442. *The Synthesis of Polysaccharides.*

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Three non-dialysable branched glucans have been synthesised by the polymerisation of molten glucose. Details of their preparation and some of their properties are given.

In a preliminary communication [1] it was reported that molten glucose can be polymerised readily in the presence of strongly acidic cationic resins. Recently [2] the investigation of the disaccharides formed in the initial stages of the melt polymerisation was described. This Paper describes three non-dialysable branched glucans synthesised by this technique; details of their preparation and some of their properties are listed in the Table. In each

Preparation of non-dialysable branched glucans from glucose.

	Glucose : resin	Time of heating (hr.)	Temp.	Yield (%)	$[\alpha]_D$ in H_2O	D.P.[3]
Glucan 1 ...	5 : 8	9	150°	36	+73·0	50
Glucan 2 ...	1 : 1	0·75	150	25	+64·3	24
Glucan 3 ...	1 : 1	15	100	13	+85·0	18

case the polymer was obtained as a white powder when the aqueous extract of the reaction mixture was dialysed and then concentrated under reduced pressure. It was shown by paper chromatography that the polymers were free from lower saccharides.

Analysis of the hydrolysate of glucan 1 showed that it contained 91% of glucose; it consumed 1·42 moles of periodate and yielded 0·47 moles of formic acid per anhydroglucose unit. The presence of unoxidised units in the oxoglucan was detected by phenylhydrazine degradation [4] to yield glucosazone, and by the determination of 6·2% of glucose in the hydrolysate of the oxoglucan.

[1] O'Colla and Lee, *Chem. and Ind.*, **1956**, 522.
[2] O'Colla, Lee, and McGrath, *J.*, **1962**, 2730.
[3] Shaffer-Somogyi, *J. Biol. Chem.*, **1933**, **100**, 695.
[4] Barry and Mitchell, *J.*, **1954**, 4020.

Hydrolysis of glucan 1 and its methyl ether by acids yielded material identical with the resin used as catalyst. As solvents did not separate the insoluble resin from the soluble polysaccharide it is probable that the compounds were combined chemically. The presence of the resin accounted in part for the low methoxyl content (38·3%) of the methylated glucan (tri-*O*-methylanhydroglucose contains 45·6% OMe). Other workers have reported [5] that highly branched synthetic glucans are difficult to methylate fully, and absorption due to hydroxyl groups in the infrared spectrum of methylated glucan 1 indicated incomplete methylation in this case as well.

A quantitative assay of the hydrolysate of methylated glucan 1 gave mono-, di-, tri-, and tetra-*O*-methylglucose in the molecular ratio 2 : 4 : 11 : 5. As the glucan was incompletely methylated only qualitative deductions are permissible, but the high yield of tetra-*O*-methylglucose indicated a highly branched structure for the glucan. Chromatographic and ionophoretic evidence indicated the presence of 2,3,4-, 2,4,6-, and 2,3,6-tri-*O*-methylglucose in the tri-*O*-methyl fraction, pointing to a randomly linked glucan.

Analysis of the hydrolysate of methylated glucan 2 showed that it was essentially the same as glucan 1 in linkages and degree of branching.

Experimental

Paper chromatography was carried out on Whatman No. 1 paper in: A, n-butanol–pyridine–water (6 : 4 : 3 v/v); B, ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4 v/v); C, n-butanol–ethanol–water (5 : 1 : 4 v/v) (upper phase); D, n-butanol (40%)–ethanol (10%)–water (49%)–ammonia (1%). Evaporations were carried out on a water-bath below 50°.

Glucan 1.—A mixture of D-glucose (25 g.) and Amberlite IR-120(H) (40 g.) was heated at 145—150°/20 mm. for 9 hr. The temperature of the glycerol bath in which the reaction flask was immersed was quickly elevated to 100°; the pressure was reduced to 20 mm. and the temperature was increased and maintained at 145—150° for 9 hr. When cool, the melt was exhaustively extracted with cold water, and the aqueous extract dialysed against running water for 3 days. The residue inside the membrane was isolated as a white powder (8·82 g.) after evaporation to dryness.

Glucan 2.—A mixture of D-glucose (10 g.) and Amberlite IR-120(H) (10 g.) was heated at 145—150°/20 mm. for 45 min. (as described above); 2·3 g. of the amorphous product were isolated.

Glucan 3.—A mixture of D-glucose (10 g.) and Amberlite IR-120(H) (10 g.) was heated on a boiling-water bath for 15 hr. at a pressure of 20 mm. The glucan was isolated as a light yellow powder (1·2 g.).

