203. *Polysaccharides. Part XXXI. Constitution of Wheat Starch and Horse-chestnut Starch.*

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The starch molecule is considered to contain a repeating unit composed of α-glucopyranose residues linked through positions 1 and 4. The chain length of this repeating unit can be measured by estimation of the proportion of tetramethyl glucose obtained on hydrolysis of the methylated derivative of the starch. It is known that in the instances of potato, maize, waxy maize and canna starches, the chain length of the repeating unit is 24—30 glucose residues. The present work shows that wheat and horse-chestnut starches contain a repeating unit of similar chain length.

THE methyl derivatives of the amylose and amylopectin fractions of potato starch gave on hydrolysis 4·5—5% of tetramethyl glucopyranose (Hirst, Plant, and Wilkinson, J., 1932, 2375) and a similar proportion of tetramethyl glucose was obtained from the methyl derivative of the soluble potato starch examined by Baird, Haworth, and Hirst (J., 1935, 1201). These methylated derivatives differed markedly in particle size and in the viscosity of their solutions, yet each of them gave on hydrolysis the same yield of end-group represented by tetramethyl glucose. These observations were interpreted as indicating the presence of a repeating unit in potato starch composed of some 24—30 glucopyranose residues linked together through their 1 : 4-positions. The differences in particle size, viscosity, and other properties were ascribed to the different states of aggregation of these repeating units, many such units of 24—30 glucose residues each being combined to form the highly viscous and difficultly soluble methylated amylopectin (Hirst, Plant, and Wilkinson, *loc. cit.*). At that time evidence was lacking concerning the exact nature of the linkage between the repeating units, but in various papers (*e.g.*, Haworth, Hirst, and Oliver, J., 1934, 1917; Haworth, *Monatsh.*, 1936, **69**, 920) possible modes of aggregation, ranging from micellar structures to union by primary valency, were described, but at no time has the view been held by us that highly viscous solutions of methylated starch contain particles of molecular weight corresponding to 24—30 glucose residues. Recent work has given more detailed information concerning the nature of the linkages which join together the repeating units (Hirst, *Chem. and Ind.*, 1938, **57**, 1130) and the evidence now available indicates that these bonds are much stronger than was formerly considered to be the case and that they are primary valencies of the glucosidic type.

The work mentioned above was concerned mainly with potato starch and in view of the great differences known to exist between starches of varied origin, it is necessary to enquire whether repeating units containing a terminal group are present in other starches also. In the case of waxy maize starch, the presence of a repeating unit similar to that in potato starch has been substantiated (Haworth, Hirst, and Woolgar, J., 1935, 177) and the same repeating unit of 24—30 glucose residues occurs in maize starch (Averill, Haworth, and Hirst, forthcoming publication). More recently, the existence of the same unit in the widely different canna starch was proved by the work of Hassid and Dore (*J. Amer. Chem. Soc.*, 1937, **59**, 1503). Other varieties of starch are under examination and we now present the results of investigations concerned with starches from the wheat grain and from the cotyledon of the horse-chestnut. As shown in detail in the experimental section, it has been found that methylated wheat starch gives 4·2% of tetramethyl glucose on hydrolysis, and methylated horse-chestnut starch 3·8%, corresponding respectively to repeating units of chain length 24 and 28. Whether or not the difference between these figures is significant cannot at present be stated, but it is clear that both starches fall exactly into line with all the others so far examined in possessing repeating units of *ca.* 24—30 glucopyranose residues.

Special precautions were taken during the preparation of the methylated derivatives to ensure that the methylation was carried out under mild conditions leading to the isolation of highly viscous methyl derivatives, and in the absence of oxygen (which, however, does not appear to have any such effect on the course of the methylation of starch as it does in the case of cellulose). One of the specimens of methylated wheat starch showed in *m*-cresol a viscosity corresponding to a molecular weight of 300,000, calculated on the basis of Staudinger's equation with $k_m = 1{\cdot}6 \times 10^{-4}$. The exact value of k_m for these methyl derivatives is still somewhat uncertain, and the figure used is obtained from the data of Carter and Record (this vol., p. 670) (see Haworth, *Monatsh.*, 1936, **69**, 921). It is, in any case, clear that the particle size is extremely large and what is of special significance is that another sample of lower viscosity, corresponding to about half this particle size, gave precisely the same value for the size of the repeating unit. The method of preparation and purification of both samples ensured that they were free from any small-size breakdown products and it follows immediately that the results cannot be explained on any hypothesis of random hydrolysis of linkages in a long chain of similarly united residues (contrast Richardson, Higginbotham, and Farrow, *J. Text. Inst.*, 1936, **27**, T 131). The new results therefore confirm and emphasise the fundamental importance of the repeating units of 24—30 glucose residues in the architecture of the starch molecule.

