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Nonoxidative and Oxidative Alkaline Degradation of Mannan

LARS LÖWENDAHL, LARS-ÅKE LINDSTRÖM and OLOF SAMUELSON

Department of Engineering Chemistry, Chalmers University of Technology, S-412 96 Gothenburg, Sweden

3-Deoxy-2-*C*-hydroxymethylpentonic, 3,4-dideoxypentonic and 1,4-anhydro-3-deoxypentitol-2-carboxylic acids were the most abundant polyhydroxy acids produced by alkali treatment of ivory nut mannan. This shows that 1,4-glycosidic linkages predominated. The formation of all diastereomeric forms of 3-deoxyhexonic acid during treatment with sodium hydroxide indicates that the endwise degradation from the reducing end proceeded to such an extent that a large proportion of the mannan molecules was brought completely into solution and confirms that, in addition to non-reducing mannose end groups, non-reducing galactose end groups were present. In experiments under oxygen pressure *trans*-2,5-dihydroxy-3-pentenoic acid was produced.

The loss of a large proportion of the mannan molecules was confirmed by determinations of the reducing sugar end groups and terminal aldonic and deoxyaldonic acid groups in the remaining mannan.

Chromatography on ion exchange resins combined with gas chromatography-mass spectrometry makes it possible to determine carboxylic acid and reducing sugar end groups in polysaccharides and to analyze the complex mixture of soluble aliphatic acids formed during alkaline treatments of polysaccharides. Alkaline treatments of polysaccharides and model compounds have shed light on the complex reactions which occur during alkaline pulping of wood and during bleaching of wood pulp, and can also be used for elucidation of the structure of polysaccharides.

No quantitative determinations of the end groups produced during alkaline treatments of ivory nut mannan have been reported, and the soluble acids formed have been investigated by gas chromatography only.[1] In this paper, we report on the end groups and the soluble hydroxy acids formed during treatment of mannan with sodium hydroxide in the absence and presence of oxygen, and during treatment with sodium hydrogencarbonate under oxygen pressure.

RECOVERED MANNAN AFTER ALKALI TREATMENT

Except for a trace amount of arabinonic acid, no aldonic or deoxyaldonic acids were recorded after hydrolysis of the isolated mannan before the treatments in alkaline media. Determinations of alditol end groups after reduction with borohydride (35.0 mmol of mannitol and 2.4 mmol of glucitol per 100 g mannan) showed that mannose was the predominant reducing end group. The proportion of glucitol can be ascribed to isomerization of the reducing mannose end groups. On the assumption that one alditol end group was present per molecule in the reduced mannan, its estimated number average degree of polymerization is equal to 16.

The large number of reducing sugar units shows that mannan should be susceptible to endwise degradation in alkaline media. Accordingly, the weight of the fraction of relatively high molecular mass material recovered by ultrafiltration after treatment with 0.4 M sodium hydroxide for 5 h at 97 °C was only 19 % of the weight of the polysaccharide. The recovered fraction contained an appreciable number of reducing hexose end groups. A balance based on the results given in Table 1 shows that, calculated per 100 g of the starting material, the number of reducing sugar end groups decreased by approximately 97 %. Only a small fraction of the molecules contained carboxylic acid end groups which, upon acid hydrolysis, were split

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Table 1. Carboxylic acids and alditols (determined after borohydride reduction) derived from end groups in mannan[a] treated with alkali at 97 °C in the presence and absence of oxygen. The amounts (mmol) refer to 100 g alkali treated mannan.

Acid or alditol	No O_2; 5 h 0.4 M NaOH mmol	p_{O_2} = 0.9 MPa; 2 h 0.2 M $NaHCO_3$ mmol	0.2 M NaOH mmol
3-Deoxy-*arabino*-hexonic	0.78		
3-Deoxy-*ribo*-hexonic	0.61		
2-C-Methylglyceric	0.17		
Mannonic	0.41	0.07	0.97
Gluconic	0.05	0.06	0.65
Arabinonic	0.24	0.25	3.07
Threonic }	0.29	0.01	0.34
Erythronic }		0.13	1.77
Mannitol	4.06	21.5	5.55
Glucitol	2.09	10.6	3.01
Total	8.70	32.6	15.36

