Carbohydrate Research, 144 (1985) 241–249
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

ALKALINE DEGRADATION OF ALGINATES TO CARBOXYLIC ACIDS

KLAUS NIEMELA AND EERO SJOSTROM
Laboratory of Wood Chemistry, Helsinki University of Technology, SF-02150 Espoo (Finland)
(Received April 22nd, 1985; accepted for publication, June 17th, 1985)

ABSTRACT

Various dicarboxylic acids related to saccharinic acids, as well as several monocarboxylic acids, formed on treatment of alginates with alkali at 95° and 135° have been identified by capillary g.l.c.–m.s. Higher concentrations of alkali facilitated the formation of glucoisosaccharinaric, anhydroisosaccharinaric, and 2-deoxy-3-*C*-methyltetraric acids, whereas 2,3-dideoxypentaric acid was the major product at lower concentrations. The presence of Ca^{2+} promoted the formation of α-glucoisosaccharinaric, 3,4-dideoxyhexaric, and 2-hydroxybutanoic acids, especially at 135°. The nature of the reaction products indicates that the alginate chains are degraded end-wise either directly or after cleavage of internal linkages.

INTRODUCTION

Although it is well-known that alginic acid is extensively degraded by alkali at higher temperatures[1–5], very little attention has been paid to the identification of the products. Whistler and BeMiller[3] reported the formation of 3-deoxy-2-*C*-hydroxymethylpentaric acid from both ionic and esterified alginates, but the formation of other dicarboxylic acids of the saccharinic acid type is probable.

Recent investigations on the degradation of pectic acid[6] and galacturonic acid[7] by alkali have demonstrated that capillary g.l.c.–m.s. is excellent for the analysis of complex mixtures of dicarboxylic acids. This technique has now been applied in the identification of the products of the degradation of alginic acid with alkali.

EXPERIMENTAL

Treatment with alkali. — Commercial alginic acid (Fluka, 300 mg) was treated for 2.5 h under nitrogen at 95° and 135° with 0.1 [with and without 0.01M $Ca(OH)_2$], 0.5, and 2M NaOH (at 135° only) (50 mL) in a rotating autoclave.

Additional treatments were carried out with a calcium hydroxide suspension corresponding to 0.1M $Ca(OH)_2$.

Analytical methods. — After cooling each autoclave, a sample (0.5 mL) of the filtered liquor was taken for identification of dicarboxylic and hydroxy mono-

