Carbohydrate Research, 106 (1982) 83–91
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

FORMATION OF "GLUCOMETASACCHARINOLACTONES" IN THE PYROLYSIS OF CURDLAN, A (1→3)-β-D-GLUCAN

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(Received January 18th, 1982; accepted for publication, February 9th, 1982)

ABSTRACT

The volatile products of vacuum pyrolysis of curdlan are very much influenced by the presence of small proportions of sodium chloride in the polysaccharide during pyrolysis. In the absence of contamination by this salt, the major, volatile products are the 1,6-anhydro-D-glucoses (pyranose and furanose) in 49% yield. Addition of increasing proportions of sodium chloride decreases the yield of these anhydro-D-glucoses, and causes formation of increasing proportions of 3-deoxy-D-*ribo*-hexono-1,4-lactone and its D-*arabino* epimer (*i.e.*, the "glucometasaccharinolactones") in a combined yield of up to 38%. It is concluded that the pyrolysis of (1→3)-glycans can lead to a "peeling" reaction of the same type as that already known for alkaline degradation. The effect of other salts was also studied.

INTRODUCTION

The pyrolysis of cellulose [(1→4)-β-D-glucan] has been extensively studied, and, especially with the pure polysaccharide under vacuum, high yields of a mixture of 1,6-anhydro-D-glucopyranose and -furanose can be obtained[1a], but the mechanism of their formation has not yet been determined. It is known that (1→4)-α-D-glucans also yield up to 44% of the same anhydro-D-glucoses, and that the pyrolysis of (1→4)-β-D-mannan yields the analogous 1,4-anhydro-D-mannoses[2]. The pyrolysis products from the (1→3)-β-D-glucan laminaran yielded 20% of the 1,6-anhydro-D-glucoses, but, in this case, a further, unidentified, major product was detected by gas–liquid chromatography (g.l.c.)[1b]. The (1→3,1→4)-α-D-glucan nigeran gave the same, unidentified product, in this case, apparently in a yield similar to that of the 1,6-anhydro-D-glucoses[1b].

The foregoing results suggested that a careful study of the major pyrolysis products of a (1→3)-linked D-glucan might throw some light on the mechanism of pyrolysis of polysaccharides in general. Laminaran was not considered the most

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0008-6215/82/0000–0000/$ 02.75,

suitable (1→3)-D-glucan for such a study, because of the complexity introduced by (1→6) linkages and by D-mannitol end-residues. We therefore chose to use curdlan, an extracellular D-glucan, produced by *Alcaligenes faecalis*, that was known to contain >99% of (1→3)-β-D-glucopyranose linkages[3].

RESULTS AND DISCUSSION

In view of previous observations of the major effects of small proportions of salts on the pyrolysis of cellulose[4], attempts were made to remove the known sodium chloride contaminant from the curdlan before pyrolysis. Cold water was used for prewashing, in order to avoid the extensive swelling of the polysaccharide particles that occurs in hot water. Curdlan prewashed in this way and then vacuum-pyrolyzed at 300° gave a condensate ("tar") that, when per(trimethylsilyl)ated and the ethers examined by g.l.c., showed mainly 1,6-anhydro-D-glucoses (pyranose and furanose, typical ratio ~6:1) plus the unidentified compound at slightly greater retention-time, as previously reported for the pyrolysis of laminaran and nigeran[1b]. Subsequently,

