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DETERMINATION AND IDENTIFICATION OF ALDEHYDE END-GROUPS IN CELLULOSE

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ABSTRACT

To determine the end-groups in cellulose, the sample is reduced with sodium borohydride, and, after acid hydrolysis and removal of the mineral acid, ethanol is added and part of the D-glucose is removed by crystallization. The solution is chromatographed on ion-exchange resins, and the alditols are recorded as discrete bands separated from D-glucose and various artefacts formed during the preceeding treatments.

INTRODUCTION

The aldehyde end-groups in cellulose can be conveniently reduced to alditol end-groups by reduction with sodium borohydride[1]. Acid hydrolysis of the cellulose, followed by determination of the alditols, would permit the determination and identification of the original end-groups. Several methods are available for the determination of alditols in the presence of glucose[2–6], but no attempts seem to have been made to determine trace amounts of alditols in the presence of large amounts of glucose and products formed during acid hydrolysis.

In earlier papers, it was shown that partition chromatography on anion exchangers in the sulphate form[7] and cation exchangers in the lithium form[8] can be used for the determination of alditols in the presence of monosaccharides. On the basis of this work, a method has been devised for the determination of trace amounts of alditols in solutions of D-glucose and applied to the determination of aldehyde end-groups in cellulose.

MATERIALS

The alditols were the same as used previously[8]. With the amounts applied to the chromatographic column, no significant amounts of impurities were detected. D-Glucose (analytical reagent grade) was applied in much larger amounts and, in agreement with earlier observations[9], the sample was found to contain impurities which were recorded under the present working conditions.

The cotton sample, which was of Peruvian origin, was purified by solvent

extraction and boiling[10] with mild alkali. The sample was washed with acetic acid and water before drying, and therefore contained lactone groups[11]. Samples of cotton degraded[12] by aging as alkali cellulose were also investigated. These samples were not treated with acid before drying.

The reduction with sodium borohydride was carried out in 0.2M solution for 90 h at 25° at a cellulose–liquid ratio of 1:50. A pretreatment in an Ultra Turrax had no influence upon the results.

PROCEDURE

The chromatographic equipment was the same as described previously[7]. Elutions were effected with 85% (w/w) ethanol at 75° on a column (4.5 × 930 mm) of a cation-exchange resin (Dowex 50W-x8, 14–17 μm) in the lithium form. The flow rate was 3.3 $ml.cm^{-2}min^{-1}$. Other experiments were run at 82° on a column (4.0 × 760 mm) of a strongly basic resin (T5C, 8–14 μm) in the sulphate form, at a flow rate (85% ethanol) of 4.4 $ml.cm^{-2}min^{-1}$.

The eluate was analysed automatically in a two-channel analyser. In one channel, the sugars were determined by the orcinol method[13], and in the second channel, the alditols were determined by the periodate–formaldehyde method[7]. In the chromatograms, the results obtained in the orcinol channel are given as a broken line, whereas full lines refer to the periodate–formaldehyde channel. The pH during the periodate oxidation was kept at 2.0. Calibration tests showed that there was a linear relationship between the absorbance and the amounts of added alditol. The chromatograms were evaluated quantitatively by determining the products of peak height and width at half-peak height[14]. The hydrolysis of the cellulose (10 g) was carried out in hydrochloric acid in two steps[12]. Since large amounts of reversion products were present, the solution was diluted to a D-glucose concentration of 2% and boiled for 20 h. The hydrochloric acid concentration was 0.1M during this final step. An insoluble residue constituting less than 0.5% of the cellulose was obtained.

