CCCXXIX.—*Polysaccharides. Part V. Glycogen.*

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In the present communication preliminary results are described concerning the properties of glycogen, more particularly its complete hydrolysis to glucose and its behaviour when subjected to acetylation or methylation. Glycogen from one uniform source was employed in the course of this investigation, although other experiments, which will be communicated, have been conducted on glycogen from a variety of sources. The specimen utilised was a good representative sample and contained traces only of nitrogen and phosphorus products, and 2·7% of ash, which is a comparatively low figure for commercial samples of glycogen. Even so, as a preliminary, it was deemed advisable to purify the specimen by electro-dialysis, with the result that the ash content diminished to 0·37% but the traces of phosphorus were not removed; in other respects the purified glycogen retained the properties of the original substance. The device was followed of precipitating the glycogen from aqueous solution by alcohol, since in parallel experiments on starch (Haworth, Hirst, and Webb, J., 1928, 2681) this was shown to lead to a more finely divided form of the polysaccharide which rendered it more open to attack by reagents. Using this prepared material in experiments involving acetylation with acetic anhydride, in the presence of either chlorine and sulphur dioxide as catalysts or of pyridine, we obtained, in almost quantitative yield, glycogen triacetate as a white powder. Apart from the fact that this had a denser quality than starch triacetate prepared under similar conditions, there would appear to be little to distinguish the triacetates

of glycogen and of starch, which showed respectively $[\alpha]_D^{20°}$ + 163° and 170° in chloroform solution.

Deacetylation of the glycogen triacetate gave quantitatively a regenerated glycogen having all the characteristic properties of the original polysaccharide which had been purified by electro-dialysis. By the agency of methyl-alcoholic hydrogen chloride the glycogen triacetate was quantitatively converted into methylglucoside, a result which demonstrates the presence of glucose units only in the polysaccharide. In all the above respects the behaviour of glycogen seems to be parallel to that of potato starch.

A further method of distinguishing the comparative behaviour of the two polysaccharides was by the simultaneous deacetylation and methylation of glycogen triacetate under conditions which were determined after numerous trials. Under the devised procedure with glycogen triacetate in acetone solution in contact with methyl sulphate and potassium hydroxide, the introduction of methyl residues amounting to 40% methoxyl was accomplished in one operation, and after five repetitions of this treatment a trimethyl glycogen was isolated in a yield of 90%. There was no evidence of any arrest of the methylation process at any stage. The trimethyl glycogen was indistinguishable in appearance and properties from trimethyl starch which had been prepared as a white powder by the application of similar methods :

Trimethyl glycogen.	Trimethyl starch.
$[\alpha]_D^{20°}$ in chloroform +208°.	$[\alpha]_D^{20°}$ in chloroform +208°.
M. p. 147° (sintering at 135°).	M. p. 145° (with earlier sintering).
C, 52·6%.	C, 52·4%.
H, 8·0%.	H, 7·9%.
OMe, 43·7%.	OMe, 44·0%.

The hydrolysis of trimethyl glycogen led to the isolation of crystalline 2 : 3 : 6-trimethyl glucose in a yield of 76%. This yield represents almost the maximum amount that could be obtained from a completely methylated glycogen when the difficulty of isolating this form of trimethyl glucose is taken into account.

Earlier preliminary experiments on the direct methylation of glycogen by Karrer (*Helv. Chim. Acta*, 1921, **4**, 994) and by Macbeth and Mackay (J., 1924, **125**, 1513) led to the formation, in unrecorded yields, of partly methylated glycogens having respectively OMe 32% and 37% (trimethyl glycogen requires OMe, 45·6%). The results which are now communicated show that glycogen can be transformed into a completely methylated trimethyl derivative in a yield of about 90% by only six or seven treatments with methylating agents. In our earlier experiments on starch a similar result was obtained with this polysaccharide. The parallel behaviour of glycogen and

starch extends also to the ease with which they can be converted, by the agency of acetyl bromide, into acetobromomaltose in a yield of about 60% (Karrer and Nägeli, *Helv. Chim. Acta*, 1921, **4**, 263), and it is also reported that glycogen is transformed into maltose by enzyme action (Karrer, "Polymere Kohlenhydrate," 1925, p. 95). There seems no reason to doubt the view supported by Karrer that starch and glycogen are similarly constituted, both structurally and configurationally. The difference in the colour reaction with iodine may be distinctive, but it is possible to prepare a starch fraction which gives the same colour reaction as glycogen. We are thus led to the view that glycogen is constituted on the basis of continuous maltose units, that is, of a conjugated chain of α-glucose units. The conformation of these glucose units in the total assemblage in the glycogen model may be different from that which obtains in starch, but any such difference is so far not detectable by the chemical methods we have applied. It would, indeed, appear to be more reasonable, in the light of the evidence at present available, to attribute to a difference in the size of their respective micelles the divergences in properties and behaviour which are shown by starch and glycogen.

EXPERIMENTAL.

