Methylation study of Hemicelluloses of *Morus nigra*

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ABSTRACT

The Methylation of purified non-cellulosic polysaccharide was done by Partial and Drastic methylation. The Partial methylation of hemicelluloses was carried out using improved Haworth method and Drastic methylation by Purdic method. The completely methylated products of hemicelluloses were hydrolysed into their constituents methylated sugar components using methanolic hydrogen chloride. The RG value and Molar ratio of methylated hydrolysates also determined volumetrically as well as by paper chromatographic technique. Various data obtained from the above investigations made it possible to determine the chemical nature and structure of hemicellulose.

Key words: Non-cellulosic polysaccharide, Partial methylation, Drastic methylation and Molar ratio.

INTRODUCTION

The methyl derivatives of wood carbohydrates¹ were considered to be the most suitable derivative for characterization, because of their relatively better stability towards alkali and acids. Determination of structural configuration specially the mode of linkage of the sugar units in the chain, the technique is very useful and important tool for investigation.

At first, completely methylated sugars were prepared by treating a methanolic solution of carbohydrate with methyl iodide and silver oxide by Purdic and Irvine². This method was observed to be restricted only to non-reducing derivatives like glycosides, due to the fact that silver oxide, oxidizes reducing sugars to the ester of carboxylic acids. The use of methanol in this procedure was also found to be high. The above mentioned drawback in the procedure was overcome by Haworth³, who discovered that more satisfactory initial methylation could be done in an aqueous solution with less expensive dimethyl sulphate and 30% of aqueous sodium hydroxide. Reducing as well as non-reducing sugars can be methylated when temperature is mentioned low and excess of alkali is avoided at initial stage.

Various improvements have been introduced in Haworth’s and Purdic’s method to suit the methylation of a particular derivative, Menzies⁴ has introduced Thallous hydroxide and methyl iodide for the methylation of non-reducing sugars; Glen⁵ introduced acetone as solvent with dimethyl sulphate and sodium hydroxide as methylation reagents; Falconer used tetrahydrofuran as solvent system during methylation; Tristler⁶ used sodium metal and methyl iodide and Kuhn⁷ introduced barium hydroxide with methyl iodide and N,N-dimethyl formamide as solvent for complete methylation of carbohydrates. Kerrer⁸ and Irvine – Macdonald⁹ gave the procedure of methylation of starch. Shrivastava, *et. al.*¹⁰ have modified the Haworth’s procedure by using dimethyl sulphate and solid sodium hydroxide for methylation of starch in
dimethyl sulphoxide solvent system, the methylation reaction system was kept strictly anhydrous. The yield of methoxyl content of the starch was forty three percent in six repeated treatments.

The complete methylation study of analysis of polysaccharide is usually performed with dilute hydrochloric acid and methanolysis is performed with about four percent methanol. The paper chromatography generally employed for quantitative separation of monosaccharides derivatives, obtained from the hydrolysis of methylated polysaccharides and for the separation of micro amount of sugar units which are identified by RG value, viz the ratio of the distance travelled by the sugar to that travelled by 2,3,4,6-tetra-O-methyl-D-glucose. In this way it is possible to propose the structure of polysaccharide.

MATERIAL AND METHODS

First step
The partial methylation of purified hemicellulose fraction was carried out by its treatment with dimethyl sulphate, potassium hydroxide, 10 N sulphuric acid, than washed with chloroform. The chloroform extracted solution was dried over anhydrous sodium sulphate. In this way partially methylated hemicellulose is obtained. For complete methylation of partially methylated hemicellulose, the Purdic procedure was used.

Second step
The methanolysis of complete hemicelluloses was carried out by its treatment with chloroform & petroleum and than by 4% methanolic hydrogen chloride. Than reaction solution was neutralized with silver carbonate and hydrogen sulphide. The small quantity of barium hydroxide was added into the filterate, and CO₂ gas passed through the filterate solution. The filterate was than treated with 10 ml of methanol. The precipitates so obtained washed with methanol. Thus it was preserved for the identification of acidic component and final filterate was further concentrated.

Third step
Determination of RG value
The concentrate semi solid filterate solution obtained from second step as a reference sugar 2,3,4,6-tetra-O-methyl-D-glucose was spotted on three Whatman Chromatographic Paper No.1. It leaves various spots on the papers. The RG values calculated by this procedure were recorded in Table 1. and graphically represented in Fig.1.

Determination of Molar ratio
The sugar solutions containing about 1.5 mg of methylated sugars were spotted using a fine capillary on a Whatman Paper No.1. Two drops of the same sugar were also spotted on the margin of the strips. The methylated sugar samples were allowed to run for about 16 hours using solvent system Ethanol: Benzene: Water and maintained the temperature at 30°C. Then strips were dried and sprayed with aniline hydrogen phthalate reagent and kept it over a temperature 105°C for 10 minutes. The spots were formed on the paper. Each individual methylated sugar extracted with 10 ml of distilled water. The iodine solution was added to each extract till 10.5 pH, the iodine liberated was treated with hypo solution and end point was determined with the help of starch indicator. Side by side a blank determination was also carried out in the similar way and the Molar ratios of methylated sugars are recorded in Table 2. and represented graphically in Fig. 2.

Table 1: RG values of methylated sugar units hydrolysate of Morus nigra

<table>
<thead>
<tr>
<th>Components</th>
<th>RG values</th>
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<tbody>
<tr>
<td>3-O-methyl-D-xylose</td>
<td>0.49</td>
</tr>
<tr>
<td>2,3-di-O-methyl-D-xylose</td>
<td>0.76</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-xylose</td>
<td>0.92</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-glucose</td>
<td>0.83</td>
</tr>
<tr>
<td>4-O-methyl-D-glucose</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Table 2: Molar ratio of methylated sugar units hydrolysates of Morus nigra

<table>
<thead>
<tr>
<th>Components</th>
<th>Molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-O-methyl-D-xylose</td>
<td>3.4</td>
</tr>
<tr>
<td>2,3-di-O-methyl-D-xylose</td>
<td>172.4</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-xylose</td>
<td>1.05</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-glucose</td>
<td>15.7</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The hydrolysate of methylated hemicelluloses obtained by the methylation of purified hemicelluloses, the RG values which are obtained with the help of paper chromatography, from it the sugar components can be identified. From results and datas of Table 1, the value obtained for Morus nigra is 0.49, it indicates the component 3-O-methyl-D-glucose. The RG value 0.76 confirms the presence of 2,3-di-O-methyl-D-xylose. The RG value 0.92 identifies 2,3,4-tri-O-methyl-D-xylose. The RG value 0.83 identifies 2,3,4-tri-O-methyl-D-glucose and the value 0.16 identifies 4-O-methyl-D-glucose components. From the values of Molar ratios of methylated hemicellulose hydrolysates as shown in Table 2, it is being concluded that in the methylated hemicellulose fraction of Morus nigra contains 3.4 units of 2,3,4-tri-O-methyl-D-xylose. It is also observed that methylated hemicellulose contains 15.7 units of 2,3,4-tri-O-methyl-D-glucose and 172.4 units of 2,3-di-O-methyl-D-xylose. Similar observation and results were reported by Harmov11, Yamada12 and Ohmiya13.

CONCLUSION

From the discussion of results obtained it can be concluded that the complete Methylation and the values of Molar ratio indicates that 2,4,4 tri-O-methyl-D-glucose may be obtained from glucuronic acid. It is also evident from the results that the plant contains sufficient quantity of 2,3-di-O-methyl-D-xylose in hemicellulose and that the 1,4-linkage between xylopyranose units build up the backbone of hemicellulose.

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