THE INTERACTION OF LINEAR, AMYLOSE OLIGOMERS WITH IODINE*

W. BANKS, C. T. GREENWOOD, AND K. M. KHAN
Department of Chemistry, The University of Edinburgh, Edinburgh EH9 3JJ (Great Britain)
(Received June 19th, 1970; accepted for publication, August 6th, 1970)

ABSTRACT

A series of linear, amylose oligomers has been synthesized enzymically to yield samples having the number-average degree of polymerisation, $\overline{DP}_n$, in the range 22 to 134. The interaction of these materials with iodine has been studied. The wavelength of maximal absorption of the iodine complex, λ_{max}, has been found to be related to $\overline{DP}_n$ by the Langmuir isotherm:

$$1/\lambda_{max} = 1.558 \times 10^{-3} + 102.5 \times 10^{-4}(1/\overline{DP}_n).$$

Differential, potentiometric titration of iodine has shown that the iodine-binding capacity of the linear oligomers is a function of both $\overline{DP}_n$ and temperatures. The enthalpy of the iodine uptake has been measured, and found to be a function of $\overline{DP}_n$ for values of $\overline{DP}_n < 200$.

INTRODUCTION

It is now widely recognized that the helix is an important structural characteristic of many biopolymers. Not so well-known is the fact that the concept was first applied over 30 years ago. In 1937, Hanes[1] suggested that the blue colour produced on the addition of iodine to an amylose solution resulted from the complex formed by the iodine entering the helical cavity of the polysaccharide. Freudenberg, *et al.*[2] independently advanced the same model. Bates, Rundle, and French[3] used a potentiometric technique to show that amylose binds iodine in amounts of up to 21%, corresponding to one iodine atom per 6 "anhydroglucose" residues, and they confirmed the correctness of the Hanes[1] model for the solid amylose–iodine complex by X-ray diffraction. Subsequently, Gilbert and Marriot[4] suggested that the blue colour might be associated with the presence of ions of the type $3I_2 \cdot 2I^-$.

The use of the enzyme phosphorylase to synthesize amylose oligomers and to study their reaction with iodine was developed by Swanson[5]. Bailey and Whelan[6] refined this approach, and related the number-average degree of polymerization ($\overline{DP}_n$) of the oligomer to the spectral characteristics of the iodine complex. Pfanne-

*Part 56 in the series "Physicochemical Studies on Starches"; for Part 55, see *Makromol. Chem.*, in the press.

müller *et al.*[7] recently used enzymically prepared oligomers to relate the $\overline{DP}_n$ to the optical rotatory dispersion and circular dichroism of the iodine complexes. We have now extended this work to investigate the iodine-binding capacity, measured by differential potentiometry, of a series of amylose oligomers in the range $22 < \overline{DP}_n < 134$.

EXPERIMENTAL

Phosphorylase was isolated from potato tubers, using the method of Hollo *et al.*[8] which is described in detail elsewhere[9]. Maltohexaose (G6) and maltoheptaose (G7) were isolated from a digest of amylose and alpha-amylase by repeated chromatography on paper (Whatman, 4MM), and freeze-drying of the pure sugar. The dipotassium salt of D-glucose 1-phosphate was obtained from BDH Ltd., and was used without further purification.

Preparation of amylose oligomers. — A master solution, which was 1.08mM with respect to both G6 and G7, was prepared in 0.1M citrate buffer (pH 6.3). Digests were prepared by dissolving D-glucose 1-phosphate (disodium salt; *ca.* 600 mg) in an aliquot of starter mixture, diluting to 10 ml with 0.1M citrate buffer (pH 6.3), adding phosphorylase solution (0.2 ml; activity = 456 units/ml; the unit being that defined by Lee[10]), and incubating at 37°. The details of the amounts of master solution, and times of incubation are shown in Table I.

TABLE I

CONDITIONS FOR THE PREPARATION OF AMYLOSE OLIGOMERS, AND VALUES OF THEIR NUMBER-AVERAGE DEGREE OF POLYMERISATION, $\overline{DP}_n$, AND WAVELENGTH OF MAXIMAL ABSORPTION OF THE IODINE COMPLEX, λ_{max}

Sample	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Master solution (ml)	10	7.5	7.5	5	2.5	2.5	2.5	2	1
Time of incubation (h)	5	3.5	5	5	5	10	18	18	18
λ_{max}(nm)	496	524	530	546	574	588	595	606	610
$\overline{DP}_n$	22.2	28.9	31.3	36.4	50.7	71	93	105	134

The products were precipitated by the addition of ethanol (4 vol.), and then washed repeatedly with 200-ml aliquots of 50% aqueous methanol in order to remove contaminating D-glucose 1-phosphate. Finally, the products were dehydrated with ethanol and dried *in vacuo* (60°, 15 h).

