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Degradation of Cellobiitol and Glucose by Oxygen-Alkali Treatment

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An important reaction of cellobiitol during oxygen – alkali treatment at 96° is an oxidation of the glucitol moiety, followed by a liberation of glucose. To a lesser extent, the glucose moiety is oxidized and glucitol liberated in a consecutive reaction. Glucose is decomposed by various reactions. The formation of arabinonic and mannonic acids decreases at high temperature, whereas the reaction paths *via* 3-deoxy-*erythro*-hexosulose become more important. Among the 23 monocarboxylic acids identified after glucose oxidation, formic, acetic, and glycolic acids were the most abundant.

In connection with the recent development of oxygen bleaching of wood pulps, studies of catalysts and inhibitors which affect the degradation of carbohydrates by oxygen in alkaline medium have become increasingly important. In these studies it is often advantageous to apply model compounds instead of cellulose. Cellobiitol has been used for this purpose [1] and since the reactions which occur have not been studied previously, an investigation of the reaction products obtained after oxygen-alkali treatment was carried out. The experiments showed that glucose was an important intermediate. Very little is known about the reaction products formed from glucose under comparable conditions and a study of these products was therefore included.

EXPERIMENTAL

Cellobiitol (6 g), prepared by reduction of cellobiose with sodium borohydride,[2] was dissolved in 300 ml of 0.5 % sodium hydroxide and treated with oxygen under pressure (6 bar) at 96° in the bubbling column described previously.[1] A small amount of hydrogen peroxide (0.67 mmol/l) was added as initiator. The time of reaction was 1.5 h. An aliquot of the solution was used for the determination of peroxide and for alkalimetric determination of organic acids. Another part of the solution was neutralized with acetic acid to pH 8 and separated into an acid fraction and a neutral fraction on an anion exchanger in the acetate form. After the sodium ions were removed on a cation exchanger, both solutions were evaporated and the fractions weighed. The acid fraction amounted to 4.5 % of the added cellobiitol.

The neutral fraction was chromatographed on a preparative scale.[3] The sub-fractions were analyzed automatically by partition chromatography in 85 % ethanol on an anion exchanger in the SO_4^{2-} form and on a cation exchanger in the Li^+ form[4]. The analyzing system contained two channels, one which recorded all sugars, and one which determined alditols and indicated the presence of ketoses.[5] The presence of glucose and glucitol was confirmed by gas chromatography and gas chromatography-mass spectrometry.[6]

The nonvolatile acids were separated quantitatively on an anion exchange column in 0.08 M sodium acetate (pH adjusted to 5.9 with acetic acid). The chromatogram was recorded automatically by means of a differential refractometer (Waters' Model R-4) and the eluate divided into nine fractions as illustrated in Fig. 1. These were analyzed on anion exchange columns in the acetate form in two media, 0.08 M sodium acetate (pH 5.9) and 0.5 M acetic acid. The chromatograms were recorded with a three-channel analyzer.[7] The volume distribution coefficients and the colour responses were compared to results obtained in previously published papers. This permitted an identification of most compounds. For additional identification, the acids were converted into fully substituted trimethylsilyl esters and identified by gas chromatography and gas chromatography-mass spectrometry.[8] Only one compound recorded on the chromatograms had properties differing from the acids studied in previous work from this laboratory. The mass spectrum revealed that the acid was a 3-deoxyheptonic acid.

A minor amount of organic acids, constituting about 5 % of the total amount of nonvolatile acids, appeared ahead of the first fraction as indicated in Fig. 1. The acids were

