

## Reducing End Groups in Birch Xylan and Their Alkaline Degradation

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**Summary.** The structure of the reducing end group in xylan can be written:



In alkaline media the reducing xylose group is easily isomerized and removed by a  $\beta$ -elimination which leads to a reducing galacturonic acid end group. The 1,2-linkage between rhamnose and the galacturonic acid explains the retarding effect on the alkaline peeling. Even under fairly mild conditions the galacturonic acid group is converted to other groups which are very stable in alkaline media. Model experiments permit the conclusion that OH-3 in the reducing group is subjected to  $\beta$ -hydroxyelimination. The 3-deoxy-2-O- $\alpha$ -L-rhamnopyranosyl-D-threo-hex-2-enuronic acid group formed is unstable in acid medium and escapes observation by the techniques employed for determination of the end groups.

Upon prolonged alkaline treatment an increased proportion of these groups is lost and a rapid peeling proceeds until a xylose group with a 4-O-methylglucuronic acid substituent is liberated. The consecutive reactions of this group are similar to those of the galacturonic acid groups.

The formation of 3-deoxyaldonic acid end groups, an important stopping reaction in cellulose, is of minor importance in xylan.

### Introduction

The high yield of pulp after alkaline pulping of hardwood is due to the high xylan content of the wood. As observed by Lindström and Samuelson [1975] xylan present in the wood contains reducing xylose end groups. This suggests that the xylan must be subjected to a rapid endwise degradation (peeling) in alkaline medium. The comparatively high yield of xylan must therefore be ascribed to competing retarding (stopping) reactions. Model experiments indicate that the 4-O-methylglucuronic acid substituents, glycosidically linked along the xylan molecule by 1→2-linkages, retard the peeling considerably [Aurell, Hartler, Persson 1963; Hartler, Svensson 1965].

Recent investigations show that galacturonic acid groups present in the xylan may also have a retarding effect [Ericsson, Samuelson 1977]. The purpose of this paper

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is to elucidate this and other questions related to the retardation of the alkaline peeling of xylan.

## Experimental

### *Isolation of xylan*

Birch meal (0.125–0.375 mm; *Betula verrucosa*) was extracted with acetone for one day and then with water for 2 days under stirring. The air-dried meal was treated with 10% KOH for 3 hours at room temperature. After filtration the solution was poured into ethanol containing excess acetic acid. The precipitated xylan was recovered and washed with ethanol and ethyl ether and dried. The crude xylan (20 g) was dissolved in 2 litres of 5% KOH. After filtration, 1.5 litres of ethanol was added slowly and the solution acidified with acetic acid to pH 4.5. The purified xylan was isolated as above.

### *Alkali treatment and borohydride reduction*

The alkali treatments were made in 0.25 M NaOH under nitrogen. The ratio liquor : xylan was 25 : 1. At 95°C the xylan dissolved in the liquor, while a large proportion was undissolved at 40°C. The reaction was interrupted by cooling and acidification with acetic acid. The xylan was recovered as above.

To determine the reducing moieties in the xylan, NaOH was added to pH 8.2. After 4 hours the slurry was neutralized, and potassium borohydride added to obtain a 0.2 M solution. After 3 days the solution was acidified with acetic acid. The xylan was isolated as above.

### *Hydrolysis and determination of acids and sugars*

The xylan was hydrolyzed for 12 hours in a boiling water bath in 0.25 M hydrochloric acid. The ratio liquor : xylan was 20 : 1. Small amounts of insolubles were filtered off. To remove chloride and organic acids, the solution was stirred overnight with an excess of an anion exchanger (Dowex 1-X8, 20–50 mesh) in its bicarbonate form. Neutral sugars and alditols were washed out with water until orcinol-sulfuric acid gave a negative test for sugars. The neutral fraction was concentrated to a small volume. Alditols and rhamnose were determined by partition chromatography in 85 per cent aqueous ethanol according to Päärt and Samuelson [1970].

The monocarboxylic acid fraction eluted with 5 M acetic acid was resolved on a preparative scale first in 0.08 M sodium acetate buffered to pH 5.9. The fractions were rechromatographed in 0.5 M acetic acid. The acids were identified by their  $D_v$  values and colour responses [Kolmodin, Samuelson 1973] and by gas chromatography-mass spectrometry of their trimethylsilyl derivatives [Petersson 1970].