The hydrolysate was treated overnight at 2° with a mixture of a weakly basic anion-exchanger in its free-base form (75 ml of wet Amberlite IR-45, sterilized with 70% ethanol) and a strongly basic resin in its hydrogen carbonate form (5 ml of Dowex 1-x8). This treatment deacidified and decolorized the hydrolysate effectively. With samples containing metallic cations, 1 ml of a strongly acid cation-exchanger (Dowex 50W-x8; H^+) was added as well. The slurry was introduced into a column containing *ca.* 10 ml of the fresh anion-exchangers. After drainage, the resin was washed with sterile water, and the combined effluent was evaporated at 40° to a syrup containing *ca.* 25% of water. A solution of the syrup in water was transferred to a 100-ml flask, and ethanol (92.4%) was added to obtain a final ethanol concentration of 85%. Crystallization of D-glucose began after less than one day, and the solution was kept at room temperature for *ca.* 1 week to complete the precipitation. About 70% of the D-glucose was removed under these conditions. Aliquots of the supernatant solutions were applied to the chromatographic columns. The reported amounts of added sugar and alditols are those present in the aliquot before crystallization.

RESULTS AND DISCUSSION

Separation of alditols from large amounts of D-*glucose*

A chromatogram obtained by elution of a sample containing 50 mg of D-glucose (before crystallization) and 25–50 μg of various alditols in a run on the lithium resin is given in Fig. 1. Parallel runs in the absence of D-glucose [*cf.* Ref. 8] showed that the retention volumes were not affected by the D-glucose present. With larger amounts of D-glucose, a lowering of the elution volumes of the alditols was observed. No impurities from the D-glucose were recorded in the periodate–formaldehyde channel in the interval within which glycerol, erythritol, arabinitol, xylitol, mannitol, and glucitol appeared. These alditols were well separated from each other and from D-glucose. The reproducibility of the areas of the elution bands was about the same as reported previously, and the deviations can be ascribed to variations in flow through the peristaltic pump[7]. Both in the presence and in the absence of D-glucose, the width at half height increased linearly with the elution volume for the peak. In analysis of unknown samples containing large proportions of D-glucose, *e. g.*, cellulose hydrolysates, it is recommended that the calibration runs be carried out with alditol solutions containing D-glucose. On the lithium resin, ribitol appears in the D-glucose band.

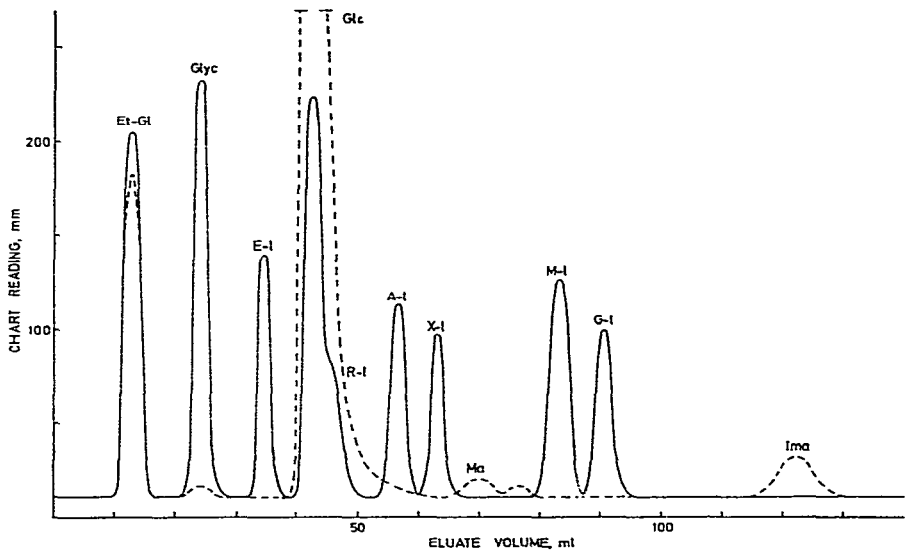

Fig. 1. Separation of 50 mg of D-glucose and various alditols on a lithium resin. *Et-Gl*, ethyl glycosides; *Glyc*, glycerol 50.0 μg; *E-l*, erythritol 25.0 μg; Glc, glucose; *R-l*, ribitol 25 μg; *A-l*, arabinitol 30.0 μg; *X-l*, xylitol 25.0 μg; *Ma*, maltose; *M-l*, mannitol 50.0 μg; *G-l*, glucitol 50.0 μg; *Ima*, isomaltose.

