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Formation of Glucopyranosylglycolic Acids During the Hydrolysis of Cellulose

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En lösning av glukos och glykolsyra i saltsyra ger vid indunstning vid 35° α- och β-formerna av D-glukopyranosylglykolsyra. Polymeriserad glykolsyra bildas också. Dessa reaktioner ger upphov till komplikationer vid analys av cellulosamaterial.

A solution of glucose and glycolic acid in hydrochloric acid gives rise to the α- and β-forms of D-glucopyranosylglycolic acid when evaporated at 35°. Polymerized glycolic acid is formed as well. These reactions give rise to complications in analyses of cellulose materials.

Eine Lösung von Glukose und Glykolsäure in Salzsäure gibt beim Eindunsten bei 35°C die α- und β-Formen der D-Glukopyranosylglykolsäure; hierbei wird auch polymere Glykolsäure gebildet. Derartige Reaktionen geben Anlass zu Komplikationen bei der Analyse von Cellulosematerialien.

In studies of uronic and aldonic acids in hydrolyzates from wood pulps and cotton cellulose the formation of reversion products with sugars gives rise to complications (1, 2). The glycosidic links formed between the sugars and the acids can be broken by acid hydrolysis, but during this treatment a destruction of uronic acids cannot be avoided.

Glycolic acid has been found to be present in all hydrolyzates from cellulose materials investigated in this laboratory and for this reason it was of interest to study whether this acid can give reversion products with glucose under the conditions applied during the hydrolysis.

Experimental

Glucose (10 g) and glycolic acid (1 g) were dissolved in concentrated hydrochloric acid (30 ml) and then most of the hydrochloric acid was evaporated under vacuum at 35° (5 hours). The remaining sirup was dissolved in water and passed through a column filled with an anion exchanger in its acetate form. The column was washed with water until the effluent gave a negative test with anthrone. The monoprotic organic acids were eluted with 5 M acetic acid whereas the chloride remained on the column. The effluent (containing mainly sugars) and the eluate were evaporated under vacuum. Both fractions were then neutralized with sodium hydroxide and were kept at pH 8 for 5 hours at room temperature [i.e. treated by the method previously recommended for the saponification of lactones (3)]. The sugar fraction was passed through an anion exchanger in its acetate form and the resin was washed with water until the sugars were removed. The eluate fraction was then passed through the same column, and after the column was washed with water the monoprotic organic acids were eluted with 0.1 M sodium acetate. After passage through a H^+ cation exchanger the solution was evaporated under reduced pressure at 35°.

The acid fraction was neutralized and kept at pH 8 as described above and then separated by anion exchange chromatography in 0.08 M sodium acetate with acetic acid added to pH 5.9. Separations were carried out on both preparative and analytical columns. After removal of the sodium ions and evaporation to dryness at 35° the bands obtained from the preparative run were rechromatographed in 0.5 M acetic acid. The D_v values (4) in both media were determined from runs on analytical columns with automatic analysis of the eluate (5). All bands gave a positive carbazole reaction. Unless otherwise mentioned the isolated fractions were kept at pH 8 as described above before being applied to the columns.

Two of the acids produced were studied by gas chromatography and mass spectrometry as trimethylsilyl (TMS) derivatives. These were prepared in dry pyridine solution with bis(trimethylsilyl)acetamide as the silylating agent (6). The reaction mixtures were kept over night at room temperature and the derivatives studied on a Perkin-Elmer 800 gas chromatograph with flame ionization detector.

The mass spectra of the TMS-derivatives were recorded at 70 eV on an LKB 9000 gas chromatograph-mass spectrometer.

Results

The chromatogram reproduced in *Fig. 1* shows that a complex mixture of acids was formed after evaporation of glycolic acid with glucose in hydrochloric acid. The fractions were numbered as indicated on the chromatogram. A parallel run in

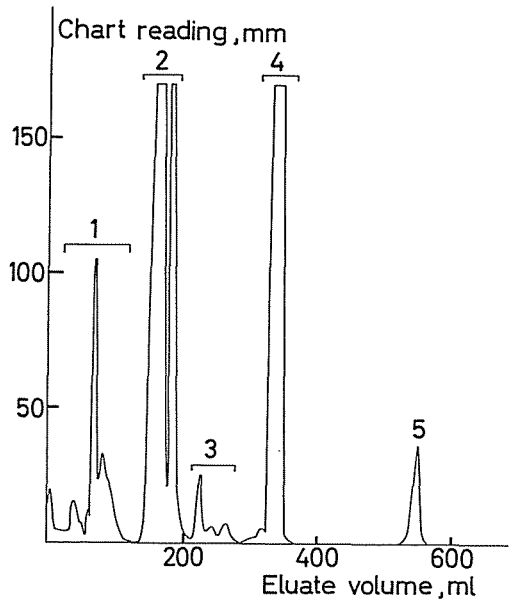

Fig. 1. Automatic analysis of organic acids from the treatment of 0.3 g glucose and 0.03 g glycolic acid with hydrochloric acid.
Resin bed: 6×725 mm; Dowex 1-X8, 13—18 μm.
Eluent: 0.08 M sodium acetate, pH 5.9.
Analysis by the chromic acid method.