[a] In addition, glyceric acid was present in the hydrolyzates (0.29, 0.11 and 0.15 mmol after treatment with NaOH, O_2-$NaHCO_3$ and O_2-NaOH, respectively). Its origin is unknown.

off and determined by the applied technique. The two diastereomeric 3-deoxyhexonic acids[2] and 2-*C*-methylglyceric acid[3] which are the most prominent terminal carboxylic acid end groups formed in hydrocellulose and cellulose after alkaline degradation in the absence of oxygen were present in the hydrolyzate from the alkali-treated mannan. The number of these groups was very low (approximately 1 %) compared to the decrease in the number of reducing sugar end groups. The results indicate that in most mannan molecules the endwise degradation continued to such an extent that all glycosidic bonds were broken, and are thus consistent with observations[4] that 1,4-β-glycosidic linkages predominate in ivory nut mannan. Accordingly, methylation analysis gave 2,3,6-tri-*O*-methyl-D-mannose as the predominant product both in the untreated and in the alkali-treated samples.

A small proportion of aldonic acid end groups was formed, although precautions were taken to avoid air oxidation. These groups can only be responsible for the stabilization of a small proportion of the mannan molecules. It is noteworthy that one reduced product (3,4-dideoxyhexonic acid) was found in the solution, indicating that redox reactions are of importance even in the absence of oxygen.

The observed total number of reducing groups, aldonic acid groups and deoxyaldonic acid groups, calculated per unit weight of mannan, was 77 % less in the alkali treated mannan than in the starting material. The large proportion of reducing sugar units compared to the carboxylic acid end groups shows that this result cannot be explained by the presence of an alkali resistant fraction of high relative molecular mass in the starting material. The types of end group found in the alkali-treated sample can be determined accurately by the applied methods. It can therefore be concluded that other types of end groups were present. Acid end groups containing ethylenic linkages[5] formed during alkali treatment of polysaccharides with a glycosidic linkage at C-2 are destroyed during the acid hydrolysis preceding the chromatographic analyses. No 3,4,6-tri-*O*-methyl-D-mannose was, however, obtained during the methylation analysis of the mannan samples. The structure of the unidentified end groups is, therefore, obscure.

When the residual mannan isolated by centrifugation and ultrafiltration was treated with an excess of 0.4 M sodium hydroxide at 97 °C for 24 h, the recovery of mannan by ultrafiltration was 63 %, corresponding to 12 % of the original mannan. Complete hydrolysis in two stages with sulfuric acid followed by partition chromatography gave 90 % yield of hexoses. The relative amounts were: mannose, 90 %; glucose, 8 % and galactose, 2 %.

The analysis shows that a polysaccharide containing a larger number of glucose units than the original mannan sample remained after these severe treatments, but that mannose was still the predomi-

nant constituent. The high alkali stability strongly supports the conclusion that, after the alkali treatment, end groups other than those listed in Table 1 were present at the potentially reducing end.

RECOVERY OF MANNAN AFTER OXYGEN-ALKALI TREATMENT

A higher yield of recovered mannan (55 %) was obtained after treatment with sodium hydroxide for 2 h under oxygen pressure and, as expected from similar experiments with hydrocellulose,[6] an appreciable proportion of aldonic end groups was formed (Table 1). Arabinonic and erythronic acids were more abundant than the hexonic acids. Under the applied conditions the relative proportion of arabinonic acid was much larger than that of erythronic acid, while after treatment at 120 °C erythronic acid predominated.[1] Only trace amounts of 3-deoxyhexonic acids were present in the hydrolyzate. The decrease in the number of reducing sugar end groups calculated per 100 g of the starting material (87 %) was large compared to the formation of aldonic acid end groups, indicating that, also under these conditions, a large proportion of the mannan molecules was degraded completely. The low total number of reducing sugar end groups and aldonic acid end groups indicates that an appreciable proportion of unknown end groups was formed at the potentially reducing end.

As expected from results obtained with other reducing saccharides, the recovery of mannan was much higher after oxygen-hydrogencarbonate treatment (86 %) than after treatment under the same conditions in sodium hydroxide. Aldonic acid end groups were not formed to any great extent and, calculated on the same basis as above, the decrease in the number of reducing sugar end groups was 26 %. Deoxyaldonic acids were virtually absent.

