523. *The Action of Cation-exchange Resins on* D-*Glucose.*

By P. S. O'Colla, E. E. Lee, and D. McGrath.

The disaccharide fraction of the mixture obtained by the action of cationic resins on molten glucose consists of isomaltose, gentiobiose, cellobiose, nigerose, laminaribiose, trehalose, and probably 5-*O*-D-glucopyranosyl-D-glucose. The quantities of the various disaccharides formed have been compared with those for the reversion of glucose in the presence of mineral acid. The most striking differences are the absence of maltose and the high incidence of 1,3- and 1,6-linked disaccharides in the resin-catalysed reversion mixture.

Cation-exchange resins have been used in several instances recently as catalysts in place of mineral acids. Wadman [1] showed that the resin Amberlite IR-120(H) is an effective catalyst in the preparation of certain glycosides and in the formation of di-*O*-isopropylideneglucose. Cadotte *et al.*[2] prepared the glycosides of pentoses, hexoses, uronic acids, and methylated sugars under the catalytic influence of a number of cation-exchange resins. Zemplen and Kisfaludy [3] found that when glucose, water, and a phenol-sulphonic acid resin were heated at 70° for 72 hours anhydro-, di-, and poly-saccharides

[1] Wadman, *J.*, 1952, 3051.
[2] Cadotte, Smith, and Spriestersbach, *J. Amer. Chem. Soc.*, 1952, **74**, 1501.
[3] Zemplen and Kisfaludy, *Acta Chim. Acad. Sci. Hung.*, 1954, **76**, 2221.

were formed, and they isolated gentiobiose. Anno *et al.*[4] reported the isolation of laminaribiose as its crystalline octa-acetate from the reversion product obtained when glucose was treated with a cation-exchange resin and mineral acid. Taufel *et al.*[5] reported chromatographic evidence that a number of sugars undergo reversion in the presence of ion-exchange resins.

In our initial report [6] on this subject we observed that polyglucans were readily formed by heating an intimate mixture of glucose and Amberlite IR-120(H) (analytical grade). During the structural investigations on three such non-dialysable polymers, it was considered desirable to get some information about the initial stages of polymerization. Consequently, glucose was heated with the cation-exchange resin at 130° for 10 minutes only, and the products of low-molecular weight were fractionated on a charcoal–Celite column.[7] The following sugars, in the order of their appearance off the column, were identified: D-glucose, lævoglucosan, trehalose, isomaltose, 5-*O*-D-glucopyranosyl-D-glucose (?), gentiobiose, nigerose, cellobiose, and laminaribiose. Isomaltose, gentiobiose, and laminaribiose, which were isolated in the approximate ratio of 10 : 5 : 2, were characterised as crystalline octa-acetates. It was found that all the disaccharides which were detected on the paper chromatogram and paper ionophoretogram with aniline hydrogen phthalate could also be detected with the alkaline triphenyltetrazolium chloride reagent which detects all reducing glucosaccharides except those with a 2-*O*-substituent.[8] The presence of these disaccharides in the initial stages of polymerization indicated that such linkages might be expected to occur in the higher polymers formed. This prediction was confirmed by investigations (forthcoming publication) on three non-dialysable polymers formed by polycondensation of molten glucose. Examination of the polysaccharides by methylation and periodate oxidation showed that the linkages were mainly of the 1,6-type; 1,4- and 1,3-links also occurred, but less frequently.

The reversion of glucose in aqueous mineral acid solution is a reversible reaction,[9] and it is probable that the cationic resin exercises some steric control in the rapid polymerization described here. Hence, it is of interest to compare the disaccharides formed in the thermodynamically controlled reaction, as reported by a number of investigators, with the products obtained in this investigation (Table). The most striking differences are the absence of maltose and the high incidence of 1,3- and 1,6-linked disaccharides in the resin-catalysed reversion mixture.

Finally, it may be noted that the identification of the disaccharide group of " Hydrol " now includes laminaribiose which has been isolated and identified as the crystalline octa-acetate by Sato *et al.*[10]

Yields (%) of disaccharides from glucose reversion mixtures.

