49. *The Conversion of Amylose into Amylopectin by the Q-Enzyme of* Polytomella coeca.*

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Samples of potato amylose have been treated with the Q-enzyme of *Polytomella coeca*, and the polysaccharide products, which stain red with iodine, have been isolated. The polysaccharides have been examined with regard to (*a*) the intensities of their iodine stains in both the visible and the ultra-violet region of the spectrum, (*b*) their behaviour when titrated potentiometrically with iodine, (*c*) their precipitability by thymol, and (*d*) their susceptibility to attack by β-amylase. In addition, they have been subjected to methylation and end-group assay, and also to molecular-weight determinations. By these methods they have been shown to be members of the amylopectin–glycogen class.

In the preceding paper it was shown that the organism *Polytomella coeca*, grown on a purely synthetic medium, contains an enzyme which is similar to potato Q-enzyme (Haworth, Peat, and Bourne, *Nature*, 1944, **154**, 236; Bourne and Peat, *J.*, 1945, 877; Bourne, Macey, and Peat, *J.*, 1945, 882) inasmuch as it converts amylose into a polysaccharide which stains red with iodine; this conversion involves the liberation of no more than traces of reducing sugar, and does not require the addition of mineral phosphate. We now report that larger amounts (*ca.* 2 g.) of potato amylose (B.V. 1·25) have been treated with three different samples of the protozoal Q-enzyme, until the values of A.V. (6800 Å) had fallen by 89, 81, and 90%, severally, and that the polysaccharide products (I, II, and III) have been isolated and examined. They have been shown to possess branched structures similar to those of amylopectin and glycogen.

Polysaccharides I, II, and III contained only very small amounts of non-carbohydrate impurities, for their protein contents were less than 0·5%, and they afforded less than 2·0% of ash on ignition. Moreover, the reducing powers of hydrolysates of the polysaccharides, determined by cuprimetric titration, corresponded to conversions into glucose of 96, 94, and 96%, respectively. The true conversions into glucose must have been slightly greater than these figures suggest, because it has been shown (Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224) that, when glucose itself is treated with sulphuric acid, under conditions similar to those obtaining during the hydrolysis of the polysaccharides, the reducing power of the solution falls to about 97% of its initial value. That glucose was, in fact, the only reducing sugar which was present in the hydrolysates of the polysaccharides was demonstrated by filter-paper chromatography; an aniline hydrogen phthalate spray revealed a single aldose component, having an R_F value identical with that of a reference glucose spot, and a resorcinol spray failed to detect a ketose sugar.

The behaviour of the three polysaccharides (I—III) towards iodine was examined in some detail; in qualitative tests they gave red stains, in contrast to the intense blue colour given by the parent amylose. Their blue values, determined by the methods of Hassid and McCready (*J. Amer. Chem. Soc.*, 1943, **65**, 1154) and of Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924), were 0·14, 0·24, and 0·12, respectively, compared with 0·10 for waxy maize starch (Bourne and Peat, *J.*, 1949, 5) and 0·11 for the amylopectin component of the native starch of *Polytomella coeca* (Bourne, Stacey, and Wilkinson, *J.*, 1950, 2694). The light-absorption curves of the iodine-stained solutions showed peaks at 5200 Å; under the same conditions, waxy maize starch and potato amylose exhibit absorption maxima at 5200 and 6400 Å, respectively (Barker, Bourne, and Peat, *J.*, 1949, 1712). Polysaccharides II and III were compared with potato amylose and potato amylopectin in other experiments, designed to measure (*a*) the dependence of A.V. (6800 Å) on iodine–iodide concentration (Fig. 1), (*b*) the changes in potential which occur during titrations of the polysaccharides with iodine–iodide solutions (Fig. 2), and (*c*) the

