CCCLIV.—*Polysaccharides. Part II. The Acetylation and Methylation of Starch.*

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EXPERIMENTS have been instituted to render more accessible and to characterise the acetyl and methyl derivatives of starch. In the present work purified potato starch has been employed. Hitherto the yields of both triacetyl and trimethyl starch do not appear to have been satisfactory.

Since Schützenberger's original experiments (*Compt. rend.*, 1865, **61**, 485; 1869, **68**, 814) many investigators have attempted to prepare triacetyl starch. If unmodified starch be employed, the transformation is generally held to be difficult and uncertain, and the small yields lead to serious doubt whether the product is an acetyl derivative of the unchanged starch or is derived from a portion only of polysaccharide which has been segregated during the process of acetylation.

More success has attended the acetylation of a starch which has been subjected to various preliminary treatments. Soluble starch,

for example, has been converted into the triacetyl derivative (Pregl, *Monatsh.*, 1901, **22**, 1049); and the acetylation of the separated amylose constituent of potato starch may readily be effected in the presence of pyridine (Bergmann and Knehe, *Annalen*, 1927, **452**, 141). Such processes, however, not only involve considerable loss during the initial treatment of the polysaccharide, but in many cases suffer also from the disadvantage that the relationship between the original starch and the final product is by no means clear.

We have endeavoured, therefore, to obtain an acetylated derivative of starch in the maximum possible yield and under the simplest experimental conditions. In this way, we consider, information respecting the acetylation of the whole of the starch may be gained, and the properties of the starch acetate may then be compared with those of the known acetylated products isolated by less direct methods and in inferior yield.

Preliminary experiments conducted by the methods of E. Peiser (*Z. physiol. Chem.*, 1926, **161**, 210) failed to give, even in small yield, the acetylated starch of high specific rotation claimed to have been obtained by that author (compare also Pringsheim and Meyersohn, *ibid.*, 1928, **173**, 211). We are in agreement, however, with Peiser that the preliminary precipitation of starch from starch paste by alcohol yields a product which is much more susceptible to acetylation than the original starch, from which it appears to differ only in being more finely divided as the result of the bursting of the granules; *e.g.*, it retains unimpaired the properties of acquiring a deep blue colour on treatment with iodine and of readily giving a paste. Whereas Bergmann and Knehe (*loc. cit.*) conducted the acetylation of separated amylose in the presence of pyridine, we have employed traces of sulphur dioxide and chlorine as catalysts, and we have obtained from precipitated starch a triacetyl derivative in a yield of 96% of the theoretical, based on the weight of the original unmodified starch. Our product is compared below with Bergmann and Knehe's triacetyl amylose :

	Triacetyl starch.	Triacetyl amylose.	Calc.
$[\alpha]_D$ (in chloroform) ...	+170° at 20°	+176° at 16°	—
C, %	49·8	50·0	50·0
H, %	5·8	5·7	5·6
$CH_3 \cdot CO$, %	44·4	44·6	44·8

The triacetyl starch differed little, if at all, in its properties from the product of Bergmann and Knehe. It gave on deacetylation a regenerated starch having all the properties of the regenerated amylose of those authors, being soluble in water and giving the characteristic blue colour with iodine. Both in the precipitated starch and in the triacetyl derivative the presence of traces of

phosphoric acid residues was detected. The deacetylation process almost necessarily involves the scission of the phosphoric ester linkages, and according to the views of Samec and Mayer (*Kolloid-chem. Beih.*, 1921, **13**, 284) on the relationship between amylose and amylopectin the loss of the phosphoric residues must result in the formation of a starch which is incapable of producing a paste with water. This we found to be the case with our regenerated polysaccharide, and the present results have therefore a bearing on the relationship of amylose to amylopectin. It appears that potato starch reacts chemically to the extent of 96% at least as if it were amylose, and our experiments tend to confirm the views of Samec and Mayer. This question will be dealt with in greater detail in a further communication.

Experiments on the methylation of starch have been described by several authors, including Karrer (*Helv. Chim. Acta*, 1920, **3**, 620) and Irvine and Macdonald (J., 1926, 1502). The method adopted by these authors was to act on the original starch with methyl sulphate and alkali and in each case the yield of methylated starch was small, Irvine and Macdonald, *e.g.*, obtaining from 24 methylation treatments a yield of 25% of trimethyl starch. We have obtained, after only six methylations, 89% of the theoretical yield of trimethyl starch by taking advantage of the solubility of triacetyl starch in acetone. The addition of methyl sulphate and dilute alkali to such a solution introduced in one operation 36% of methoxyl, and a progressive increase of the methoxyl content was obtained on each subsequent methylation.

