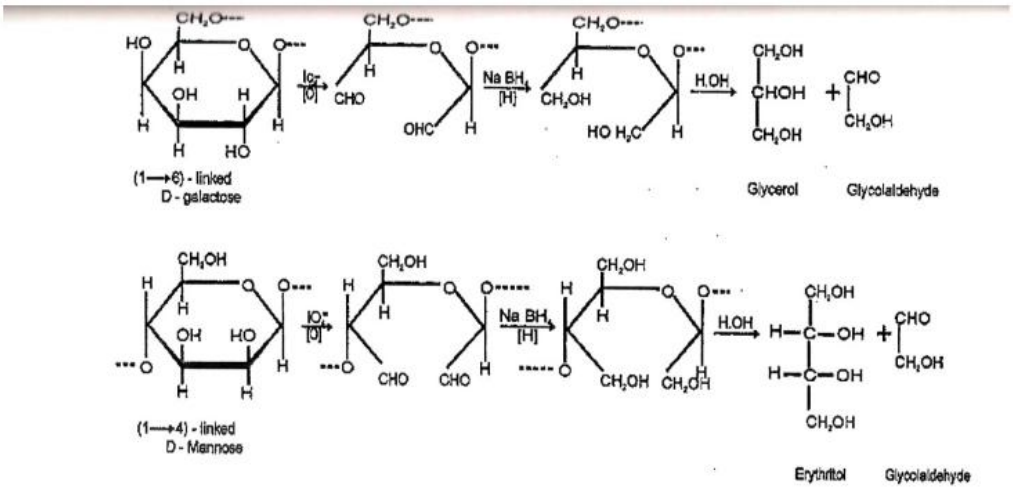


(IV) 2,3-di-O-methyl-D-mannose

METHYLATED SUGARS FRACTION OF *WRIGHTIA TINCTORIA* R.BR. (ROXB.) SEEDS POLYSACCHARIDE

SMITH DEGRADATION STUDY OF PERIODATE OXIDISED SEED POLYSACCHARIDE

Peroxidised seed polysaccharide was degraded by Smith degradation method. It was then reduced by $NaBH_4$ & hydrolysed with H_2SO_4 (IN) & then hydrolysate was neutralized with $BaCO_3$ slurry, filtered & filtrate was deionized by Amberlite Ion Exchange resins. The paper chromatographic analysis of hydrolysate on whatmann no. 3MM filter paper sheets revealed the presence of Glycerol, Erythritol, Thritol. The obtained polyalcohol fraction were purified, identified & estimated as follows.



SMITH DEGRADATION OF SUGAR UNITS OF *WRIGHTIA TINCTORIA* R.BR. (ROXB.) SEEDS POLYSACCHARIDE

(I) Glycerol (1.10 mole) glycerol-tri-o-p-nitrobenzoate, M.P & Mixed Melting Point 187-189^{0}C

(II) Erythritol (4.85 mole) tetra-o-tosyl-erythritol M.P& Mixed Melting Point 165-167^{0}C.

(III) Thritol(0.009 mole & traces) it moved as a single spot on Paper chromatography corresponding to thritol & spot is visible in UV light.

(IV)

(V) **ABSORBANCE FOR GLYCEROL & ERYTHRITOL AT DIFFERENT CONCENTRATION**

S.No.	Amount in Micrograms		Klett Reading(Absorbance) at 540 mμ	
1	2.0	2.0	28	18
2	4.0	4.0	54	37
3	6.0	6.0	77	55
4	8.0	8.0	100	73
5	10.0	10.0	126	89

