CLXXVIII.—*Polysaccharides. Part VIII. Evidence of Continuous Chains of α-Glucopyranose Units in Starch and Glycogen.*

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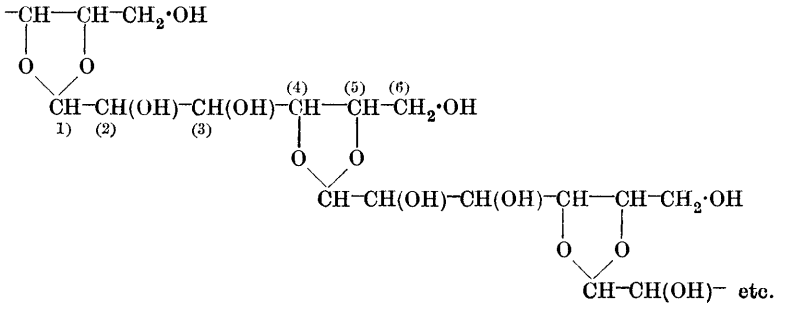
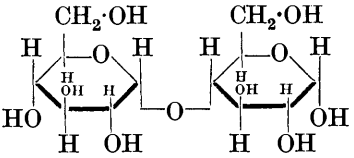
THE conception, previously advanced, that starch is composed of conjugated maltose residues is the simplest basis on which to formulate this carbohydrate (Haworth, "Constitution of Sugars," Ed. Arnold & Co.). It recognises the significant facts of the hexagonal structure of glucose units in maltose and cellobiose, and also the ease with which these disaccharides are generated from the two polysaccharides. It recognises α-glucose as the essential residue in starch, and β-glucose as the corresponding residue in cellulose. Associated with this conception is the idea that these units are joined entirely by glucosidic linkings, and the wide inference may be drawn that starch and cellulose are analogues of the simplest glucosides.

It was also pointed out that the isolation of 2 : 3 : 6-trimethyl glucose from trimethyl starch and trimethyl cellulose furnishes no evidence of the existence of glucopyranose units in these polysaccharides, since the only inference to be drawn from this result is that positions 1 : 4 : 5 in the glucose units are concerned in the internal structural arrangements of the carbohydrates. Whilst the pyranose or the furanose ring would equally explain this mode of union of glucose residues, yet it may be argued that neither of these cyclic forms is necessary to interpret these simple facts inasmuch as the following formulation is at least equally feasible :



The basis for the recognition of the glucopyranose residue in starch is the experimental observation that starch is degraded to maltose almost quantitatively, combined with the further observation that α-maltose can only be constituted as shown below (Haworth and Peat, J., 1926, 3094).



This expression is structurally identical and stereoisomeric with that allocated to cellobiose (Haworth, Long, and Plant, J., 1927, 2809) and it is this structure which is embodied in the constitution assigned to cellulose.

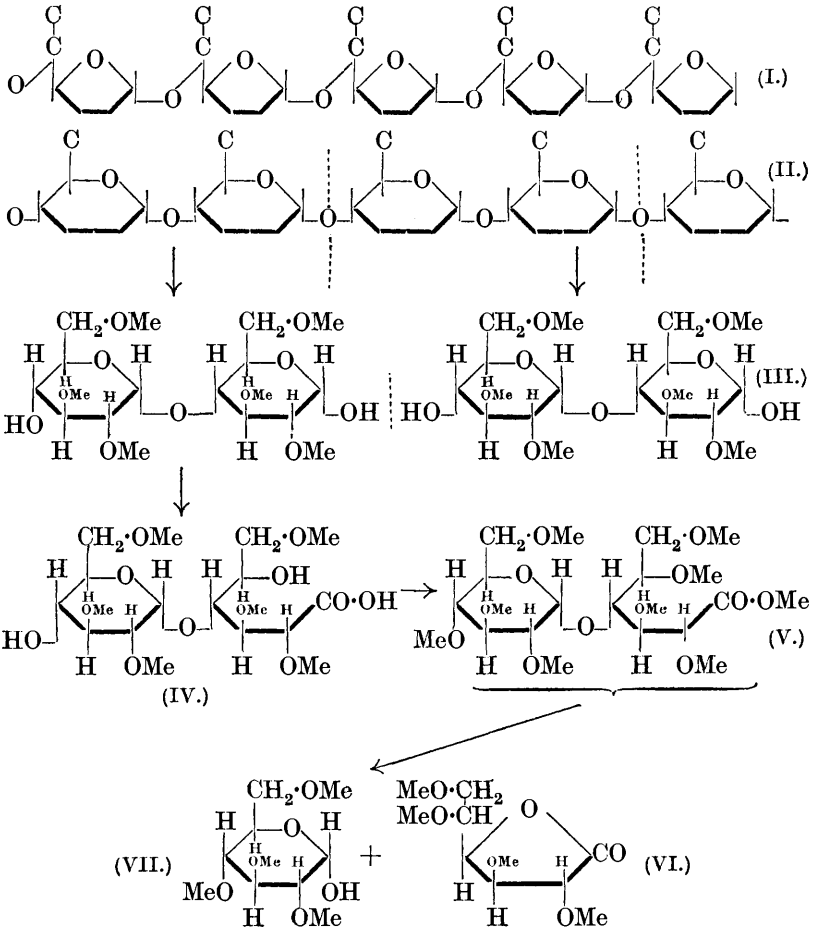
In supporting the continuous chain structure of α-glucopyranose units in starch, one has always been conscious of other factors which may intervene to render this conception untenable. One of the chief difficulties urged against the acceptance of this view is that no valid proof exists that maltose is preformed in starch. It has been suggested on many occasions that maltose may be only a reversion product of the action of enzymes on starch, and in support of this contention reference is made to the ease with which maltose can be synthesised from glucose by enzyme action. The analogy which can be drawn between the behaviour of α-methylglucoside and starch favours, however, the existence of α-glucopyranose units in the polysaccharide, as does also the observation of Karrer that starch is degraded to hepta-acetyl maltosidobromide by the action of acetyl bromide (Karrer and Nägeli, *Helv. Chim. Acta*, 1921, **4**, 263).

