166. *The Amylolytic Degradation of Starch.*

By W. N. Haworth, H. Kitchen, and S. Peat.

It is shown that β-amylase hydrolyses the amylopectin component of starch with the formation of maltose and a limit dextrin : dextrin-A or α-amylodextrin. Dextrin-A is not susceptible of further attack by β-amylase until it has been " sensitised " by contact with salivary amylase. Thereafter the action of β-amylase continues until a second resting stage—dextrin-B—is reached. Dextrin-B is not further hydrolysed by β-amylase and is not sensitised by saliva. It is, however, hydrolysed by salivary amylase with the production of maltose and dextrin-C. This dextrin is slowly hydrolysed by pancreatic amylase with the formation of dextrin-D. End-group assay on the methylated dextrins indicates that the unit chain-lengths are 7—8 (dextrin-B), 5—6 (dextrin-C), and 4—5 (dextrin-D).

The mechanism of amylolysis is explicable on the basis of the simple laminated formulation of the structure of starch propounded by Haworth and Hirst, if it be assumed (i) that the impediment to β-amylase action is represented by the polymeric links which unite the unit chains; (ii) that the polymeric links are ruptured by an enzymic constituent of saliva and of malt α-amylase; and (iii) that the unit chains so liberated immediately recombine with the formation of new polymeric (*i.e.*, 1 : 6-α-glucosidic) links with a different orientation of position on the respective chains.

It is not necessary to postulate a complex, highly ramified structure for amylopectin, such as that proposed by K. H. Meyer, to explain the facts of amylolysis.

The various dextrins produced by the amylolysis of starch have been named on no logical basis but rather according to the predilections of individual investigators. Thus the residual dextrin obtained by the action of β-amylase on starch has been named α-amylodextrin by Baker and erythrogranulose by Wijsman. In this paper are described a series of limit dextrins which are closely interrelated in that they are produced by the systematic and progressive enzyme degradation of starch. We propose, therefore, to name these dextrin-A, dextrin-B, dextrin-C, etc. Dextrin-A is identical with α-amylodextrin and was in fact so named by us in an earlier paper (Haworth, Hirst, Kitchen, and Peat, J., **1937**, 791).

In the earlier paper the limit dextrin which resulted from the completed action of β-amylase on potato starch was described. This limit dextrin (α-amylodextrin, erythrogranulose, dextrin-A) represents 40% by weight of the original starch, the remaining 60% of which appears as maltose. Dextrin-A was shown to have an apparent unit chain length (determined by end-group assay) of 11—12 glucose members and to be resistant to further attack by β-amylase.

It is known that the obstruction to the action of β-amylase shown by dextrin-A may be simply removed by submitting the dextrin to treatment with water in an autoclave at 120°, or to the action of malt α-amylase (cf. Hanes, *Canadian J. Res.*, **1935**, **13**, *B*, 185). In the further degradation of dextrin-A, we have adopted the second method and have used saliva as the enzyme source.

Preliminary experiments demonstrated that the susceptibility of dextrin-A to hydrolytic attack by β-amylase was restored when salivary amylase was allowed to act upon the dextrin for even a few seconds.

Dextrin-A, " sensitised " by contact with salivary amylase, was now submitted to the action of β-amylase at 38° for a prolonged period. A second limit dextrin, dextrin-B, was thus produced and this represented

58% by weight of dextrin-A. The only other product of hydrolysis was maltose, which was isolated as crystalline maltose hydrate, the weight of which represented 38% of dextrin-A.

Dextrin-B was easily methylated with methyl sulphate and sodium hydroxide and end-group assay on the methylated derivative indicated for the dextrin a unit chain-length of 7—8 glucose members.

Dextrin-B was not further hydrolysed by β-amylase. Moreover, it was not " sensitised " by the momentary action of salivary amylase, in the sense that the latter enzyme did not remove the impediment to the further action of β-amylase. Nevertheless, the salivary amylase itself effected further hydrolysis of dextrin-B whereby crystalline maltose (27%) and limit dextrin-C (67%) were produced. End-group assay of the methylated dextrin-C showed its unit chain to consist of 5—6 glucose members. Dextrin-C was not further hydrolysed by β-amylase, salivary amylase, malt amylase (α and β) or taka-diastase. It was, however, slowly attacked in the presence of a purified commercial pancreatic amylase. Dextrin-D (80% of dextrin-C) was thus obtained, together with a sugar which, by analytical procedure only, appeared to be maltose.* The chain-length of this dextrin, as determined by assay of the methylated derivative, was 4—5 glucose units.

