731. *Properties of Periodate-oxidised Polysaccharides. Part II.* The Structure of Some Nitrogen-containing Polymers.*

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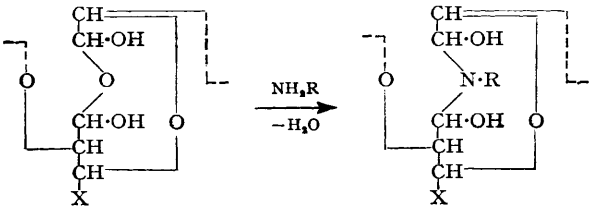
Cyclic structures are proposed for periodate-oxidised polysaccharides and for the polymers obtained on reaction with hydrazides and other bases.

IN Part I * nitrogen-containing polymers prepared from periodate-oxidised starch (oxystarch) were described. Analysis of these, and of others of a similar kind from alginic acid and inulin, has shown that they contain only one molecule of reagent per hexose unit, instead of two, as would be expected from reaction with a hexose unit containing two free aldehyde groups. The aldehyde functions of the oxy-hexose units are apparently modified in some way and it is suggested that they are united by a " hemialdal " linkage to give a seven-membered cyclic unit (I; $X = CH_2{\cdot}OH$), which reacts with the base as follows:

(I)



(II)

The mechanism of the periodate oxidation of α-glycols has been studied by various workers (Criegee, *Sitzungsber. Ges. Beford. Naturwiss. Marburg*, 1934, **69**, 25; Criegee, Kraft, and Rank, *Annalen*, 1933, **507**, 159; Price and Knoll, *J. Amer. Chem. Soc.*, 1938, **60**, 2726; Price and Knell, *ibid.*, 1942, **64**, 552; Duke, *ibid.*, 1947, **69**, 3054) and it has been suggested by Criegee that an intermediate in the reaction is a cyclic ester formed between the reagent and the α-glycol group. If this is correct it is reasonable to suppose that, throughout the reaction, the parent structure is retained and that a condensation (as hydrates) of the liberated aldehyde groups occurs through the pair of carbon atoms which formed the original α-glycol group.

A hemialdal linkage of this kind is embodied in one of the cyclic structures proposed by Hurd and his co-workers (Hurd, Baker, Holysz, and Saunders, *J. Org. Chem.*, 1953, **18**, 186) to explain the absence, as deduced from spectrophotometric data, of carbonyl groups in periodate-oxidised methyl α-D-glucopyranoside. Such a structure contains an extra molecule of water not present in the dialdehyde, and it is significant, in this connection, that the crystalline dialdehydes obtained from four different 6-deoxy-methylaldohexopyranosides (Dayton, MaClay, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1939, **61**, 1660) gave analyses agreeing with requirements for monohydrates. Furthermore, when heated in a vacuum to 65° over anhydrone, they sublimed without change in composition.

Spectrophotometric examination of oxycellulose (Rowen, Forziati, and Reeves, *ibid.*, 1951, **73**, 4484) yielded no evidence of the existence of carbonyl groups. This result does not lend support to the structure proposed for oxystarch by Michell and Purves (*ibid.*, 1942, **64**, 589), in which the aldehyde group at $C_{(3)}$ is visualised as forming a hemialdal linkage with the secondary alcoholic group at $C_{(6)}$, thus yielding a D-erythrofuranose ring, whilst the aldehyde group at $C_{(2)}$ remains free. Rowen *et al.* (*loc. cit.*) showed that light absorption due to carbonyl groups was still absent in the dialdehyde prepared from methyl 4 : 6-*O*-benzylidene-α-D-glycoside. Here there is no possibility of acetal formation, and these authors were of the opinion that the aldehyde groups were modified by hydration.