The product appears to be identical with the material described by Irvine and Macdonald, who operated with both rice and potato starch. Hydrolysis of the trimethyl starch with 2% hydrogen chloride in methyl alcohol yielded 80% of pure trimethyl methylglucoside and 8% of a mixture of the latter with dimethyl methylglucoside, leaving a residue of 6% which was not hydrolysed. Hydrolysis of the trimethyl methylglucoside gave crystalline 2 : 3 : 6-trimethyl glucose (yield, 85%) and there can therefore be no doubt that nearly the whole of the potato starch gives rise on methylation and hydrolysis to 2 : 3 : 6-trimethyl glucose.

This conclusion has considerable significance in showing that the mode of linking of the glucose units in potato starch leaves three exposed hydroxyl groups at analogous positions to those which have been determined in the case of cellulose. The specific rotations of trimethyl starch (+ 208°) and trimethyl cellulose (— 18°) are, however, widely different.

By dispensing with the use of acetone as solvent for the triacetyl starch during the methylation, we have prepared a dimethyl starch

(yield, 80% of the theoretical) having $[\alpha]_D$ + 143° in chloroform and OMe, 31·8%. This again appeared to be similar to the dimethyl starch described by Irvine and Macdonald. We were unable, however, with our product to confirm the observation of these authors that this is a homogeneous substance yielding on hydrolysis only dimethyl glucose. Our product gave on hydrolysis a mixture of trimethyl, dimethyl, and monomethyl glucose. The trimethyl glucose was present to the extent of about 20%. The results indicate that all the hydroxyl groups are exposed to simultaneous attack by the methylating agents. In this respect there would appear to be no well-marked difference between the phenomena observed in the methylation of starch and of cellulose. The problem is rather one of obtaining the polysaccharide initially in a suitable condition for combination with the methylating agent. In the present case we have found that after three methylation treatments the starch has acquired a methoxyl content of 40% or more; and similarly with cellulose, Freudenberg and Braun (*Annalen*, 1928, **460**, 288) have observed that the introduction of 42% methoxyl content can be accomplished by two treatments with methyl sulphate if the cellulose fibres used are diminished to 2—4 mm. in length. In the earliest experiments of Denham and Woodhouse (J., 1914, **105**, 2363) a methylated cellulose of about 25% methoxyl content gave an appreciable proportion of trimethyl glucose on hydrolysis. The increase in the methoxyl content of starch from 38 to 44% proceeds just as easily and regularly as from 34 to 38% (contrast Irvine and Macdonald). We are doubful, therefore, whether the claim can be substantiated that seven out of nine hydroxyl positions in starch undergo preferential methylation. If maltose is preformed in starch, then the polysaccharide is composed of glucopyranose units joined in positions 1 : 4. The alternative hypothesis is that maltose is a reversion product and that starch is composed of glucofuranose units linked in positions 1 : 5. These opposing views will be the subject of subsequent work.

Experimental.

Acetylation of Starch.—The starch used in these experiments was the purest commercial potato starch (Prime starch), the properties of which corresponded in all respects with those ascribed in the literature to pure potato starch. It contained 18% of water.

It was prepared for acetylation in the following manner. The starch (20 g.) was stirred in water (600 c.c.) on the water-bath until it formed a paste. After 30 minutes the heating was discontinued and the starch was precipitated by the addition of alcohol to the warm paste. The supernatant liquor was decanted from the white

curdy mass which soon settled, and the starch was ground in a mortar in the presence of alcohol. It was then washed with alcohol and ether and dried in a vacuum desiccator (yield, quantitative). This process resulted in the bursting of the granules of the original starch but in general respects appeared to be without effect on its properties. The ash content (0·3%, by incineration), the paste-forming capacity, the blue iodine colour reaction, and the absence of reducing power were all reproduced in the prepared starch.