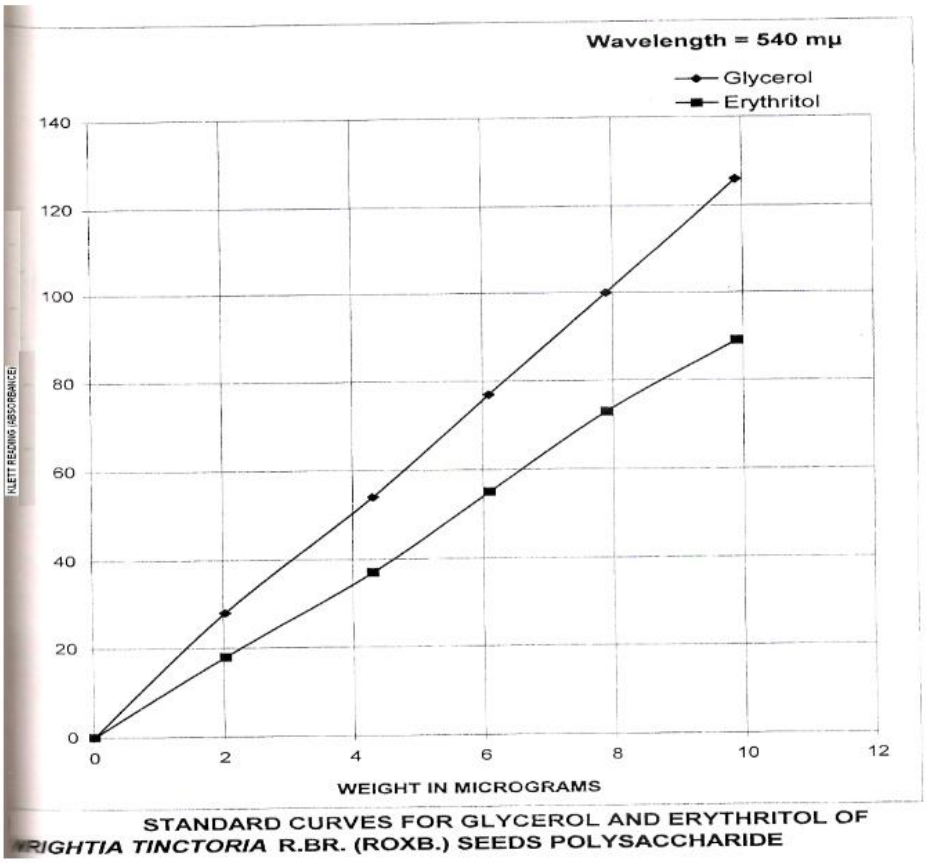


Fig: Standard curves for glycerol and erythriol of Wrightia Tinctora R.BR. (ROXB.) seed polysaccharide

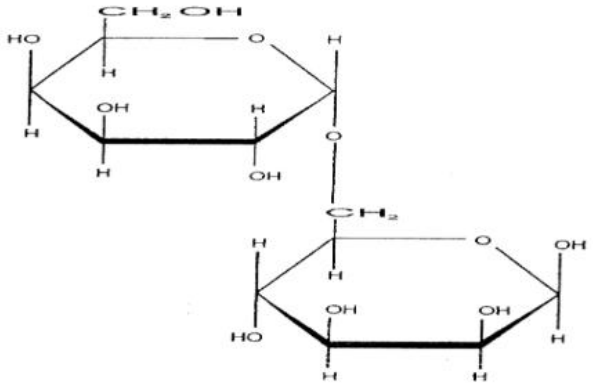
PARTIAL ACID HYDROLYSIS STUDIES OF WRIGHTIA TINCTORIA R.Br(Roxb.) SEED POLYSACCHARIDE

Partial acid hydrolysis of purified seed polysaccharide of Wrightia Tinctoria R.Br (Roxb.) with H_2SO_4 (1.5N) under suitable conditions were obtained after a number of pilot experiments gave a mixture of Oligosaccharides & Monosaccharide.

Paper Chromatographic examination of hydrolysate showed the presence of D-galactose ,D-mannose & 3 disaccharide &1 trisaccharide .Mixture of monosaccharide & oligosaccharide were separated on charcoal celite column chromatography employing gradient elution method. Each Oligosaccharide fraction were separated on whatmann no. 3mm filter paper sheet and then purified ,it obtained 5 oligosaccharide out of them 3 disaccharide & one trisaccharide .The oligosaccharide were identified on the basis of their composition ,chromatographic behaviour, specific rotation, degree of polymerization ;Acid hydrolysis & Per Iodate Oxidation. The obtained Oligosaccharide were characterized & identified as

OLIGOSACCHARIDE-I

The oligosaccharide (I) was obtained in crystalline form, had Melting point 203-205^{0}c, Lit M.p 200-202^{0}c.Optical rotation $[\alpha]^{24}_D$ +123.5^{0}c (H_2O) ,Lit $[\alpha]_D$+123-125^{0}C (H_2O)