### *Experiments with wood meal*

The wood meal was extracted with acetone and water as described above. After an additional treatment with 1% ammonium oxalate solution (pH 6.5) for one day and rinsing with water the meal was directly reduced with potassium borohydride. Acid hydrolysis was carried out in 0.125 M sulphuric acid for 15 hours at 90°C. The loss in weight during the hydrolysis was 18.0%. The hydrolyzate was analyzed as described above with the exception that the acids were chromatographed at a lower concentration of sodium acetate (0.04 M) to obtain a better separation of the higher uronic acids.

## **Results and discussion**

### *Formation of 3-deoxypentonic acid end groups*

It is well known that during alkali treatment of cellulose the formation of alkali-stable 3-deoxyhexonic (metasaccharinic) acid end groups competes with the end-wise degradation from the reducing end. It has been suggested that xylan is subjected to an analogous stopping reaction, but the corresponding 3-deoxypentonic acids have not been isolated by previous investigators, and whether this reaction really occurs has been the subject of much discussion. As seen from Table 1 only trace amounts were present in the untreated xylan. Significant but small amounts of both the *erythro* and *threo* forms of 3-deoxypentonic acid were present after the alkali treatment at 95°C. The amounts were smaller than those of the corresponding 3-deoxyhexonic acids in comparable experiments with cellulose [Johansson, Samuelson 1975]. No detectable formation occurred during alkali treatment at 40°C.

Separate experiments showed that the 3-deoxypentonic acids were stable both during acid hydrolysis and during treatment with alkali. After treatment with 0.25 M hydrochloric acid at 100°C for 10 hours the recovery was 97% for the *threo* form and 100% for the *erythro* form. Heating for 48 hours in 0.25 M sodium hydroxide at 95°C resulted in a slight interconversion (0.7%) by epimerization. The recovery was about 98%.

These results clearly show that, analogously to the well-known cellulose reaction, 3-deoxypentonic acid end groups are formed during alkali treatment of xylan but that this is a stopping reaction of minor importance.

Small amounts of 3-deoxypentonic acid groups were also formed in model experiments with xylose oligomers (312 mg) with a degree of polymerization of 7–14 (free from 4-*O*-methylglucuronic acid groups) prepared from birch xylan. After 3 hours in 0.25 M sodium hydroxide at 95°C (under nitrogen) the sugars were completely destroyed. Anion exchange chromatography showed that the expected peeling acids were present [Kolmodin, Samuelson 1973]. When sodium acetate is used as eluent, acid oligomers such as aldobionic acids appear before these acids. Although

**Table 1.** Monocarboxylic acids isolated after borohydride reduction and subsequent hydrolysis of untreated and alkali treated birch xylan in 0.25 M sodium hydroxide at 40 °C and 95 °C. The amounts are given per 100 g xylan

Acids	Untreated xylan	Alkali treated xylan		
	mg	3 h, 95 °C mg	48 h, 95 °C mg <sup>a)</sup>	48 h, 40 °C mg <sup>a)</sup>
<i>O</i> -(4- <i>O</i> -Me- $\alpha$ -D-GpA)-(1 $\rightarrow$ 2)- <i>O</i> - $\beta$ -D-Xylp-(1 $\rightarrow$ 4)-D-Xyl	384	552	398	96
2- <i>O</i> -(4- <i>O</i> -Methyl- $\alpha$ -D-glucopyranosyluronic acid)-xylose <sup>b)</sup>	7,900	8,056	5,730	6,995
2- <i>O</i> -(4- <i>O</i> -Methyl- $\alpha$ -D-glucopyranosyluronic acid)-xylitol <sup>b)</sup>	< 10	25	0	55
2- <i>O</i> -( $\alpha$ -D-Glucopyranosyluronic acid)-xylose	170	62	50	68
4- <i>O</i> -Me- $\alpha$ -D-GpA- $\beta$ -D-Xylp 1,2' : 2,1'-dianhydride	80	35	37	138
4- <i>O</i> -Me- $\alpha$ -D-GpA- $\alpha$ -D-Xylf 1,2' : 2,1'-dianhydride	210	140	120	185
4- <i>O</i> -Me- $\alpha$ -D-GpA- $\alpha$ -D-Xylp 1,2' : 2,1'-dianhydride	180	160	110	161
4- <i>O</i> -Methylglucuronic <sup>b)</sup>	1,710	1,256	1,043	1,600
L-Galactonic	82	0	0	197
Altronic	0	0	0	102
Galacturonic	350	89	45	137
3-Deoxy- <i>erythro</i> -pentonic	4	15	18	4
3-Deoxy- <i>threo</i> -pentonic	5	15	16	4
2-Deoxy-tetronic				18
Xylonic	70	41	47	72
Lyxonic	55	28	35	55
Threonic	41	22	16	30
Erythronic	2	3	1	3
Glyceric	88	83	62	85
Glycolic	70	56	34	33
2-Hydroxypropanoic	40	39	7	12