Attempts to acetylate potato starch by the usual methods revealed many unsatisfactory features, but the following process, which is a modification of Barnett's method (*J. Soc. Chem. Ind.*, 1921, **40**, 8T) for the acetylation of cellulose, was found to be eminently suitable for the routine preparation of the triacetate. Finely powdered starch (prepared in the above manner) was treated with six times its weight of glacial acetic acid through which chlorine had been bubbled for a few seconds. The mixture was stirred for 30 minutes at 20°. Acetic anhydride (20 parts by weight), containing an amount of sulphur dioxide equivalent to that of the chlorine in the acetic acid, was then added and the mixture was stirred at room temperature for 60 minutes. The temperature was then raised to 55° and stirring was continued until a clear solution was obtained (about 4 hours). The clear solution was poured into a large excess of water, and the resulting white powder was washed for some 2 days with changes of water and, when free from acid, successively with dilute alcohol, alcohol, and other; it was then dried for twenty-four hours in a vacuum (yield, 96·5% of the theoretical). This material contained neither chlorine nor sulphur. It was a white impalpable powder, without action on boiling Fehling's solution, insoluble in ether, alcohol or water, but readily soluble in chloroform and in acetone. The acetate could be reprecipitated unchanged from its solutions in the last two solvents by the addition respectively of alcohol and water. When the acetate was treated with aqueous or alcoholic sodium hydroxide solution, the regenerated starch possessed all the properties of Bergmann's regenerated amylose. Hydrolysis of the acetate by the method of Bergmann and Knehe (*loc. cit.*) gave a white powder, soluble in cold water, $[\alpha]_D^{17^\circ} + 186^\circ$ ($c = 1·0$). This gave a blue colour with iodine, but did not form a paste with hot water. For the starch acetate the following data were observed: $[\alpha]_{5461}^{20^\circ} + 197^\circ$ in chloroform ($c = 1·0$); $[\alpha]_D^{20^\circ} + 170^\circ$ in chloroform ($c = 1·0$). The air-dry acetate contained 2·2% of moisture. Acetyl estimations were carried out by treating a weighed quantity with an excess of $N/2$-alcoholic sodium hydroxide for 3 hours at 50°, with

frequent shaking (Found : C, 49·8; H, 5·8; CH_3·CO, 44·4; P, as P_2O_5, 0·05. $C_{12}H_{16}O_8$ requires C, 50·0; H, 5·55; CH_3·CO, 44·8%).

Acetylation of the prepared starch may be effected at temperatures higher or lower than the 55° mentioned, without affecting in any way the chemical properties or the specific rotation of the starch acetate. Too low a temperature, however, involves prolonged treatment. The principal difference between the various samples of acetate obtained at different temperatures appeared to lie in the greater viscosity of the acetate solutions obtained at lower temperatures.

It is to be noted that careful preparation and drying of the " prepared " starch are essential. Certain specimens of prepared starch which had been heated at 80° to complete the drying process were found to acetylate slowly and imperfectly, the retardation being due apparently to alteration of surface conditions produced by heat.

For comparison, acetylations were also carried out on the original starch (before preparation), (*a*) chlorine and sulphur dioxide, as above, and (*b*) sulphuric acid being used as catalysts. In (*a*) it was found impossible to effect complete solution of the starch even after 12 hours at 65° with high concentrations of catalyst. After removal of the undissolved starch by filtration through glass wool the acetylated starch was isolated in the manner already described. The product was essentially similar to that from prepared starch but had a lower specific rotation, $[\alpha]_D$ + 154° in chloroform (c = 1·0). The yield (15% of the theoretical) was very inferior to that of the first method.

In case (*b*) 10 g. of starch and 60 c.c. of acetic anhydride containing 12 drops of sulphuric acid were heated at 55° for 8 hours. The brown liquid was filtered through glass wool, and the product isolated as before. The resulting acetate showed $[\alpha]_D$ + 169° in chloroform (c = 1·0) and appeared to be identical with the material obtained from the prepared starch. The yield, however, was small (10—15%), much of the starch either decomposing or not undergoing acetylation.

Methylation of Potato Starch.—Preliminary experiments, following the methods of Irvine and Macdonald (*loc. cit.*), confirmed the grave manipulative difficulties encountered in the direct methylation of starch. When, however, a method of simultaneous deacetylation and methylation was used, these difficulties almost entirely disappeared. This was peculiarly the case when the operations were conducted in acetone solution. If the following procedure is adopted, methylation of starch becomes sufficiently rapid and convenient to render methylated starch the most suitable intermediate substance for the preparation of 2 : 3 : 6-trimethyl glucose.