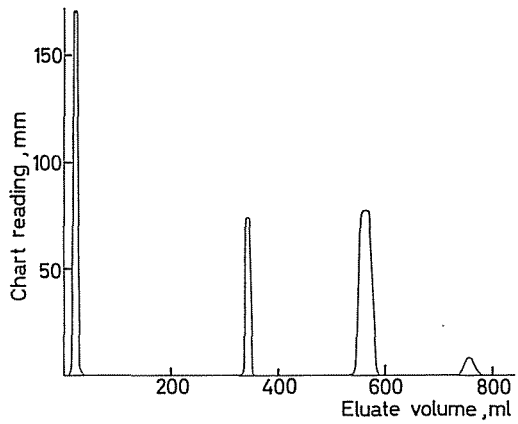

Fig. 2. Rechromatography of band 5 from Fig. 1.
Resin bed: 6×715 mm; Dowex 1-X8, 17—20 μm.
Eluent: 0.08 M sodium acetate, pH 5.9.
Analysis by the chromic acid method.

which the neutralization before the application of the acid mixture to the column was omitted was carried out on the analytical column. A new overlapping band was recorded in the tailing part of band 1. Bands 3 and 5 were found to be larger than in the run carried out with pretreatment at pH 8 [Fig. 1]. The results indicated that the acids which appeared in these positions were unstable in alkali. In a run in which the acid mixture was kept at pH 10 for 5 hours at room temperature and in a refrigerator over night, it was found that band 5 had disappeared completely and that only a trace amount of band 3 remained. The overlapping band in the tail of band 1 was also missing. The areas of bands 2 and 4 had increased.

From a run on a preparative scale after treatment at pH 8, bands were cut as indicated in Fig. 1 and characterized by various methods.

Band 4

Band 4 was identified as unchanged glycolic acid by its D_v values in sodium acetate ($D_v=16.5$) and acetic acid ($D_v=19.7$).

Band 5

As already mentioned the acid was unstable in alkali. Band 5 was therefore rechromatographed in sodium acetate without any treatment with sodium hydroxide. As can be seen from *Fig. 2* three major compounds and one minor compound were recorded indicating that the acid originally present in band 5 is unstable in acid medium as well. From the position on the chromatogram it can be concluded that no free acidic group was present in the first compound. The second band had the position of glycolic acid. The third band, which was the largest, had the same position as band 5 in Fig. 1. ($D_v=26.4$). The minor compound appeared at $D_v=37.1$.

In another experiment the mixture isolated from band 5 was kept at pH 8 by automatic titration with sodium hydroxide for 5 hours at room temperature. Sodium hydroxide was consumed during the course of the treatment indicating that a saponification of ester linkages occurred. An aliquot of the solution was then applied to an anion exchanger in the acetate form. Elution with sodium acetate gave one band with the position of glycolic acid and one band with the position of the original band 5. The areas of the bands were about equal. A trace amount of acid was eluted at $D_v=37.1$.

Boiling in 0.1 M sodium hydroxide for 3 hours resulted in a complete conversion into glycolic acid.

Evidently the acid mixture isolated from band 5 consisted of polymerized esters of glycolic acid. Both acidic and non-acidic (cyclic) products of this type are well known (7). Compounds with the same D_v values and properties as those obtained from the reversion experiment referred to above were recorded after treatment of glycolic acid with hydrochloric acid and subsequent evaporation. Likewise the compounds were found to be present in a commercial sample of glycolic acid which, according to the manufacturer, contained glycolide.

Band 2

Band 2 which gave typical sugar reactions when heated in acid medium was rechromatographed in 0.5 M acetic acid and gave rise to two major bands 2:S1 with $D_v=19.9$ and 2:S2 with $D_v=23.6$. The recovered amounts were 135 mg and 55 mg respectively, calculated on 1 g of added glycolic acid. The D_v values in 0.08 M sodium acetate (pH 5.9) were 7.6 and 8.4 respectively. A minor band was recorded at $D_v=7.9$ in acetic acid but this band was not studied further. The major compounds, which were yellowish, were dissolved in water and treated with active carbon. After having been freeze-dried both were an amorphous mass. The equivalent weights (263 and 256) were somewhat higher than that calculated for glucosidoglycolic acids (238). The titration curves indicated the presence of lactones which can explain the difference.