The synthetic preparations were dissolved in methyl sulphoxide to give 1.5% (w/v) solutions.

Estimation of $\overline{DP}_n$ *of the oligomers.* — The values of $\overline{DP}_n$ were determined by using the enzymic method of Banks and Greenwood[11]. This method depends on there being an equality between the numbers of odd- and even-membered chains; to ensure this equality, we use, as a priming material, an equimolar mixture of G6 and G7.

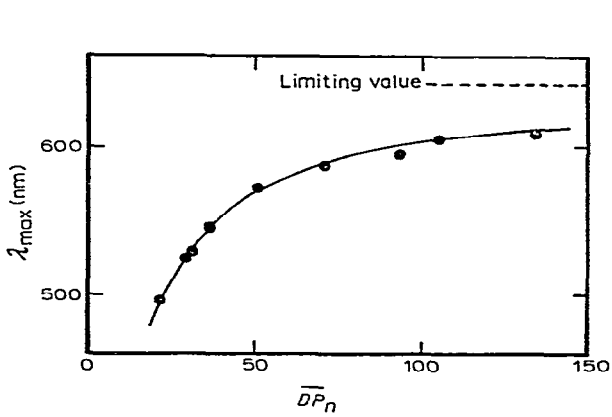
Determination of the wavelength of maximal absorption of the amylose–iodine complex (λ_{max}). — Standard iodine–iodide solution (5 g of KI, 0.2 g of I_2 in 250 ml; 0.2 ml), and polysaccharide solution (0.01 ml) were diluted with water to 10 ml. The absorption of this solution was measured in a Unicam SP 800 spectrophotometer in the wavelength region 350–700 nm, in a 1-cm cell.

Measurement of iodine-binding capacity. — The apparatus used in this work for potentiometric titration of iodine has been described in detail elsewhere[12]. Measurements were carried out at a number of temperatures in the range 1.5–20.4°.

RESULTS AND DISCUSSION

The relation between $\overline{DP}_n$ *and* λ_{max}

The measured values of $\overline{DP}_n$ and λ_{max} are shown in Table I; the relation between these parameters is demonstrated in Fig. 1.



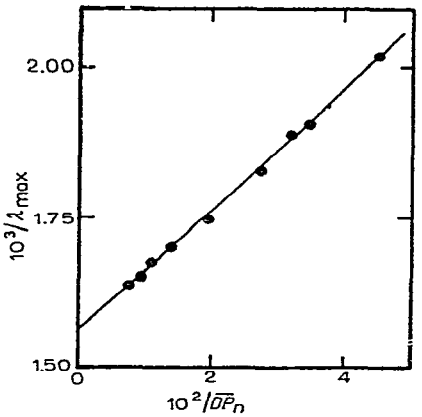


Fig. 1. Graph of λ_{max} as a function of $\overline{DP}_n$ for the synthetic, linear, amylose oligomers. The limiting value of λ_{max} for amylose of high molecular weight ($DP \sim 1500$) is shown as a dotted line.

Fig. 2. Graph of $1/\lambda_{max}$ as a function of $1/\overline{DP}_n$ for the synthetic, linear, amylose oligomers.

With increasing $\overline{DP}_n$, the value of λ_{max} moves towards higher wavelengths; the form of Fig. 1 is similar to that obtained by previous investigators[6,7]. We have restricted our studies to a comparatively narrow range of $\overline{DP}_n$, as this is the area in which the major changes occur. According to Bailey and Whelan[6], there is a sharp break in the relation between $\overline{DP}_n$ and λ_{max} at $\overline{DP}_n = 72$. Szejtli *et al.*[13,14] concluded that the break occurred at $\overline{DP}_n = 120$, a value subsequently confirmed by Pfannemüller *et al.*[7]. However, we regard this apparently good agreement as likely to be fortuitous in view of the difference in the distribution functions for the amylose samples. Szejtli *et al.*[13,14] employed a series of amyloses obtained by mechanochemical degradation of natural amylose, and which therefore possess a broad molecular-weight distribution, and estimated $\overline{DP}_n$ by the relation of Cowie and Greenwood[15],