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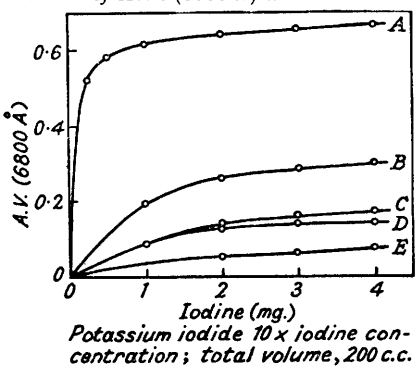
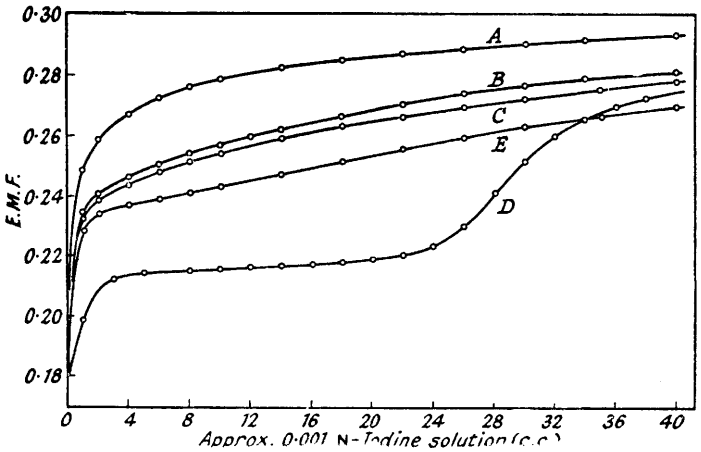
ultra-violet absorption spectra of their iodine-stained solutions (Fig. 3). All of these methods demonstrated that the polysaccharides under examination were markedly different from the parent amylose and were, in fact, very similar to potato amylopectin in their reactions with iodine. In support of this, they did not exhibit the property, characteristic of amyloses, of forming insoluble complexes with thymol (Tables 1 and 3). As was expected, since it represented an intermediate stage in the action of Q-enzyme on amylose, polysaccharide II showed less resemblance to potato amylopectin than did the other two polysaccharides.

FIG. 1. *Variation of A.V.* (6800 Å) *with iodine concentration.*



A, Potato amylose (*B.V.* 1·25; 1 *mg.*). *B, Polysaccharide II* (2 *mg.*). *C, Potato amylopectin* (*B.V.* 0·15; 2 *mg.*). *D, Polysaccharide III* (2 *mg.*). *E, Glycogen* (25 *mg.*).

FIG. 2. *The iodine titration of polysaccharide solutions.*



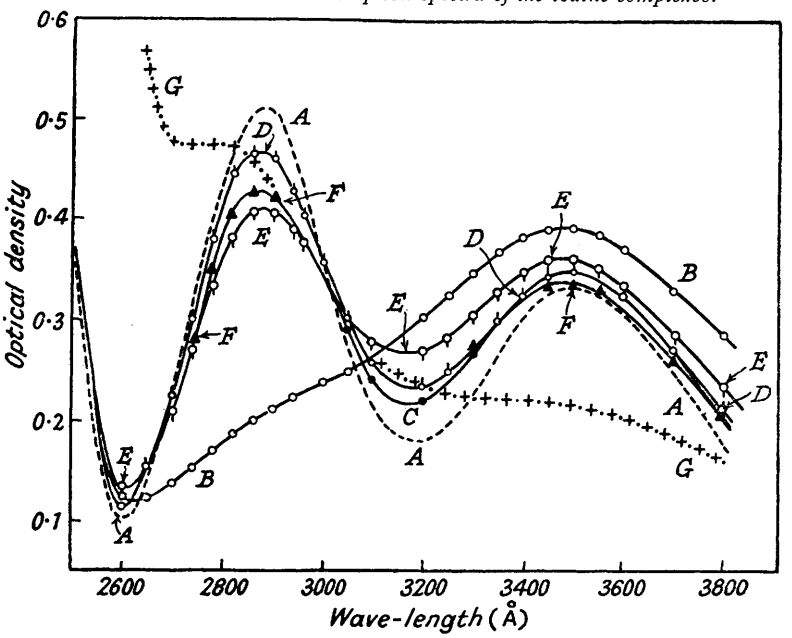
A, Iodine blank. B, Potato amylopectin (40 *mg.*). *C, Polysaccharide III* (40 *mg.*). *D, Potato amylose* (20 *mg.*). *E, Polysaccharide II* (40 *mg.*).