At the same time it must be confessed that it is always open to dispute whether an independent proof has been furnished of the precise mode of linking of the constituent hexose units of starch. If the polysaccharide is made to undergo cleavage whilst its hydroxyl groups remain unprotected and therefore free to participate in subsequent secondary changes, the inferences drawn from these results may lead to invalid conclusions.

We have endeavoured in the course of the present work to overcome this objection and have provided, by a method which is free from dubiety, a proof of the structure of two contiguous glucose units of starch.

Apart from the chain formula given on p. 1342 there are two competing structures ascribable to starch, according as a furanose or a pyranose constitution is allocated. These are given in skeleton

form below, and it will be seen that in the furanose formula the mutual linking of units occurs at position 5 in the hexose residue, ring formation occurring at positions 1 : 4. In the pyranose formula the linking of contiguous glucose units is represented at position 4 and the ring closure occurs at positions 1 : 5.



We have previously observed in our experiments with triacetyl starch that no essential constitutional change is introduced during the process of acetylation, since we have regenerated the polysaccharide from this triacetyl derivative and found it to behave exactly as the amylose portion of starch (Haworth, Hirst, and Webb, J., 1928, 2681). Similarly we have developed a procedure by which triacetyl starch is simultaneously deacetylated and methyl-

ated in alkaline solution, and it is clear that amylose is unaffected by treatment with alkali. The trimethyl starch obtained by this means is recognisable as a genuine derivative of amylose and we are left in no doubt that the molecular arrangement of the original starch has been unimpaired.

We have now degraded the trimethyl amylose by contact, at the ordinary temperature (15—16°), with a solution of acetyl bromide in chloroform for a period of about 4 hours (since chloroform is also a solvent for the trimethyl amylose). The methylated polysaccharide breaks down under these conditions to give derivatives of (a) monosaccharide, (b) disaccharide, (c) trisaccharide, leaving a small part only of the trimethyl amylose unchanged.

The disaccharide derivative formed by this cleavage was evidently the bromide and monoacetyl derivative of a hexamethyl biose (III), from which the bromine was eliminated by shaking with moist silver carbonate in ether. This monoacetyl hexamethyl biose was oxidised with bromine water in the presence of barium benzoate and the derived bionic acid (IV) was further methylated and esterified. The methyl octamethyl bionate (V) was then hydrolysed with mineral acid and gave rise to two cleavage fragments, one of these being isolated as crystalline tetramethyl glucopyranose (VII) (yield, 95%) and the other as 2 : 3 : 5 : 6-tetramethyl-γ-gluconolactone (VI) (yield, 80%), which was identified both by its m. p. and rotation as well as by the m. p. of the crystalline phenylhydrazide of the 2 : 3 : 5 : 6-tetramethyl gluconic acid.

The conclusions to be drawn from these results are that, since 2 : 3 : 5 : 6-tetramethyl gluconic acid and its lactone are isolated from this cleavage, only the 4-position can be involved in the union of this member with the adjoining residue, namely, tetramethyl glucopyranose, in the octamethyl bionic ester. The 4-position is therefore recognised as being involved in the union of the contiguous units in amylose. That the 5-position must have been liberated as a hydroxyl group during the oxidation of the biose derivative was evident from the fact that when the oxidation stage was omitted, only 2 : 3 : 6-trimethyl glucose was obtained on hydrolysis. This conclusion is verified by the characterisation of the tetramethyl glucopyranose as the second cleavage product of the octamethyl bionic ester, thus establishing the six-atom ring in this half of the molecule. The 1-position of this tetramethyl glucopyranose could only have been involved in union with the 4-position of the gluconic acid component, from which it follows that the constitution of the hexamethyl biose furnished as a cleavage product of trimethyl amylose is that given in formula (III).