The two minor bands recorded in the orcinol channel at positions between the peaks for xylitol and mannitol were obtained in blanks with the D-glucose sample as well. The same holds true for the last peak recorded in Fig. 1. The first and last of

these peaks had the positions of maltose and isomaltose, respectively. The total amount of the three compounds was less than 0.1% of the added D-glucose. A trace amount of an unknown impurity in the D-glucose was recorded in the orcinol channel at the position of glycerol. The first peak on the chromatogram contained ethyl D-glucosides which are formed in appreciable proportions when D-glucose is kept for about one week in ethanol at room temperature, even in the absence of acid. Their formation has no influence upon the determination of the alditols.

On the sulphate resin, D-glucose appears later than all investigated alditols[7]. The elution volumes for peaks of glycerol, erythritol, xylitol, ribitol, and arabinitol were not affected significantly by the presence of D-glucose, whereas a decrease in the elution volumes for the alditols which appear nearer to D-glucose was observed. Both on the lithium and on the sulphate column, the elution volumes in the presence of D-glucose were reproducible (to $\gtrsim \pm 2\%$). The positions are characteristic on both columns, and, in the present work, the runs on the sulphate column were used as a check of the identity of the alditols. Among the investigated alditols, ribitol and arabinitol exhibited overlapping elution bands on the sulphate resin.

Separate experiments showed that no detectable losses of alditols occurred during the crystallization of D-glucose. In these experiments, a standard solution of alditols in 85% ethanol was mixed with saturated and supersaturated solutions of D-glucose. The supersaturated solutions were allowed to crystallize before being applied to the chromatographic column. The chromatograms from these runs showed no systematic deviations from those obtained with the saturated solution.

Artefacts produced during acid hydrolysis

Alditols are known to be very stable during acid hydrolysis, a fact which was confirmed in the present work. No new compounds were detected on the chromatograms in model experiments with the alditols carried out under conditions simulating those applied during the hydrolysis of cellulose. No losses were detected, and the areas of the chromatographic bands were reproducible within $\pm 2\%$.

D-Glucose is severely attacked in acid medium and gives rise not only to appreciable amounts of 5-hydroxymethyl-2-furaldehyde, levulinic acid, and 1,6-anhydro derivatives, but also to trace amounts of several monosaccharides[15]. Experiments in which D-glucose was subjected to treatment with hydrochloric acid, under the same conditions as used in the hydrolysis of cellulose, with subsequent chromatography on a sulphate column (Fig. 2), showed that peaks were recorded having the positions of mannose (Man, 60 μmoles per 100 g of D-glucose), fructose (Fru, 550 μmoles per 100 g of D-glucose), 1,6-anhydro-β-D-glucofuranose (*Lg,f*) and 1,6-anhydro-β-D-glucopyranose (*Lg,p*). These compounds have been isolated and identified in previous work, in which the acid treatment was carried out with sulphuric acid. A significant peak was recorded at the position of arabinose (Ara, 120 μmoles per 100 g of D-glucose). The presence of arabinose and the two anhydro sugars was confirmed in a parallel run on a lithium column (see also Fig. 3). As can be seen from Fig. 2, two unknown compounds were recorded between the peaks for 1,6-anhydro-