a) Second batch of xylan

b) Including C-2 epimer

no distinct peaks were recorded within this range, the eluate was collected and hydrolyzed. The hydrolyzate contained xylose and 0.6 mg of 3-deoxypentonic acid. Evidently, only about 0.2% of the xylose groups were converted to terminal 3-deoxypentonic acid groups.

#### *Destruction of reducing xylose end groups*

The alditol determinations in the reduced xylan (Table 2) show that in the isolated xylan samples a large proportion of the molecules contained terminal reducing pentose groups, predominantly xylose end groups. The arabinitol (lyxitol) was derived

**Table 2.** Yield and analysis of birch xylan after treatment in 0.25 M sodium hydroxide and subsequent borohydride reduction

Alkali treatment	Yield	Intrinsic viscosity in CEDA	Xylitol	Arabinitol	2-O-(4-O-Methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylitol <sup>b</sup>	Rhamnose
	%	dm <sup>3</sup> /kg	mg/100 g	mg/100 g	mg/100 g	mg/100 g
0	100	92.6	421	31	<10	862
3 h, 95 °C	88	88.1	51	13	25	530
6 h, 95 °C	84	85.8	35	8	< 5	478
10 h, 95 °C	82	84.0	31	9	< 5	472
0 <sup>a</sup> )	100	80.0	352	12	< 5	944
48 h <sup>a</sup> ), 95 °C	73	67.6	4	1	0	438
48 h <sup>a</sup> ), 40 °C	99		55	18	55	850

a) Second batch of xylan

b) Including C-2 epimer

from both lyxose and xylulose groups formed by isomerization during the isolation of the xylan [Johansson, Samuelson 1976]. The proportion of arabinitol was higher for the alkali treated samples than for the untreated xylan samples.

Table 2 shows that 2-O-(4-O-methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylitol constituted a considerable proportion of the alditols obtained from the xylan subjected to alkali treatment at 40 °C for 48 h. As shown by Roy and Timell [1968] the substituent is in part split off during the acid hydrolysis. The ratio between substituted and unsubstituted reducing xylose end groups must therefore be higher than reflected in the relative amounts of the alditols. This alditol was also obtained from the xylan treated at 95 °C for 3 hours. No significant amount was obtained from the untreated xylan.

The total number of pentitol end groups, i. e. the total number of reducing pentose end groups in the unreduced xylan, decreased dramatically during the alkali treatment and after the most severe treatments only trace amounts were present. These results show that the alkaline peeling is retarded when the endwise degradation has proceeded so far that a reducing xylose group substituted with a 4-O-methylglucuronic acid group is liberated. The small number of these groups present after severe alkali treatment shows, however, that these groups are converted to new retarding end groups. These escape observation by the techniques employed. Viscosity measurements in copper ethylenediamine [Glaudemans, Timell 1958] show that no appreciable depolymerization occurs within the xylan chains. Since, on the average, every tenth xylose residue carries a 4-O-methylglucuronic acid substituent, the observed yields show that under severe conditions even the new retarding end groups are eliminated by endwise degradation.

Pentonic, tetronic and glyceric acid end groups are formed during alkali treatment of xylan in the presence of oxygen but no detectable amounts of these acids were obtained from wood meal [Lindström, Samuelson 1975]. Evidently, these acid groups (Table 1) were formed from the xylose end groups during the isolation of the xylan. No formation occurred during the alkali treatment.

*Formation of reducing galacturonic and talaronic acid end groups at low temperature*

The isolation of large amounts of 4-*O*-( $\alpha$ -D-galactopyranosyluronic acid)-D-xylose by Samuelson and Wictorin [1966] and fractionation studies [Shimizu, Samuelson 1973] of the isolated xylan (*Betula verrucosa*) indicate that a major portion of the non-reducing galacturonic acid is a constituent of xylan. This has been confirmed for *Betula platyphylla* by studies of the products obtained by enzymatic hydrolysis [Shimizu, Ishihara, Ishihara 1976]. It was found that galacturonic acid is linked to a rhamnose group which in its turn is linked to a xylose group in the following manner:



Analysis of the monocarboxylic acids formed by treatment of xylan with hypochlorite showed that this structure also occurs in the xylan present in *Betula verrucosa* [Andersson and Samuelson 1977]. Reducing xylose end groups are present in the wood, while reducing galacturonic acid moieties are absent [Ericsson and Samuelson 1977].