In the cyclic structure (I) $C_{(2)}$ and $C_{(3)}$ are asymmetric. Reactions taking place with the aldehyde groups in this cyclised form might be expected to be accompanied by rotational changes such as are observed when a solution of oxystarch is treated with various reagents. When ammonia or sodium *p*-aminobenzoate was added to a solution of oxystarch, marked

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* Part I, *J.*, 1953, 3610.

changes in the rotation of the solution occurred. With both reagents, an initial rapid fall from a *dextro-* to a *lævo*-value was followed by a slower return to a dextrorotatory value. From the oxystarch solution to which sodium *p*-aminobenzoate had been added and in which such rotational changes had taken place, a polymeric nitrogen-containing substance was isolated. These changes in rotation were in contrast to those obtained when oxystarch was treated with sodium hydroxide (cf. Head, *J. Text. Inst.*, 1947, **38**, 1395). An initial rise followed by a slow fall in rotation was accompanied by the disappearance of the potential aldehyde functions, possibly through the medium of an internal Cannizzaro reaction (Fry, Wilson, and Hudson, *J. Amer. Chem. Soc.*, 1942, **64**, 873), and after an hour no polymer was obtained on addition of isoniazid to the neutralised solution.

Evidence in support of structure (II) for the nitrogen-containing polymers has been obtained by degradation. When the oxystarch–isoniazid polymer was boiled with 50% acetic acid, crystalline glyoxal bis*iso*nicotinoylhydrazone separated. [The monohydrazone of glyoxal which is, presumably, formed first has been shown to be converted, under the above conditions, into the bishydrazone (Barry and Mitchell, *J.*, 1953, 3610)]. Degradation with phenylhydrazine and *cyclo*hexylamine proceeded in different ways. The former reagent yielded glyoxal bisphenylhydrazone and the latter, glyoxal bis*iso*nicotinoyl hydrazone. These different products could arise from the proposed cyclic structure (II) by scission at the $C_{(2)}$–N and $C_{(3)}$–N bonds, respectively. A somewhat similar result was obtained with the isoniazid polymer of alginic acid which may be formulated as (II; $X = CO_2H$). Degradation with acetic acid gave, in this case, glyoxal bis*iso*nicotinoylhydrazone, whereas phenylhydrazine and *cyclo*hexylamine yielded glyoxal bisphenylhydrazone, and the double Schiff base of glyoxal and *cyclo*hexylamine, respectively. No derivatives of the other fragment of the molecule separated during these degradations, but this is not surprising in view of the experiences of other workers. The D-erythrose part of oxidised starch or cellulose has only been characterised by conversion of the dialdehydes into diacids or dialcohols before hydrolysis (Jayme and Maris, *Ber.*, 1944, **77**, 383; Abdel-Akher, Hamilton, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1952, **74**, 4970).

The polymer obtained from the reaction of oxyinulin with isoniazid proved to be quite resistant to the methods used to degrade those derived from starch and alginic acid. The composition of this polymer was again approximately that required for the addition of one isoniazid molecule to each hexose unit. In contrast to the 7-membered ring which arises from the pyranose structure of starch, the furanose structure of inulin may form a six-membered cyclic structure both in the dialdehyde and in the isoniazid polymer. This may be a reason for the increased stability of the polymer although construction of molecular models showed that both rings are apparently strainless. In the larger seven-membered ring, however, an element of conformational instability is introduced because the substituents on two adjacent carbon atoms must be in the true *cis*-position (for a review, see Reeves, *Adv. Carbohydrate Chem.*, 1952, **6**, 108).

EXPERIMENTAL

Oxyinulin–Isoniazid Polymer.—Inulin {2 g.; $[\alpha]_D^{21}$ −35·3° (*c*, 0·957 in H_2O)} was added to an aqueous solution (1·25%; 250 c.c.) of sodium metaperiodate. The polysaccharide dissolved to give a clear solution which, after 48 hr., was reduced, while cooled in ice-water, with sulphur dioxide. The solution was neutralised with aqueous sodium hydrogen carbonate and made slightly acid by the addition of dilute acetic acid. Addition of isoniazid (2 g.) in water (20 c.c.) gave a white precipitate which was separated in the centrifuge and washed with water, methanol, and ether. The white amorphous *polymer* (2·12 g.) was dried in the vacuum-oven at 100° (P_2O_5) for 2 hr. (Found: C, 43·6; H, 5·6; N, 12·1. $C_{12}H_{13}O_5N_3,H_2O$ requires C, 48·5; H, 5·1; N, 14·1%).