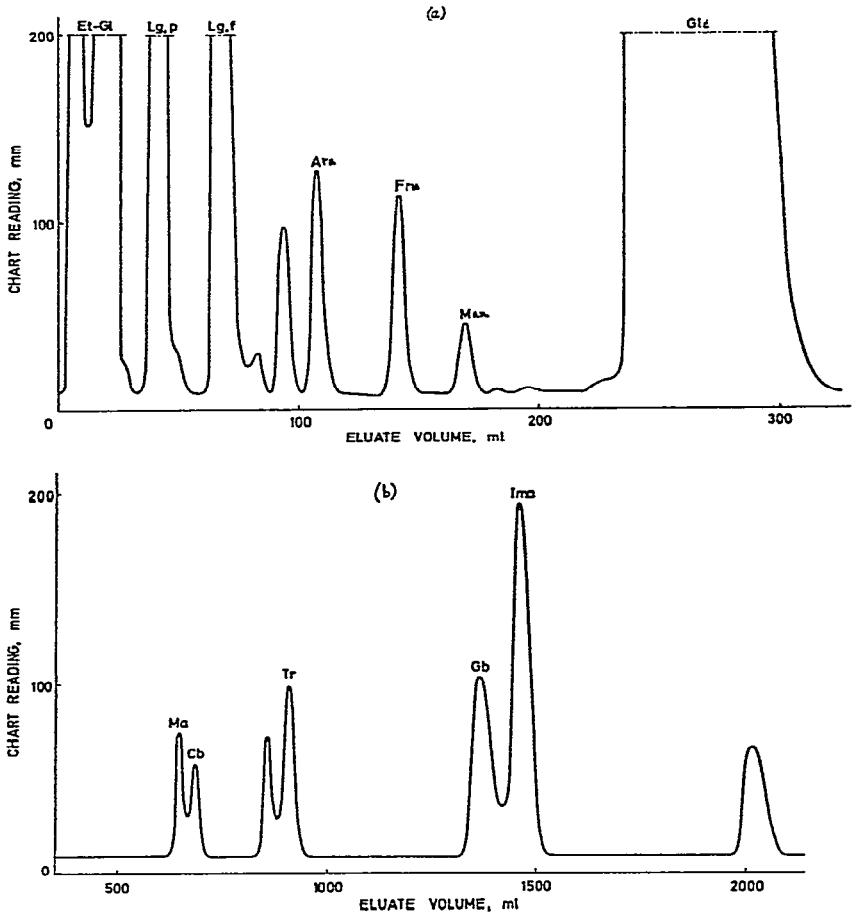

Fig. 2. Separation on a sulphate resin of products obtained from 40 mg of D-glucose after treatment with hydrochloric acid. Orcinol method. (*a*) *Et-Gl*, ethyl glucosides; *Lg,p*, 1,6-anhydro-β-D-glucopyranose; *Lg,f*, 1,6-anhydro-β-D-glucofuranose; Ara, arabinose 7.7 μg; Fru, fructose 41.6 μg; Man, mannose 4.5 μg; Glc, glucose; (*b*) *Ma*, maltose; *Cb*, cellobiose; *Tr*, trehalose; *Gb*, gentiobiose; *Ima*, isomaltose.

β-D-glucofuranose and arabinose. In addition, seven peaks corresponding to various reversion products were recorded after the D-glucose band. Several reversion products have been isolated and identified by previous research workers[16,17]. Authentic samples of only five of these compounds were available, and peaks corresponding to these [maltose (*Ma*), cellobiose (*Cb*), gentiobiose (*Gb*), trehalose (*Tr*), and isomaltose (*Ima*)] were recorded on the chromatograms from both columns.

The chromatogram given in Fig. 3 refers to an experiment in which a mixture of D-glucose and alditols was treated under conditions simulating acid hydrolysis of the cellulose, with subsequent separation of the mixture on a lithium column. It is seen that the expected artefacts were recorded in the orcinol channel. In runs on this type