During the alkaline extraction procedure used for the isolation of the xylan, the reducing xylose end group is subjected to an endwise degradation (peeling) involving isomerization to a xylulose group followed by a  $\beta$ -elimination. This reaction proceeds until a reducing galacturonic acid group is formed. The number of reducing galacturonic acid groups in the xylan samples studied in the present work (Table 1), determined as L-galactonic acid after borohydride reduction, was much lower than in a sample studied previously. On the other hand the number of reducing pentose groups was correspondingly higher (Table 2). A lower temperature during the alkali extraction explains this difference.

After alkali treatment at 40°C in 0.25 M sodium hydroxide for 48 hours the number of reducing pentose groups decreased drastically. A very large number of reducing galacturonic and talaronic acid groups (determined as galactonic and altronic acid in the sample subjected to borohydride reduction) were present after the alkali treatment. Evidently these groups effectively retard the peeling at low temperature. Under the applied conditions 1 $\rightarrow$ 4- and 1 $\rightarrow$ 3-linked polysaccharides are subjected to extensive peeling while for 1 $\rightarrow$ 2-linked species the reducing moiety will epimerize but suffer little degradation. Hence, the observed liberation and high stability (except for epimerization) of reducing galacturonic acid groups is consistent with the structure referred to above. The observation that the loss in yield during the alkali treatment was only about one per cent suggests that only one xylose group was lost by peeling before a reducing galacturonic acid group was formed and that,

before the alkali treatment, 1→2-linked galacturonic acid moieties glycosidically linked to xylose by 1,4-bonds are located close to the reducing end in the isolated xylan.

To establish the location of the galacturonic acid groups linked to xylan in the wood, birch meal was reduced with borohydride and subjected to mild acid hydrolysis. Table 3 shows that 4-*O*-methylglucuronic acid and its oligomers with 1–5 xylose moieties derived from xylan, constituted the major proportion of acids. 2-*O*-( $\alpha$ -D-Galactopyranosyluronic acid)-L-rhamnose previously found in crude birch xylan [Shimizu, Samuelson 1973] is probably derived from pectic substances. The most striking result is the high yield of 4-*O*-( $\alpha$ -D-galactopyranosyluronic acid)-D-xylitol. Although great efforts were made to detect it, no traces of the corresponding aldobiouronic acid were detected. Hence, the reducing xylose group in the xylan present in the wood is linked directly to the galacturonic acid by a 1,4-glycosidic bond. This result is consistent with those referred to above and confirms that only one xylose group is removed by peeling before the retarding rhamnose-(1→2)-galacturonic acid end group is formed.

Even after mild alkali treatment of the isolated xylan an appreciable proportion of the galacturonic acid groups was non-reducing (Table 1). Since galacturonic acid suffers a severe degradation during acid hydrolysis the true number of galacturonic acid groups must be higher than the observed number. On the other hand aldonic acid end groups suffer little degradation. In addition, these end groups are stable during mild alkaline treatments. Their number (Table 1) was greater than the observed number of nonterminal (nonreducing) galacturonic acid groups for all alkali

**Table 3.** Uronic acids and alditols obtained after partial hydrolysis of purified wood meal from birch for 15 h at 90 °C in 0.125 M sulfuric acid. The weights refer to products isolated from 100 g of wood meal