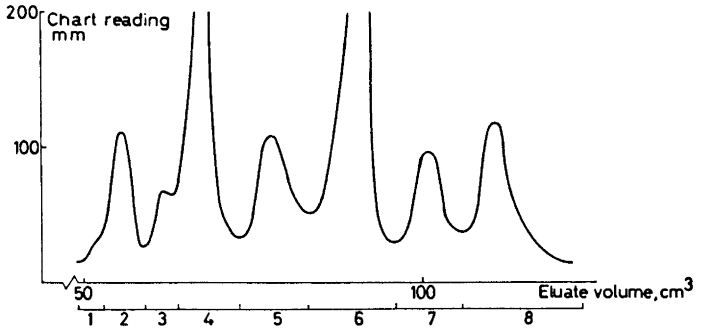

Fig. 1. Group separation of monocarboxylic acids obtained by oxygen-alkali treatment of cellobiitol. Column: 10 × 935 mm, Dowex 1-X8, 25 – 32 μ. Eluent: 0.08 M sodium acetate, pH = 5.9. Detection: Differential refractometer.

rechromatographed on the analysis columns and found to be a complex mixture of aldobionic and similar acids containing one glucose moiety. The amounts were too small for identification.

Formic acid was determined after being extracted with ether from the reaction solution which had been acidified to pH 1. The ether solution was titrated with aqueous 0.1 M NaOH and the aqueous solution chromatographed in 0.3 M sodium acetate on an anion exchange column. The formic acid was recorded automatically by chromic acid oxidation. Acetic acid was obtained as a sodium acetate solution by the same extraction method. The sodium ions were removed by a cation exchange resin and the solution was analyzed by gas chromatography.[9] As a check, the acetic acid was distilled from the reaction solution after acidification with phosphoric acid to pH 2. The distillate was studied by gas chromatography. The values obtained by the two methods differed by less than 10 %.

In the experiment with glucose, the oxygen-alkali treatment was carried out for 15 min. No hydrogen peroxide was added, but in other respects the conditions were the same as those applied with cellobiitol. The pH of the solution was 8.5 at the end of the experiment. The solution was analyzed by the same methods as used in the experiment with cellobiitol.

RESULTS AND DISCUSSION

Oxidation of glucose. The reaction during oxygen-alkali treatment of glucose is very rapid and the treatment was carried out only for 15 min, during which time the sodium hydroxide was consumed completely. In addition to organic acids, a number of neutral sugars were formed by isomerization and fragmentation. Besides glucose (52 %), fructose (37 %) and mannose (7 %) were the most abundant sugars. The presence of psicose, allose, and altrose (1 % of each) showed that not only the substituents at C-1 and C-2, but also those at C-3 were involved in Lobry de Bruyn – Alberda van Ekenstein rearrangements. As expected on the basis of previous results,[10] a small amount of arabinose was formed (0.5 %). It is noteworthy that ribose, the C-2 epimer of arabinose was present in about an equal amount.

A comparison between the organic acids formed by oxygen-alkali treatment at high temperature (96°) in dilute sodium hydroxide (0.5 %) and high

Table 1. Organic acids obtained after oxygen-alkali treatment of glucose and cellobiitol. The amounts given as g acid per 100 g of starting material. The nonvolatile compounds are reported as a percentage of the total weight of isolated nonvolatile acids. p = present but not determined.

Acid	From glucose				From cellobiitol	
	0.5 % NaOH, 96°		18 % NaOH, 25°		0.5 % NaOH, 96°	
	g/100 g	%	g/100 g	%	g/100 g	%
Glycolic	2.67	18.6	0.67	11.0	0.52	15.0
Glyceric	2.22	15.5	0.97	15.9	0.93	26.9
Erythronic	1.56	10.8	0.38	6.2	0.28	8.1
3-Deoxy-*arabino*-hexonic	1.46	10.2	0.08	1.4	0.14	4.0
2-Deoxy-*erythro*-pentonic	1.38	9.6	0.11	1.7	0.05	1.4
Arabinonic	1.15	8.0	3.03	49.7	0.08	2.3
2-Hydroxypropionic	0.65	4.5	0.21	3.4	0.31	9.0
3-Deoxytetronic	0.50	3.5	0.06[a]	1.0	0.32	9.2
3-Hydroxypropionic	0.45	3.1				
Threonic	0.42	2.9			0.04	1.2
3-Deoxy-*ribo*-hexonic	0.33	2.3	0.08	1.4	0.05	1.4
Ribonic	0.33	2.3			0.03	0.9
2-Deoxytetronic	0.23	1.6	0.06	1.0	0.11	3.2
Gluconic	0.21	1.5	0.04	0.7	0.05	1.4
3-Deoxy-*threo*-pentonic	0.21	1.5	p		0.37	10.7
Mannonic	0.21	1.4	0.36	5.9	0.03	0.9
2-Deoxy-*lyxo*-hexonic	0.15	1.1				
3-Deoxyheptonic	0.14	1.0				
Xylonic	0.05	0.3				
3-Deoxy-*erythro*-pentonic	0.05	0.3	0.04	0.7	0.11	3.2
2-Deoxy-*threo*-pentonic	0.01	0.1				
3-Deoxy-*xylo*-hexonic					0.03	0.9
2-*C*-Methyl-ribonic					0.01	0.3
Acetic	4.1				0.04	
Formic	4.5		p		0.32	
Carbonic	1.2				0.2	