The glycogen was supplied by C. A. F. Kahlbaum. It was readily soluble in cold water and the slightly opalescent solution was coloured red by iodine. $[\alpha]_D^{20°}$ + 192° in water (c = 0·23). The air-dried glycogen contained moisture, 5·0% (determined by heating a sample at 110° in a vacuum); nitrogen, 0·35%; phosphorus, 0·14% (calculated as P_2O_5); and ash, 2·7% (by incineration). On analysis, 4·112 mg. gave 2·529 mg. H_2O and 6·245 mg. CO_2. These figures, when corrected for moisture and ash, correspond to C, 44·8; H, 6·9 (Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2%).

Purification of Glycogen by Electro-dialysis.—A solution containing 3 g. of glycogen in 50 c.c. of water was placed in a glass cylinder closed at one end with a parchment membrane. Into this was lowered a smaller cylinder closed at the bottom by a similar parchment membrane, and containing a square platinum electrode, 2 cm.^{2} in area. The outer cylinder was immersed in water and a platinum electrode, 4 cm.^{2} in area, was placed immediately below the parchment membrane. The latter electrode served as anode and a potential difference of 220 volts was placed across the centre compartment. The water in the innermost and outermost compartments was removed from time to time and the experiment was continued until the electrical resistance of the centre compartment remained constant. No flocculation of the glycogen occurred, and

the quality of the parchment membranes was such that no glycogen escaped. The product was isolated in the usual manner by the addition of alcohol. After being dried in a vacuum at 50°, it was indistinguishable from the glycogen originally used, except that the ash content had dropped to 0·37%. The phosphorus had not been removed. Electro-dialysis had therefore effected considerable reduction of the ash content without altering the chemical properties of the material. The purified glycogen showed $[\alpha]_D^{22^\circ}$ + 191·4° in water (c = 0·4) and gave a red colour with iodine (Found : C, 44·6; H, 6·5. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2%).

Acetylation of Glycogen.—(a) *With chlorine and sulphur dioxide as catalysts.* Glycogen (8 g.) was dissolved in water (80 c.c.), and absolute alcohol (800 c.c.) added with stirring. The precipitated glycogen was kept for 1 hour under alcohol and then ground with more alcohol. After being washed with ether, it was dried for 15 hours at room temperature in a vacuum desiccator. Glacial acetic acid (50 c.c.) containing a little chlorine was then added, and the mixture stirred for 30 minutes. After the addition of acetic anhydride (80 c.c.) containing a weight of sulphur dioxide equal to that of the chlorine, the mixture was stirred for 1 hour at 15° and then at 55°, until a clear solution was obtained (3 hours). This was poured into an excess of cold water and the precipitated glycogen triacetate was washed successively with water, alcohol, and ether and dried in a vacuum at 50° for several hours (yield, 95% of the theoretical). Glycogen triacetate was thus isolated as a heavy white powder, markedly different in bulk from the same weight of the light impalpable starch triacetate obtainable from starch under precisely similar conditions. It was neutral to litmus and had no action on boiling Fehling's solution. It decomposed at about 177° without showing a definite m. p. and had $[\alpha]_D^{22^\circ}$ + 163° in chloroform (c = 1·0). It was soluble in chloroform or acetone, but insoluble in ethyl alcohol, methyl alcohol, or water (Found : C, 49·9; H, 5·8. Calc. for $C_{12}H_{16}O_8$: C, 50·0; H, 5·6%).

Acetylation of glycogen by the above method, but with omission of the preliminary precipitation with alcohol, proceeded much more slowly, required an excessive quantity of catalyst, and gave inferior yields. The properties of the resulting acetate were identical with those described above.

(b) *In presence of pyridine.* The acetylation was carried out by Brigl and Schinle's method (*Ber.*, 1929, **62**, 99). When freshly precipitated glycogen (2 g.) was heated with pyridine (32 c.c.) and acetic anhydride (20 c.c.) at 80°, a clear solution was obtained after 20 hours, whereas the untreated material did not dissolve completely in 3 days. The products were isolated by pouring the clear solution

(filtered through glass wool if necessary) into water, the subsequent treatment being similar to that given above. The properties of the acetates were identical with those of the glycogen triacetate obtained by method (*a*). Yield, 87—90% of the theoretical.

Regeneration of Glycogen from the Acetate.—Glycogen triacetate (4 g.) was shaken with *N*/2-alcoholic potassium hydroxide (100 c.c.) at 20° for 30 minutes. The excess of alkali was then exactly neutralised with acetic acid and the regenerated glycogen was removed by filtration and ground with alcohol. The washing, with alcohol containing a little acetic acid, was repeated until the whole of the alkali had been removed; the glycogen was then dissolved in 100 c.c. of water, reprecipitated with alcohol (400 c.c.), washed with ether, and dried in a vacuum at 50° (yield, quantitative). This material possessed the characteristic properties of glycogen and in particular it gave with iodine a red-brown colour of the same intensity as did the original glycogen. The phosphorus content was small, but in other respects it was impossible to distinguish between this material and the glycogen which had been purified by electro-dialysis. $[\alpha]_D^{23°}$ + 191° in water (c = 0·4) (Found: C, 44·4; H, 6·5%).

