CHALMERS TEKNISKA HÖGSKOLA


CHALMERS UNIVERSITY OF TECHNOLOGY
GÖTEBORG
SWEDEN

INSTITUTIONEN FÖR TEKNISK KEMI
DEPARTMENT OF ENGINEERING CHEMISTRY

# THE ALKALINE DEGRADATION OF CARBOHYDRATES

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1976

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by

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Akademisk avhandling som med tillstånd av sektionen för kemi vid Chalmers Tekniska Högskola, framlägges till offentlig granskning för avläggande av teknisk doktorsexamen i teknisk kemi den 31 maj 1976 kl 10.15 i biblioteket, institutionen för teknisk kemi, Chalmers Tekniska Högskola.

Göteborg 1976

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Mats H. Johansson

This thesis is based on the following papers:

I The Formation of End Groups in Cellulose During Alkali Cooking
by Mats H. Johansson and Olof Samuelson
Carbohydrate Research, 34, 33 (1974)

II End-Wise Degradation of Hydrocellulose During Hot Alkali Treatment
by Mats H. Johansson and Olof Samuelson
Journal of Applied Polymer Science, 19, 3007 (1975)

III End-Wise Degradation of Hydrocellulose in Bicarbonate Solution
by Mats H. Johansson and Olof Samuelson
submitted for publication

IV Kinetics of the Isomerization and Degradation of Xylotetraose in Alkaline Medium
by Mats H. Johansson and Olof Samuelson
Chemica Scripta, in press

V Epimerization and Degradation of 2-O-(4-O-Methyl--α-D-glucopyranosyluronic acid)-D-xylitol in Alkaline Medium
by Mats H. Johansson and Olof Samuelson

# ALKALINE DEGRADATION OF CARBOHYDRATES

In most processes used in the production of wood pulp, the carbohydrates are subjected to alkaline degradation, resulting in losses in yield and in depolymerization. The losses lead to increased consumption of alkali during cooking and bleaching.

Although the attack on the carbohydrates is of very great technical and economical importance, the chemical reactions are still not completely understood. A common feature of most alkaline degradation reactions is that an end-wise degradation starts at reducing end groups present in wood or pulp (1) or at reducing end groups formed after the cleavage of the polysaccharide chains (2). In 1,4-linked carbohydrates the reducing end group is subjected to a Lobry de Bruyn-Alberda van Ekenstein rearrangement (3), and the ulose group formed as an intermediate is eliminated. A new reducing group is then formed which is subjected to the same attack (peeling reaction). The eliminated ulose moiety is rearranged to isosaccharinic and other soluble peeling acids.

Competing with these reactions are stopping reactions which give rise to terminal stopping acids e.g. metasaccharinic (3-deoxyhexonic) and glucosaccharinic (2-C-methylpentonic) acids (1). Stopping reactions resulting in terminal alditol groups have also been proposed (4).

In addition, side groups present in hemicellulose and pectic material are subjected to degradation in alkaline media. An important example is the attack on 4-O-methyl-α-D-glucopyranosyluronic acid moieties linked to xylan (5).

In the following, summaries of the different papers are given.

## The formation of end groups in cellulose during alkali cooking (I)

The purpose of paper I was to investigate the end groups formed in cotton cellulose after alkali cooking at 170°C, using methods developed in recent years. A sample of the alkali-cooked cellulose was hydrolyzed and analysed for alditol groups by partition chromatography in aqueous ethanol (6). No alditols were detected, which shows that alditol end groups must constitute less than 0.0005% of the cellulose. Thus alditols are not formed as stopping groups in cellulose during alkali cooking (4).

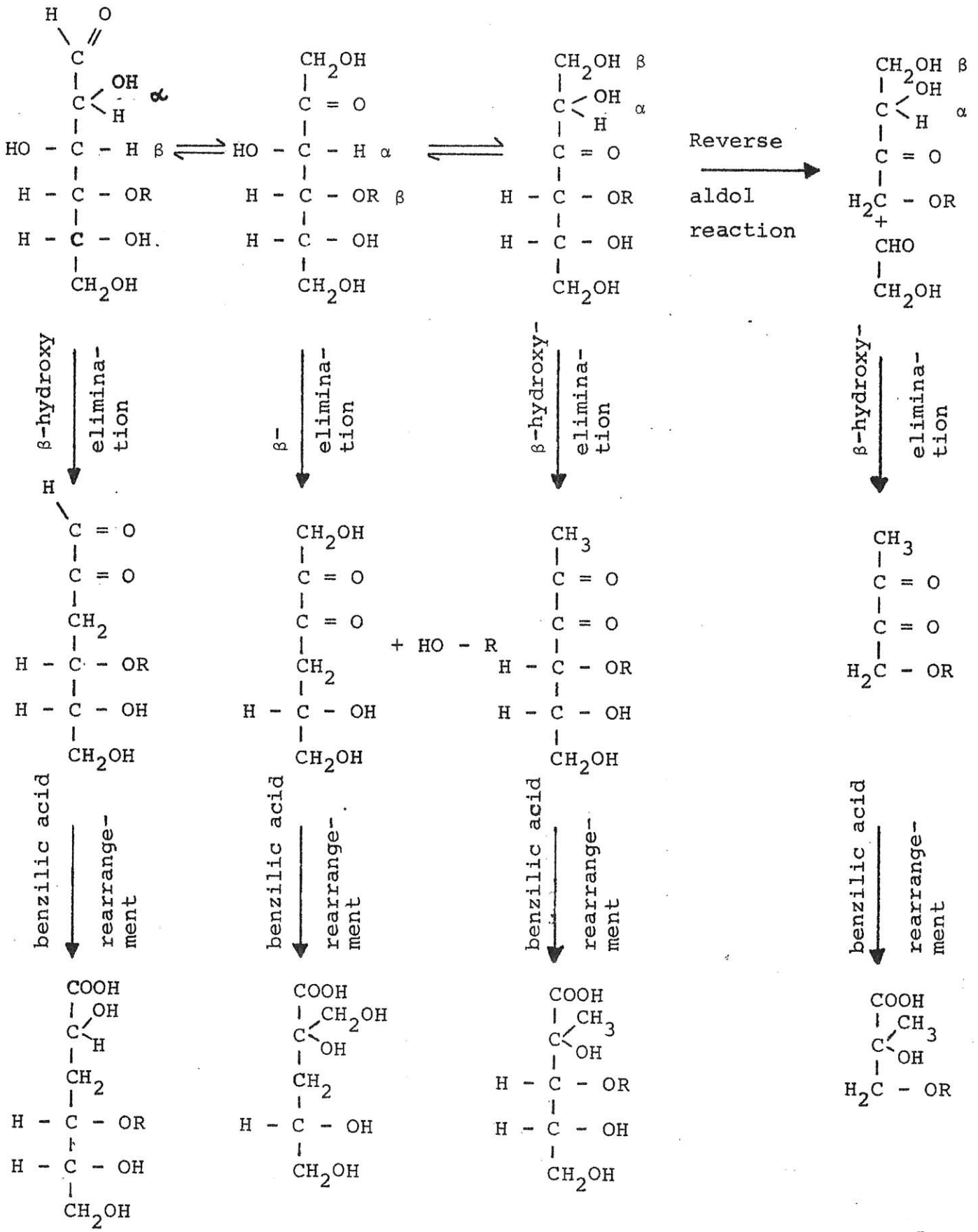
Another sample of the cellulose, after splitting of lactones, was reduced with borohydride before hydrolysis. The analysis showed that 8 mg glucitol was present per 100 g of alkali-cooked cellulose. This small amount of reducing end groups is mainly derived from intermediate glucose end groups formed during the peeling reaction.

To further investigate the stopping groups, a third sample of the alkali-cooked cotton cellulose was hydrolyzed, and the monocarboxylic acids were isolated by an anion exchange technique. The individual acids were then identified by anion exchange chromatography in two media on separate columns which were coupled to three channel analysers (7). Final confirmation of the identity of the acids was obtained by converting them into trimethylsilyl derivatives and by subsequent performing gas chromatographic and mass-spectrometric analysis (8).

In an earlier investigation with a less advanced technique, 3-deoxyhexonic (metasaccharinic) acids were isolated after hydrolysis of alkali-cooked cotton cellulose (9). The results obtained in the present work show that in addition to major proportions of 3-deoxy-ribo-hexonic and 3-deoxy-arabino-hexonic (metasaccharinic) acids, a large amount of 2-C-methyl-glyceric acid was formed. A minor proportion of 2-C--methylribonic (glucosaccharinic) acid was also present together with small amounts of aldonic acids. To confirm that these acids were derived from the terminal moiety, a fourth sample of the cellulose was reduced with lithium aluminium hydride in tetrahydrofuran before hydrolysis and analysis by partition chromatography. The analysis showed that 3-deoxy-ribo-hexitol, 3-deoxy-arabino-hexitol, 2-C-methylglycerol, and a small amount of 2-C-methylribitol were present.

The isolated 2-C-methylglyceric acid had no optical rotation, indicating that both D and L forms were formed. This important stopping acid has not been observed in earlier investigations. The suggested route of its formation starts with a Lobry de Bruyn-Alberda van Ekenstein rearrangement (3) of the terminal moiety to a 3-hexulose moiety, which is subjected to a reverse aldol reaction, resulting in a loss of glycolaldehyde. The hydroxyl group on C-1 is then eliminated, and the dicarbonyl moiety is converted into 2-C-methylglyceric acid via a benzilic acid rearrangement. The routes for the formation of stopping acids and peeling acids from the glucose end group are given in Fig. 1.

Fig. 1. Reactions for the degradation and the formation of stopping acids in cellulose during alkali cooking




| 3-Deoxy-hexonic acid (Metasaccharinic acid) end group | 3-Deoxy-2-C-hydroxymethyl-pentonic acid (Isosaccharinic acid) + other peeling acids | 2-C-Methyl-pentonic acid (Glukosaccharinic acid) end group | 2-C-Methyl-glyceric acid end group |
|---|---|---|---|
| Per cent of acid end groups: 49 | 1 | 23 | |

End-wise degradation of hydrocellulose during hot alkali treatment (II)

The objective of this investigation was to determine the stopping reactions at a lower temperature. Hydrocellulose was treated at 95°C in 0.25 M sodium hydroxide for varying periods of time. The reducing end groups in the cellulose were determined by the alditol method after borohydride reductions and the carboxylic acid groups by alkalimetric titration (10). The number of carboxylic acid groups increased throughout the treatment, while the number of reducing groups decreased. Hydrolysis and determination of the individual nonvolatile monocarboxylic acids showed that 3-deoxyhexonic and 2-C-methylglyceric acids were predominant. Small amounts of 2-C-methylpentonic, 2-deoxypentonic, and aldonic acids were also found. Calculations of the total number of end groups showed a decrease throughout the treatment, indicating that some molecules were brought completely into solution. The degree of polymerization of the lost molecules was 53 after alkali treatment for 3 hrs, and it increased during prolonged treatment. The ratio between the number of lost glucose moieties and the carboxylic groups formed in the hydrocellulose was 170. This ratio was not appreciably affected by the duration of the treatment. In Fig. 2 the natural logarithm of the number of reducing groups, $q_r$, and carboxylic groups, $q_a$, is plotted versus reaction time. In a first order reaction [cf. (IV)], these relations should follow straight lines.

The curved lines show that the rate constant decreases throughout the treatment, indicating that the solid structure of the hydrocellulose retards the overall reaction.

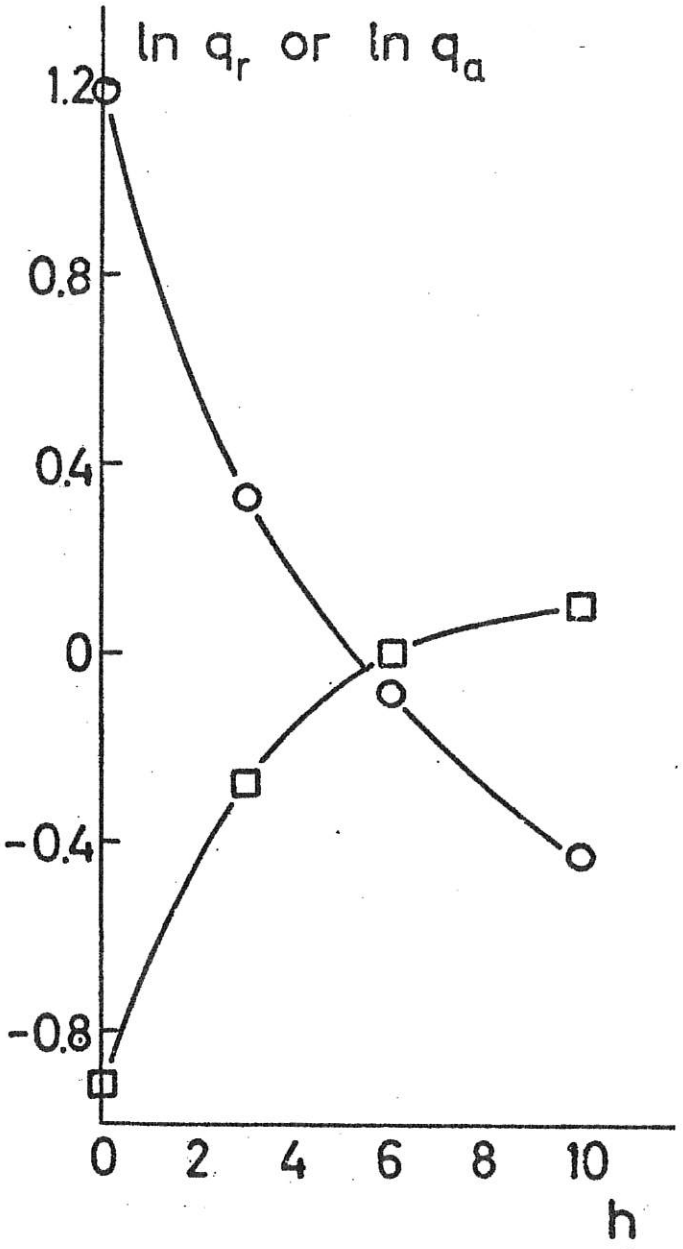



Fig. 2. Natural logarithm for the number of reducing groups, $q_r$, and carboxylic groups, $q_a$, versus time. Hot alkali treatment of hydrocellulose at 95°C in 0.25 M sodium hydroxide solution.

End-wise degradation of hydrocellulose in bicarbonate solution (III)

In connection with the development of sulphur-free methods for production of pulp, the behaviour of polysaccharides

under mildly alkaline conditions, with and without the presence of oxygen, has gained great interest. The degradation of hydrocellulose in 0.2 M sodium bicarbonate solution at 120°C was therefore investigated. The reaction was much slower under these conditions than in sodium hydroxide. The loss in yield after 8 hrs at this high temperature was similar to that after alkali treatment of hydrocellulose for 3 hrs in 0.25 M sodium hydroxide solution at 95°C. In agreement with the behaviour of hydrocellulose in sodium hydroxide solution, the number of reducing end groups decreased after the treatment, while the number of carboxylic groups increased. Hydrolysis and analysis of the acid end groups showed that 3-deoxyhexonic and 2-C-methylglyceric acids were the most important stopping groups and that small amounts of 2-C-methylpentonic, 2-deoxypentonic, 2-deoxyhexonic, and aldonic acids were formed as well. The relative amounts of 2-C-methylglyceric acid and 3-deoxyhexonic acids were similar to those found in the experiments with sodium hydroxide solution.

As already mentioned, the formation of 2-C-methylglyceric acid end groups requires that C-3 in the terminal moiety is involved in a Lobry de Bruyn-Alberda van Ekenstein rearrangement. Since 3-hexulose end groups lack the ability to form pyranose rings, they must be rather unstable and their formation must be depressed by competing reactions. Careful analysis of the reducing

end groups after borohydride reduction and hydrolysis revealed that 1.3% of the alditols were allitol and altritol. These alditols must be derived from the 3-hexulose end groups. Routes for the formation of stopping and peeling acids from these groups are given in Fig. 3.

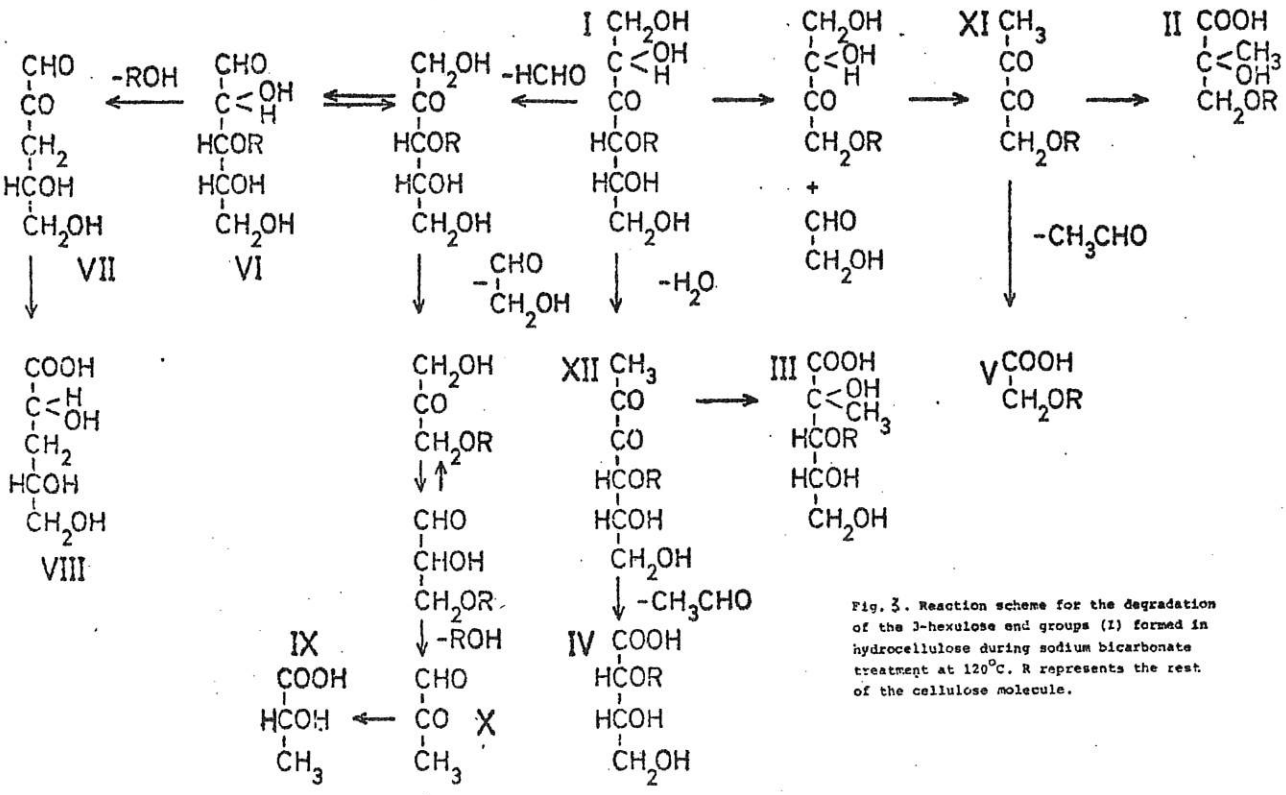



Fig. 3. Reaction scheme for the degradation of the 3-hexulose end groups (I) formed in hydrocellulose during sodium bicarbonate treatment at 120°C. R represents the rest of the cellulose molecule.

