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Periodate oxidation studies of seeds polysaccharide from *Wrightia tinctoria* R.Br. (Roxb.) plant

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ABSTRACT

Periodate oxidation is one of the most important reactions which is used in the structural study of non-ionic seeds polysaccharides in carbohydrate chemistry. Periodate oxidation was done using sodium metaperiodate as oxidant and it was proposed by Fluery & Lange's method. The mole of periodate consumed during periodate oxidation reaction of seeds polysaccharide of Wrightia tinctoria R.Br. (Roxb.) was determined volumetrically. The composition and probable polysaccharide structure have also been elucidated with the information obtained from the periodate oxidation on purified fraction of seeds polysaccharide. It consumed 1.848 moles of oxidation and liberated 0.1452 moles of formic acid per mole of anhydrohexose sugar unit after 75 hrs.

Keywords: Periodate oxidation, formic acid liberation, periodate consumption, *Wrightia tinctoria* seeds polysaccharide

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INTRODUCTION

Wrightia tinctoria R.Br. (Roxb.) plant [1] belongs to the family- Apocynaceae and commonly called as *Indrajau.* It occurs in Northern India particularly in Garhwal region and others places in India, Australia, Malaysia, Thailand, Nepal, Myanmar, Sri Lanka. It is an evergreen medicinal deciduous tree usually 1.8-7.5 m in height and 60 cm in girth. Powdered seeds contain a water soluble sugar extract [2] as D-galactose and D-mannose in 1:3 molar ratio by GLC, TLC, Column and Paper chromatographic analysis. Isolation, purification and preliminary analysis [2] and methylation studies [3] of seeds polysaccharide have already been studied for the determination of proposed polysaccharide structure. Present manuscript mainly deals with the periodate oxidation in the purified seeds polysaccharide. The reaction of periodate oxidation was first discovered by Malaprade [4]. Fluery & Lange [5] have given a better method for more extensive use of periodic acid for the oxidation of glycol. Perlin [6] have given two important reagents are periodic acid and lead tetra acetate which showed that the glycol groups undergo cyclic ester formation with oxidation and reaction considered to be dialdehyde type of oxidation. While Chatterjee [7], Kumar [8] and Sarkar [9] have used periodate oxidation to determine the polysaccharide structure. The central atom of the oxidation reagent must be able to coordinate at least two hydroxyl group.

MATERIALS AND METHODS

The purified seeds polysaccharide (0.25 gm) of *Wrightia tinctoria* R.Br. (Roxb.) was oxidized with distilled water (50 ml) and sodium metaperiodate solution (0.125 M, 100 ml) then the volume of the reaction flask was made upto 250 ml with distilled water. The reaction flask was kept in refrigerator for 48 hrs at 4-8^{0}C. Aliquot (5 ml) was pipetted out from reaction mixture at different intervals of time to determine the amount of periodate consumption and formic acid liberation [10].

Determination of periodate consumption :

Reaction mixture (5 ml) was pipetted out from reaction flask then added standard sodium bicarbonate solution (2 ml), sodium arsenite solution (0.01 N, 25 ml) and potassium iodide solution (20 %, 2 ml). The reaction mixture was shaken for 30 minutes and added iodine solution (0.01 N, 5 ml) then add boric acid (2 gm). The excess iodine was titrated against sodium thiosulphate (hypo) solution (0.1 N), using starch as an indicator near the end point. A blank titration was also carried out in a similar way. The difference between blank and experiment gives the periodate consumption of 1.848 moles after 75 hrs and results are given in Table- 1.

Table- 1: Periodate consumption of polysaccharide per anhydrohexose sugar unit

S. No.	Time of oxidation (hrs)	Vol. of hypo used with blank (ml) V_2	Vol. of hypo used with sample (ml) V_1	Vol. of hypo used V_2-V_1 (ml)	Moles of periodate consumed per anhydrohexose sugar unit (moles)
1.	10	10.70	9.90	0.80	1.056
2.	20	10.70	9.75	0.95	1.254
3.	30	10.70	9.65	1.05	1.386
4.	40	10.70	9.55	1.15	1.518
5.	50	10.70	9.45	1.25	1.650
6.	60	10.70	9.35	1.35	1.782
7.	65	10.70	9.30	1.40	1.848
8.	70	10.70	9.30	1.40	1.848
9.	75	10.70	9.30	1.40	1.848

The moles of periodate consumption of per anhydrohexose sugar unit are derived by the following equation :

Moles of periodate consumption per anhydrohexose sugar unit = $\frac{N(V_2-V_1) \times 132 \times A.F.}{1000 \times w \times 2}$

Where:

0.1 N = N = Normality of hypo solution

V_2 = Volume of hypo used with blank

V_1 = Volume of hypo used with sample

0.25 g = w = Weight of polysaccharide

50 = A.F. = Aliquot Factor = $\frac{\text{Total volume of periodate taken}}{\text{Periodate volume withdrwan for each titration}}$

$= \frac{250}{5} = 50$

Moles of periodate consumed per anhydrohexose unit = $\frac{0.1\,(10.70 - 9.30) \times 132 \times 50}{1000 \times 0.25 \times 2}$

$= \frac{924}{500}$

= 1.848 moles

Determination of formic acid liberation :

The formic acid released was carried out by Halsall and Coworkers [11] and Brown [12]. Aliquot about 5 ml were taken out from each flask and ethylene glycol (10 ml) was added to destroy the excess of periodate present in the reaction mixture for 45 min. The formic acid produced was estimated by titration against sodium hydroxide solution (0.1 N) and using methyl red dye as an indicator near the end point. A blank titration was also carried out in a similar way. It liberated 0.1452 moles of formic acid per mole of anhydrohexose sugar unit after 75 hrs and results are given in Table-2.

