I.—STUDIES ON DEXTRINS. PART I.

Action of amylase from Cholam (*Sorghum vulgare*) on potato starch.

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The dextrins form an important group of carbohydrates occurring under various conditions and invariably formed as intermediate products during enzyme-hydrolysis of starch; they possess considerable interest from the academic and the nutritional point of view, but our knowledge of them is comparatively obscure. The only criteria at present available for establishing their identity and purity are reducing power, specific rotation and behaviour on enzyme-hydrolysis. It is therefore not possible to state from our present knowledge whether any of the dextrins so far prepared is a single substance or a mixture.

Musculus (*Compt. rend.*, 1860, **50**, 785) was the first to show that sugar and dextrins were formed simultaneously during the hydrolysis of starch. O'Sullivan (*J.C.S.*, 1872–76), in his systematic studies on the breakdown of starch by diastase showed conclusively that maltose was the sugar formed in the hydrolysis and was always accompanied by dextrins. He stated that the formation of dextrin increased with rise of temperature, more dextrin than maltose being formed between 60° and 70°.

Brown and Heron (*J.C.S.*, 1879, **35**, 596) stated that diastatic hydrolysis of starch takes place by stages, one molecule of maltose being set free at each step accompanied by a lower dextrin. Brown and Morris (*J.C.S.*, 1889, **55**, 449) isolated an amylodextrin by the action of dilute mineral acids on ungelatinised starch in the cold, and showed that it differed from soluble starch. Lintner (*Ber.*, 1893, **26**, 2533) observed that by the action of diastase on starch three dextrins were formed, besides maltose and isomaltose. He named them amylodextrin, $(C_{12}H_{20}O_{10})_{54}$, erythrodextrin, $(C_{12}H_{20}O_{10})_{17}\cdot(C_{12}H_{22}O_{11})$ and achroodextrin, $(C_{12}H_{20}O_{10})_5\cdot(C_{12}H_{22}O_{11})$ respectively. Amylodextrin did not reduce Fehling solution, but gave a blue colour with iodine: erythrodextrin reduced Fehling solution and gave a brown colour with iodine, while achroodextrin reduced Fehling solution, but gave no colour with iodine. Ling and Baker (*Proc. C. S.*, 1896–1897, 3) separated two dextrins, maltodextrin-α and maltodextrin-β from the reaction mixture of starch and diastase at 70°. Brown and Millar (*J.C.S.*, 1899, **75**, 315) obtained a 20 per cent. yield of stable dextrin and 80 per cent. of maltose by the action of diastase on starch at 60°.

The dextrin had a reducing power (R), 5·5–5·9 (maltose, 100) and specific rotation, $[\alpha]_D$ 195–196°. When hydrolysed by diastase this dextrin yielded equal quantities of maltose and glucose, the hydrolysis being very slow. Zulkowski and Franz (*J.C.S.*, *Abstr.*, 1896, **i**, 120) showed that dextrins were also formed by heating starch with glycerol at temperatures between 190° and 210°. The action of *Bacillus macerans* on starch (Schardinger, *J.C.S.*, *Abstr.*, 1911, **i**, 181, from *Centr. Bakt.*, 1911, **29**, ii, 188) produced bodies resembling dextrins; some were crystalline and others amorphous, but all were unfermentable by yeast.

Latterly Gatin-Gruzewska (*Compt. rend.*, 1908, **146**, 540, and *ibid*, 1911, **152**, 785) claimed to have separated amylopectin and amylose from the granules of starch. Amylose was soluble in cold water, while amylopectin only gelatinised in hot water. Pringsheim and Eissler (*Ber.*, 1913, **46**, 2959) isolated two kinds of dextrin by the action of Schardinger's bacillus on starch, naming them dextrin-α or tetra-amylose and dextrin-β or hexa-amylose; underlying this nomenclature was the idea that polyamyloses formed the structural basis of the starch molecule. They subjected the polyamyloses to the action of malt diastase, taka-diastase, emulsin, *Penicillium africanum* and yeast. Pringsheim and his collaborators devoted much attention in later years to the chemical constitution of polyamyloses, mention of which will be made in a later paper dealing with that particular aspect.