It is interesting to note the different effects which branched and unbranched structures have on the ultra-violet absorption spectrum of an iodine–iodide solution. In the absence of polysaccharide, the solution possesses two absorption peaks (at λ 2880 and 3500 Å), which have been attributed by Awtrey and Connick (*J. Amer. Chem. Soc.*, 1951, **73**, 1842) to the I_3^- ion. Potato amylopectin causes a slight decrease in the height of the peak at 2880 and a slight increase in that at 3500 Å (cf. Schlamowitz, *J. Biol. Chem.*, 1951, **190**, 519). On the other hand, potato amylose has a more pronounced influence on both peaks; indeed, the one at 2880 Å is eliminated completely.

There is a second respect in which the ultra-violet absorption spectra shown in Fig. 3 are important; this concerns Beckmann and Roger's claim (*J. Biol. Chem.*, 1951, **190**, 467) that potato Q-enzyme is an artifact, and that the red-staining product of its inter-

action with amylose is an amylose–soap complex, similar to that formed from amylose and potassium linoleate. There is already abundant evidence to be found in our earlier papers, published in collaboration with Professor S. Peat, that the active fraction isolated from potatoes is in fact an enzyme and not a salt of a fatty acid, and the claims made in those papers have been fully substantiated recently by Nussenbaum and Hassid (*J. Biol. Chem.*, 1951, **190**, 673). However, since Beckmann and Roger, in collaboration with Forster (*Amer. Chem. Soc. Symp. Abstracts*, September 1949), have themselves advocated the use of ultra-violet absorption spectra of the type described in order to distinguish

FIG. 3. *The ultra-violet absorption spectra of the iodine complexes.*



A, Iodine and potassium iodide. B, Potato amylose (B.V. 1·25). C, Potato amylopectin (B.V. 0·15) (coincident with D below ca. 3000 Å). *D, Polysaccharide III. E, Polysaccharide II. F, Synthetic polysaccharide (B.V. 0·22) from glucose-1 phosphate. G, Amylose–potassium linoleate complex.*

TABLE 1. *Characteristics of polysaccharides* I—III.

	Polysaccharide		
	I	II	III
Ash content (%)	0·8	1·4	2·0
Protein content (approx. %)	<0·5	<0·5	<0·5
Conversion (%) into glucose	96	94	96
Proportion (%) precipitated by thymol	—	4·0	1·2
Blue value	0·14	0·24	0·12
Wave-length (Å) of peak absorption of iodine stain in visible region	5200	5200	5200
Limiting conversion (%) into maltose by β-amylase	34	34	33
Blue value of limit dextrin	—	—	0·06
Average chain length	—	44	23, 39
Degree of polymerisation	—	—	128

between amylopectin and an amylose–soap complex, we wish to emphasise that in Fig. 3 there are very striking differences between the curves for polysaccharides II and III and potato amylopectin, on the one hand, and a red-staining amylose–potassium linoleate complex, on the other. In the same manner, a polysaccharide synthesised by Barker, Bourne, Peat, and Wilkinson (*J.*, 1950, 3022) from glucose-1 phosphate, by the agency of a mixture of potato phosphorylase and potato Q-enzyme, has been distinguished sharply from an amylose–soap complex. As supplementary evidence to support the contention that the Q-enzyme of *Polytomella coeca* is not a fatty acid, we have demonstrated its

extreme sensitivity to freeze-drying (see previous paper) and to inhibitors, such as mercuric chloride (Table 2).