[a] Band 3S1 identified from original chromatograms.

oxygen pressure and those obtained in 18 % solution at room temperature and atmospheric pressure [10] shows that the working conditions exert a great influence. The results obtained in both the present and the previous investigation are given in Table 1. Arabinonic acid was the preponderant acid and mannonic acid was among the major acids obtained at low temperature, whereas the relative amounts produced under the conditions used in the present work were much less. In general, the formation of acids of low molecular weight is favoured under the conditions applied in the present work. A similar influence of an increased temperature was observed by de Wilt [11] and by Malinen and Sjöström.[12]

The formation of arabinonic and mannonic acids, as well as arabinose, can be ascribed to the formation of D-glucosone followed by a benzilic acid rearrangement or a fragmentation between the carbonyl groups.[10,13] These and other reactions of glucosone have recently been studied by Malinen and Sjöström.[12]

The fact that the relative amounts of arabinonic and mannonic acids were lower under the present working conditions than in the earlier experiments indicates that the reaction path *via* glucosone is less important at high temperature. This is not unexpected since, even in the absence of oxygen, glucose is decomposed very rapidly in hot sodium hydroxide solution and gives rise to 2-hydroxypropionic, 3-deoxy-*ribo*- and 3-deoxy-*arabino*-hexonic acids as the preponderant reaction products.[12,14] It is worth mentioning that ribonic acid was more abundant than mannonic acid and that a new reaction path for the formation of ribonic acid from methyl β-glucopyranoside during oxygen-alkali treatment has been suggested recently.[15]

Formic, acetic, and glycolic acids were the three most abundant acids isolated in the present work. Reactions paths which give rise to formic and glycolic acids have been discussed earlier,[10,12,16] whereas acetic acid has escaped observation in previous investigations. Glucosone is one precursor responsible for the formation of formic and glycolic acids, but since this intermediate seems to be less important at high than at low temperature, the reaction path *via* glucosone cannot explain the large amount of formic acid.

Among the most striking differences between the results obtained by alkali treatment of glucose at high temperature in the absence of oxygen and the results obtained under oxygen pressure is that 2-hydroxypropionic acid is the most abundant acid in the absence of oxygen, whereas the amount formed in the presence of oxygen is fairly small.[12] Instead, very large amounts of acetic acid are formed during oxygen-alkali treatment. It has been reported [17] that pyruvaldehyde, formed after cleavage of glucose between C-3 and C-4, is a precursor of 2-hydroxypropionic acid, *i.e.* that 2-hydroxypropionic acid is formed by a benzilic acid type rearrangement of this dicarbonyl compound. As pointed out previously, an oxidative cleavage of dicarbonyl compounds between the carbonyl groups is a very important reaction during oxygen-alkali treatment of carbohydrates [18] and it can be concluded therefore that the intermediate pyruvaldehyde is a precursor to acetic acid, which, in this reaction, is formed together with an equimolar amount of formic acid.[19]

No detectable amounts of 2-C-methylpentonic acids were present in the reaction mixture after the oxygen-alkali treatment of glucose. It is possible

that the intermediate, 1-deoxy-2,3-hexodiulose, which is a precursor to these saccharinic acids, [20] is formed, but that it is subjected to an oxidative cleavage of the linkage between the carbonyl groups, which produces equimolar amounts of acetic and erythronic acids. The fact that erythronic acid was among the most abundant acids, but still present in much smaller amounts than acetic acid, can be taken as an indication that this reaction is of importance, but by no means solely responsible for the formation of acetic acid.