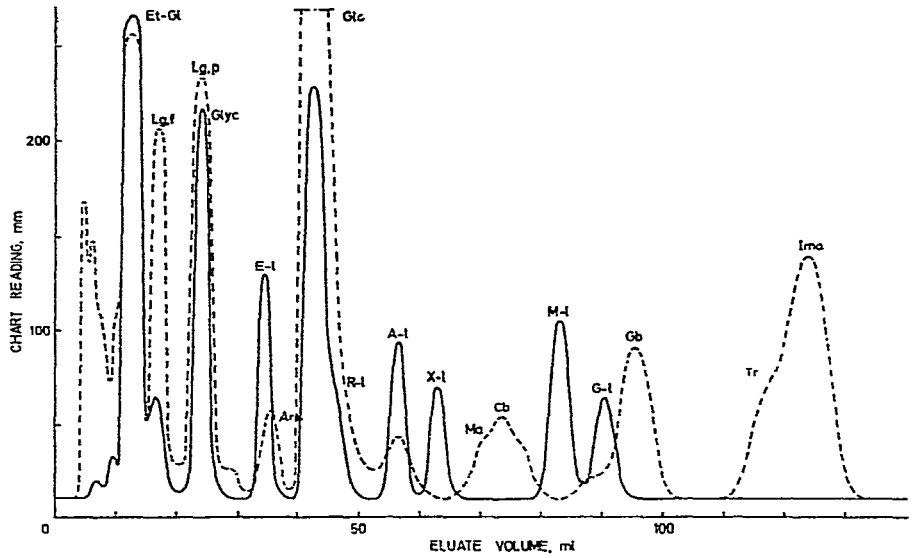

Fig. 3. Separation on a lithium resin of a reaction mixture obtained from 40 mg of D-glucose and microamounts of various alditols after treatment with hydrocloric acid. *Glyc*, glycerol 42.1 μg; *E-l*, erythritol 20.0 μg; *A-l*, arabinitol 24.0 μg; *X-l*, xylitol 19.2 μg; *M-l*, mannitol 40.3 μg; *G-l*, glucitol 39.6 μg. For other symbols, see Fig. 2.

of resin, the peaks corresponding to fructose and mannose are hidden under the glucose peak. Under the applied working conditions, the artefacts do not interfere with the determination of the alditols.

Application of the method to cellulose

To study the application of the suggested technique to the determination of end-groups in cellulose, experiments were carried out with cotton cellulose subjected to aging as alkali cellulose. Model experiments with cellobiose show that oxygen treatment in strongly alkaline medium gives rise to the formation of D-glucose, D-mannose, D-fructose, D-arabinose, 4-*O*-β-D-glucopyranosyl-D-fructose, 4-*O*-β-D-glucopyranosyl-D-mannose, and 3-*O*-β-D-glucopyranosyl-D-arabinose[18]. Moreover, a study of the carboxylic acid end-groups formed during the aging of alkali cellulose strongly indicates that D-glucose, D-fructose, (D-mannose), and D-arabinose end-groups are formed as intermediates[12].

A chromatogram obtained with a hydrolysate of the aged alkali-cellulose in a run on the lithium column is reproduced in Fig. 4. The same peaks as obtained with D-glucose after acid treatment were recorded in the orcinol channel. An additional peak having the position of xylose (X) appeared. This was expected since, as shown previously[19], xylose is present as an impurity in cotton cellulose purified by the applied technique. Likewise, the chromatograms recorded from the sulphate column were almost identical, except for the presence of a xylose peak in the run with the aged cotton.

