161. *Polysaccharides. Part XXV. α-Amylodextrin.*

By W. N. Haworth, E. L. Hirst, H. Kitchen, and S. Peat.

In an extended study, now proceeding, of the products of the action of amylolytic enzymes on starch occasion has arisen to examine more closely the limit dextrin produced by the action of β-amylase, the description applied by R. Kuhn to the enzyme of ungerminated grain. This dextrin, called variously α-amylodextrin and erythrogranulose, is formed, together with maltose, when the action of β-amylase on starch is allowed to proceed to

completion. The experiments recorded here support the observations of Hanes (*Canadian J. Res.*, 1935, **13**, 185), who found that α-amylodextrin is formed in 40% yield (calculated on the weight of starch), that the dextrin is homogeneous, is not degraded by β-amylase, and that the only other product of the amylolysis is maltose. Haworth, Hirst, and Waine (J., 1935, 1299), in an examination of α-amylodextrin, also record the striking fact that there is a complete cessation of the action of β-amylase on starch when the limit represented by 60% hydrolysis (calculated in terms of maltose produced) is reached. A gravimetric assay of methylated α-amylodextrin by the latter authors indicated a chain length of 16—17 glucose units for the dextrin. It was recognised that a discrepancy existed here in that a dextrin of this chain length could not be produced in 40% yield from a starch with a chain length of 24—30 glucose units if every chain had been uniformly degraded and it seemed possible that the discrepancy lay in the estimated chain length of the α-amylodextrin. A value of 11—12 units for the dextrin would more nearly represent the weight relationship between starch and the dextrin.

In view of the fact that a better yield of β-amylase is obtainable from wheat than from barley (von Klinkenberg, *Z. physiol. Chem.*, 1932, **209**, 253) the former source of β-amylase was used in this investigation. The enzyme was extracted by means of aqueous alcohol and was used in the form of an unbuffered aqueous solution. Although this solution (containing a crystal of thymol as preservative) lost very little of its amylolytic power on keeping over a period, a fresh preparation was usually made for each series of experiments. A commercial soluble starch was employed as substrate, investigation having shown that the cold aqueous acid treatment used in its preparation does not appreciably degrade the 24—30 unit glucose chain. Further slight variations in the experimental procedure of Haworth, Hirst, and Waine were introduced. The starch enzyme mixture was incubated at 38° instead of at 55°, no buffer was employed, and the adjustment of acidity to p_H 6 was not made. The small amount of mineral acid contained in the soluble starch was sufficient to maintain the hydrolysis mixture slightly on the acid side of neutrality. The course of the hydrolysis was followed by estimation of the increase in reducing power of the solution (by a copper reduction method). At completion, the copper number corresponded to the liberation of 62—63% of maltose. From the hydrolysis mixture were isolated α-amylodextrin and crystalline maltose and no other product, the yields by weight being respectively 40% and 50%.

The α-amylodextrin so prepared is identical with that prepared by Haworth, Hirst, and Waine in respect of solubility, colour with iodine, iodine number, phosphorus content and in resistance to the further action of β-amylase. Nevertheless a difference is observed in the optical activity of the two dextrins: the dextrin now described has $[\alpha]_D^{20°}$ + 167·0° in water (+ 133·0° in aqueous sodium hydroxide), whereas that prepared by Haworth, Hirst, and Waine shows $[\alpha]_D^{20°}$ + 200° in water (+ 144° in aqueous sodium hydroxide). Moreover, this difference persists in the methylated dextrins. Haworth, Hirst, and Waine record for methylated α-amylodextrin, $[\alpha]_{5780}^{19°}$ + 222°, whereas here the value found is $[\alpha]_D^{25°}$ + 197·2° (maximum). Further investigation may reveal the cause of this difference.

The gravimetric assay of the end group has been made on three specimens of methylated α-amylodextrin, each prepared by a different method. The first was obtained by the simultaneous deacetylation and methylation of a triacetate which had been prepared by the action of pyridine and acetic anhydride on α-amylodextrin. Acetylation of the dextrin in the presence of chlorine and sulphur dioxide yielded an acetate which differed from the first only in that it gave more viscous solutions in chloroform. Deacetylation and methylation of this acetate proceeded smoothly and gave methylated α-amylodextrin. The third specimen was prepared by the direct treatment of α-amylodextrin with the methylating agents, the intermediate formation of an acetate being omitted. The methylations were all carried out at room temperature and the three specimens of methylated α-amylodextrin were closely similar in properties. Each was separately hydrolysed by boiling with methyl-alcoholic hydrogen chloride and the proportions of tetramethyl methylglucoside so formed were estimated as 9·8, 10·5, and 10·4% respectively of the weight of methyl derivative. The chain length of the methylated dextrin is thus, in each case, 11—12 glucose units. Obviously the α-amylodextrin suffers no degradation under the action of the acetylating agents, since direct methylation in alkaline solution, where no hydrolysis of glucosidic

