Carbohydrate Research, 83 (1980) 93–101
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

THE STRUCTURAL ANALYSIS OF SOME AMYLODEXTRINS*

R. STUART HALL AND DAVID J. MANNERS
Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh EH1 1HX (Great Britain)

(Received August 20th, 1979; accepted for publication, October 15th, 1979)

ABSTRACT

Methylation and enzymic analysis of various amylodextrin samples shows that a proportion of the molecules, having an average d.p. of ~25, contain two branch-points. This finding is related to the resistance of the amylodextrin to debranching by pullulanase, and to the overall molecular structure of the original amylopectin.

INTRODUCTION

Amylodextrins are prepared by the prolonged action of acid on granular starches; they retain some of the characteristics of the original starch, *e.g.*, solubility and crystallinity, even though the molecular size is so much smaller, with d.p. values in the range 15–30. Although the amylodextrins have been known for more than 100 years[2], it is only in the last decade that progress on their structural analysis has been reported. Kainuma and French[3] prepared amylodextrins from several types of starch. Since the amylodextrin from two-component starches contained material derived from both the amylose and amylopectin, later work[4] has concentrated on the amylodextrins from waxy-maize starch. This amylodextrin was heterogeneous, and was separated by gel filtration into three fractions. The fraction of lowest molecular weight was linear, and had a d.p. of 12; the intermediate fraction had a d.p. of 25 and was believed to be singly branched, whilst the fraction of highest molecular weight was multiply branched.

Robin and co-workers[5] prepared amylodextrins from granular potato-starch, and obtained two major fractions by gel filtration. One of these, with a d.p. of 25, was singly branched, whilst the other, with a d.p. of 15, was linear. The latter product was believed to originate from the crystalline regions of the amylopectin, and the overall results suggested that the macromolecule contained clusters of highly ordered chains of d.p. 15. More recently, Maningat and Juliano[6] prepared amylodextrins from various samples of rice starch and obtained linear material of d.p. 14–17, and a singly branched fraction of d.p. 29–34.

Amylodextrin has been used as a substrate for Q-enzyme[7]. Although Q-enzyme

*α-(1→4)-D-Glucans, Part XXIII. For Part XXII, see ref. 1.

0008-6215/80/0000–0000/$ 02.25,

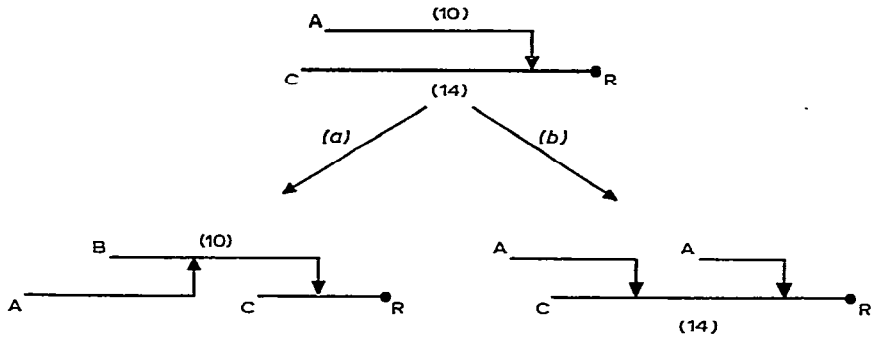

Fig. 1. The action of Q-enzyme on amylodextrin, from ref. 7. The number of D-glucose residues in the A and C chains is shown in brackets: (*a*) denotes transfer of a segment of the C chain to the A chain; (*b*) denotes transfer in the opposite direction; R represents the single, free reducing-group in the molecule; and ↓ represents an α-(1→6)-linkage.

had no action on linear chains of d.p. 10–14, it acted readily on a singly branched amylodextrin, prepared by French and Kikumoto[4,7], in which a side chain (A chain) of ~10 D-glucose residues was attached near to the reducing end of a main chain (C chain) of ~14 D-glucose residues. It was suggested[7] that the close association of the A and C chains, most probably in a double helix[4], provided a favourable substrate for Q-enzyme to introduce a second branch-point into the molecule (Fig. 1).

The above evidence of Q-enzyme action on a complex of two relatively short chains has been extrapolated[7] to amylose, which is believed to exist as a double helix[4]. Random attack by Q-enzyme would give rise to an amylopectin having an elongated structure containing features of both the laminated[8] and comb[9] structures first postulated in 1937.