Disaccharide link	1,1-	1,2-α-	1,2-β-	1,3-α-	1,3-β	1,4-α	1,4-β	1,5-	1,6-α	1,6-β
Thompson *et al.** ...	0·036	—	0·086	0·111	—	0·202	0·161	—	2·12	1·745
Sowden *et al.*†	0·160	—	—	—	—	0·165	0·040	—	1·66	1·0
		0·044				0·054				
Peat *et al.*‡				0·025	0·008			—	0·105	0·071
Present work	Trace	—	—	2·40	1·45	Nil	0·48	0·30	7·0	5·0

* Thompson, Anno, Wolfrom, and Anatome, *J. Amer. Chem. Soc.*, 1954, **76**, 1309. † Sowden and Spriggs, *J. Amer. Chem. Soc.*, 1956, **78**, 2503. ‡ Peat, Whelan, Edwards, and Owen, *J.*, 1958, 586.

Experimental

Polymerization.—Amberlite IR-120(H) (20 g.) and D-glucose (20 g.) were intimately mixed and heated in an open-flask at 130° for 10 min. The light yellow viscous mixture was allowed

[4] Anno, Seno, Nakamura, Saito, and Hoshi, *Bull. Agric. Chem. Soc. Japan*, 1959, **23**, 67.
[5] Taufel, Steinbach, and Grunert, *Nährung*, 1961, **5**, No. 1, p. 66.
[6] O'Colla and Lee, *Chem. and Ind.*, 1956, 522.
[7] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.
[8] Wallenfels, *Naturwiss.*, 1950, **37**, 491.
[9] Frahm, *Ber.*, 1941, **74**, 622.
[10] Sato, Wanatabe, and Aso, *Chem. and Ind.*, 1958, 887.

to cool to room temperature and then repeatedly extracted with cold water, to yield a yellow acidic solution.

Fractionation of the Products.—The neutralized solution was evaporated to dryness under reduced pressure, and the resulting syrup added in water to a charcoal–Celite column (1 : 1 by wt.; 5 × 81 cm.). The column was irrigated under pressure, applied by a constant head of eluant 6 ft. above the top of the column, first with water and then by stepwise elution with aqueous ethanol. Fractions of 500 ml. were collected and evaporated to dryness under reduced pressure; each residue was examined on paper chromatograms which were irrigated in each of the solvent systems butanol–acetic acid–water (4 : 1 : 5 v/v) and butanol–pyridine–water (6 : 4 : 3 v/v). The chromatograms were developed with alkaline permanganate [11] (reducing and non-reducing sugars) and aniline hydrogen phthalate [12] (reducing sugars).

The column was washed with water (15 l.; fraction 1), 2% ethanol (10 l.; fraction 2), 5% of ethanol in water (3 + 14 l.; fractions 3 and 4), 7½% of ethanol (14 l.; fraction 5), 10% of ethanol (10 l.; fraction 6), the change in concentration being made when the effluent reacted negatively to Benedict's reagent. Each fraction was evaporated under reduced pressure to a syrup and purified by repeated dissolution in methanol followed by evaporation to dryness.

Fraction 1. Analysis by paper chromatography and paper ionophoresis [13] indicated the presence of glucose and a trace of lævoglucosan.

Fraction 2. Paper chromatography showed the presence of a trace of both glucose and a non-reducing disaccharide, tentatively identified as trehalose by comparison with the authentic disaccharide.

Fraction 3. Chromatographic analysis indicated the presence of isomaltose only; this was confirmed by examination on the paper ionophoretogram. The amorphous material [0·501 g.; $[\alpha]_D$ +120·5° (*c* 1·52 in H_2O)] with anhydrous sodium acetate and acetic anhydride yielded the octa-acetate as a syrup (0·44 g.). Crystallization from ethanol gave the crystalline acetate, m. p. and mixed m. p. 145—146°, $[\alpha]_D$ +96·1° (*c* 0·52 in $CHCl_3$).