Acids	mg
2- <i>O</i> -(4- <i>O</i> -Methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylopentaoses	134
2- <i>O</i> -(4- <i>O</i> -Methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylotetraoses	170
2- <i>O</i> -(4- <i>O</i> -Methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylotrioses	309
<i>O</i> -(4- <i>O</i> -Me- $\alpha$ -D-GpA)-(1→2)- <i>O</i> - $\beta$ -D-Xylp-(1→4)-D-Xyl	1,041
<i>O</i> - $\beta$ -D-Xylp-(1→4) [ <i>O</i> -(4- <i>O</i> -Me- $\alpha$ -D-GpA)-(1→2)]-D-Xyl	84
2- <i>O</i> -( $\alpha$ -D-Galactopyranosyluronic acid)-L-rhamnose	28
$\alpha$ -D-GalpA- $\beta$ -L-Rhap 1,2' : 2,1'-dianhydride	6
4- <i>O</i> -( $\alpha$ -D-Galactopyranosyluronic acid)-D-xylitol	231
2- <i>O</i> -(4- <i>O</i> -Methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylose	502
6- <i>O</i> -( $\beta$ -D-Glucopyranosyluronic acid)-D-galactose	8
2- <i>O</i> -( $\alpha$ -D-Glucopyranosyluronic acid)-D-xylose	13
4- <i>O</i> -Methyl- $\alpha$ -D-glucuronic	57
Galacturonic	70
Glucitol	4
Mannitol	9
Xylitol	135
Arabinitol	<1

treated xylan samples. Evidently, the aldonic acid end groups in the isolated xylan (formed by air oxidation of the reducing xylose end group) must protect the non-reducing galacturonic acid moieties in the xylan chain from alkaline degradation. Although pectic substances containing galacturonic acid moieties are present in the wood, and possibly also in minor amounts in the crude xylan, it is reasonable to assume that after the alkaline treatments (even at 40 °C) all galacturonic acid groups were derived from the xylan. While the determinations of galacturonic acid are inaccurate, rhamnose determinations are more reliable. It is therefore tempting to calculate the average number of xylose groups per molecule (DP) of the isolated xylan on the assumption that one rhamnose group is present per molecule and that every tenth xylose group in the chain is linked to a 4-*O*-methylglucuronic acid substituent. For the two isolated samples the calculated values are 125 and 115 respectively. The DP of xylan in birch meal has previously been calculated from the determinations of xylitol in reduced wood meal on the assumption that it was formed exclusively from the reducing end in the xylan and that each xylan molecule contained one reducing xylose group. For two different samples of birch meal the estimated DP was 120 and 130 respectively [Lindström, Samuelson 1975]. Hence, the DP values calculated from the rhamnose contents of the xylan are in good agreement with those calculated from the reducing xylose groups in wood meal.

A calculation of the DP of the isolated xylan based instead on the determinations of reducing pentose groups (2.97 mmol/100 g), reducing galacturonic acid groups (0.46), 3-deoxypentonic (0.07) and aldonic (2.03) acid end groups gives a total number of 5.53 mmol per 100 g which corresponds to a DP of 120.

From experiments on alkaline degradation of xylotetraose [Johansson, Samuelson 1976] it can be estimated that after the alkali treatment at 40 °C for 48 hours the terminal reducing xylose groups linked to galacturonic acid should be completely removed. The presence of xylitol, arabinitol and 2-*O*-(4-*O*-methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylitol in the reduced sample and the large proportion of the latter compounds indicates that in some molecules not only was the terminal reducing xylose group removed by endwise degradation but also the adjacent galacturonic acid and rhamnose groups. If the conversion of reducing 2-*O*-(4-*O*-methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylose end groups to other end groups is disregarded under these mild conditions, the sum of the rhamnose groups and alditol end groups can be used for the determination of DP. This method gives a value of 115. Since the loss in yield during this treatment was only about one percent, the calculated value should not differ much from that of the untreated xylan sample (DP = 115). The results strongly suggest that all reducing end groups in the xylan have the structure:



#### *Destruction of galacturonic acid and rhamnose groups*

During alkali treatment in 0.25 M sodium hydroxide for 3 hours at 95 °C, the reducing galacturonic acid end groups were completely destroyed. Moreover, the num-



ber of non-reducing galacturonic acid groups decreased by about 75 %, although the loss in yield was only 12 %. Model experiments with 2-*O*-(4-*O*-methyl- $\alpha$ -D-glucopyranosyluronic acid)-xylose described below show that this compound was decomposed at 95 °C and that the C-2 substituent at the reducing group was liberated as 4-*O*-methylglucuronic acid, which, as shown by Löwendahl, Petersson and Samuelson [1976], was converted to 3-deoxy-2-C-hydroxymethylpentaric acids.

If the same reaction route applies to the reducing galacturonic acid group in xylan, a reducing rhamnose end group should be liberated. Since this end group is linked to the xylan by a (1  $\rightarrow$  3)-linkage, the rhamnose group is removed more rapidly than (1  $\rightarrow$  4)-linked xylose groups and gives rise to the *ribo* and *arabino* forms of 3,6-dideoxyhexonic acid in high yield. Their presence in the liquor after hot alkali treatment of birch xylan [Kolmodin, Samuelson 1973] supports the suggested scheme. The observed decrease in the rhamnose content and the absence of reducing rhamnose end groups in all samples is consistent with this mechanism.