The properties of the dextrins formed by the amylolytic breakdown of starch are given in Tables I and II, together with those of potato starch and a commercial acid-solubilised starch.

TABLE I.

Degradation Products of Potato Starch.

	Potato starch.[1]	Soluble starch.[1]	Dextrin-A (α-amylodextrin).[2]	Dextrin-B.	Dextrin-C.	Dextrin-D.
Solubility	Boiling H_2O	Hot H_2O	Hot H_2O	Cold H_2O	Cold H_2O	Cold H_2O
Retrogrades?	Yes	Yes	Yes	No	No	No
Reducing? (Cu number)	Slightly (0·04)	Slightly (0·2)	Slightly	Slightly	Yes	Yes
Iodine value	1·1	1·3	2·7	8	13	14·2
Iodine colour	Blue	Blue	Purple	Red	Nil	Nil
$[\alpha]_D$ in H_2O	+187°	—	+167°	+156°	+148°	+116°
$[\alpha]_D$ in 5% NaOH	+150°	+148°	+133°	+127°	+116°	+111° → +95°
P content % (as P_2O_5)	0·14	0·22	0·40	0·59	1·0	1·25

TABLE II.

Methylated Products.

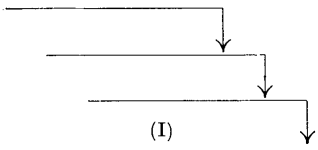
	Potato starch.[1]	Soluble starch.[1]	Dextrin-A.[2]	Dextrin-B.	Dextrin-C.	Dextrin-D.
OMe %	44·5	44·0	44·0	43·1	44·5	46·0
$[\alpha]_D$ in $CHCl_3$	+205°	+204°	+194°	+185°	+182°	+176°
$\eta_{sp./c}$ in *m*-cresol	0·9	0·5	0·43	0·075	0·09	0·10
Mol. wt. (osmotic pressure)	—	28,000 [3]	79,000 [4]	16,000 [4]	—	—
Chain-length by end group assay (glucose units)	24—26	27—28	11—12	7—8	5—6	4—5

[1] H. Kitchen, Ph.D. Thesis, Birmingham, 1937. [2] Haworth, Hirst, Kitchen, and Peat, *loc. cit.* [3] Carter and Record, *Chem. and Ind.*, 1936, 218. [4] W. T. Chambers, Ph.D. Thesis, Birmingham, 1939.

DISCUSSION.

The immediate problem is to reconcile the facts of amylolysis with the structure which has been assigned to starch on chemical grounds.

The laminated formulation (I) was proposed for starch in 1937 (Haworth, Hirst, and Isherwood, J., 1937, 577), based on the model we had already suggested as best representing the constitution of xylan (Haworth, Hirst, and Oliver, J., 1934, 1917).



The observations now recorded were published in thesis form in 1937 (H. Kitchen, Ph.D. Thesis, University of Birmingham) and we have long had in mind the possibility that the impediment to β-amylase action is presented by the linkages [represented by the arrow heads in (I)] which unite the basal chains. No novelty is claimed for this view of the nature of the obstruction to the action of β-amylase, and it has, of course, occurred to others (cf. Hanes, *New Phytol.*, 1937, **36**, 229).

Wider publication of our results is necessitated by the appearance of a series of papers by K. H. Meyer and his associates describing investigations which follow substantially the same lines as our own (see *Helv. Chim. Acta*, 1940, **23**, 875). Dextrin-A (called dextrin-I by Meyer) was treated for an arbitrary period (14 hours) with yeast maltase, whereby glucose (17%) was liberated and dextrin-II (75%) formed. Dextrin-II was now susceptible to the action of β-amylase, which effected further hydrolysis to dextrin-III (64% of dextrin-I). It appears likely that Meyer's dextrin-III and our dextrin-B are identical in that both represent approximately the same fraction by weight of dextrin-A and both give a red colour with iodine. No end-group assay of dextrin-III is reported, so a comparison in this respect is not possible.

* In all other cases the weight of isolated crystalline maltose is quoted. We do not favour the facile dependence on titration methods for the estimation of maltose in solution, since the result may be due to more than one reducing sugar.

