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Influence of Oxygen upon Glucose and Cellobiose in Strongly Alkaline Medium

OLOF SAMUELSON and LARS THEDE

Department of Engineering Chemistry, Chalmers Tekniska Högskola, Göteborg, Sweden

Thirteen monoprotic carboxylic acids were isolated from the reaction mixture obtained by oxidation of glucose with oxygen in 18 % sodium hydroxide whereas nineteen acids were obtained from cellobiose under the same conditions. The main products from glucose were formic, aldonic, glycolic and glyceric acids. In addition to these acids substantial amounts of aldobionic, 3,4-dihydroxybutyric, 3-deoxypentonic and glucoisosaccharinic acids were isolated from the experiment with cellobiose.

The aging of alkali cellulose is an important technical process in which cellulose, impregnated with strong sodium hydroxide solution, is subjected to air oxidation at moderate temperatures. To elucidate the chemical reactions which occur during this process, model experiments were carried out with glucose and cellobiose. The results are presented in this paper; those obtained in parallel experiments with cellulose will be described elsewhere.

PROCEDURE

Two flasks, each containing 1 liter of an 18 % sodium hydroxide solution, were connected to a large reservoir with oxygen at a constant pressure of 1 atmosphere. Intimate mixing of gas and liquid was achieved by means of magnetic stirrers run at such a high speed that the gas was distributed throughout the solution in the form of small bubbles. After one hour D-glucose (4.75 g) or D-cellobiose (9.00 g) was added and the oxidation was carried out at 25°C.

The reaction of the sugars was followed by withdrawal of samples (200 ml) after various reaction times. The samples were passed through columns containing 1 liter of Dowex 50−X8 cation exchanger in the H^+ form and washed out with distilled water to neutral reaction of the effluent.

One tenth of the effluent was neutralized with sodium hydroxide by means of an autotitrator and the lactones formed were saponified by keeping the pH at 8 for 4 h. After concentration under vacuum at 35°C samples containing about 0.20 mequiv. of acids were chromatographed in 0.08 M sodium acetate at pH 5.9 and 0.50 M acetic acid on an analytical column with automatic recording as previously described.[1,2] Before elution with sodium acetate the sugars were washed out with water.

The rest (90 %) of the oxidized solutions was concentrated under vacuum at 35°C without foregoing titration. Consequently volatile acids were lost in this step. The concentrated solutions were chromatographed on a preparative column (15×900 mm)

filled with Dowex 1—X8, 40—60 μm in the acetate form and the eluate analyzed and worked up as usual.[3]

For further identification, the aldonic acids were studied as fully trimethylsilyl (TMS) substituted 1,4-lactones [4] by gas chromatography-mass spectrometry. Some other acids were studied by the same method using the TMS-ethers of lactones and TMS-esters.

The sugar fraction was examined by partition chromatography on an anion exchanger in the sulfate form.[5] The same method was used for sugars formed during hydrolysis of aldobionic acids and disaccharides. These hydrolyses were made in 0.1 N sulfuric acid at 130°C and a concentration of 2—5 mg organic substance per ml of mineral acid. The time of hydrolysis was 1 h for the disaccharides, otherwise 5 h.

SEPARATION AND IDENTIFICATION OF ACIDS

The organic acids obtained from glucose after 26 h treatment were chromatographed on anion exchange columns using 0.08 M sodium acetate as eluent. On the chromatogram from the automated column seven well separated bands were recorded (Fig. 1), whereas on the preparative column which was over-loaded to obtain sufficient amounts for identification, a serious overlapping occurred between some neighbouring elution curves. The fractions from the preparative column, cut as indicated in Fig. 1, were rechromatographed in 0.5 M acetic acid. The first fraction gave a chromatogram with six separated bands (Table 1). Two of these appeared much ahead of the others indicating that these contained 2-deoxy-acids.[2] The D_v-values indicated that the first compound was 2-deoxy-*erythro*-pentonic acid. This was confirmed by gas chromatography and mass spectrometry of both the TMS-substituted lactone and the TMS-ester. The D_v-values of the second compound were in agreement with those obtained in parallel runs with an authentic sample of 3,4-dihydroxybutyric acid and the identity of this compound was confirmed by paper chromatography.