The number of rhamnose groups in the alkali treated xylan samples was much higher than the number of galacturonic acid groups. For the samples treated at 95 °C for 3 and 48 hours the molar ratio rhamnose : galacturonic acid was 7.0 and 11.5, respectively, while for the sample treated at 40 °C for 48 hours the corresponding value (including galacturonic, galactonic and altronic acid) was 2.3. The results indicate that the peeling is effectively retarded before the rhamnose group is reached. Hence, we conclude that the galacturonic acid groups are converted to new end groups during the alkali treatment, and that these end groups escape observation by the techniques employed.

This conclusion is confirmed by a calculation of the DP for the alkali treated samples from the total number of reducing pentose groups, reducing galacturonic and taluronic acid groups, 3-deoxypentonic and aldonic acid groups. For the sample treated at 40 °C for 48 hours this method gave a DP value of 155 instead of 115 obtained by the previous method. The large number of rhamnose groups in this mildly treated sample indicates that the missing end groups were formed principally by conversion of the reducing galacturonic acid groups to new end groups which escaped observation. A rough calculation based on these assumptions shows that their number was 1.5 mmol per 100 g of the alkali treated xylan compared to 1.11 mmol of reducing galacturonic and 0.57 mmol of reducing taluronic acid groups.

For the samples treated at 95 °C for 3 and 48 hours the calculation of DP by the same method gave the values 300 and 420, respectively. These unrealistically high values show that the number of missing end groups was greater than the number of end groups included. The observed loss in yield and loss of rhamnose groups suggests that the missing end groups for these samples were derived both from reducing galacturonic acid groups and from terminal 2-*O*-(4-*O*-methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylose groups formed during the alkaline peeling. The high yield of xylan (73 %) even after 48 hours at 95 °C shows that these groups retard the endwise degradation very effectively and are very stable in alkaline medium. The slow decrease in the number of rhamnose groups upon prolonged alkali treatment at 95 °C

indicates that a large proportion of the retarding groups are formed from galacturonic acid groups with a rhamnose group linked at C-2 by a glycosidic linkage.

### Comments on retarding reactions

A common feature of retarding groups derived from xylose and galacturonic acid end groups is that OH-2 is blocked by a glycosidic linkage (to 4-*O*-methylglucuronic acid and rhamnose respectively). It is well known that sugars with a substituent (ether linkage or glycosidic bond) at OH-2 are much more stable in alkaline media than the corresponding OH-3 and OH-4 substituted compounds. Experiments with 2-*O*-methylglucose show that OH-3 is subjected to a  $\beta$ -hydroxyelimination which leads to 3-deoxy-2-*O*-methyl-D-*erythro*-hex-2-*enose*. In acid medium this compound was decomposed to tarry substances [Klemer, Lukowski, Zerhusen 1963].

To elucidate the retarding effect of the reducing end groups in xylan, model experiments were made with 2-*O*-(4-*O*-methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylose (*I*). Fig. 1 confirms that the compound is epimerized in alkaline medium and shows that the degradation of the aldobiouronic acids was very slow at 40°C. After 22 hours in 0.026 M NaOH the recovery of the epimeric aldobiouronic acids was 90%, while at the higher alkali concentration the figure was 80%. Parallel experiments with *O*-(4-*O*-methyl- $\alpha$ -D-glucopyranosyluronic acid)-(1  $\rightarrow$  2)-*O*- $\beta$ -D-xylopyranosyl-(1  $\rightarrow$  4)-*O*- $\beta$ -D-xylopyranosyl-(1  $\rightarrow$  4)-D-xylose showed that after 24 hours both the starting material and the aldotriouronic acid formed as an intermediate had disappeared completely. As expected, compound *I*, and the epimeric aldobiouronic