Since two glucopyranose units constitute the biose derived from

trimethyl amylose, we conclude that the essential structure of the original polysaccharide is that represented by formula (II), namely, the conjugated maltose residues comprising a chain of α-glucopyranose units. It may be added that the octamethyl bionic ester (V) has the physical constants of octamethyl methylmaltobionate, which gives also the above cleavage products on hydrolysis (Haworth and Peat, *loc. cit.*).

We have also carried out a parallel series of experiments with trimethyl glycogen, prepared from triacetyl glycogen as described by Haworth, Hirst, and Webb (J., 1929, 2479). In the case of glycogen the methylated polysaccharide yielded 22·9% of methyl octamethylmaltobionate which was characterised by its hydrolysis to crystalline 2 : 3 : 4 : 6-tetramethyl glucopyranose (VII) (yield, 96%) and to crystalline 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone (VI) (yield, 87%). The latter was also identified by its crystalline phenylhydrazide. It is evident, then, that the formulations ascribed above to trimethyl amylose and to each of its conversion products apply equally to trimethyl glycogen. From this it follows that glycogen is constituted on the same general plan as starch and consists largely of α-glucopyranose residues joined in a continuous chain formation as represented by the conjugated maltose skeleton (II). In a series of control experiments with 2 : 3 : 6-trimethyl glucose we have shown that under the same experimental conditions as those which were followed in the above investigation this cleavage product of trimethyl amylose does not undergo any re-synthesis or reversion to the disaccharide derivative (III).

Experimental.

The Action of Acetyl Bromide on Trimethyl Amylose.—The trimethyl amylose was prepared as described by Haworth, Hirst, and Webb (*loc. cit.*). The reaction was carried out on three portions of trimethyl amylose (3 g. in chloroform, 9 c.c.) and acetyl bromide (9 c.c. in chloroform, 36 c.c.), that is, 9 g. in all of trimethyl starch. The reagents were mixed in small flasks fitted with calcium chloride tubes and allowed to react for 4 hrs. 20 mins. The contents of each flask were then poured on to crushed ice, more chloroform added, and the solution washed with ice-water and dilute sodium bicarbonate solution until freed from acid. The three samples were then united and dried over magnesium sulphate, and the chloroform removed at 45° under reduced pressure. The product was taken up in ether (200 c.c.) and shaken with silver carbonate (12 g.) and water (5 c.c.) and kept in contact with these reagents over-night or until the solution was devoid of bromine. The silver residues were extracted with boiling ether and the combined ethereal ex-

tracts yielded 12 g. of a light brown syrup (reducing). A portion of the trimethyl amylose was recovered unchanged (1·4 g.).

Oxidation of the Biose Derivative.—To an ice-cold solution of this syrup, there were added barium benzoate (16 g.) and water (450 c.c.) (solid insoluble in water, 1·4 g.). Bromine (1·6 c.c.) was now introduced, and the mixture kept in the dark. After 23 hours, more bromine (0·5 c.c.) was added and the oxidation was complete after 2 days. The barium was precipitated by the addition of sodium sulphate (8 g.) and the bromine was removed by aeration : filtration removed barium sulphate and benzoic acid. The solution was then made slightly alkaline with sodium carbonate and concentrated to 100 c.c. at 50°. Cautious acidification of the cold solution precipitated more benzoic acid, which was removed. The filtrate was again made alkaline and concentrated to 50 c.c. in readiness for methylation.

Methylation of the Mixed Sodium Salts.—Methylation was carried out at an initial temperature of 35°, which was raised finally to 65°, methyl sulphate (25 c.c.) and sodium hydroxide (30%; 67 c.c.) being used in the presence of acetone (200 c.c.). The methylated mixture was cooled to 0° and rendered acid to Congo-red with 5*N*-sulphuric acid in the presence of ice and was extracted six times with chloroform (1200 c.c.). The aqueous layer was rendered alkaline and evaporated to dryness at 60°, and the residue was extracted with boiling 90% alcohol; this extract yielded on evaporation under diminished pressure a mixture of sodium salts. These were dissolved in water and remethylated, together with the residue from the chloroform extraction, by methyl sulphate and alkali. On acidification and extraction of the solution with chloroform there was obtained a viscid syrup (8·5 g.).

