Acta Chemica Scandinavica B 29 (1975) 975—980

Oxygen—Alkali Treatment of Cellobiose

LARS LÖWENDAHL, GÖRAN PETERSSON and OLOF SAMUELSON

Chalmers University of Technology, Department of Engineering Chemistry, Fack, S-402 20 Göteborg 5, Sweden

C-(2,3-Dihydroxypropyl)tartronic acid is a major dicarboxylic acid formed during O_2-NaOH and O_2-$NaHCO_3$ treatment of cellobiose. The observation that this acid is the main reaction product after alkali treatment of ascorbic acid in O_2-free medium supports the conclusion that it is formed *via* an aglycon moiety related to ascorbic acid. Other dicarboxylic acids from cellobiose are oxalic, tartronic, deoxytetraric, *C*-(hydroxymethyl) tartronic, and succinic acids.

The temperature, pH and additions of iron and cobalt salts strongly influence the product composition. The formation of aldobionic acids from cellobiose parallels the formation of aldonic acid end groups during oxygen bleaching of cellulose.

Determinations of monocarboxylic acids and sugars formed by treating cellobiose with oxygen in sodium hydroxide solution have given valuable information about reactions during oxygen-alkali treatment of cellulose.[1–2] We now report on the dicarboxylic acids formed from cellobiose and on the effect of catalysts upon the product composition. Since the cellulose reactions with oxygen in bicarbonate solution have attracted attention in recent years, a study of the behaviour of cellobiose in this medium is included.

RESULTS AND DISCUSSION

Sugars and monocarboxylic acids. In the experiments with sodium hydroxide, the amount of alkali was chosen so that the sodium hydroxide was consumed. In the experiments carried out for 6 min at 97 °C the final pH was about 9. Among non-volatile compounds, monocarboxylic acids constituted the major fraction, but large amounts of sugars were also formed (Table 1). The presence of a large

Table 1. Recovery of non-volatile solutes (g per 100 g cellobiose) after oxygen-alkali treatment under various conditions.

	0.125 M Sodium hydroxide					$NaHCO_3$
	No addn 6 min 97 °C	Fe 6 min 97 °C	Co 6 min 97 °C	Co 10 min 50 °C	No addn 60 min 97 °C	$+Na_2CO_3$ No addn 90 min 97 °C
4-*O*-(β-D-Glucopyranosyl)-D-glucose	6.0	3.5	1.8	12.9	2.6	25.2
4-*O*-(β-D-Glucopyranosyl)-D-mannose	2.5	1.4	1.1	7.5	1.8	17.2
4-*O*-(β-D-Glucopyranosyl)-D-fructose	tr[a]	tr	tr	0.8	tr	0.8
D-Glucose	4.8	3.7	4.6	6.4	6.4	20.7
D-Mannose	2.0	1.7	2.0	0.2	2.9	0.5
D-Fructose	2.7	0.7	1.5	1.0	2.0	2.2
Monocarboxylic acids	66.8	62.4	55.0	46.8	66.0	20.2
Dicarboxylic acids	4.9	5.8	8.2	3.7	5.4	3.3
Total recovery	89.7	79.2	74.2	79.3	87.1	90.1

[a] tr = less than 0.2.

amount of glucopyranosylmannose shows that Lobry de Bruyn-Alberda van Ekenstein rearrangements occurred. As expected with regard to its lower stability much smaller amounts of glucopyranosylfructose were present. The results confirm that following the introduction of a carbonyl group at C-2 in the reducing glucose moiety by isomerization or oxidation, glucose is liberated by β-elimination. Glucose is further attacked and converted mainly to monocarboxylic acids.[3] It is noteworthy that an increased reaction time from 6 min to 60 min resulted in an increased glucose concentration demonstrating that in weakly alkaline solution the β-elimination is more rapid than the following destruction of glucose. This was confirmed by an experiment in bicarbonate-carbonate solution. Under these conditions the hexoses constituted the largest fraction of the products. From the amounts of hexoses and disaccharides it was estimated that 0.84 mol of hexoses were present per mol of destructed disaccharide. Since hexoses are not formed from the reducing glucose moiety, this experiment shows that in weakly alkaline medium β-elimination and conversion of the reducing moiety to carboxylic acids occur much more rapidly than the attack of oxygen on the liberated glucose.