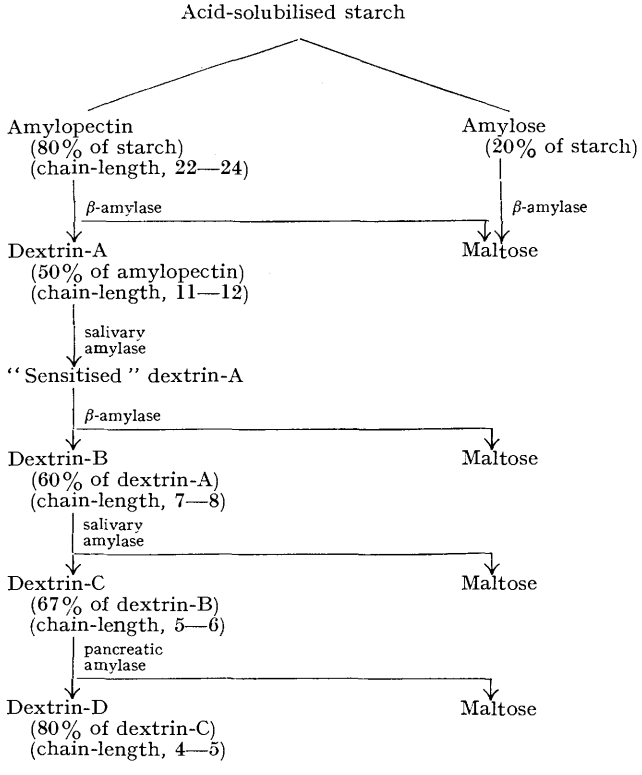
Natural starch is now considered to be a mixture of two components which are different in respect of the length of their unit chains (K. H. Meyer *et al., loc. cit.*; Hassid and McCready, *J. Amer. Chem. Soc.*, **1943, 65,** 1157) and of the degree of aggregation of these chains. The short-chain, highly aggregated component is described as " amylopectin "; the long-chain component as " amylose."

In the experiments described above, Meyer used amylopectin as substrate for the amylases, whereas we used an acid-solubilised whole starch. Inasmuch as amylose is completely hydrolysed to maltose by β-amylase, it is clear that our dextrin-A is derived, like Meyer's dextrin-I, entirely from the amylopectin component, which constitutes, according to the most recent evidence, 80% of the whole. With the readjustment of the weight yield of dextrin-A in accordance with this development, our observations may be summarised as in Table III.

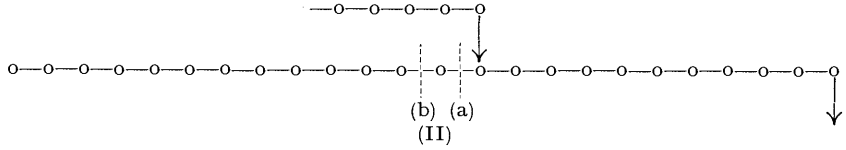
If it be accepted that the impediment to β-amylase action on amylopectin lies in the polymeric linkages between unit chains, it is necessary to assume that such linkages unite the reducing end-group of one chain with, as an average, the middle glucose member of the second chain, for otherwise it is not possible to account for the formation in 50% weight yield of a dextrin with a unit chain of half the length. It must be clearly understood that we are necessarily speaking in terms of averages only. The figures quoted for unit chain-lengths are statistical averages and the actual length of any one unit chain may be widely different from the mean—how widely we have no way of judging at present. A similar reservation attaches to the statement concerning the position of the polymeric bonds in amylopectin.

β-Amylase effects the progressive removal of maltose residues from the non-reducing ends of the unit chains and it is to be expected that this activity will continue so long as there remain two glucose units mutually combined in the configuration of maltose and united by a 1 : 4-α-glucosidic link (at which the actual fission will take place) to the remainder of the chain. The glucose unit involved in the polymeric link clearly cannot

TABLE III.



form part of a free maltose configuration, because it is substituted at C_6 and therefore β-amylase hydrolysis will not pass along the chain beyond the polymeric link. This is illustrated in (II), which shows, diagrammatically, an intermediate chain-unit in amylopectin.