Examination of Glucan 1.—*Reducing sugars liberated during acidic hydrolysis.* Glucan 1 (20 mg.) was hydrolysed on a boiling-water bath for 10 hr. with N-sulphuric acid (2 ml.); the solution was diluted with water and freed from ions. The hydrolysate was evaporated to dryness and examined on paper chromatograms, with solvents A and B, for 24 hr. Only one sugar component (R_F same as glucose reference spot) was revealed on development with aniline hydrogen phthalate. The glucan (20 mg.) was hydrolysed for 12 hr. with N-sulphuric acid at 100°, and the volume of the neutralised hydrolysate was adjusted to 50 ml. The glucose present was determined with the Schaffer-Somogyi reagent.[3] The reducing power of the hydrolysate corresponded to a conversion into glucose of 90—91%.

Periodate oxidation. The glucan (0·1164 g.) was oxidised in 0·99N-sodium metaperiodate (15 ml.) for 2 days. Samples (0·5 ml.) were removed at intervals and the periodate uptake and formic acid liberation determined by the methods of Malaprade [6] and Halsall, Hirst, and Jones.[7] Oxidation was complete after 20 hr. and formic acid ceased to be released after 40 hr. 1·42 moles of periodate were consumed and 0·46 moles of formic acid were produced per anhydroglucose unit.

Degree of polymerisation. Preliminary experiments indicated that glucan 1 developed maximum reducing power when heated for 80—90 min. with the Shaffer-Somogyi reagent.[3] The reducing equivalent of each glucan was determined on the assumption of one reducing

[5] Micheel and Böckmann, *Angew. Chem.*, 1960, **72**, 209.

[6] Malaprade, *Compt. rend.*, 1928, **186**, 382; Schwarz, *Chem. and Ind.*, 1954, 1000; Rankin and Jeanes, *J. Amer. Chem. Soc.*, 1954, **76**, 4435.

[7] Halsall, Hirst, and Jones, *J.*, 1947, 1427.

group per molecule (Table). However, alkaline methods are not reliable in measurements of the degree of polymerisation of carbohydrates.[8]

Treatment of the periodate-oxidised glucan with phenylhydrazine. A solution containing the glucan (0·10 g.) in sodium metaperiodate (2N; 8 ml.) was left at room temperature for 2 days, after which excess of periodate was reduced with ethylene glycol. After removal of iodate as the barium salt, the filtrate was treated with phenylhydrazine–acetic acid–water (2 : 2 : 5 v/v) (4 ml.). The yellow precipitate was separated, and heated at 95° for 20 min. with phenylhydrazine in acetic acid (50%; 4 ml.); the material dissolved with frothing. The clear wine-coloured solution was poured into ethanol (5 vol.). On evaporation to small bulk a mixture of osazones crystallised. Paper chromatography as described by Barry and Mitchell [4] revealed glucosazone.

Hydrolysis of the oxidised glucan. The periodate-oxidised glucan 1 (130 mg.) was hydrolysed with sulphuric acid and neutralised. The residual glucose was estimated by the method of Flood *et al.*[9] with rhamnose as reference, and corresponded to 6·2% of the glucan.

Methylation. The glucan (4 g.) was methylated according to Haworth, Hirst, and Webb.[10] After three such methylations the crude methyl ether was purified by extraction with chloroform, and the chloroform extract was evaporated, to yield a product having OMe, 31·54%. Five further methylations were carried out with methyl iodide and silver oxide under anhydrous conditions. The methylated glucan (1·09 g.) showed $[\alpha]_D$ +65° (*c* 0·20 in $CHCl_3$) (Found: OMe, 38·3. Calc. for $C_9H_{16}O_5$: OMe, 45·6%).

Identification of the O-*methyl sugars obtained from the methylated glucan.* The glucan ether (0·5 g.) was boiled for 7½ hr. with 8% methanolic hydrogen chloride (14 ml.) and then, after removal of the solvent, with 4% hydrochloric acid at 100° for 6 hr. The solution was freed from ions, and evaporated to a syrup. Paper chromatograms of the hydrolysate, irrigated for 27—30 hr. in solvents C and D, showed seven components after being sprayed with aniline hydrogen phthalate. The R_G values of the methyl sugars (in solvent C); and the colour of the stain, are as follows: 2,3,4,6-tetra-*O*-methylglucose—red, 2,3,4-tri-*O*-methylglucose 0·87 red, 2,4,6-tri-*O*-methylglucose 0·77 red, 2,3,6-tri-*O*-methylglucose 0·80 brown, 2,3-di-*O*-methylglucose 0·59 brown, di-*O*-methylglucose 0·54 red, monomethyl glucose 0·29; there was also a trace of glucose.