Moreover, the glucose was converted to mannose and fructose whereas other reactions known to occur in alkaline medium [4] occurred only to a slight extent. The results are similar to those obtained by oxygen-alkali cooking of wood meal in bicarbonate solution where the depolymerization of the carbohydrates results in a liberation of terminal reducing end groups which suffer little oxidation [5] in comparison to that occurring during oxygen-sodium hydroxide treatment of cellulose [6] and xylan.[7]

Anion exchange chromatography of the non-volatile monocarboxylic acids produced during the treatment in bicarbonate solution showed that the *erythro* and *threo* forms of 3-deoxy-2-*C*-(hydroxymethyl)pentonic acids together constituted 15 % (by weight) of this fraction. Evidently the benzilic acid rearrangement of 4-deoxy-2,3-hexodiulose formed from the reducing glucose moiety was of great importance although the experiment was made under oxygen pressure. Other acids derived in part from this dicarbonyl intermediate were 3,4-dihydroxybutanoic (28 %) and glycolic (39 %) acids. The presence of 3-deoxypentonic acid (*threo* and *erythro*, together 12 %) indicated an oxidation of the reducing glucose moiety in cellobiose to a hexosulose moiety at low alkalinity.[8] Glyceric acid which is an important oxidation product formed from glucose in sodium hydroxide[3] was obtained in small amounts (4 %) during the oxidation of cellobiose in bicarbonate solution. These results confirm that the hexoses were only slightly attacked at low pH and that the monocarboxylic acids were mainly formed from the reducing glucose moiety.

Dicarboxylic acids. As can be seen in Table 1 the dicarboxylic acids constituted about 10 % of the non-volatile carboxylic acids formed. No attempt to determine and identify the dicarboxylic acids had been previously made. All dicarboxylic acid fractions were therefore isolated and analysed.

C-(2,3-Dihydroxypropyl)tartronic acid was the major dicarboxylic acid formed during oxygen-sodium hydroxide treatment of cellulose. The large amount of this acid from cellobiose both in hydroxide and bicarbonate media (Table 2) was therefore expected. The results indicate that the acid decomposes on prolonged treatment. According to the reaction scheme proposed for its formation,[6] the acid is formed *via* terminal 2-hexulosonic acid (or the corresponding lactone) groups. The keto group at C-2 gives rise to a β-elimination followed by a benzilic acid rearrangement. A fragmentation of the intermediate dicarbonyl compound leads to the formation of oxalic and 3,4-dihydroxybutanoic acids which were both found after treatment of cellulose and cellobiose.

If the postulated reaction scheme is correct, it should be possible to prepare *C*-(2,3-dihydroxypropyl)tartronic acid from ascorbic acid in alkaline medium (Fig. 1). An experiment with ascorbic acid was therefore made in lime-water. A suspension of calcium hydroxide in 15 ml of boiled water was covered with paraffin oil to prevent dissolution of oxygen. Ascorbic acid (100 mg) in boiled water (4 ml) was then injected into the suspension. After 89 h at 95 °C, the solution contained the expected acid together with unreacted ascorbic acid and its diastereomer (molar ratio ≈2:2:1).

Table 2. Relative amounts (mol %) and retention in gas chromatography (trimethylsilyl derivatives) of dicarboxylic acids from cellobiose.

Acids	Retention rel. to the glucitol derivative QF-1, 120 °C	0.125 M Sodium hydroxide					$NaHCO_3$ + Na_2CO_3
		No addn 6 min 97 °C %	Fe 6 min 97 °C %	Co 6 min 97 °C %	Co 10 min 50 °C %	No addn 60 min 97 °C %	No addn 90 min 97 °C %
C-(2,3-Dihydroxypropyl)-tartronic	1.365	28	17	15	24	15	16
C-(3-Hydroxypropyl)-tartronic [a]	0.689			3	20		
Deoxytetraric	0.280	13	7	5	5	15	7
C-(Hydroxymethyl)tartronic	0.275	6	3	9	3	3	3
Tartronic	0.187	19	44	30	25	22	8
Succinic	0.141	6	4	3	3	4	11
Oxalic	0.075	28	25	38	21	41	55

[a] Tentative identification based on mass spectrum of the Me_3Si derivative.