(a) *Methylation in presence of water only.* Powdered starch triacetate (20 g.) was treated with 30% sodium hydroxide solution (224 c.c.) and methyl sulphate (80 c.c.), added gradually and simultaneously with vigorous mechanical stirring. The temperature was maintained at 50° for 1¼ hours and thereafter at 70° for a further 1¼ hours until the whole of the reagents had been added, care being taken to avoid acidity at any stage. After being heated for 30 minutes at 100°, the solution was cooled, rendered almost neutral by addition of 30% sulphuric acid, and filtered. The solid residue was extracted with boiling chloroform. The slightly alkaline aqueous portion was treated with excess of carbon dioxide and evaporated to dryness in the presence of barium carbonate, and the solid residue extracted with chloroform. The united chloroform extracts yielded (when evaporated) a white flaky solid, which was slightly soluble in water or chloroform and had a methoxyl content only slightly lower than that required by a dimethyl starch. Yield, 80% of the theoretical for dimethyl starch. $[\alpha]_D$ + 143° in chloroform (c = 1·0) (Found: OMe, 31·8. Dimethyl starch requires OMe, 32·7%).

Hydrolysis of dimethyl starch. The methylated starch (4·0 g.) was dissolved in methyl alcohol (50 c.c.) containing 2% of hydrogen chloride and the solution was boiled for 24 hours. The acid was neutralised by addition of lead carbonate, and the product isolated in the usual way. It was a viscous syrup (4·55 g.) which, on distillation, gave (I) 0·9 g., b. p. 110—117°/0·05 mm., $n_D^{16°}$ 1·4611 (Found: OMe, 47·7%); (II) 1·45 g., b. p. 117—130°/0·04 mm., $n_D^{16°}$ 1·4670 (Found: OMe, 41·5%); (III) 1·48 g., b. p. 130—180°/0·03 mm., $n_D^{16°}$ 1·4800 (Found: OMe, 37·2%); (IV) still residue, 0·6 g. Dimethyl methylglucoside and trimethyl methylglucoside require respectively OMe 41·9% and 52·6%. The distillates were glucosidic in character; (I) appeared to consist mainly of trimethyl methylglucoside, and (II) and (III) mainly of dimethyl methylglucoside admixed with some monomethyl methylglucoside. This diagnosis was confirmed in the case of (I) by hydrolysing it with boiling 5% aqueous hydrochloric acid for 4 hours. The acid was neutralised with barium carbonate, the neutral solution evaporated to dryness at 50°/15 mm., and the product of hydrolysis extracted from the solid residue by boiling ether. Removal of the ether left a syrup which crystallised almost completely (yield, 80%). Recrystallisation from ether removed some adhering gum and gave in good yield 2 : 3 : 6-trimethyl glucose, m. p. 117—118°; mixed m. p. with a specimen of 2 : 4 : 6-trimethyl glucose of m. p. 124°, 90—95°. No tetramethyl glucose was present.

Further methylations by the same method gave a pro-

gressive increase in the methoxyl content. This applied also to the simultaneous deacetylation and methylation of the acetylated methylated starch obtained by heating dimethyl starch with acetic anhydride and fused sodium acetate at 100° for 1½ hours (compare Irvine and Macdonald, *loc. cit.*). Methylation by means of Purdie's reagents was ineffective and was known to lead to changes in the nature of the methylated starch.

(b) *Methylation with acetone as solvent.* The process (a) yielded a dimethyl starch readily and in good yield, but constituted no improvement on known methods for proceeding from dimethyl starch to the fully substituted derivative. The following method gives in one operation a methylated starch with a methoxyl content of 36%, greatly reduces the manipulative difficulties, and facilitates the preparation of trimethyl starch in almost quantitative yield. It consists essentially in the simultaneous deacetylation and methylation of starch triacetate dissolved in acetone in a large flask provided with a reflux condenser, through which the spindle of the stirrer is passed. Efficient stirring is necessary. The methoxyl content of the product may be varied by employing a smaller quantity of acetone and in this way methylated starches containing from 28% to 36% OMe have been obtained.