Ling and Nanji (*J.C.S.*, 1923, **123**, 2666) determined the ratio of amylose to amylopectin in the molecule of starch and found it to be 1 : 2. They found that when alcohol-dried amylase from ungerminated barley was allowed to act on starch-paste at 50°, amylose was converted quantitatively to maltose, while amylopectin was left entirely unaffected. Undried barley amylase, on the other hand, was observed to attack amylopectin and give αβ-hexa-amylose. Ling and Nanji (*J.C.S.*, 1925, **127**, 629) also studied the stable dextrin isolated by Brown and Morris in 1885. The dextrin had the following constants:—R, 14; $[\alpha]_D$ 185°·0 and molecular weight, 1923; it could be hydrolysed by malt diastase, emulsin and maltase to give mixtures of glucose, maltose and isomaltose.

Baker and Hulton (*J.C.S.*, 1929, 1655) observed that precipitated amylase from ungerminated oats hydrolysed potato starch to maltose only, but that when the reaction was stopped before completion, α-amylodextrin (R, 1·5 and $[\alpha]_D$ 184°·1) identical with that obtained by the action of amylase from ungerminated rye on potato starch (Baker and Hulton, *J.C.S.*, 1921, **119**, 805) was the product. Amylase from germinated oats gave a dextrin with R, 9·3 and $[\alpha]_D$ 185°·9,

while germinated rye amylase gave a dextrin with the constants R, 10·8 and $[\alpha]_D$ 181°·9. These results of Baker and Hulton do not fit in with the theory first brought forward by Maquenne and Roux (*Compt. rend.*, 1905, **140**, 1303) and later, elaborated by Ling and Nanji (*loc. cit.*), namely, that starch is not a homogeneous substance but is built up of two substances, polymerised amylose and amylopectin.

The work in this laboratory supports the views of Baker and Hulton. During the hydrolysis of potato starch by amylase from cholam (*Sorghum vulgare*) four different dextrins were isolated. To avoid confusion of nomenclature they have been tentatively named Dextrins–I, II, III and IV. Dextrin–I was isolated from the reaction mixture containing cholam malt amylase and potato starch when it just failed to give a purple colour with iodine. Dextrin–II represents the stable dextrin of Brown and Morris. It was obtained when the reaction between cholam malt amylase and potato starch had reached an equilibrium, which was after about 80 per cent. starch had been converted into maltose. Alcohol-dried amylase from ungerminated cholam saccharified starch to the extent of 30 per cent. in sixteen hours; nearly 66 per cent. of non-saccharine solids could be precipitated from the reaction mixture and were separated into two fractions, Dextrin–III and Dextrin–IV. The former was non-reducing and the latter only feebly reducing.

The crude dextrins were purified by redissolving and reprecipitating from water several times, using 95 per cent. alcohol as the precipitant, till reducing power and specific rotation were found to remain unchanged by subsequent treatment. They were then subjected to prolonged extraction with dry methyl alcohol and later, with 80 to 95 per cent. ethyl alcohol to ensure complete removal of sugar. The dextrins as finally obtained were white amorphous powders soluble in water. Dextrins–I and II dissolved in cold water giving clear solutions, while the other two dissolved only on boiling. The constants for the pure dry substances were as follows :—

Dextrin	I	II	III	IV
Reducing power (glucose 100) ...	3·0	5·0	0	less than 1
Specific rotation, degrees ...	174·0	155·9	170·6	184·2

The constants for Dextrin–IV suggested that it was probably identical with α-amylodextrin of Baker and Hulton.

The dextrins were submitted to hydrolysis by amylases from malted and ungerminated cholam. Dextrin–II was resistant to the action of malted cholam amylase at P_H 7·0, while at P_H 4·67 it was hydrolysed to the extent of about 20 per cent. The other dextrins

were rapidly hydrolysed by both the enzymes at the optimal temperature and hydrogen-ion concentration (P_H 4·67). The enzyme from ungerminated cholam attacked all four dextrins more effectively than that from malted cholam and carried the hydrolysis much further. In no case, however, did hydrolysis proceed to completion; under the most favourable circumstances the dextrins were hydrolysed as follows :—

Dextrin	I	II	III	IV
Hydrolysis per cent.	61·6	35 8	66·3	74·8

EXPERIMENTAL.

Preparation and hydrolysis of Dextrin–I.

Malted cholam powder (10 gms.) was extracted with cold water for two hours with frequent shaking, the filtered extract being diluted to 100 c.c. Potato starch (100 gms.) was made into paste with three litres of boiling water and after boiling for a few minutes cooled to 50°, at which temperature the paste and the enzyme were mixed. The reaction was allowed to proceed at the same temperature and stopped by boiling the mixture after it ceased to give a purple colour with iodine. The mixture was then concentrated on the water bath and the dextrin precipitated with 2½–3 volumes of 95 per cent. alcohol. The precipitate was purified in the manner described already.