links can occur, yields a methylated dextrin of the same chain length as those prepared from the acetates. It is reasonable to conclude, therefore, that the α-amylodextrin from which these derivatives are prepared is also composed of a chain of 11—12 glucopyranose units. We are of the opinion that the specimen forming the subject of the present work was the genuine α-amylodextrin and that the material described by Haworth, Hirst, and Waine (*loc. cit.*) may not have been identical with it. The viscosity data indicate that the molecules of α-amylodextrin are aggregated in the manner observed with starch and other polysaccharides (compare Haworth, Hirst, and Isherwood, this vol., p. 577) and discussion of the mode and degree of aggregation will be the subject of future communications.

Experimental.

Preparation of β-Amylase.—The procedure of Hanes (*Biochem. J.*, 1936, **30**, 168) was adopted for the extraction of β-amylase from ungerminated wheat. The wheat, ground to a fine flour, was twice extracted by stirring with 50% aqueous alcohol, and the enzyme precipitated from the clear extract by increasing the alcohol concentration to 80%. The precipitate, separated on the centrifuge and freed from alcohol by suction, was dissolved in water (450 c.c. for the extract from 200 g. of flour) and filtered. The clear solution, to which a crystal of thymol was added as a preservative, was utilised as the source of β-amylase in the following experiments. The solution gave a negative Wijsman test (*Rec. trav. chim.*, 1890, **9**, 1) for α-amylase.

Preparation of α-Amylodextrin.—The starch was a commercial soluble starch which had undergone no degradation in the process of solubilisation. The starch gave a blue colour with iodine. The iodine number was 1·3; phosphorus (as P_2O_5), 0·22%; $[\alpha]_D^{21°}$ + 148·0° in 5% aqueous sodium hydroxide (*c*, 1·5, dried sample); moisture content, 12% and ash, 0·35%. The soluble starch contained a small amount of mineral acid equivalent to 20·0 c.c. of 0·1*N*-sodium hydroxide per 100 g.

The following is a typical example of the method used: Soluble starch (50 g.) was stirred into cold water (100 c.c.) and the thin cream so produced was poured into boiling water (400 c.c.). The resulting gel was cooled and mixed with the standard solution of β-amylase (200 c.c.), and the mixture incubated at 38°. The starch paste rapidly liquefied and after 30 minutes a very small amount of a flocculent precipitate separated. This precipitate appeared to be of protein nature and was discarded. The course of the hydrolysis was followed by a copper-reducing method (*J. Soc. Chem. Ind.*, 1925, **44**, 150T). After 24 hours no further increase occurred in the reducing powder of the solution, which corresponded to a conversion of 62—63% of the starch into maltose. Alcohol (2 l.) was added to the solution and the grey sticky precipitate was hardened by refluxing with absolute alcohol. The crude dextrin so obtained was purified by solution in water (400 c.c.), precipitation by addition of alcohol (2 l.), filtration, and washing with alcohol and ether. Finally it was dried in a vacuum. The α-amylodextrin was a white non-reducing powder which was soluble in cold water. The solution was turbid but became clear on boiling. No gel formation was observed. The α-amylodextrin gave a purple coloration with iodine and showed iodine number 2·7; P_2O_5, 0·40%; $[\alpha]_D^{20°}$ + 167·0° in water (*c*, 2·0) and $[\alpha]_D^{24°}$ + 133·0° in 5% aqueous sodium hydroxide (*c*, 1·4). Numerous experiments demonstrated that β-amylase was without action on this dextrin.

The combined filtrates from the precipitation and purification of the α-amylodextrin were concentrated under diminished pressure to a thick syrup, which was repeatedly extracted with boiling methyl alcohol. A small insoluble residue was added to the dextrin fraction. The methyl-alcoholic extract was concentrated to a syrup, which crystallised on standing for 12 hours in contact with one-tenth of its volume of water. The crystalline product showed $[\alpha]_D^{20°}$ + 11·7° ⟶ + 127·4° in water (*c*, 0·6); m. p. 110° (decomp.), and iodine number 55·3 (calc. for maltose hydrate, 55·5) (Found: C, 40·5; H, 7·0. Calc. for maltose hydrate: C, 40·0; H, 6·7%). It formed an osazone, m. p. 200° (decomp.); $[\alpha]_D^{20°}$ + 58·0° in methyl alcohol (*c*, 1·4), which was microscopically identical with maltosazone (Found: N, 10·3. Calc. for $C_{24}H_{32}O_9N_4$: N, 10·8%). The yields, by weight, of α-amylodextrin and crystalline maltose were respectively 40% and 50% of the original starch (corrected for moisture content).