O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose

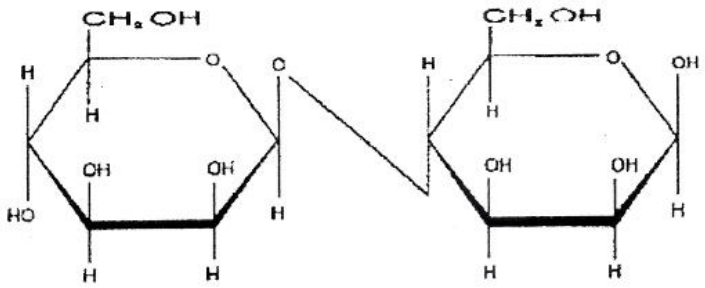
STRUCTURE OF OLIGOSACCHARIDE (A) OBTAINED FROM *WRIGHTIA TINCTORIA*

The degree of polymerization found to be 1.79.Acid hydrolysis of oligosaccharide (I) showed the presence of D-galactose & D-mannose inequimolar proportion .On treatment with phenyl hydrazine yielded osazone derivative having m.p 176^{0}c. .It showed that the

low rotation of disaccharide indicate that biose linkage are of (16) α type. On the basis of above result the disaccharide has been identified as O-α-galactopyranosyl- (16)-O-α-D-mannopyaranose.

This Oligosaccharide was found in the form of white amorphous powder and had optical rotation $[\alpha]^{24}_{D}$ -130^{0}C(H_2O),Lit$[\alpha]_{D}$-131^{0}C(H_2O) & $[\alpha]^{24}_{D\text{-}21.0^{0}C}$ (C_2H_5OH),R_{gal} 0.57 in solvent (e) & R_{glu} 0.43 .

OLIGOSACCHARIDE (II):



O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose

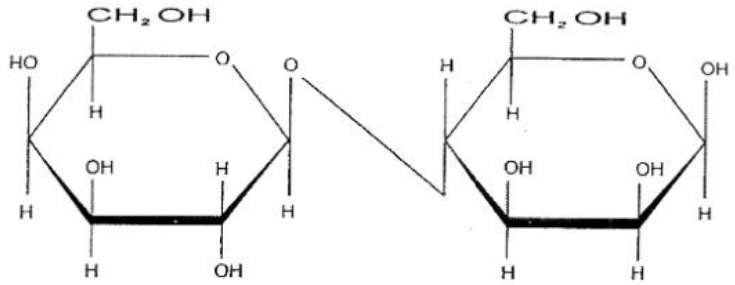
STRUCTURE OF OLIGOSACCHARIDE (B) OBTAINED FROM *WRIGHTIA TINCTORIA* R.BR. (ROXB.) SEEDS

Degree of Polymerization was found to be 2.20 as determined by Timell's method corresponding to disaccharide .Acid hydrolysis of Oligosaccharide (II) gave D-mannose only. It was converted to crystalline derivative as O-β-D mannopyranosyl-Dmannopyaranose-octa-acetate having m.p & mixed m.p 151-152^{0}C& Lit m.p-152-153^{0}C.The low rotation of disaccharide indicate that biose linkage was (1 4)β type linkage on the above basis oligosaccharide has been identified as O-β-D-Mannopyranosyl-(14)-O-β-d mannopyranose

OLIGOSACCHARIDE (III)

The Oligosaccharide fraction was obtained in the form of crystalline, had $[\alpha]^{24}_{D}$ +16.5^{0}C(H_2O),Lit$[\alpha]_{D}$-17^{0}C(H_2O).The degree of polymerization was found to be 1.90 corresponding to disaccharide .Acid hydrolysis revealed the presence of D-galactose & D-

Mannose in equal amount by paper chromatography.



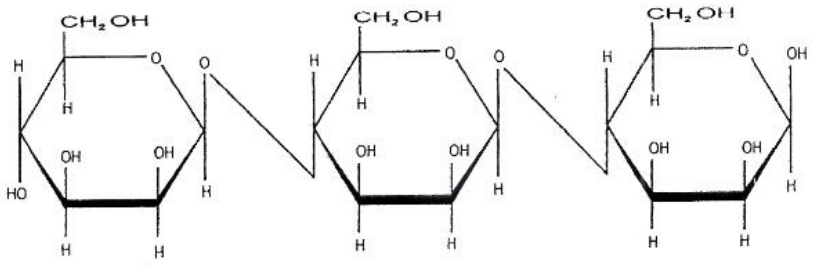
O-β-D-galactopyranosyl-(1→4)-O-β-D-mannopyranose

