725. *Properties of Periodate-oxidised Polysaccharides. Part I. The Preparation of Polymeric Substances containing Nitrogen from Oxidised Starches.*

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When *cyclo*hexylamine is added to an aqueous solution of periodate-oxidised starch, the double Schiff base of glyoxal separates. No such degradation takes place with *iso*nicotinoylhydrazine or thiosemicarbazide, or with a number of other reagents examined. With reagents of this kind, polymeric condensation products are formed. Some polymers formed from oxidised starch are described.

POLYSACCHARIDES containing α-glycol groups, after oxidation by periodate, can be degraded by treatment with phenylhydrazine (Barry, *Nature*, 1943, **152**, 537) and this process has been employed successfully in elucidating the fine structure of polysaccharides for which, in certain cases, the methylation procedure has proved ambiguous (Barry and Dillon, *Proc. Roy. Irish Acad.*, 1945, **50**, *B*, 349 ; Dillon and O'Colla, *ibid.*, 1951, **54**, *B*, 51 ; O'Colla, *ibid.*, **55**, *B*, 165, 321 ; Dillon, O'Ceallacháin, and O'Colla, *ibid.*, p. 331). Further studies have now been carried out in these laboratories on the properties of periodate-oxidised polysaccharides. In the first instance it has been shown that aqueous solutions of oxidised starch, on treatment in the cold with *cyclo*hexylamine, readily deposit the crystalline, double Schiff base of glyoxal, $(C_6H_{11}\cdot N\colon CH\cdot)_2$. Since *cyclo*hexylamine is a more stable reagent than phenylhydrazine and gives cleaner products it is possible that it may replace phenylhydrazine in the degradation mentioned above. This possibility is being examined. Simpler aliphatic bases, *e.g.*, ethylenediamine, bring about slow changes of the oxidised polysaccharide molecule without causing the separation of insoluble products.

In contrast to these degradative processes, however, we have now found that a large number and variety of other reagents condense with periodate-oxidised polysaccharides to form polymeric molecules. Readily isolatable polymers have been obtained on the addition, to aqueous solutions of oxidised starch, of *iso*nicotinoylhydrazine (isoniazid), thiosemicarbazide, *p*-aminobenzoic acid, the phenylenediamines, *p*-chloroaniline, etc. With hydrazine itself no precipitate is obtained until the solution is made faintly acid and then the solution sets to a tough colourless gel. The polymers formed from oxidised potato starch with isoniazid, thiosemicarbazide, and *p*-aminobenzoic acid are described in this paper.

The product (A), which separates when a dilute aqueous solution containing excess of isoniazid is added to an aqueous solution of periodate-oxidised starch can be obtained as a pale yellow amorphous powder, insoluble in water and in organic solvents, but soluble in ammonia and dilute alkali to pale yellow solutions. Decomposition takes place in these solutions and is particularly rapid in alkali and, if the alkaline solution is kept for a few minutes and then acidified, the precipitate always contains crystalline material. (A) is also soluble in dilute mineral acid and in moderately dilute acetic acid. After thorough drying, it gives analyses fairly correct for the polymeric dialdehyde in which each modified hexose unit is combined with one molecule of isoniazid.

When an aqueous solution of thiosemicarbazide is used, a similar polymeric product (B) is obtained. This is a very pale yellow amorphous powder which proved on analysis to contain approximately one thiosemicarbazide residue for each α-glycol grouping in the original starch molecule. When thoroughly dried, it swells and dissolves gradually in dilute alkali to a pale yellow viscid solution which on acidification gives a flocculent colloidal precipitate. (B) does not dissolve in dilute ammonia or in 50% acetic acid. It is also insoluble in dilute mineral acid at ordinary temperatures; it dissolves on boiling but cannot be reprecipitated by neutralisation. The reduced solubility and increased stability of this polymer, as compared with (A), are probably due to the formation of cross-linkages. A similar stability was encountered in other polymers in which the formation of cross-linkages might be expected, *e.g.*, those formed from *o*-phenylenediamine and *p*-aminobenzaldehyde thiosemicarbazone.

In the preparation of the compound (C) from *p*-aminobenzoic acid and oxidised starch, an aqueous solution of the acid was at first used. The yellow material separating under these conditions contained much less nitrogen than expected. On the assumption that the polymer separated because of its insolubility when the condensation had proceeded only to a limited extent, the experiment was repeated with an aqueous solution of sodium *p*-aminobenzoate. The reaction mixture was kept for 24 hours and then made faintly acid with acetic acid. The resulting precipitate had a much higher nitrogen content and approximated more nearly to that required for one aminobenzoic acid residue per α-glycol group. On account of its content of free carboxyl groups, (C) dissolved readily in sodium hydrogen carbonate and in ammonia solutions.

