

## Demethylation and Degradation of Sugars in Acid Hydrolysis

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The magnitude of demethylation and degradation of some methylated sugars during conditions of acid hydrolysis has been studied. The conditions were typical for various procedures used for the hydrolysis of methylated polysaccharides. The investigation thus throws some light upon possible sources of error when the structure of a polysaccharide is studied by the methylation technique.

The methylation technique involving the complete methylation of a carbohydrate, hydrolysis and identification and quantitative determination of the methylated sugar obtained is an important method in structural carbohydrate chemistry. A prerequisite for the correct interpretation of the results especially for polysaccharides, which may give mixtures of several methylated sugars, is that the methylation is complete and that the methylated sugars are not degraded or demethylated during the acid hydrolysis of the methylated polysaccharide. Although some polysaccharides are difficult to methylate completely it is usually possible to obtain a product with a methoxyl content rather close to the theoretical value. No systematic studies of the demethylation and degradation of methylated sugars under the conditions used in acid hydrolysis seem to have been made, but some observations have been recorded in the literature.

Freudenberg and Boppel<sup>1</sup> studied the demethylation of 2,3,6-tri-*O*-methyl-D-glucose on treatment with 36 % aqueous hydrochloric acid at +5°, the conditions used for the hydrolysis of methylated starch, and found that 2 % or more of di-*O*-methyl-D-glucoses was formed. They believed that one of these, presumably the 2,6- and definitely not the 2,3-derivative, was formed preferentially. Chanda *et al.*<sup>2</sup> studied the demethylation of 2,3-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-xylose on treatment with 1 % methanolic hydrogen chloride and found 0.2 and 2 % demethylation, respectively. Croon and Lindberg<sup>3</sup> observed that the treatment of seven partially methylated glucoses, first with 72 % sulphuric acid at 0° and then with 12 % sulphuric acid at 100° did not produce any apparent demethylation but that the sugars showed between 3 and 6 % degradation. In a recent publication, Goldstein

Table 1. Acid hydrolysis conditions.

| Conditions | Acid                     | Temp.      | Time  |
|------------|--------------------------|------------|-------|
| 1          | 2 % HCl in water         | 100°       | 13 h  |
| 2          | a) 2 % HCl in methanol   | 100°       | 13 h  |
|            | b) 0.5 N HCl in water    | 100°       | 8 h   |
| 3          | a) 72 % sulphuric acid   | Room temp. | 0.5 h |
|            | b) 12.4 % sulphuric acid | 100°       | 4 h   |
| 4          | a) 98 % formic acid      | 100°       | 6 h   |
|            | b) 0.5 N sulphuric acid  | 100°       | 14 h  |

Table 2. Degradation of methylated sugars under hydrolytic conditions.

| Conditions used | 2,3-Di-O-Me-xylose | 2,3-Di-O-Me-glucose | 2,3,6-Tri-O-Me-glucose |
|-----------------|--------------------|---------------------|------------------------|
| 1               | 6–7 %              | 8 %                 | 7 %                    |
| 2               | 1–3 %              | —                   | 7 %                    |
| 3               | 2–4 %              | 7 %                 | 5 %                    |
| 4               | ~ 20 %             | 23 %                | 17 %                   |

Table 3. Demethylation during acid hydrolysis, expressed as mole % of ethers formed having a lower degree of methylation.

| Substance          | Conditions           | Demethylation |
|--------------------|----------------------|---------------|
| 2,3-X <sup>a</sup> | 1                    | 2.1           |
| »                  | 2 <sup>b</sup>       | 1.7           |
| »                  | 3                    | 0.6           |
| »                  | 4                    | Traces        |
| 2,3-G              | 4 % aq HCl 100, 12 h | 2.5           |
| 2,3,6-G            | 2                    | 0.9           |

a) 2,3-X = 2,3-Di-O-methyl-D-xylose, 2,3-G = 2,3-Di-O-methyl-D-glucose and so on.

b) The methanolic HCl was heated for 13 h under reflux and not in a sealed tube.

*et al.*<sup>4</sup> report that 1,4-di-O-methylerythritol was demethylated to 1.3 % when refluxed with 3 % methanolic hydrogen chloride for 18 h.

The present paper is a report of studies on the degradation and demethylation of 2,3-di-O-methyl-D-xylose, 2,3-di-O-methyl-D-glucose and 2,3,6-tri-O-methyl-D-glucose under different conditions. The conditions used are listed in Table 1; they were selected as representative of those various procedures used in the hydrolysis of methylated polysaccharides.

The extent of degradation was determined by measuring the decrease in optical rotation after the hydrolytic treatment. This method is subject to serious limitations as some reversion might occur and small mechanical losses

are not excluded. The observed effects were generally rather small and the values in Table 2 should be regarded as highly approximate. They do, however, allow a comparison of the different methods of hydrolysis.

Formolysis in 98 % formic acid involves severe degradation, and the reaction solutions become very dark. The use of 90 % formic acid is often reported and is probably less harmful; Isbell *et al.*<sup>5</sup> report good results with this mixture. In the other treatments the degradation is less, usually of the same order of magnitude as that observed for the free sugars during the total hydrolysis of polysaccharide material.