$$\overline{DP}_n = 7.4\,[\eta], \qquad (1)$$

where $[\eta]$ is the limiting-viscosity number of amylose (in ml/g) in M potassium hydroxide. This relation was established only for $140<[\eta]<560$; Szejtli *et al.*[14] have employed it in the range $7.5<[\eta]<190$, hence the potential error is large. Also, the use of an equation in which the experimentally accessible parameter, $[\eta]$, is a weight-average value to deduce number-average degrees of polymerisation must be suspect. Pfannemüller *et al.*[7], on the other hand, used enzymically synthesized amylose fractions for which $\overline{DP}_w/\overline{DP}_n \sim 1.001$, and determined $\overline{DP}_n$ by the same enzymic technique used in this work.

Our own results do not show any sharp break at either $\overline{DP}_n = 72$, or $\overline{DP}_n = 120$. In fact, a smooth curve may be drawn through the points. The value of λ_{max} for a linear amylose ($\overline{DP}_n = 1500$) is also shown in Fig. 1; this figure (642 nm) may be taken as the asymptotic limit to the curve for the oligomer series.

Pfannemüller *et al.*[7] have suggested that the $\overline{DP}_n$–λ_{max} relation takes the form of a Langmuir adsorption isotherm, and may therefore be written as

$$\lambda_{max} = (A \times \overline{DP}_n)/(\overline{DP}_n + B), \quad (2)$$

where A is the asymptotic limit at high $\overline{DP}_n$, and B is a constant. Equation (2) can be inverted to give

$$1/\lambda_{max} = 1/A + (B/A)(1/\overline{DP}_n) \quad (3)$$

Fig. 2 shows the results graphically presented according to equation (3). Within experimental error, the data conform to a linear relation. Moreover, extrapolation gives $1/A = 1.588 \times 10^{-3}$ (nm)$^{-1}$, from which $A = 642$ nm. This is precisely the value obtained experimentally for natural amylose of high molecular weight (see above). The fact that this linear relation correctly predicts the asymptotic value of λ_{max} gives confidence in its use.

The results of Bailey and Whelan[6] can also be fitted to equation (3). The parameters derived from that work, together with those quoted by Pfannemüller *et al.* and those from the present-work, are compared in Table II.

TABLE II

COMPARISON OF THE CONSTANTS A AND B OBTAINED BY VARIOUS WORKERS

Constant	$(1/A) \times 10^3$	$(B/A) \times 10^4$	$B \times 10^5$
Bailey and Whelan[6]	1.56	88	1.37
Pfannemuller *et al.*[7]	1.637	92.5	1.51
Present work	1.558	102.5	1.60

The same value of $1/A$ is obtained from the data of Bailey and Whelan as in the present work. There is, however, a considerable difference in the values of the parameter B which is not easily explained. The results of Pfannemüller *et al.*[7] are quite different; the extrapolated value of $1/A$ corresponds to λ_{max} of 612 nm. This rather large discrepancy was at first thought to be due to the different conditions used

in measuring λ_{max}, for Pfannemüller *et al.* used 0.4 g of oligomer/l (2.47 mM monomer), 0.1045 g of I_2/l (0.412 mM), and 0.1045 g of KI/l (0.63 mM), *i.e.*, a molar ratio of iodine–"anhydroglucose" of 1:6. We have employed conditions similar to those of Bailey and Whelan[6], *i.e.*, 0.015 g of amylose/l (0.093 mM), 0.040 g of I_2/l (0.157 mM), and 0.40 g of KI/l (2.41 mM), *i.e.*, a molar ratio of iodine–"anhydroglucose" of 1.5:1, a factor of 9 greater than that employed by Pfannemüller *et al.* However, on measuring λ_{max} of the linear amylose by both methods, no difference could be detected (it was necessary to decrease the polysaccharide concentration when using the conditions of Pfannemüller *et al.*, in order to avoid precipitation of the amylose–iodine complex).

The iodine-binding capacities of the amylose oligomer series

The iodine-binding capacities, defined as mg of iodine bound/100 mg of polysaccharide at zero free-iodine concentration, are recorded in Table III; the experimental results from which these values were obtained are shown in Fig. 3 for measurements at 1.4°, and in Fig. 4 for measurements at 20.4°.

