*The Structure of the Oligosaccharides produced by the Enzymic Breakdown of Pectic Acid. Part II.**

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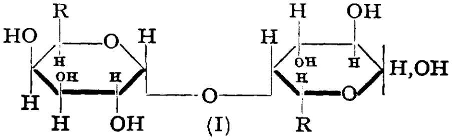
The disaccharide produced by enzymic hydrolysis of apple pectic acid has been converted into 4-*O*-α-D-galactopyranosyl-D-galactopyranose, and its structure thereby proved.

In Part I * it was shown that the mixture of oligosaccharides produced by the action of the enzymes of *Aspergillus foetidus*, Thom and Raper, on apple pectic acid contained a disaccharide and a trisaccharide. Evidence was advanced which indicated that the disaccharide consisted of two D-galactopyruronic acid residues joined by an α-glycosidic linkage (I; $R = CO_2H$). This diuronide has now been converted into the corresponding galactobiose (cf. Whistler and Conrad, *J. Amer. Chem. Soc.*, 1954, **76**, 1673).



(I)

The major difficulty involved in this transformation was the reduction of the carboxyl groups of the disaccharide without concomitant reduction of the aldehyde group. Protection was required for the aldehyde group during reduction of the carboxyl groups, which could yet be removed later under conditions which did not break the glycosidic linkage. Orthoesters possess such properties : they are apparently not affected by lithium aluminium hydride (cf. Claus and Morgenthau, *J. Amer. Chem. Soc.*, 1951, **73**, 5005) and are hydrolysed with great ease by cold dilute acids (Bott, Haworth, and Hirst, *J.*, 1930, 1395).

Sugar orthoesters are formed under certain conditions from acetobromo-sugars but they are more readily prepared by reaction of the sugar with an orthoester in the presence of an acid catalyst (Astle, Zaslowsky, and Lafyatis, *Ind. Eng. Chem.*, 1954, **46**, 788; Schwarz, *J.*, 1954, 2644; Freudenberg and Jakob, *Ber.*, 1941, **74**, 162). When the disaccharide was shaken with trimethyl orthoformate in the presence of a little hydrochloric acid, it rapidly dissolved and the solution then gave a negative Fehling's test. Removal of the catalyst and esterification of the carboxyl groups was effected by ethereal diazomethane. The resulting syrupy methyl ester derivative was reduced with lithium aluminium hydride, and neutral disaccharide isolated after hydrolysis of the orthoformyl groupings with cold dilute acid. The crystalline disaccharide was identical in all respects with the disaccharide isolated by Whistler and Conrad (*loc. cit.*) from Okra mucilage and is therefore correctly represented by (I; $R = CH_2{\cdot}OH$), and the digalacturonic acid by (I; $R = CO_2H$).

Experimental

The following solvent systems were used in chromatographic separations : (*a*) ethyl acetate–acetic acid–water (9 : 2 : 2, v/v), (*b*) *n*-butanol–pyridine–water (10 : 3 : 3, v/v), and (*c*) *n*-butanol–ethanol–water (4 : 1 : 2, v/v). *p*-Anisidine hydrochloride (1%, w/v) in *n*-butanol or silver nitrate solution in acetone followed by sodium hydroxide in ethanol was used to locate the sugars. Optical rotations were determined at 20° ± 2°. Solvents were evaporated under reduced pressure.

Preparation of the Orthoester.—The crystalline diuronide (1·95 g.) was added to trimethyl orthoformate (40 c.c.) to which concentrated hydrochloric acid (7 drops) had been added. The solid slowly dissolved when the mixture was shaken, and dissolution was complete in 15 hr. The cooled solution was neutralized by the addition of diazomethane in ether, and the resultant lemon-yellow solution then evaporated to a syrup which was dissolved in dioxan (10 c.c.). This

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* Part I, *J.*, 1954, 1361.

solution was added with stirring to a solution of lithium aluminium hydride (2 g.) in dioxan. After 2 hr. excess of the hydride was destroyed by the addition, first, of ethyl acetate and then of water. The slurry was filtered and the filtrate evaporated to a syrup which was extracted with acetone. The acetone extracts were evaporated to a syrup, and the residue extracted with chloroform. Removal of the solvent left an oil (1·6 g.). Chromatographic examination (solvents *b* and *c*) indicated that it was a complex mixture. The syrup dissolved in water to yield a milky neutral solution containing derivatives of the required galactobiose.

Hydrolysis of the Galactobiose Derivatives.—The syrup was dissolved in 0·1N-hydrochloric acid (20 c.c.) and the solution kept at room temperature for 24 hr. It was not possible to follow any change in optical rotation but chromatographic examination indicated that hydrolysis was taking place with formation of traces of galactose and a sugar which had the chromatographic characteristics of a disaccharide. After 24 hr. the solution was passed down a column of Amberlite resin IR-4B (acetate form), and the effluent from the column concentrated to a syrup. Addition of acetone–methanol caused the syrup to dissolve and in a day or so crystals of 4-*O*-α-D-galactopyranosyl-D-galactose separated. The total yield of product after recrystallisation from methanol–*n*-butanol–water mixture was 0·31 g. This substance moved at the same rate as an authentic specimen which was kindly provided by Dr. R. L. Whistler of Purdue University. Its rate of movement relative to lactose was 1·0 in solvent (*b*) and 1·1 in solvent (*a*). [The corresponding 1 : 3-β-linked isomer kindly provided by Dr. A. S. Perlin moved at the same rate as lactose in solvent (*a*).] It had m. p. 210°, with sintering at 200°, not altered on admixture with an authentic specimen, and $[\alpha]_D + 186 \longrightarrow + 173°$ (*c*, 1·2) (6 hr., equilib.). The *X*-ray picture was identical with that of an authentic specimen (Found : C, 42·2; H, 6·4. Calc. for $C_{12}H_{22}O_{11}$: C, 42·1; H, 6·4%). Attempts to make an osazone yielded oils.

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