An unusual feature of the amylodextrin was that it was not completely debranched by pullulanase or isoamylase[7]. This property was believed to be due to the strong association between the A and C chains, which prevented the normal action of the debranching enzymes.

In view of the implications that have been derived from the structure of the amylodextrin, we have carried out methylation and enzymic analyses on various samples of amylodextrin, including two prepared by Professor D. French (samples DF II and DF III). The results lead to the conclusion that a significant proportion of the molecules are, in fact, doubly branched, so that some of the above implications require further examination.

MATERIALS AND METHODS

Analytical methods. — Reducing sugars, usually maltose, were determined by using the Nelson–Somogyi reagents, as described by Robyt and Whelan[10]. Total carbohydrate was determined by the phenol–sulphuric acid method[11]. The d.p. of amylodextrins was measured by a modified version[12] of the D-glucitol dehydroge-

nase method[13]. The average chain-length was determined by the enzymic estimation of glycerol following periodate oxidation, borohydride reduction, and acid hydrolysis[14]. Methylation analysis was performed by a modified Hakomori procedure[15]. Beta-amylolysis limits were determined by using Wallerstein barley beta-amylase[16]. Bacterial isoamylase and pullulanase from *Aerobacter aerogenes* were the preparations used by Evans and co-workers[17], and were assayed against glycogen and pullulan, respectively; it should be noted that their respective units of activity are not directly comparable.

Preparation of amylodextrin. — Amylodextrin was prepared by suspending waxy-maize starch (40 g) in 15% sulphuric acid (2 litres) at 30°. The mixture was resuspended daily by hand-shaking. After 43 days, the resistant material was allowed to settle, the acid siphoned off, water added, and the mixture centrifuged. The amylodextrin was resuspended in water, and following centrifugation, the supernatant solution was discarded. The water-washing was repeated twice, and the amylodextrin finally neutralised by the addition of 2M sodium hydroxide. Following neutralisation, the volume was made up to 300 ml with distilled water, ethanol (3.5 vol.) was then added, and the mixture stored overnight at 4°. The resulting, white precipitate was collected by centrifugation, washed once with distilled water to remove residual alcohol, and then dissolved in distilled water (250 ml) at 65°. The resulting solution

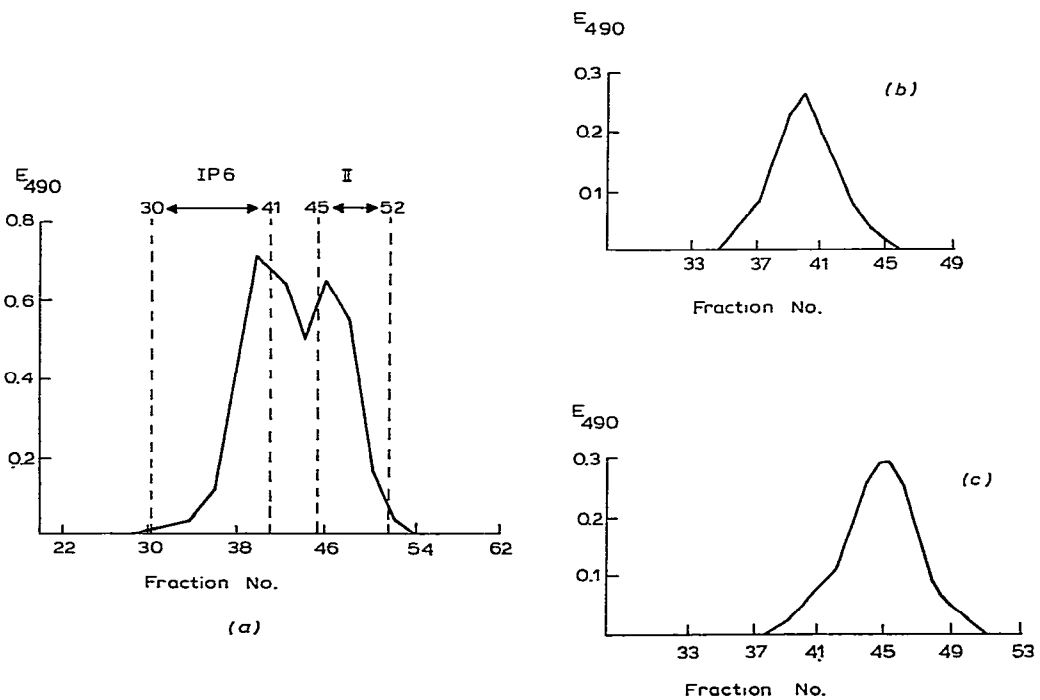

Fig. 2. Fractionation of amylodextrin on Bio-Gel P6: (*a*) elution profile of initial amylodextrin; (*b*) elution profile of Fraction IP6; and (*c*) elution profile of Fraction II. E_{490} represents the carbohydrate content of column fractions determined with the phenol–sulphuric acid reagent.