Table-2 : Determination of formic acid released during periodate oxidation

S. No.	Time of oxidation	Time of alkali used with blank V_2 (ml)	Vol. of alkali used with sample V_1 (ml)	Vol. of alkali used with V_2-V_1 (ml)	Formic acid released/100 gm of polysaccharide sample (ml)
1.	10	10.20	9.75	0.45	0.0594
2.	20	10.20	9.60	0.60	0.0792
3.	30	10.20	9.45	0.75	0.0990
4.	40	10.20	9.35	0.85	0.1122
5.	50	10.20	9.25	0.95	0.1254
6.	60	10.20	9.15	0.05	0.1386
7.	65	10.20	9.10	1.10	0.1452
8.	70	10.20	9.10	1.10	0.1452
9.	75	10.20	9.10	1.10	0.1452

Formic acid released per anhydrohexose sugar units = $\frac{N(V2 - V1) \times 132 \times A.F.}{100 \times w \times 2 \times 100}$

$$= \frac{0.10 \times 1.10 \times 132 \times 50}{100 \times 0.25 \times 2 \times 100}$$

$$= \frac{726}{5000}$$

= 0.1452 moles

Reaction of periodate oxidation of seeds polysaccharide :

The periodate oxidation study showed that the consumption of 1.848 moles of periodate ions per anhydrohexose sugar units as determined volumetrically. The probable reaction by which the consumption of seeds polysaccharide (galactomannan) are occurs. Reaction showed that the 90 % of D-galactopyranose units were containing adjacent free hydroxyl groups, resulting in the consumption ranges of periodate ions during periodate oxidation reaction. It is concluded from the above mentioned facts that probably one branch point occurs per 8 repeating unit of the galactomannan. The consumption of moles of periodate increases from 1.056 to 1.848 but from 65-75 hrs, the consumption of moles of periodate became constant (1.848 moles).

The formic acid appeared is to be originating from reducing as well as non-reducing terminal units of D-galactopyranose units. It may be concluded that the terminal D-galactopyranose units of the seeds polysaccharide are not substituted from the above reaction. It liberated 0.1452 moles of formic acid per anhydrohexose sugar units after 75 hours. Amount of released formic acid increases from 0.0594 to 0.1452 moles but from 65-75 hrs. The amount of released formic acid becomes constant (0.1452 moles).

RESULTS AND DISCUSSION

Wrightia tinctoria R.Br. (Roxb.) seeds polysaccharide was oxidized with sodium metaperiodate by usual manner. The periodate oxidized polysaccharide was liberated 0.1452 moles of formic acid per equivalent of polysaccharide with concomitant consumption of 1.848 moles of periodate for each anhydrohexose sugar units of the polymer after 75 hrs. The presence of (1→4)-β-type and (1→6)-α-type linkages are also confirmed by the free hydroxyl groups resulting in the consumption of periodate ions during periodate oxidation reaction. It is concluded from the above facts that the probably one branching point are occurs 8 repeating units of the galactomannan in the polysaccharide structure (Figure-1). The formic acid appears is to be originating from the reducing as well as non-reducing terminal units of the D-galactopyranose sugar units.

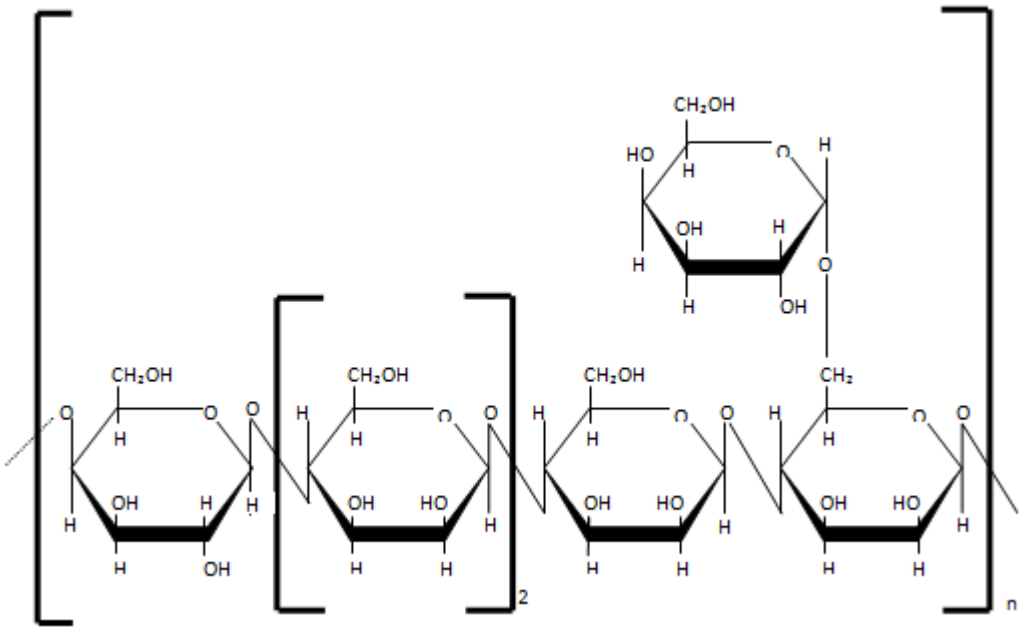

FIGURE- 1: Polysaccharide structure from *Wrightia tinctoria* R.BR. (Roxb.) seeds galactomannan

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