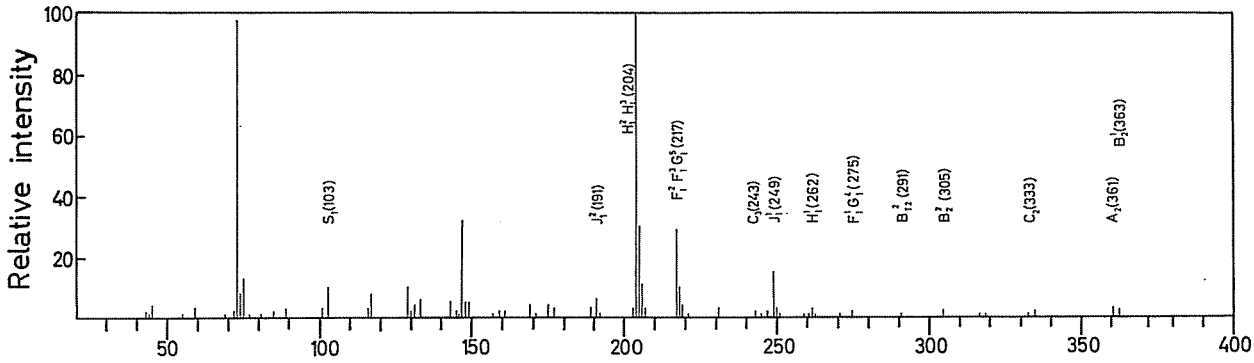

Fig. 3. Mass spectrum of the fully trimethylsilylated acid from band 2:S2 (β-D-glucopyranosylglycolic acid).

Both compounds were studied as TMS-derivatives by gas chromatography at 180° on a column with 3 % DC QF-1 on 100—120 mesh Gas Chrom Q. One predominant peak was recorded for each of the compounds indicating that the reducing group in the sugar units was blocked by a glycosidic bond. The retention times relative to that of 2,3,5,6-tetra-o-trimethylsilylglucono-1,4-lactone were 1.27 for 2:S1 and 1.51 for 2:S2.

Band 2:S1 was treated with 18 % sodium hydroxide at room temperature for 2 hours. The sodium hydroxide was removed by cation exchange and the recovered sample chromatographed in sodium acetate. About 90 % of the acid was recovered, which shows that the acid contained in band 2:S1 is quite stable in alkaline medium. Three minor products were recorded, one of which with the position of glycolic acid.

Heating of band 2:S1 in 0.1 N sulfuric acid at 130° for 3 hours gave rise to a complete hydrolysis to glycolic acid and glucose in about equal amounts. Hydrolysis for 2 hours at 110° gave an incomplete hydrolysis.

Band 2:S2 was treated with alkali as described above and exhibited an almost identical behavior. Treatment with 0.1 N sulfuric acid at 120° for 2 hours gave an almost complete hydrolysis to glycolic acid and glucose.

These results strongly indicate that the major acids contained in band 2 where the two expected isomers of glucosidoglycolic acid. The β-isomer has been described by Fischer and Helferich (8) [see also ref. (9)], whereas the α-isomer does not seem to have been isolated previously.

Investigations of the mass spectra of TMS-derivatives of partially methylated, ethylated and hydroxyethylated aldohexopyranoses (10, 11) revealed that the general fragmentation pattern is influenced to a slight extent only by the exchange of substituents. It therefore was deemed possible to obtain additional confirmation of the structure of the compounds contained in band 2 from the mass spectra of the TMS-derivatives. As in previous work the ion denotations of Kochetkov and Chizhov (12) will be applied.

The mass spectra of the two compounds were found to be almost identical. This confirms that the acids were diastereomers. The spectrum of band 2:S2 is reproduced in *Fig. 3*. The main features of the spectrum suggest a pyranose ring as basic structure. The two large peaks at $m/e=204$ and 217 are in full agreement with the observations made with TMS-substituted aldohexopyranoses (10) and indicate trimethylsilylated hydroxyls at C-2, C-3 and C-4. The structure of the ions are given in *Fig. 4*. The third large ion, recorded at $m/e=249$, is consistent with the formation of a very abundant J^1_1 ion with all the derivatives and indicates mass 147 for the substituent at C-1. The peaks at $m/e=103$ and $m/e=117$ obtained from the hexopyranose derivatives with a trimethylsiloxyl group at C-6 (10) are found in the spectrum in Fig. 3 as well. In addition to the peak at $m/e=249$ (191+58), peaks were obtained at $m/e=262$ (204+58) and $m/e=275$ (217+58) evidently arising from ions containing the substituent at C-1 (10). As could be anticipated from the bulkiness of this group, the ions ($m/e=361$) resulting from loss of the group at C-1 and elimination of trimethylsilanol give rise to the peak of highest intensity in the upper mass range of the spectrum.

