63. *Synthesis of Trehalose-type Disaccharides.*

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Fusion of acetohalogeno-sugars in air with molten sodium leads to the production of non-reducing disaccharides of the trehalose type. These have been synthesised by alternative methods, and the constituent monosaccharides appear to have α β-configuration.

FOLLOWING the methods described previously (Gilbert, Stacey, and Smith, *J.*, 1946, 622) for the direct constitutional synthesis of cellobiose and gentiobiose, we have made numerous unsuccessful attempts to synthesise octa-acetyl maltose by the condensation of 4-sodio-D-glucopyranose 1 : 2 : 3 : 6-tetra-acetate and β-acetofluoro-D-glucopyranose. There appeared to be much deacetylation and acyl migration, and there was no evidence that any significant amount of disaccharide, other than traces of non-reducing products, had been synthesised. Similar attempts to synthesise directly a lactose derivative by condensing α-acetobromogalactose with 4-sodio-D-glucopyranose 1 : 2 : 3 : 6-tetra-acetate were unsuccessful. In this reaction, however, there was produced an appreciable amount of disaccharide material which, after separation on an alumina column, was obtained crystalline. This was a non-reducing disaccharide derivative which was shown to be an octa-acetyl galactobiose and it evidently resulted from the action of sodium on the α-acetobromogalactose. It is well known that trehalose-type disaccharides have been made before in condensation reactions, as for example in the attempted synthesis of sucrose (for references, see Pigman and Goepp, " Chemistry of the Carbohydrates," Academic Press, 1948, p. 451). We decided to investigate the action of sodium on acetohalogeno-sugars. In one example α-acetobromogalactose in the liquid state was heated with sodium in the presence of essentially oxygen-free nitrogen and even after several hours of heating there was no reaction, the sodium globules remaining bright and unchanged. However, in a similar reaction in air, the sodium dissolved gradually and there was a slow separation of sodium bromide. The product of this reaction, obtained in about 50% yield, was an octa-acetyl disaccharide which did not reduce Fehling's solution. Deacetylation produced a crystalline non-reducing disaccharide which yielded only D-galactose on acid hydrolysis. This was apparently identical with Vogel and Debowska-Kurnicka's galactobiose (*Helv. Chim. Acta*, 1928, **11**, 910), which was shown by them to be α-D-galactopyranosyl-β-D-galactopyranoside. Their product was made by the action of phosphoric oxide in dry chloroform on β-D-galactose 2 : 3 : 4 : 6-tetra-acetate. In our disaccharide, the presence of the glycosidic link was shown by the ease of acid hydrolysis of the galactobiose to form galactose, and it was of interest to find that this type of ether linkage and not a C–C linkage had been formed by the action of the sodium on the glycosyl bromide. No condensation could be achieved by the action of dry sodium hydroxide on the glycosyl bromide, and sodium oxide was probably the effective condensing agent.

The non-reducing disaccharide produced during the attempted synthesis of maltose mentioned above could also be obtained in small yield by the action of sodium in air on β-acetofluoro-D-glucose alone. A much more convenient synthesis of the same compound was, however, achieved by the action on β-acetofluoroglucose of the reagents described by McCloskey, Pyle, and Coleman (*J. Amer. Chem. Soc.*, 1944, **61**, 349) for the synthesis of gentiobiose, etc. The method consisted in shaking the acetofluoroglucose for 48 hours in the dark with a mixture of silver carbonate, anhydrous magnesium perchlorate, and iodine, followed by chromatographic separation of the products on a silica gel column. The separation of the disaccharide component was controlled by the determination of the molecular weight of the separated crystalline fractions. For this determination the method normally used was that of Rast, employing as solvent either camphor or the less well-known but very useful solvent, the lactam of *cis*-p-aminohexahydrobenzoic acid (Wendt, *Ber.*, 1942, **75**, 425). After purification, the non-reducing disaccharide acetate was identified, as described in the Experimental section, as the octa-acetyl derivative of α-D-glucopyranosyl-β-D-glucopyranoside (αβ-trehalose) which was smoothly converted into the crystalline sugar, αβ-trehalose. Theoretically there are three non-reducing trehalose-type disaccharides for each dihexose. For example, with a diglucose type linked through the reducing positions there could be the αα-, the αβ-, and the ββ-trehalose.