was freeze-dried, giving 8.02 g of amylodextrin. During the protracted treatment with acid, it was noted that the starch gelatinised after 23 days, and that very little further hydrolysis took place thereafter.

Preparative-scale fractionation of amylodextrin, using Bio-Gel. — Amylodextrin (100 mg) was dissolved in 0.05M sodium acetate buffer (pH 5.0, 5 ml) and applied to a column of Bio-Gel P6 (98 cm × 2 cm) or Bio-Gel P4 (90 cm × 2 cm) previously equilibrated with the same buffer. The column was then eluted with this buffer, and fractions (4 or 7 ml) were collected. The carbohydrate content of the fractions was measured on 1-ml samples by the phenol–sulphuric acid method. The elution profile from the P-6 column is shown in Fig. 2(*a*). Fractions 30–41 were combined to give amylodextrin Fraction IP6, and fractions 45–52 to give Fraction II. These two amylodextrin fractions were re-chromatographed on P-6, and then gave reasonable distribution curves, Fig. 2 (*b* and *c*).

RESULTS

Analysis of unfractionated amylodextrin. — Amylodextrin was obtained in a 20% yield from waxy-maize starch. It has a d.p. of 27, by the D-glucitol dehydrogenase method[12,13], and a beta-amylolysis limit of 90%. Methylation analysis gave 6.3 mole % of 2,3,4,6-tetra-, 90.0% of 2,3,6-tri-, and 3.7% of 2,3-di-*O*-methyl-D-glucose. These results show the amylodextrin to be composed of (1→4)-linked D-glucose residues with (1→6) inter-chain linkages. The overall ratio of tetra- to di-*O*-methyl glucose was 1.7:1. Since some of the amylodextrin molecules were expected to be linear, it follows that a proportion of the molecules must contain more than one inter-chain linkage.

Amylodextrin (5 mg) was incubated with pullulanase (1 unit) at 30° for 24 h. The enzyme was inactivated by heating, and the digest chromatographed on Bio-Gel P6. The elution profile (Fig. 3*a*) shows a change in the relative amounts of large and small molecules, and the presence of a significant amount of pullulanase-resistant carbohydrate.

Amylodextrin (5 mg) was also incubated with isoamylase (10 units) at 37° for 48 h. The elution profile of the heat-inactivated digest is shown in Fig. 3(*b*). The proportion of large and small molecules had again been changed, but the amount of material that was resistant to debranching was smaller than with pullulanase.

Fractionation of amylodextrin, using Bio-Gel P6 and P4. — Gel filtration on Bio-Gel P6 yielded two main fractions, IP6 and II. Their beta-amylolysis limits are shown in Table I; the results show that Fraction II was linear, and therefore it was not examined further.

Fraction IP6 had a d.p. of 26 by the D-glucitol dehydrogenase method[12,13], and an average chain-length of 7.7 by the periodate-oxidation method[14]. The effects of pullulanase and isoamylase on the beta-amylolysis limits of amylodextrin and Fraction IP6 are shown in Table I.