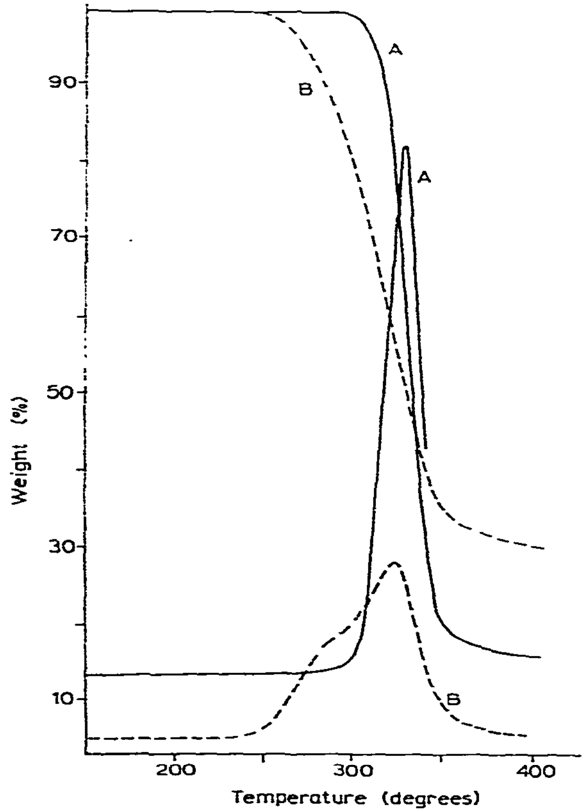
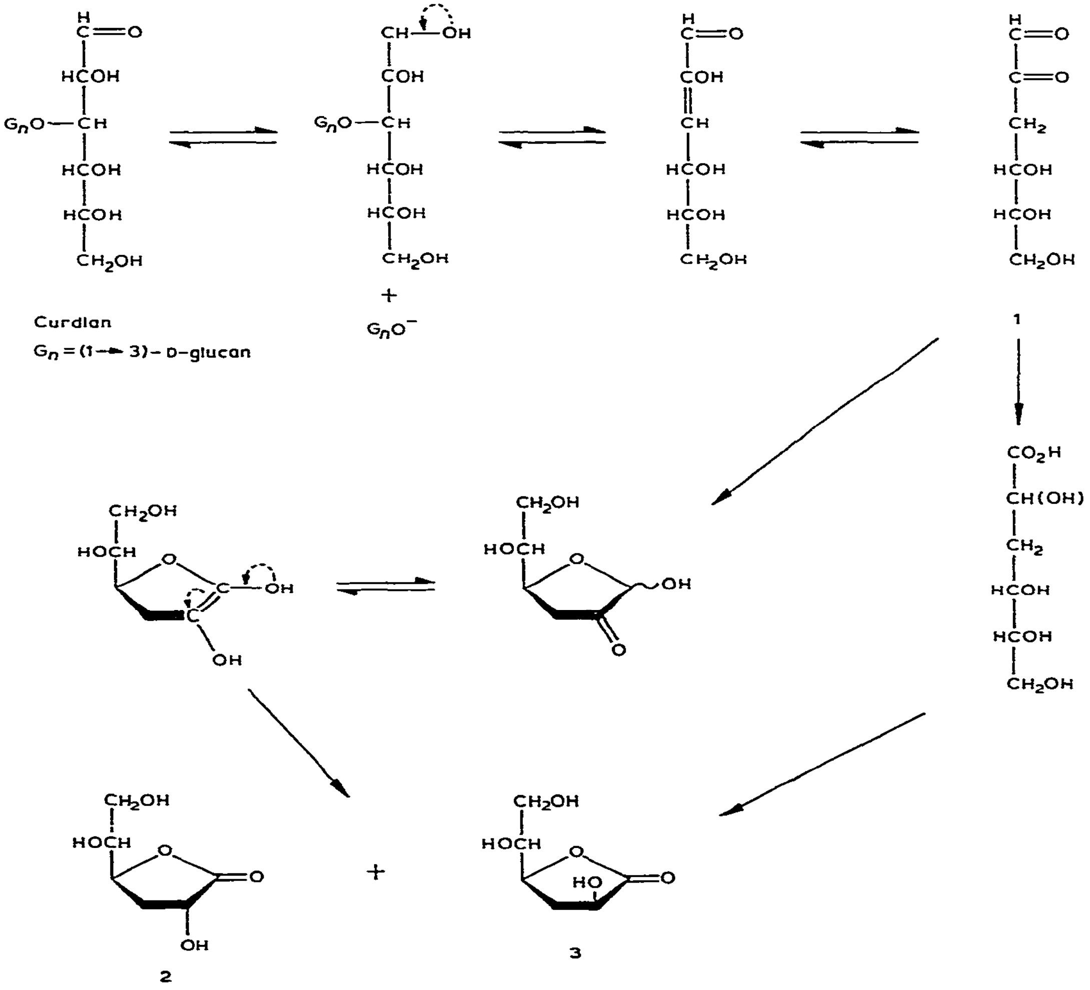


Fig. 1. Thermogravimetry and derivative thermogravimetry of curdlan (N_2, 15°.min^{-1}). (A, Salt-free; B, containing 10% of sodium chloride.)

it was found that prolonged washing with hot water is necessary in order to remove all of the sodium chloride (cold-water washing left 0.5% in the polysaccharide), and the effects thereof on the pyrolysis were then dramatic. Thermal analysis (see Fig. 1) showed that the effect of sodium chloride was to induce the onset of weight loss at a lower temperature, but extending over a wider range of temperature and resulting in an increased residue. The derivative thermogravimetry (d.t.g.) curves suggest that, in the presence of sodium chloride, some different reaction may occur at ~270° that does not take place in its absence. Samples having an intermediate content of sodium chloride, such as the original curdlan (3.5% of sodium chloride), gave curves intermediate between A and B in Fig. 1.