Preparation of methylated starch (OMe, 36%). Powdered starch triacetate (25 g.) was dissolved in acetone (250 c.c.) and treated with 30% sodium hydroxide solution (280 c.c.) and methyl sulphate (100 c.c.). The methylating reagents were added (with the usual precautions) in ten equal portions at intervals of 10 minutes, the temperature being maintained at 56°. After being heated for 30 minutes at 100°, during which the acetone was allowed to evaporate, the mixture was almost neutralised with sulphuric acid, treated with excess of carbon dioxide, and filtered. The aqueous portion was extracted four times with chloroform ; * the solid was extracted with boiling chloroform. The combined extracts gave on evaporation a light yellow, glassy solid (16·8 g.; 96% of the theoretical). Further purification was effected by dissolving it in a little chloroform and boiling with excess of ether. This yielded a white impalpable powder which was dried at 100°/15 mm. The loss during this treatment was inappreciable. The dry substance showed $[\alpha]_D$ + 170° in chloroform. Ash (mainly sodium sulphate), 1·6%

* When methylated starches of methoxyl content 34% or more are being dealt with, the chloroform separates readily from the aqueous layer. Below 34% an intractable emulsion usually forms and more tedious methods have to be adopted (see above). With increasing methoxyl content manipulative difficulties of this kind progressively diminish.

(Found : C, 50·8; H, 7·7; OMe, 36·0. Calc. for methylated starch : C, 51·2; H, 7·6; OMe, 36·0%).

Two more methylations under similar conditions raised the methoxyl content by 4%. After three further methylations (being six in all) a product corresponding very closely to trimethyl starch was obtained. The final product was purified by dissolving it in a little chloroform and boiling the solution with excess of light petroleum. The crisp white powder obtained was treated again with light petroleum, separated by filtration, and dried in a vacuum (Found : OMe, 44·0; ash, 0·04%). The loss in yield on each methylation was 0·1 g., and the final yield of methylated starch from starch triacetate was thus 92% of the theoretical. This represents a yield of 89·4% of the theoretical from the original starch.

No advantage could be gained by introducing an acetylation process after the first methylation, the solubility of the methylated starch in acetone being apparently the decisive factor in facilitating the etherification. For instance, in one experiment the product from the first treatment of starch triacetate in acetone solution (yield, 16·4 g. from 25·0 g. Found : C, 50·4; H, 7·8; OMe, 34·4; ash, 0·9%) was acetylated by heating it with 8 parts of acetic anhydride and 1 part of fused sodium acetate for 1½ hours at 100°. The mixture was then poured into an excess of cold water, the acid neutralised with sodium bicarbonate, and the product extracted from the solution by chloroform (yield, 10 g. from 10 g. Found : CH_3·CO, 12; OMe, 30·6%). Simultaneous deacetylation and methylation in acetone, followed by a further acetylation and combined deacetylation and methylation, raised the methoxyl content to 36·4%. Four successive methylations in acetone solution then gave trimethyl starch (Found : OMe, 45%).

Trimethyl Starch and its Hydrolysis.—The methylated starch finally obtained was a white powder, m. p. 145° with previous softening, $[\alpha]_D$ + 208° in chloroform (c = 1·0), which appeared to be identical with the material described by Irvine and Macdonald (*loc. cit.*) (Found : C, 52·4; H, 7·9; OMe, 44·0; * ash, 0·04. Calc. for $C_9H_{16}O_5$: C, 52·9; H, 7·8; OMe, 45·5%). When boiled for 24 hours with methyl alcohol containing 2% of hydrogen chloride, it gave (*a*) pure trimethyl methylglucoside, b. p. 95—97°/0·02 mm., $n_D^{24°}$ 1·4550 (yield, 80%) (Found : OMe, 51·8. Calc. : OMe, 52·6%); (*b*) a mixture of trimethyl methylglucoside and dimethyl methylglucoside having $n_D^{24°}$ 1·4630, OMe 44% (yield, 8%); (*c*) a small

* As in the case of cellulose, the final 1% of methoxyl can be introduced only with great difficulty. In the present experiments methylation has not been carried beyond the stage 44—45%.

quantity of solid which appeared to be unhydrolysed material (yield, 6%). Fraction (*b*) corresponded to the small amount of dimethyl starch in the original material, the presence of which was indicated by the slight deficiency in the OMe value. Hydrolysis of (*a*) with boiling 5% aqueous hydrochloric acid gave only 2 : 3 : 6-trimethyl glucose (yield, 85%), which, after one recrystallisation, had m. p. 117° alone or when mixed with an authentic specimen of the same m. p. Tetramethyl glucose was definitely absent.

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