Analysis of the spent liquor showed that formic, 3,4-dideoxypentonic, and 3-deoxy-2-C-hydroxymethylpentonic acids were the most abundant acids. Evidently, fragmentation reactions of the intermediates were much more important than during treatment of hydrocellulose in sodium hydroxide solution (11).

The yield of peeling acids is low in sodium bicarbonate (66%) compared to that in sodium hydroxide solution (89%) ref (11). Obviously, material was lost in the analyses, due to irreversible sorption on the ion exchangers. This was confirmed by the following experiment. An aliquot of the acidified spent liquor was run through an Amberlite XAD-1 (styrene-divinylbenzene) resin. Chromic acid oxidation of the effluent showed that 19.2% of oxidizable material was retained in the column. The eluted material exhibited high carbon content (C 61.0, H 7.0%) and a strong chromophore at 260 nm. The equivalent weight was 527. Ultrafiltration of another aliquot of the spent liquor showed that 20% (by weight) of the material was retained on the filter. The results show that cyclic compounds of fairly high molecular weight were formed.

The coloured solutes were extracted almost completely from the acidified spent liquor with ethylacetate after saturation with ammonium sulphate. The extractable material calculated on degraded cellulose was 14.3%. Gas chromatographic analysis of trimethylsilyl derivatives revealed that at least 60 volatile derivatives were present. Mass-spectrometric analysis combined with gas chromatography showed that the major compound was 1,2-dihydroxybenzene. This compound, together with other aromatic compounds of a more complicated structure, has been isolated after alkali treatment of glucose and other carbohydrates (12,13).

Kinetics of the isomerization and degradation of xylotetraose in alkaline medium (IV)

Partition chromatography of oligomeric sugars on ion exchange resins in aqueous ethanol (14) was found to be a useful method for studies of the kinetics of the isomerization and degradation of carbohydrates in alkaline solution. The degradation of xylotetraose (X4) starts with a rearrangement of xylose end groups to ulose groups (X3U), which are eliminated. Xylotriose (X3) is formed and subjected to the same attack. Ultimately xylose (X1) is formed. In a parallel slower reaction, the xylose end groups are isomerized to lyxose (L). This group is also rearranged to the ulose group and eliminated. The experiments were performed at 40.0°C in sodium hydroxide solutions of various concentrations. Samples were withdrawn at intervals and analysed. An example of the analysis is given in Fig. 4. The two isomerized species (U and L) are

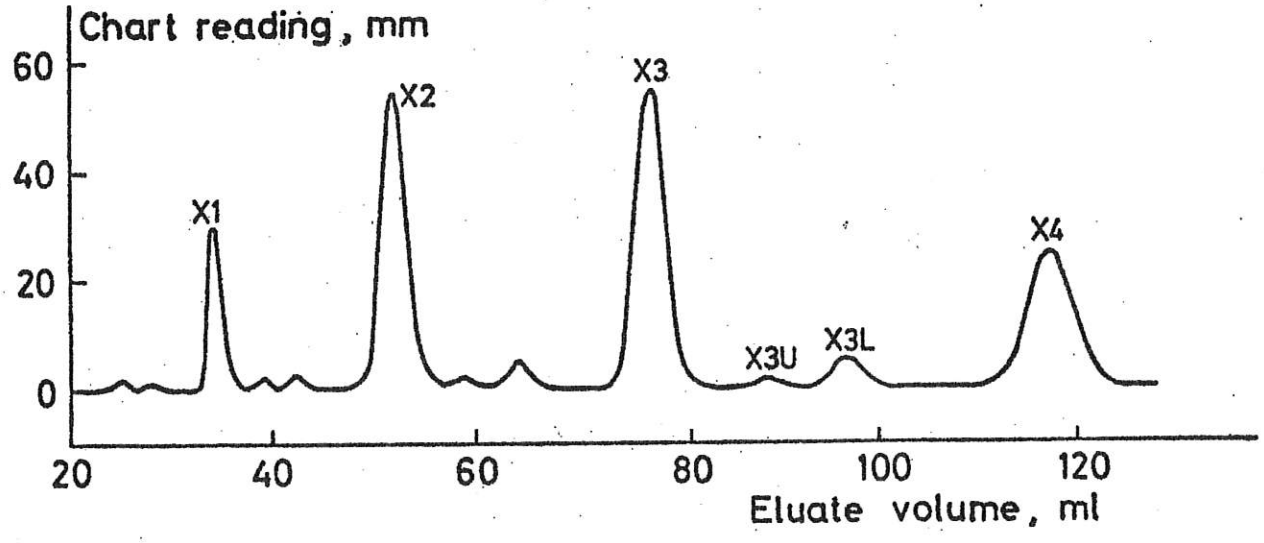



Fig. 4. Separation by partition chromatography in 75% aqueous ethanol of products obtained after treatment of xylotetraose (X4) in 0.045 M sodium hydroxide solution for 4.25 h at 40.0°C. Resin bed: 4 × 750 mm, Technicon T5A, $SO_4^{2-}$, 10-15 µm. Flow rate: 3.1 cm $min^{-1}$. (X1) xylose; (X2) -bi-; (X3) -tri-; (X4) xylotetraose. The two small peaks ahead of each major saccharide refer to isomers with terminal lyxose (L) and ulose (U) moieties.

eluted as minor peaks ahead of each major compound (X1-X4). The plots of the logarithm of the X4 concentration versus time gave a linear relationship not only during the initial period of alkali treatment but also during prolonged treatment (Fig. 5) The pseudo--first-order rate constant was calculated from the slope

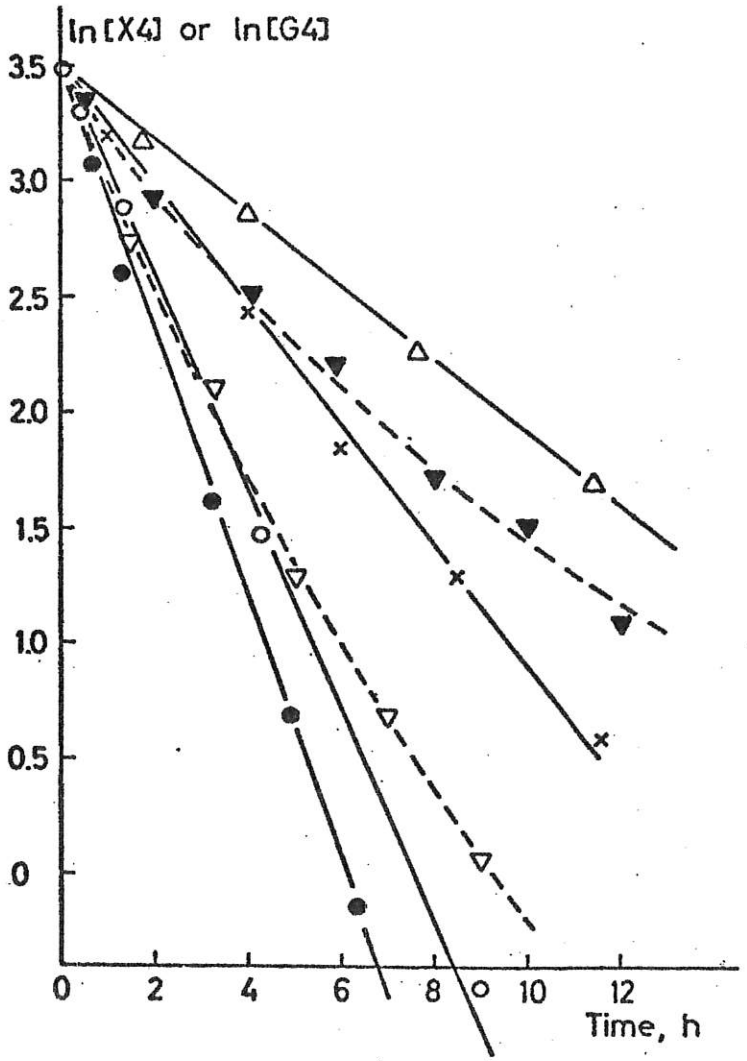



**Fig. 5.** Relationship between the nat. logarithm of the xylotetraose and cellotetraose concentration (μmol/l) and the time of reaction. Experiments with xylotetraose (full line) in ● 0.45 M; ○ 0.045 M; × 0.0091 M and △ 0.0045 M NaOH and cellotetraose (dashed line) in ▽ 0.045 M and ▼ 0.0091 M NaOH.

of the straight line in experiments at different sodium hydroxide concentrations. The rate constant for the degradation of X3U was also calculated. As expected, the rate constant for this β-elimination was much higher than that for the isomerization of the terminal xylose moiety in the tetrasaccharide to a xylulose moiety. It is noteworthy that the ratio between the rate constants was almost constant, independently of the sodium hydroxide concentrations. Both rate constants increased markedly when the sodium hydroxide concentration was raised from 0.0045 M to 0.045 M, while the change was fairly small when the concentration was further increased by a factor of ten. This behaviour indicates that terminal anionic xylose and xylulose moieties are intermediates and that their decomposition is the rate determining step. The rate constant for the decomposition of the anionic moiety was determined (0.595 $h^{-1}$). Assuming that its decomposition is a first-order reaction and that the terminal moieties behave as monoprotonic acids, we calculated the $pK_a$ to 11.6.

Calculations of the formation and degradation of X3 and X2, with the assumption that their degradation has the same rate constant as X4 gave results in excellent agreement with the experimental results (Fig. 6). Obviously, the rate constants are independent of the length of the chain.

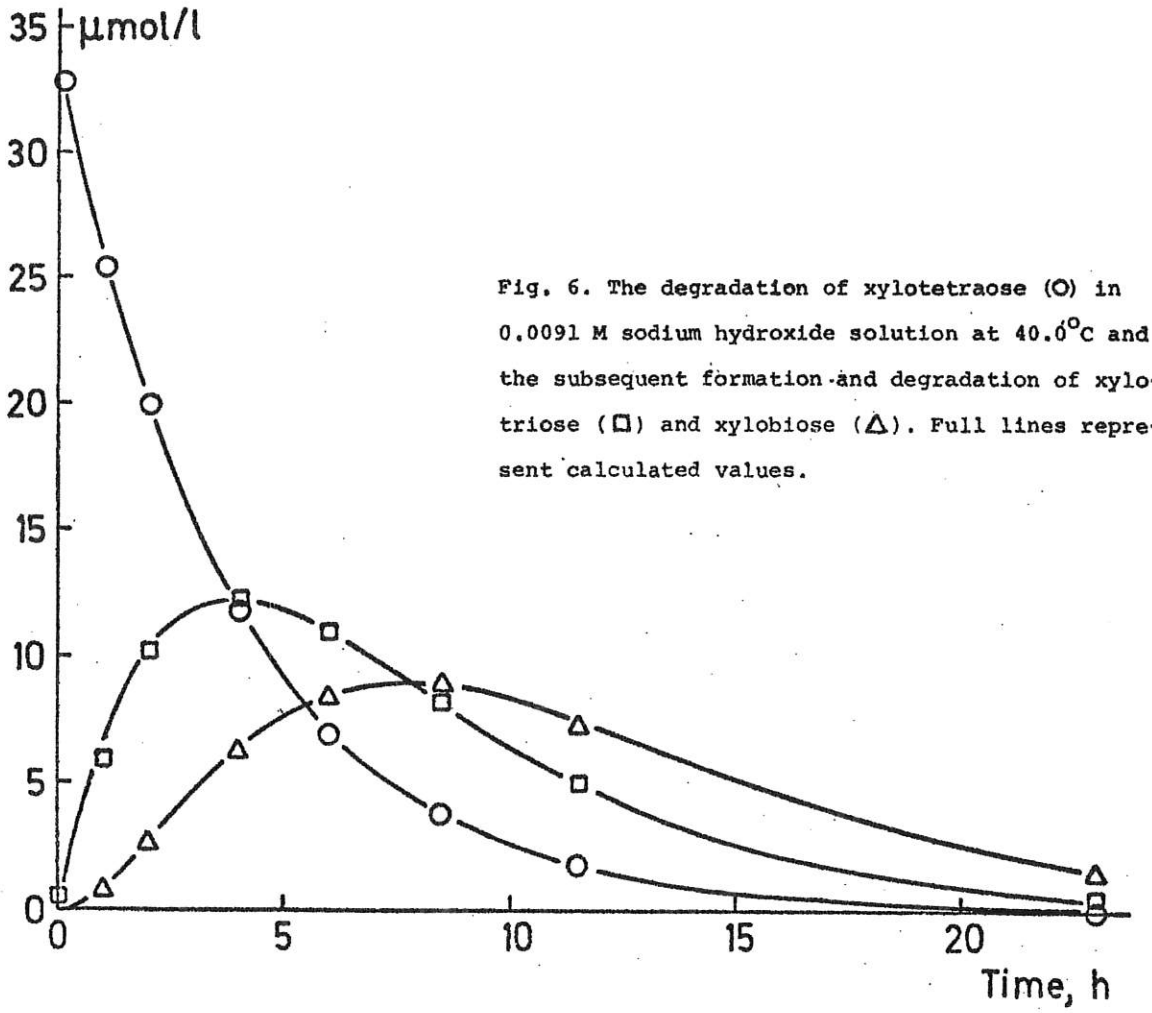



Fig. 6. The degradation of xylotetraose (O) in 0.0091 M sodium hydroxide solution at 40.0°C and the subsequent formation and degradation of xylotriose (□) and xylobiose (△). Full lines represent calculated values.

Epimerization and degradation of 2-O-(4-O-methyl-α-D--glucopyranosyluronic acid)-D-xylitol in alkaline medium (V)

2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylitol (I) was treated in 1 M sodium hydroxide at 150°C for various reaction times. The products obtained were analysed by ion exchange chromatography on columns which were coupled to UV-detectors and three channel analysers (7). A typical chromatogram is reproduced in Fig. 7.

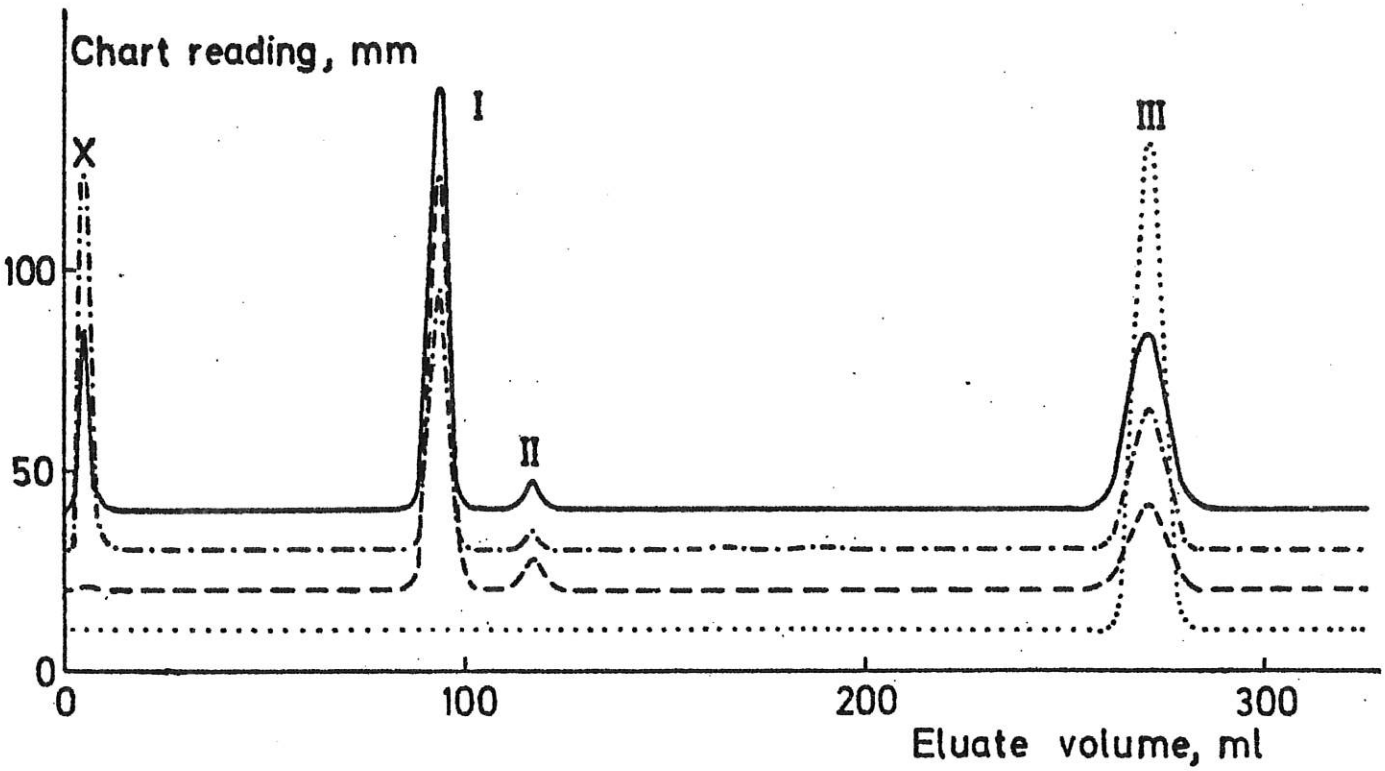



Fig. 7. Separation of products obtained after treatment of 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylitol (I) in 1 M sodium hydroxide for 1 h at 150°C. Column: Dowex 1-X8, 13-16 μm, $Ac^-$, 4 × 820 mm. Eluent: 0.02 M sodium acetate with acetic acid added to pH 5.9. Linear flow rate: 1.09 cm $min^{-1}$.

The first compound (X) that was not retained by the resin was identified as xylitol and the second as unreacted I. Analysis showed that the third compound (II) was 2-O-(4-O-methyl-β-L-idopyranosyluronic acid)-D-xylitol. The last compound with a strong UV absorption was identified as 2-O-(4-deoxy-β-L-threo-hex-4-enopyranosyluronic acid)--D-xylitol (III).