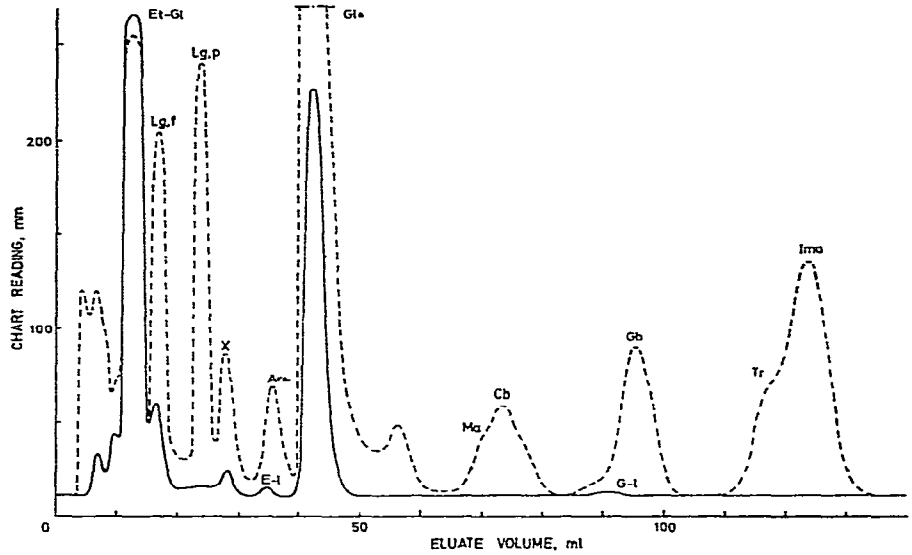

Fig. 4. Separation on a lithium resin of hydrolysis products from 42 mg of cellulose obtained after aging of alkali cellulose. Symbols, see Figs. 1 and 2.

With this sample which had not been subjected to any reduction, only trace amounts of alditols were indicated on the chromatograms. Mannitol and arabinitol were absent, but small amounts of D-glucitol (*G-l*, 10 μmoles per 100 g of cellulose) and erythritol (*E-l*, 20 μmoles per 100 g) were indicated in the runs on both columns.

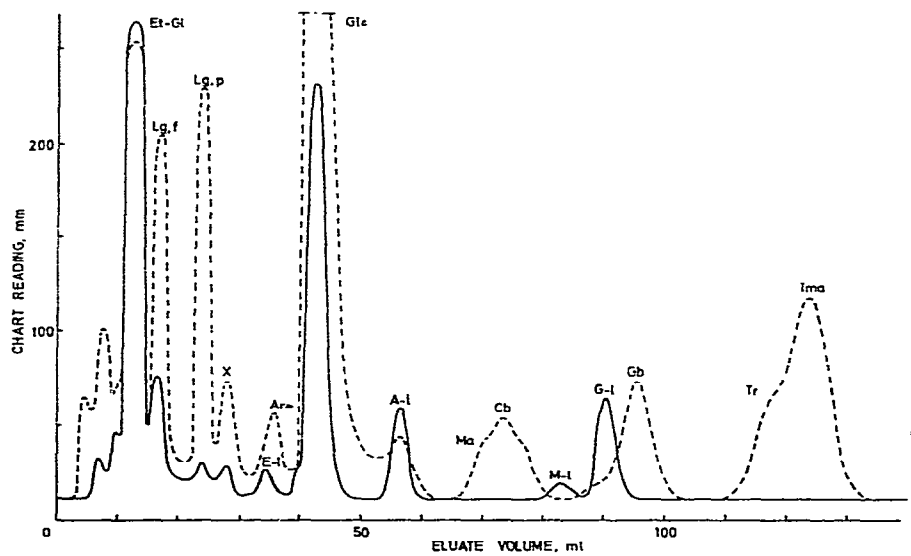

Fig. 5. Separation on a lithium resin of hydrolysis products from 42 mg of cellulose obtained after aging of alkali cellulose and subsequent reduction with sodium borohydride. Symbols, see Figs. 1 and 2.

Fig. 5 shows a chromatogram obtained on the lithium column with the same alkali cellulose after reduction with sodium borohydride. A comparison with Fig. 4 shows that the chromatograms recorded in the orcinol channel are almost identical, whereas those recorded in the periodate–formaldehyde channel differ markedly. As expected from the investigations referred to above, significant peaks appeared at the positions corresponding to glucitol (*G-I*), mannitol (*M-I*), and arabinitol (*A-I*) in the run with the reduced sample. Moreover, the erythritol (*E-I*) peak was larger than that obtained in the run with the non-reduced cellulose. Evidently, D-glucitol and D-arabinitol originated from the D-glucose and D-arabinose end-groups formed during the aging of the alkali cellulose. D-Mannitol is formed by reduction of D-mannose end-groups and, together with D-glucitol, from D-fructose end-groups. The results strongly indicate that D-erythrose end-groups are formed during the aging as well. A final confirmation of the presence of the above-mentioned alditols was obtained from chromatographic runs on the sulphate column. The amounts of alditols found by the two methods are listed in Table I. It is seen that good agreement was obtained between the determination made from these chromatographic runs which were both carried out with the same hydrolysate. For the major alditols, duplicate analyses on separate samples of the same cellulose showed variations of $\pm 5\%$ from the mean or less. These variations include those which occur as a result of all operations from the reduction of the end-groups to the final evaluation of the chromatogram.