Quantitative analysis of the condensate from vacuum pyrolysis at 300° of



Scheme 1

curdlan free from sodium chloride showed that the 1,6-anhydro-D-glucoses were the only major products. However, similar pyrolysis of the original curdlan sample (containing 3.5% of sodium chloride) yielded only the single "new" peak in g.l.c. of the trimethylsilyl ethers, but, in g.l.c. of the acetylated condensate, two peaks were observed. The infrared spectrum of the condensate suggested the presence of a γ-lactone (1772 cm^{-1}, s), and, ultimately, the two g.l.c. peaks were found to correspond exactly to the acetates of the "α- and β-D-glucometasaccharinolactones", *i.e.*, the 2,5,6-triacetate of 3-deoxy-D-*ribo*-hexono-1,4-lactone (**2**) and of its D-*arabino* epimer (**3**). The mass spectra of these two compounds are indistinguishable, and correspond exactly with those of the pyrolysis products. The identification was confirmed by comparison with the authentic compounds by thin-layer chromatography (t.l.c.) and by g.l.c. of the per(trimethylsilyl) ethers (which did not resolve the two epimers). From the g.l.c. peak-areas, the ratio of D-*ribo* to D-*arabino* isomers was 1.0:1.5, and this corresponds well with the ratio of 1.0:1.4 for the two corresponding acids previously obtained[5] by treatment of 3-deoxy-D-*erythro*-hexos-2-ulose with alkali.

We therefore concluded that, in the presence of sodium chloride, curdlan is mainly pyrolyzed by a "peeling" mechanism of the same type as that already proved for alkaline degradation of the same type of polysaccharide[6] (shown in Scheme 1). As each reducing-end group is removed from the polysaccharide, and ultimately converted into lactones **2** and **3**, the next D-glucose unit in the chain is exposed and can undergo the same reaction. There is no doubt that, in aqueous alkaline degradation, the 3-deoxy-D-*erythro*-hexos-2-ulose intermediate (**1**) undergoes the benzilic acid rearrangement to the "metasaccharinic" acids, as shown in Scheme 1, and this may also be the mechanism involved in pyrolysis. The rearrangement requires water, but this could be provided by competing, nonspecific, thermal-degradation reactions such as those that produce char. However, under pyrolysis conditions, an alternative mechanism, proceeding *via* rearrangement of the furanose form of **1** (as shown), may also be postulated.

The yield of **2** and **3** is lower than that of the corresponding acids that would be anticipated from alkaline degradation of curdlan, probably because of char-forming reactions and because competing trans-glycosylation reactions may occur in the pyrolysis, with resultant introduction of non-(1→3) linkages. Conversely, when an attempt was made to block the formation of lactones **2** and **3** by reducing the end groups of the curdlan with borohydride before pyrolysis, a 30% combined yield of the lactones was still obtained (plus 2% of the 1,6-anhydro-D-glucoses). This suggests that, during the pyrolysis, some scission of mid-chain glycosidic linkages can occur, thus producing new reducing end-groups that can undergo the peeling reactions.

The thermal analysis (see Fig. 1) suggests that the peeling reactions that are favored by the presence of sodium chloride (shown in Scheme 1), probably occur at a temperature lower than that for the reactions that produce the 1,6-anhydro-D-glucoses. Table I shows that, as the sodium chloride content is increased (up to 1.5%),

TABLE I

PRODUCTS FROM VACUUM PYROLYSIS OF CURDLAN CONTAINING VARIOUS PROPORTIONS OF SODIUM CHLORIDE

Sodium chloride (%)	*"Tar" (%)*[a]	*"Char" (%)*[b]	*1,6-Anhydro-D-glucoses (%)*[a,b]	*"Metasaccharino"-lactones (%)*[a]
0.0	50	15	49	n.d.[c]
0.1	51	20	40	0.7
0.2	36	32	12	21
1.5	39	33	<1	37
3.7	38	31	<1	38
10	38	29	<1	38

[a]Corrected for sodium chloride. [b]Sum of pyranose and furanose forms. [c]n.d., not detected.