After a short duration of the treatment, III was the major reaction product. The concentration of III reached its maximum after about 1.5 hours and then decreased. The formation of the unsaturated acid is explained by a β-elimination of the methoxy group at C-4 as illustrated in Fig. 8. According to a generally

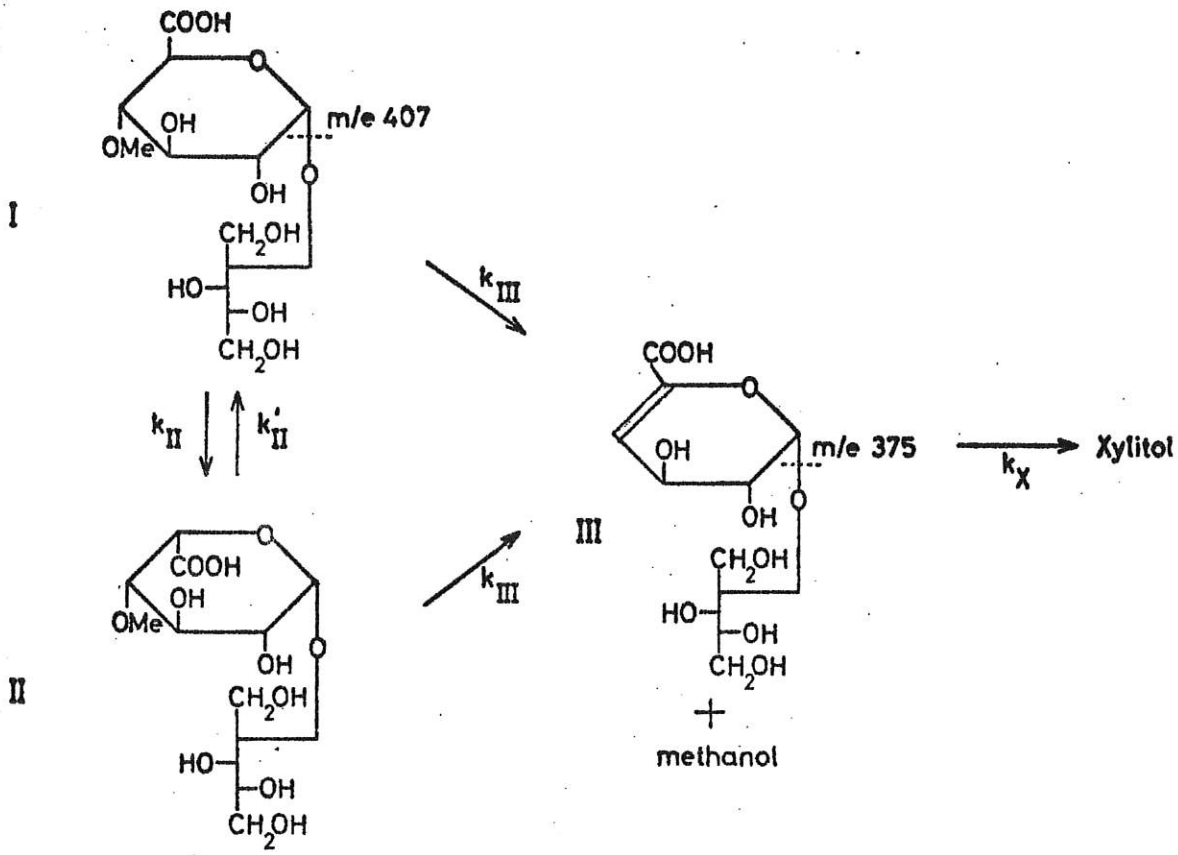



Fig. 8. Simplified reaction scheme for the alkaline degradation of I and mass-spectrometric fragmentation of the $(Me)_3Si$-derivatives of I and III.

accepted reaction scheme (15), the loss of methanol is preceded by the removal of the proton at C-5. It is therefore reasonable to assume that both epimers (I and II) are decomposed at the same rate.

The decreased concentration of III on prolonged treatment, together with the continuous increase in the xylitol concentration, shows that xylitol is formed from this intermediate.

Calculations showed that the degradation of I and II was a pseudo-first-order reaction with the rate constant 0.91 $h^{-1}$. The rate constant for the formation of xylitol from III was 0.52 $h^{-1}$.

Hydrolysis of III released xylitol, but no traces of the unsaturated uronic acid were observed. The destruction of this acid in the hydrolysis explains why this acid has not been observed in analyses of hydrolyzates of sulfate pulps. Since the number of uronic acid groups decreases (5) during alkaline pulping and 4-O-methylliduronic acid (16) has been isolated from pulps, it is concluded that the scheme for the epimerization and degradation of the 4-O-methylglucuronic acid moieties (Fig. 8) is valid for xylan during alkaline pulping at high temperature.

ACKNOWLEDGEMENTS

This thesis is based on research performed at the Department of Engineering Chemistry, Chalmers University of Technology, during 1971 to 1976.

I wish to express my special thanks to Professor Olof Samuelson for his continuos support and encouragement. I am also indebted to Miss Mary Lundin who performed the gas chromatographic analysis, to Miss Eva Asplund for typing the manuscripts, and to all other friends in the department for valuable discussions and stimulating collaboration.

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Reprinted from

*Carbohydrate Research*, 34 (1974) 33–43


# THE FORMATION OF END GROUPS IN CELLULOSE DURING ALKALI COOKING

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(Received October 22nd, 1973; accepted for publication December 3rd, 1973)

## ABSTRACT

Cotton that had been subjected to alkali cooking at 170° was hydrolysed to determine the carboxylic acid end-groups. Large proportions of 3-deoxy-*ribo*-hexonic, 3-deoxy-*arabino*-hexonic, and 2-*C*-methylglyceric acids, together with a minor proportion of 2-*C*-methylribonic acid, were isolated and identified. Reduction of the cellulose end-groups and subsequent analysis of the hydrolysate revealed 3-deoxy-*ribo*-hexitol, 3-deoxy-*arabino*-hexitol, 2-*C*-methylglycerol, and a small proportion of 2-*C*-methylribitol. It is concluded from these results that, in addition to 3-deoxyhexonic acid end-groups, significant quantities of terminal 2-*C*-methylglyceric and minor amounts of 2-*C*-methylribonic acid groups are formed during the alkali cooking. No alditol end-groups were detected in the unreduced cellulose.

## INTRODUCTION

Although the manufacture of kraft pulp is an important industrial process, the cellulose reactions occurring during the cooking of kraft pulp are incompletely understood. The presence of 3-deoxy-*ribo*- and 3-deoxy-*arabino*-hexonic acid end-groups has been demonstrated qualitatively by various chromatographic methods[1,2], but quantitative determination has not been attempted. We now report methods for the study of the formation of end groups under conditions simulating the cooking of kraft pulp.

## EXPERIMENTAL AND RESULTS

*Alkaline treatment of cotton and subsequent hydrolysis with acid.* — Unbleached cotton (170 g), purified by solvent extraction, kier boiling[3], and extraction in 6% aqueous sodium hydroxide at 2° for 2 min, was introduced into an autoclave (10 l) containing 8.5 l of 5% aqueous sodium hydroxide through which nitrogen had been bubbled for 30 min. The autoclave was evacuated and the temperature raised to 170° during 3 h in the following manner: at 40° and 80° the autoclave was evacuated, and at 120° it was vented. After a cooking-time at 170 ±3° of 7 h, the autoclave was

cooled, and the liquor was filtered off. The cotton was then washed with water, soaked for 1 h in 1% acetic acid, washed with water again, and dried in circulating air at 30°. The yield was 73.5%, and the intrinsic viscosity in copper ethylenediamine[4] was 148 $cm^3/g$.

The cellulose (130 g) was placed in a glass vessel (2 l) and cooled to −22°, and hydrochloric acid (1.8 l, 43%) at the same temperature was added. The vessel was sealed, kept at 23° for 10 h, and again cooled to −22°, at which the gas was vented. The hydrochloric acid was largely removed by evaporation at 35°. To decompose the reversion products, the residue was diluted with water to a hydrochloric acid concentration of 0.35M and kept at ~90° for 23 h.

The hydrolysate was stirred with Dowex-1 x8 ($HCO_3^-$) resin (50–100 mesh; 750 ml) for 16 h to remove chloride ions, as well as organic acids and lactones. The resin slurry was transferred to a column and washed with water until the effluent gave a negative anthrone test. The effluent containing the sugars was concentrated *in vacuo* and analysed by partition chromatography[5]. The resin was transferred to a beaker, and 5M acetic acid was added to liberate carbon dioxide. This slurry was poured into a column which contained a short bed of Dowex-1 x8 ($AcO^-$) resin. The monocarboxylic acids were eluted with 0.2M sodium acetate (15 l). The cations were exchanged for hydrogen, and the eluate was evaporated to dryness to give a residue of 763 mg.

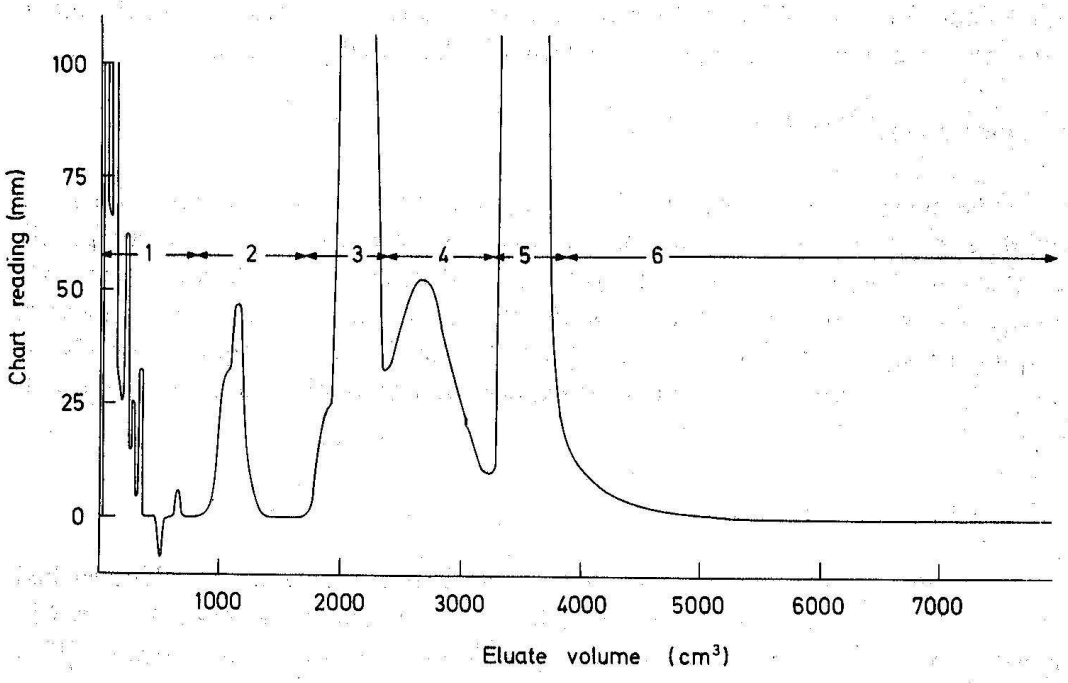


Fig. 1. Group separation of monocarboxylic acids isolated after hydrolysis of cotton cellulose that had been cooked with hot alkali. Eluent: 0.08M sodium acetate (pH 5.9). Column (20 × 800 mm): Dowex-1 x8, 25–32 μm. Nominal flow: 0.8 $cm.min^{-1}$. Analysis by Waters R 401 differential refractometer.

*Separation and identification of acids.* — The non-volatile, organic acids were separated into groups by anion-exchange chromatography[6], and bands were cut as indicated in Fig. 1. After removal of sodium ions, the fractions were evaporated to dryness and weighed.

The position of band 3 (the first major fraction) suggested that it contained 3-deoxy-*ribo*- and 3-deoxy-*arabino*-hexonic acids. This was substantiated by pre-

TABLE I

DISTRIBUTION COEFFICIENTS ($D_v$) IN ACETIC ACID AND SODIUM ACETATE MEDIA, AND COLOUR RESPONSES IN CHROMIC ACID (Cr), PERIODATE–FORMALDEHYDE (P–f), AND CARBAZOLE (K) CHANNELS

| *Band number* | *mg/130 g* | *Acids* | $D_v$ *in ion-exchange chromatography* | | *Colour response*[a] | | |
|---|---|---|---|---|---|---|---|
| | | | *0.08M NaOAc* | *0.5M HOAc* | *Cr* | *P–f* | *K* |
| 3:S1 | 3.4 | 2-*C*-Methylribonic | 6.70 | 5.53 | + | + | – |
| 3:S2 | 86 | 3-Deoxy-*ribo*-hexonic | 7.12 | 6.85 | + | + | (+) |
| 3:S3 | 82 | 3-Deoxy-*arabino*-hexonic | 7.64 | 9.50 | + | + | (+) |
| 3:S4 | 7.1 | Unknown 1 | 6.54 | 24.3 | + | + | (+) |
| 4:S1 | 13 | 3-Deoxy-*ribo*-hexonic levulinate | 6.65[b] | 5.89 | + | + | (+) |
| 4:S2 | 10 | 3-Deoxy-*ribo*-hexonic | 7.20 | 6.80 | + | + | (+) |
| 4:S3 | 24 | 3-Deoxy-*arabino*-hexonic levulinate | 9.08 | 8.43 | + | – | (+) |
| 4:S4 | 10 | 3-Deoxy-*arabino*-hexonic | 7.60 | 9.67 | + | + | (+) |
| 4:S5 | 6.8 | Gluconic | 7.21 | 12.5 | + | + | – |
| 4:S6 | 5.7 | Arabinonic | 8.93 | 14.2 | + | + | – |
| | 8.2 | 2-*C*-Methylglyceric | 11.1 | 14.2 | + | + | – |
| 4:S7 | 1.3 | Anhydrosaccharinic | | 17.2 | | | |
| 4:S8 | 1.4 | Mannonic | 9.50 | 17.5 | + | + | – |
| 4:S9 | 7.7 | Erythronic | 10.5 | 18.9 | + | + | – |
| 4:S10 | 9.8 | Anhydrosaccharinic | 10.4 | 24.2 | + | – | + |
| 5:S1 | 312 | Levulinic | 12.9 | 3.25 | + | – | – |
| 5:S2 | 8.8 | 2-*C*-Methylglyceric levulinate | 7.12 | 12.0 | + | + | – |
| 5:S3 | 45 | 2-*C*-Methylglyceric | 11.1 | 14.0 | + | + | – |
| 5:S4 | 9.0 | Glyceric | 12.0 | 19.8 | + | + | – |
| 5:S5 | 11 | 2-*C*-Methylglyceric levulinate | 13.4 | 25.6 | + | – | – |
| 6:S1 | 11 | Levulinic | 13.1 | 3.50 | + | – | – |
| 6:S2 | 2.7 | 2-*C*-Methylglyceric | 11.1 | 14.4 | + | + | – |
| 6:S3 | 2.3 | Lactic | 13.9 | 15.0 | + | – | (+) |
| 6:S4 | 3.0 | Glycolic | 14.7 | 17.8 | + | (+) | + |
| 6:S5 | 12.0 | 2-*C*-Methylglyceric levulinate | 13.4 | 25.8 | + | – | – |
| 6:S6 | 6.0 | Unknown 2 | — | 38.6 | | | |

[a]Key: +, positive reaction; (+), weak reaction; –, no reaction. [b]Two peaks were recorded in the acetate run, one with the values in the Table, and one with $D_v = 8.93$ and colour responses + – (+).

parative rechromatography, using 0.5M acetic acid, and by determinations of the $D_v$ values [Table I] on analytical columns coupled with a three-channel analyzer[7]. Final confirmation was obtained by g.l.c.–m.s. of the trimethylsilyl-substituted trimethylsilyl (TMS) esters[8]. Unless stated otherwise, the acids discussed below were identified by the same methods. In addition to 3-deoxyhexonic acids, band 3 contained a minor amount of 2-*C*-methylribonic acid as well as a small amount of an unknown acid.

Band 4 constituted only a minor part of the organic acids. Due to overlapping, it also contained both the 3-deoxyhexonic acids present in band 3. In addition, gluconic, arabinonic, 2-*C*-methylglyceric, mannonic, and erythronic acids were present in small amounts. An anhydrosaccharinic acid, which is formed as an artefact during acid hydrolysis[9], was obtained together with a minor amount of another acid (4:S7) that gave an almost identical mass spectrum. The results indicated that this acid was the diastereomer. Two of the major peaks obtained when band 4 was rechromatographed in 0.5M acetic acid contained 4-oxovaleric (levulinic)esters of the two 3-deoxyhexonic acids. This fact was established by saponification and identification of the products by the methods described above. The results indicate that these esters, as well as those present in band 5, are formed during the removal of the acetic acid by evaporation.

Similarly, band 5, which contained levulinic acid (artefact) and 2-*C*-methylglyceric acid as the main components, also contained appreciable amounts (see Fig. 2

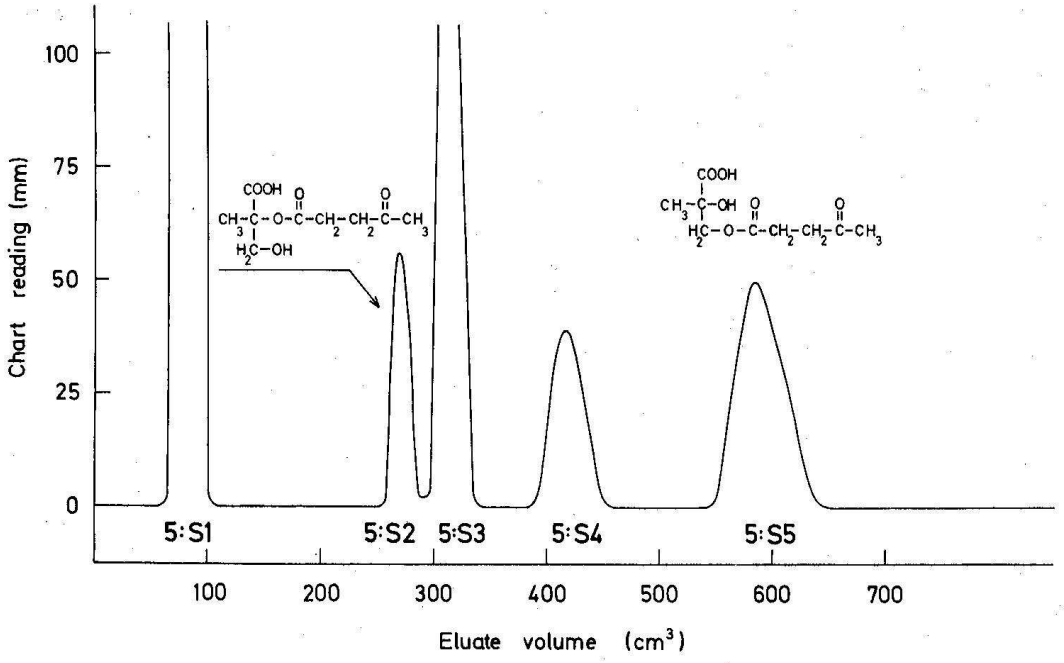


Fig. 2. Separation of monocarboxylic acids from band 5. Eluent: 0.5M acetic acid. Column (6 × 780 mm): Dowex-1 x8, 37–41 $\mu$m. Nominal flow: 0.21 cm.min$^{-1}$. Analysis by Waters R 401 differential refractometer.

and Table I) of two compounds which, after saponification at pH 10 for 4 h at room temperature, gave levulinic and 2-*C*-methylglyceric acids. Evidently, these compounds were esters. The structure of the compound 5:S5 was deduced by g.l.c.–m.s. of the TMS derivative[8] and is shown in Fig. 2. The structure of the ester contained in band 5:S2 is most probably that shown in Fig. 2.

