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The Occurrence of 3-Oxoglucose Units in Oxidised Cellulose

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D-Allose has been isolated from the hydrolysate of a cellulose which was first oxidised with chromate and subsequently reduced with borohydride. The mannose content was roughly the same as found in the hydrolysate of an unmodified cellulose. These results strongly indicate that most of the keto-groups in the oxidised cellulose are present as 3-oxoglucose units and that the contribution from 2-oxoglucose units is rather insignificant.

The oxidation of cellulose is technically an important although usually most undesirable reaction, which takes place during the bleaching of pulp, aging of paper etc. The alcoholic hydroxyl groups of the cellulose are converted to oxo or carboxyl groups, and more drastic oxidation also cleaves carbon-carbon linkages. Fairly satisfactory methods are available for the analytical determinations of these groups, but these are complicated by the heterogeneous nature of the reactions upon which they are based. Meesook and Purves [1] have also developed methods which distinguish between aldo and keto groups. The assignment of definite structures to different oxidised units in the modified celluloses is, with few exceptions, based upon assumptions only. The structure of periodate oxidised cellulose, however, has been proved both by further oxidation and by reduction of the material and subsequent identification of the products of hydrolysis [2]. The isolation of 3,5-dioxo-tetrahydro-4-pyrone [3] after acid hydrolysis of a cellulose oxidised with nitrogen dioxide, indicated the presence of 2,3-dioxoglucose units.

Whistler *et al.*[4] have recently studied a hypochlorite oxidised starch; after hydrolysis they isolated glyoxylic acid and D-erythronic acid, which demonstrates that the link between $C_{(2)}$ and $C_{(3)}$ in the glucose units has been broken. Purves *et al.*[5,6] have investigated chromate and hypochlorite oxidised starch. The modified polysaccharides were first subjected to a cyanohydrin synthesis and then to hydrolysis and reduction with hydrogen iodide. 4-Hydroxy-2-methylhexanoic acid was isolated from the reaction product and is obviously formed from 2-oxoglucose units. As no products derived from potential 3-oxoglucose units were obtained, they draw the conclusion that the main part of the keto-groups in these modified starches were due to 2-oxoglucose units.

When methyl β-D-glucopyranoside was oxidised with chromate, the 6-oxoglucoside was the dominating oxo-derivative, follwed by the 3-oxoglucoside [7]. We have since found that the 2-oxoglucoside is also formed, but in a much lower yield [8]. From these results one would, by analogy, expect to find more 3- than 2-oxoglucose units in chromate oxidised cellulose.

The present paper describes an investigation of cellulose, cotton linters, oxidised with chromate; the modified product contained 6.9 oxo groups per 100 glucose units, as determined by the borohydride method [9]. The oxidised cellulose was reduced with borohydride. By reduction under similar conditions [8] methyl β-D-2-oxoglucoside yielded glucoside and mannoside in the proportions 41:59 and methyl β-D-3-oxoglucoside yielded glucoside and alloside in the proportions 48:52. In neither case were other sugars formed in detectable amounts, which indicates that no isomerisations took place. It is reasonable to assume that the reduction of potential 2- and 3-oxoglucose units in the cellulose should follow a similar course. The reduced polysaccharide was then hydrolysed to a mixture of glucose and other sugars, and the glucose was selectively removed by enzymatic oxidation to gluconic acid with the aid of notatin and subsequent treatment with ion exchange resins. In the residue, the presence of mannose, xylose, allose and traces of glucose could be demonstrated by paper chromatography and by paper electrophoresis in borate buffer. Glucose and xylose, however, were not separated by the last method. A weak spot, probable due to arabinose, was also obtained both in the modified and unmodified sample. Mannose and xylose were also found in the hydrolysate of the unmodified cellulose. The quantitative determination of the sugars was complicated by the presence of unknown material, possibly proteins or peptides from the enzyme preparation which interfered with various reactions tried. These difficulties were finally overcome by estimating the sugars from the sizes of the spots in comparison with those of reference spots, using anisidine hydrogen chloride as spraying reagent. Fairly reproducible results were obtained and the agreement between the values determined by paper chromatography and by paper electrophoresis was satisfactory (Table 1). The analytical figures were corrected for sugar decomposition during the acid hydrolysis and also for the formation of 1,6-anhydro-D-allose [10]. Losses during the manipulations were also corrected for by adding a known amount of rhamnose to the hydrolysate and determining rhamnose in the final sugar mixture. The oxidation of sugars other than glucose by notatin was considered insignificant and was not corrected for (*cf.* Ref.[11]) In a separate experiment, the losses of mannose during this treatment were found to be less than 1 % of the total amount.