Conversion of Glycogen Triacetate into Methylglucoside.—Glycogen triacetate (2·950 g.) was heated at 125° for 16 hours in a sealed tube containing 1% methyl-alcoholic hydrogen chloride (45 c.c.). The acid was then neutralised with silver carbonate, and the neutral solution evaporated to a syrup under diminished pressure. At this stage coagulation of colloidal silver occurred and, in order to eliminate this, the syrup was dissolved in ethyl alcohol and the solution filtered. On evaporation, a clear colourless syrup now remained which soon crystallised. The crystalline mass consisted exclusively of α- and β-methylglucosides (yield, 1·95 g., *i.e.*, 98% of the theoretical). M. p. 110—130°. $[\alpha]_D^{18°}$ + 96° in methyl alcohol (c = 0·6); $[\alpha]_D^{18°}$ + 106° (equilibrium value in methyl-alcoholic hydrogen chloride). These figures are in agreement with those recorded for the equilibrium mixture of α- and β-methylglucosides (Found: C, 43·1; H, 7·2; OMe, 15·9. Calc. for $C_7H_{14}O_6$: C, 43·25; H, 7·2; OMe, 16·0%). When the solid was recrystallised from absolute alcohol, pure α-methylglucoside, m. p. 165°, was readily obtained.

Simultaneous Deacetylation and Methylation of Glycogen Triacetate.—The glycogen triacetate (10 g.) was dissolved in acetone (100 c.c.) and treated at 50° during 90 minutes with 30% aqueous sodium hydroxide (112 c.c.) and methyl sulphate (40 c.c.), with vigorous stirring. After being heated for 30 minutes at 100°, the solution was cooled in ice, sulphuric acid added until the solution

was faintly alkaline, and the neutralisation of the sodium hydroxide completed by the addition of an excess of carbon dioxide. The mixture was then filtered and the liquid and solid portions were separately extracted with chloroform. The united chloroform extracts, when evaporated, gave methylated glycogen as a colourless glass, exactly similar in appearance and properties to methylated starch of equal methoxyl content. After one methylation the methoxyl content ranged from 35—40% according to the conditions of methylation, the latter figure being obtained by the use of potassium hydroxide in place of sodium hydroxide. No evidence was obtained of a definite arrest of the methylation process at any stage. The yield of methylated glycogen was 97—98% of the theoretical, based on the weight of glycogen triacetate employed.

Further methylations under similar conditions resulted in a regular rise in the methoxyl content and, after 6 or 7 treatments in all, the methylated glycogen gave analytical figures in close agreement with those required by the trimethyl derivative. The chloroform extract from the final methylation gave, on evaporation, trimethyl glycogen as a light yellow glass. This could be obtained as a white powder by dissolving it in acetone, evaporating the solution to a syrup, and boiling the residue with light petroleum (b. p. 40—60°). The petroleum was then removed by filtration, and the solid dried at 100° in a vacuum (yield, 90% of the theoretical, based on the weight of glycogen triacetate employed).

Trimethyl glycogen was indistinguishable in appearance and properties from trimethyl starch. It did not reduce Fehling's solution. It was soluble in chloroform or acetone, became gelatinous, without dissolving, in alcohol or water, and was insoluble in ether or light petroleum. M. p. 147°, with previous softening at 135°. $[\alpha]_D^{20°}$ + 208° in chloroform ($c = 1{\cdot}0$) (Found: C, 52·6; H, 8·0; OMe, 43·7. Calc. for $C_9H_{16}O_5$: C, 52·9; H, 7·8; OMe, 45·6%).

Hydrolysis of Trimethyl Glycogen.—Trimethyl glycogen (4·35 g.) was boiled for 24 hours with 2% methyl-alcoholic hydrogen chloride. The product (4·82 g.) was isolated in the usual way (see previous papers) and gave, on distillation, 2 : 3 : 6-trimethyl methylglucoside, b. p. about 100°/0·03 mm., $n_D^{16°}$ 1·4578 (yield, 80%) (Found: OMe, 51·8. Calc.: OMe, 52·6%). A viscid undistillable residue (15%) appeared to consist either of incompletely hydrolysed or of recondensed material. The 2 : 3 : 6-trimethyl methylglucoside, when hydrolysed by boiling with 5% aqueous hydrochloric acid, gave exclusively the crystalline 2 : 3 : 6-trimethyl glucose, which was obtained as a solid mass of needle-shaped crystals, m. p. 100°, free from adhering syrup and consisting of a mixture of the α- and β-forms

of 2 : 3 : 6-trimethyl glucose (yield, 95% of the theoretical). One recrystallisation from ether sufficed to give the α-form, m. p. 118°, alone or in admixture with an authentic specimen. $[\alpha]_D^{20^\circ}$ + 67° (equilibrium value in water; $c = 1{\cdot}0$).

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