Among the nonvolatile acids, 3-deoxyhexonic and 2-deoxy-*erythro*-pentonic acids were much more abundant after the treatment at 96° than at room temperature. The two 3-deoxyhexonic acids mentioned above, which are among the major reaction products after alkaline treatment of glucose and fructose in the absence of oxygen, are formed *via* 3-deoxy-*erythro*-hexosulose by a benzilic acid rearrangement.[21] The results presented in the table indicate that this reaction is of great importance during oxygen-alkali treatment at high temperature. Like other dicarbonyl compounds this intermediate can be subjected to fragmentation between the carbonyl groups. Oxidation will give formic and 2-deoxy-*erythro*-pentonic acid, which were both present in much larger amounts after the high-temperature oxidation than after the reaction at low temperature. The observation by Malinen and Sjöström [12] that the 2-deoxypentonic acid was not formed in the absence of oxygen indicates that the cleavage of the dicarbonyl intermediate by hydrolysis is of little or no importance.

A 3-deoxyheptonic acid was among the minor acids formed in the present work. The formation of acids with a larger number of carbon atoms than the original sugar has previously been observed by Ishizu, Lindberg, and Theander [22] in studies of alkaline treatment of xylose. An aldol condensation is very probably responsible for the formation of a heptose, which then rearranges to a 3-deoxyheptosulose and is converted, by a benzilic acid rearrangement, to 3-deoxyheptonic acids. Like other dicarbonyl sugars, 3-deoxyheptosulose should be subject to fragmentation, which, in this case, should give rise to some 2-deoxyhexonic acid. This reaction path explains the presence of 2-deoxy-*lyxo*-hexonic acid after oxygen-alkali treatment of glucose.

Oxidation of cellobiitol. Peroxide is formed during oxygen bleaching of cellulose, and since cellobiitol is very stable towards the attack of oxygen in alkaline medium,[1] a small amount of hydrogen peroxide (0.67 mmol/l) was added to initiate the reaction. It is interesting to note that the final peroxide concentration was the same as that at the beginning of the oxygen treatment. In view of the instability of hydrogen peroxide in hot alkaline solution, it can be concluded that peroxide was formed during the oxygen treatment. It is likely that intermediate carbonyl compounds or enolate anions formed during the degradation of cellobiitol are involved in the reactions which give rise to peroxide.[23]

To simulate the reactions which occur during oxygen-alkali treatment of cellulose, a short time of reaction (1.5 h) was chosen. This means that only a fraction (about 10 %) of the added cellobiitol was attacked. The major portion was recovered in the nonelectrolyte fraction which, in addition, contained glucose and glucitol as major products together with minor amounts of 3-*O*-(α-D-glucopyranosyl)-D-arabinose and 2-*O*-(α-D-glucopyranosyl)-D-erythrose.

The amount of glucose found in the reaction mixture was 3.2 %, calculated as a percentage (by weight) of the organic acids formed during the oxygen-alkali treatment. Since glucose is unstable under the applied working conditions and is rapidly converted into organic acids, it can be concluded that glucose is a very important intermediate.

Glucitol was present in a larger amount (5.3 % calculated on the same basis). Glucitol is much more stable than glucose in the reaction mixture. A separate experiment in which glucitol was treated under the same working conditions as cellobiitol revealed that the rate of formation of acids was roughly the same as that observed with cellobiitol. Evidently, the liberation of glucose from cellobiitol is a much more important reaction than the formation of glucitol.