Direct chemical evidence regarding the structures of polysaccharides II and III has been obtained by methylation and end-group assay. The two polysaccharides were methylated with sodium and methyl iodide in liquid ammonia, as described by Freudenberg and Boppel (*Ber.*, 1938, **71**, 2505), and hydrolysates of the resulting ethers were analysed quantitatively by filter-paper chromatography (cf. Hirst, Hough, and Jones, *J.*, 1949, 928). In each case, an aniline hydrogen phthalate spray revealed four components, three of which had R_F values identical with those of 2 : 3-dimethyl glucose, 2 : 3 : 6-trimethyl glucose, and 2 : 3 : 4 : 6-tetramethyl glucose, severally. The fourth spot had an R_F value slightly smaller than that of 2 : 3-dimethyl glucose, and was probably a second dimethyl glucose arising wholly or in part from incomplete methylation of the polysaccharide. Similar fourth spots have been found by Hirst, Hough, and Jones (*loc. cit.*) and by Barker, Bourne, and Wilkinson (*J.*, 1950, 3027) during examinations of hydrolysates of the methyl ethers of waxy maize starch and potato amylopectin. The relative proportions of the various methylated glucoses derived from polysaccharide II revealed that this polysaccharide had an average chain length of 44 units (Table 4). The methyl ether of polysaccharide III was divided into two fractions by extraction with ether; the average chain lengths of the larger and the smaller fraction were 23 and 39 units, respectively (Tables 5 and 6). Since the average chain length of the parent potato amylose, as determined by methylation and end-group assay, is *ca.* 200 units (Bourne, Fantes, and Peat, *J.*, 1949, 1109), it is clear that the action of the protozoal Q-enzyme on the linear polysaccharide had caused a marked decrease in chain length, the value finally reached, in the case of polysaccharide III, being very similar to that usually found for amylopectin.

The molecular size of polysaccharide III was determined by the method of Meyer and his colleagues (*J. Phys. Coll. Chem.*, 1949, **53**, 319), involving determination of the reducing end-group by treatment with an alkaline solution of 3 : 5-dinitrosalicylic acid. The degree of polymerisation was *ca.* 130 units (*M*, 21,000), which was appreciably higher than the average chain length of this polysaccharide, but lower than that of a natural amylopectin. However, if Q-enzyme is in fact a trans-glucosidase, then it is possible that any product of its action on amylose would not have a molecular weight higher than that of the linear parent polysaccharide. On this hypothesis, a product of higher molecular weight would result only when polysaccharide synthesis by phosphorylase is proceeding simultaneously with Q-enzyme action. This point will be examined further.

The behaviour of polysaccharides I, II, and III towards soya bean β-amylase furnished additional evidence that they possessed branched structures. However, the limiting conversions into maltose (33—34%) were somewhat lower than that (54%) observed when potato amylopectin (B.V. 0·15) was treated with the same enzyme sample. In addition, the B.V. of the limit dextrin, which was isolated only in the case of polysaccharide III, was 0·06, compared with 0·12 for that derived from potato amylopectin. These figures are more in keeping with those reported for glycogen (for summary, see Barker, Bourne, and Stacey, *J.*, 1950, 2884), the low limiting conversion of which has been attributed by Meyer and Jeanloz (*Helv. Chim. Acta*, 1943, **26**, 1784) to the inability of β-amylase to penetrate the more complex regions of the polysaccharide molecule. The same reason may apply in the present case, or, alternatively, the distribution of the branch points in polysaccharide III may be somewhat different from that in potato amylopectin (cf. Barker, Bourne, Peat, and Wilkinson, *loc. cit.*; Cori and Illingworth, *J. Biol. Chem.*, 1951, **190**, 679).

Experimental

Isolation of Polysaccharides formed from Potato Amylose by the Q-Enzyme of Polytomella coeca.—A sample of amylose (2·0 g.; B.V. 1·25), which had been prepared from potato starch by fractional precipitation with thymol (Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687), was dissolved in warm 0·5N-sodium hydroxide (100 c.c.). The cooled solution was diluted with water (1 l.), neutralised with 0·5N-sulphuric acid, mixed with a solution of Q-enzyme in 0·2M-citrate buffer (pH 6·8; 200 c.c.), and diluted further to 2 l. The enzyme had been prepared from a mass culture (16 l.) of *Polytomella coeca* by the standard method described