TABLE III

VALUES OF IODINE-BINDING CAPACITIES FOR AMYLOSE OLIGOMERS AT VARIOUS TEMPERATURES

Sample (as in Table I)	*Iodine-binding capacity (mg of I_2/100 mg of amylose)*			
	1.4°	*7.7°*	*14.2°*	*20.4°*
1	7.8	n.d.[a]	n.d.	—[b]
2	9.4	n.d.	n.d.	—
3	12.5	n.d.	n.d.	—
4	19.2	n.d.	n.d.	3.6
5	19.6	18.8	14.8	11.1
6	20.7	n.d.	n.d.	n.d.
7	21.0	20.2	18.9	16.2
8	21.4	20.5	19.5	16.4
9	21.8	20.9	20.1	17.3
Amylose	22.2	21.7	20.7	19.5

[a]n.d., not determined. [b]Not possible to extrapolate experimental values to positive iodine affinities.

From the results shown in Table III, two conclusions are immediately obvious, namely (*a*) as $\overline{DP}_n$ increases, so also does the iodine-binding capacity, and (*b*) at any given $\overline{DP}_n$, the iodine-binding capacity increases with decreasing temperature. However, the effect of temperature on iodine-binding capacity becomes much more pronounced as $\overline{DP}_n$ decreases. Defining a function $F(I)$, such that $F(I)$ = (Iodine-binding capacity at 20.4°)/(Iodine-binding capacity at 1.4°), the values shown in Table IV are obtained.

The values of $F(I)$ in Table IV emphasise very clearly the profound changes which occur in iodine binding as the $\overline{DP}_n$ and temperature of measurement are varied. It should be noted that sample 4 ($\overline{DP}_n$ = 36.4) was the lowest $\overline{DP}_n$ to give a positive

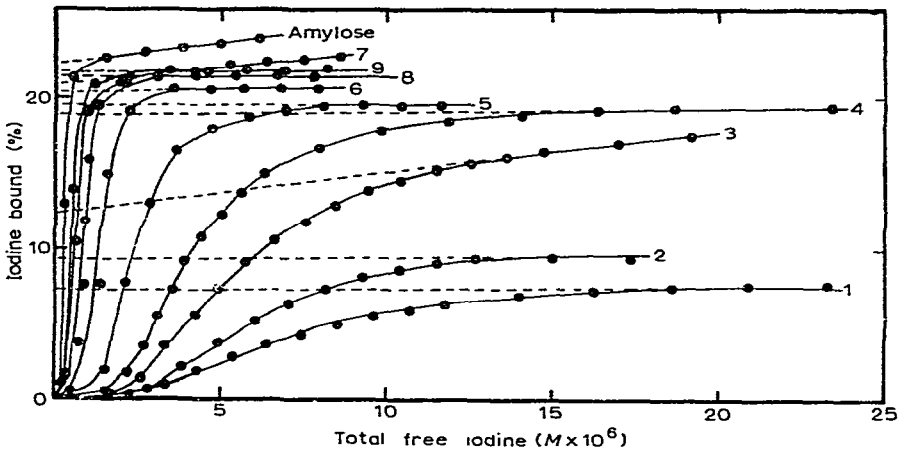


Fig. 3. Potentiometric iodine-binding curves at 1.4° for synthetic, linear, amylose oligomers (number on curve corresponds to sample in Table II), and amylose.

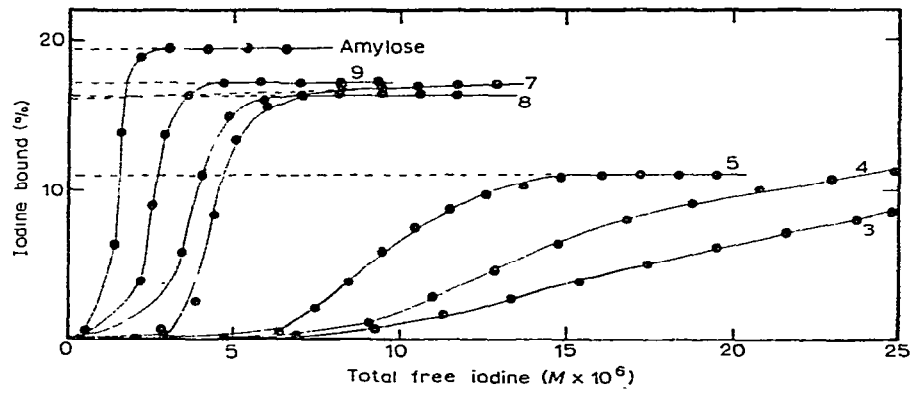


Fig. 4. Potentiometric iodine-binding curves at 20.4° for synthetic, linear, amylose oligomers (number on curve corresponds to sample in Table II), and amylose.