These acids were identified by GC and GC-MS The remaining material (15 %) consisted of a wide range of other products. At 23 °C the reaction was very slow and even after 310 h the starting material was virtually unchanged. The results indicate that hexulosonic acid end groups are formed during oxygen-alkali treatment of cellulose and cellobiose and that *C*-(2,3-dihydroxypropyl)tartronic acid is derived from these.

Tartronic, deoxytetraric, and *C*-(hydroxymethyl)tartronic acids were also produced during oxygen-alkali treatment of cellulose.[6] With cellobiose the relative amount of oxalic acid was much larger in the experiment in bicarbonate medium than in the short-time treatments in sodium hydroxide whereas with tartronic acid the opposite holds true. These observations indicate that oxalic acid is formed mainly from the reducing end group while an oxidation of the liberated glucose is mainly responsible for the formation of tartronic acid.

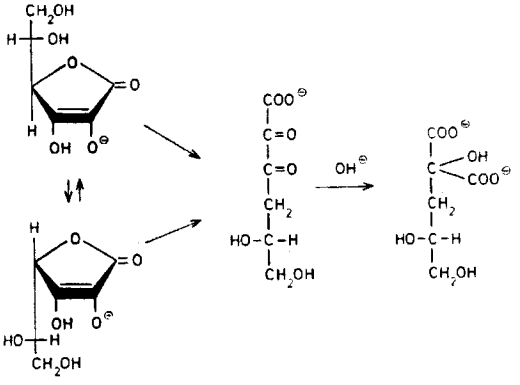

Fig. 1. Schematic representation of the formation of *C*-(2,3-dihydroxypropyl)tartronic acid from ascorbic acid in alkaline medium.

This hypothesis was confirmed in a separate experiment with glucose (0.53 % in 0.125 M NaOH, 97 °C, 30 min). The dicarboxylic acid fraction obtained in a yield of 4.6 % (compared to 74.0 % non-volatile monocarboxylic acids and 8.3 % remaining monosaccharides) was studied by GC and GC-MS. Tartronic and oxalic acids in a molar ratio of 6:1 were the major dicarboxylic acids. A large number of other acids together constituting about 25 % of the fraction were recorded. Among these deoxytetraric and *C*-(hydroxymethyl)tartronic acids were identified.

Effect of catalysts on the product composition. During oxygen-alkali treatment of cellobiose at low temperature and high alkali concentration aldobionic acids are the dominant products.[1] By lowering the alkali concentration and increasing the temperature, their yield decreases[2] whereas the fraction of acids formed after β-elimination increases markedly. Similarly, the number of aldonic acid end groups in cellulose relative to the amount of soluble carboxylic acids is lower after oxygen-alkali

Table 3. Aldobionic acids produced by oxygen-alkali treatment of cellobiose in 0.125 M sodium hydroxide. Weights refer to 100 g cellobiose.

Acids	No addn 6 min 97 °C g	Fe 6 min 97 °C g	Co 6 min 97 °C g	Co 10 min 50 °C g
4-*O*-(β-D-Glucopyranosyl)-D-mannonic	0.50	0.29	0.68	0.37
4-*O*-(β-D-Glucopyranosyl)-D-gluconic	0.17	0.22	0.35	0.22
3-*O*-(β-D-Glucopyranosyl)-D-arabinonic	2.29	1.72	2.71	3.67
2-*O*-(β-D-Glucopyranosyl)-D-erythronic	0.75	0.30	0.31	0.86
2-*O*-(β-D-Glucopyranosyl)-D-threonic	0.19	0.07	0.15	0.08
Total	3.9	2.6	4.2	5.2

treatment of cellulose under conditions simulating oxygen bleaching[6] (low alkali concentration, high temperature) than that observed after ageing of alkali cellulose. The results given in Table 3 show that, as expected, a fairly small fraction of the cellobiose was converted to aldobionic acids during the oxygen treatment in 0.5 % sodium hydroxide at 97 °C, whereas at 50 °C the abundance of aldobionic acids was significantly larger. The results confirm that under the applied conditions cleavage of the glycosidic bond by β-elimination is favoured compared to oxidation. In accordance with earlier findings, glucopyranosylmannonic acid was formed in larger amounts than glucopyranosylgluconic acid.