Fraction 4. This fraction contained isomaltose and less of an unknown disaccharide which was probably 5-*O*-D-glucopyranosyl-D-glucose (total yield 1·09 g.). The mixture was fractionated further by partition chromatography on Whatman No. 17 paper in butanol–acetic acid–water. A clearly defined separation could not be achieved, because of the closeness of the chromatographic mobilities of the two disaccharides. However, by eluting the main portion of the upper fraction with water, chromatographically pure isomaltose was isolated (0·368 g.). The amorphous material with sodium acetate–acetic anhydride yielded a syrup (0·310 g.) which, when recrystallized from ethanol, gave crystalline β-isomaltose octa-acetate, m. p. 145—146°, $[\alpha]_D$ +96° (in $CHCl_3$). By elution of a portion of the lower fraction with water and concentration of the eluate under reduced pressure, an amorphous product was obtained (0·307 g.), $[\alpha]_D$ +89° (in H_2O). Examination of this product on the paper ionophoretogram showed that the unknown disaccharide (M_G 0·46) was contaminated with some isomaltose (M_{iso} 0·71). This mixture (12 mg.) was hydrolysed with N-sulphuric acid (2 ml.) for 2 hr. on a boiling-water bath. The hydrolysate was neutralized and concentrated under reduced pressure to a syrup which, when examined on the paper chromatogram in each of the two solvents described above, gave one spot only, corresponding to glucose. Acetylation of the mixture (0·30 g.), as described above, resulted in the isolation of a syrup which, despite repeated attempts at crystallization from ethanol, failed to yield a crystalline acetate.

Fraction 5. The first 10 l. of the eluate from which this fraction was obtained were shown by paper chromatography and ionophoresis to contain both nigerose and gentiobiose. The mixture of sugars obtained {1·47 g.; $[\alpha]_D$ +50·4° (*c* 1·03 in H_2O)} was refractionated on Whatman no. 17 paper. From the upper fraction gentiobiose was isolated as an amorphous powder (0·445 g.). Acetylation of the powder and recrystallization of the product from ethanol yielded β-gentiobiose octa-acetate (0·378 g.), m. p. 194—195°, $[\alpha]_D$ −5·8° (*c* 4·46 in $CHCl_3$). Extraction of the lower fraction on the paper with water and evaporation of the solvent yielded an amorphous product (0·492 g.), $[\alpha]_D$ +56° (in water). Chromatography showed the presence of nigerose and gentiobiose, and acetylation of the mixture yielded a syrupy acetate (0·48 g.). Fractional crystallization from ethanol separated gentiobiose octa-acetate (0·03 g.). No further crystallization took place. Continued elution of the column with 7·5% of ethanol in

[11] Lemieux and Bauer, *Analyt. Chem.*, 1954, **26**, 920.
[12] Partridge, *Nature*, 1949, **164**, 443.
[13] Foster, *J.*, 1953, 982.

water yielded 4 l. of eluate which on chromatographic examination was found to contain gentiobiose, cellobiose, and a trace of nigerose. This mixture of sugars was isolated as an amorphous powder (0·132 g.), $[\alpha]_D$ +28° (in H_2O). From the rotations of the various fractions and the relative densities of the spots on paper chromatograms, it was calculated nigerose and cellobiose were present in the ratio of 5 : 1.

Fraction 6. This fraction was shown, chromatographically and ionophoretically, to contain laminaribiose together with some trisaccharide {total yield, 0·650 g.; $[\alpha]_D$ +47° (*c* 0·130 in H_2O)}. Pure laminaribiose was isolated by chromatography on Whatman no. 3 paper (18¼ × 22½ in.) as described above, being obtained as an amorphous powder (0·210 g.), $[\alpha]_D$ +16·7° (*c* 0·840 in H_2O), that with sodium acetate–acetic anhydride yielded a syrupy octa-acetate (0·180 g.). Crystallization from ethanol yielded the crystalline β-acetate, m. p. and mixed m. p. 159—160°, $[\alpha]_D$ −28·8° (*c* 0·77 in $CHCl_3$).

CHEMISTRY DEPARTMENT, UNIVERSITY COLLEGE,
GALWAY, IRELAND.

[*Received, January 15th*, 1962.]