The third band from the elution with acetic acid (1:S3) was rechromatographed in sodium acetate and gave three well separated peaks; each of the three last bands gave rise to two peaks. Some overlapping with neighbouring

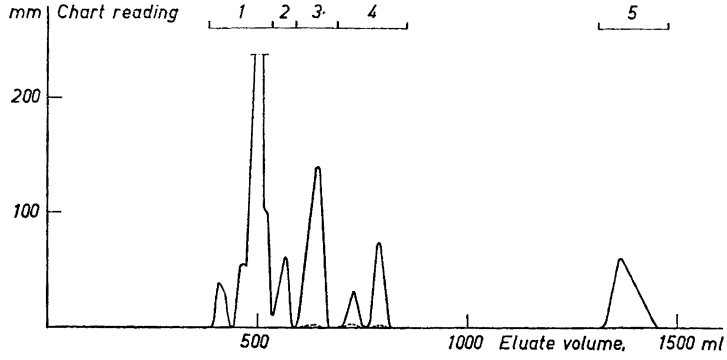

Fig. 1. Chromatographic separation of organic acids formed from glucose. Bands cut according to the figure. Added amount of acids: 0.20 mequiv. Analysed by chromic acid (full line) and carbazole method (broken line). Column: 6 × 1350 mm, Dowex 1—X8, 26—32 μm. Eluent: 0.08 M sodium acetate (pH 5.90). Flow rate: 5.2 ml cm^{-2} min^{-1}.

Table 1. Ion exchange chromatography and paper chromatography on chromatographic bands (s) and reference substances (r). Experiments with glucose.

Band number	Mg	Reference substance	D_v in ion exchange chromatography 0.08 M NaAc s	0.08 M NaAc r	0.5 M HAc s	0.5 M HAc r	Paper chromatography R_F s	Paper chromatography R_F r
1:S1	5	2-Deoxy-D-*erythro*-pentonic	8.45	8.44	2.67	2.68		
1:S2	3	3,4-Dihydroxybutyric	9.12	9.31	3.42	3.39	0.33	0.34
1:S3:A1	4	β-D-Glucometasaccharinic	7.53	7.59	9.5	9.52		
1:S3:A2	2	3-Deoxy-*erythro*-pentonic	8.39	8.27	9.5	9.56		
1:S3:A3	4	D-Ribonic	9.14	9.24	9.5	9.17		
1:S4:A1	2	D-Gluconic	7.26	7.21	12.3	12.5		
1:S4:A2	4	D-Arabinonic	8.93	8.89		14.2		
1:S5:A1	2	D-Gluconic	7.25	7.21		12.5		
1:S5:A2	140	D-Arabinonic	8.91	8.89	14.1	14.2	0.07, 0.25	0.06, 0.27
1:S6:A1	17	D-Mannonic	9.38	9.49	17.9	17.5	0.10	0.10
1:S6:A2	3		9.97		17.9			
2:S1	0.5		10.4		15.9			
2:S2	18	D-Erythronic	10.4	10.4	18.9	18.8	0.10, 0.25, 0.40	0.11, 0.25, 0.39
3:S1	3		12.0		15.4			
3:S2	46	Glyceric	12.0	11.9	19.7	20.2	0.27	0.27
4:S1	10	Lactic	13.8	13.8	15.3	15.1	0.65	0.65
4:S2	32	Glycolic	14.9	14.8	17.6	17.6	0.48	0.47
5		Formic	26.5	26.5	42.5	42.4		

$D_v = \overline{V}/x - 0.39$ where $\overline{V}$ = eluate volume and x = column volume (*cf.* Ref. 27).
R_F = Migrations relative to the front in ethyl acetate–acetic acid–water (100:13:10). Spray reagent permanganate–periodate.

bands from the acetic acid elution explains the fact that gluconic and arabinonic acids appeared in two fractions in the final separation with sodium acetate. A minor compound (1:S6:A2) was not identified. The identity of the other compounds was established from the chromatographic data given in the table and from the mass spectra of their TMS-derivatives. The same methods were used for the identification of erythronic acid which was the predominant component in band 2 from the preparative acetate column. Glyceric, lactic, glycolic, and formic acids were identified from the chromatographic data listed in the table and by typical color tests.[6] Minor amounts of unknown acids were present in bands 2:S1 and 3:S1. The isolated amount of unidentified acids was less than 3 % of the total amount of non-volatile monoprotic acids.