The figures in Table 1 show that the cellulose contained small but detectable amounts of mannose and xylose. The modified cellulose contained comparable amounts of these sugars, and no increase in the mannose content was observed. Allose, which of course was not present in the linters, was found in the modified cellulose. In a separate and essentially analogous experiment, crystalline D-allose was isolated from an oxidised and reduced cellulose and

Table 1. Sugars other than glucose, before and after oxidation reduction of the cellulose. E and C, determined by paper electrophoresis and paper chromatography, respectively.

Sample	Mannose %		Xylose %		Allose %	
	E	C	E	C	E	C
Cotton linters,	0.18	0.13	—	0.26	0	0
» » modified	0.16	0.14	—	0.24	0.17	0.17

proved to be identical with an authentic sample. These results, which are in agreement with our earlier model experiments, demonstrate the presence of 3-oxoglucose units in chromate oxidised cellulose. There may be 2-oxogroups also present, but to a much lesser extent. If practically no mannose is formed by the reduction of the 2-oxoglucose units, the percentage of the latter could of course be quite high, but the results of model experiments[8] make this assumption improbable. It is also possible that the original mannose in the sample, as present in the form of a highly accessible hemicellulose, was destroyed during the oxidation and that the mannose found in the modified cellulose should come from 2-oxoglucose units. This assumption, however, seems highly improbable as the xylose content is the same in both samples, and xylose units, by the same argument, should also be preferentially oxidised. Thus, of the oxogroups present, 6.9 in 100 glucose units, only 0.34 or 5 % should be in 3-oxoglucose units. This, of course, is a very crude estimation, which is valid only for the actual sample investigated, since the proportions between different groups may vary considerably with the conditions of preparation.

EXPERIMENTAL

Preparation of the modified cellulose. Cotton linters (20 g) was treated with a solution of potassium dichromate (7.86 g) in 0.4 N sulphuric acid (1 600 ml) at 25° for 120 h and was then carefully washed with water and dried in air. The oxogroups were determined by the borohydride method as 6.9 groups in 100 glucose units. The copper number, determined according to Hägglund[12], was 18.9. The modified cellulose (10 g), reduced by treatment for 6 hours at 25° with an aqueous solution (400 ml) containing potassium borohydride (0.56 g), buffered to pH 9.6 by boric acid (3.70 g) and sodium hydroxide (1.60 g) was filtered off, washed and air-dried.

Hydrolysis and enzymatic oxidation of the hydrolysate. Unmodified or modified cellulose (3 g) was treated with 72 % sulphuric acid (7.5 ml), a vacuum being applied and released a number of times to obtain a better contact with the solvent. After 17 h at room temperature the solution was diluted with water (105 ml) and refluxed 3 h. The cooled solution was then neutralised with barium carbonate using vigorous agitation and keeping the temperature below 15°. After filtering and washing, the solution was deionised, using the ion exchange resins Amberlite IR 120 and IR 4B and concentrated under reduced pressure to an almost colourless syrup (3.5 g). The syrup was dissolved in 0.5 M acetate buffer (90 ml) at pH 5.5 and the enzyme preparation (Dee 0 Takamine Laboratory) (30 mg) was added. Oxygen was bubbled through the solution, which was kept at 35° and vigorously agitated. At intervals the pH was adjusted to 5.5 by addition of solid sodium hydrogen carbonate. After one day further enzyme preparation (30 mg) was added and after 2 ½ days the oxidation was stopped. Rhamnose monohydrate (30 mg) was added and the solution was deionised as above and concentrated to a syrup under reduced pressure.