TABLE IV

THE RATIOS, $F(I)$, OF IODINE-BINDING CAPACITY AT 20.4° TO THOSE AT 1.4° AS A FUNCTION OF $\overline{DP}_n$

Sample[a]	$\overline{DP}_n$	F(I)
4	36.4	0.187
5	50.7	0.566
7	93	0.772
9	134	0.794
Amylose	1,500	0.880

[a]As in Table I.

value of iodine affinity at 20.4°. However, as may be seen from Fig. 4, a fairly lengthy extrapolation has to be made, and the linear region has an anomalously high slope. Thus, the iodine affinity of 3.6 may be in considerable error, and consequently the $F(I)$ value of 0.186 for this sample should be regarded with caution.

The enthalpy of the amylose-iodine interaction

A comparison of Figs. 3 and 4 shows that the binding of iodine is a complex function of $\overline{DP}_n$, concentration of free iodine, and temperature. For a given temperature, Szejtli *et al.*[14] have suggested that the interaction of amylose with iodine is represented by the equilibrium

$$[I_f]+[Am_f^*] \rightleftharpoons [I_b\, Am_b^*], \tag{4}$$

where $[I_f]$ and $I_b]$ are, respectively, the concentration of free and bound iodine, and $[Am_f^*]$ and $[Am_b^*]$ are functions of the amylose concentration expressed in terms of iodine-binding sites[14]. Therefore,

$$K = [I_b\, Am_b^*]/[I_f]\,[Am_f], \tag{5}$$

where K is the equilibrium constant. Denoting the point of half-saturation by the subscript v, it follows that

$$[Am_f^*]_v = [I_b\, Am_b^*]_v, \tag{6}$$

and

$$K = 1/[I_f]_v. \tag{7}$$

The parameter $[I_f]_v$, which may be obtained directly from such graphs as those shown in Figs. 3 and 4, is thus a reciprocal measure of the equilibrium constant K.

Szejtli *et al.*[14] then related the equilibrium constant to the temperature of measurement** by

$$K = A \exp(-\Delta H/RT). \tag{8}$$

Expressing equation (*8*) in logarithmic form, and substituting for K from equation (*7*) yields

$$\log_{10}[I_f]_v = -\log_{10} A + \Delta H/2.30\, RT. \tag{9}$$

The values of $[I_f]_v$ as a function of temperature and $\overline{DP}_n$ are shown in Table V, together with the ΔH-values derived from graphs of $\log_{10}[I_f]_v$ as a function of reciprocal temperature (see Fig. 5).

For amylose, we obtain the value $\Delta H = -15.7$ Kcal/mole iodine. This figure is comparable to other literature values[4,14,16–18]. Of more interest is the fact that ΔH decreases with decreasing $\overline{DP}_n$, *i.e.*, at low $\overline{DP}_n$, the amylose–iodine interaction is less exothermic. The asymptotic limit for ΔH will be attained, within experimental

**In fact, these authors used the relation $[I_f]_v = \exp(-(E_a/RT)+A)$, where E_a is the energy of activation for formation of the iodine complex. It is, of course, possible to obtain an activation energy only if the *rate constant* is related to the temperature. The use of an equilibrium constant, as in the present instance, must yield an enthalpy.

TABLE V

VALUES OF FREE-IODINE CONCENTRATION AT HALF-SATURATION, $[I_f]_v$, AS A FUNCTION OF TEMPERATURE AND $\overline{DP}_n$; AND THE DERIVED ENTHALPIES

Sample	$\overline{DP}_n$	$[I_f]_v \times 10^6$				$-\Delta H$ (kcal/mole)
		1.4°	*7.7°*	*14.2°*	*20.4°*	
5	50.7	2.44	3.70	5.86	9.08	11.4
7	93	0.90	1.61	2.61	4.40	13.4
8	105	0.70	1.35	2.36	3.75	14.4
9	134	0.50	0.91	1.73	2.60	14.8
Amylose	1,500	0.25	0.50	0.92	1.50	15.7