The methylated horse-chestnut starch prepared under these conditions has the highest viscosity of any methyl starch we have hitherto examined, the estimated molecular weight being approximately 700,000 on the basis of viscosity figures using $k_m = 1{\cdot}6 \times 10^{-4}$. Here again the repeating unit was found to have a chain length of about 28 glucose residues. These methylated derivatives were obtained by direct methylation of the free starch without the intervention of an acetyl derivative. For purposes of comparison, the acetates of both wheat starch and horse-chestnut starch were prepared by the action of acetic anhydride and pyridine on the starches. The products obtained were generally similar in their properties to corresponding acetates from potato starch and rice starch, but were less readily soluble in organic solvents and showed high viscosity in solution. The fully acetylated derivatives could not be obtained by the use of pyridine and acetic anhydride under conditions which readily yield the fully acetylated derivative of rice starch.

Experimental.

The sample of wheat starch used in this work had the following properties: (a) blue coloration with iodine; (b) $[\alpha]_D^{20°}$ +161° in N-sodium hydroxide (*c*, 0·6); (c) distillation with 12% hydrochloric acid gave no furfural; (d) with the same reagents under the conditions used for the estimation of uronic acid groups, 0·40% of carbon dioxide was evolved; no structural significance can be attached to yields of this order (see Campbell, Hirst, and Young, *Nature*, 1938, **142**, 912) (Found: moisture content of air-dried sample, 13·0; P_2O_5, 0·15, determined by a modification of Neumann's method; N, 0·1%; acid number, 0·2 c.c. of N/10-alkali required to neutralise 1 g. of starch).

Acetylation of Wheat Starch.—Air-dried starch (30 g.) was made into a 3% paste with hot water, and alcohol (2 vols.) added. The precipitate was washed with alcohol and with ether and shaken with pyridine (300 c.c.) for 36 hours; acetic anhydride (300 c.c.) was then added gradually with stirring, the temperature rising to 105°. The mixture was kept at room temperature for 18 hours and then stirred at 60° for 72 hours. The viscous mass was diluted with glacial acetic acid and poured into water. The white precipitate was washed with water for 2 days until free from acid and then with alcohol, and finally with ether and dried at 80° in a vacuum (yield, quantitative). $\eta_{sp.}^{20°}$ 0·82 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 560,000 ($k_m = 1{\cdot}0 \times 10^{-4}$) (Found: $CH_3{\cdot}CO$, 41·5%).

The conditions required for acetylation are considerably more drastic than those which suffice for potato or rice starch. For instance, preliminary experiments in which the reactants were stirred at room temperature for 8 hours gave a product having $CH_3{\cdot}CO$ 30·6%, and after 11 hours' stirring at 45°, followed by 12 hours at 15°, an acetate having $CH_3{\cdot}CO$ 37% was obtained (Calc. for the triacetate: $CH_3{\cdot}CO$, 44·8%).

Methylation of Wheat Starch.—Wheat starch (30 g.) was made into a cream with water (250 c.c.), and 30% sodium hydroxide solution (100 c.c.) added, with formation of a viscid paste. This was stirred rapidly in an atmosphere of nitrogen, and methyl sulphate (200 c.c.) and 30% sodium hydroxide solution (400 c.c.) added gradually at room temperature with vigorous stirring. After 12 hours, the alkali was partially neutralised by 50% sulphuric acid, the solution saturated with carbon dioxide and concentrated almost to dryness, and the mixture of partially methylated starch and mineral salts again treated with the methylating reagents. After this second methylation, sodium sulphate was removed by extraction with boiling water and the insoluble product was dissolved in acetone and remethylated in the usual manner. After eight methylations, the methoxyl content of the product was 43·0%. A solution of the crude methylated starch in acetone was poured into an equal volume of ice-cold water, and the acetone distilled off from the clear yellow solution, which was then heated to 100°. The methylated starch which separated was filtered off, dried at 100° under reduced pressure, and extracted with boiling ether, which removed a small amount of impurity, mainly condensation products of acetone (yield, 80% of the theoretical).