The oxidised-starch solutions employed in these experiments were prepared in about 2% strength on the water-bath, complete dissolution being obtained in 1½—2 hours. If the solutions were boiled, there was considerable degradation, and polymer (A) prepared from such a solution proves on microscopic examination to be accompanied by crystalline material. The structure of these polymers is discussed in the following paper.

It is of interest that (A) and (B) are strongly inhibitory of the growth of *Mycobacterium tuberculosis in vitro*, and (A), in particular, is highly active in experimental mouse infections. Detailed biological results will be published elsewhere.

EXPERIMENTAL

Oxidised Starch.—Potato starch (4·5 g.), dried *in vacuo* over phosphoric oxide, was kept in the dark for 48 hr. with sodium metaperiodate (6·18 g. in 200 c.c. of water). The consumption of periodate corresponded to about 0·9 mol. per glucose anhydride unit. The oxidised starch was separated and washed with water until free from periodate and iodate, then washed with alcohol and ether, and dried in a vacuum-desiccator over concentrated sulphuric acid. The yield was quantitative. The oxidised starch may be dissolved by warming it on the water-bath for 1½—2 hr. with 50 parts of water.

Degradation with cyclo*Hexylamine.*—Oxidised starch solution (40 c.c.; 1%) was shaken with *cyclo*hexylamine (2 c.c.) for 24 hr. The mixture became milky immediately and colourless crystals separated gradually, accompanied by some gum. The Schiff base was separated and recrystallised twice from aqueous alcohol, giving colourless glistening leaflets (0·15 g.), m. p. 149—150°, undepressed on admixture with the crystalline product separating from a mixture of glyoxal and *cyclo*hexylamine (Found: C, 76·2; H, 10·8; N, 12·9. $C_{14}H_{24}N_2$ requires C, 76·4; H, 10·9; N, 12·7%).

Isoniazid Polymer (A).—An aqueous solution of oxidised starch (5 g.; 500 c.c.) was stirred with a solution of isoniazid (9 g. in 100 c.c. of water). It became milky and after 1 hr. the precipitate was coagulated by the addition of 1 c.c. of glacial acetic acid. The product was separated on a sintered-glass filter, washed well with water, alcohol, and ether, and dried in a vacuum-desiccator to a pale yellow powder (7·1 g.). After further drying *in vacuo* at 100° (P_2O_5), the *product* (Found: C, 47·8, 46·4; H, 5·3, 5·4; N, 15·4, 15·4. $C_{12}H_{13}N_3O_5,H_2O$ requires C, 48·5;

H, 5·0; N, 14·1%) had $[\alpha]_D^{23}$ + 122° (in 0·1N-NaOH; *c* 0·796) (22 min.) falling to +49·8° (100 min.).

Thiosemicarbazide Polymer (B).—An aqueous solution of thiosemicarbazide (6 g. in 200 c.c.) was added to oxidised starch solution (5 g. in 500 c.c.) with stirring and the mixture kept for 1 hr. The *product* (7·4 g.) was isolated, washed, and dried as above (Found: C, 32·7; H, 5·7; N, 17·9, 16·6; S, 14·2, 13·8. $C_7H_{11}O_4N_3S,H_2O$ requires C, 33·4; H, 5·2; N, 16·8; S, 12·8%) and had $[\alpha]_D^{21}$ + 104° (in 5% NaOH; *c* 0·781) (18 min.) falling to + 107° (72 min.).

p-*Aminobenzoic Acid Polymer* (C).—Oxidised starch solution (1 g. in 50 c.c.) was added to aqueous sodium *p*-aminobenzoate (2 g. in 50 c.c.) and the mixture kept overnight. The clear solution on acidification with dilute acetic acid gave a lemon-yellow gelatinous precipitate which was difficult to filter. It was separated and washed on the centrifuge, dissolved in alcohol, and reprecipitated by ether. In this condition it can be separated by filtration through a sintered-glass filter. It was obtained as a bright yellow powder which was dried in the vacuum-desiccator (H_2SO_4) and for 3 hr. at 100°/20 mm. (Found: C, 50·6; H, 5·6; N, 3·8. $C_{13}H_{13}O_6N,H_2O$ requires C, 52·5; H, 5·1; N, 4·7%). When dissolved in 0·1N-NaOH this *compound* showed negligible rotation. When the condensation was effected with aqueous *p*-aminobenzoic acid in place of its sodium salt, the product contained only 3·0% of nitrogen.

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