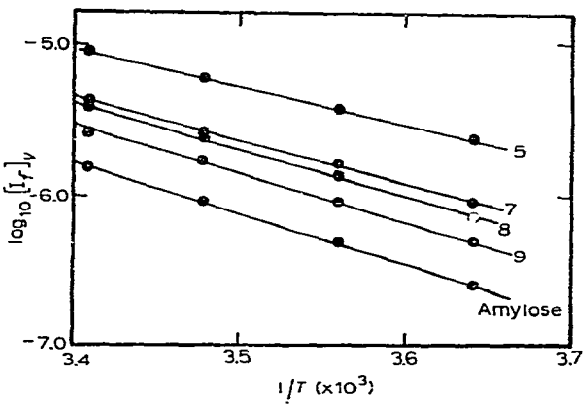


Fig. 5. Variation of $\log_{10}[I_f]_v$ (see text) with $1/T$ (number on curve corresponds to sample in Table I)

error, when $\overline{DP}_n > 200$. Kuge and Ono[17] examined the temperature dependence of $[I_f]_v$ for three amylose samples of different $\overline{DP}_n$, and showed that the same ΔH was obtained for all three. However, the values of limiting-viscosity number for these samples show that the minimum $\overline{DP}_n > 500$. Thus, there is no contradiction between the present results and those of Kuge and Ono.

Two points regarding the values of ΔH shown in Table V should be stressed. First, the experimental difficulty of ensuring that equilibrium has been attained after each addition of iodine. At low temperatures, an apparent equilibrium is reached within a minute. As the temperature is increased, however, the time necessary for equilibration also increases, especially at low $\overline{DP}_n$; for sample 5, periods of up to 30 min were required at 20.4°. In fact, this was the factor which restricted our measurements to the comparatively narrow temperature range of 19°.

Second, there is a theoretical doubt as to the correct function of $[I_f]_v$ to be used. We have employed the total concentration of free iodine, as did Szejtli *et al.*[14], but others[4,17] have preferred to use the concentration of molecular iodine. This may be obtained from the equilibrium constant K' of the reaction $I_2 + I^- \rightleftharpoons I_3^-$, where $K' = [I_2]\,[I^-]/[I_3^-] = 1.4 \times 10^{-3}$ at 25°, and the heat of the reaction is −4.22 Kcal/

mole[19]. This type of approach would considerably modify the values of ΔH. However, our own (unpublished) work on iodine-binding capacity as a function of concentration of iodide ion suggests that neither the concentration of molecular iodine nor that of the I_3^- ion is, of itself, the factor controlling the equilibrium.

REFERENCES

1 C. S. HANES, *New Phytologist*, 36 (1937) 189.
2 K. FREUDENBERG, E. SCHAAF, G. DUMPERT, AND T. PLOETZ, *Naturwissenschaften*, 27 (1939) 850.
3 F. L. BATES, D. FRENCH, AND R. E. RUNDLE, *J. Amer. Chem. Soc.*, 65 (1943) 142.
4 G. A. GILBERT AND J. V. R. MARRIOT, *Trans. Faraday Soc.*, 44 (1948) 84.
5 M. A. SWANSON, *J. Biol. Chem.*, 172 (1948) 825.
6 J. M. BAILEY AND W. J. WHELAN, *J. Biol. Chem.*, 236 (1961) 969.
7 B. PFANNEMÜLLER, H. MAYERHÖFFER, AND R. C. SCHULZ, *Makromol. Chem.*, 121 (1969) 147.
8 J. HOLLÓ, E. LÁSZLÓ, AND A. HOSCHKE, *Stärke*, 16 (1964) 243.
9 W. BANKS, C. T. GREENWOOD, AND K. M. KHAN, *Stärke*, 22 (1970) 292.
10 Y. P. LEE, *Biochim. Biophys. Acta*, 43 (1960) 18.
11 W. BANKS AND C. T. GREENWOOD, *Carbohyd. Res.*, 6 (1968) 177.
12 W. BANKS, C. T. GREENWOOD, AND D. D. MUIR, *Stärke*, in press.
13 J. SZEJTLI AND S. AUGUSTAT, *Stärke*, 18 (1966) 38.
14 J. SZEJTLI, M. RICHTER, AND S. AUGUSTAT, *Biopolymers*, 5 (1967) 5.
15 J. M. G. COWIE AND C. T. GREENWOOD, *J. Chem. Soc.*, (1957) 2862.
16 R. W. KERR, *Chemistry and Industry of Starch*, Academic Press, New York, 1950.
17 T. KUGE AND S. ONO, *Bull. Chem. Soc. Japan*, 33 (1960) 1273.
18 J. HOLLÓ AND J. SZEJTLI, *Stärke*, 9 (1957) 109.
19 G. JONES AND B. B. KAPLAN, *J. Amer. Chem. Soc.*, 50 (1928) 1845.