TABLE I

AMOUNTS OF ALDITOLS IN CELLULOSE DEGRADED BY AGING, AND SUBSEQUENTLY REDUCED WITH SODIUM BOROHYDRIDE

Alditols	*Dowex 50W-x8 (Li^+)*		*Dowex 1-x8 (SO_4^{2-})*	
	*Found** (*μg*)	*mmole per 100 g of cellulose*	*Found** (*μg*)	*mmole per 100 g of cellulose*
D-Glucitol	33.8	0.45	35.5	0.46
D-Mannitol	3.2	0.04	3.1	0.04
D-Arabinitol	14.9	0.23	14.0	0.22
Erythritol	3.3	0.06	2.6	0.05

*Amount of cellulose applied in the analysis, 42 mg.

As can be seen from Table I, the total number of alditol end-groups in the investigated sample was about 0.8 mmole per 100 g. Since the sample was free from lactone groups, this figure would be equal to the amount of carbonyl end-groups, provided that the reduction was complete. An independent determination of the total carbonyl content was carried out by the spectrophotometric, hydrazine method described previously[20]. The results were within the range 0.8–0.9 mmole per 100 g, *i.e.*, in good agreement with the determination of the alditol groups. In the determinations carried out by the spectrophotometric method, a correction was applied for the absorbance obtained with the original cellulose sample dissolved in sulphuric acid.

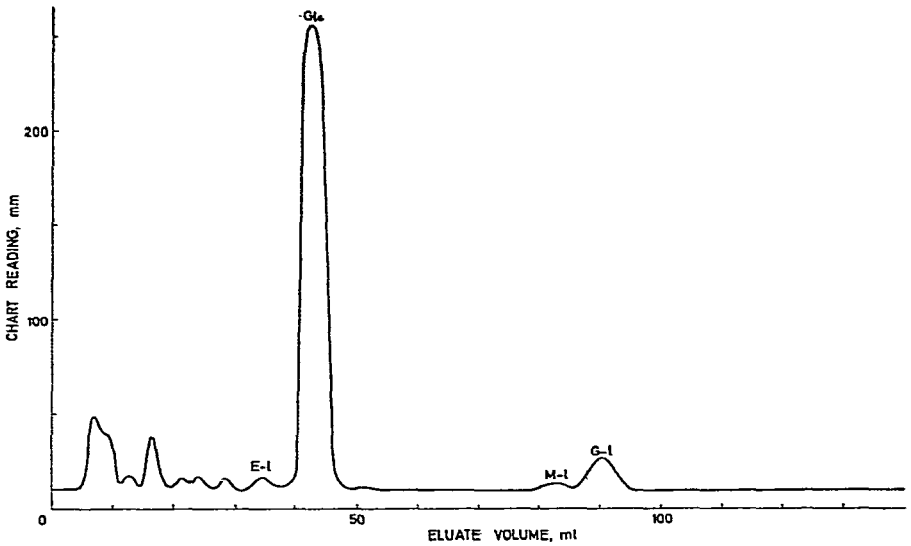

Fig. 6. Separation on a lithium resin of hydrolysis products from 59 mg of purified raw-cotton, after reduction with sodium borohydride. Symbols, see Fig. 1.