Hydrolysis of Dextrin–I at 50°.—Flasks containing 50 c.c. of dextrin solution (2 per cent.), 20·85 c.c. of malted cholam enzyme (0·24 per cent.) with 19·15 c.c. of buffer (McIlvaine's and 1 c.c. of toluene were kept in a thermostat. Sugar was determined at intervals.

TABLE I.

Time in hours	Mgms. maltose	
	P_H 4·67	P_H 7·05
1	23·8	...
2	31·2	17·3
4	40·0	...
24	59·6	32·7
48	65·0	37·5

Preparation and hydrolysis of Dextrin–II.

Starch paste and enzyme solution were prepared as before; the reaction proceeded at 45° with 5 c.c. of toluene for 54 hours. The dextrin was obtained and purified as stated above.

Hydrolysis of Dextrin–II at 37° and P_H 4·67.—Four flasks were arranged, each containing 25 c.c. dextrin solution (2 per cent.) and 15 c.c. of buffer solution with (1) 10 c.c. of malted cholam enzyme (0·2 per cent.), (2) 10 c.c. of ungerminated cholam enzyme (0·2 per cent.), (3) 10 c.c. of mixture of equal volumes of malted and ungerminated cholam enzymes and (4) 10 c.c. of water. The dextrin being very resistant to hydrolysis sugar was determined only after 48 and 90 hours. At the end of the latter period, the percentages of hydrolysis in the different cases were as follows :—(1), 20·8 ; (2), 35·8 ; (3), 29·8 ; (4), 0.

Preparation and hydrolysis of Dextrins–III and IV.

Alcohol-dried amylase (0·5 gm.) was shaken with cold water for half an hour and made up to 100 c.c. after filtration ; 100 gms. of potato starch were made into a paste with three litres of water and the paste cooled to 50°. The solutions were mixed and the reaction allowed to proceed at 50° after adding 5 c.c. of toluene. In sixteen hours the paste was observed to be completely liquefied and the coloration with iodine was purple. The mixture was raised to the boiling-point and concentrated on the water-bath to 800 c.c. when 1300 c.c. of 95 per cent. alcohol was added, the precipitate being allowed to settle for 24 hours. The precipitate was observed to be in two layers, a stiff, horny mass below surmounted by a white, fluffy powder; these were separated and dehydrated. On further treatment and purification they were found to be two different dextrins, the horny mass on complete dehydration giving a white powder (Dextrin–III). The yields of crude substances were 35 and 33 gms. respectively ; there was not much loss during the purification.

Dextrins–III and IV were only slightly soluble in cold water, but dissolved on boiling, and remained in solution on cooling.

Hydrolysis of Dextrin–III.—Three flasks each containing 50 c.c. of dextrin solution (2 per cent.) and 25 c.c. of buffer solution together with, in flasks 1 and 2, 25 c.c. malted cholam enzyme, and in the third flask 25 c.c. of ungerminated cholam enzyme, were kept in a thermostat at 30°, 0·5 c.c. toluene being added to each flask. Sugar was determined at intervals.

TABLE II.

Time in hours	Mgms. of maltose		
	Cholam Malt		Ungerminated cholam
P_H	7·05	4·67	4·67
24	21·1	31·1	46·6
48	27·6	39·4	56·5
72	31·2	44·5	60·6
92	...	..	70·3

Hydrolysis of Dextrin-IV.—Reaction mixtures were prepared as described in the previous trial except that only two flasks were arranged for malted and ungerminated enzymes at P_H 4·67. The reaction was conducted in a thermostat at 30°.

TABLE III.

Time in hours	Mgms. of maltose	
	Cholam malt	Ungerminated cholam
5	25·6	30·1
22	36·5	55·9
92	54·4	79·0

The composition of these dextrins is unknown, but attempts are being made to determine their nature.

SUMMARY.

1. Four dextrins were isolated from the products of hydrolysis of potato starch, and their reducing power and specific rotation determined; one dextrin appears to be identical with the α-amylodextrin of Baker and Hulton.

2. The dextrins were hydrolysed by the enzymes from malted and ungerminated cholam. The latter attacked the dextrins more readily and carried the hydrolysis farther than the former.

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