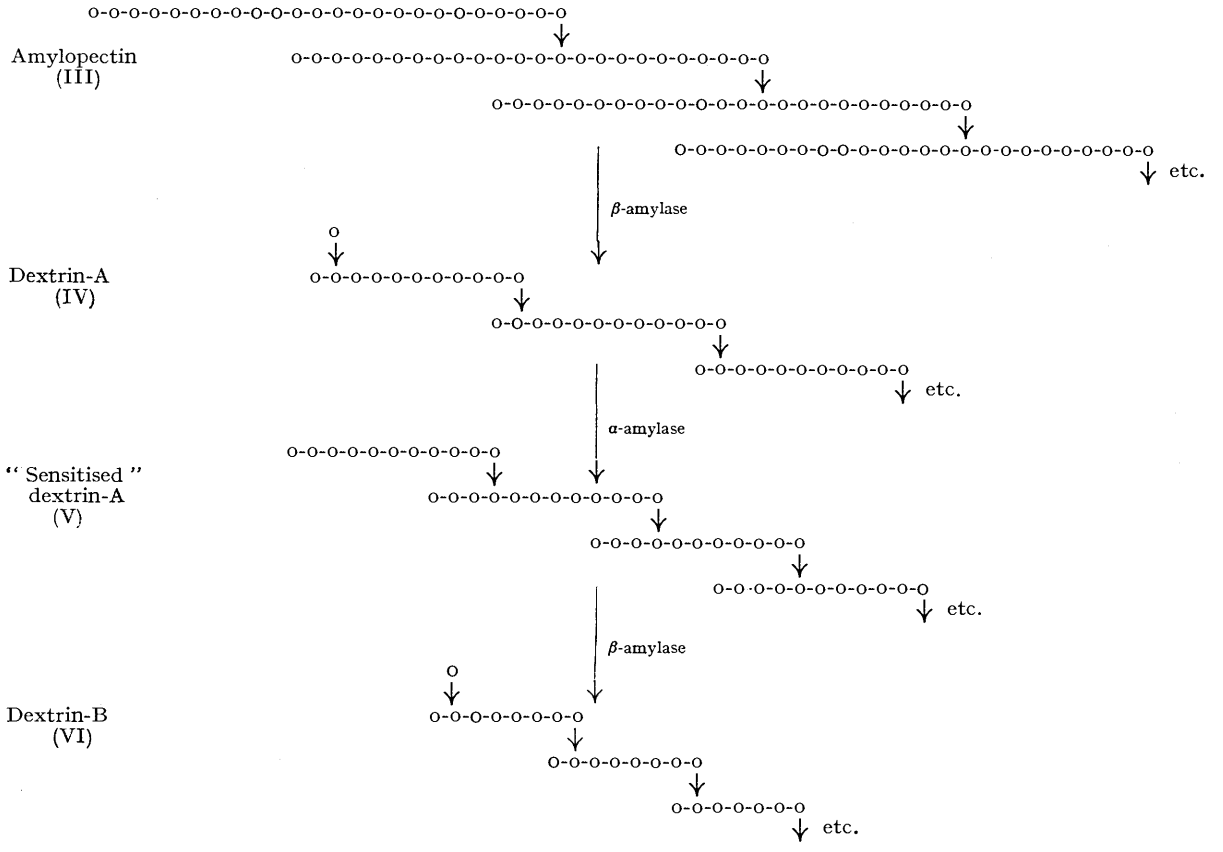


(II)

(Circles represent glucose residues; straight lines, the 1 : 4-glucosidic links; arrow heads, the 1 : 6-polymeric bonds.)

It is furthermore apparent that the final scission must take place at (*b*) and not at (*a*), for otherwise there would be no tetrahydroxy terminal group left in the chain, which is now a chain-unit of dextrin-A, and end-group assay would indicate only one end-group in the whole molecule of the dextrin. As one glucose residue in every 12 is an end-group, the dextrin must, like starch, be constituted of unit chains aggregated by polymeric bonds. This could not be the case if β-amylase carried hydrolysis to the point (*a*).

On this view, the degradation of amylopectin to dextrin-A is shown diagrammatically by the change (III) ⟶ (IV).



This view implies that the polymeric linkages are not disturbed and it is to be expected that dextrin-A will possess a degree of aggregation of the same order as that of the amylopectin from which it is derived. Dextrin-A is in fact highly aggregated, the molecular weight of the methylated dextrin determined by osmotic pressure measurement being 79,000 (Table II). This figure corresponds to 400 glucose residues.

It is in the conversion of dextrin-A into dextrin-B that real difficulties of interpretation arise. It would seem that salivary amylase (and also the α-glucosidase of Meyer, *loc. cit.*) attacks the 1 : 6-α-glucosidic linkages which constitute the " block " to further action of β-amylase on dextrin-A. Whereas α-glucosidase in bringing about the same result is allowed to act on dextrin-A for a prolonged period and causes the liberation of glucose at the same time, the salivary amylase appears to act instantaneously. In fact, its action on dextrin-A forcibly recalls its action in liquefying a starch paste, which also takes place almost instantaneously.