0008-6215/85/$ 03.30

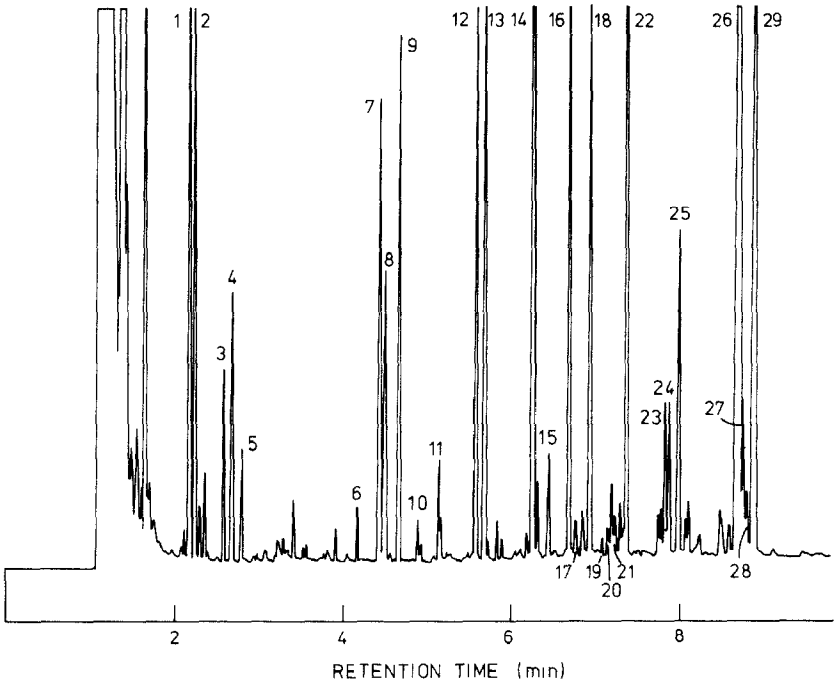


Fig. 1. Separation on an OV-101 fused-silica capillary column of the trimethylsilylated carboxylic acids obtained after treatment of alginic acid with alkali (135°, 2M NaOH): 1, lactic; 2, glycolic; 3, oxalic; 4, 2-hydroxybutanoic; 5, 3-hydroxypropanoic; 6, succinic; 7, 2-*C*-methylglyceric; 8, glyceric; 9, *C*-methyltartronic; 10, tartronic; 11, 3-deoxytetronic; 12, 2-deoxy-3-*C*-methyltetraric; 13, malic; 14, 2,3-dideoxypentaric; 15, erythraric; 16, β-anhydroisosaccharinaric; 17, threaric; 18, α-anhydroisosaccharinaric; 19, 3-deoxy-*erythro*-pentaric; 20, 3-deoxy-*threo*-pentaric; 21, *C*-(3-hydroxypropyl)tartronic; 22, xylitol (internal standard); 23, 3,4-dideoxy-*threo*-hexaric; 24, 3,4-dideoxy-*erythro*-hexaric; 25, 2,5-dihydroxy-3-hexenedioic (either or both of the *erythro* and *threo* isomers); 26, β-glucoisosaccharinaric (+ 3-deoxy-*ribo*-hexaric); 27, 3-deoxy-*xylo*-hexaric + 3-deoxy-*arabino*-hexaric; 28, 3-deoxy-*lyxo*-hexaric, and 29, α-glucoisosaccharinaric acids

carboxylic acids. These compounds were converted into their trimethylsilyl derivatives and analysed[8] by capillary g.l.c. Xylitol (0.2 mg) was added as the internal standard.

As shown in Fig. 1, satisfactory separations of most of the compounds were obtained by using an OV-101 fused-silica capillary column[8], with the exception of the trimethylsilylated isomers of 3-deoxyhexaric acid, the peaks of which overlapped with each other and with that of trimethylsilylated β-glucoisosaccharinaric acid. Trimethylsilylated 3-deoxy-*ribo*-hexaric acid and β-glucoisosaccharinaric acid could be separated by using an OV-1701 fused-silica capillary column (*cf.* ref. 9). The proportion of trimethylsilylated 3-deoxy-*xylo*-hexaric acid in the peak 27 (Fig. 1) was calculated on the basis of a previously observed ratio[7] for the formation of the *xylo* and *lyxo* isomers.

Compounds were identified by m.s. using a Hewlett–Packard 5992 instrument (70 eV) fitted with an SE-54 fused-silica capillary column (0.32 mm i.d. × 25 m).

The temperature programme was 1 min at 40°, 15°/min to 230°, and 32 min at 230°. The interpretation of the mass spectra was based on previous studies[6–8].

Formic and acetic acids were determined by g.l.c., essentially as described elsewhere[10].

RESULTS AND DISCUSSION

Depending on the conditions of treatment with alkali, the amounts of carboxylic acids formed corresponded to 30–70% of the initial alginic acid (Tables I and II). A major part of these compounds were identified previously, after treatment[6] of pectic acid with alkali.

Dicarboxylic acids. — The variety of dicarboxylic acids indicates the presence of several competing routes of degradation. Essentially, these routes can be illustrated as shown in Scheme 1 for the reducing D-mannuronic acid end-group (*cf.* ref. 6).