In order to study the demethylation the products obtained after hydrolytic treatment were fractionated by carbon column chromatography and the lower ethers isolated were characterised by paper chromatography and paper electrophoresis. The percentages of lower methylated products obtained after different treatments are given in Table 3. These values also are probably not very accurate, but they give the correct order of magnitude. Comparable amounts of 2- and 3-*O*-methyl-D-xylose were formed during the demethylation of 2,3-di-*O*-methyl-D-xylose. Similarly, comparable amounts of 2- and 3-*O*-methyl-D-glucose and of 2,3-, 2,6- and 3,6-di-*O*-methyl-D-glucose were formed from 2,3-di-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-glucose, respectively.

Methylated polysaccharides are generally insoluble in hot aqueous solutions, and for that reason methanolysis, formolysis or treatment with 72 % sulphuric acid is performed before hydrolysis with dilute, aqueous acid. It is evident from the present investigation that hydrogen chloride, either in water or methanol, causes a significant amount of demethylation and that the use of sulphuric acid is therefore preferable. Formolysis in 98 % formic acid causes almost no demethylation but is accompanied by considerable degradation. Of the methods investigated, the pretreatment with 72 % sulphuric acid followed by hydrolysis with 12 % sulphuric acid offers the best compromise. It is possible, however, that equally good or better results may be obtained by formolysis in 90 % formic acid, followed by acid hydrolysis. Aspinall *et al.*<sup>6</sup> have hydrolysed a methylated polysaccharide that was soluble in cold water, by carrying out the reaction in dilute sulphuric acid and raising the temperature only slowly, so as to keep the material in solution. This method seems to have definite advantages.

The substances studied in the present investigation did not show any selective demethylation and the methoxyl groups in different positions seem to show comparable reactivities. This was to be expected; smaller differences in reactivity are reasonable but a highly selective demethylation seems most improbable.

#### EXPERIMENTAL

*Paper chromatography* and *paper electrophoresis* were carried out on Whatman No. 1 filter paper.

Solvent systems: Ethyl acetate-acetic acid-water, 3:1:3.  
Butanone, saturated with water.  
Buffer: 0.1 M borate buffer at pH 10.

*Starting materials.* 2,3-Di-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-glucose were prepared by conventional methods. 2,3-Di-*O*-methyl-D-xylose was prepared by methylation, detritylation and hydrolysis of methyl-5-*O*-trityl-D-xylofuranosides ( $\alpha$ - and  $\beta$ -mixture) analogously to the synthesis by Robertson and Gall<sup>7</sup>, of the same substance from methyl-5-*O*-benzoyl-D-xylofuranosides. The substance was purified by carbon column chromatography and distillation under reduced pressure. It did not crystallise but was chromatographically and electrophoretically pure and indistinguishable from 2,3-di-*O*-methyl-D-xylose, obtained as the main product of hydrolysis of fully methylated xylans.

### Estimation of the extent of degradation

The optical rotation of an aqueous solution of the methylated sugar (*c* 2.0) was determined, 5.00 ml of this solution was concentrated to dryness in a rotating laboratory evaporator and the residue was treated according to one of the four different alternatives described below.

1. It was dissolved in 2 % aqueous hydrochloric acid (4 ml), refluxed for 13 h, diluted to 5.00 ml and the optical rotation was determined (2 dm tube).

2. It was dissolved in 2 % methanolic hydrogen chloride (5 ml) and heated in a sealed tube to 100° for 13 h. The methanol was replaced with water by distillation under reduced pressure, the volume was adjusted to 4.5 ml and the solution was refluxed for 8 h. The volume was then adjusted to 5.00 ml and the optical rotation was determined.

3. It was dissolved in 72 % sulphuric acid (0.44 ml) and kept at room temperature for 30 min. Water was added (3.52 ml) and the solution was heated to 100° for 4 h, diluted with water to 5.00 ml and the optical rotation was determined.

4. It was dissolved in 98 % formic acid (6 ml) and heated to 100° for 6 h, then concentrated to a syrup under reduced pressure, dissolved in 0.5 N sulphuric acid (4.5 ml) and heated to 100° for 14 h. It was then diluted to 5.00 ml and the optical rotation was determined.

For treatments 1, 2 and 3 the decrease in optical rotation was only between 0.02° and 0.05°, duplicate experiments showing a fairly good agreement. The concentrations during the acid treatments were somewhat too high, and some reversion probably occurred.

The formolysis gave a larger decrease in rotation, about 0.2°

### Estimation of the demethylation

A sample of the methylated sugar (about 1.0 g) was subjected to hydrolytic treatment as described above. The acid was removed by filtration through a column of Amberlite IR 4 B and the aqueous solution was concentrated to a small volume and added to the top of a carbon-Celite column (4 × 40 cm). The sugars were eluted with aqueous ethanol, using gradient elution. For the dimethyl ethers, elution with 5 000 ml, 0 → 25 % ethanol was sufficient. The products from the hydrolytic treatment of 2,3,6-tri-*O*-methyl-D-glucose were eluted with 8 000 ml, 0 → 50 % ethanol. Pure starting material was recovered together with smaller amounts of partially demethylated sugars. The fractionation of the latter was incomplete and they were therefore combined and the total amount present was determined by weighing, hypiodite titration and determination of the optical rotation. The components of these fractions were characterised by paper chromatography and paper electrophoresis and their proportions were estimated from the intensities of the spots. In the experiments with 2,3-di-*O*-methyl-D-xylose, very small amounts of xylose were observed in the hydrolysate in addition to the mono-*O*-methyl-D-xyloses and starting material.

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