TABLE II

THERMAL ANALYSIS OF CURDLAN CONTAINING VARIOUS PROPORTIONS OF SODIUM CHLORIDE

Sodium chloride (%)	*DTG maximum*	*Initiation of weight loss*[a]	*Residue at 400° (%)*[b]	*DSC endotherm maximum*
0.0	330	305	16	325
0.2	340	284	19	339
1.5	323	270	25	325
3.7	318	269	26	306
10	325	262	30	320

[a]Loss in weight, 1%. [b]Corrected for sodium chloride.

the peeling reactions gradually replace the 1,6-anhydro-D-glucose reactions in the pyrolysis. Presumably, the heterolytic scissions involved in Scheme 1 are favored by the higher dielectric constant induced by the presence of sodium chloride in the polysaccharide sample.

A more detailed examination of the effect of the content of sodium chloride on the thermal analysis is shown in Table II. The d.t.g. peak is symmetrical in the absence of sodium chloride, but develops a lower temperature inflection as the content of sodium chloride is increased (see Fig. 1). It is, therefore, not surprising that, although the d.t.g. maxima are reproducible for a given sample, they are not a reliable indicator of the content of sodium chloride. However, the temperature of initiation of weight loss is very sensitive to small proportions of sodium chloride up to 1–2%, as is the residue remaining at 400°. The d.t.a. (differential thermal analysis) endotherms are complex. Increasing the content of sodium chloride causes increasing broadness in the endotherm peak, and the appearance of a shoulder at

TABLE III

PRODUCTS FROM VACUUM PYROLYSIS OF CURDLAN CONTAINING ADDED SALTS (5%)

Additive	*"Tar"*[a] *(%)*	*"Char"*[a] *(%)*	*1,6-Anhydro-D-glucoses*[a,b] *(%)*	*"Metasaccharino"-lactones*[a] *(%)*
NaCl	36	26	<1	38
NaH_2PO_4	6.2	45	3.3	0.1
$ZnCl_2$	7.1	47	2.8	0.3
$NaHCO_3$	31	30	<0.1	31
$Ca(OH)_2$	23	42	<0.1	22
NaOH	32	36	<0.1	32
$Na_2B_4O_7$	0.7	61	<0.1	0.1

[a]Corrected for added salt. [b]Sum of pyranose and furanose forms.

TABLE IV

THERMAL ANALYSIS OF CURDLAN CONTAINING ADDED SALTS (5%)

Additive	*Initiation of weight loss*[a]	*DTG maximum*	*Residue at 400°* *(%)*[b]
NaCl	264	317	26
NaH_2PO_4	267	288	46
$NaHCO_3$	188	258	36
$ZnCl_2$	186	257	51
$Na_2B_4O_7$	260	325	57
$Ca(OH)_2$	221	250	39
NaOH	193	237	44

[a]Loss in weight, 1%. [b]Corrected for added salt.

~300°, but there is no systematic relationship between the d.t.a. maxima and the content of sodium chloride. Neither is there any systematic relationship between the area enclosed by the d.t.a. endotherm-peak and the content of sodium chloride or the yield of product for the different samples (compare ref. 1). The area under the endotherm peak was very dependent on sample preparation, especially the temperature of the wash water and the method of drying the samples; these variations probably result from differences in the density (the fineness) of the polysaccharide samples. The initiation temperature of the endotherms showed a decrease with increase in sodium chloride content, especially up to 1.5%.

Tables III and IV show the effects of a range of added salts on the pyrolysis of curdlan, and the results led to the following general conclusions. "Acidic" additives (NaH_2PO_4; $ZnCl_2$) do not favor either the peeling or the 1,6-anhydro-D-glucose