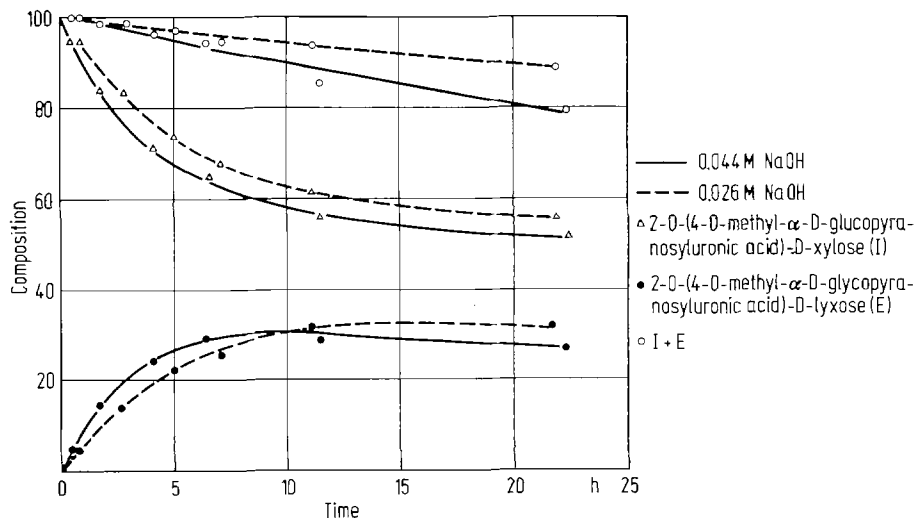


Fig. 1. Composition of the solution obtained by treatment of 2-*O*-(4-*O*-methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylose at 40.0°C (mol per 100 mol of starting material)

acid and acids formed by endwise degradation of the two 1→4-linked xylose moieties were the principal products.

As shown by Aurell, Hartler and Persson [1963] *I* is decomposed completely during treatment in 1 M sodium hydroxide for 1 hour at 100 °C. No attempt was made, however, to determine and identify the reaction products. In the present work 3% of *I* and 2% of its epimer were recovered after treatment of *I* for 30 min at 95 °C in 0.25 M NaOH. Studies of the reaction products by chromatography on anion exchange resins revealed that in addition to the epimerization product other monocarboxylic acids containing a 4-*O*-methylglucuronic acid substituent were present in large amounts and that these were extremely sensitive towards acids. Treatment with the free acid form of a cation exchange resin of sulphonic acid type (Dowex-50) at room temperature which is a standard procedure for removal of excess alkali and of metal cations from eluents, resulted in a rapid liberation of 4-*O*-methylglucuronic acid. In addition non-identified UV-absorbing solutes were formed (derived from the reacted xylose group).

Anion exchange chromatography in 0.04 M sodium acetate of reaction solutions obtained after treatments of *I* in 0.25 M NaOH for 15 min at 95 °C and treated instead with a carboxylic acid resin (Amberlite IRC-50) to decrease the pH to 8.5 showed that in addition to *I* and its epimerization product two major peaks were recorded. One of these (A1) was eluted before the aldobiouronic acids while the larger peak (A4) appeared after these acids. No appreciable amount of 4-*O*-methylglucuronic acid was recorded while in experiments in which the sodium hydroxide was removed with Dowex-50 the predominant peak was 4-*O*-methylglucuronic acid and the area of A4 was much smaller.

Reduction with borohydride of the reaction mixture followed by anion exchange chromatography in sodium acetate showed that both A1 and A4 disappeared. However, the reduction products were not separated from the reduced aldobiouronic acids. An attempt was instead made to isolate the compounds contained in the major peak (A4) by chromatography on a preparative scale. The eluate fraction obtained after elution with 0.04 M sodium acetate was treated with Amberlite IRC-50 and concentrated to a small volume by evaporation at 35 °C. Chromatography of one part of the solution under the same conditions showed that in addition to the starting material (A4) a significant amount of 4-*O*-methylglucuronic acid was present. Evidently A4 was not stable even under these mild conditions. Another part was subjected to acid hydrolysis in 0.025 M sulphuric acid at 60 °C for 11 hours. As expected, 4-*O*-methylglucuronic acid was the predominant monocarboxylic acid, while A4 had disappeared completely.