Separate experiments carried out with cellobiitol in the absence of oxidant showed that neither glucose nor glucitol was formed. The most likely explanation for the formation of glucitol during oxygen-alkali treatment is that an oxidation of the glucose moiety in cellobiitol, resulting in the introduction of a keto group at C-3, occurs and is followed by a β-elimination at C-1 in the oxidized glucose moiety. Experiments carried out by Theander strongly indicate that oxidation at C-2 or C-3 will give the same reaction pattern since the two carbonyl compounds are rapidly interconverted.[24]

The oxidized glucose unit is unstable in alkali and may also be further oxidized. The consecutive reactions give rise to various fragmentation acids as main end products. Since, as already mentioned, the amount of glucitol was comparatively small, it can be concluded that this reaction path is not very important.

Only a small amount of gluconic acid was present in the reaction mixture (Table 1) indicating that no direct oxidative attack occurs upon the glycosidic bond during oxygen-alkali treatment. Similar observations have been made for aging of alkali cellulose,[25] oxygen bleaching of cellulose,[26] and oxygen alkali treatment of cellobiose.[10,12] In the experiment with glucitol, it was found that gluconic acid was the most abundant acid formed during the oxidation. The working conditions seem to have a great influence upon the products formed from glucitol.[27,28] These results confirm that, in the experiment with cellobiitol, the major part of the liberated glucitol remained unattacked in the reaction mixture.

From the results given above, it can be concluded that a major reaction during the oxygen-alkali treatment results in the liberation of glucose. In analogy with the reactions which seem to be predominant during oxygen-alkali treatment of cellulose [26] and xylan,[29] it can be assumed that the glucose is liberated by β-elimination after the introduction of a carbonyl group in the glucitol moiety.

The introduction of a keto group at C-2 in the glucitol moiety or an oxidation at C-1 followed by a Lobry de Bruyn – Alberda van Ekenstein transformation, would lead to a β-elimination with the formation of glucose and 4-deoxy-D-*glycero*-2,3-hexodiulose, according to generally accepted reaction schemes. In the absence of oxygen, this compound rearranges to 3-deoxy-2-*C*-hydroxymethyl-*ribo*- and *arabino*-pentonic acids, whereas oxidation in alkaline medium results in the formation of 2-deoxytetronic

and glycolic acids.[30] Both the rearranged acids and these oxidation products were formed in large amounts when cellobiose was subjected to oxygen-alkali treatment.[10,12] Moreover, these products belonged to the main compounds recovered from the solution when hydrocellulose was subjected to the same treatment.[31] No detectable amounts of isosaccharinic acids were produced from cellobiitol under the applied conditions indicating that this reaction path is of little or no importance as far as the degradation of cellobiitol is concerned.

Another possibility of explaining the formation of glucose would be an oxidation at C-6 in the glucitol moiety followed by a β-elimination at C-4 with the formation of glucose and 3-deoxy-*threo*-hexosulose. An expected rearrangement product (3-deoxy-*xylo*-hexonic acid) was recorded, but only in trace amounts. Evidently, these reaction schemes cannot explain the main reactions which occur during the oxygen-alkali treatment of cellobiitol.

As shown previously [1] and confirmed in the present work 3-*O*-(β-D-glucopyranosyl)-D-arabinose was formed during the oxygen-alkali treatment of cellobiitol. This intermediate is very unstable in the alkaline medium. A β-elimination results in the liberation of glucose. A subsequent rearrangement of the intermediate dicarbonyl compound gives rise to the two diastereomeric 3-deoxypentonic acids [10] which were among the major reaction products derived from the glucitol moiety. These results as well as the observation that an appreciable amount of the expected fragmentation acid, 2-deoxytetronic acid, was present permit the conclusion that oxidation of the glucitol moiety to an arabinose moiety is a very important reaction path.