A separation of the acids formed from cellobiose after 19.5 h treatment gave a chromatogram with 13 peaks on the automated column (Fig. 2) while the run on the preparative column resulted in seven peaks, which were cut as indicated in the figure and rechromatographed in 0.5 M acetic acid.

The first fraction, which showed chromatographic behavior and a carbazole reaction characteristic of aldobionic acids, gave a chromatogram with two overlapping and one well separated band. After acid hydrolysis the two overlapping bands (1:S1—2) were rechromatographed in acetic acid and sodium acetate and were found to contain the following acids: D-gluconic (4 mg), D-arabinonic (92 mg), and D-mannonic acid (30 mg). Only one acid, erythronic acid (14 mg), was found in the hydrolyzate from the last band (1:S3). The identity of the simple aldonic acids was established as described above. The only sugar formed in the hydrolyzates was D-glucose. The positions of 3-*O*-(β-D-glucopyranosyl)-D-arabinonic and 4-*O*-(β-D-glucopyranosyl)-D-gluconic acid corresponded to those of authentic samples.

Band 2 contained the two glucoisosaccharinic acids which were identified as shown in Table 2. The chromatographic data used for the identification of the acids in band 3 are listed in the same table. In addition mass spectrometric identification was used for the three acids in band 3:S4. An unknown acid, 3:S8, gave a carbazole reaction. The other acids listed in the table were identified as described above.

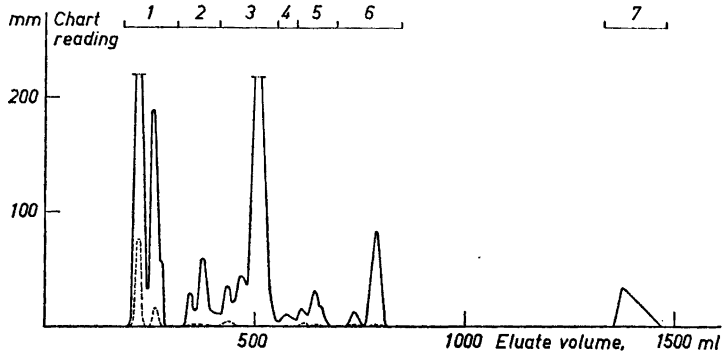

Fig. 2. Organic acids formed from cellobiose, separated under the same conditions as in Fig. 1.

Table 2. Ion exchange chromatography and paper chromatography on chromatographic bands (s) and reference substances (r). Experiments with cellobiose.

Band number	Mg	Reference substance	D_v in ion exchange chromatography 0.08 M NaAc s	0.08 M NaAc r	0.5 M HAc s	0.5 M HAc r	Paper chromatography R_F s	Paper chromatography R_F r
1:S1	60	4-*O*-(β-D-Glucopyranosyl)-D-mannonic	4.19		5.44			
1:S2	185	3-*O*-(β-D-Glucopyranosyl)-D-arabinonic	3.7	3.66	5.9	6.00		
	8	4-*O*-(β-D-Glucopyranosyl)-D-gluconic	3.7	3.71	5.9	5.86		
1:S3	35	2-*O*-(β-D-Glucopyranosyl)-D-erythronic	3.58		8.52			
2:S1	11	α-D-Glucoisosaccharinic	6.05	6.06	6.15	6.09	0.05, 0.34	0.05, 0.34
2:S2	26	β-D-Glucoisosaccharinic	6.59	6.46	14.9	14.8	0.05, 0.34	0.05, 0.34
3:S1	2		7.49		2.17			
3:S2–3:A1	3	2-Deoxy-D-*erythro*-pentonic	8.44	8.27	2.67	2.65		
3-S2–3:A2	80	3,4-Dihydroxybutyric	9.34	9.31	3.37	3.39	0.33	0.33
3:S2–3:A3	2		10.0		3.43			
3:S4:A1	2	β-D-Glucometasaccharinic	7.55	7.59	9.5	9.56		
3:S4:A2	9	3-Deoxy-*erythro*-pentonic	8.42	8.41	9.5	9.52		
3:S4:A3	3	D-Ribonic	9.06	9.24	9.5	9.17		
3:S5	40	3-Deoxy-*threo*-pentonic	9.05	9.17	11.7	11.8	0.15, 0.40	0.15, 0.40
3:S6	50	D-Arabinonic	8.88	8.89	14.2	14.2	0.06, 0.28	0.06, 0.27
3:S7	4	D-Mannonic	9.39	9.49	17.8	17.5	0.10	0.10
3:S8	12		7.62		22.6			
4	3	D-Erythronic	10.4	10.4	18.9	18.8		
5:S1	21	Glyceric	11.9	11.9	20.1	20.2	0.27	0.27
5:S2	14		11.2		28.5			
6:S1	1				9.58			
6:S2	5	Lactic	13.7	13.8	15.3	15.1	0.68	0.68
6:S3	80	Glycolic	14.9	14.8	17.6	17.6	0.52	0.52
7		Formic	26.5	26.5	42.0	42.5		