The methylated wheat starch was separated into fractions by successive additions of light petroleum to a chloroform solution: (a) 11 g., $[\alpha]_D^{20°}$ +208° in chloroform (*c*, 0·316), $\eta_{sp.}^{20°}$ 1·0 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 300,000 ($k_m = 1{\cdot}6 \times 10^{-4}$) (Found: OMe, 44·8%); (b) 53 g., $[\alpha]_D^{20°}$ +207° in chloroform (*c*, 0·577), $\eta_{sp.}^{20°}$ 1·0 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 300,000 (Found: OMe, 42·7%); (c) 18 g., $\eta_{sp.}^{20°}$ 0·94 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 290,000 (Found: OMe, 45·5%); (d) 10 g., $[\alpha]_D^{20°}$ +207° in chloroform (*c*, 0·581), $\eta_{sp.}^{20°}$ 0·55 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 170,000 (Found: OMe, 44·0%).

Hydrolysis of Methylated Wheat Starch.—The methylated starch [fractions (a) and (c) above] was boiled with 1% methyl-alcoholic hydrogen chloride for 7 hours. The mixed methylglucosides were isolated in the usual manner (97% yield) and on fractional distillation the following fractions were finally obtained (Table I). For the method of estimation see Hirst and Young (J., 1938, 1247). In all cases methoxyl estimations were carried out as checks, but these results were not used in calculating the amount of " tetramethyl " derivative. Absorption measurements showed the absence of furfural in the various fractions.

TABLE I.

Fraction.	Wt., g.	$n_D^{16°}$.	Constants used in estimation.*	" Tetra," %.	" Tetra," g.	" Tri," g.	" Di," %.	" Di," g.
1	0·55	1·4475	(a) 1·4430 (b) 1·4560	65	0·36	0·19	—	—
2	0·26	1·4503	(a) 1·4440 (b) 1·4570	52	0·14	0·12	—	—
3	0·08	1·4560	(a) 1·4445 (b) 1·4570	8	0·01	0·07	—	—
4	0·88	1·4571	—	0	—	0·88	—	—
5	4·61	1·4577	—	—	—	4·61	—	—
6	2·05	1·4582	—	—	—	2·05	—	—
7	3·09	1·4624	—	—	—	2·16	30	0·93
8	0·71	1·4747	—	—	—	—	100	0·71
	12·23				0·51	10·08		1·64

* (a) and (b) are the $n_D^{16°}$ values of the " tetra " and the " tri " portions respectively present in these fractions, as estimated from rotational data (see Hirst and Young, *loc. cit.*).

The relative proportions of the fission products, represented as a percentage of the total recovery of glucosides, were therefore: 2 : 3 : 4 : 6-Tetramethyl methylglucoside (end-group), 4·2%; 2 : 3 : 6-trimethyl methylglucoside, 83%; dimethyl methylglucosides, 13%. This proportion of end-group corresponds to a repeating unit of chain-length approximately 24 glucose residues.

Hydrolysis of Methylated Wheat Starch of Lower Viscosity.—The methylated starch used was fraction (d) above, having $\eta_{sp.}^{20°}$ 0·55 in *m*-cresol, corresponding to a molecular weight of 170,000 ($k_m = 1·6 \times 10^{-4}$). It was hydrolysed precisely as described above, and the following fractions were finally obtained.

TABLE II

Fraction.	Wt., g.	$n_D^{16°}$.	Constants used in estimation.*	" Tetra," %.	" Tetra," g.	" Tri," g.	" Di," %.	" Di," g.
1	0·30	1·4461	(a) 1·4440 (b) 1·4560	83	0·25	0·05	—	—
2	0·10	1·4561	(a) 1·4445 (b) 1·4568	6	0·01	0·09	—	—
3	0·55	1·4568	—	0	—	0·55	—	—
4	4·74	1·4583	—	—	—	4·74	—	—
5	1·07	1·4682	—	—	—	—	100	1·07
	6·76				0·26	5·43		1·07

* See note under Table I.

The composition of the fission products, expressed as a percentage of the total recovery, was therefore: 2 : 3 : 4 : 6-Tetramethyl methylglucoside, 3·9%; 2 : 3 : 6-trimethyl methylglucoside, 80%; dimethyl methylglucosides, 16%. This amount of end-group corresponds to a repeating unit of chain length 26 glucose residues.

Identification of 2 : 3 : 4 : 6-*tetramethyl methylglucoside.* The syrup was hydrolysed by 2N-hydrochloric acid on a boiling water-bath for 15 hours. The acid was neutralised with barium carbonate, and the water removed in a vacuum. The dried residue was extracted with boiling chloroform, which was then removed, leaving a syrup (yield, nearly quantitative) which crystallised on inoculation with 2 : 3 : 4 : 6-tetramethyl glucose. After recrystallisation from light petroleum (b. p. 60—80°) the crystals had m. p. and mixed m. p. 88°.