TABLE I

YIELDS[a] OF DICARBOXYLIC ACIDS OBTAINED ON TREATMENT OF ALGINIC ACID WITH SODIUM HYDROXIDE

Dicarboxylic acid	*95°*			*135°*			
	*0.1*M[b]	*0.1*M	*0.5*M	*0.1*M[b]	*0.1*M	*0.5*M	*2*M
Oxalic	3	3	4	5	5	9	8
Succinic	3	7	5	4	5	3	2
C-Methyltartronic	3	3	5	4	4	8	17
Tartronic	3	4	2	2	+	4	2
2-Deoxy-3-*C*-methyltetraric	15	7	66	2	2	50	36
Malic	9	14	23	10	21	31	34
2,3-Dideoxypentaric	70	62	71	91	86	77	64
β-Anhydroisosaccharinaric	3	+[c]	23	1	1	18	32
α-Anhydroisosaccharinaric	2	+	11	+	+	12	21
Erythraric	–	1	1	–	–	1	2
Threaric	–	–	–	+	+	+	1
3-Deoxy-*erythro*-pentaric	3	3	1	3	5	1	1
3-Deoxy-*threo*-pentaric	5	6	2	6	10	2	1
C-(3-Hydroxypropyl)tartronic	–	1	1	1	1	1	1
3,4-Dideoxy-*erythro*-hexaric	3	3	1	+	1	2	5
3,4-Dideoxy-*threo*-hexaric	3	4	2	+	1	2	5
2,5-Dihydroxy-3-hexenedioic	–	+	3	3	1	4	12
β-Glucoisosaccharinaric	54	40	123	47	53	140	174
α-Glucoisosaccharinaric	36	15	39	31	23	49	71
3-Deoxy-*ribo*-hexaric	–	–	2	1	1	3	14
3-Deoxy-*arabino*-hexaric	–	–	1	2	+	1	6
3-Deoxy-*xylo*-hexaric	–	–	+	–	–	+	1
3-Deoxy-*lyxo*-hexaric	–	–	1	1	+	2	3
Total amount (mg)	211	173	387	214	220	422	510

[a]Weights (mg) are from 1 g of alginic acid. [b]Also containing 0.01M $Ca(OH)_2$. [c]Key: +, trace; –, not detected.

TABLE II

YIELDS[a] OF MONOCARBOXYLIC ACIDS OBTAINED ON TREATMENT OF ALGINIC ACID WITH SODIUM HYDROXIDE

Monocarboxylic acid	*95°*			*135°*			
	0.1M[b]	*0.1M*	*0.5M*	*0.1M*[b]	*0.1M*	*0.5M*	*2M*
Formic	16	21	48	22	27	49	63
Acetic	2	+	10	8	7	13	16
Lactic	42	60	33	80	85	50	48
Glycolic	5	7	5	10	13	12	20
2-Hydroxybutanoic	4	4	4	5	5	5	11
3-Hydroxypropanoic	+[c]	+	1	1	1	1	4
2-*C*-Methylglyceric	2	2	6	+	1	7	13
Glyceric	+	1	1	1	1	3	7
3-Deoxytetronic	1	2	3	2	1	2	3
2-Deoxytetronic	1	1	1	+	1	+	+
Total amount (mg)	73	98	112	129	142	142	185