$D_v = \overline{V}/x - 0.39$ where $\overline{V}$ = eluate volume and x = column volume (*cf.* Ref. 27).
R_F = Migrations relative to the front in ethyl acetate–acetic acid–water (100:13:10). Spray reagent permanganate-periodate.

Two unknown peaks were recorded in addition to the one described above. One of these (5:S2) gave a carbazole reaction and contained an appreciable amount of acid. The fully TMS-substituted TMS-ester was resolved by gas chromatography into one major and several minor peaks. The weight of the identified acids corresponded to about 95 % of the total amount of isolated non-volatile acids.

DISCUSSION

Degradation of glucose. A study of the sugar composition in the reaction mixture obtained after oxygen had been passed through the alkaline glucose solution for 26 h showed that, as expected, small amounts of mannose and fructose were present in addition to glucose. This is explained by the well-known Lobry de Bruyn-Alberda van Ekenstein transformations. More interesting is the fact that an appreciable amount of arabinose was formed. As shown by Ishizu, Lindberg and Theander,[7] arabinose is formed in small amounts when fructose is kept in calcium hydroxide solution at room temperature even in the absence of oxygen. Likewise, it is known that treatment of amylopectin with peroxide in alkaline medium,[8] as well as the attack of alkaline hypochlorite upon methyl-β-D-glucopyranoside [9] and cellulose,[3] gives rise to arabinose and arabinose end groups, respectively.

The results obtained from studies of the various monoprotic acids formed are given in Table 1. The formation of large amounts of formic acid during the treatment of glucose with oxygen in alkaline medium has been well established in previous investigations by various authors [10,11] and was confirmed in the present work. As shown by Spengler and Pfannenstiel,[12] formic acid is formed by a cleavage of the bond between carbon atoms 1 and 2 with the formation of arabinonic acid, which can be prepared in high yield under conditions suitable for this purpose. In the present work arabinonic acid constituted about 50 % of the fraction of nonvolatile acids isolated from the reaction mixture.

Ribonic acid seems to have escaped observation in previous investigations with more primitive isolation methods. In the present work it was found that an amount of about 2 % was present in the acid fraction. The occurrence of small amounts of this acid can be explained either by a C-2 epimerization of arabinonic acid or by a common precursor which gives rise to both pentonic acids.

The presence of erythronic acid was observed in a paper chromatographic study by Dubourg and Naffa [10] and its presence was confirmed in our work. Since arabinonic acid was found to be stable against oxidation under their working conditions, the authors concluded that erythronic acid was formed, together with glycolic acid, by a cleavage of the bond between carbon atoms 2 and 3 in the glucose molecule. The amount of glycolic acid formed both in their work with fructose and in our experiments with glucose was larger than that of erythronic acid, which indicates that glycolic acid must be formed in other reaction paths as well.