Acetylation of α-Amylodextrin.—(a) *By pyridine and acetic anhydride.* The air-dried dextrin (50 g.) was shaken for 12 hours with pyridine (200 g.) and a cold mixture of pyridine (200 g.) and acetic anhydride (300 g.) was then added with stirring. In 3 hours at room temperature complete solution of the dextrin was attained and the acetate was precipitated by stirring the solution slowly into cold water (10 vols.). The white granular precipitate was collected on cloth, washed with hot water until acid-free, and dried in the usual way. The acetate was readily and

completely soluble in acetone, chloroform, *m*-cresol and anisole, was non-reducing, and showed $[\alpha]_D^{20°}$ + 155·0° in chloroform (*c*, 0·9); iodine number, 1·4; P_2O_5, 0·20% (Found: CH_3·CO, 39·0. Calc. for $C_{12}H_{16}O_8$: CH_3·CO, 44·8%). The specific viscosity was 0·190 (*c*, 0·8) in *m*-cresol, corresponding to an apparent molecular weight of 6840 ($K_m = 1 \times 10^{-3}$) or 24 glucose units.

(b) *By acetic anhydride in the presence of sulphur dioxide and chlorine.* α-Amylodextrin (70 g.), 'activated' by precipitation from an aqueous solution by alcohol, was suspended in glacial acetic acid (450 g.) containing a little chlorine. After ½ hour, acetic anhydride (750 g.) containing an amount of sulphur dioxide equivalent to the chlorine was added with stirring. The mixture was maintained at 60° for 5 hours and was then poured into cold water (10 vols.). The precipitated acetate was separated, washed, and dried in the usual way. Yield, 20 g. The acetate was non-reducing and gave no coloration with iodine solution; $[\alpha]_D^{20°}$ + 162·0° in chloroform (*c*, 5·0); iodine number, 1·6; $\eta_{sp.}^{20°}$ 0·180 (*c*, 0·4), corresponding to an apparent molecular weight of 13,000 or 45 glucose units (Found: CH_3·CO, 44·0. Calc. for $C_{12}H_{16}O_8$: CH_3·CO, 44·8%).

Methylated α-Amylodextrin.—(a) *Methylation of the acetate prepared by the pyridine method.* The α-amylodextrin acetate (50 g.) was dissolved in acetone (700 c.c.) and treated in the cold with methyl sulphate (300 c.c.) and 30% aqueous sodium hydroxide (800 c.c.) over a period of 5 hours. The mixture was then neutralised and boiled, the partly methylated dextrin being precipitated. This methylation process was repeated nine times, and the product finally treated once with methyl iodide and silver oxide. The following properties were recorded:

No. of methylations:	1.	2.	4.	6.	8.	9.	10.
$[\alpha]_D^{25°}$ in $CHCl_3$	+160°	+159·2°	+195·0°	+180·0°	+197·2°	+184·5°	+190·8°
OMe, %	24·2	32·8	39·6	42·5	43·7	44·0	44·1
$\eta_{sp.}^{20°}$ in *m*-cresol (*c*, 0·4) ...	—	0·130	0·126	0·096	0·086	0·097	0·113
M, from viscosity ($K_m = 10^{-3}$)	—	6500	6300	4900	4400	4800	5700

The final product was purified by fractionation in a chloroform–light petroleum mixture. A small first fraction was discarded; the main fraction was dried in a vacuum and used in the experiments to be described, where it is referred to as methyl dextrin A.

(b) *Methylation of the acetate prepared by using chlorine and sulphur dioxide as catalysts.* The acetate (20 g.) was dissolved in acetone (600 c.c.) and treated with the methylating reagents (see above) at room temperature. After eight methylations the product—methyl dextrin B—showed (after purification by precipitation, chloroform–light petroleum) OMe, 44·8%; $[\alpha]_D^{17°}$ + 194·9° in chloroform (*c*, 1·3); $\eta_{sp.}^{20°}$ 0·153 in *m*-cresol (*c*, 0·4), corresponding to *M*, 7800. Yield, 20 g.

(c) *Methylation of α-amylodextrin directly.* α-Amylodextrin (35 g.) was dissolved in dilute aqueous sodium hydroxide and treated in the cold with methyl sulphate (300 c.c.) and 30% aqueous sodium hydroxide (800 c.c.) in the manner described above. After eight methylations, the product had OMe, 45·0%; $[\alpha]_D^{18°}$ + 194·3° in chloroform (*c*, 2·0) and $\eta_{sp.}^{20°}$ 0·195, corresponding to an apparent molecular weight of 10,000. Yield, 35 g. The product was fractionated by solution in chloroform and precipitation with light petroleum. The main fraction—methyl dextrin C—showed OMe, 44·4%; $[\alpha]_D^{18°}$ + 194·0° in chloroform (*c*, 1·5) and $\eta_{sp.}^{20°}$ 0·200 in *m*-cresol (*c*, 0·4), corresponding to *M*, 10,200.