It is seen that the addition of catalysts had a significant effect on the relative amounts of the two diastereomers with terminal hexonic acid groups. This was also observed with cellulose where in experiments without added catalysts the number of mannonic acid end groups was much larger than that of gluconic acid groups while the opposite was found in experiments with additions of cobalt and iron.[6] These results together with those given in Table 3 indicate that with both cellulose and cellobiose the formation of *arabino*-hexosulose end groups is less important in the presence of cobalt and iron than in their absence. It is reasonable to assume that in the presence of catalysts the major portion of gluconic acid end groups is formed by some other reaction mechanism than by benzilic acid rearrangement of *arabino*-hexosulose moieties.

As can be seen from Tables 1 and 2 the amounts of other products were also affected by the presence of cobalt and iron. In the experiments made under otherwise unchanged conditions, the total amount of recovered non-volatile compounds was significantly lower than in the blank and the amount of dicarboxylic acids was higher. Among these, tartronic acid was formed in larger amounts in the presence of the metal compounds. In the experiments with cobalt at 50 °C a large amount of an acid tentatively identified as *C*-(3-hydroxypropyl)tartronic acid was recorded. This acid was also present after the experiment with cobalt at 97 °C but was neither detected in the presence of iron nor in the blank. Inspection of the tables shows that in addition there are several other differences between cobalt (*e.g.* larger formation of volatile compounds and oxalic acid) and iron (*e.g.* larger relative amount of tartronic acid). Moreover, the amount of fructose was much lower in the presence of iron than in the run with cobalt or in the blank. Evidently, metal compounds exert a great influence not only on the rate of cleavage of the glycosidic bond[9] but also on the consecutive reactions.

EXPERIMENTAL

The oxygen-alkali treatments were performed in a Teflon reactor.[10] A preheated cellobiose solution (1 g in 10 ml water) was injected into 90 ml of a preheated sodium hydroxide solution in equilibrium with oxygen at a gauge pressure of 0.6 MPa. The concentration of sodium hydroxide in the reaction mixture was 0.125 M and that of transition metals 0.05 mM. The metal compounds [$FeSO_4$ and $Co(NO_3)_2$] were added to the preheated cellobiose solution. In one experiment the sodium hydroxide was

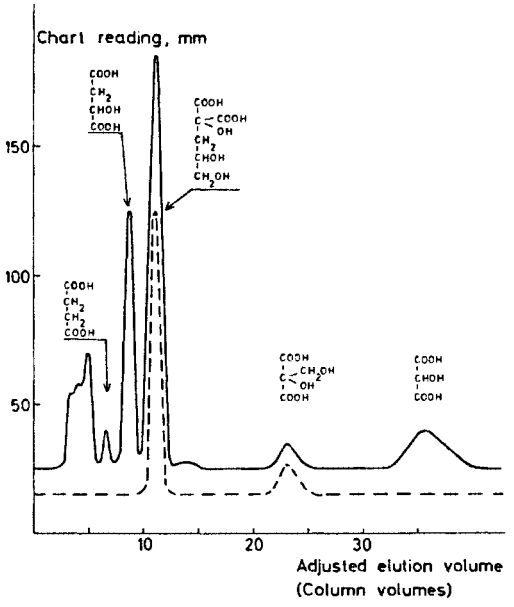

Fig. 2. Ion exchange chromatography of dicarboxylic acids (0.05 mmol) in 0.3 M sodium acetate in 2 M acetic acid at 30 °C. Column: 4 × 815 mm, Dowex 1 − X8; 14 − 17 μm. Nominal linear flow: 4.5 cm min^{-1}. (————), chromic acid channel, (– – – –), periodate-formaldehyde channel (succinic acid recorded in the chromic acid channel by oxidation in the presence of silver sulfate).

replaced by a mixture of sodium bicarbonate (0.043 M) and sodium carbonate (0.009 M). After the experiments the reaction mixture was cooled in ice-water and kept at −18 °C.