Band 6 contained only minor amounts of acids and, as expected from the tailing of band 5, levulinic acid, 2-*C*-methylglyceric acid, and one of the levulinic esters of 2-*C*-methylglyceric acid were present. As in the corresponding fraction of all other polysaccharide hydrolysates studied in this laboratory, band 6 contained small proportions of lactic and glycolic acids. A minor amount of an unknown acid, which was held strongly during column chromatography in acetic acid was also present.

Bands 1 and 2, which contained only minor amounts of material, had positions corresponding to those of oligomeric acids. Hydrolysis of the material in band 1 in 0.1M sulphuric acid at 130° for 3 h released glucose, the two diastereomeric 3-deoxyhexonic acids (5 mg of each), and 2-*C*-methylglyceric acid (4 mg). Minor amounts of gluconic, arabinonic, erythronic, glyceric, and levulinic (artefact) acids were also detected.

Rechromatography of band 2 in 0.5M acetic acid showed that it contained at least six different organic acids with positions similar to those recorded for aldobionic acids. After hydrolysis, glucose and appreciable amounts of the two 3-deoxyhexonic and 2-*C*-methylglyceric acids were obtained. These results strongly indicate that the acids contained in bands 1 and 2 are mainly reversion products. The amounts of acids found in the hydrolysates from bands 1 and 2, as well as the amounts of acids recovered after saponification of the levulinic esters, are added to the amounts of the acids reported in Table II.

TABLE II

SACCHARINIC AND ALDONIC ACIDS IN THE HYDROLYSATE

| *Acids* | *Weight (mg)*[a] |
|---|---|
| 3-Deoxy-*ribo*-hexonic | 100 |
| 3-Deoxy-*arabino*-hexonic | 105 |
| 2-*C*-Methylribonic | 3.4 |
| 2-*C*-Methylglyceric | 64 |
| Gluconic | 5.3 |
| Mannonic | 1.1 |
| Arabinonic | 4.4 |
| Erythronic | 5.9 |

[a]Calculated on 100 g of alkali-cooked cotton.

Furthermore, trace amounts (probably artefacts) of 3-deoxy-2-*C*-(hydroxymethyl)pentonic acids (*erythro* and *threo*) were present in band 3 and in the hydrolysate of band 1.

The results in Table II confirm the previous findings that the formation of terminal 3-deoxyhexonic acid groups is an important reaction occurring during the alkali-cooking at high temperatures[1], and that these groups are the most abundant, acidic end-groups. Moreover, the results strongly indicate that, in addition, a large number of 2-*C*-methylglyceric acid end-groups are formed. The small amount of 2-*C*-methylribonic acid isolated from the hydrolysate can also be attributed to terminal groups formed during the cooking. The reactions which give rise to these acids will be briefly discussed in the Discussion section.

The small proportions of aldonic acids found in the hydrolysate are derived from aldonic acid end-groups present in the raw cotton[10] and formed by oxidation of cellulose in alkaline medium during purification. These acids will, therefore, not be discussed in detail.

*Determinations of monosaccharides and alditols.* — The sugar fraction obtained after removal of the organic acids and the hydrochloric acid from the hydrolysate was chromatographed on a cation exchanger in the lithium form (Aminex A6; 10–19 $\mu$m; Bio Rad) and also on an anion exchanger in the sulphate form (T5A; 15–20 $\mu$m; Technicon), with automatic analysis of the eluate by the orcinol and periodate–formaldehyde methods[5]. In addition to glucose, trace amounts of xylose, and the usual artefacts formed during the hydrolysis (fructose, mannose, arabinose, 1,6-anhydroglucoses, and reversion products) were present. Alditols were not found on the chromatograms; since the method is very sensitive, alditol end-groups must constitute less than 0.0005% of the cellulose.

Determinations of alditols by the same techniques were made on alkali-cooked samples which, before the acid hydrolysis, had been reduced with sodium borohydride. Experiments were carried out on the original sample (dried after soaking in acetic acid before the final washing with water) and on the same sample after pretreatment with an alkaline salt solution to open the lactone rings[11].

In the lactone-containing sample, significant peaks were recorded in the periodate–formaldehyde channel with positions corresponding to 3-deoxy-*ribo*-hexitol (8 mg), 3-deoxy-*arabino*-hexitol (16 mg), and glucitol (10 mg) for the sulphate resin. These and all other amounts of products reported here are calculated per 100 g of dry, alkali-cooked cotton. The presence of glucitol was confirmed by chromatography on the lithium column. The deoxyhexitols appeared together with glucose, and therefore could not be detected with the latter type of resin. The distribution coefficients of the alditols are given in Table III, together with those of xylitol, which served as a marker. The sample subjected to alkaline pretreatment to open the lactones gave a significant peak for glucitol (8 mg). No peaks could be observed at the positions of the 3-deoxyhexitols. The results from this sample show that only a very small fraction of the end groups in the alkali-cooked cotton are reducing glucose end-groups. It is not clear whether any glucose groups are actually present at the end of the alkali-cooking, since it is possible that a small proportion of glucosidic bonds were cleaved during subsequent treatments, which then gave rise to the glucitol end-groups subsequently detected.

TABLE III

VOLUME DISTRIBUTION COEFFICIENTS OF SOME ALDITOLS AT 75° AND 85% ETHANOL

| *Alditol*[a] | *$Li^+$ resin* | *$SO_4^{2-}$ resin* |
|---|---|---|
| 3-Deoxy-*ribo*-hexitol | 2.51 | 3.13 |
| 3-Deoxy-*arabino*-hexitol | 2.68 | 3.78 |
| 2-*C*-Methylribitol | 1.30 | 2.53 |
| 2-*C*-Methylarabinitol | 1.39 | 2.15 |
| 2-*C*-Methylglycerol | 0.65 | 0.78 |
| Xylitol | 4.21 | 5.34 |

[a]The four first alditols were prepared by reduction with sodium borohydride of the lactones of the corresponding saccharinic acids. 2-*C*-Methylarabinonic acid was kindly supplied by Professor O. Theander. 2-*C*-Methylglyceric acid was reduced with lithium aluminium hydride, and the structure of the alditol confirmed by g.l.c.–m.s. of its TMS derivative.

The higher value of glucitol obtained with the lactone-containing sample may be ascribed to reduction of terminal gluconolactone units. The presence of 3-deoxy-*ribo*- and 3-deoxy-*arabino*-hexitols confirms that the 3-deoxyhexonic acids found in the hydrolysate are present as end groups and shows that they have the ability to form ester linkages (probably 1,5-lactones). The amount of alditols was much less than the amount of 3-deoxyhexonic acids isolated from the hydrolysate. This finding indicates that the formation of esters is not extensive.

To increase the yield of alditols, the reduction was carried out with lithium aluminium hydride for 14 days at room temperature in tetrahydrofuran with ultrasonic treatment. Before adding the solvent, the alkali-cooked sample was treated in succession with methanol, ethyl ether, and tetrahydrofuran. Determinations of alditols by the same chromatographic methods gave the results presented in Table IV. As expected, large quantities of 3-deoxy-*ribo*-hexitol and 3-deoxy-*arabino*-hexitol

TABLE IV

AMOUNTS OF ALDITOLS[a] RECORDED IN HYDROLYSATES AFTER REDUCTION WITH LITHIUM ALUMINIUM HYDRIDE

| *Alditols* | *$Li^+$ resin* | *$SO_4^{2-}$ resin* |
|---|---|---|
| 3-Deoxy-*ribo*-hexitol | | 28 |
| 3-Deoxy-*arabino*-hexitol | | 29 |
| 2-*C*-Methylribitol | | 1.4 |
| 2-*C*-Methylglycerol | 21 | |
| Glucitol | 68 | 70 |
| Mannitol | 1.9 | 1.9 |
| Arabinitol | 2.9 | 2.6 |
| Erythritol[b] | 8.5 | |

[a]Mg/100 g of alkali-cooked cotton. [b]Erythritol has the same position as 3-deoxy-*ribo*-hexitol on the sulphate resin. The amount of 3-deoxy-*ribo*-hexitol is therefore corrected for the amount of erythritol recorded in the run on the lithium resin.

were obtained, but the amounts were only about 25% of those expected for a quantitative reduction of the 3-deoxyhexonic acid end-groups. The results clearly show that the reduction was incomplete, probably as a result of low accessibility of the terminal groups.

A striking confirmation of the conclusion that 2-*C*-methylglyceric acid end-groups are responsible for the presence of this acid in the hydrolysate followed from the fact that 2-*C*-methylglycerol was one of the more abundant terminal groups in the reduced sample. The absence of this alditol, following reduction with sodium borohydride after lactonisation, is explained by the inability of the corresponding acid end-groups to give lactones.

A small, but unfortunately overlapping, peak with the position of 2-*C*-methylribitol indicates that the 2-*C*-methylribonic acid found in the hydrolysate was derived from terminal units. The overlapping compound was not identified.

Since aldonic acid end-groups were detected, the presence of small amounts of glucitol, mannitol, arabinitol, and erythritol was expected. The results in Table IV show, however, that the amount of glucitol detected was considerably larger than the sum of the gluconic acid end-groups (Table II) and the maximum amount of glucose end-groups estimated after reduction with borohydride. This finding indicates that some glucosidic bonds must have been cleaved during the treatment with lithium aluminium hydride.

*Stability of the saccharinic acids towards acid hydrolysis and alkali-cooking.* — In separate experiments, samples of 2-*C*-methylglyceric, 2-*C*-methylribonic, 3-deoxy-*ribo*-hexonic, and 3-deoxy-*arabino*-hexonic acids (~10 mg) were subjected to the treatment with hydrochloric acid used for the hydrolysis of cotton. After evaporation, the organic acids and chloride ions were adsorbed on an anion exchanger in its hydrogencarbonate form, as described above. The organic acids were eluted with 5M acetic acid, and the eluates were evaporated to dryness and weighed. In none of the experiments was the loss in weight (including that due to lactone formation) larger than 8%. Samples of the recovered acid were chromatographed on analytical columns, using sodium acetate and acetic acid as described above. From the $D_v$ values and the colour responses (compared with those of authentic samples), no degradation effects were detected. For further confirmation, the sodium salts of the acids were converted into TMS derivatives; their g.l.c. behaviour was identical with that of the authentic samples.

Similar experiments were made with alkali treatment (5% sodium hydroxide) at 170° for 7 h. After removal of the alkali with a cation-exchange resin, the same methods, including the treatment with anion-exchange resin, were applied to check possible degradation and rearrangements. The recovery of 2-*C*-methylribonic and 2-*C*-methylglyceric acids was ~98%, and no traces of new products could be detected.

In agreement with the earlier observation[12], it was found that the *ribo* and *arabino* forms of the 3-deoxyhexonic acids were interconverted. In the experiment with the *ribo* form, the ratio between areas of the peaks corresponding to the *arabino*

and *ribo* forms was 1.09, whereas in the run with the *arabino* form the ratio was 1.26. The results show that equilibrium was not attained within 7 h. No degradation products were recorded, and the total yield was at least 95%.

## DISCUSSION

The degradation of the cellulose molecule during alkali-cooking at high temperature starts by a cleavage of D-glucosidic bonds along the molecular chain. Terminal D-glucose end-groups are formed and these end groups are then attacked[13] by a stepwise degradation ("peeling") of the same type as that occurring during the treatment of hydrocellulose with alkali at lower temperatures[14].

The formation of carboxylic acid end-groups renders the terminal group almost stable towards further attack[14,15] and, as shown by Machell and Richards[14], the formation of 3-deoxyhexonic acid end-groups explains the stopping of the peeling reaction during treatment of hydrocellulose with alkali. As already mentioned, the same stopping-reaction occurs during alkali-cooking[1] and during kraft-pulp cooking of wood[2,16]. No attempt to determine quantitatively the terminal acid groups formed during alkali cooking has been made previously. The results in Table II show that large proportions of the 3-deoxyhexonic acids are present in the hydrolysate. The determinations of the alditols, referred to above, confirm that these acids are derived from the terminal groups. Hence, additional support is obtained for the importance of this type of stopping reaction.

The possible formation of the 2-*C*-methylribonic acid end-group has been discussed by Machell and Richards[14], who tentatively identified it by paper chromatography in a hydrolysate of cotton hydrocellulose that had been treated with alkali at 100°. A minor quantity of this acid was also tentatively identified in an Eucalypt kraft pulp[2]. The results of the present research confirm that terminal 2-*C*-methylribonic acid units are formed under conditions simulating kraft-pulp cooking, but that the reaction path which gives rise to this terminal unit is of minor importance.

A more important reaction is the formation of 2-*C*-methylglyceric acid end-groups. This reaction has not been observed by earlier investigators. 2-*C*-Methylglyceric acid has previously been detected in hydrolysates from unbleached Eucalypt and pine pulps[2]. No attempts were made, however, to determine whether this acid is derived from end groups or from impurities, or is simply an artefact. The results given here show that this acid is derived from the end groups in the cellulose molecules, and hence that terminal, 2-*C*-methylglyceric acid end-groups are present in commercial pulps. The formation of 2-*C*-methylglyceric acid end-groups may be initiated by Lobry de Bruyn–Alberda van Ekenstein rearrangements of a D-glucose end-group, which give rise to terminal moieties having a keto group at C-3 (see Fig. 3). This terminal unit is then subject to a reverse aldol reaction, which leads to a splitting off of glycolaldehyde and the formation of an end group which then gives rise to elimination of the $\beta$-hydroxyl group at C-1 accompanied by the formation of an unstable dicarbonyl moiety. By a benzilic acid type rearrangement, this group is

converted to a terminal 2-*C*-methylglyceric acid group. The isolated acid exhibited no detectable optical rotation, which shows that, as expected, both D and L forms were formed.

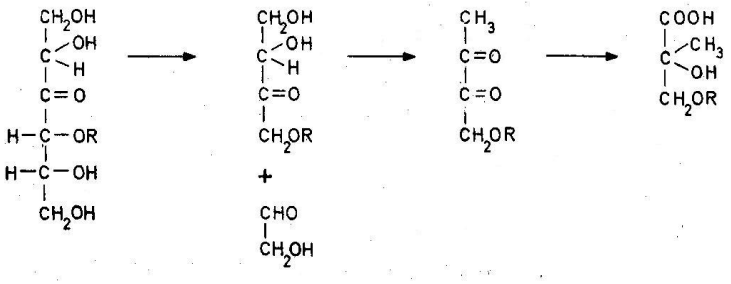

Fig. 3. Proposed route for the formation of 2-*C*-methylglyceric acid end-groups during alkali cooking of cotton cellulose.

When 3-deoxyhexonic, 2-*C*-methylribonic, and 2-*C*-methylglyceric acids were treated with alkali, no degradation could be detected after 7 h at 170°. The 3-deoxyhexonic acids were, however, slowly interconverted by epimerization, but the acids lacking an acidic hydrogen at C-2 were unchanged.

The total number of carboxylic acid end-groups in the alkali-cooked sample, as determined by alkalimetric titration[11], was 2.31 mmoles per 100 g. The total amount of 3-deoxyhexonic acids recovered in the hydrolysate was 1.14 mmoles per 100 g, *i.e.*, 49% of the carboxyl number. The amount of 2-*C*-methylribonic acid corresponds to 1%, and that of 2-*C*-methylglyceric acid to 23% of the carboxyl number. The sum of the aldonic acids listed in Table II is equal to 0.11 mmole, which corresponds to 5%. Hence, 78% of the carboxylic acid end-groups were accounted for by these determinations. The losses of acids during the hydrolysis and removal of mineral acid are very small. On the other hand, losses cannot be avoided during chromatography. It can therefore be concluded that, in addition to the carboxylic acid end-groups discussed above, only minor amounts of other acid end-groups can be present after the alkali cooking.

From molecular weight determinations by osmosis, and from determinations of the total number of carboxylic acid groups in alkali-cooked cotton, Franzon and Samuelson[15] concluded that ~70% of the cellulose molecules contained a carboxylic acid end-group. Since D-glucose end-groups were virtually absent, some other, non-ionic, terminal unit must be present. It has been suggested that arabinitol and erythritol end-groups are formed by cleavage of formic and glycolic acids, respectively, from the terminal D-glucose residue[17]. The possibility of the formation of D-glucitol end-groups has also been reported[14]. These hypotheses are disproved by the observation that no alditol end-groups are present in the alkali-cooked sample. The alditol determinations were repeated with another sample of alkali-cooked cotton and again no traces were recorded.

The results of the present work do not permit further conclusions about the non-ionic, terminal units, except that they confirm that D-glucose end-groups, and other reducing sugar moieties, are virtually absent. From model experiments with cellobiitol, Dryselius, Lindberg, and Theander[18] concluded that terminal 1,6-anhydro-D-glucose residues are formed during the alkali digestion, but, unfortunately, analytical techniques are not available for the direct determination of these groups in the cellulose.

ACKNOWLEDGMENTS

The authors thank the Cellulosaindustriens Forskningsstiftelse för Teknisk och Skoglig Forskning samt Utbildning for financial support, and Dr. Göran Petersson for the mass-spectrometric investigations.

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JOURNAL OF APPLIED POLYMER SCIENCE VOL. 19, 3007–3013 (1975)

# End-wise Degradation of Hydrocellulose During Hot Alkali Treatment

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## Synopsis

Determinations of reducing and carboxylic acid end groups in hydrocellulose treated with hot alkali showed that the end-wise degradation proceeded to such an extent that some of the molecules were brought completely into solution. The major carboxylic acid end groups formed in reactions competing with the end-wise degradation were 3-deoxyhexonic and 2-C-methylglyceric acids; but 2-deoxypentonic, 2-C-methylpentonic, and minor amounts of aldonic acids were also formed. The formation of these stable end groups explains the observed increase in number-average degree of polymerization during the treatment. The ratio between the number of lost glucose moieties and the number of carboxylic acid groups formed in the solid phase was about 170 and was only slightly affected by the duration of the hot alkali treatment.

## INTRODUCTION

During hot alkali treatment of cellulose containing reducing end groups (e.g., sulphite pulp), the cellulose molecules are subjected to end-wise degradation[1] (peeling) which results in a severe loss of material. The reaction paths derived from model experiments[2] are compatible with the results obtained in experiments with hydrocellulose.[3]

Competing reactions (stopping) give rise to carboxylic acid end groups[4] that are stable in alkaline medium. The purpose of this work is to determine and identify these acid groups and to evaluate the relative importance of peeling and stopping reactions.