There is some evidence for the view that the liquefaction of starch paste is due to a specific enzyme (the so-called amylophosphatase of Waldschmidt-Leitz and Mayer, *Z. physiol. Chem.*, 1935, **236**, 168), which is distinct from both α- and β-amylase and it may indeed be the constituent of saliva which is responsible for the " sensitisation " of dextrin-A.

It is not, however, essential to the argument to distinguish between the amylolytic, liquefying and " sensitising " activities of saliva. We have referred to the enzyme as an α-amylase, but this has been for convenience only and does not commit us to the view that the same enzyme is responsible for all three functions. We confine ourselves to the assumption that saliva contains an enzyme system which is capable of hydrolysing the polymeric linkages of starch and dextrin-A.

K. H. Meyer (*loc. cit.*) finds an explanation of the step-wise degradation of amylopectin by β-amylase in the assumption, for this component of starch, of a highly ramified structure constituted of chains of different length so inter-linked that a single chain may show as many as four branchings (see *Helv. Chim. Acta*, 1940, **23**, 880, Fig. I). It is not possible to attach a quantitative significance to the structure proposed by Meyer and indeed

it is doubtful if the author intended to express thereby any other than a qualitative interpretation of the facts. It must be emphasised, nevertheless, that the weight yields, taken in conjunction with the chain lengths, of dextrins-A and -B are all important. On no system of multiple branching of the Meyer type does it seem possible to account for the formation of as much as 50% by weight of dextrin-A. Furthermore, in such a formulation, the assumption is implicit that the sensitising enzyme, like β-amylase, makes an end-wise attack on the unit chains and that only those polymeric links are hydrolysed which may be approached by the enzyme directly from a free, non-reducing chain termination.

Another explanation is, however, possible and indeed seems to us to be the more probable, inasmuch as it interprets the quantitative, as well as the qualitative, data and does not postulate a needlessly complex and irregular system of multiple branchings for the structure of amylopectin.

The hypothesis which we propose attempts to reconcile the facts of amylolysis with the simple, laminated structure which we regard as best representing the chemical properties of the amylopectin component of starch (I). The hypothesis is dependent upon two assumptions : (1) the " sensitising " enzyme effects the rupture of *all* polymeric links, irrespective of their positions in the laminated structure; (2) the unit chains so liberated are incapable of independent existence for a measurable time and consequently a laminated structure is re-constituted by the re-establishment of polymeric linkages between the unit chains. The probability is very small that the new 1 : 6-linkages between the chains will occupy the same positions relative to the long axes of the chains as they formerly occupied. Kinetic considerations indicate, moreover, that the net effect of the " sensitisation " of dextrin-A (IV) will be a shift in the mean position of the 1 : 6-glucosidic links nearer to the mid-points of the unit chains of dextrin-A (V). This sensitised dextrin-A presents an appropriate substrate for the further action of β-amylase and dextrin-B (VI) is produced thereby.

The unit chain-length of dextrin-B being 7—8, it follows that the mean positions of the re-established polymeric linkages in (V) involve the 7th or 8th glucose residue, counting from the reducing ends of the chains. In terms of averages only, there is in the sensitisation process a displacement of the polymeric linkages from the 11th to the 7th glucose member, counting from the reducing end.

Although the ratio of the unit chain-lengths of dextrin-A and dextrin-B is less than 2 (see Table II), the molecular weight of dextrin-A is five times that of dextrin-B. Clearly dextrin-B is not so highly aggregated as dextrin-A from which it is derived, an observation which lends support to the view that sensitisation implies initial depolymerisation, followed by a reaggregation of the unit chains, the number of polymeric links so established being fewer than the number present before depolymerisation.

It is not proposed at this stage to discuss the formation of the dextrins-C and -D. These are clearly the products of the action of α-amylase, about which much less is known than about β-amylase. It is proposed to continue the investigation of the mechanism of α-amylase activity when circumstances permit.

Experimental.

Preparation of α-Amylase from Saliva and β-Amylase from Wheat.—After the mouth had been rinsed out with water, early morning saliva (30 c.c.) was collected and diluted with twice its volume of water, and the mucin separated in a centrifuge. The supernatant liquid was used as the source of α-amylase in the following experiments and could be preserved by the addition of a crystal of thymol. The aqueous solution contained ionised phosphorus and gave positive ninhydrin and xanthroproteic tests for protein. The preparation contained no β-amylase (negative Wijsmann test) but occasionally contained maltase. A new preparation was always tested for maltase by incubation with 5% maltose solution and if found present, the preparation was discarded. The β-amylase was extracted from ungerminated wheat by the method described previously (J., 1937, 793) and the concentration of enzyme solution used there was employed in the present work.