SOLUBLE HYDROXY ACIDS AFTER ALKALI TREATMENT

In agreement with the observations by Malinen and Sjöström[1] the diastereomeric 3-deoxy-2-*C*-hydroxymethylpentonic acids and 3,4-dideoxy-

Table 2. Nonvolatile monocarboxylic acids in spent liquors after alkali treatments of mannan at 97 °C in the presence and absence of oxygen. The weights (g) refer to 100 g degraded mannan.

Acid	0.4 M NaOH; no oxygen		p_{O_2} = 0.9 MPa; 2 h	
	5 h	Additional 24 h	0.2 M $NaHCO_3$	0.2 M NaOH
3-Deoxy-2-*C*-hydroxymethyl-*threo*-pentonic	19.3	14.5	3.2	7.0
3-Deoxy-2-*C*-hydroxymethyl-*erythro*-pentonic	5.6	6.1	0.6	2.4
1,4-Anhydro-3-deoxypentitol-2-carboxylic acid	3.3	2.2	<0.1	1.5
3,4-Dideoxypentonic	7.5	6.4	0	0
3-Deoxy-*threo*-pentonic	0.2	1.7	5.0	11.1
3-Deoxy-*erythro*-pentonic	0.1	0.7	2.2	4.9
2-Deoxytetronic (3,4-dihydroxybutanoic)	0.4	0.4	43.0	25.8
3-Deoxy-*arabino*-hexonic	1.9	0.5	0	0
3-Deoxy-*ribo*-hexonic	0.5	0.3	0	0
3-Deoxy-*xylo*-hexonic	0.3	0.3	0	0
3-Deoxy-*lyxo*-hexonic	0.2	0.3	0	0
3-Deoxytetronic (2,4-dihydroxybutanoic)	1.4	0.7	0	0.2
2-Hydroxypropanoic	5.7	5.8	0	2.2
Arabinonic	0	0	0	0.1
Threonic	0	0	0	0.3
Erythronic	0	0	0	0.2
Glyceric	0.1	0.1	0.5	1.5
Glycolic	0.4	3.3	33.8	24.2
3,4-Dideoxyhexonic	0.4	0.5	0	0
trans-2,5-Dihydroxy-3-pentenoic	0	0	0.7	0.7
Total	47.3	43.8	88.4	81.9

pentonic acid were among the most abundant hydroxy acids formed during the sodium hydroxide treatment in the absence of oxygen (Table 2). In addition, we found a large amount of 1,4-anhydro-3-deoxypentitol-2-carboxylic acid. It is noteworthy that the relative amounts of these acids, which belong to the most abundant hydroxy acids formed during treatment of 4-deoxy-2,3-hexodiulose with sodium hydroxide,[7] was only slightly lower after the second alkali treatment for 24 h than after the first treatment of the mannan. Isomerization of a reducing mannose end group in mannan to a fructose end group and β-elimination will lead to this intermediate and to a mannan molecule containing one mannose moiety less than before the alkaline attack. This molecule contains a reducing mannose end group. The endwise degradation can, therefore, continue until the whole mannan molecule is degraded. The high yields of the four hydroxy acids derived from 4-deoxy-2,3-hexodiulose both after the first and the second alkali treatment confirm that 1,4-glycosidic bonds predominate in mannan.

The 3-deoxyhexonic acids are not formed from 4-deoxy-2,3-hexodiulose [7] but are important products after alkali treatment of aldohexoses. The formation of the *ribo* and *arabino* forms shows that the endwise degradation proceeds so far that terminal non-reducing mannose moieties are converted to mannose. The liberated sugar is subjected to β-hydroxy-elimination followed by benzilic acid rearrangement of the 3-deoxyhexosulose intermediate to these deoxyhexonic acids.

In addition, 2-hydroxypropanoic and 3-deoxytetronic acids were produced. Both hexoses [8] and 4-deoxy-2,3-hexodiulose [7] yield these acids. The high yield of 2-hydroxypropanoic acid supports the conclusion that a large proportion of the mannan chains are degraded completely by alkaline peeling.