The yields of non-electrolytes, monocarboxylic and dicarboxylic acids were determined after group separation of an aliquot of the reaction solution (pH 9) on an anion exchanger in the acetate form (Dowex, 1-X8). The non-electrolytes were displaced with 7 column volumes of water, the monocarboxylic acids eluted with 17.5 column volumes of 2 M acetic acid and the dicarboxylic acids with 15 column volumes of 0.3 M magnesium acetate. After cation exchange (Dowex 50 − X8, H^+) the fractions (Table 1) were evaporated to dryness.

The non-electrolyte fraction was analysed by partition chromatography on an anion exchanger in the sulfate form.[11] The monocarboxylic acid fraction was chromatographed on an analytical anion exchange column[12] in 0.5 M acetic acid and in 0.08 M sodium acetate. The glucosylaldonic acids were isolated by applying an aliquot of the alkaline reaction solution to a preparative anion exchange column and eluting the monocarboxylic acids with 0.08 M sodium acetate (pH 5.9). The aldobionic acids which appeared in a single band before the other acids[1] were hydrolyzed[13] and the resulting aldonic acids analysed by GC as trimethylsilyl (Me_3Si) derivatives.

The dicarboxylic acid fraction obtained from the experiment in sodium hydroxide (6 min) without catalysts was studied by anion exchange chromatography (Fig. 2). The chromatographic bands from a preparative run were further analyzed by GC.[14] Only GC was used for the separation of the individual acids in the other experiments. Each of the acids (Table 2) was identified by MS in at least one of the experiments. Deoxytetraric and *C*-(hydroxymethyl)tartronic acids were not resolved on QF-1 but could be analyzed on OV-17. In addition to the major products listed in Table 2 at least five minor compounds together constituting less than 15 % of the total fraction were recorded by GC. The quantitative determinations were based on the assumption that the GC peak areas were proportional to the weight of the derivatives.

To confirm the identity of *C*-(2,3-dihydroxypropyl)tartronic acid, a sample isolated by anion exchange chromatography was dissolved in water and evaporated to dryness in 3 M HCl. The resulting esters (including lactones) were reduced with borohydride.[15] Analysis of the reaction products by GC-MS gave 3-deoxy-2-*C*-hydroxymethyl-*erythro*-pentonic acid, 3-deoxy-2-*C*-hydroxymethyl-*threo*-pentonic acid, and 3-deoxy-2-*C*-(hydroxymethyl)pentitol (molar ratio ≈3:1:2). Less than 1 % of the starting material remained unchanged. The results confirm (*cf.* Ref. 6) that the acid was *C*-(2,3-dihydroxypropyl)tartronic acid. The presence of the corresponding alditol among the reduction products indicates that, in addition to lactones, intermolecular esters were formed during the evaporation in acid medium.

Acknowledgements. The authors wish to thank the 1959 Års Fond för Teknisk och Skoglig Forskning samt Utbildning for financial support.

REFERENCES

1. Samuelson, O. and Thede, L. *Acta Chem. Scand. 22* (1968) 1913.
2. Malinen, R. and Sjöström, E. *Pap. Puu 54* (1972) 451.
3. Samuelson, O. and Stolpe, L. *Acta Chem. Scand. 27* (1973) 3061.
4. Sowden, J. C. *Advan. Carbohyd. Chem. 12* (1957) 35.
5. Lindström, L.-Å. and Samuelson, O. *Tappi 58* (1975) 146.
6. Löwendahl, L. and Samuelson, O. *Sv. Papperstidn. 77* (1974) 593.

7. Kolmodin, H. and Samuelson, O. *Sv. Papperstidn. 76* (1973) 71.
8. Kolmodin, H. and Samuelson, O. *Sv. Papperstidn. 75* (1972) 369.
9. Ericsson, B., Lindgren, B. and Theander, O. *Sv. Papperstidn. 74* (1971) 757.
10. Samuelson, O. and Stolpe, L. *Sv. Papperstidn. 77* (1974) 16.
11. Strömberg, H. and Samuelson, O. *Z. Anal. Chem. 236* (1968) 506.
12. Carlsson, B., Isaksson, T. and Samuelson, O. *Anal. Chim. Acta 43* (1968) 47.
13. Samuelson, O. and Thede, L. *Tappi 52* (1969) 991.
14. Petersson, G. *Carbohyd. Res. 33* (1974) 47.
15. Perry, M. and Hulyalkar, R. *Can. J. Biochem. 43* (1965) 573.

Received March 17, 1975.