Another possible reaction path is the formation of erythronic acid *via* arabinose by a reaction mechanism analogous to that responsible for the forma-

tion of arabinonic acid from glucose. As shown by Hardegger, Kreis and El Khadem [13] erythronic acid is formed in a rather high yield from arabinose and since appreciable amounts of arabinose were present in the reaction mixture, this reaction path is probably important. It is likely that a detailed kinetic study would permit a final decision concerning the relative importance of the reaction paths responsible for the formation of erythronic acid.

The formation of mannonic and gluconic acids does not seem to have been observed by earlier investigators. Interestingly, mannonic acid is present in much larger amounts than gluconic acid. Since mannose was present only in small amounts in the reaction mixture and the oxidation of mannose seems to be slower than that of glucose,[10] the reaction path *via* mannose can probably be disregarded. Gluconic acid is partially converted into mannonic acid in alkaline medium by C-2 epimerization and vice versa. The equilibrium composition in strong sodium hydroxide is not known, but in all experiments reported in the literature gluconic acid predominates. It can be concluded therefore that mannonic acid is not formed *via* gluconic acid, but rather that the gluconic acid can be formed from mannonic acid by epimerization. Another and more likely explanation is that both are formed *via* a common precursor, probably D-glucosone, which in a recent paper by Abenius, Ishizu, Lindberg and Theander [14] was postulated to be a primary oxidation product when glucose is oxidized with polysulfide in strongly alkaline solution. Unpublished investigations by Lindberg and Theander show that decomposition of D-glucosone in alkaline medium results in much larger amounts of mannonic than of gluconic acid. It is noteworthy that also in the decomposition of glucosone in sodium hydroxide arabinonic acid was the predominant acid and that substantial amounts of erythronic acid and arabinose were formed as well. Hence, the results given above support the assumption that D-glucosone is an intermediate also under our working conditions.

Glyceric acid which was found in large amounts has been observed previously in paper chromatographic studies,[10] although other authors reported the absence of this acid.[15] The results given in the table confirm the previously reported observations that lactic acid is formed when glucose is treated with oxygen in alkaline medium.[15] It is worth mentioning that lactic acid is formed in the absence of oxygen also.[16,17] Possible reaction paths are discussed in a review by Sowden.[18]

In the absence of an oxidant the reaction between glucose and alkali gives rise to glucometasaccharinic acid.[17] The amount obtained by treatment of glucose with oxygen in the presence of strong alkali (Table 1) was very small, however. Only the β-form could be isolated in weighable amounts. As shown by Machell and Richards [19] 3-deoxy-D-glucosone, an intermediate in the formation of glucometasaccharinic acids is oxidized by hydrogen peroxide in alkaline medium and gives rise to 2-deoxy-D-*erythro*-pentonic and formic acids. The oxidation of 3-deoxy-D-glucosone explains both the presence of this deoxypentonic acid in the reaction mixture and the low yield of glucometasaccharinic acid.

Glucoisosaccharinic and glucosaccharinic acids are known to be formed by rearrangements of glucose in the presence of alkali.[16] These acids were not recorded in our experiments in the presence of oxygen. If these had been

formed in appreciable amounts they should not have escaped observation. A likely explanation is that various intermediates which appear in the reaction mixture are oxidized. As shown by Machell and Richards [20] 4-deoxy-D-*glycero*-2,3-hexodiulose, which is an intermediate in the formation of these acids, is oxidized by peroxide in alkaline medium. The reaction gives rise to 3,4-dihydroxybutyric and glycolic acids which can explain the fact that small amounts of this dihydroxybutyric acid were detected in the reaction mixture. The amount of glycolic acid formed in this reaction (together with the amount which can be formed in the reaction giving rise to erythronic acid as discussed above) is much smaller than that observed by analysis. This shows that glycolic acid is formed in other fragmentation reactions as well.

A trace amount of 3-deoxypentonic acid was isolated and identified by mass spectrometry. The chromatographic behavior showed that it was the *erythro* form. Machell and Richards [20] suggested that the formation of 3-deoxypentonic acids from 4-deoxy-D-*glycero*-2,3-hexodiulose is associated with the formation of formic acid. Another possibility under our working conditions would be that the deoxypentonic acids are formed *via* a β-hydroxyelimination from arabinose, *i.e.* in a reaction path analogous to that which gives rise to glucometasaccharinic acid.