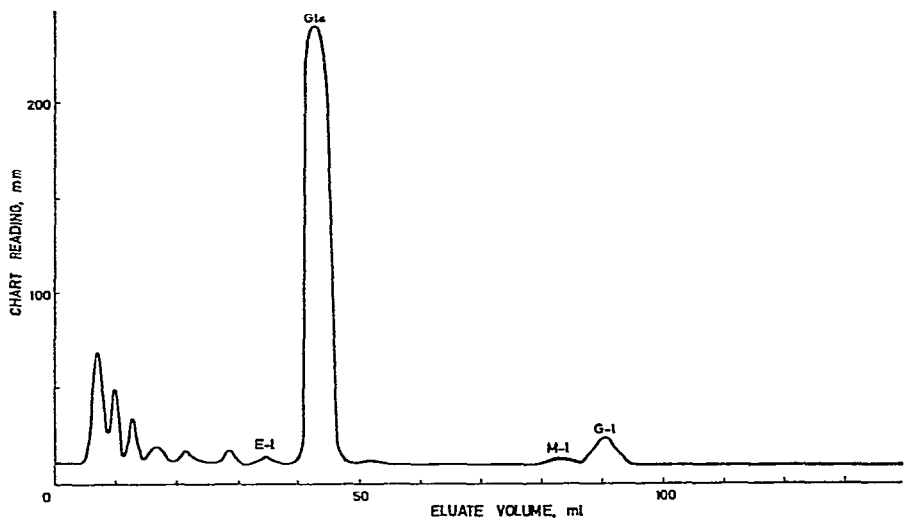

Fig. 7. Separation on a lithium resin of hydrolysis products from 59 mg of purified raw-cotton, after saponification of lactones and subsequent reduction with sodium borohydride. Symbols, see Fig. 1.

The results indicate that the chromatographic method can be applied both for the characterization of the individual end-groups in cellulose, and for the determination of the total number of carbonyl groups.

Influence of lactone groups in cellulose

Finally, experiments have been carried out with purified, raw cotton to study the influence of lactone groups, and to demonstrate the application of the method to cellulose samples containing a very low number of carbonyl groups. The total carboxyl content (including the lactones) was 0.96 mmole per 100 g.

The chromatogram obtained after reduction of the lactone-containing sample is given in Fig. 6. As expected, a significant peak was recorded at the position of glucitol (*G-l*, 79 μmoles per 100 g). Moreover, a minor peak appeared at the position of mannitol (*M-l*, 9 μmoles per 100 g), which can be explained by an isomerization of D-glucose end-groups to D-fructose and D-mannose end-groups during the alkaline purification of the cotton. Arabinitol was absent, whereas a small peak appeared at the position of erythritol (*E-l*). A peak having the same position appeared in a blank run with the non-reduced cotton, and it is possible that this peak is an artefact. A very small amount of glucitol was recorded in this run as well (5 μmoles per 100 g).

To study the influence of lactone groups upon the determination of carbonyl groups, the same cotton cellulose was treated with an alkaline sodium chloride solution to saponify the lactone groups[11] before reduction with sodium borohydride. A chromatogram obtained with this sample is reproduced in Fig. 7. The chromatogram exhibited the same peaks as those recorded with the lactone-containing sample. A decrease (*ca.* 30%) in the amount of glucitol is the most significant difference observed. It can be concluded that the lactones must be saponified before the reduction with borohydride, in order to obtain a reliable estimate of the carbonyl groups present in the cellulose.

The fact that the saponification of the lactones resulted in a decrease in the D-glucitol formation shows that the untreated sample contained D-gluconolactone groups which were reduced to D-glucitol end-groups. This result was expected since, as shown by Larsson and Samuelson[21], D-gluconic acid end-groups are present in unbleached cotton cellulose. In principle, it should be possible to calculate the number-average degree-of-polymerization of the cellulose from the sum of the number of D-gluconic acid groups and the number of alditol end-groups determined after saponification of the lactones. A serious source of error is that non-cellulosic carbohydrates cannot be removed without seriously affecting the cellulose molecules, for example, by transforming D-glucose end-groups to metasaccharinic acid end-groups, and for this reason no attempt was made to calculate the molecular weight.

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