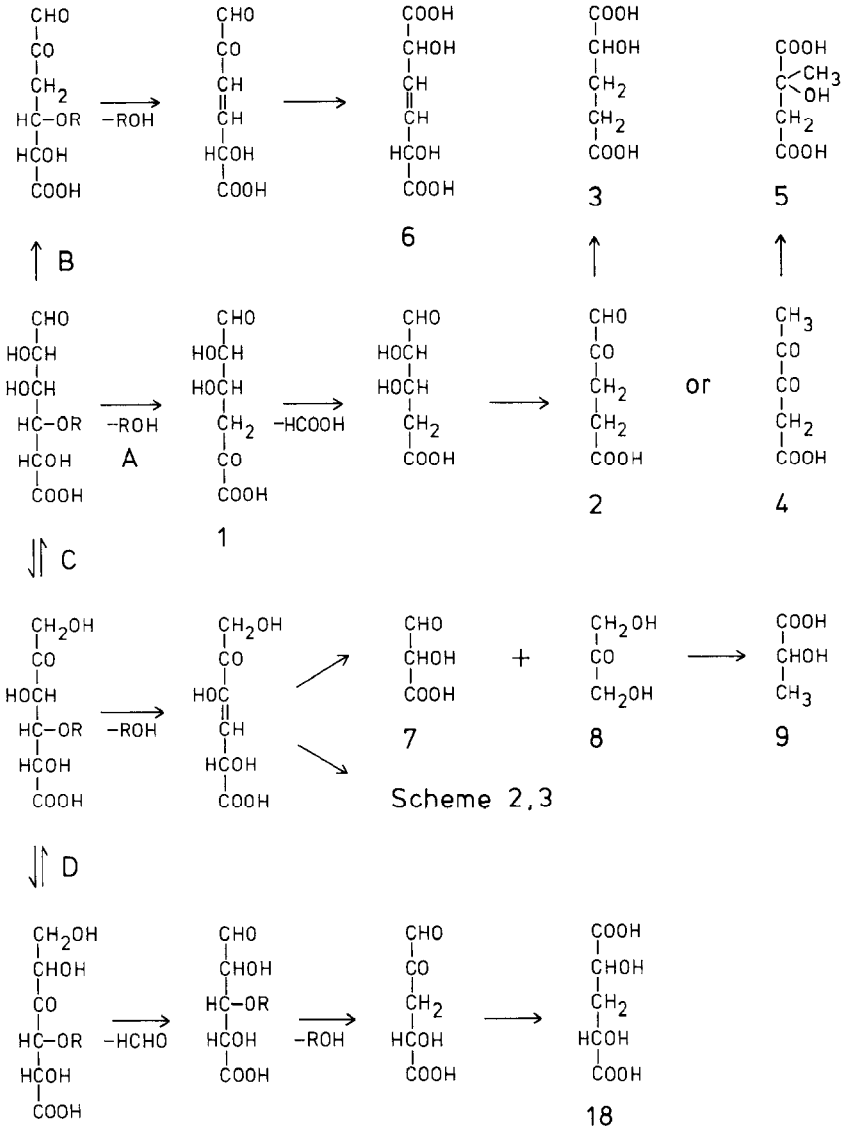
[a]Weights (mg) are from 1 g of alginic acid. [b]Also containing 0.01M $Ca(OH)_2$. [c]Key: +, trace; −, not detected.

According to Haug *et al.* [4], β-elimination is important in the degradation of alginic acid. For the reducing hexuronic acid end-group, this reaction path would yield 4-deoxyhex-4-enuronic acid, which should rearrange to 4-deoxy-5-hexulosuronic acid **1** (route A). The loss of C-6 as formic acid followed by β-elimination at C-3 converts **1** into the dicarbonyl compound **2**, which rearranges into 2,3-dideoxypentaric acid (**3**). Alternatively, 3,4-dioxopentanoic acid **4** may be formed, which could[6] be a precursor of 2-deoxy-3-*C*-methyltetraric acid (**5**). An alternative (and more probable) route for the formation of **3** is shown in Scheme 2, but we have been unable to discover any other mechanism for the formation of **5**. No mass spectra indicating the presence of 5-hydroxy-2-oxoadipic acid and supporting the proposed cleavage of the C-5–C-6 bond could be obtained.

By analogy, when chain cleavage occurs by a similar reaction, a terminal 4-deoxyhex-4-enuronic acid end-group will be generated[4], which is liberated when the chain is subjected to an exhaustive peeling reaction. The formation of **5** will be more favoured at higher concentrations of alkali and at lower temperatures.

A β-elimination at position 3 of the reducing hexuronic acid end-group yields an unstable structure (route B), which can undergo a β-elimination to give an unsaturated compound and thence 2,5-dihydroxy-3-hexenedioic acid (**6**) by a benzilic acid rearrangement[6]. The acid **6** was most abundant after treatment of alginic acid with 2M sodium hydroxide. Although no end-group determinations were made, it is reasonable to suggest that rearangement of the dicarbonyl structure into 3-deoxyhexaric acid end-groups is favoured by analogy with the formation of 3-deoxyhexonic acid end-groups in cellulose[11].