Chromatograms run on the reaction mixture after various times of reaction showed that after a comparatively short time of reaction (7 h) the percentages of β-metasaccharinic and lactic acids in the acid fraction were higher than after the longest time of reaction (50 h). After 7 h a significant peak was recorded at the position corresponding to α-metasaccharinic acid whereas (with the same total amount of acids applied to the chromatographic column) no significant peak at this position was recorded with the final reaction mixture. These results indicate that there is a time-lag before the oxidation reactions reach their maximum rates which is compatible with the assumption [10] that at least some of these oxidations occur *via* peroxides.

Degradation of cellobiose. Glucose is known to be formed when cellobiose is treated with lime water [21] or sodium hydroxide solution.[22] The chromatogram given in Fig. 3 shows that under our working conditions large amounts

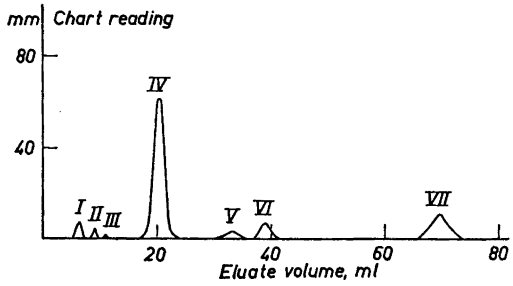

Fig. 3. Chromatographic analysis of the carbohydrates formed from cellobiose. Column: 4×870 mm, Dowex 1−X8, in the sulfate form, 20−24 μm. Eluent: 85 % ethanol, 94°C. Flow rate: 3.9 ml cm^{-2} min^{-1}. I: Arabinose. II: Fructose. III: Mannose. IV: Glucose. V: 3-*O*-(β-D-Glucopyranosyl)-D-arabinose and 4-*O*-(β-D-Glucopyranosyl)-D-fructose. VI: 4-*O*-(β-D-Glucopyranosyl)-D-mannose. VII: Cellobiose.

of glucose were present in the reaction mixture after 19.5 h. As could be expected from the results presented in the previous paragraph, fructose, mannose, and arabinose were recorded as well. The last elution band corresponded to unreacted cellobiose. In addition two other elution bands with positions expected for disaccharides were recorded. The fractions were isolated and hydrolyzed. The first band gave rise to D-arabinose (35 %), D-fructose (15 %) and D-glucose (50 %) and should therefore contain a mixture of 3-*O*-(β-D-glucopyranosyl)-D-arabinose and 4-*O*-(β-D-glucopyranosyl)-D-fructose. The second band gave equal amounts of D-glucose and D-mannose and should hence be 4-*O*-(β-D-glucopyranosyl)-D-mannose. The disaccharides isolated from the reaction mixture were those expected from the results obtained with glucose.

Like the reaction with glucose, that between cellobiose and oxygen in alkaline medium gave rise to large amounts of formic acid. The aldobionic acids constituted the predominant fraction of the non-volatile acids, calculated on a weight basis. Within this group 3-*O*-(β-D-glucopyranosyl)-D-arabinonic acid was formed in much larger amounts than any of the other acids. This acid has been prepared previously in high yields from cellobiose by oxidation with oxygen in barium hydroxide solution.[11] As can be seen from Table 2 appreciable amounts of 4-*O*-(β-D-glucopyranosyl)-D-mannonic and 2-*O*-(β-D-glucopyranosyl)-D-erythronic acids were isolated. The amount of cellobionic acid was small. A comparison of these results with the formation of aldonic acids in the reaction with glucose and oxygen in alkaline solution indicates that the same reaction sequence is responsible for the formation of both the aldobionic acids and the simple aldonic acids from glucose. There are some differences between the ratios of the individual aldonic acids formed from glucose and those of the corresponding aldobionic acids formed from cellobiose. These can be ascribed in part to reaction paths which compete with the formation of aldobionic acids. As an example, the decomposition of 3-*O*-(β-D-glucopyranosyl)-D-arabinose mentioned below competes with the formation of 2-*O*-(β-D-glucopyranosyl)-D-erythronic acid. Likewise the liberation of glucose from 4-*O*-(β-D-glucopyranosyl)-D-fructose by β-alkoxyelimination competes with the formation of aldobionic acids.