The Action of Salivary Amylase, followed by β-Amylase, on α-Amylodextrin (Dextrin-A).—In each of a series of flasks in a thermostat at 38° were placed 200 c.c. of a 2% aqueous solution of α-amylodextrin (prepared from potato starch by the method of Haworth, Hirst, Kitchen, and Peat, *loc. cit.*) and 5 c.c. of α-amylase solution. After intervals of time varying from a few seconds to 30 minutes the solution was raised quickly to boiling point to destroy the enzyme; after cooling to 38°, β-amylase solution (10 c.c.) was added, and each mixture incubated for 20 hrs. The extent of hydrolysis of the α-amylodextrin was in each case determined by estimation of the reducing power (R.P.) in terms of maltose of the solution after incubation. It was found that, whereas β-amylase alone has no action on α-amylodextrin, the susceptibility of the latter to β-amylase action reappears when the salivary amylase is allowed to act on the dextrin for even the shortest possible time (a few seconds).

Advantage was taken of this effect of α-amylase to continue the hydrolysis of starch by β-amylase beyond the α-amylodextrin stage. α-Amylodextrin (60 g.) was dissolved in boiling water (900 c.c.), cooled to 38°, mixed with salivary amylase solution (50 c.c.), and then immediately raised to boiling again. After boiling for 15 mins., the solution was cooled, mixed with β-amylase solution (500 c.c.), and incubated at 38° for 30 hrs. Thereafter a further quantity of the enzyme solution (250 c.c.) was added, and the incubation continued for 6 hrs.

The solution (1700 c.c.) was poured into alcohol (8 l.), and the dextrin so precipitated collected on a filter, hardened by grinding under absolute alcohol, washed with ether, and dried in a vacuum. This product will be referred to as dextrin-B (yield, 35 g.).

The alcoholic filtrate was evaporated under diminished pressure to a thin syrup, which cyrstallised on keeping. The crystalline material proved to be maltose hydrate (21 g.), $[\alpha]_D^{18°}$ +114·8° ⟶ +129·8° (*c*, 1·66). It gave an osazone, m. p. 200° (Found : N, 10·3. Calc. for $C_{24}H_{32}O_9N_4$: N, 10·8%). The hydrolysis of α-amylodextrin by β-amylase after the momentary action of α-amylase thus yields dextrin-B (58·3%) and maltose (38·3%).

Properties of Dextrin-B.—Dextrin-B was a white gritty solid soluble to a slightly opalescent solution in warm water. β-Amylase was without action on it, although further hydrolysis occurred with salivary amylase and malt amylase. The aqueous solution of dextrin-B was quite stable and no evidence of retrogradation was observed. The solution gave a blood-red coloration with iodine. The dextrin had a slight reducing action on Fehling's solution (1 g. ≡ 0·061 g. of maltose); iodine number, 8; $[\alpha]_D^{18°}$ +156·2° in water (*c*, 2·5) and +126·7° in 5% sodium hydroxide solution (*c*, 1·4)

with no mutarotation. The whole of the phosphorus of the original starch was present in dextrin-B (Found : P, 0·58—0·60% as P_2O_5).

The Methylation of Dextrin-B.—Dextrin-B (15 g.), dissolved in 5% sodium hydroxide solution (300 c.c.), was treated at room temperature with methyl sulphate (200 c.c.) and 30% sodium hydroxide solution in ten portions at intervals of ½ hr.; the mixture was neutralised and evaporated to dryness. The partially methylated product extracted from the dry residue by chloroform was submitted to further methylation. After four methylations, the methylated dextrin was insoluble in boiling water. The methylation was repeated eight times in all and two lots of 15 g. were combined, boiled with fresh changes of water, dissolved in chloroform, and the solution dried (anhydrous magnesium sulphate). This was concentrated to a syrup, the latter dissolved in ether, and the filtrate after removal of a small residue treated with light petroleum. The precipitate, methylated dextrin-B, was dried at 60° in a vacuum for 2 days. It was a glassy solid, fairly soluble in cold water, insoluble in hot, which showed $[\alpha]_D^{18°}$ +185·0° in chloroform (*c*, 2·0) and $\eta_{sp.}^{20°}$ 0·03 (*c*, 0·4%) [Found : OMe, 43·1; P(as P_2O_5), 0·40%].