As expected, the relative amounts of the hexonic, pentonic, and tetronic acids were lower in the reaction mixture obtained from cellobiitol than after the treatment of glucose. On the other hand, glyceric and 2-hydroxypropionic acids were much more abundant in the run with cellobiitol. Very probably these acids are formed not only from liberated glucose, but also from the glucitol moiety.

An obvious difference, compared to the reactions which occur with sugars, cellulose and the dicarbonyl compounds referred to above, is that with alditols, straight chain compounds are the primary oxidation products. The results indicate that these are very reactive and give rise to other reaction products than those formed from the corresponding ring-closed compounds.

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REFERENCES

1. Samuelson, O. and Stolpe, L. *Svensk Papperstid.* **72** (1969) 662; **74** (1971) 545.
2. Marchessault, R. H. and Timell, T. E. *J. Polym. Sci. Part C* **2** (1963) 49.
3. Larsson, L.-I. and Samuelson, O. *Acta Chem. Scand.* **19** (1965) 1357.
4. Samuelson, O. and Strömberg, H. *Acta Chem. Scand.* **22** (1968) 1252; *Carbohyd. Res.* **3** (1966) 89.
5. Havlicek, J., Petersson, G. and Samuelson, O. *Acta Chem. Scand.* **26** (1972) 2205.
6. Petersson, G. *Tetrahedron* **25** (1969) 4437.

7. Carlsson, B., Isaksson, T. and Samuelson, O. *Anal. Chim. Acta* **43** (1968) 47.
8. Petersson, G. *Tetrahedron* **26** (1970) 3413.
9. Rexfelt, J. and Samuelson, O. *Svensk Papperstid.* **75** (1972) 299.
10. Samuelson, O. and Thede, L. *Acta Chem. Scand.* **22** (1968) 1913.
11. de Wilt, H. G. J. *Thesis*, University of Technology, Eindhoven 1969.
12. Malinen, R. and Sjöström, E. *Pap. Puu* **54** (1972) 451.
13. Lindberg, B. and Theander, O. *Acta Chem. Scand.* **22** (1968) 1782.
14. Bamford, C. H., Bamford, D. and Collins, J. R. *Proc. Roy. Soc. Ser. A* **204** (1950) 62.
15. Ericsson, B., Lindgren, B. O., Theander, O. and Petersson, G. *Carbohyd. Res.* **23** (1972) 323.
16. Dubourg, J. and Naffa, P. *Bull. Soc. Chim. France* **1959** 1353.
17. Sowden, J. C. *Advan. Carbohyd. Chem.* **12** (1957) 35.
18. Rowell, R. M. *Pulp Pap. Mag. Can.* **72** (1971) T 236.
19. Friedeman, T. E. *J. Biol. Chem.* **73** (1927) 331.
20. Ishizu, A., Lindberg, B. and Theander, O. *Carbohyd. Res.* **5** (1967) 329.
21. Isbell, H. S. *J. Res. Natl. Bur. Std.* **32** (1944) 45.
22. Ishizu, A., Lindberg, B. and Theander, O. *Acta Chem. Scand.* **21** (1967) 424.
23. Entwistle, D., Cole, E. H. and Wooding, N. S. *Text. Res. J.* **19** (1949) 527.
24. Theander, O. *Acta Chem. Scand.* **12** (1958) 1897.
25. Samuelson, O. and Thede, L. *Tappi* **52** (1969) 99.
26. Kolmodin, H. and Samuelson, O. *Svensk Papperstid.* **73** (1970) 93.
27. Minor, J. L. and Sanyer, N. J. *Polym. Sci. Part C* **36** (1971) 73.
28. Malinen, R., Sjöström, E. and Ylijoki, J. *Pap. Puu* **55** (1973) 1.
29. Kolmodin, H. and Samuelson, O. *Svensk Papperstid.* **74** (1971) 301; **76** (1973) 71.
30. Machell, G. and Richards, G. M. *J. Chem. Soc.* **1960** 1932.
31. Kolmodin, H. and Samuelson, O. *Svensk Papperstid.* **75** (1972) 369.

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