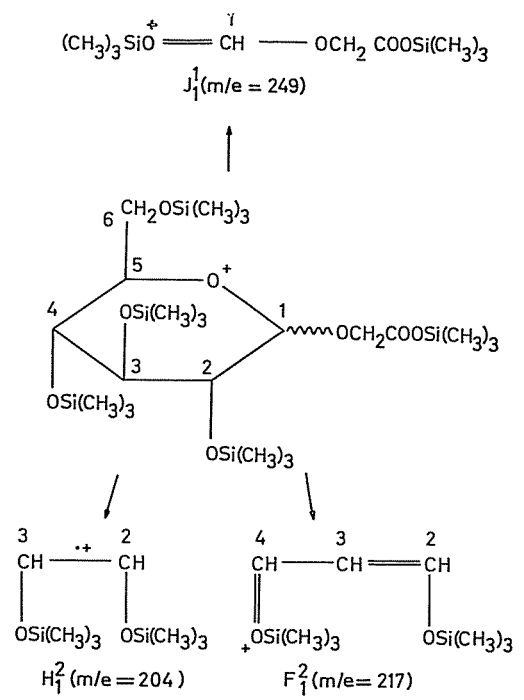

Fig. 4. Schematic representation of the origin of prominent ions from fully trimethylsilylated glucopyranosylglycolic acids.

The results confirmed that the acids contained in band 2 were the two anomeric forms of glucopyranosylglycolic acid. Determinations of the optical rotation in water gave the following results: Band 2:S1 $[\alpha]_D^{20} = +185^\circ$ (c=0.941); band 2:S2 $[\alpha]_D^{20} = -45^\circ$ (c=1.00). Fischer and Helferich found $[\alpha]_D^{21} = -44$ for the β-anomer. Evidently the acid present in the largest amount (2:S1) was α-D-glucopyranosylglycolic acid and the other acid (2:S2) the β-anomer.

Band 3

Band 3 constituted only a minor fraction of the acids. Acid hydrolysis (0.1 N H_2SO_4, 130°, 3 hours) gave rise to glycolic acid and glucose as the only hydrolysis products. Other experiments, in which both the time of contact of the reaction mixture (glucose+glycolic acid) with hydrochloric acid and the acid concentration were varied, showed that there was a correlation between the areas of bands 3 and 5 (polymerized glycolic acid). In a run in which band 5 was absent, band 3 was absent as well. These results, together with the observation already mentioned that band 3 disappeared and the amount of glucopyranosylglycolic acids increased when the original reaction mixture was kept at pH 10, strongly indicated that the acids contained in band 3 were esters of glucopyranosylglycolic and glycolic acids. This was confirmed in an experiment in which α-D-glucopyranosylglycolic and glycolic acids (1:50 w/w) in 5 M acetic acid were evaporated to dryness. Chromatography of the reaction mixture in sodium acetate showed a significant peak appearing at the same position as the major peak in band 3.

Band 1

Band 1 contained a complex mixture of higher reversion products (1) comprised of more than one glucose unit. No detectable amounts of band 1 were recorded in an experiment in which the ratio glucose: glycolic acid was 1:10 (instead of 10:1 in the experiment referred to in Fig. 1), whereas bands 2—5 were recorded in this run as well. No attempts were made to isolate the higher reversion products.

Conclusions

The results given above show that appreciable amounts of glucopyranosylglycolic acids are formed when a solution of glucose and glycolic acid in hydrochloric acid is evaporated under conditions similar to those applied in analyses of hydrolyzates from cellulose materials. Both α-D-glucopyranosylglycolic acid and β-D-glucopyranosylglycolic acid are comparatively stable toward acid hydrolysis. Unless their formation be considered, the results from the analyses can be misinterpreted. Moreover, minor amounts of the known polymerization products of glycolic acid were formed under the applied conditions. The ester linkages are rather stable towards alkali. Under the very mild conditions for lactone splitting (5 hours at pH 8 at room temperature) recommended to avoid an interfering destruction of sugars and uronic acids, their saponification is not complete. With fractions containing glycolic acid and its polymerization products but which are free from alkali labile compounds, a higher pH or temperature is recommended to complete the saponification of the ester linkages.

Esters of glucosidoglycolic acid with glycolic acid were also present. Complications due to the presence of these acids can be avoided if the compounds are saponified before being chromatographed.

Acknowledgements

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