From Table 2 it is seen that in addition to aldobionic acids, appreciable amounts of aldonic acids were formed from cellobiose. Among these, arabinonic acid was predominant, but ribonic, mannonic, and erythronic acids were present as well. There was probably a slight amount of gluconic acid formed, but the amount was too small for identification. In a separate experiment in which cellobionic acid was treated with oxygen in 18 % sodium hydroxide for 50 h and subsequently treated in exactly the same way as in the run with cellobiose, it was found that no aldonic acids or other reaction products could be detected on the chromatograms. Obviously, the cellobionic acid was not affected under these conditions nor under the passage through the cation exchanger. This does not necessarily mean that cellobionic acid is stable when treated with oxygen in the presence of carbonyl compounds which in the case of cellulose are known to catalyze the oxidation.[23] When cellobionic acid was kept in sodium hydroxide containing 1 % hydrogen peroxide for 25 h appreciable amounts of acidic oxidation products were

recorded on the chromatogram from a run in 0.08 M sodium acetate. Some acids were not recovered in this medium but were easily eluted with 0.2 M magnesium acetate. A substantial amount of monosaccharides was recorded.

It is most likely that in the run with cellobiose the simple aldonic acids originate from the glucose formed as an intermediate. Considering the great difficulties in the determination of minor aldonic acids in the complex reaction mixture, the relative amounts of aldonic acids are in fairly good agreement with those obtained in the experiment with glucose.

One reaction path for the formation of glucose is indicated by the presence of appreciable amounts of α- and β-glucoisosaccharinic acids. As mentioned these acids were not detected in the experiments with glucose. Corbett and Kenner have shown, however, that they belong to the main acidic degradation products obtained when cellobiose is treated with alkali in the absence of oxygen.[21] These acids are formed by a rearrangement of cellobiose to 4-*O*-(β-D-glucopyranosyl)-D-fructose which, as mentioned, was present in the reaction mixture. This rearrangement is followed by a cleavage of the glycosidic bond by a β-alkoxyelimination. This results in the formation of one glucose molecule per molecule of glucoisosaccharinic acid. Instead of being transformed into isosaccharinic acids, the fructose unit can be subjected to fragmentation reactions, both in the absence and presence of oxidizing agents.

It is well known that in the absence of oxidizing agents an alkoxyl group or a glycosidic bond at C-4 favors the formation of isosaccharinic acids and depresses the relative amount of fragmentation products such as lactic acid. This explains the fact that despite the competing oxidation of the intermediate dicarbonyl compound, appreciable amounts of isosaccharinic acids were obtained when the reaction was carried out in the presence of oxygen. If, under our conditions, the oxidation of the 4-deoxy-D-*glycero*-2,3-hexodiulose intermediate is as important as the results obtained with glucose indicate, the amount of 3,4-dihydroxybutyric acid should be larger in the experiments with cellobiose than that of isosaccharinic acids. This is confirmed by the results given in Table 2. This reaction scheme would also require an appreciable formation of glycolic acid (in addition to that connected with the formation of erythronic and 2-*O*-(β-D-glucopyranosyl)-D-erythronic acids). The experiments confirm that just as in the run with glucose the amount of glycolic acid is larger than required by the two types of reaction. Calculated as the mole percentage of all acids formed, the excess is less than that obtained with glucose. This indicates that at least the main part of the glycolic acid not accounted for in these reaction schemes is formed by fragmentation of liberated glucose molecules.

From the results obtained with glucose trace amounts of metasaccharinic and 2-deoxypentonic acids and small amounts of lactic acid were expected. The results given in Table 2 confirm this assumption. The fact that the relative amounts of lactic and glyceric acids were much smaller than in the experiment with glucose indicates that in the run with cellobiose these acids were formed mainly from liberated glucose.