Oxyalginic Acid–Isoniazid and –Thiosemicarbazide Polymers.—Sodium alginate ("Manucol"; 2 g.) was treated with a solution of sodium metaperiodate (1·25%; 214 c.c.). A clear solution was obtained and after 48 hr. 0·008 mole of periodate had been consumed. Ethylene glycol (5 c.c.) was added to the solution which was then dialysed against running water for 11 days. The dialysed solution was found, by evaporation of an aliquot portion, to contain 6·45 g./l. of oxidised material and had $[\alpha]_D^{22}$ +133°. Two 50-c.c. portions of this solution were treated with aqueous solution of isoniazid (50 c.c.; 1·15%) and thiosemicarbazide (50 c.c.; 1·12%). After 16 hr., acidification with dilute acetic acid gave amorphous precipitates which were isolated as above. The pale yellow isoniazid polymer weighed 0·394 g. (Found: C, 43·1, 45·5, 43·2; H, 5·2, 4·0, 5·0; N, 11·9, 13·8, 13·1. $C_{12}H_{11}O_6N_3,H_2O$ requires C, 46·5; H, 4·1; N, 13·6%). The *thiosemicarbazide* polymer (0·196 g.) was white (Found: C, 30·9, 29·7; H, 4·4, 4·5; N, 17·5, 17·5; S, 13·5, 13·0. $C_7H_9O_5N_3S,H_2O$ requires C, 31·7; H, 4·2; N, 15·9; S, 12·1%).

Degradation of Oxyalginic Acid–Isoniazid Polymer.—(a) *With acetic acid.* The polymer (0·5 g.) was refluxed with 50% acetic acid (15 c.c.) for 10 min. A clear brown solution was obtained which, on cooling, gave well-defined colourless rectangular crystals of glyoxal bis*iso*nicotinoylhydrazone, m. p. $>360°$, $\lambda_{\text{max.}}$ 366 mμ in 0·1N-sodium hydroxide (Barry and Mitchell, *loc. cit.*).

(b) *With phenylhydrazine.* The polymer, dissolved in sodium hydrogen carbonate solution, was treated with aqueous phenylhydrazine acetate. The yellow precipitate obtained was dissolved by warming the solution to which had been added a small quantity of ethanol. On cooling, characteristic saw-shaped yellow crystals of glyoxal bisphenylhydrazone separated, having m. p. 167—169° and mixed m. p. 168°.

(c) *With* cyclo*hexylamine.* A solution of the polymer (100 mg.) in saturated sodium hydrogen carbonate solution (5 c.c.) was treated with *cyclo*hexylamine (1 c.c.) in ethanol (3 c.c.). After 2 hr., solid material which had separated was redissolved by warming, and, on cooling, colourless spear-shaped crystals of the double Schiff base of glyoxal and *cyclo*hexylamine were obtained (m. p. 147—179°; mixed m. p. 148—149°).

Degradation of Oxystarch–Isoniazid Polymer.—(a) *With acetic acid.* The polymer described in Part I (0·5 g.) was refluxed with 50% acetic acid for 2 hr. The dark brown solution, on cooling, gave colourless rectangular plates (136 mg.) of glyoxal bis*iso*nicotinoylhydrazone, m. p. $>360°$, $\lambda_{\text{max.}}$ 366 mμ in 0·1N-sodium hydroxide.

(b) *With phenylhydrazine.* The polymer was dissolved in warm 50% acetic acid, and phenylhydrazine added to the cooled solution. After 24 hr., a reddish-brown product was obtained which on recrystallisation from aqueous methanol had m. p. 169°, unchanged on admixture with authentic glyoxal bisphenylhydrazone.