## EXPERIMENTAL

Unbleached cotton, purified by solvent extraction and kier boiling,[5] was boiled under reflux conditions for 5 hr in 0.05*M* $H_2SO_4$ at a cotton to liquid ratio of 1:25. The hydrocellulose was treated for 4 min in 6% sodium hydroxide at +2°C, rinsed with water, soaked in 1% acetic acid for 30 min, rinsed with water again, and finally dried in circulating air at 30°C.

The hot alkali treatment was carried out in 1% sodium hydroxide at 95°C under nitrogen. An aqueous suspension (400 ml) containing 15 g of hydrocellulose and heated to the reaction temperature, was mixed with 400 ml of preheated 2% sodium hydroxide solution. After the desired reaction time, the cellulose was filtered off and washed with water. The sample was then treat-

ed with 500 ml of 0.2*M* potassium borohydride solution to convert the terminal reducing sugar moieties to alditol end groups. The reduction was carried out for five days at room temperature under stirring. A separate study showed that the copper number (according to Braidy) of the hydrocellulose dropped from 3.27 to 0.07 after one day and that a further decrease to 0.04 and 0.02 was obtained after three and five days. The results indicate that under the applied conditions [see ref. 6) the reducing end groups were converted completely into alditol end groups. The alditols were determined after hydrolysis by partition chromatography on ion-exchange resins in the lithium and sulphate forms.[7] The carboxyl number was determined by alkalimetric titration.[8] Viscosity determinations were made in copper ethylene diamine solution.[9]

Determinations of individual carboxylic acids were made on a larger sample of hot-alkali-treated (nonreduced) hydrocellulose (100 g). The treatment was carried out for 6 hr under identical conditions. The cellulose was hydrolyzed; and the nonvolatile monocarboxylic acids, isolated as a group by anion exchange, were separated by chromatography in sodium acetate. The peaks were rechromatographed on a preparative scale with the use of 0.5*M* acetic acid.[10]

The distribution coefficients were compared with those obtained with authentic samples. The values for all identified acids have been previously reported[10] except for 2-C-methylarabinonic acid ($D_v$ = 8.8 in 0.08*M* sodium acetate with acetic acid added to pH 5.9; and $D_v$ = 13.4 in 0.5*M* acetic acid). These values were in agreement with those of an authentic sample kindly supplied by Professor O. Theander.

Final confirmation of the identity of all acids was obtained by gas chromatography and gas chromatography–mass spectrometry of the trimethylsilyl-substituted trimethylsilyl esters.[11] The acids present in amounts greater than 2 mg were also analyzed on anion-exchange columns coupled with a three-channel analyzer.[12] The amounts reported for the individual acids were obtained from the weights of the isolated acids or lactones. Exceptions are the minor acids (<2 mg) that were estimated from the chromatograms.

Levulinic and anhydroisosaccharinic acids that are artifacts formed from glucose during the acid hydrolysis were recorded as usual.[10] These acids will not be further discussed.

## RESULTS AND DISCUSSION

### Peeling and Stopping Reactions

Table I shows that glucose was the predominant reducing end group in the hydrocellulose. The observation that a slight amount of mannitol was obtained from the reduced sample shows that isomerized moieties were also present (probably both mannose and fructose). Although the cotton was subjected to careful purification, the hydrocellulose contained 4-O-methylglucuronoxylan and pectic substances (see ref. 13), as reflected by the presence of carboxyl groups and xylose end groups. The observation that the xylose end groups were lost after a 3-hr hot alkali treatment, and that during this period the increase in the number of carboxyl groups in the solid cellu-

TABLE I
Yield and Analyses of Cellulose Samples after Borohydride Reduction

| Alkali treatment, hr | Yield, % | Glucitol, mmole/ 100 g | Mannitol, mmole/ 100 g | Xylitol, mmole/ 100 g | Carboxyl groups mmole/ 100 g | Intrinsic viscosity $dm^3/kg$ | $(DP)_n$ |
|---|---|---|---|---|---|---|---|
| 0 | 100 | 3.15 | 0.11 | 0.009 | 0.40 | 161 | 189 |
| 3 | 79.3 | 1.59 | 0.16 | 0.000 | 0.96 | 160 | 228 |
| 6[a] | 73.1 | 1.12 | 0.14 | 0.000 | 1.34 | 160 | 237 |
| 10 | 68.9 | 0.84 | 0.10 | 0.000 | 1.59 | 159 | 244 |

[a] The following results were obtained with the sample used for characterization of the carboxylic acid groups: glucitol, 1.10 moles; mannitol, 0.14 mmole; carboxyl groups (before reduction), 1.36 mmoles.

lose was small in comparison with the loss of reducing hexose moieties, indicates that most of the impurities containing carboxyl groups were removed during an early stage of the treatment. Analysis of the acids obtained after acid hydrolysis of the sample subjected to a 6-hr hot alkali treatment showed that 13 μmoles of 4-O-methylglucuronic acid, 23 μmoles of 2-O-(4-O-methylglucopyranosyluronic acid)-xylose and 2 μmoles of galacturonic acid (calculated per 100 g of the hot-alkali-treated cellulose) were present in the hydrolyzate. The results show that hemicellulose was still present, but that the amount was very small. No carboxyl groups are formed in cellulose during acid hydrolysis, and the number present as end groups in native cotton is small.[14] With the low molecular weight hydrocellulose studied, the number-average degree of polymerization $[(DP)_n]$ was, therefore, calculated from the number of reducing hexose moieties determined by the alditol method.

During the hot alkali treatment the end-wise attack starting at the reducing hexose group resulted in a large decrease in yield (Table I). The total number of reducing hexose moieties in the solid phase decreased; and among the remaining moieties, reducing glucose groups were by far the most abundant. These results are in agreement with the generally accepted theory. According to this theory, the reducing glucose groups are isomerized to fructose end groups that give rise to a β-elimination at C—4 with the liberation of a new glucose end group. In competing reactions that occur at lower rates, the reducing glucose groups are converted to carboxylic acid end groups that render the cellulose stable in alkaline medium. The decreased number of reducing hexose moieties and the increased number of carboxyl groups on prolonged hot alkali treatment is in agreement with this reaction scheme. A calculation of the $(DP)_n$ of the hot-alkali-treated cellulose was made on the assumption that the number of cellulose molecules was equal to the sum of reducing hexose moieties and carboxylic acid groups. The small fraction of carboxylic acid groups caused by remaining impurities in the cellulose was thus neglected. In this connection it should be mentioned that no alditols were detected in hydrolyzates of hot-alkali-treated samples that had not been subjected to borohydride reduction. The postulated[4] formation of alditol end groups during hot alkali treatment can therefore be disregarded.

With the hydrocellulose sample used, the viscosity was not affected by the hot alkali treatment. Evidently, the end-wise degradation of the cellulose

TABLE II
Cellulose Reactions and Lost Glucose Moieties after Hot Alkali Treatment for 3, 6, and 10 hr

| | 3 hr | 6 hr | 10 hr |
|---|---|---|---|
| Stopping reactions, $q_a$, mmole | 0.76 | 0.98 | 1.10 |
| Remaining reducing hexose groups, $q_r$, mmole | 1.39 | 0.92 | 0.65 |
| Lost cellulose molecules, $q_c$, mmole | 1.11 | 1.36 | 1.51 |
| Total loss of glucose moieties, $L$, mmole | 128 | 166 | 192 |
| $L$: $(q_a + q_c)$ | 68 | 71 | 74 |
| $L$: $q_a$ | 168 | 169 | 175 |
| $(DP)_{lost}$ | 53 | 57 | 62 |

molecules was compensated by a loss of short cellulose molecules. The results show that the polymolecularity of the cellulose decreased significantly during the hot alkali treatment. This was confirmed by the observation that $(DP)_n$ increased on prolonged duration of the alkali treatment. The results show that no detectable cleavage in the cellulose molecules occurred under the applied conditions (95°C).

The results given in Table I permit a calculation of the relative importance of the reactions that occurred during the hot alkali treatment. As a basis for the calculation, 100 g of hydrocellulose was arbitrarily chosen. The number of stopping reactions, $q_a$, was calculated as the product of carboxyl content and yield, and the number of remaining cellulose molecules $(q_r)$ containing reducing hexose moieties was calculated as the product of reducing hexose groups and yield. The number of cellulose molecules brought into solution, $q_c$, was obtained by subtracting the sum $q_a + q_r$ from the number of cellulose molecules present in the hydrocellulose (3.26 mmoles). The total number of lost glucose moieties, $L$, was calculated from the decrease in yield.

Table II shows that both the stopping $(q_a)$ and the peeling reactions were rapid during an early period of the hot alkali treatment. The observations that there was little change in the ratio $L/q_a$ during the course of the reaction and that both values decreased markedly with a decreasing content of reducing end groups, indicate that when accessibility factors can be disregarded the rates of both reactions are proportional to the number of reducing sugar moieties. The probability that the end-wise attack proceeds to such an extent that the whole cellulose molecule is brought into solution increases with decreasing molecular weight of those molecules that contain reducing end groups.[15] The observation that $q_c$ was already very high after a reaction period of 3 hr agrees with the high polymolecularity found in the hydrocellulose. In fact, the number of lost cellulose molecules was, throughout the reaction period, greater than the number of stopping reactions in the solid phase. In a previous study[15] of hydrocellulose samples of varying $(DP)_n$ subjected to hot alkali treatment for a constant time, $q_c$ was determined osmometrically whereas $q_a$ was determined by the same method as used in this paper. The results obtained in comparable experiments are in good agreement. As in the earlier paper, we have calculated the ratio $L/(q_a + q_c)$. In Table II this ratio is shown to be only slightly affected by the duration of the alkali treatment. The numerical values were very close to those calculated in the previous work for comparable samples. The results showed that on the average, about 70

glucose moieties were lost per cellulose molecule brought into solution or subjected to a stopping reaction in the solid phase. As expected, the ratio $L/q_a$ which increases markedly with decreasing length of the molecules with reducing end groups,[15] was only slightly affected by the duration of the hot alkali treatment. From the earlier studies[15] of hydrocellulose of different $(DP)_n$ it can be concluded that, under such conditions that the loss of cellulose molecules can be disregarded, the ratio between the rate of peeling to that of the formation of carboxylic acid end groups is about 90:1. If it is assumed that this ratio holds true under the conditions applied in the present work, the $(DP)_n$ of the hydrocellulose molecules lost during the hot alkali treatment $(DP)_{\text{lost}}$ can be calculated from the equation

$$L = q_a \cdot 90 + q_c(DP)_{\text{lost}}$$

The results included in Table II indicate that the $(DP)_n$ of the lost hydrocellulose molecules was low and, as expected, increased somewhat for a prolonged duration of the hot alkali treatment.

The results given in Table II show that all reaction rates decrease rapidly with increasing reaction time. A plot of the logarithm of the reducing hexose moieties in the solid phase against time showed that with prolonged time the attack on the reducing ends was slower than expected for a first-order reaction. Since the decrease in alkali concentration was small and it is known that this concentration has only a slight effect on the reaction rates,[16] the decreased rate of attack should be ascribed to a decreased accessibility. This conclusion had been drawn from earlier studies of yield as a function of time.[17] It has even been suggested that the decreased accessibility should be the primary stopping reaction and that the formation of carboxylic acid end groups occurs mainly after the end-wise degradation has proceeded to a point where the reducing end group is inaccessible to further peeling.[18,19] This hypothesis is incompatible with the results given in Table II, which indicate that the relative importance of the peeling and stopping reactions is only slightly affected by the duration of the treatment.

## Stopping Reactions

As indicated by model experiments[2] and confirmed by qualitative analysis of hydrocellulose subjected to hot alkali treatment,[4] the formation of 3-deoxyhexonic ("metasaccharinic") acid end groups is an important stopping mechanism. In the present work, an attempt was made to determine the carboxylic acid end groups quantitatively. The amounts of aldonic and deoxyaldonic acids isolated from the hydrolyzate of hydrocellulose subjected to hot alkali treatment for 6 hr are given in Table III. In agreement with previous investigations, large amounts of 3-deoxy-*ribo*-hexonic and 3-deoxy-*arabino*-hexonic acids were present. Since no interconversion occurs during acid hydrolysis,[10] both isomers must be formed as end groups during alkali treatment. According to the reaction scheme postulated by Machell and Richards,[4] 3-deoxyhexosulose end groups are formed as precursors.

Appreciable amounts of the two isomeric 2-deoxypentonic acids were also present. Since vicinal dicarbonyl compounds are easily cleaved between the carbonyl groups, it can be concluded that the 2-deoxypentonic acids were

TABLE III
Nonvolatile Organic Acids Isolated from the Hydrolyzate of 100 g of Hot-Alkali-Treated Hydrocellulose

| Acids | μmole |
|---|---|
| 3-Deoxy-*ribo*-hexonic | 205 |
| 3-Deoxy-*arabino*-hexonic | 163 |
| 2-Deoxy-*threo*-pentonic | 30 |
| 2-Deoxy-*erythro*-pentonic | 52 |
| 2-C-Methylribonic | 8 |
| 2-C-Methylarabinonic | 5 |
| Gluconic | 41 |
| Mannonic | 7 |
| Ribonic | 3 |
| Arabinonic | 67 |
| 2-C-Methylglyceric | 207 |
| Erythronic | 39 |
| Threonic | 3 |

present as end groups and were formed from the same precursor. The presence of the *threo* form is explained by a Lobry de Bruyn–Alberda van Ekenstein rearrangement of the intermediate.

The formation of 2-C-methylribonic acid end groups has been predicted and verified tentatively by paper chromatography.[4] The results given in Table III confirm this prediction, but show that this reaction is of minor importance. In addition, a very small amount of the 2-C-methylarabinonic acid was isolated. This acid has not been observed in previous investigations.

The formation of 2-C-methylglyceric acid end groups after the hydrolytic degradation of cotton cellulose in alkaline solution at 170°C (conditions simulating sulphate pulping) was recently demonstrated by two independent methods.[10] The observation that large amounts of this acid were present after alkali treatment of hydrocellulose at 95°C indicates that the postulated reaction scheme is valid also under conditions when the cellulose is subjected exclusively to end-wise degradation. In agreement with this reaction scheme both the *D* and *L* forms were produced (no optical rotation observed). Evidently, the postulated reverse aldol reaction occurs also under much milder conditions than those used in the previous work. The results support the conclusions that the depolymerization of the cellulose during alkali cooking at high temperature results in the formation of a reducing glucose moiety and that an end-wise degradation starts at this end group.[10,20]

In addition to these deoxyaldonic acids, small amounts of hexonic, pentonic, and tetronic acids were present as end groups after the hot alkali treatment. These acids are partially derived from the starting material;[14] but from the amounts recovered, an oxidation occurring during the hot alkali treatment is indicated. The dicarbonyl moieties that are precursors to the deoxyaldonic acid end groups are extremely sensitive to oxidation. Traces of oxygen present in the hydrocellulose at the beginning of the alkali treatment can contribute to the formation of these groups. Moreover, studies of the soluble organic acids formed during the peeling showed that both oxidized and reduced species are formed.[21] Formation of aldonic acid end groups even in the absence of oxygen might therefore be an additional explanation.

It is unlikely that the acids listed in Table III could be derived from any source other than the terminal carboxylic acid moieties in the cellulose. The total amount of acid groups corresponded to 0.83 mmole per 100 g. Glycolic acid can be present as end groups,[22] and the total amount found was 0.12 mmole per 100 g. The formation of these end groups is explained by an oxidative cleavage of the terminal dicarbonyl moiety that is the precursor to 2-C-methylglyceric acid. Glycolic acid is probably also obtained as an artifact during acid hydrolysis. Lactic (0.04 mmole) and glyceric (0.08 mmole) acids were also found, but their origin is still obscure. Minor amounts of three unknown acids, together constituting 6.4 mg per 100 g, were also isolated. The total carboxylic acid content in the alkali-treated hydrocellulose was 1.36 mmoles per 100 g. If a correction is applied for the amounts of observed 4-O-methyluronic and galacturonic acid groups, the total amount of acid end groups would be 1.32 mmoles. Some destruction of the acids occurs during acid hydrolysis;[10] and losses of acids, especially those forming stable lactones and intermolecular ester linkages, cannot be avoided in the separations. It is therefore difficult to decide whether the deoxyaldonic and aldonic acids discussed above are the only acid end groups present in the hot-alkali-treated cellulose or if, in addition, other acid end groups were present.

The financial support of 1959 Års Fond för Teknisk och Skoglig Forskning samt Utbildning is gratefully acknowledged.

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Received March 19, 1975

# Endwise Degradation of Hydrocellulose in Bicarbonate Solution

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## Synopsis

Determinations of individual terminal carboxylic acid endgroups and terminal reducing sugar moieties together with analysis of spent liquor revealed that the same reactions occur during treatment of hydrocellulose with hot sodium bicarbonate as with sodium hydroxide solution. Some fragmentation reactions, of little importance in the presence of sodium hydroxide, are favored in bicarbonate medium while benzilic acid rearrangements are less favored. Hence, the formation of 2-deoxyerythropentonic acid endgroups is more important. Among the soluble reaction products, 3,4-dideoxypentonic acid formed via 3-deoxypentulose and cyclic compounds formed via the same precursor are much more abundant in bicarbonate medium while 3-deoxy-2-hydroxymethylpentonic (isosaccharinic) acids are less abundant.

## INTRODUCTION

Polysaccharide reactions in mildly alkaline solutions at elevated temperature, both in the presence and in the absence of oxygen, have become of great interest in connection with efforts to develop sulfur-free methods for the production of wood pulp.[1] We now report on the endwise degradation of hydrocellulose in sodium bicarbonate solution in the absence of oxygen.

## EXPERIMENTAL

Unbleached cotton, purified by solvent extraction and kier boiling,[2] was refluxed for 5 hr in 0.05*M* sulfuric acid at a cotton:liquor ratio of 1:25. The hydrocellulose was treated for 4 min in 6% sodium hydroxide at +2°C, rinsed with water, soaked in 1% acetic acid for 30 min, rinsed with water again, and finally dried in circulating air at 30°C.

The hydrocellulose (150 g) was introduced into an autoclave containing 0.2*M* sodium bicarbonate (8.5 liters) solution. Nitrogen was bubbled through the suspension, and the autoclave was then closed and evacuated. The temperature was raised to 120°C over a period of 1 hour and kept at this temperature for 8 hr. During this treatment, the autoclave was vented at intervals of 30 min to release carbon dioxide. After cooling, the hydrocellulose was washed and dried as above.

The reducing endgroups in the cellulose were determined as alditols after reduction with 0.2*M* potassium borohydride[3] and the carboxyl number, by alkalimetric titration.[4] The viscosity was measured in copper ethylenediamine solution.[5] The coumption of sodium bicarbonate was determined by titrating the boiling liquor with hydrochloric acid to pH 7.