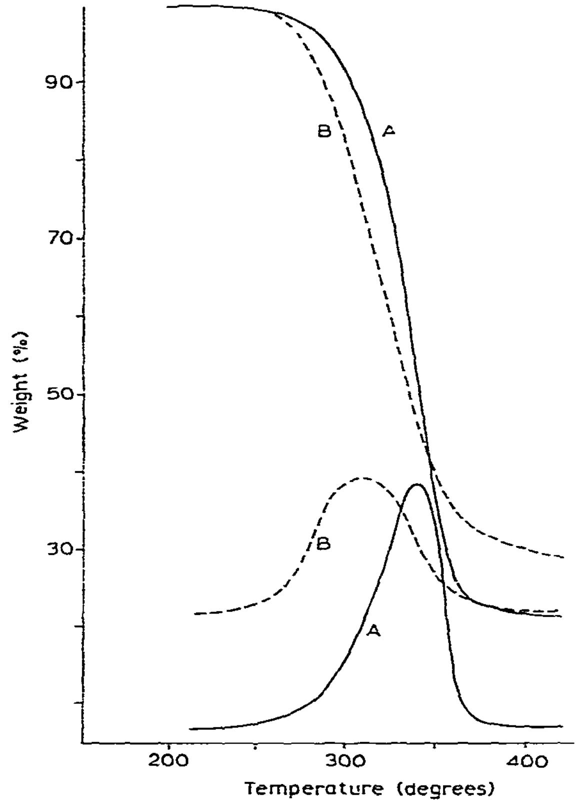


Fig. 2. Thermogravimetry and derivative thermogravimetry of lichenan (N_2, 15°.min^{-1}). (A, Salt-free; B, containing 5% of sodium chloride.)

reactions; they favor condensation/char reactions, with the formation of considerable proportions of volatile products, especially water. The "alkaline" additives (except borate) favor the peeling reactions, presumably because alkali catalyzes all of the reactions shown in Scheme 1, although they are no more effective in this respect than the "neutral" sodium chloride. Presumably, had larger proportions of alkali been used, the "metasaccharinic" acid products would have been "trapped" as salts, and the yields of volatile lactones would have been lessened. Conversely, it may be possible to increase or optimize the yield of lactones by using smaller proportions of the alkaline salts. Borate excels in directing the pyrolysis to condensation/char reactions. The sodium dihydrogenphosphate sample gave a particularly clean thermogram having a narrow, symmetrical, d.t.g. peak, and this system probably merits further investigation.

It is possible that the detection of "metasaccharinolactones" as major products in the vacuum pyrolysis of polysaccharides might have occasional use in structural analysis, even for mixed-linkage polysaccharides. To investigate this possibility, we studied the pyrolysis of lichenan, a linear (1→3,1→4)-β-D-glucan having the respective linkages in the ratio of 3:7. Addition of sodium chloride, before pyrolysis, again induced the onset of weight loss at a lower temperature, and increased the amount of final residue (see Fig. 2). In this case, however, the d.t.g. curves became more symmetrical in the presence of sodium chloride. The condensate from the vacuum pyrolysis of lichenan containing 5% of sodium chloride yielded, after 1 h at 300°, 19% of "tar", composed mostly of the "metasaccharinolactones" (6.4%) and the 1,6-anhydro-D-glucoses (5.9%). Pyrolysis of lichenan in the absence of sodium chloride gives higher yields of the anhydro-D-glucoses and much lower yields of the lactones, and it seems that the sodium chloride suppresses the anhydro-D-glucose reactions, as well as favoring the peeling reactions. The yields of the two types of product are not well related to the proportions of linkages in the polysaccharide, and it is unlikely that such a procedure would ever be useful for quantitative, linkage analysis. It may, however, be speculated that the vacuum pyrolysis, in the presence of sodium chloride, of any hexan that contains a major proportion of (1→3) linkages should yield the "metasaccharinolactones" as major components of the tar.

EXPERIMENTAL

General methods. — Thermal analysis (t.g., d.t.g., and d.t.a.)[7] and vacuum pyrolysis in a flow of nitrogen[8] at 1.5 mm Hg were performed as previously described. G.l.c. analysis was conducted, with flame ionization detection, and nitrogen as the carrier gas, in steel columns: column *1* (1.8 m × 2.2 mm) packed with 3% of SE-52 on Gas-Chrom Q (100–120 mesh); column *2* (2.0 m × 2.2 mm) packed with 3% of OV-225 and 2.5% of HI-EFF-9BP on Gas-Chrom Q (80–100 mesh). Column *1* was used for (trimethylsilyl)ated compounds at 150° + $10°.\mathrm{min}^{-1}$ with the injector at 280°; column *2* was used for acetylated compounds at 220° with the injector at 240°. Acetylation was conducted with 1-methylimidazole and acetic anhydride[9]. In the quantitative analysis of pyrolysis products by g.l.c., methyl eicosanoate (arachidate) was added as the internal standard before per(trimethylsilyl)ation.