Identification of 2 : 3 : 6-*trimethyl methylglucoside.* The methylglucoside was hydrolysed with 5% sulphuric acid on a boiling water-bath for 12 hours. The product was isolated in the usual manner and crystallised on standing (yield, nearly quantitative); m. p. 117° after recrystallisation from ether.

Extraction of Starch from Horse Chestnut.—Freshly gathered nuts were peeled and the inner portions were finely ground, covered with 1% sodium sulphite solution, and left at room temperature for several hours. The starch granules were then washed through a fine cloth with water, and allowed to settle. After thorough washing by decantation, the starch was filtered off and washed with alcohol and then ether. Horse-chestnut starch was thus obtained as a white powder consisting of tiny granules similar in size and shape to those of rice starch (yield, 10—20% of the weight of the original nuts). In hot water the starch gave a clear paste (viscosity comparable with that of rice starch) which gave a deep blue colour with iodine. It was non-reducing to Fehling's solution. $[\alpha]_D^{20°}$ +151° in N-sodium hydroxide (*c*, 0·668). Hydrolysis with taka-diastase left 4% of residue. No furfural was obtained on distillation with 12% hydrochloric acid. With the same reagent, under the conditions used for uronic acid determinations, 0·50% of carbon dioxide was evolved (compare wheat starch, above) [Found : P_2O_5, 0·045 (Neumann's method); N, 0·06; S, nil; moisture on air-dried sample, 12·5%; acid number, 0·15 c.c. of N/10-alkali required to neutralise 1 g. of starch].

Acetylation of Horse-chestnut Starch.—The procedure was similar to that described for wheat starch. After addition of the acetic anhydride, the temperature rose to 70° and then fell, and the stirring was continued for 8 hours. On pouring into water, a viscous, partially acetylated mass separated; the product was therefore treated again with pyridine (100 c.c.) and acetic anhydride (100 c.c.), and the mixture stirred at 50° for a further 6 hours. The acetate was then precipitated and purified in the usual manner (Found : $CH_3{\cdot}CO$, 40·9%). $\eta_{sp.}^{20°}$ 0·78 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 540,000 ($k_m = 1{\cdot}0 \times 10^{-4}$).

Methylation of Horse-chestnut Starch.—The methylation was carried out as described for wheat starch (yield, 60%). On precipitation of the methylated product from chloroform by light petroleum, the following fractions were obtained : (a) 22 g., $[\alpha]_D^{20°}$ +205° in chloroform (*c*, 0·537), $\eta_{sp.}^{20°}$ 2·38 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 700,000 ($k_m = 1{\cdot}6 \times 10^{-4}$) (Found : OMe, 43·9%); (b) 6 g., $\eta_{sp.}^{20°}$ 2·10 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 650,000 (Found : OMe, 43·8%); (c) 9 g., $[\alpha]_D^{20°}$ +204° in chloroform (*c*, 0·836), $\eta_{sp.}^{20°}$ 1·4 in *m*-cresol, corresponding to an apparent molecular weight of 430,000 (Found : OMe, 43·9%).

Hydrolysis of Methylated Horse-chestnut Starch.—The methylated starch [fraction (a) above] was hydrolysed in the usual manner with 1% methyl-alcoholic hydrogen chloride. The syrup was isolated as before (yield, 90%) and was fractionally distilled at 0·001 mm. (see Table III).

TABLE III.

Fraction.	Wt., g.	$n_D^{16°}$.	Constants used in estimation.*	"Tetra," %.	"Tetra," g.	"Tri," g.	"Di," %.	"Di," g.
1	0·29	1·4458	(a) 1·4432 (b) 1·4575	82	0·24	0·05	—	—
2	0·29	1·4484	(a) 1·4445 (b) 1·4575	70	0·21	0·08	—	—
3	1·04	1·4563	(a) 1·4445 (b) 1·4575	9	0·09	0·95	—	—
4	0·92	1·4574	—	0	—	0·92	—	—
5	9·02	1·4601	—	—	—	9·02	—	—
6	2·88	1·4610	—	—	—	2·59	10	0·29
7	0·84	1·4690	—	—	—	0·25	70	0·59
	15·28				0·54	13·86		0·88

* See note under Table I.

The composition of the fission products, calculated on the total recovery, is therefore : 2 : 3 : 4 : 6-Tetramethyl methylglucoside, 3·5%; 2 : 3 : 6-trimethyl methylglucoside, 91%; dimethyl methylglucosides, 5·7%. This amount of end-group corresponds to a chain-length of 28 glucose units. The identity of the hydrolysis products was proved as in the case of wheat starch by the isolation of crystalline 2 : 3 : 4 : 6-tetramethyl glucose and 2 : 3 : 6-trimethyl glucose from the corresponding fractions.

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