The Chain-length of Dextrin-B.—Dry, ash-free, methylated dextrin-B (21·3 g.) was hydrolysed by being boiled for 6 hrs. with 2% methyl-alcoholic hydrogen chloride (300 c.c.). After neutralisation with silver carbonate and filtration, the solution was evaporated, the residue dissolved in ether and filtered, and the solution again evaporated. The dried mixture of glucosides so obtained was distilled into a Widmer flask until the refractive index of the distillate indicated that only trimethyl methylglucoside was distilling. The contents of the Widmer flask were then separated by distillation at 0·1 mm. pressure into the following fractions :

Fraction.	Weight (g.).	$n_D^{22°}$.	% OMe.	Fraction.	Weight (g.).	$n_D^{22°}$	% OMe.
1	2·298	1·4429	61·6	4	0·458	1·4552	52·8
2	0·914	1·4482	57·5	5	6·720	1·4569	52·3
3	2·465	1·4540	53·4	6 (residue)	1·960	1·4630	48·2

(Fractions 4 and 5 were partially crystalline.)

From these figures it was estimated that fractions 1, 2, 3, and 4 contained respectively 2·3, 0·54, 0·38, and 0·03 g. of tetramethyl methylglucoside, the total yield of which was thus 15·3% of the weight of methylated dextrin. The value becomes 15·8% after the usual correction is applied, corresponding to a chain length of 7—8 units for the dextrin. The identity of the tetramethyl methylglucoside was established by the conversion of fraction 1 into crystalline 2 : 3 : 4 : 6-tetramethyl glucopyranose, m. p. 88° (alone or in admixture with an authentic specimen); $[\alpha]_D^{20°}$ +102·0° ⟶ +80·0° in water (*c*, 0·5). Similarly fraction 5 was converted into 2 : 3 : 6-trimethyl glucopyranose, m. p. 118°; $[\alpha]_D^{20°}$ +98·0° ⟶ +70·2° in water (*c*, 1·0).

The Hydrolysis of Dextrin-B by Means of Salivary Amylase.—Dextrin-B, unlike α-amylodextrin, was not affected by β-amylase after the momentary action on it of α-amylase. It was, however, hydrolysed by the more prolonged action of α-amylase or of malt extract. Dextrin-B (15 g.) in water (250 c.c.) was mixed with saliva solution (50 c.c.) and incubated at 38° for 48 hrs. Thereafter, when the solution was poured into alcohol (2 l.), dextrin-C was precipitated; it was separated in the centrifuge and dried in the manner described for dextrin-B (yield, 10 g.). The alcoholic mother-liquor was concentrated to a thin syrup, which crystallised on keeping. The crystalline material (4 g.) was recognised as maltose hydrate; iodine value, 52; m. p. 108°; $[\alpha]_D^{20°}$ +127·0° (equilibrium value) in water (*c*, 3·0). It formed maltosazone, $[\alpha]_D^{20°}$ +56·4° in methyl alcohol (*c*, 1·2). No glucose could be detected. Thus, by the action of α-amylase on dextrin-B, there are formed dextrin-C (67%) and maltose (27%).

Properties of Dextrin-C.—Dextrin-C was a white gritty solid, soluble in cold water to a clear solution which showed no retrogradation on standing. The dextrin reduced Fehling's solution, had iodine value 13, and showed $[\alpha]_D^{17°}$ +147·6° in water (*c*, 1·2) with no mutarotation. The whole of the phosphorus in the original starch was present in the dextrin [Found : P (as P_2O_5), 0·98%]. The dextrin gave no colour with iodine and was not further hydrolysed by β-amylase, α-amylase (saliva), malt extract, or taka-diastase, but was slowly attacked by pancreatin.

The Methylation of Dextrin-C.—The procedure adopted for the methylation in the cold of dextrin-C was the same as that used for dextrin-B. The product of eight methylations was a brown-white solid, which could not be purified by the usual method, since it was soluble in light petroleum. It contained OMe, 44·5; ash, 0·50; P (as P_2O_5), 0·23% and showed $\eta_{sp.}^{20°}$ 0·073 (*c*, 0·8 in *m*-cresol) and $[\alpha]_D^{17°}$ +182·3° in chloroform (*c* 1·3). The methylated dextrin was soluble in ether, alcohol, light petroleum, acetone, chloroform, and was partly soluble in water.