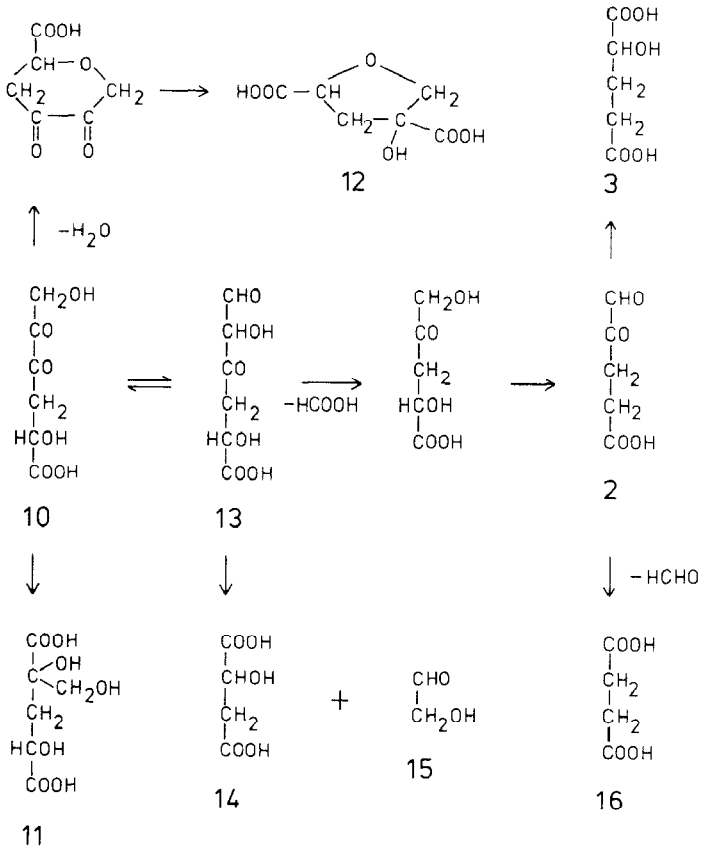
Most of the remaining compounds not accounted for by routes A and B can



Scheme 1

be explained by route C, according to the well-known peeling mechanism[3,6]. Isomerisation of the reducing hexuronic acid end-group yields a 5-hexulosonic acid structure, which is readily eliminated and degraded to tartronaldehydic acid (**7**) plus dihydroxyacetone (**8**) (Scheme 1), or rearranged into 3-deoxy-4,5-hexodiulosonic acid (**10**, *cf.* ref. 12).

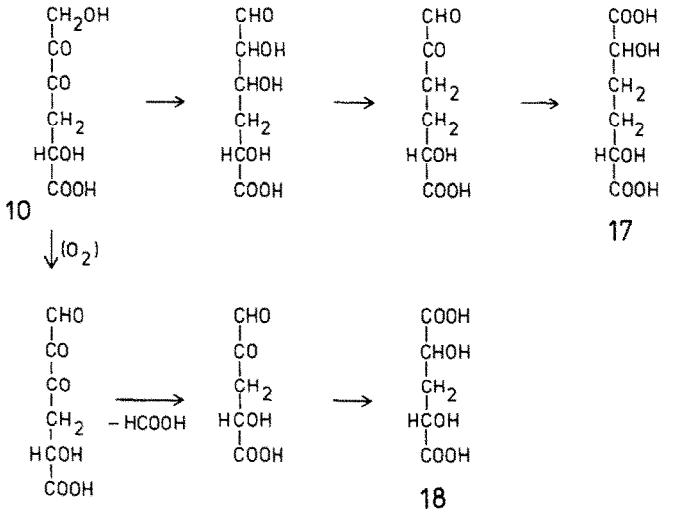
Benzilic acid rearrangement of **10** (Scheme 2) explains the formation of the isomers of glucoisosaccharinaric (**11**, 3-deoxy-2-*C*-hydroxymethylpentaric) acid in