Fraction IP6 was de-ionised by chromatography on a column (45 × 2 cm) of

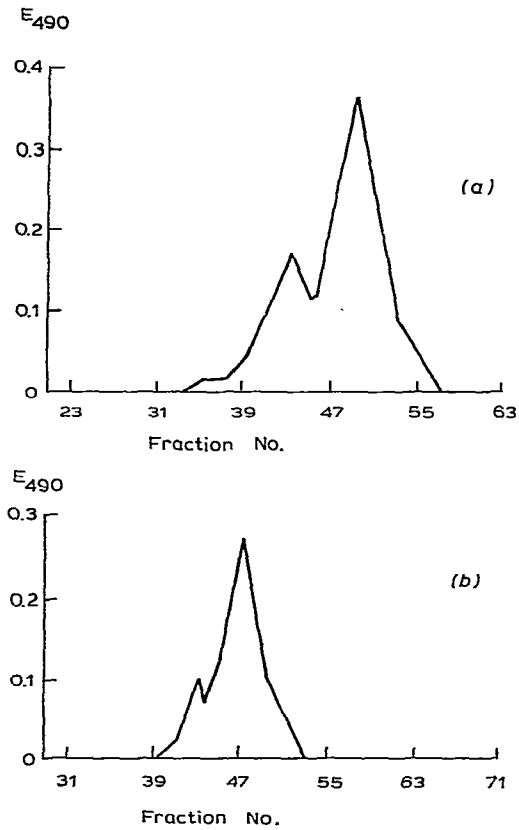

Fig. 3. Elution profiles of the initial amylodextrin after treatment with (*a*) pullulanase and (*b*) isoamylase.

TABLE I

BETA-AMYLOLYSIS LIMITS OF AMYLODEXTRINS

Sample	*Beta-amylolysis limit (%)*		
	Initial	*After pretreatment with pullulanase*	*isoamylase*
Amylodextrin	90	92	101
Fraction IP6	81	84	100
Fraction II	102	—	—

Sephadex G-25 equilibrated and eluted with distilled water. A sample (2.4 mg) was then subjected to methylation[15]; the results (see Table II) show the presence of a high proportion of molecules containing an average of two inter-chain linkages, since the molar ratio of tetra- to di-*O*-methyl-D-glucose was 2.8:2.

Since the homogeneity of any amylodextrin sample must be established before the results of structural analysis can be assessed, a sample of Fraction IP6 was

TABLE II

METHYLATION ANALYSIS OF AMYLODEXTRINS

Sample	*D.p.*	*O-Methyl-D-glucose (mole %)*			
		Tetra-	*Tri-*	*Di-*	*Tetra:Di ratio*[a]
Fraction IP6	26	10.9	81.3	7.8	2.8:2
Fraction IP4	25	13.5	78.2	8.3	3.2:2
Fraction DFII	25	9.2	85.5	5.3	3.5:2

[a]For singly branched molecules, the ratio is 2:1 or 4:2, and for doubly branched molecules, 3:2.

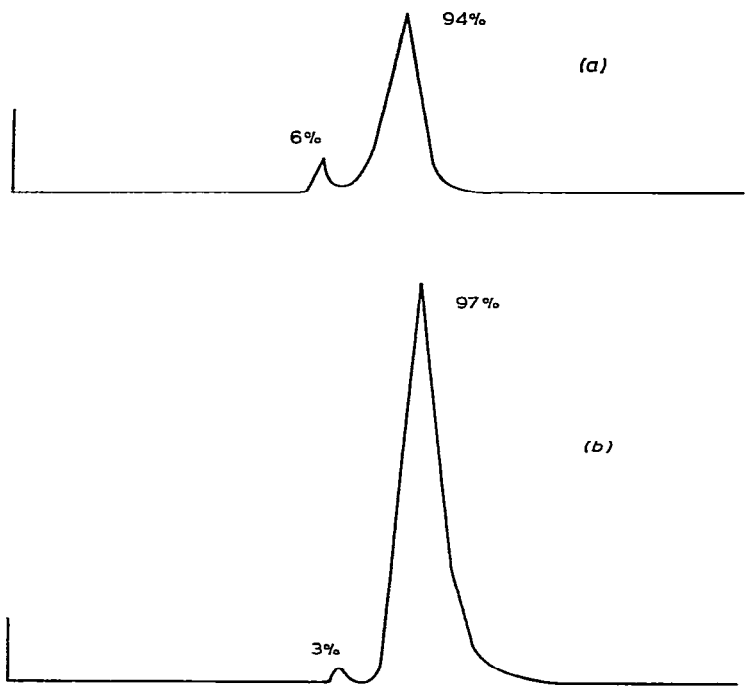

Fig. 4. Sacchromat analysis of (*a*) Fraction IP4 and (*b*) Fraction DFII. The vertical axis represents relative carbohydrate content, and the horizontal axis, the rate of elution from a column of Bio-Gel P4. (The instrument settings were different in the two analyses.)

subjected (by Dr. B. S. Enevoldsen) to quantitative gel-filtration analysis on Bio-Gel P4, using a Sacchromat[18]. This analysis showed the presence of 67% of a major component having d.p. 25, ~14% of material of lower molecular weight (d.p. ranging from 9 to 19), and ~19% of a component of higher molecular weight, which was excluded from Bio-Gel P4.