Preparation of the Bionic Ester (Methyl Octamethyl Maltobionate).—The above product was now methylated twice with silver oxide (16 g.) and methyl iodide (25 c.c.) and yielded 9·1 g. of a mobile syrup, which was subjected to fractional distillation : (1) b. p. 100—180°/0·05 mm., 4·7 g., $n_D^{15°}$ 1·4410; (2) b. p. 180—210°/0·05 mm., 2·0 g., $n_D^{15°}$ 1·4620; (3) b. p. 210—250°/0·05 mm., 0·2 g., $n_D^{15°}$ 1·4695; residue, 0·3 g.

The second fraction was redistilled and showed the following properties : $[\alpha]_D^{20°}$ + 116°, $n_D^{15°}$ 1·4620 (Found : C, 52·4; H, 8·3; OMe, 56·5; CO_2Me, 12·0. Calc. for $C_{21}H_{40}O_{12}$: C, 52·1; H, 8·3; OMe, 57·6; CO_2Me, 12·2%). These analyses and constants, together with the subsequent behaviour of the product, indicated that it was mainly methyl octamethyl maltobionate (compare Haworth and Peat, J., 1926, 3094). Yield, 22·4% of the theoretical.

Hydrolysis of the Ester.—2·78 G. in 52·8 c.c. of 5% hydrochloric

acid were heated at 95° on the water-bath. Polarimetric readings were taken at intervals : $[\alpha]_D^{20°}$ 115° (initial value); 88·2° (30 mins.); 73° (80 mins.); 66·4° (175 mins.); 61·2° (220 mins.); 60·2° (335 mins.); 55·9° (420 mins.); 55·7° (440 mins.; constant value).

Isolation of Tetramethyl Glucose.—The solution was neutralised with barium carbonate at 50°, air drawn through it for an hour, and the mixture kept at the ordinary temperature during 2 days. The neutral filtered solution was then evaporated at 40° under reduced pressure, and the solid residue extracted ten times with ether (500 c.c.). The ethereal extracts yielded on evaporation a syrup which crystallised spontaneously in colourless needles; these were purified from light petroleum and identified as 2 : 3 : 4 : 6-tetramethyl glucopyranose (yield, 1·35 g.), m. p., and mixed m. p. with an authentic specimen, 86°; $[\alpha]_D^{18°}$ + 91·0° (after 10 mins.), 83·2° (90 mins.) (*c*, 1·04).

Isolation of Tetramethyl γ-Gluconolactone.—The dried barium salts remaining after the ether extraction were dissolved in water, and the solution acidified with *N*-hydrochloric acid. The aqueous solution was then evaporated as before, the solid residue repeatedly extracted with boiling ether, the extracts evaporated, and the residual syrup heated for ½ hour at 100° to complete lactonisation (yield, 1·2 g., *i.e.*, 96% of the theoretical). Distillation in a high vacuum at a bath temperature of 115—120°/0·01 mm. gave a clear mobile liquid, $n_D^{15°}$ 1·4490 (yield, 1·0 g., *i.e.*, 80% of the theoretical). Nucleation of the liquid with a crystal of 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone resulted in complete crystallisation. M. p. and mixed m. p. with an authentic specimen 26° : $[\alpha]_D^{18°}$ + 61·0° (30 mins.) (*c*, 1·1); 58·2° (1 day); $[\alpha]_D^{20°}$ + 57·0° (2 days) (*c*, 1·1). M. p. of the phenylhydrazide 134—136°.

The Action of Acetyl Bromide on Trimethyl Glycogen.—The trimethyl glycogen was prepared from triacetyl glycogen as described by Haworth, Hirst, and Webb (*loc. cit.*). The procedure followed for the acetyl bromide treatment of the methylated polysaccharide was identical with that given above in the case of trimethyl amylose. The yield of methyl octamethyl maltobionate obtained after the oxidation stage, followed by that of remethylation and esterification, was 22·9% of the theoretical, as compared with the yield of 22·4% from trimethyl amylose. This was a colourless syrup distilling at about 190°/0·012 mm., having $n_D^{15°}$ 1·4610, $[\alpha]_D^{20°}$ + 119° (*c*, 0·86) (Found : C, 51·8; H, 8·5; OMe, 57·3. Calc. for $C_{21}H_{40}O_{12}$: C, 52·1; H, 8·3; OMe, 57·6%).

Hydrolysis of the Bionic Ester (*Methyl Octamethyl Maltobionate*).—This process was complete after 2·58 g. of the ester had been heated with 5% hydrochloric acid at 95°. The rate of hydrolysis was

identical with that of the bionic ester from trimethyl amylose and similar polarimetric data were observed. Isolation of the products from this treatment yielded crystalline 2 : 3 : 4 : 6-tetramethyl glucopyranose (1·25 g.) corresponding in amount to 93% of the theoretical, and also crystalline 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone (1 g.) corresponding to 87% of the theoretical. This gave the crystalline phenylhydrazide of 2 : 3 : 5 : 6-tetramethyl gluconic acid, m. p. 135—136° (Haworth and Peat, *loc. cit.*).

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