(c) *With* cyclo*hexylamine.* Treatment of a solution of the polymer in 50% acetic acid with a few drops of *cyclo*hexylamine gave, after 24 hr., colourless needles of glyoxal bis*iso*nicotinoylhydrazine, m. p. $>360°$, $\lambda_{\text{max.}}$ 366 mμ in 0·1N-sodium hydroxide. The degradation was not caused by the method of solution of the polymer since in a parallel experiment in which addition of *cyclo*hexylamine was omitted no solid separated.

Rotational Measurements.—The following Tables give the changes in the rotation of oxidised polysaccharide solutions on addition of dilute aqueous ammonia, sodium *p*-aminobenzoate, and sodium hydroxide. Measurements were made at 22° in 2-dm. tubes at intervals, until the solutions became too dark. 0·0585M-Sodium metaperiodate was used throughout.

(i) *With ammonia.* (*a*) Amylose (1·139 g.) was treated with periodate solution (144 c.c.) and after 44 hr., 0·0063 mole of oxidant had been consumed. The oxyamylose was separated by filtration and washed with water until free from iodate and periodate. The product was dissolved in water (100 c.c.) on the water-bath in 1 hr. The concentration of the solution of oxyamylose thus obtained was determined by evaporation to dryness of an aliquot portion. To 10 c.c. of this solution (1·088%), 10% aqueous ammonia (0·5 c.c.) was added and the change in rotation of the solution followed:

t (min.) ...	0	2	5	10	15	20	30	35
α	+0·52°	−0·71°	−0·61°	−0·46°	−0·35°	−0·26°	−0·15°	−0·05°
t (min.) ...	45	50	60	80	145	210	260	330
α	+0·13°	+0·17°	+0·24°	+0·34°	+0·55°	+0·69°	+0·72°	+0·80°

(*b*) Amylopectin (2 g.) was treated with periodate solution (252 c.c.) and after 44 hr. 0·0114 mole of oxidant had been consumed. From the product, treated as above, a solution (1·666%) of oxyamylopectin was obtained and the rotational changes on addition of ammonia (0·5 c.c.; 10%) to 10 c.c. of the solution were measured:

t (min.) ...	0	1	2	5	10	15	20	26
α	+0·70°	−1·07°	−0·97°	−0·77°	−0·53°	−0·36°	−0·29°	−0·09°
t (min.) ...	31	40	65	76	90	100	120	130
α	−0·05°	+0·19°	+9·45°	+0·56°	+0·58°	+0·66°	+0·68°	+0·69°

(*c*) Waxy maize starch (2 g.), treated with sodium metaperiodate solution (252 c.c.), consumed 0·0115 mole of oxidant in 44 hr. A 1·999% solution of the oxidised polysaccharide was obtained and treated with ammonia as above:

t (min.) ...	0	2	5	10	15	20	25	30	35	40
α	+0·81°	−0·26°	−1·14°	−0·87°	−0·67°	−0·47°	−0·36°	−0·22°	−0·11°	−0·04°
t (min.) ...	45	50	60	70	100	150	220	310	360	
α	+0·06°	+0·14°	+0·24°	+0·34°	+0·52°	+0·82°	+0·96°	+1·05°	+1·07°	

(*d*) Floridean starch was isolated from *Dilsea edulis* by the method described by Barry, Halsall, Hirst, and Jones (*J.*, 1949, 1468). The starch (2 g.) was oxidised with periodate solution (252 c.c.) and consumed in 44 hr. 0·0115 mole of oxidant. The polysaccharide dissolved and, after addition of ethylene glycol (5 c.c.) to the solution, it was dialysed against running water for 12 days. The addition of ammonia was made as before (*c* 0·84):

t (min.)	0	2	3	5	10	20	30
α	+0·24°	−0·59°	−0·61°	−0·57°	−0·39°	−0·37°	−0·23°
t (min.)	38	105	124	233	297	308	540
α	−0·21°	+0·04°	+0·05°	+0·17°	+0·20°	+0·21°	+0·29°