Quantitative assay of the O-*methyl sugars present in the hydrolysate.* A sample (20 mg.) of the above hydrolysate was subjected to paper chromatography as described by Hirst, Hough, and Jones.[11] The following approximate molar proportions were indicated: 2,3,4,6-tetra-*O*-methylglucose, 5; the mixture of tri-*O*-methylglucoses, 11; the di-*O*-methylglucose mixture, 4; monomethylglucose, 2.

The remainder of the hydrolysate was fractionated on Whatman No. 3 paper (18 × 22 in.) using solvent C. Fraction 1 was identical with a spot of 2,3,4,6-tetra-*O*-methylglucose, on paper chromatography in solvents C and D. The syrup (24 mg.), treated with aniline (18 mg.) in ethanol under reflux for 2 hr., gave 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine, m. p. 134—135°. Fraction 2 was subjected to paper chromatography for 30 hr. in solvent C, and sprayed with aniline hydrogen phthalate. The principal component had a colour similar to, and R_F equal to, that of 2,3,4-tri-*O*-methylglucose. The two other components, of approximately equal intensity, had staining properties and R_F values identical with those of 2,3,6- and 2,3,4-tri-*O*-methyl glucose. Analysis of fraction 3 by paper chromatography indicated a mixture of di-*O*-methyl ethers. One component had the same R_F and staining properties as 2,3-di-*O*-methylglucose; the second had R_G 0·58 (red stain) in solvent D. When an aqueous solution of this mixture (18 mg.) was oxidised with sodium metaperiodate under buffered conditions as described by Bell,[12] crystals of the dimedone–formaldehyde condensation product were obtained, m. p. and mixed m. p. 188—189°. Demethylation [13] of this fraction yielded glucose, detected by paper chromatography. Fraction 4 gave a brown coloration with aniline hydrogen phthalate and had R_G 0·29 in solvent C. The M_G value agreed with that reported [14] for 3-*O*-methylglucose.

[8] Launer and Tomimatsu, *Analyt. Chem.*, 1961, **33**, 79.
[9] Flood, Hirst, and Jones, *J.*, 1948, 1679.
[10] Haworth, Hirst, and Webb, *J.*, 1929, 2483.
[11] Hirst, Hough, and Jones, *J.*, 1949, 928.
[12] Bell, *J.*, 1948, 994.
[13] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[14] Foster, *J.*, 1953, 982.

Examination of Glucan 2.—*Acidic hydrolysis.* The glucan (20 mg.) was hydrolysed with N-sulphuric acid (3 ml.) at 100° for 7 hr. After the removal of sulphate ions with barium carbonate, the neutral hydrolysate was examined by paper chromatography, as described above; the single component had an R_F value equal to that of the glucose reference spot.

Periodate oxidation. Oxidation of glucan 2 (0·103 g.) in 0·93N-sodium metaperiodate (10 ml.) was complete in 22 hr.; 1·54 moles of periodate were consumed per mole of anhydroglucose.

Methylation. The glucan (0·91 g.) was subjected to a single methylation, following the procedure of Haworth, Hirst, and Webb.[10] The chloroform-soluble product was re-methylated with methyl iodide in dimethylformamide as described by Kuhn *et al.*[15] The polysaccharide ether was isolated as a white, friable solid (0·54 g.; OMe, 39·3%). A paper chromatogram of this water-soluble methyl ether, in solvent C (27 hr.), revealed no trace of free *O*-methyl-sugars. The methyl ether (24 mg.) was heated with formic acid [16] (90%; 1 ml.) in a sealed tube at 100° for 8 hr. and then, after removal of the formic acid, with water at 90° for ½ hr. When the aqueous solution was evaporated to dryness, a clear syrup was obtained; this was treated once more with water to complete the hydrolysis of formyl esters. A paper chromatogram of this hydrolysate, in solvent D (24 hr.), sprayed with aniline hydrogen phthalate, revealed the presence of seven *O*-methylglucoses corresponding to the seven *O*-methyl-derivatives of glucose which were detected by paper-chromatographic examination of a hydrolysate of the methyl ether of glucan 1. The infrared spectrum of the glucan 2 methyl ether also showed a small absorption peak at 3400 $cm.^{-1}$ (OH).

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[15] Kuhn, Löw, and Trischmann, *Chem. Ber.*, 1955, **88**, 1502.
[16] Jones and Painter, *J.*, 1959, 578.