The Chain-length of Dextrin-C.—Methylated dextrin-C (11·54 g.) was hydrolysed, and the resulting methylglucoside mixture fractionally distilled as previously described for dextrin-B. The following fractions were obtained :

Fraction	1	2	3	4	5	6
Weight (g.)	1·389	0·469	0·5765	0·853	5·054	0·606
$n_D^{21·5°}$	1·4422	1·4440	1·4468	1·4518	1·4582	1·4678
% OMe	61·4	60·5	59·1	55·7	52·2	44·2

(Fraction 5 was crystalline.)

It was estimated that fractions 1, 2, 3, and 4 contained respectively 1·39, 0·41, 0·38, and 0·30 g. of tetramethyl methylglucoside, a total of 2·48 g. from 11·5 g. of methylated dextrin-C. After correction, the yield becomes 22%, corresponding to a chain length of 5—6 units.

Fraction 1, on hydrolysis, gave 2 : 3 : 4 : 6-tetramethyl glucopyranose, m. p. 88°; $[\alpha]_D^{16°}$ +102·2° ⟶ +79·4° in water (*c*, 0·8) (Found : OMe, 52·2. Calc. for $C_{10}H_{20}O_6$: OMe, 52·6%). Fraction 5 was converted into 2 : 3 : 6-trimethyl glucose, m. p. 118°; $[\alpha]_D^{16°}$ +98·0° ⟶ +70·0° in water (*c*, 1·6) (Found : OMe, 41·5. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

The Action of Pancreatin on Dextrin-C.—Commercial pancreatin (5 g.), which contained glucose, was purified by shaking with water (30 c.c.), filtering and pouring the filtrate into alcohol (300 c.c.). The flocculent precipitate formed was collected in a centrifuge, washed with alcohol, and dried in a vacuum.

Dextrin-C (1 g.), dissolved in water (4 c.c.), was incubated for 48 hrs. at 38° with purified pancreatin (30 mg.) and a trace of sodium chloride. The solution, which reduced Fehling's solution, was poured into an excess of alcohol. Dextrin-D separated as a flocculent white precipitate, which was purified by reprecipitation, washed with alcohol, acetone and ether, and dried in a vacuum (yield, 0·8 g.). In the aqueous-alcoholic mother-liquors the only sugar detected was maltose.

It was found that a dextrin with identical properties was prepared in this way by the action of commercial pancreatin. In subsequent preparations the purification of the pancreatin was omitted.

Properties of Dextrin-D.—The dextrin reduced Fehling's solution, had iodine number 14·2, but gave no colour with iodine. It was easily soluble in cold water, in which it showed $[\alpha]_D^{18°}$ +116·0° (*c*, 0·7). In 5% sodium hydroxide solution it had $[\alpha]_D^{18°}$ +110·9° ⟶ +95·3° in 12 hrs. (*c*, 0·6). The solution in alkali became brown, but this coloration disappeared on exposure to air for several hours. No phosphorus was lost during the hydrolysis (Found : P_2O_5, 1·25%).

The Methylation of Dextrin-D.—The dextrin (2 g.) was methylated as before by seven treatments with the methylating reagents. After each treatment, the product was extracted with chloroform. Yield, 1 g. The methylated dextrin (OMe, 46·0%) had $[\alpha]_D^{19°}$ +175·6° in chloroform (*c*, 0·4%); ash, 0·24%; $\eta_{sp.}^{20°}$ 0·080 (*c*, 0·8% in *m*-cresol).

Chain-length of Dextrin-D.—The methylated dextrin (0·90 g.), hydrolysed under the usual conditions with methyl-alcoholic hydrogen chloride, gave a mixture of methylglucosides (1·03 g.), which was separated by distillation at 0·04—0·07 mm. pressure.

Fraction	1	2	3	4	Residue
Weight (g.)	0·235	0·227	0·111	0·056	0·24
$n_D^{18°}$	1·4462	1·4562	1·4578	1·4600	—
OMe %	59·9	54·0	50·8	49·0	40·1

It was estimated that fractions 1 and 2 contained respectively 0·202 and 0·027 g. of tetramethyl methylglucoside. This yield corresponds to a chain-length for dextrin-D of 4·8 units (without correction for loss) or 4·6 units (with correction). Tetramethyl glucose and 2 : 3 : 6-trimethyl glucose were obtained in crystalline form by the hydrolysis of fractions 1 and 3 respectively.

A. E. Hills Laboratories,
The University, Edgbaston, Birmingham, 15.

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