A third part of the reaction mixture was reduced with borohydride. After acidification and removal of the borate with methanol, the solution was analyzed by anion exchange chromatography. No peak with the position of A4 was recorded, while a new compound appeared, located close to that of the reduced reaction mixture. Interestingly, this peak gave a strong response not only with carbazole but (in contrast to A1 and A4) also in the periodate-formaldehyde channel. This result shows

that the aldehyde group in A4 was reduced. The ring opening makes the vicinal terminal hydroxyl groups at C-5 and C-6 amenable to periodate oxidation which leads to the formation of formaldehyde. As expected, the 4-*O*-methylglucuronic acid liberated during the isolation of the fraction was reduced to 3-*O*-methylgulonic acid, identified by its peak position and color responses. A minor but significant peak with the position and color responses of 4-*O*-methylglucuronic acid was also recorded, indicating that the reduced A4 was partially hydrolyzed during the procedures used for removal of the borate. In addition to these monocarboxylic acids, a large unretained peak which gave color responses typical of alditols with two terminal diol groups was obtained. This fraction was isolated. Gas chromatography showed that it contained the expected 3-deoxypentitols (both diastereomers).

These results permit the conclusion that, like 2-*O*-methylglucose, *I* is subjected to a  $\beta$ -hydroxyelimination which primarily leads to the formation of 2-*O*-(4-*O*-methyl- $\alpha$ -D-glucopyranosyluronic acid)-3-deoxy-pent-2-ene (*II* in Fig. 2). It can be predicted that *II* is isomerized to *III* and *IV*. Additional studies are necessary to distinguish these isomers.

In alkaline medium *III* should be decomposed to 4-*O*-methylglucuronic acid and an unstable intermediate *V* giving rise mainly to UV-absorbing compounds which are not determined by the techniques used. As already mentioned, 4-*O*-methylglucuronic acid is converted to 3-deoxy-2-C-hydroxymethylpentaric acid during alkali treatment. Accordingly, this dicarboxylic acid was formed in high yield after a severe alkali treatment of *I* (20% by weight after 30 min at 95 °C in 0.25 M NaOH). The very low yield of hydroxy acids derived from the xylose group is consistent with this scheme.

It is reasonable to assume that in xylan the liberated terminal xylose groups with 4-*O*-methylglucuronic acid substituents are subjected to analogous reactions and hence converted to groups with structures corresponding to *II*, *III* and *V*. (*IV* cannot be formed since OH-4 in the xylose end group is blocked by a glycosidic linkage).

As shown above, the retarding effect of the galacturonic acid groups predominates under mild conditions. The analogous retarding reactions can be written as shown

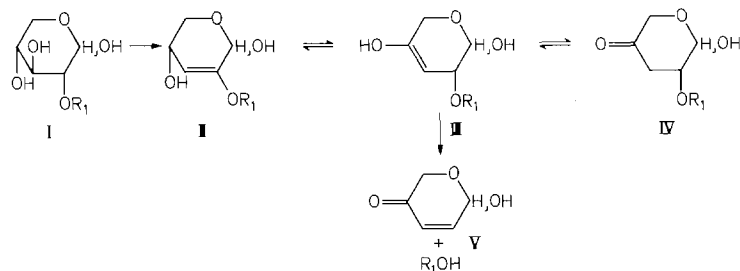
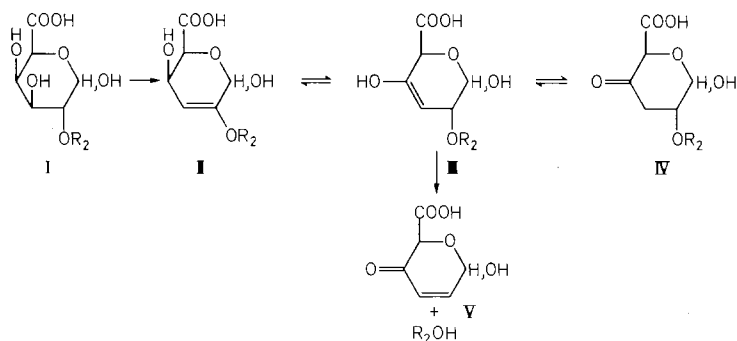


Fig. 2. Proposed route for the alkaline degradation of 2-*O*-(4-*O*-methyl- $\alpha$ -D-glucopyranosyluronic acid)-D-xylose.  $R_1$  represents the uronic acid substituent. For simplicity only one isomer has been included



**Fig. 3.** Proposed route for the alkaline degradation of 2-O-( $\alpha$ -L-rhamnopyranosyl)-D-galacturonic acid. R<sub>2</sub> represents the rhamnose group in the xylan chain. For simplicity only one isomer has been included

in Fig. 3. The high yield of 3,6-dideoxyhexonic acids derived from the rhamnose moiety under severe conditions and the instability of the groups with ethylenic linkages in acid medium are consistent with these reaction schemes.

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