Scheme 2

large amounts, especially after treatment with alkali at the higher concentration. The β isomer (*threo* form) preponderated, although the presence of small amounts of calcium ions facilitated the formation of the α isomer (*erythro* form), in accordance with earlier findings concerning the formation of α-glucoisosaccharinic acid from 4-*O*-methylglucose[13]. With a 0.1M $Ca(OH)_2$ suspension at 135°, alginic acid gave equal amounts (33 mg) of the *threo* and *erythro* isomers. Benzilic acid rearrangement of the anhydro derivative of **10** gives the isomeric anhydroisosaccharinaric acids (**12**, 1[1],4-anhydro-3-deoxy-2-*C*-(hydroxy methyl) pentaric acids). Higher concentrations of alkali favoured their formation.

Further degradation reactions of the diulose **10** start by isomerisation[6,14] to 4-deoxy-3-hexulosuronic acid (**13**), which is then cleaved to malic acid (**14**) and glycolaldehyde (**15**). Markedly less of **14** was formed in the presence of calcium ions. A more favoured reaction of **13** involves the formation of **2** and its rearrangement into 2,3-dideoxypentaric acid (**3**, *cf.* Scheme 1). Compound **3** was the main degradation product after treatment of alginic acid with 0.1M sodium hydroxide. Only small proportions of **3** were detected when 0.1M $Ca(OH)_2$ was



Scheme 3

used. An alternative reaction of **2** involves cleavage to succinic acid (**16**) which, however, is formed only in moderate amounts.

As can be seen from Table I, several additional dicarboxylic acids were formed in minor amounts. Of these, 3,4-dideoxyhexaric acid (**17**) might be formed from **10** by reduction, β-elimination, and benzilic acid rearrangement (Scheme 3), as in the formation of 3,4-dideoxyhexonic acid from cellulose[15]. Surprisingly, **17** was a major product after treatment of alginic acid with 0.1M $Ca(OH)_2$ at 135°, strongly indicating an (unknown) alternative route for its formation. It is noteworthy that **17** is the main dicarboxylic acid formed on treatment of cellulose[15,16] and mannan[17] with hot alkali, but its route of formation has been neglected.

Traces of oxygen possibly present in the autoclaves may be responsible for the oxidation of **10** to a tricarbonyl compound (Scheme 3), which gives rise to the diastereomeric 3-deoxypentaric acids (**18**), but more probably these compounds are formed *via* the 4-hexulosonic acid end-groups[14] (route D). In agreement with previous studies with pectic acid[6] and 4-*O*-methylglucuronic acid[18], the *threo* form preponderated. Somewhat larger amounts of **18** were formed with the lower concentrations of alkali, as with the formation of 3-deoxypentonic acids from cellobiose[19].

All the diastereomers of 3-deoxyhexaric (metasaccharinaric) acid were detected. Their presence indicated that the degradation of alginic acid chains can proceed so far that terminal non-reducing hexuronic acid moieties are liberated. The subsequent β-elimination of these hexuronic acids to give 3-deoxy-2-hexulosuronic acid followed by benzilic acid rearrangement gives these deoxyhexaric acids[7]. The *ribo* and *arabino* forms are derived from D-mannuronic

acid, whereas L-guluronic acid gives the *lyxo* and *xylo* forms. Their ratios reflect the preponderance of D-mannuronic acid in alginic acid.

Under the conditions of alkaline degradation used, not more than one-third of the hexuronic acids liberated were converted into 3-deoxyhexaric acids because of several competing fragmentation reactions[7]. Such reactions might explain the generation at least of oxalic, tartronic, *C*-methyltartronic, and tartaric acids in small amounts. On the other hand, some of these low-molecular-weight acids could be formed by recombination of fragmentation products. For example, aldol condensation of tartronaldehydic acid (**7**) and formaldehyde to give 2-tetrulosonic acid, followed by a β-elimination and benzilic acid rearrangement, could explain, in part, the formation of *C*-methyltartronic acid. It is not clear whether *C*-(3-hydroxypropyl)tartronic acid is derived from the liberated hexuronic acids[7] or whether it is formed from **1** or **10** by an unknown route.