In agreement with the results reported by Aspinall *et al.*[4] methylation analysis of the untreated mannan yielded appreciable proportions of 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,4,6-tetra-*O*-methyl-D-galactose. The same sugars were obtained from the alkali-treated samples. The results indicate that, in addition to nonreducing mannose end groups, non-reducing galactose end groups are present in ivory nut mannan. This structure was confirmed by the observation that in addition to 3-deoxy-*ribo*-hexonic and 3-deoxy-*arabino*-hexonic acids small amounts of the *xylo* and *lyxo* forms were produced both during the first and the second alkali treatment of the mannan.

The yield of monocarboxylic hydroxy acids amounted to 47.3 g per 100 g degraded mannan after the treatment with sodium hydroxide for 5 h. Formic (4.6 g) and acetic (1.5 g) acids were also produced. A small fraction which probably contained polar dicarboxylic acids (1.8 g) was eluted with aqueous 0.5 M magnesium acetate solution. The results suggest that a large proportion of cyclic compounds were produced (*cf.* Ref. 7). The higher yields of hydroxy acids in the experiments under oxygen support this conclusion.

SOLUBLE ACIDS AFTER OXYGEN-ALKALI TREATMENT

4-Deoxy-2,3-hexodiulose, hexose end groups and hexoses are oxidized by oxygen in alkaline media. This explains the observation that the presence of oxygen led to markedly decreased proportions, or to the absence of the most prominent hydroxy acids formed when oxygen was excluded. The predominant products from oxygen treatment of the dicarbonyl compound in the presence of sodium hydroxide or sodium hydrogencarbonate are 2-deoxytetronic and glycolic acids.[7] Both were the most prominent hydroxy acids formed during oxygen treatment of mannan in these media. Like 4-deoxy-2,3-hexodiulose, mannan yielded larger proportions of these fragmentation acids in hydrogencarbonate than in hydroxide solution. The 3-deoxypentonic acids formed in large amounts have also been obtained in high yield from the same dicarbonyl intermediate, but they are also formed during oxygen-alkali treatment of hexoses.[9] In agreement with experiments with the dicarbonyl intermediate, the largest amounts of 3-deoxypentonic acids were formed after oxygen treatment in sodium hydroxide. The oxidation of a reducing mannose end group to an *arabino*-hexosulose group followed by β-elimination is another reaction path which seems to be of great importance in sodium hydroxide.[10] Glycolic and 2-deoxytetronic acids have also been obtained after oxidation of a terminal hexose end group [10] and after oxygen-alkali treatment of hexoses.[9] Arabinonic and tetronic acids present after treatment of mannan with oxygen in sodium hydroxide are probably formed by oxidation of liberated mannose.

An acid, *trans*-2,5-dihydroxy-3-pentenoic acid, which has not been reported in previous studies on alkaline treatments of polysaccharides, was isolated

and identified after oxygen treatment in both media. A small amount was also found after alkaline degradation of 4-deoxy-2,3-hexodiulose under oxygen pressure. It is likely that *trans*-3-pentenose-2-ulose is a precursor which, by benzilic acid rearrangement, is converted to this acid. Cleavage of the glycosidic linkage by β-elimination and β-elimination of OH-3, together with oxygen oxidation leading to a loss of C-1, can explain the formation of the dicarbonyl intermediate. The preferred sequence of these reactions is still unclear. Isbell [11] has proposed that this unsaturated dicarbonyl compound participates in the formation of reductic acid and 2-furaldehyde during acid treatment of pentoses.

EXPERIMENTAL

Ivory nut (*Phytelephas macrocarpa*) meal was extracted with ethanol/benzene 1:2 and subsequently treated with 5.7 % sodium hydroxide solution at 2 °C for 72 h. The residue was filtered off and mannan was precipitated by pouring the solution into ethanol acidified with acetic acid. Acid hydrolysis followed by partition chromatography on an anion exchange resin in the sulfate form using 85 % ethanol as eluent [12] yielded: Arabinose 0.2 %, galactose 1.3 %, glucose 0.8 % and mannose 97.7 % (by difference).