in the preceding paper. The digest was incubated at 25°; at intervals, aliquot portions (1 c.c., equivalent to 1 mg. of amylose) were stained with iodine (2 mg.) and potassium iodide (20 mg.) in a total volume of 100 c.c. In addition, the reducing power of the digest was measured periodically by cuprimetric titration with the Shaffer–Hartmann reagent (*J. Biol. Chem.*, 1921, **45**, 377), a known amount of maltose being introduced during the determination in order to overcome the insensitivity of the reagent to small amounts of sugar (<0·4 mg. of maltose). When the A.V. (6800 Å) had reached an appropriate value, the digest was inactivated at 100° and the precipitated protein was removed by filtration. The filtrate and washings were concentrated under diminished pressure to 150 c.c. and dialysed for 48 hours. The polysaccharide product was precipitated with alcohol (500 c.c.), collected in the centrifuge, triturated with alcohol and then with ether, and finally dried in a vacuum over phosphoric oxide.

From three experiments of this type, in which different samples of Q-enzyme were employed, and in which the final values of A.V. (6800 Å) were about 11, 19, and 10% of the initial value, polysaccharides I, II, and III were isolated in yields of 65, 61, and 80%, respectively. In each case, the "apparent conversion into maltose" at the end of the incubation period was only 1—4%.

Purities of Polysaccharides I—III.—(a) *Ash contents.* Each polysaccharide (20 mg.) was heated in a platinum boat in a micro-muffle furnace until there was no further change in weight.

(b) *Products of acidic hydrolysis.* Each polysaccharide (40 mg.) was heated with 2N-sulphuric acid (1·0 c.c.) for 5 hours at 100° in a sealed tube. The hydrolysate was freed from inorganic material by means of an apparatus of the type described by Consden, Gordon, and Martin (*Biochem. J.*, 1947, **41**, 590). Part of the solution was examined for sugars by filter-paper chromatography; the solvent was the upper layer of a mixture of *n*-butanol (40%), ethanol (10%), water (49%), and ammonia (1%), and the spraying reagents were aniline hydrogen phthalate (Partridge, *Nature*, 1949, **164**, 443) and a 3% solution of resorcinol in 5% alcoholic hydrogen chloride. The former spray, which is more sensitive for aldoses than for ketoses, revealed only one component in each of the three hydrolysates, and this component had an R_F value identical with that of a glucose reference spot. The latter spray, which is more sensitive for ketoses, failed to detect any sugar component.

A second portion of each hydrolysate was examined, by filter-paper chromatography, for amino-acids, a saturated aqueous solution of phenol being used as the solvent and a 0·2% solution of ninhydrin in *n*-butanol as the spraying reagent. By comparison of the intensities of the spots with those obtained from amino-acid solutions of known concentration, it was shown that each of the three polysaccharides contained less than 0·5% of proteinaceous impurity. Moreover, negative reactions were obtained when the polysaccharides were tested for nitrogenous impurities by fusion with sodium and for proteins by treatment with Millon's mercuric nitrite reagent.

(c) *Degrees of conversion into glucose.* Polysaccharides I, II, and III were hydrolysed for 6 hours with hot 7% sulphuric acid, and the amounts of glucose produced were determined by cuprimetric titration, as described by Bourne, Donnison, Haworth, and Peat (*loc. cit.*). The conversions were 96, 94, and 96%, respectively.

Intensities of Polysaccharide–Iodine Stains.—Details of the methods employed for the determination of blue value (B.V.) and absorption value (A.V.) were given by Bourne, Haworth, Macey, and Peat (*loc. cit.*).