Monocarboxylic acids. — Lactic acid (**9**) was the main hydroxy monocarboxylic acid (Table II), as expected on the basis of the previous study with pectic acid[6]. Both the peeling reaction (Scheme 1) and liberated hexuronic acids[7] are responsible for its formation. 2-Hydroxybutanoic acid, previously detected after treatment of pectic[6] and galacturonic[7] acids with alkali, was also formed in small amounts from alginic acid. The most probable route involves decarboxylation of the enolate of **2** followed by isomerisation to 2-oxobutanal and benzilic acid rearrangement (*cf.* ref. 20). Substantially larger proportions of 2-hydroxybutanoic acid (25 mg) were found after the treatment of alginic acid with 0.1M $Ca(OH)_2$ suspension. Several other hydroxy monocarboxylic acids were also identified and the liberated hexuronic acid moieties are important sources for their formation[7]. Various fragmentation reactions explain the presence of formic and acetic acids.

ACKNOWLEDGMENTS

Mrs. Sirpa Ristlakki (Technical Research Center of Finland) is thanked for recording the mass spectra, and the Ministry of Trade and Industry for financial support.

REFERENCES

1 B. VOLLMERT, *Makromol. Chem.*, 5 (1950) 110–127.
2 J. K HAMILTON AND N. S. THOMPSON, *Pulp. Pap. Mag. Can.*, 61 (1960) T263–T272.
3 R. L. WHISTLER AND J. N. BEMILLER, *J. Am Chem. Soc.*, 82 (1960) 457–459.
4 A. HAUG, B. LARSEN, AND O. SMIDSROD, *Acta Chem. Scand.*, 21 (1967) 2859–2870
5 J. N. BEMILLER AND G. V. KUMARI, *Carbohydr. Res.*, 25 (1972) 419–428.
6 K. NIEMELA AND E. SJOSTROM, *Carbohydr Res.*, 144 (1985) 87-92
7 K. NIEMELA AND E. SJOSTROM, *Carbohydr. Res.*, 144 (1985) 93–99.
8 R. ALÉN, K. NIEMELA AND E SJOSTROM, *J. Chromatogr.*, 301 (1984) 273–276.
9 G. PETERSSON, *J Chromatogr. Sci.*, 15 (1977) 245–255.
10 K NIEMELA, R. ALEN, AND E SJOSTROM, *Holzforschung*, 39 (1985) 167–172.
11 M. H. JOHANSSON AND O. SAMUELSON, *Carbohydr. Res.*, 34 (1974) 33–43.
12 E. SJOSTROM, *Tappi*, 60 (9) (1977) 151–154.
13 M. STEN AND T. MUSTOLA, *Cellul. Chem. Technol*, 7 (1973) 359–369.

14 L. LOWENDAHL, G. PETERSSON, AND O. SAMUELSON, *Tappi*, 59 (9) (1976) 118–121.
15 O. SAMUELSON AND L.-A. SJOBERG, *Cellul. Chem. Technol.*, 12 (1978) 463–472.
16 L. LOWENDAHL, G. PETERSSON, AND O. SAMUELSON, *Cellul. Chem. Technol.*, 10 (1976) 471–477.
17 K. NIEMELA AND E. SJOSTRÖM, *Holzforschung*, in press.
18 L. LOWENDAHL, G. PETERSSON, AND O. SAMUELSON, *Carbohydr. Res.*, 43 (1975) 355–359.
19 L. LOWENDAHL AND O. SAMUELSON, *Acta Chem. Scand., Ser. B*, 33 (1979) 531–536.
20 L.-Å. LINDSTRÖM, *Carbohydr. Res.*, 69 (1979) 269–271.