Determination of the Chain Length of α-Amylodextrin by the End-group Method.—(a) *Hydrolysis of methyl dextrin A.* The dry methylated α-amylodextrin (10·0 g.) was heated, under reflux, with methyl alcohol (150 c.c.) containing dry hydrogen chloride (2% by weight) until the optical rotation became constant (4½ hours). The clear solution was neutralised with dry silver carbonate, filtered, and the solvent removed, under diminished pressure, from the filtrate. The residual syrup was purified by solution in ether, filtration, and removal of the solvent. Yield, 10·3 g. of mixed glucosides (90% of the theoretical). The syrup was now fractionated by slow distillation at 0·05 mm. pressure from a Widmer flask bearing a short vacuum-jacketed fractionating column. The properties of the fractions were as follows:

Fraction:	1.	2.	3.	4.	5.	6.
Weight, g.	0·81	0·40	0·78	1·41	2·96	1·92
$n_D^{20°}$	1·4453	1·4510	1·4559	1·4580	1·4584	1·4612
OMe, %	60·0	56·2	53·0	52·0	51·1	49·2

The methoxyl content justifies the assumption that fraction 4 consists entirely of trimethyl methylglucoside (Calc.: OMe, 52·6%) and that fraction 1 is not entirely tetramethyl methyl-

glucoside (OMe, 62·0%). The refractive indices of the fractions indicate that the content of tetramethyl methylglucoside ($n_D^{20°}$ 1·4430) in fractions 1, 2, and 3 are respectively 84·7, 46·6, and 1·4%. The total weight of tetramethyl methylglucoside (including a 10% correction for losses; see Haworth and Machemer, J., 1932, 2270) is 0·977 g., consistent with a chain length of 12 glucose units for methyl dextrin A.

(b) *Hydrolysis of methyl dextrin B.* The procedure adopted in the hydrolysis of methyl dextrin B (13·7 g.) was the same as that described under (a). The greater part (11·3 g.) of the mixed glucosides (14·0 g.) was distilled into a Widmer flask and this part (containing the whole of the tetramethyl methylglucoside) was carefully fractionated by slow distillation at 0·10 mm. pressure, as follows :

Fraction :	1.	2.	3.	4.	5.
Weight, g.	0·68	0·33	0·56	1·01	8·69
$n_D^{19°}$	1·4430	1·4462	1·4511	1·4553	1·4572
OMe, %	61·8	59·3	55·8	53·0	52·4

The methoxyl contents indicate that fractions 1 and 5 consist respectively of pure tetramethyl methylglucoside and pure trimethyl methylglucoside and from the refractive indices it was estimated that fractions 2, 3, and 4 contained respectively 77·5, 43·0, and 13·4% of tetramethyl methylglucoside. The total yield of the latter glucoside (with the 10% correction) was 1·440 g., *i.e.*, 10·5% by weight of the original methyl dextrin, the chain length of which is thus shown to be 11—12 glucose units.

(c) *Hydrolysis of methyl dextrin C.* The dried dextrin (20·0 g.), submitted to the action of methyl-alcoholic hydrogen chloride under the conditions described above, yielded 20·2 g. of the mixed glucosides, which, on distillation from a Widmer flask at 0·01 mm. pressure, were separated into the following fractions :

Fraction :	1.	2.	3.	4.	5.
Weight, g.	1·30	0·40	0·46	1·6	7·8
$n_D^{20°}$	1·4428	1·4462	1·4494	1·4554	1·4560
OMe, %	61·6	59·7	57·3	53·0	52·3

Fraction 1 consists solely of tetramethyl methylglucoside, fraction 5 of pure trimethyl methylglucoside, and fractions 2, 3, and 4 contain respectively 74·3, 50·0, and 4·6% of tetramethyl methylglucoside, the yield of which (with the 10% correction) is 10·45% of the weight of methyl dextrin. This value corresponds to a chain length of 11—12 units. In all three experiments the identity of the products was established by acid hydrolysis of the appropriate fractions and isolation of crystalline 2 : 3 : 6-trimethyl glucopyranose and crystalline 2 : 3 : 4 : 6-tetramethyl glucopyranose.

UNIVERSITY OF BIRMINGHAM, EDGBASTON. [*Received, March 24th*, 1937.]