Potentiometric Titration of the Polysaccharides with Iodine.—The method employed was essentially that of Bates, French, and Rundle (*J. Amer. Chem. Soc.*, 1943, **65**, 142), as modified by Wilson, Schoch, and Hudson (*J. Amer. Chem. Soc.*, 1943, **65**, 1380). The polysaccharide sample (20 mg. in the case of amylose and 40 mg. in the case of a branched polysaccharide) was dispersed in 0·5N-potassium hydroxide (10 c.c.). The solution was neutralised (methyl-orange) with 0·5N-hydrochloric acid, mixed with 0·5N-potassium iodide solution (10 c.c.), and diluted with water to 100 c.c. A solution, which was approx. 0·001N in iodine, 0·05N in potassium iodide, and 0·05N in potassium chloride, was added to the polysaccharide solution in 1—2 c.c. portions. After each addition the potential was measured with the aid of a 3M-calomel cell and a bright platinum electrode. The results are shown graphically in Fig. 2.

Ultra-violet Absorption Spectra of Polysaccharide–Iodine Solutions.—(a) *The reference iodine–potassium iodide solution.* The absorption spectrum of a solution containing iodine (0·001%) and potassium iodide (0·01%), measured in a "Unicam" spectrophotometer with 10-mm. quartz cells, is shown in Fig. 3.

(b) *Modifications of the reference curve by various polysaccharides.* The polysaccharide (25 mg.) was suspended in water (5 c.c.), and N-sodium hydroxide solution (1 c.c.) was added.

The suspension was warmed until dissolution was completed, cooled, neutralised with 0·5N-sulphuric acid, and diluted to 25 c.c. A portion (5 c.c.) of the solution was stained with iodine (1 mg.) and potassium iodide (10 mg.) in a final volume of 100 c.c. The absorption spectrum of this solution was determined as in (a). The results obtained with various polysaccharides are shown in Fig. 3. In a control experiment, in which the polysaccharide was omitted, it was demonstrated that, under these conditions, sodium sulphate alone had a negligible effect on the absorption spectrum of the iodine–iodide solution. The amylose–potassium linoleate solution referred to in Fig. 3 contained amylose (5 mg., dissolved as before), potassium linoleate (17 mg.), and sufficient of the iodine–iodide solution to give a permanent red stain; excess of iodine–iodide solution produced a blue colour.

Inhibition of Q-Enzyme by Mercuric Chloride.—A sample of Q-enzyme, which had been prepared by the standard method from a mass culture of *Polytomella coeca*, was incorporated in four digests, each of which contained potato amylose (B.V. 1·25; 20 mg.), 0·02M-citrate buffer (pH 6·8; 6·0 c.c.), and Q-enzyme solution [containing the enzyme from a 400 c.c. culture, dissolved in 0·02M-citrate buffer (pH 6·8; 2·0 c.c.)] in a total volume of 20 c.c. In addition, three of the digests contained mercuric chloride, the concentrations being 0·1, 0·01, and 0·001%, respectively. The digests were incubated at 25°; at intervals aliquot portions (1 c.c.) were stained with iodine (2 mg.) and potassium iodide (20 mg.) in a total volume of 100 c.c. for the measurement of A.V. (6800 Å). The results are given in Table 2. The two digests containing

TABLE 2.

Concn. of $HgCl_2$ in digest (%)		Nil	0·001	0·01	0·1
Fall (%) in A.V. (6800 Å) in	45 min.	67·1	0·0	0·0	0·0
	100 min.	87·6	0·9	1·7	2·6

the highest concentrations of mercuric chloride developed a slight turbidity; the other two remained clear.

β-*Amylolysis of Polysaccharides* I—III.—The limiting conversions into maltose recorded in Table 1 were determined by the method of Barker, Bourne, Peat, and Wilkinson (*loc. cit.*), which was based on cuprimetric titration. The β-amylase employed had been isolated from soya beans (Bourne, Macey, and Peat, *loc. cit.*).

Isolation of the Limit Dextrin from Polysaccharide III.—The polysaccharide (200 mg.) was incubated at 20° with M-acetate buffer (pH 7·0; 10 c.c.), N-acetic acid (10 c.c.), and 0·2% β-amylase solution (20 c.c.) in a total volume of 100 c.c. When the digest, which had pH 4·7, attained constant reducing power (equivalent to a 33% conversion into maltose), it was heated to 100°, and the precipitated protein was removed by filtration. The filtrate was dialysed for 4 days and concentrated to 30 c.c. Alcohol (3 vols.) was added to precipitate the dextrin, which was collected in the centrifuge, ground with alcohol, then with ether, and dried at 60° in a vacuum over phosphoric oxide. The product (100 mg.) had B.V. 0·06 and contained 0·9% of ash.