Another interesting difference in the composition of the reaction mixture is that large amounts of 3-deoxy-pentonic acids were formed in the experiment with cellobiose. The *threo*-form dominated, but appreciable amounts

of the *erythro*-form were isolated as well. It has earlier been shown that these acids are formed rapidly by the influence of alkaline solutions upon 3-*O*-substituted pentoses such as 3-*O*-methyl-D-xylose [24] and D-ribose-3-phosphate [25] after β-elimination. It is likely that the 3-*O*-(β-D-glucopyranosyl)-D-arabinose obtained as an intermediate in our experiments reacts in a similar manner and gives rise to 3-deoxypentonic acids and glucose. These deoxypentonic acids are formed by alkaline treatment of hexoses as well,[7] but the amounts are small and it is likely that the reaction path *via* the disaccharide predominates.

The presence of diprotic acids in the reaction mixture was established in a chromatographic study of a reaction mixture obtained by oxidation for 50 h. After elution of the monoprotic acids with sodium acetate an elution was made with 0.2 M magnesium acetate which is an effective eluent for dibasic acids that complex with magnesium ions.[26] At least eight different compounds were eluted. The consumption of chromic acid by these fractions was only about 5 % of that recorded for the isolated monoprotic acids. No attempts were made to isolate and identify these acids.

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REFERENCES

1. Johnson, S. and Samuelson, O. *Anal. Chim. Acta* **36** (1966) 1.
2. Samuelson, O. and Thede, L. *J. Chromatog.* **30** (1967) 556.
3. Norstedt, I. and Samuelson, O. *Svensk Papperstid.* **68** (1965) 565.
4. Petersson, G., Samuelson, O., Anjou, K. and von Sydow, E. *Acta Chem. Scand.* **21** (1967) 1251.
5. Larsson, L. I. and Samuelson, O. *Acta Chem. Scand.* **19** (1965) 1357.
6. Feigl, F. *Spot Tests in Organic Analysis*, Elsevier, Amsterdam 1960.
7. Ishizu, A., Lindberg, B. and Theander, O. *Acta Chem. Scand.* **21** (1967) 424.
8. Whistler, R. L. and Schweiger, R. *J. Am. Chem. Soc.* **81** (1959) 3133.
9. Theander, O. *Svensk Papperstid.* **61** (1958) 581.
10. Dubourg, J. and Naffa, P. *Bull. Soc. Chim. France* **1959** 1353.
11. Hardegger, E., Kreis, K. and El Khadem, H. *Helv. Chim. Acta* **35** (1952) 618.
12. Spengler, O. and Pfannenstiel, A. *Z. Wirtschaftsgruppe Zuckerindustrie, Tech. Tl.* **85** (1935) 546.
13. Hardegger, E., Kreis, K. and El Khadem, H. *Helv. Chim. Acta* **34** (1951) 2343.
14. Abenius, P. H., Ishizu, A., Lindberg, B. and Theander, O. *Svensk Papperstid.* **70** (1967) 612.
15. Warshawsky, B. and Sandstrom, W. M. *Arch. Biochem. Biophys.* **37** (1952) 46.
16. Kenner, J. and Richards, G. N. *J. Chem. Soc.* **1954** 1784.
17. Green, J. W. *J. Am. Chem. Soc.* **78** (1956) 1894.
18. Sowden, J. C. *Advan. Carbohydrate Chem.* **12** (1957) 35.
19. Machell, G. and Richards, G. N. *J. Chem. Soc.* **1960** 1938.
20. Machell, G. and Richards, G. N. *J. Chem. Soc.* **1960** 1932.
21. Corbett, W. M. and Kenner, J. *J. Chem. Soc.* **1955** 1431.
22. Lindberg, B., Theander, O. and Uddegård, J. E. *Svensk Papperstid.* **69** (1966) 360.
23. Entwistle, D., Cole, E. H. and Wooding, N. S. *Textile Res. J.* **19** (1949) 609.
24. Corbett, W. M., Richards, G. N. and Whistler, R. L. *J. Chem. Soc.* **1957** 11.
25. Brown, D., Hayes, F. and Todd, A. *Chem. Ber.* **90** (1957) 936.
26. Lee, K. S. and Samuelson, O. *Anal. Chim. Acta* **37** (1967) 359.
27. Samuelson, O. *Ion Exchange Separations in Analytical Chemistry*, Almqvist & Wiksell, Stockholm; Wiley, New York 1963.

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