As it was possible that Fraction IP6 contained 19% of highly branched material, which could account for the overall, observed molar ratio of tetra- to di-*O*-methyl-D-glucose, a sample of amylodextrin was subjected to gel filtration on Bio-Gel P4.

This second sample of Fraction I (Fraction IP4) was also examined on a Sacchromat[18]. It contained 94% of carbohydrate having a d.p. range of 24–28, with a maximum d.p. of 26, and only 6% of material of higher molecular weight (Fig. 4).

Methylation analysis of Fraction IP4 (see Table II) indicated a molar ratio of tetra- to di-*O*-methyl-D-glucose of 1.6 to 1 (*i.e.*, ~3:2), indicating the presence of a high proportion of doubly branched molecules.

Fraction IP4 was treated by Dr. B. S. Enevoldsen with a large excess of pullulanase, and the digest analysed by using a Sacchromat. The amount of enzyme was 150 times that required to hydrolyse an equivalent weight of pullulan.

The results showed that, in spite of a 22-h incubation period, 75% of Fraction IP4 had not been hydrolysed by pullulanase. The material that had been hydrolysed gave rise to a series of maltosaccharides with d.p. 10–17. These are the products to be expected from a singly branched amylodextrin of d.p. 25.

Structural analysis of amylodextrin DFII and DFIII. — Analysis by the D-glucitol dehydrogenase method[12,13] gave d.p. values of 25 and 17, in good agreement with the findings of Professor D. French. Methylation analysis (see Table II) showed that amylodextrin DFII did not analyse as a singly branched molecule of d.p. 25. This would have given mole % values of tetra-, tri-, and di-*O*-methyl-D-glucose of 8.0, 88.0, and 4.0, respectively. The analytical results agree with those expected for a mixture of one doubly branched molecule and two singly branched molecules. Sacchromat analysis of sample DFII showed that 97% of the carbohydrate had an average d.p. of 25, and contained only 3% of a minor component of higher molecular weight (Fig. 4). Beta-amylolysis of this sample gave maltose (72%) and a range of beta-amylase-resistant dextrins with d.p. ranging from 4 to 10, of which 24% had d.p. 6, and 28% had d.p. 7.

On methylation analysis, sample DFIII gave 6.8% of tetra- and 93% of tri-*O*-methyl-D-glucose, and no other products. These results confirm the linear nature of DFIII, and suggest a d.p. of ~15.

DISCUSSION

The present results confirm the heterogeneous nature of Nägeli amylodextrins, as previously described by French[3,4] and Robin[5] and their co-workers. The slow acid-degradation of amylopectin yields a mixture of branched molecules (Fractions IP4 and IP6) and linear molecules (Fraction II), and the former include molecules containing more than one branch-point. The linear molecules presumably arise from the exterior chains of amylopectin, whilst the branched molecules arise from regions in the vicinity of the inter-chain linkages. The absence of methylated sugars other than those shown in Table II shows that transglucosylation reactions resulting in the formation of (1→2)- and (1→3)-glucosidic linkages have not taken place during the prolonged treatment of waxy-maize starch with acid.

The branched molecules (Fractions IP4, IP6, and DFII) are still heterogeneous, and all analytical figures represent average values for a family of molecules of closely

related structures. Whilst there is no unique structure for a branched amylodextrin molecule, the Sacchromat tracings (Fig. 4) show a relatively narrow range of d.p. values, and some indication of an "average" molecular structure can be obtained.

The branched nature of Fraction IP6 is shown by the d.p. value of 26, the average chain-length of 8, and the methylation results in Table II. Fraction IP6 therefore contains an average of two branch-points per molecule, although individual molecules may contain one, two, or more branch-points. Since the beta-amylolysis limit was 81%, the beta-limit dextrin must be a pentasaccharide. The two inter-chain linkages must therefore be in close proximity. This conclusion may be related to the isolation of small quantities of isomaltotriose from partial, acid hydrolysates of glycogen[19]. The above conclusions were also confirmed by the methylation analysis of Fraction IP4 (Table II).