STRUCTURE OF OLIGOSACCHARIDE (C) OBTAINED FROM *WRIGHTIA TINCTORIA* R.BR. (ROXB.) SEEDS

The Phenyl Hydrazone derivative was prepared by usual manner had m.p & mixed m.p 192-194^{0}c,Lit m.p194-195^{0}c.Thus it has been identified as O-β-D-galactopyranosyl-(14)-O-β-D- mannopyranose

OLIGOSACCHARIDE (IV)

The Oligosaccharide was chromatographically purified, having R_{gal} 0.31 in & $R_{glu\ 0.18}$ $[\alpha]^{24}_D$ -21^{0}C(H_2O),Lit$[\alpha]_D$-22^{0}C having m.p 169^{0}c-170^{0}c.Lit m.p 169. ^{0}c .The degree of polymerization of Oligosaccharide was found to be 3.10 determined by Timell's method to approve that this oligosaccharide is a Trisaccharide .Acid Hydrolysis of trisaccharide with usual manner revealed the presence of D-Mannose .Partial acid hydrolysis of trisaccharide (IV) revealed the presence of O-β-D-mannopyranosyl-(14)-O-β-D- annopyranosyl(1 4)-O-β-D- mannopyranose.

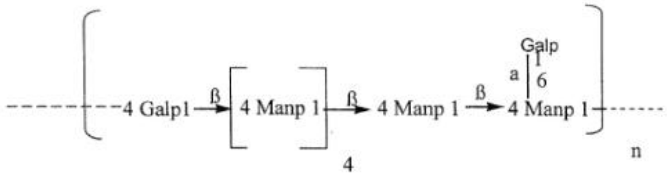


O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose

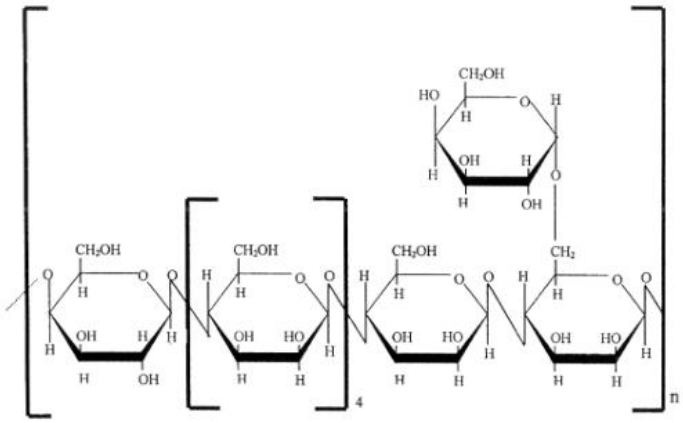
STRUCTURE OF OLIGOSACCHARIDE (D) OBTAINED FROM *WRIGHTIA TINCTORIA* R.BR. (ROXB.) SEEDS POLYSACCHARIDE

4.0 RESULT & DISCUSSION

The proposed Polysaccharide structure was supported by Per iodate oxidation studies showed the liberation of 0.1452 moles of formic acid for each anhydrhexose unit with consumption of 1.848 moles of periodate for rich anhydrohexose unit of polymer after 75 hrs at 4-8^{0}C in refrigerator. The Oxogalactomanan was degraded by smith degradation method & it on reduction with sodium boro hydride gave a mixture of glycerol, Erythritol, Thritol. The molar ratio of polyalcohol was found to be 1.20:4.95:0.009.The molar ratio of erythritol to that of amount of glycerol indicate one branching point on average after every 8 hexose unit main chain. The result is also in fair agreement with result of periodate oxidation & methylation studies within the limit of experimental error. The above finding result indicate that the branching point on average of seven hexose units are the backbone & one hexose unit are in the non reducing end for the support below proposed polysaccharide structure of Wrightia tinctoria R.Br(Roxb.)



Where Galp=D-galactopyranose, Manp= D-mannopyranose



POLYSACCHARIDE STRUCTURE FROM *WRIGHTIA TINCTORIA* R.BR. (ROXB.) SEEDS GALACTOMANNAN

Purified seed Polysaccharide upon partial acid hydrolysis followed by charcoal celite column chromatography & paper chromatography separation on whatmann no. 3MM filter paper sheet obtained hydrolysate afforded 3 disaccharide & one trisaccharide in authentic form. So on the basis of the above studies i.e. Periodate Oxidation, Smith Degradation, Optical Rotation &Partial acid hydrolysis we led to the conformation of proposed structure of Wrightia tinctoria R.Br(Roxb.)

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