Analysis of the hydrolysate. The syrup above was dissolved in water and the volume adjusted to 5 ml. A qualitative chromatographic analysis revealed the presence of significant amounts of rhamnose, xylose, mannose and allose, the latter, however, only in the hydrolysate from the modified cellulose. Smaller amounts of glucose and arabinose were also found together with some substances that were obviously oligosaccharides, since they gave spots with low R_F-values and positive reactions with anisidine hydrogen chloride. A fast spot, which gave a weak reaction with the silver nitrate-sodium ethoxide reagent, and which had the R_F-value of 1,6-anhydroallose, was also obtained from the hydrolysate of the modified cellulose.

For the quantitative estimations, aliquot amounts of the hydrolysate were spotted on sheets of Whatman No. 1 filter papers, together with varying amounts of reference substances (5—75 μg). The chromatograms were run, first with solvent A, ethyl acetate-acetic acid-water, 3:1:3 (upper phase), for 16 h, dried and then run with solvent B, butanol-pyridine-water, 3:1:1.5, for 24 h. The spots were developed with the anisidine hydrogen chloride reagent, the areas of the reference spots were plotted against the amounts of sugars and the amounts of sugars in the hydrolysates determined by interpolation. The electrophoretic separations were carried out on Whatman No. 3 filter paper in a 0.1 M borate buffer at pH 10 for 2 h at 1 500 V. The quantitative estimations were made as described above. The results of analyses are given in Table 1.

Isolation of D-*allose.* A sample of the modified cellulose (10 g) was hydrolysed, enzymatically oxidised and worked up as described above. A rather large amount of enzyme preparation, 0.50 g, was used. The syrup (525 mg) was fractionated as above on ten sheets of Whatman No. 3 filter paper (width 23 cm), which had previously been carefully washed with water. The bands containing the allose, mixed with mannose, were cut out, the sugars were eluted and the solution concentrated to a syrup (70 mg) under reduced pressure. This syrup was now fractionated on three sheets of the same paper, run for 24 h with solvent B. A fraction (42 mg) containing allose as the only sugar was obtained. The syrup was extracted with boiling 75 % aqueous ethanol, and the extract was concentrated to dryness, dissolved in water, decolourised with carbon, deionised and concentrated again. When anhydrous ethanol was added to the syrup, it crystallised. The crystals (8 mg) were recrystallised from ethanol, yielding pure D-allose (4.2 mg) m. p. 127—128°, undepressed on admixture with authentic material. In its behaviour on paper chromatography in various solvents and in paper electrophoresis, the substance was undistinguishable from authentic D-allose.

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REFERENCES

1. Meesook, B. and Purves, C. B. *Paper Trade J.* **123** (1946) 35.
2. Purves, C.B., in Wise, L. E. and Jahn, E. C. *Wood Chemistry*, Vol. I, Reinhold, New York 1952, p. 168.
3. Battenberg, E. and Berg, A. *Ber.* **86** (1953) 640.
4. Whistler, R. L., Linke, E. G. and Kazeniac, S. *J. Am. Chem. Soc.* **78** (1956) 4704.
5. Ellington, A. C. and Purves, C. B. *Can. J. Chem.* **31** (1953) 801.
6. McKillican, Marg. E. and Purves, C. B. *Can. J. Chem.* **32** (1954) 312.
7. Lindberg, B. and Theander, O. *Acta Chem. Scand.* **8** (1954) 1870.
8. Theander, O. *Unpublished results.*
9. Lindberg, B. and Theander, O. *Svensk Papperstidning* **57** (1954) 83.
10. Pratt, J. W. and Richtmyer, N. K. *J. Am. Chem. Soc.* **77** (1955) 1906.
11. Whittaker, V. P., in Klyne, W. *Progress in Stereochemistry*, Vol. I, Butterworth, London 1954, p. 297.
12. *Central Laboratory of the Swedish Cellulose Industry Standards, CCA* **3** (1940).

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