Curdlan samples. — The curdlan was kindly supplied by T. Harada of Osaka University as a free-flowing, white powder, isolated[3] from *Alcaligenes faecalis* var. *myxogenes* IFO 13140. It had $\overline{\mathrm{d.p.}}$ 455 (by periodate end-group analysis) and contained 99% of β-D-(1→3)-glucopyranosyl linkages. The ash content was 3.5%, almost entirely due to sodium chloride; this was progressively removed by washing with water as follows. Washing with successive batches of water in a centrifuge tube at room temperature with intermediate centrifuging (4 × 5 min for each wash) gave a dried product containing 0.5% of ash. A sample of this product was boiled with a large volume of water, and the extract was analyzed by atomic absorption spectrometry (a.a.s.) (Na 0.21%, corresponding to 0.53% of sodium chloride; no other metals detected). A further sample of curdlan (0.564 g) that had been washed with cold water was stirred with boiling water (150 mL) for 5 min. The highly swollen, gel particles were separated by centrifuging, washed successively with hot water and acetone (×2), and dried (a.a.s., <0.1% of NaCl).

For the addition of controlled amounts of sodium chloride, and of other salts, an aqueous solution of the salt was added to a sample of the foregoing hot-water-washed curdlan, and the slurry of the gel particles was lyophilized. For zinc chloride, methanol was used as the solvent, and it was removed in a rotary evaporator.

Identification of products from the pyrolysis of curdlan containing sodium chloride. — "Unwashed" curdlan containing 3.5% of sodium chloride was heated in a stream of nitrogen at 1.5 mm Hg pressure for 1 h at 300°. After per(trimethylsilyl)-ation, the condensate (water-cooled condenser) showed a single g.l.c. peak with column *1*; this peak corresponded to the unknown component previously detected in the pyrolysis of laminaran and nigeran[1b], and was also indistinguishable, in co-chromatography, from both 3-deoxy-D-*ribo*-hexono-1,4-lactone and 3-deoxy-D-*arabino*-hexono-1,4-lactone. After acetylation, and g.l.c. of the acetates in column *2*, the condensate gave two peaks (9.80, 10.46 min), in the area ratio of 1.0:1.5, corresponding exactly with those afforded by authentic samples of the diacetates of the same respective lactones. G.l.c.–m.s. (electron impact) gave the same spectrum across the incompletely resolved, acetate peaks: *m/z* 288 (M^+, trace), 229 ($M-CH_3CO_2$, trace), 215 ($M-CH_2OCOCH_3$, trace), 145 (4), 143 (10), 115 (11), 83 (9), 82 (11), 54 (28), and 43 (100). The same m.s. results were given by each of the foregoing authentic lactones. The pyrolysis products were also shown to correspond with the lactones by t.l.c., before and after acetylation, and by infrared spectroscopy.

ACKNOWLEDGMENTS

The authors thank the National Science Foundation for supporting this work, through Grant No. PFR8023854, and Mr. Thomas T. Stevenson for his technical assistance.

REFERENCES

1 (a) F. Shafizadeh, R. H. Furneaux, T. G. Cochran, J. P. Scholl, and Y. Sakai, *J. Appl. Polym. Sci.*, 23 (1979) 3525–3539; (b) F. Shafizadeh, R. H. Furneaux, T. T. Stevenson, and T. G. Cochran, *Carbohydr. Res.*, 67 (1978) 433–447.

2 R. H. Furneaux and F. Shafizadeh, *Carbohydr. Res.*, 74 (1979) 354–360.

3 T. Harada, in *Extracellular Polysaccharides: A.C.S. Symp. Ser.*, 45 (1977) 265; and in J. M. V. Blanshard and J. R. Mitchell (Eds.), *Polysaccharides in Food*, Butterworths, London, 1979, p. 283.

4 F. Shafizadeh, *Adv. Carbohydr. Chem.*, 23 (1968) 419–474.

5 G. Machell and G. N. Richards, *J. Chem. Soc.*, (1960) 1938–1944.

6 R. L. Whistler and J. N. BeMiller, *Adv. Carbohydr. Chem.*, 13 (1958) 289–330.

7 F. Shafizadeh and P. P. S. Chin, *Carbohydr. Res.*, 46 (1976) 149–154.

8 A. G. W. Bradbury, Y. Sakai, and F. Shafizadeh, *J. Appl. Polym. Sci.*, 23 (1979) 3271–3280.

9 C. C. Chen and G. D. McGinnis, *Carbohydr. Res.*, 90 (1981) 127–130.