The mannan (15 g) was treated with sodium hydroxide in a 750 ml volumetric flask. The suspension of mannan in boiled water was boiled for 2 min and then covered with a layer of paraffin oil. Sodium hydroxide (12 g) dissolved in water was injected into the magnetically stirred suspension which was kept at 97 °C. The total volume of the solution was 750 ml. After 5 h, undissolved mannan was separated from the liquor by centrifugation and washed with water. A mannan fraction retained by ultrafiltration (separation limit at a relative molecular mass of about 500) was added to the undissolved mannan.

The oxygen treatments were made in a Teflon-lined autoclave with magnetic stirring. Before introducing the mannan, (5 g) the alkaline solutions (500 ml 0.2 M $NaHCO_3$ and 0.2 M NaOH respectively) were heated to 97 °C and saturated with oxygen at a gauge pressure of 0.9 MPa. When this temperature had been reached, the mannan sample previously kept in an inlet port was blown into the solution with a stream of oxygen. During the reaction period a stream of oxygen was passed through the solution so that the carbon dioxide liberated in the experiment with $NaHCO_3$ was removed from the autoclave. After 2 h at 97 °C and 0.9 MPa, a high molecular mass fraction was separated from low molecular mass products as described above.

Carboxylic acid end groups were determined by ion exchange chromatography in acetic acid and sodium acetate,[13] gas chromatography [14] and gas chromatography-mass spectrometry [15] after total hydrolysis. Reducing sugar end groups were determined after reduction to alditol end groups using potassium borohydride.[16] Methylation analysis [17] was carried out by Viveka Eriksson of the Institute of Organic Chemistry, Stockholm University.

The analytical methods used for determination of the acid end groups were also employed for determination of the carboxylic acids in the ultrafiltrate. Table 3 shows that a complete separation of the four diastereomeric 3-deoxyhexonic acids can hardly be achieved by these methods. After a separation in acetic acid the fraction containing the overlapping *lyxo* and *ribo* forms, together with a small amount of 3-deoxy-2-*C*-hydroxymethyl-*erythro*-pentonic acid, was rechromatographed in 0.1 M potassium borate solution. As expected from the distribution coefficients determined separately with authentic samples, a complete resolution was obtained. The *xylo* and *arabino* forms present in the subsequent fraction eluted with acetic acid were well-separated from each other and from 3-deoxy-*erythro*-pentonic acid by rechromatography in 0.08 M sodium acetate (with acetic acid added to pH 5.9).

Anion exchange chromatography in sodium acetate was used to isolate *trans*-2,5-dihydroxy-3-pentenoic acid on a preparative scale. The adjusted

Table 3. Chromatographic retention data for the diastereomeric 3-deoxyhexonic acids.

Form	Volume distribution coefficients			MU in gas chromatography	
	0.5 M acetic acid	0.08 M sodium acetate	0.1 M potassium borate	OV-101	OV-17
lyxo	7.1	6.6	15.0	19.11	18.57
ribo	6.8	7.2	18.0	19.21	18.68
xylo	8.8	6.9		19.15	18.77
arabino	9.6	7.6		19.18	18.75

retention volume was 16.6 bed volumes. The corresponding value in 0.5 M acetic acid was 38. Temperature programmed gas chromatography on OV-101 and OV-17 gave a single peak with MU values of 15.45 and 16.10 respectively (*cf.* Ref. 14). The mass spectrum of the Me_3Si derivative indicated a molecular weight of 438, and that the acid was a dihydroxypentenoic acid. The definite structure,

$$\mathrm{DO-\overset{\delta}{C}H_2-\overset{\gamma}{C}H=\overset{\beta}{C}H-\overset{\alpha}{C}H(OD)-COOD}$$

was derived from the ^1H NMR spectrum of its sodium salt in D_2O [Bruker WH 270, δ units: 4.12 (2 H, d, $J = 5.2$ Hz; H_δ), 4.51 (1 H, d, $J = 6.0$ Hz; H_α), 5.83 (1 H, dd, $J = 6.0$ and 15.5 Hz; H_β), 5.96 (1 H, dt, $J = 5.2$ and 15.5 Hz; H_γ)]. The large J value (15.5 Hz) for the coupling between the vinyl protons is consistent with the *trans* configuration.

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