Journal of Applied Polymer Science, Vol. 22, 615–623 (1978)

0021-8995/78/0022-0615$01.00

The terminal carboxylic acid moieties were determined after acid hydrolysis as described previously.[6] In addition to the acids reported below, levulinic and 1,4-anhydro-3-deoxypentitol-2-carboxylic acids (artifacts formed from glucose) were obtained. The acids present in the spent liquor were liberated in an aliquot by cation exchange and taken up by stirring with an anion exchanger in its bicarbonate form. After neutralization with acetic acid to liberate carbon dioxide, the slurry was transferred to a column with anion exchanger in the acetate form. A nonelectrolyte fraction was isolated after washing with water and a monocarboxylic acid fraction was isolated after elution with 2*M* acetic acid. The dicarboxylic acids were subsequently eluted with 0.5*M* magnesium acetate solution. The nonvolatile acids contained in these fractions were separated and identified as previously described.[6] Formic and acetic acids were determined in a separate aliquot.[7]

The monosaccharides present in the nonelectrolyte fraction were determined by partition chromatography on an anion exchanger in the sulfate form.[8] Anion exchange chromatography in 0.075*M* potassium borate[9] confirmed their identities. Gas chromatography was used for determination of methanol and ethanol.

## RESULTS AND DISCUSSION

### Peeling and Stopping Reactions

Table I shows that bicarbonate treatment of cellulose, containing reducing sugar endgroups, results in an endwise attack (peeling) starting at the reducing terminal moiety. The loss in yield (19.9%) after treatment in 0.2*M* sodium bicarbonate at 120°C for 8 hr was similar to that recorded after treatment in 0.25*M* sodium hydroxide for 3 hr at 95°C for a sample with approximately the same degree of polymerization.[10] Only a slight decrease in intrinsic viscosity was obtained, indicating that no attack occurred along the cellulose chains and that short molecules present in the starting material were lost during the treatment.

Analysis of the bicarbonate-treated hydrocellulose before borohydride reduction showed that no alditol endgroups were present. As in the case of sodium hydroxide treatment, the bicarbonate treatment resulted in a decreased number of reducing endgroups and the formation of carboxylic groups (Table I). The acid groups determined after acid hydrolysis are listed in Table II. The results show that reactions which give rise to carboxylic acid endgroups of high stability (stopping reactions) compete with the peeling. As in alkali treatment in sodium hydroxide, formation of terminal 3-deoxyhexonic (metasaccharinic) acid groups is the major reaction. It has been shown by Machell and Richards[11] that these are formed by benzilic acid rearrangement of a terminal 3-deoxyerythrohexosulose precursor. The ribo form was sterically favored over the arabino form. At 170°C in strong alkali, the arabino form is more abundant. The observation that an interconversion of these acids occurs at high temperature[6,12] explains this difference.

As discussed below, the relative importance of fragmentation reactions compared to benzilic acid rearrangement is greater in bicarbonate solution than in sodium hydroxide. It can therefore be anticipated that the cleavage of the ter-

TABLE I
Yield and Analyses of Cellulose Samples

| | Yield, % | Alditols after $KBH_4$ reduction | | Carboxyl number, mmoles/100 g | Intrinsic viscosity, $dm^3/kg$ |
|---|---|---|---|---|---|
| | | Glucitol, mmoles/100 g | Mannitol mmoles/100 g | | |
| Untreated | — | 3.15 | 0.18 | 0.26 | 171 |
| $NaHCO_3$ treated | 80.1 | 1.77 | 0.23 | 1.03 | 164 |

TABLE II
Nonvolatile Organic Acids Obtained from Hydrocellulose[a] and Cellulose[b] Hydrolyzates

| Acids | Hydrocellulose $NaHCO_3$ 120°C, μmoles | Hydrocellulose NaOH 96°C, μmoles | Cellulose NaOH 170°C, μmoles |
|---|---|---|---|
| 3-Deoxyribohexonic | 125 | 205 | 617 |
| 3-Deoxyarabinohexonic | 81 | 163 | 648 |
| 2-Deoxyarabinohexonic | 26 | 25 | 0 |
| 2-Deoxyerythropentonic | 56 | 52 | 0 |
| 2-Methylribonic | 6 | 8 | 21 |
| 2-Methylarabinonic | 2 | 5 | 0 |
| 2-Methylglyceric | 111 | 207 | 533 |
| Gluconic | 43 | 41 | 29 |
| Mannonic | 8 | 7 | 6 |
| Arabinonic | 36 | 67 | 29 |
| Ribonic | 0 | 3 | 0 |
| Erythronic | 22 | 39 | 49 |
| Threonic | 2 | 3 | 0 |
| Glyceric | 76 | 80 | 65 |
| Glycolic | 93 | 120 | 30 |
| Lactic[c] | 28 | 40 | 20 |
| Deoxytetraric[c] | 6 | d | d |
| Threaric[c] | 4 | d | d |

[a] 100 g Hydrocellulose treated for 8 hr at 120°C in 0.2*M* sodium bicarbonate solution or hot alkali.
[b] 100 g Cellulose cooked in alkali.
[c] Origin unknown.
[d] Not determined.

minal 3-deoxyhexosulose moiety between the carbonyl groups will be favored in bicarbonate solution. As expected, the end group produced in this reaction (2-deoxyerythropentonic acid) was more abundant than observed after treatment with sodium hydroxide. The fact that no detectable amount of the threo form was formed supports this reaction path. The acid erroneously reported as 2-deoxythreopentonic acid in a previous report[10] is 2-deoxyarabinohexonic acid, which was also formed under the conditions applied in the present work. This acid has also been found in hydrolyzates of cellulose subjected to oxygen-alkali treatment.[13]

Another important stopping reaction during alkali cooking of cellulose[6] and hot alkali treatment of hydrocellulose[10] is the formation of 2-methylglyceric acid endgroups (compound II in Fig. 1). The yield of this acid relative to that of 3-deoxyhexonic acid was about the same under the conditions used in the present work. The proposed route of formation of this acid[6] requires that C-3 in the terminal moiety is involved in a Lobry de Bruyn-Alberda van Ekenstein rearrangement. Since 3-hexulose endgroups (I) must be unstable and their formation depressed by competing reactions, their number must be much lower than that of the reducing glucose moieties. This was confirmed by an experiment in which the reducing endgroups were determined after reduction to alditols and chromatographic analysis of the hydrolyzed sample. The chromatogram (Fig. 2) shows that small but significant amounts of the expected alditols (allitol, 0.015 mmole/100 g, and altritol, 0.011 mmole/100 g) were present in the bicarbonate-treated and reduced hydrocellulose. This rearrangement is also a prerequisite

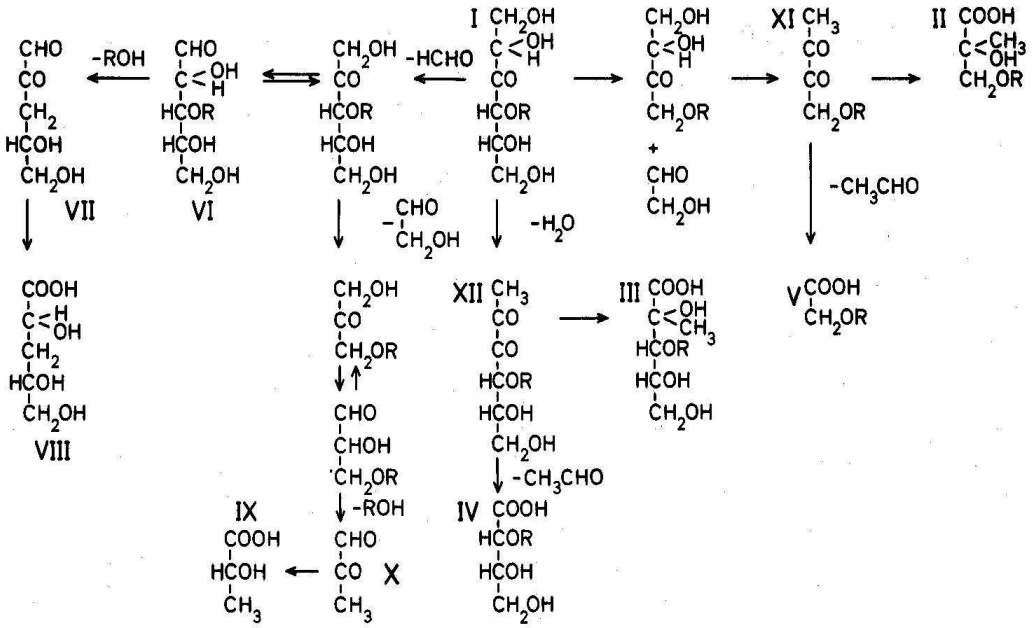


Fig. 1. Reaction scheme for the degradation of the 3-hexulose endgroups (I) formed in hydrocellulose during sodium bicarbonate treatment at 120°C; R represents the rest of the cellulose molecule.

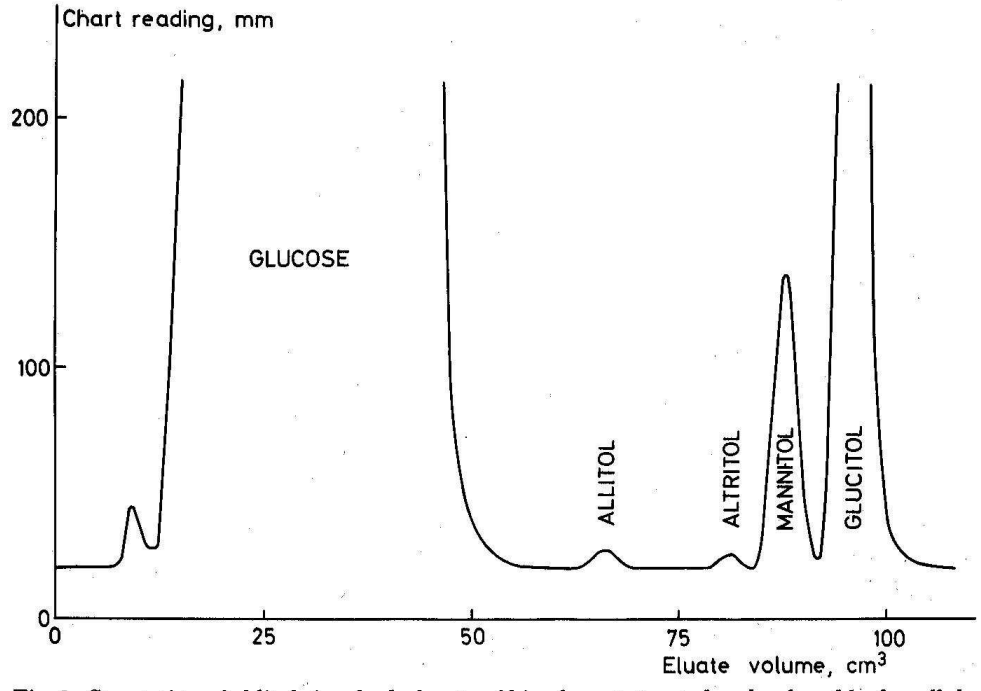


Fig. 2. Separation of alditols in a hydrolyzate of bicarbonate-treated and reduced hydrocellulose (150 mg) by partition chromatography in 85% (w/w) ethanol on a cation exchanger in the lithium form, Aminex A5, 13 ± 2 $\mu$m [4 × 910 mm]. Automatic analysis by the periodate–formaldehyde method.

for the formation of the observed 2-methylribonic and 2-methylarabinonic acid endgroups (III). Only minor amounts of these compounds were formed. A cleavage of the intermediate 1-deoxy-2,3-hexodiulose endgroup will contribute to the formation of erythronic acid endgroups (IV).

Other acids derived exclusively from the terminal groups are hexonic and ar-

abinonic acids formed by air oxidation during the purification of the cellulose and during the bicarbonate treatment. The total yield of isolated carboxylic acids which with certainty were derived from terminal acid moieties (0.52 mmole) and uronic acids derived from hemicellulose present after the treatment (0.09 mmole) was lower than the number of carboxylic acid groups found by titration (1.03 mmole). This corresponds to a recovery of 59%. The loss of uronic acids during acid hydrolysis is high, while aldonic and deoxyaldonic acids suffer little degradation.[6,14] There is insufficient information about the origin of the other acids, but it is most probable that the glyceric and glycolic acids observed are derived in part from terminal carboxylic acid moieties. Hence, a cleavage of the 1-deoxy-2,3-tetrodiulose endgroup between the keto groups may give rise to glycolic acid endgroups (V).

Calculations of the relative importance of the major reactions which occur during the hot alkali treatment were made with the assumptions previously used.[10] As a basis for the calculation, 100 g hydrocellulose was arbitrarily chosen. For the bicarbonate treatment, the number of stopping reactions, $q_a$, calculated from the product of yield and carboxyl number, was 0.83 mmole. The number of remaining cellulose molecules containing reducing endgroups ($q_r$) was 1.60; and the number of cellulose molecules brought into solution, calculated by subtracting $q_a + q_r$ from the number of molecules present in the hydrocellulose (3.33 mmoles), was equal to 0.90. The number of glucose moieties lost, calculated from the loss in yield, was 123 mmoles. The ratio of the number of glucose moieties lost to the number of carboxylic acid endgroups formed in the solid phase (123/0.83 = 148) was slightly lower than during hot alkali treatment in sodium hydroxide. The number of glucose moieties lost per cellulose molecule either brought into solution or subjected to a stopping reaction in the solid phase [123/(0.83 + 0.90) = 71] was the same as that observed after treatment in sodium hydroxide and so was the calculated average degree of polymerization ($D.P_n$ = 54) of cellulose molecules brought completely into solution.

## Spent Liquor

The consumption of sodium bicarbonate during the treatment corresponds to 1.42 moles per mole of glucose moieties brought into solution. The alkali consumption was approximately the same as in previous studies of sodium hydroxide treatment.[15] The isolated acids listed in Table III account for 87% of the observed consumption. The weight of the isolated acids was 12.9 g calculated per 100 g hydrocellulose, which corresponds to a recovery of 64.9% calculated on the weight of cellulose brought into solution. A calculation on a carbon basis shows that only 57% was recovered as carboxylic acids.

Determinations of the acids present in the monocarboxylic acid fraction (Table III) showed that these were essentially the same as observed after sodium hydroxide treatment of hydrocellulose.[16] On the other hand, the product distribution differed markedly. Hence, the benzilic acid rearrangement of liberated 4-deoxy-2,3-hexodiulose which results in the formation of 3-deoxy-2-hydroxymethylpentonic (isosaccharinic) acids[17] is less important in bicarbonate medium, while 3,4-dideoxypentonic acid, which belongs to the minor products in sodium hydroxide, was the second most abundant acid after bicarbonate treatment. The results are in agreement with observations[18] that both types of acids are formed

TABLE III
Carboxylic Acids in Spent Liquor after Sodium Bicarbonate Cooking of Hydrocellulose at 120°C

| Acids | Weight per 100 g degraded cellulose, g |
|---|---|
| 3-Deoxy-2-hydroxymethylerythropentonic | 2.15 |
| 3-Deoxy-2-hydroxymethylthreopentonic | 11.21 |
| 1,4-Anhydro-3-deoxypentitol-2-carboxylic | 2.16 |
| 3-Deoxyribohexonic | 0.13 |
| 3-Deoxyarabinohexonic | 1.62 |
| 2,5,6-Trihydroxy-3-hexenoic | 0.54 |
| 2-Deoxyerythropentonic | 0.13 |
| 3-Deoxyerythropentonic | 0.45 |
| 3-Deoxythreopentonic | 0.90 |
| 3,4-Dideoxypentonic (2,5-dihydroxypentanoic) | 14.10 |
| 2-Deoxytetronic (3,4-dihydroxybutanoic) | 4.98 |
| 3-Deoxytetronic (2,4-Dihydroxybutanoic) | 0.98 |
| 2-C-Methylglyceric | 0.04 |
| Glyceric | 0.21 |
| Lactic | 0.69 |
| Glycolic | 1.71 |
| Acetic | 5.72 |
| Formic | 16.85 |
| 3-Deoxy-2-hydroxymethylerythropentaric | 0.04 |
| 3-Deoxy-2-hydroxymethylthreopentaric | 0.16 |
| 3-Deoxythreopentaric | 0.06 |
| Deoxytetraric (malic) | 0.09 |

in competing reactions from the same intermediate. The fragmentation reaction which gives rise to formic acid and 3-deoxypentulose (precursor to 3,4-dideoxypentonic acid) is favored over benzilic acid rearrangement. Other striking differences are that larger amounts of 1,4-anhydro-3-deoxypentitol-2-carboxylic (anhydroisosaccharinic) and 2-deoxytetronic acids were formed in bicarbonate medium. Most likely, the latter is formed together with glycolaldehyde by cleavage of 4-deoxy-2,3-hexodiulose.

A reverse aldol reaction of terminal 3-hexulose moieties in the cellulose (I in Fig. 1) will result in the elimination of formaldehyde and the formation of unstable arabinose and ribose endgroups (VI). A rapid $\beta$-elimination followed by a benzilic acid rearrangement of the dissolved dicarbonyl intermediate (VII) explains the formation of the two diastereomeric 3-deoxypentonic acids (VIII). This reaction, which competes with the stopping reaction, giving rise to 2-methylglyceric acid endgroups, is favored in bicarbonate medium. A reaction path which may in part be responsible for the formation of 2-hydroxypropanoic acid (IX) via pyruvic aldehyde (X) is included in Figure 1. Acetic acid is probably formed by a hydrolytic cleavage of the terminal dicarbonyl intermediates XI and XII.

The 3-deoxy-2-hydroxymethylpentaric acids present in the spent liquor are the major dicarboxylic acids formed during alkali treatment of 4-O-methylglucuronoxylan.[19] Their presence confirms that the cotton cellulose was not completely freed from hemicellulose during the purification procedures employed. Only minor amounts of other dicarboxylic acids were present. Evidently, the formation of dicarboxylic acids from the cellulose is favored at high alkalinity in the solution.[20] Only trace amounts of methanol (61 mg per 100 g degraded cellulose) and ethanol (27 mg) were formed.

The nonelectrolyte fraction that was not retained by the ion exchangers cor-

responded to 6.5% of the cellulose brought into solution. While no detectable amounts of monosaccharides were present in spent liquors from alkali treatment in sodium hydroxide, significant amounts (Table IV) were present at the end of the bicarbonate treatment. Hexoses do not belong to the soluble compounds formed from the reducing end during alkaline peeling. Since hexoses are much more stable in bicarbonate solution than (1 → 4)-β-linked oligosaccharides, we conclude that the small amounts of glucose, mannose, and fructose found to be present were derived from the nonreducing end in cellulose molecules brought completely into solution.