(*e*) Sodium alginate (2 g.) was treated with periodate (214 c.c.) and consumed in 46 hr. 0·008 mole of oxidant. The alginate dissolved to give a clear solution, which was dialysed for 11 days after addition of ethylene glycol (5 c.c.). A 0·645% solution of oxyalginate was obtained, 10 c.c. of which were treated with ammonia as above:

t (min.) ...	0	1	5	10	19	25	29	38
α	+1·75°	+1·49°	+1·38°	+1·29°	+1·12°	+1·03°	+1·01°	+0·90°
t (min.) ...	45	51	60	71	99	112	156	
α	+0·84°	+0·80°	+0·70°	+0·65°	+0·46°	+0·41°	+0·18°	

(ii) *With sodium* p-*aminobenzoate.* Potato starch (5 g.), treated with periodate solution (580 c.c.), consumed 0·026 mole of oxidant. The product, washed with water until free from periodate and iodate, was dissolved in warm water to give a 2% solution. To 10-c.c. quantities of this solution was added sodium *p*-aminobenzoate which dissolved readily giving clear solutions whose rotations in a 1-dm. tube were followed:

(*A*) Sodium *p*-aminobenzoate added: 0·249 g.

t (min.) ...	0	3	5	10	18	24	34	44	57	67
α	+0·91°	+0·51°	+0·44°	+0·34°	+0·28°	+0·27°	+0·26°	+0·26°	+0·31°	+0·37°
t (min.) ...	74	83	103	117	137	147	177	192	388	
α	+0·39°	+0·42°	+0·48°	+0·49°	+0·55°	+0·58°	+0·60°	+0·63°	+0·74°	

(*B*) Sodium *p*-aminobenzoate added: 0·500 g.

t (min.) ...	0	1·5	2	3	5	11	14	24	37
α	+0·91°	+0·16°	+0·07°	−0·03°	−0·10°	−0·11°	−0·09°	−0·07°	−0·02°
t (min.) ...	59	72	81	91	101	111	119	145	325
α	+0·07°	+0·12°	+0·12°	+0·17°	+0·18°	+0·20°	+0·25°	+0·30°	+0·42°

(*C*) Sodium *p*-aminobenzoate added: 0·750 g.

t (min.) ...	0	1·5	2·5	4·5	6	10	15	20	25	28
α	+0·91°	−0·26°	−0·40°	−0·45°	−0·44°	−0·38°	−0·34°	−0·31°	−0·29°	−0·29°
t (min.) ...	35	54	60	65	70	131	143	150	217	390
α	−0·29°	−0·24°	−0·21°	−0·20°	−0·16°	+0·05°	+0·09°	+0·09°	+0·24°	+0·24°

After 48 hr., the dark solution was acidified with dilute acetic acid, and the yellow precipitate obtained was separated in the centrifuge. The amorphous *product* was washed with water and precipitated from solution in ethanol with ether. A yellow powder (250 mg.) was obtained (Found: C, 50·6; H, 5·6; N, 3·8. $C_{13}H_{15}O_7N$ requires C, 52·5; H, 5·1; N, 4·7%).

(*D*) Sodium *p*-aminobenzoate added: 1·250 g.

t (min.) ...	0	2·5	5	10	15	20	25	30
α	0·91°	−0·79°	−0·76°	−0·65°	−0·62°	−0·53°	−0·50°	−0·46°
t (min.) ...	35	41	51	57	128	150	300	352
α	−0·44°	−0·44°	−0·35°	−0·32°	−0·10°	−0·09°	−0·01°	+0·08°

(iii) *With sodium hydroxide.* 2N-Sodium hydroxide (1 c.c.) was added to a 2% solution of oxystarch (20 c.c.) and the rotational changes of the mixture in a 2-dm. tube were noted :

t (min.) ...	0	3·5	5	5·5	6·5	7·5	8	20·5	22	97
α	+1·10°	+1·74°	+1·89°	+1·87°	+1·84°	+1·90°	+1·90°	+1·86°	+1·92°	+1·68°

Next morning, the dark solution when acidified with dilute acetic acid, gave no precipitate with isoniazid.

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