In a second experiment, potato amylopectin (B.V. 0·15; 2·50 g.) was treated with the same enzyme sample. The limiting conversion into maltose was 54%. The limit dextrin (0·90 g.) had B.V. 0·12 and contained 0·8% of ash.

Treatment of Polysaccharides II *and* III *with Thymol.*—The polysaccharides were dissolved in alkali, neutralised, and treated with thymol according to the procedure of Barker, Bourne, Peat, and Wilkinson (*loc. cit.*); the results are given in Table 3.

Methylation of Polysaccharides II *and* III.—The polysaccharides were methylated with sodium and methyl iodide in liquid ammonia, as described by Freudenberg and Boppel (*loc. cit.*). The

TABLE 3. *Treatment of polysaccharides* II *and* III *with thymol.*

Polysaccharide	Fraction pptd. by thymol: Yield (%)	B.V.
II	4·0	0·17
III	1·2	0·07

modifications introduced by Barker, Bourne, and Wilkinson (*loc. cit.*) in the method used for the isolation of the products were again employed.

(a) *Methylation of polysaccharide* II. The polysaccharide (758 mg.) was treated eight times with the methylating reagents [one sixth of the quantities employed by Freudenberg and Boppel (*loc. cit.*)]. The crude product obtained when the ammonia was removed was stirred with water (200 c.c.), a small amount of insoluble material being separated by the centrifuge and discarded.

After being dialysed in " Cellophane " until iodide ions could no longer be detected by the silver nitrate test, the methylated polysaccharide was freeze-dried. The residue was extracted with ether for 3 hours (Soxhlet). The ether-insoluble portion (650 mg.) had OMe, 44·5% (Calc. for trimethyl starch : OMe, 45·6%). Evaporation of the ethereal extracts afforded a small residue (70 mg.) having OMe, 35·0%; this was not examined further.

(b) *Methylation of polysaccharide* III. The polysaccharide (616 mg.) was treated 16 times with the methylating reagents, and the product (428 mg.) was isolated as in (a). The ether-insoluble portion (209 mg.; fraction A) had OMe, 40·0% and ash content, 4·0%. The ethereal solution was concentrated to a syrup, to which chloroform (2 c.c.) was added. After the removal of some insoluble material, light petroleum (b. p. 100—120°; 40 c.c.) was added. The precipitate (80 mg.; fraction B) had OMe, 45·1%.

Analysis of the Hydrolysates of the Methylated Polysaccharides by Filter-paper Chromatography.—The hydrolysates were prepared and analysed by a method which was essentially that used by Flood, Hirst, and Jones (*J.*, 1948, 1679) and by Hirst, Hough, and Jones (*loc. cit.*). The methylated polysaccharide (*ca.* 60 mg.) was heated with 4% methanolic hydrogen chloride (1·0 c.c.) for 8 hours in a sealed tube at 100°. The glucosides produced were hydrolysed with 4% hydrochloric acid (5·0 c.c.) at 100° for 5 hours. The hydrochloric acid was removed by treatment with silver carbonate.

The components of the neutral hydrolysate were determined by filter-paper chromatography, the modifications introduced by Barker, Bourne, and Wilkinson (*loc. cit.*) being included. The blank strips used for the determination of reducing substances in the paper were cut from a second sheet of the same filter-paper which had been irrigated with the solvent and on which no hydrolysate spots had been placed. The sizes and positions from which the blank strips were cut were identical with those of the corresponding strips from the main chromatogram.