More interesting is the observation that an appreciable amount of 3-deoxypentulose was present. This comparatively unstable sugar which, as already mentioned, is a precursor of 3,4-dideoxypentonic acid, was found in large amounts and can give rise to a complex mixture of cyclic compounds.[21] Their properties are such that a large proportion should be retained irreversibly by the ion exchange resins under the applied working conditions. Reactions of this type, together with condensation reactions involving aldehydes split off during bicarbonate treatment (Fig. 1), explain the fact that the total recovery in the isolated acid and nonelectrolyte fractions amounted only to 73% of the weight of the cellulose brought into solution. It is worth mentioning that in a previous study of alkali treatment in sodium hydroxide[16] the recovery of acids amounted to 85%. The results permit the conclusion that the fragmentation of 4-deoxy-2,3-hexodiulose in bicarbonate medium is favored even more over benzilic acid rearrangement than is apparent from the yield of 3,4-dideoxypentonic acid relative to 3-deoxy-2-hydroxymethylpentonic acids.

To characterize the products which escaped identification by the methods employed, an aliquot of the spent liquor was subjected to ultrafiltration (separation limit corresponding to a molecular weight of 500). The organic material in the fraction retained corresponded to 20% of the weight of degraded cellulose. Another aliquot was acidified to pH 3 and passed through a column containing a macroreticular styrene–divinylbenzene resin (Amberlite XAD-1). Chromic acid oxidation showed that 19.2% of the oxidizable material was retained. After washing with water, adsorbed solutes were eluted with 50% aqueous ethanol. The recovery was 14.7% of the weight of the degraded cellulose. The solution exhibited a strong absorbtion in the UV, with an absorbance maximum at 260 nm. The absorptivity at this wavelength was 19.6 l./(g)(cm). Combined with elemental analysis (C, 61.0%; H, 7.0%), these results showed that cyclic compounds of fairly high molecular weight constituted the major portion of the material retained irreversibly on the ion exchange resins. The equivalent weight was 527, determined by titration to pH 8. Borohydride reduction decreased the sorption on XAD-1 markedly and decreased the consumption of sodium hydroxide for neutralization to pH 8 by 20%, indicating the presence of carbonyl (or enol) groups.

TABLE IV
Monosaccharides Isolated from the Spent Liquor after Sodium Bicarbonate Cooking of Hydrocellulose at 120°C

| Monosaccharides | Weight per 100 g dissolved cellulose, mg |
|---|---|
| Glucose | 95 |
| Mannose | 17 |
| Fructose | 32 |
| 3-Deoxypentulose | 266 |

Extraction of the acidified liquor with ethyl acetate after saturation with ammonium sulfate showed that colored solutes were extracted almost completely. The extractable material corresponded to 14.3% of the degraded cellulose. Thin-layer chromatography[21] indicated that a large number of solutes including 1,2-cyclopentanedione were present. Gas chromatography of the trimethylsilyl derivatives before and after reduction with borohydride revealed that at least 60 volatile derivatives were present in the material converted. Gas chromatography and gas chromatography–mass spectrometry showed that the compound contained in the largest peak was 1,2-dihydroxybenzene, previously identified after alkali treatment of monosaccharides.[22,23]

The financial support of 1959 Års Fond för Teknisk och Skoglig Forskning samt Utbildning is gratefully acknowledged.

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Received March 20, 1976
Revised August 10, 1976

Chemica Scripta. 1976, 9, 151–154

# Kinetics of the Isomerization and Degradation of Xylotetraose in Alkaline Medium

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*Received February 24, 1976*

## Abstract

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*Chemica Scripta* (*Sweden*) 1976, 9 (4), 151–154.

Determinations of the sugars produced by isomerization and end-wise degradation of xylotetraose show that the over-all rate of degradation is predominantly determined by the rate of conversion of the anionic reducing xylose moiety to a terminal xylulose moiety. In the consecutive end-wise degradation, a rearrangement of the anionic xylulose end group which preceeds the cleavage of the glycosidic bond is the rate-determining step. This reaction step is slower with cellotetraose than with xylotetraose. The reaction rates are independent of the number of xylose moieties in the oligomer.

## Introduction

The Lobry de Bruyn–Alberda van Ekenstein interconversion among aldoses and ketoses in alkaline medium is among the most important reactions in carbohydrate chemistry. However, little kinetic data has been published [1–3] and the interpretation of results is still controversial. Research in this field is complicated due to the degradation of the sugars in consecutive reactions, and difficulties in analyses of solutions containing oligomeric sugars. As shown recently, partition chromatography on anion exchange resins is a useful method for separation of oligomers [4, 5]. In the present work this technique is applied for the determination of the products formed by isomerization and end-wise degradation of xylotetraose in alkaline medium.

## Experimental

A Teflon tube provided with a Teflon coated stirrer was filled with 50 ml sodium hydroxide solution. The solution was boiled and immediately covered with a layer of purified paraffin oil to exclude air. The Teflon tube was placed in a water bath at 40.0°C. Chromatographically pure tetrasaccharide (1.0 mg) was dissolved in 5 ml deaerated water and injected into the solution with a plastic syringe. Samples (5 ml) were withdrawn at intervals. After treatment with a cation exchanger ($H^+$) at +2°C the acids formed in the reaction were neutralized with sodium hydroxide. The sample solution was evaporated to dryness at reduced pressure, diluted with ethanol and then analysed by partition chromatography in aqueous ethanol (75–78% w/w) on an anion exchanger in the sulphate form [4, 5]. The volume distribution coefficient, $D_v$, is defined as the adjusted retention volume calculated in bed volumes.

### *Identification of reaction products*

A chromatogram from the analysis of the sugars present after alkali treatment of xylotetraose is given in Fig. 1. Four prominent peaks denoted X1–X4 were recorded, together with two minor peaks ahead of each major peak. The $D_v$-values for the major peaks were in close agreement with those of authentic samples of xylose (X1), xylobiose (X2), xylotriose (X3) and xylotetraose (X4).

It has previously been demonstrated [4–6] that for oligomeric sugars (and alditols) with the same mode of glycosidic linkages, each sugar moiety of a given structure (e.g. xylose) gives the same incremental change in log $D_v$ independent of the structure of the terminal moiety. The relationship is valid for disaccharides and higher saccharides and in general the monomers cannot be included. The positions of the first and second peaks on the chromatogram correspond to that of xylulose and lyxose. It can therefore be predicted that the first and second minor sugars (X1U and X1L) eluted ahead of xylobiose are the isomers containing a xylulose moiety [4-O-(β-D-xylopyranosyl)-D-*threo*-pentulose] and the epimer of xylobiose [4-O-(β-D-xylopyranosyl)-D-lyxose]. Similarly the first and second compounds ahead of xylotriose and xylotetraose should be the corresponding isomerization products of these oligomers. The plots of log $D_v$ of the oligomers with terminal ulose and lyxose moieties against the predicted number of monomeric units given in Fig. 2 confirm the identity of these oligomers.

Similarly the major reaction products from the alkali treatment of cellotetraose were identified as cellotriose, cellobiose and glucose. Mannose, fructose and the corresponding isomerized oligosaccharides containing reducing terminal mannose and fructose moieties were also present.

## Results and discussion

The alkaline degradation of polysaccharides containing 1,4-glycosidic linkages and terminal reducing aldose moieties starts by an isomerization of the reducing end [3]. Epimeric aldose moieties and isomeric ulose groups are formed. The ulose end groups are rearranged to 2,3-enediol groups which give rise to a β-elimination at C-4. This results in the formation of a new terminal reducing aldose moiety and several carboxylic acids as the main reaction products [7].

The formation of xylotriose, xylobiose and xylose as important products (Fig. 1) during the alkali treatment of xylotetraose is in agreement with this reaction scheme and so is the observation that the concentration of the tetrasaccharide with a ulose end group (X3U) first increased rapidly and then decreased continuously during prolonged treatment (Fig. 3). The amounts of oligomers with ulose and lyxose end groups were small compared to those with xylose end groups.

A simplified reaction scheme in which enediols and other intermediates are omitted can therefore be written

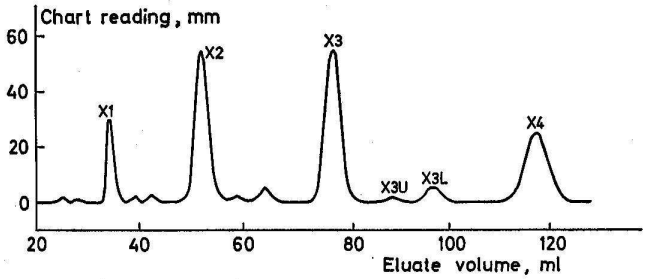
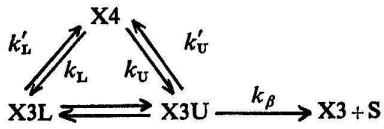



*Fig. 1.* Separation by partition chromatography in 75% aqueous ethanol of products obtained after treatment of xylotetraose (X4) in 0.045 M sodium hydroxide solution for 4.25 h at 40.0°C. Resin bed: 4×750 mm, Technicon T5A, $SO_4^{2-}$, 10–15 μm. Flow rate: 3.1 cm $min^{-1}$. (X1) xylose; (X2) -bi-; (X3) -tri-; (X4) xylotetraose. The two small peaks ahead of each major saccharide refer to isomers with terminal lyxose (L) and ulose (U) moieties.




where $k_U$ and $k_L$ are the rate constants for the formation of ulose and lyxose end groups from xylose end groups, $k'_U$ and $k'_L$ those of the reverse reactions and $k_\beta$ the rate constant for the $\beta$-elimination which gives rise to xylotriose and "peeling" acids (S).

With first order reactions the following equation applied.

$$d[X4]/dt = -(k_L + k_U)[X4] + k'_L[X3L] + k'_U[X3U]$$

Fig. 4 shows that plots of the logarithm of the xylotetraose concentration versus time gave a linear relationship not only in the initial period of alkali treatment but also during prolonged treatments. This was not unexpected since, as already mentioned, the concentrations of tetrasaccharides with lyxose and ulose end groups were small. This implies that the second and third terms on the right-hand side in the equation can be neglected. The pseudo-first-order rate constant $k_4 = k_L + k_U$ calculated from experiments at different concentrations of sodium hydroxide are given in Table I.

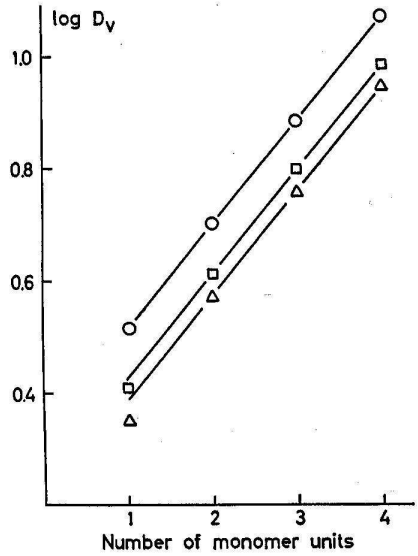



*Fig. 2.* Relationship between log $D_V$ and number of monomeric units for the saccharides present after alkali treatment of xylotetraose. Sugars containing ○ xylose, □ lyxose and △ ulose end groups.

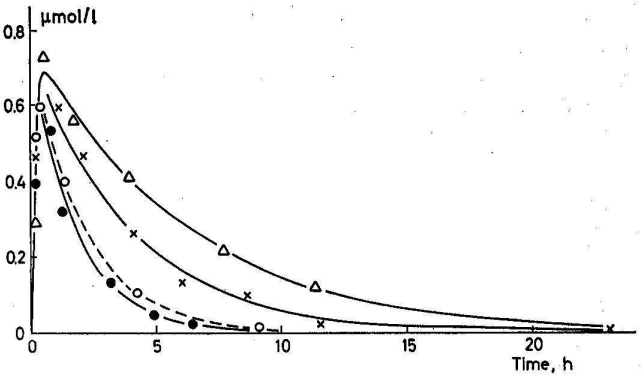



*Fig. 3.* Concentrations of tetrasaccharide with a terminal ulose moiety (X3U) after treatment of xylotetraose at 40.0°C at different sodium hydroxide concentrations: ●, 0.45 M; ○, 0.045 M; ×, 0.0091 M and △, 0.0045 M. The lines represent calculated values.

An estimate of $k_L$ from determinations of the tetrasaccharide with a lyxose end group during the first period of the alkali treatment showed that $k_L$ was about 200 times less than $k_U$. This means that $k_4$ can be taken as the rate constant for the isomerization of a terminal xylose moiety to a xylulose moiety. With the assumption that the $\beta$-elimination follows a pseudo-first-order reaction scheme we can therefore calculate the concentration of the tetraose with ulose end groups (X3U) from the equations

$$d[X4]/dt = -k_4[X4]$$

$$d[X3U]/dt = k_4[X4] - k_\beta[X3U]$$

which upon integration gives

$$[X3U] = \frac{k_4[X4]_0}{k_\beta - k_4}[e^{-k_4 t} - e^{-k_\beta t}]$$

where $[X4]_0$ is the initial concentration of xylotetraose.

The second term exerts an influence only during a short initial period and $k_\beta$ was therefore determined from [X3U] after the maximum concentration had been reached. The average values are given in Table I. The calculated concentration of the tetrasaccharide with ulose end groups was in good agreement with the observed values (Fig. 3).

As expected the rate constant for the $\beta$-elimination was much higher than that for the isomerization of the terminal xylose moiety in the tetrasaccharide to a xylulose moiety. It is noteworthy that the ratio $k_\beta/k_4$ was almost constant, independent of the sodium hydroxide concentration. Both rate constants increased markedly when the sodium hydroxide concentration was raised from 0.0045 M to 0.045 M while the change was fairly small when the concentration was further increased by a factor of ten. These results indicate that terminal anionic xylose and xylulose

Table I. *Rate constants for the degradation of xylotetraose at 40.0°C at different sodium hydroxide concentrations*

| Conc. (M) | $k_4$ $h^{-1}$ | $k_\beta$ $h^{-1}$ | $k_\beta/k_4$ |
|---|---|---|---|
| 0.0045 | 0.16 | 7.1 | 44 |
| 0.0091 | 0.26 | 11.6 | 45 |
| 0.0125 | 0.30 | 14.0 | 47 |
| 0.045 | 0.46 | 21.3 | 46 |
| 0.45 | 0.57 | 25.7 | 45 |

moieties are intermediates and that their decomposition is the rate determining step both as far as the isomerization of the xylose end group to xylulose and the consecutive reactions resulting in a $\beta$-elimination are concerned.

Assuming that the decomposition of the anionic terminal moiety is a first-order reaction we obtain

$$d[X4]/dt = -k_4^* \alpha[X4]$$

where $\alpha$ is the degree of ionization of the terminal moiety and $k_4^* \alpha = k_4$. If the terminal moiety is considered as a monoprotic acid with ionization constant $K_a$, the rate constant $k_4^*$ can be determined graphically from the relationship:

$$\frac{1}{k_4} = \frac{1}{k_4^*}\left(\frac{K_w}{[OH]K_a} + 1\right)$$

where $K_w$ is the ionic product of water.

The linear relationship given in Fig. 5 supports the validity of the predicted reaction scheme. The $k_4^*$ value determined graphically (0.585) was only slightly higher than the value of $k_4$ determined at the highest sodium hydroxide concentration, indicating that ionization was virtually complete in 0.45 M sodium hydroxide. The value of the ionization constant determined from the slope of the straight line corresponds to a $pK_a$-value of 11.6. This value is slightly lower than the $pK_a$-value of xylose determined by other methods [8].

The results suggest that the rate-determining step in the complex reaction scheme for the isomerization of aldoses to ketoses in alkaline medium is the rearrangement of the anion of the aldose to an enediolate anion. Evidence for the validity of this reaction scheme for the transformation of glucose to fructose has been presented by Lai [3]. According to this author both mono- and di-anionic intermediates are involved in the rate-determining reaction at high alkali concentration but it is doubtful if the experimental data is accurate enough to permit this conclusion. The results obtained in the present work are consistent with the assumption that only mono-anions of xylose are formed.

According to the generally accepted reaction scheme the cleavage of the glycosidic bond by $\beta$-elimination is preceeded by the formation of an unstable 2,3-enediolate ion. The observation that the ratio $k_\beta/k_4$ was virtually independent of the hydroxide concentration suggests that the rate-determining step is the rearrangement of an anionic ulose moiety to a 2,3-enediolate anion. A similar reaction scheme has previously been postulated for the end-wise degradation of (1→3)-$\beta$-D-glucans [9]. Our results indicate that the acid strength of the terminal xylose and xylulose moieties is approximately the same.

According to the reaction scheme given above the concentration of xylotriose in the reaction solution can be calculated from the equation

$$d[X3]/dt = k_\beta[X3U] - k_3[X3]$$

After the first initial period this equation can be approximated by

$$d[X3]/dt = \frac{k_\beta k_4[X4]_0}{k_\beta - k_4} e^{-k_4 t} - k_3[X3]$$

Since in the case of xylotetraose, $k_4$ is small compared to $k_\beta$ this equation reduces to

$$d[X3]/dt = k_4[X4]_0 e^{-k_4 t} - k_3[X3]$$

or $d[X3]/dt = k_4[X4] - k_3[X3]$

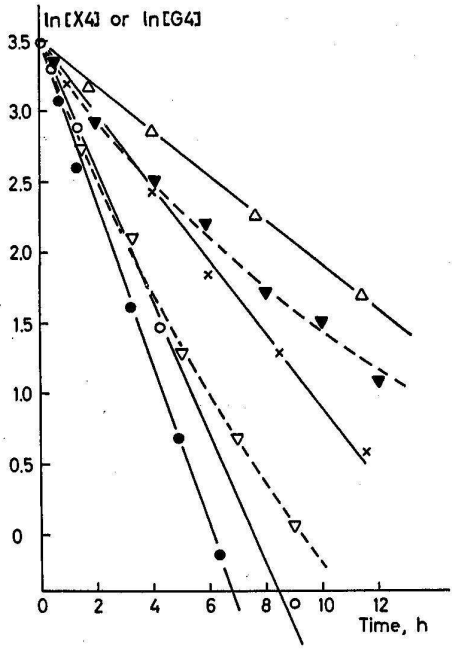



*Fig. 4.* Relationship between the nat. logarithm of the xylotetraose and cellotetraose concentration ($\mu$mol/l) and the time of reaction. Experiments with xylotetraose (full line) in ●, 0.45 M; ○, 0.045 M; ×, 0.0091 M and △, 0.0045 M NaOH and cellotetraose (dashed line) in ▽, 0.045 M and ▼, 0.0091 M NaOH.