The resistance of amylodextrin to debranching by pullulanase was first noted by Whelan[7] and is confirmed in Table I. However, isoamylase could debranch amylodextrin more completely. These observations parallel the ability of isoamylase to debranch glycogen completely, whereas pullulanase action on this substrate is incomplete. Whelan[7] has suggested that an association between the A and C chains of amylodextrin, involving a double helix, could be sufficiently strong to impede the action of pullulanase. An alternative explanation is that the presence in an amylodextrin molecule of two branch-points in such close proximity to each other could hinder normal debranching activity. It should also be noted that any association between an A chain and a C chain does not appear to prevent beta-amylase or iso-amylase from acting normally, and that the lengths of A and C chains available for double-helix formation (see Fig. 1) are too short to permit even two complete helices to exist.

These conclusions were confirmed with a sample of amylodextrin (DFII) similar to that used by Whelan[7]. Methylation analysis indicated that about one-third of the molecules contained two branch-points, and because of the high beta-amylolysis limit (81%), these must be present in a "pentasaccharide" beta-limit dextrin.

Although amylodextrin is undoubtedly a good substrate for Q-enzyme, the extrapolation of this action to a two-chain complex of amylose, thereby producing an elongated, branched structure for amylopectin[7], is not justified, in the absence of additional experimental evidence. A more satisfactory formulation for amylopectin appears to be the cluster model first proposed by French[4] and later by Robin and co-workers[5]. These workers have studied in detail the lintnerization of starch, in which hydrolysis of the more amorphous regions of the amylopectin molecules occurs, whilst the more crystalline regions are resistant to acid, and are isolated as a mixture of linear molecules, and branched molecules having a d.p. of ~25. Our results are in general agreement with these findings, and we therefore conclude that, at present, the cluster model is the most accurate representation of the overall structure of amylopectin.

ACKNOWLEDGMENTS

We are greatly indebted to Dr. B. S. Enevoldsen for his collaboration in part of this work, and to Professor D. French for a helpful exchange of information and the provision of amylodextrin samples. We also thank the Science Research Council for a research studentship (to R.S.H.).

REFERENCES

1 D. J. Manners and J. R. Stark, *Staerke*, 26 (1974) 78–81.
2 W. Nägeli, *Justus Liebigs Ann. Chem.*, 173 (1874) 218–227.
3 K. Kainuma and D. French, *Biopolymers*, 10 (1971) 1673–1680.
4 D. French, *J. Jpn. Soc. Starch Sci.*, 19 (1972) 8–25; and D. French, K. Kainuma, and W. Watanabe, unpublished work cited in this review.
5 J. P. Robin, C. Mercier, R. Charbonniere, and A. Guilbot, *Cereal Chem.*, 51 (1974) 389–406.
6 C. C. Maningat and B. O. Juliano, *Staerke*, 31 (1979) 5–10.
7 W. J. Whelan, *J. Jpn. Soc. Starch Sci.*, 23 (1976) 101–115.
8 W. N. Haworth, E. L. Hirst, and F. A. Isherwood, *J. Chem. Soc.*, (1937) 577–581.
9 H. Staudinger and E. Husemann, *Justus Liebigs Ann. Chem.*, 527 (1937) 195–236.
10 J. F. Robyt and W. J. Whelan, in J. A. Radley (Ed.), *Starch and its Derivatives*, Chapman and Hall, London, 4th edn., 1968, pp. 432–433.
11 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
12 S. Hall and R. J. Sturgeon, unpublished work.
13 D. J. Manners, A. J. Masson, and R. J. Sturgeon, *Carbohydr. Res.*, 17 (1971) 109–114.
14 D. W. Noble and R. J. Sturgeon, *Carbohydr. Res.*, 12 (1970) 448–452.
15 S. Hall and D. J. Manners, *Carbohydr. Res.*, 66 (1978) 295–297.
16 A. M. Liddle and D. J. Manners, *J. Chem. Soc.*, (1957) 3432–3436.
17 R. M. Evans, D. J. Manners, and J. R. Stark, *Carbohydr. Res.*, 76 (1979) 203–213.
18 F. Schmidt and B. S. Enevoldsen, *Carlsberg Res. Commun.*, 41 (1976) 91–110.
19 M. L. Wolfrom and A. Thompson, *J. Am. Chem. Soc.*, 78 (1956) 4182.