(a) *Analysis of methylated polysaccharide* II. The chromatogram obtained from a hydrolysate of methylated polysaccharide II showed four components which stained with aniline hydrogen phthalate. Three of these had R_F values identical with those of 2 : 3-dimethyl glucose, 2 : 3 : 6-trimethyl glucose, and 2 : 3 : 4 : 6-tetramethyl glucose, used as standards on the same chromatogram. The fourth component had an R_F value slightly smaller than that of 2 : 3-dimethyl glucose and appeared to be a second dimethyl glucose. Owing to the close proximity of the two dimethyl glucose spots, it was not possible to determine them separately and the figures quoted for the dimethyl glucose fraction in Table 4 represent the sum of both components. The mean value for the molecular proportion of 2 : 3 : 4 : 6-tetramethyl glucose corresponded to an average chain length of 44 glucose units.

TABLE 4. *End-group assay of methylated polysaccharide* II.

	Molecular composition (%) indicated by assay number				
Probable derivative of glucose	I	II	III	IV	Mean
Dimethyl fraction	2·37	2·60	2·31	2·84	2·53
2 : 3 : 6-Trimethyl	95·2	94·6	95·8	95·2	95·2
2 : 3 : 4 : 6-Tetramethyl	2·48	2·76	1·89	1·99	2·28

(b) *Analysis of methylated polysaccharide* III (*fraction A*). A chromatogram of a hydrolysate of methylated polysaccharide III (fraction A) showed four components having R_F values identical with those described in (a). The results obtained in three independent analyses are shown in Table 5. The results corresponded to an average chain length of 23 glucose units.

(c) *Analysis of methylated polysaccharide* III (*fraction B*). Chromatographic examination of a hydrolysate of methylated polysaccharide III (fraction B) revealed four components having R_F values identical with those reported in (a). Because of the small amount of the

TABLE 5. *End-group assay of methylated polysaccharide* III (*fraction A*).

	Molecular composition (%) indicated by assay no.			
Probable derivative of glucose	I	II	III	Mean
Dimethyl fraction	4·33	4·61	4·49	4·48
2 : 3 : 6-Trimethyl	91·2	91·0	91·1	91·1
2 : 3 : 4 : 6-Tetramethyl	4·49	4·41	4·43	4·44

polysaccharide ether available, only two end-group determinations could be made; these are shown in Table 6. The mean value for the molecular proportion of 2 : 3 : 4 : 6-tetramethyl glucose corresponded to an average chain length of 39 glucose units.

The Molecular Weight of Polysaccharide III.—A determination of the molecular size of one of the synthetic polysaccharides (III) was made by a method which was essentially that of Meyer, Bernfeld, Boissonnas, Gürtler, and Noelting (*J. Phys. Coll. Chem.*, 1949, **53**, 319). It involved the colorimetric estimation of the reducing end-group with 3 : 5-dinitrosalicylic acid in alkaline solution.

TABLE 6. *End-group assay of methylated polysaccharide* III (*fraction B*).

Probable derivative of glucose	Molecular composition (%) indicated by assay no. I	II	Mean
Dimethyl fraction	4·90	5·97	5·44
2 : 3 : 6-Trimethyl	92·5	91·5	92·0
2 : 3 : 4 : 6-Tetramethyl	2·63	2·49	2·56

The polysaccharide (51·8 mg.) was suspended in water (2 c.c.), and complete solution attained by the addition of 6N-sodium hydroxide solution (1 c.c.). An aliquot portion (1 c.c.) of a 1·5% solution of the reagent (3 : 5-dinitrosalicylic acid in 0·2N-sodium hydroxide solution) was added, and the mixture was heated at 65° for 30 minutes. After cooling, the volume was adjusted to 25 c.c. with water, and the intensity of the colour was compared with that of a blank (polysaccharide absent), the Spekker photoelectric absorptiometer being used with a green filter and 4-cm. cells. In order to correct for any turbidity due to the polysaccharide, a blank containing polysaccharide and alkali, but no 3 : 5-dinitrosalicylic acid, was treated as described above, and its absorption measured. The reagent had been previously standardised with solutions of maltose hydrate, so that the degree of polymerisation of the polysaccharide could be calculated directly from its absorption and the weight of maltose exhibiting the same absorption. A degree of polymerisation of 128 was obtained, corresponding to a molecular weight of approximately 21,000 for polysaccharide III.

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