Similarly the concentration of xylobiose can be calculated from the equation

$$d[X2]/dt = k_3[X3] - k_3[X2]$$

According to the postulated reaction scheme each reaction step involves the same type of reaction and it is reasonable to assume that the rate constants should be the same, independent of the number of non-reducing xylose moieties present in the oligomer. Hence the concentration of oligomers in the solution should be given by the equations

$$[X4] = [X4]_0 e^{-k_4 t}$$

$$[X3] = k_4 t[X4]$$

$$[X2] = k_4 t[X3]/2$$

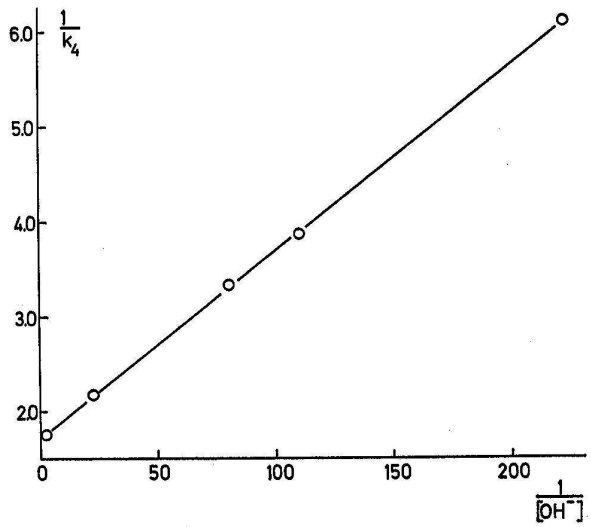



*Fig. 5.* Relationship between $1/k_4$, calculated for xylotetraose, and $1/[OH^-]$.

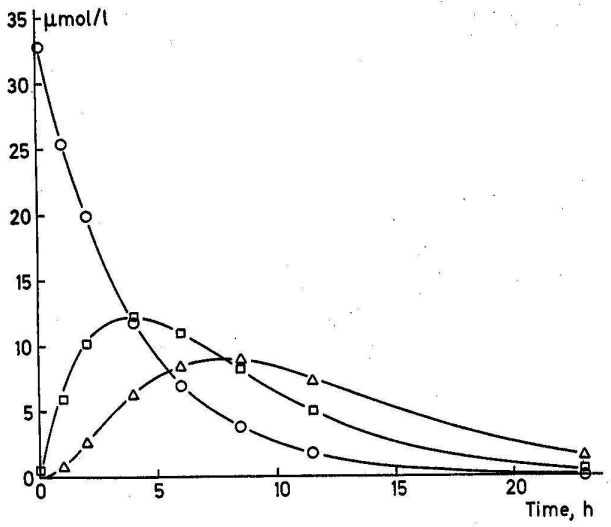



*Fig. 6.* The degradation of xylotetraose (○) in 0.0091 M sodium hydroxide solution at 40.0°C and the subsequent formation and degradation of xylotriose (□) and xylobiose (△). Full lines represent calculated values.

In Fig. 6 the calculated concentrations are compared with the experimental results obtained in 0.0091 M sodium hydroxide. Equally good agreement was obtained in the experiments with xylotetraose at the other hydroxide concentrations. The results permit the conclusion that in a homogeneous medium the rate of the end-wise attack on the oligomers is independent of the number of xylose moieties and that the reaction step which determines the over-all reaction rate is the conversion of an anionic reducing xylose moiety to a xylulose end group.

In contrast to xylotetraose the decomposition of cellotetraose in sodium hydroxide followed pseudo-first-order reaction kinetics only during a short initial period (Fig. 4). Interestingly, the initial rate constants were almost the same as those determined for xylotetraose at the same sodium hydroxide concentration.

On prolonged reaction the observed loss of cellotetraose was much less than that calculated for a first-order reaction. These results are explained by the observation that the oligomers with fructose end groups were much more stable than those with xylulose end groups and therefore accumulated in the solution. Appreciable amounts of oligomers with mannose end groups were also present. Evidently, the reconversion of the isomeric tetrasaccharides to cellotetraose cannot be disregarded. These results are in agreement with observations by MacLaurin and Green [10] on the isomerization and degradation of cellobiose in 1 M sodium hydroxide at 25°C. These authors found that an appreciable amount of cellobiulose was accumulated in the system and that the conversion of this sugar to cellobiose is a fairly rapid reaction with rate constant of about 30% of that of the reverse reaction.

The deviation from a linear relationship for cellotetraose, and the presence of larger amounts of oligomers with ulose end groups in the reaction solutions in these experiments than in parallel experiments with xylotetraose shows that the consecutive reactions are significantly slower with species containing terminal fructose groups than with those containing terminal xylulose groups. A plausible explanation is that the comparatively stable pyranose form of the fructose end group is favoured while the only cyclic form possible for the xylulose end group is the less stable furanose form.

## Acknowledgement

The sample of cellotetraose was kindly supplied by Dr Kelvin Hamilton, Rayonier Inc., Shelton, USA. Financial support from the 1959 Års Fond för Teknisk och Skoglig Forskning samt Utbildning is gratefully acknowledged.

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*Carbohydrate Research*, 54 (1977) 295–299


## Note

# Epimerization and degradation of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylitol in alkaline medium

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(Received June 7th, 1976; accepted for publication, August 3rd, 1976)

4-*O*-Methylglucuronoxylan is an important constituent of wood and wood pulps. An appreciable portion of the uronic acid moieties are split off during a kraft cook[1] by reactions which are still obscure. Moreover, other methylated uronic acid moieties formed[2] in the pulp during the cooking have been identified as 4-*O*-methyl-L-iduronic acid[3].

We now report on model experiments with 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylitol (**1**).

## EXPERIMENTAL

Deaerated solutions of **1** in M sodium hydroxide (10 ml) were heated in Teflon-coated autoclaves for various times, then cooled, decationised, and analyzed by anion-exchange chromatography. The eluates were monitored automatically at 254 nm and by using a three-channel analyzer[4]. A typical chromatogram is shown in Fig. 1. The quantitative determinations were based on the response in the periodate–formaldehyde channel.

The products were isolated by preparative anion-exchange chromatography (Table I) and identified by g.l.c. and g.l.c.–m.s. of the trimethylsilyl ($Me_3Si$) derivatives[5]. The compound (X) eluted first was xylitol and the second was unreacted **1**. The product in peak **2** gave colour tests very similar to those of **1**, indicating it to be the epimerization product 2-*O*-(4-*O*-methyl-β-L-idopyranosyluronic acid)-D-xylitol. The mass spectra of **1** and **2** were almost identical, and **2** gave xylitol and 4-*O*-methyl-L-iduronic acid on acid hydrolysis.

The compound eluted last gave a weaker, relative response in the carbazole channel, but had very strong u.v. absorbance ($\lambda_{max}$ 230 nm, ε 5910) characteristic of α,β-unsaturated uronic acids[6]. The acid was retained very strongly by the anion-exchanger, both in sodium acetate solution and in acetic acid. These data suggest the

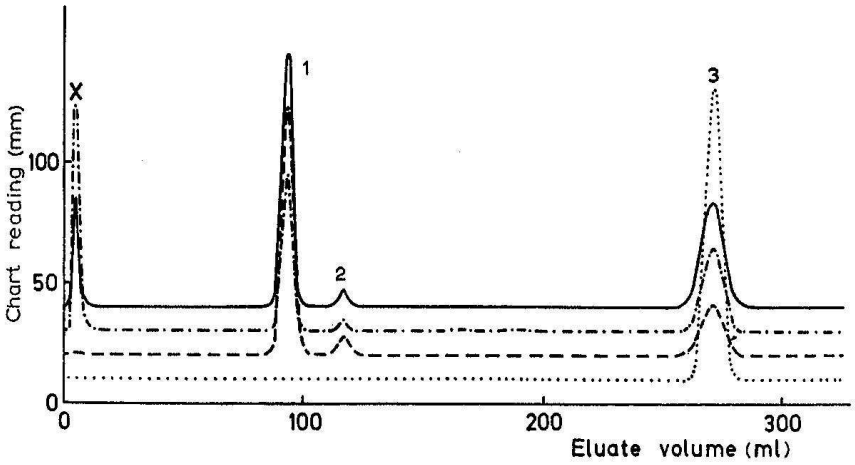



Fig. 1. Separation of products obtained after treatment of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylitol (**1**) in M sodium hydroxide for 1 h at 150°. Column (4 × 820 mm) of Dowex 1-X8($AcO^-$) resin (13–16 μm) eluted with 0.02M sodium acetate (with acetic acid added to pH 5.9) at a linear flow-rate of 1.1 cm/min: ———, chromic acid method; —·—·, periodate–formaldehyde method; – – –, carbazole method; ·····, u.v. absorption at 254 nm.

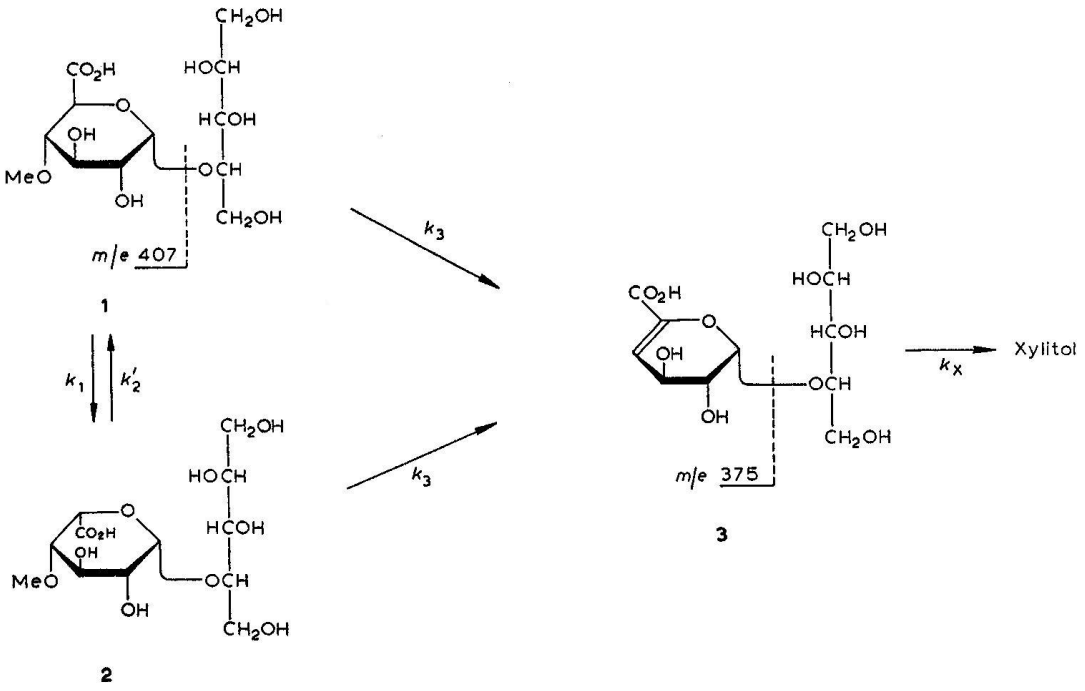
TABLE I

CHROMATOGRAPHIC DATA OF THE ACIDS PRESENT IN THE REACTION MIXTURE

| | *Volume distribution coefficient ($D_v$)* | | | *Relative retention*[a] | | |
|---|---|---|---|---|---|---|
| | *0.02M NaOAc (pH 5.9)* | *0.08M NaOAc (pH 5.9)* | *0.5M HOAc* | *OV-1 240°* | *OV-17 240°* | *SP-2401 200°* |
| 2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylitol (**1**) | 8.7 | 2.2 | 11.2 | 0.537 | 0.797 | 0.643 |
| 2-*O*-(4-*O*-Methyl-β-L-idopyranosyluronic acid)-D-xylitol (**2**) | 10.9 | 2.7 | 9.3 | 0.415 | 0.600 | 0.515 |
| 2-*O*-(4-Deoxy-β-L-*threo*-hex-4-enopyranosyl-uronic acid)-D-xylitol (**3**) | 25.9 | 6.5 | 33.8 | 0.403 | 0.616 | 0.502 |

[a]Retention times of the $Me_3Si$ derivatives relative to those of the *O*-β-D-glucopyranosyl-(1→4)-D-glucitol derivative.

structure **3** which was confirmed by mass spectrometry of the $Me_3Si$ derivative. The most prominent difference between the spectra of the $Me_3Si$ derivatives of **1** and **3** was that the characteristic fragment ion (*m/e* 407) from the glycosyl moiety of **1** (see formula) was not recorded for **3** which gave a corresponding abundant ion with *m/e* 375.




The $^1$H-n.m.r. spectra ($D_2O$, 270 MHz) of **1** and **3** contained doublets for H-1 at $\delta$ 5.17 ($J$ 4 Hz) and 5.43 ($J$ 2.7 Hz), respectively. A characteristic doublet for H-4 at $\delta$ 6.15 ($J$ 3.2 Hz) was recorded for **3**.

## RESULTS AND DISCUSSION

The results given in Fig. 2 show that **1** was degraded rapidly in M sodium hydroxide at 150°. An appreciable amount of 2-*O*-(4-*O*-methyl-β-L-idopyranosyluronic acid)-D-xylitol (**2**) was formed rapidly by epimerization and both epimers were decomposed to give 2-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-D-xylitol (**3**) and xylitol. The concentration of **3** reached a maximum after ~1.5 h and then decreased with increasing formation of xylitol. The formation of **3** from **1** or **2** presumably involves[7,8] β-elimination of MeO-4 following loss of H-5. It is therefore reasonable to assume that **1** and **2** are decomposed at the same rate.

No uronic or aldonic acids were detected in the reaction, but anion-exchange chromatography in 0.08 and 0.3M sodium acetate revealed small amounts of several fragmentation acids, mainly formic acid (0.7 mol per mol of degraded **3**). Condensation products held irreversibly by the ion-exchangers were also formed.

If pseudo-first-order reactions apply for the depicted formulae scheme, then

$$-d[\mathbf{1}]/dt = (k_1+k_3)[\mathbf{1}]-k'_2[\mathbf{2}],$$
$$-d[\mathbf{2}]/dt = k'_2[\mathbf{2}]-k_1[\mathbf{1}]+k_3[\mathbf{2}],$$
$$d[\mathbf{3}]/dt = k_3\{[\mathbf{1}]+[\mathbf{2}]\}-k_X[\mathbf{3}],$$

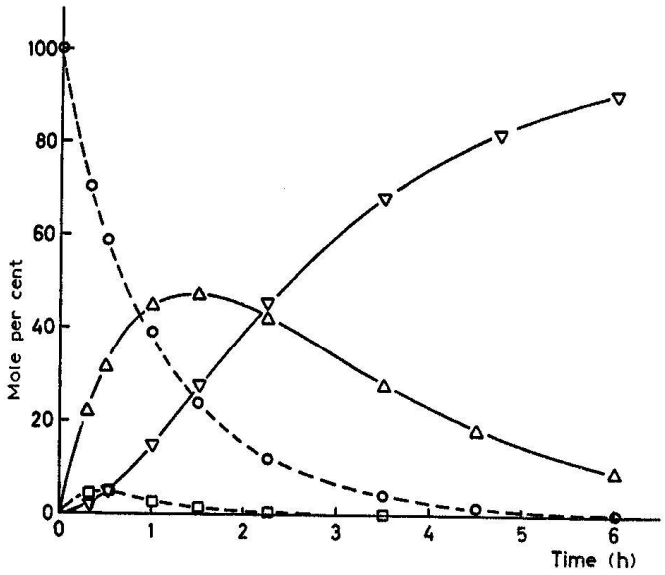



Fig. 2. Composition of the reaction mixture: 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylitol (—○—); 2-*O*-(4-*O*-methyl-β-L-idopyranosyluronic acid)-D-xylitol (—□—); 2-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-D-xylitol (—△—); xylitol (—▽—).

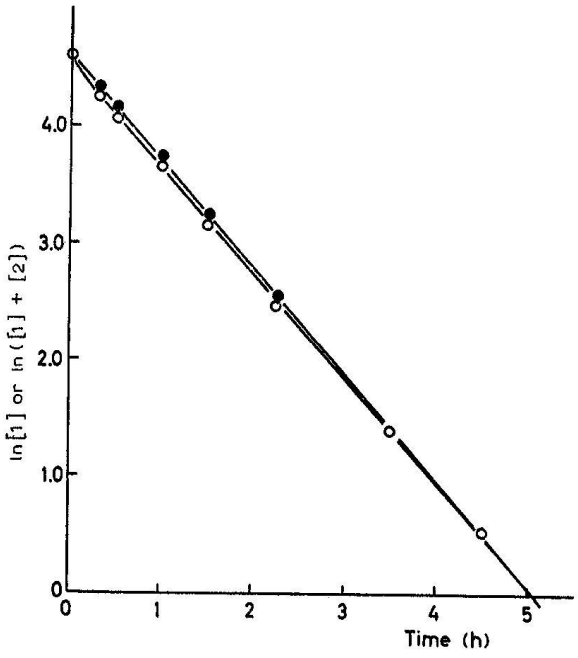



Fig. 3. Relationship (—●—) between ln{[1]+[2]} and the time of reaction used for determination of $k_3$; ln [1] versus time (—○—) for comparison.

and

$$d[X]/dt = k_X[3].$$

As predicted from the first two equations, a plot of ln {[1]+[2]} versus time (Fig. 3) gave a straight line. The pseudo-first-order rate constant $k_3$ was equal to 0.91 $h^{-1}$. The rate constant $k_X$ for the formation of xylitol from **3** was calculated from the following equation,

$$[3] = \frac{k_3 \cdot [1]_0}{k_X - k_3} [e^{-k_3 t} - e^{-k_X t}],$$

which was obtained by integration. The full lines drawn in Fig. 2 represent the concentrations of **3** and xylitol (X) calculated by application of the rate constant $k_X = 0.52\ h^{-1}$. The results are in agreement with the postulated reaction scheme.

Most probably, a reaction scheme analogous to that discussed above is also responsible for the removal of 4-*O*-methylhexuronic acid moieties from xylan during a kraft cook. Hence, demethylation of the xylan should occur more rapidly than the loss of the uronic acid groups. This situation was observed by Clayton[8] in a study of the alkaline degradation of hardwood xylans. From the comparatively low degradation rate of **3**, it must be concluded that 4-deoxy-L-*threo*-hex-4-enopyranosyluronic acid moieties are linked to the xylan present in kraft pulps. After acid hydrolysis of kraft pulps, large proportions of 4-*O*-methylglucuronic acid and appreciable proportions of 4-*O*-methyliduronic acid are present in the hydrolysate[2], whereas no unsaturated uronic acids have been observed. A plausible explanation is that the unsaturated uronic acid moieties are decomposed during the acid hydrolysis[9]. This was confirmed in experiments with **3**, which showed that hydrolysis (2 and 3 h, 0.05M $H_2SO_4$, 130°) gave tar in addition to xylitol. No uronic acids were present in the hydrolysate. Hence, the above reaction scheme is valid for the epimerization and destruction of the 4-*O*-methylglucuronic acid moieties in xylan during alkaline pulping at high temperature.

ACKNOWLEDGMENT

The financial support of the 1959 Ars Fond för Teknisk och Skoglig Forskning samt Utbildning is gratefully acknowledged.

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