The naturally-occurring non-reducing disaccharide trehalose, isolated from ergot or from young mushrooms (for references, see Harding, *Sugar*, 1923, **25**, 476), has been identified as α-D-glucopyranosyl-α-D-glucopyranoside (αα-trehalose) (Schlubach and Maurer, *Ber.*, 1925, **58**, 1179) but it has not yet been synthesised.

Several syntheses of the ββ-trehalose from α-acetobromo-D-glucose and/or D-glucose 2 : 3 : 4 : 6-tetra-acetate have been claimed (Fischer and Delbruch, *Ber.*, 1909, **42**, 2776; Schlubach and Schetelig, *Ber.*, 1925, **58**, 1178; McCloskey, Pyle, and Coleman, *loc. cit.*). Derivatives of αβ-trehalose have previously been synthesised from β-D-glucose 2 : 3 : 4 : 6-tetra-acetate, *e.g.*, (*a*) by condensing two molecules by means of phosphoric oxide and zinc chloride (Vogel and Debowska-Kurnicka, *loc. cit.*), (*b*) by fusion with zinc chloride (Schlubach and Maurer, *loc. cit.*), and (*c*) by heating it with 1 : 2-anhydro-D-glucose 3 : 4 : 6-triacetate in benzene (Haworth and Hickinbottom, *J.*, 1931, 2947). The melting point and rotations of these derivatives have been variously quoted as shown in the table.

Compound.	M. p.	$[\alpha]_D$.	Reference.
αα-Trehalose octa-acetate	100—102°	+162·3° in chloroform	Hudson and Johnson, *J. Amer. Chem. Soc.*, 1915, **37**, 2748.
ββ-Trehalose octa-acetate	181	−18·6° in chloroform	Schlubach and Schetelig, *Z. physiol. Chem.*, 1932, **213**, 83.
αβ-Trehalose octa-acetate	68—70	+68·1 in chloroform	Vogel and Debowska-Kurnicka, *loc. cit.*
αβ-Trehalose (*neo*trehalose) octa-acetate	140—141	+82° in chloroform	Haworth and Hickinbottom, *loc. cit.*
αα-Trehalose,$2H_2O$	97	+178° in water	Schukoff, *Z. deutsch. Zuckerindustrie*, 1900, **2**, 818.
αα-Trehalose, anhydrous	203	+197° in water	Lippmann, *Ber.*, 1921, **54**, 3431.
ββ-Trehalose	130—135	−41·5° in water	Schlubach and Schetelig, *loc. cit.*
αβ-Trehalose	85	+67·1° in water	Vogel and Debowska-Kurnicka, *loc. cit.*
αβ-Trehalose (*neo*trehalose)	*ca.* 145	+95° in water	Haworth and Hickinbottom, *loc. cit.*

The non-reducing disaccharide obtained in low yield by Haworth and Hickinbottom (*loc. cit.*) was termed *neo*trehalose and the authors pointed out that it differed markedly in its properties from the αβ-trehalose of Vogel and Debowska-Kurnicka (*loc. cit.*), the specific rotation of which did, however, approximate very closely that calculated ($[\alpha]_D$ +70°) by Hudson's statistical rules (*J. Amer. Chem. Soc.*, 1916, **38**, 1571). The rotations of our products prepared above appeared to resemble more closely those recorded by Vogel and Debowska-Kurnicka. In this connection it should be pointed out that recent investigations on syntheses involving the opening of ethylene oxide rings reveals that two products can be formed, so that it is theoretically possible that in addition to the *neo*trehalose, which is a " glucose 1 : 1-glucose " disaccharide, Haworth and Hickinbottom's method could also give rise to a " glucose 1 : 2-hexose " disaccharide, though this would of course have been strongly reducing.

In our synthesis of αβ-trehalose outlined above, it should be noted that on combination of the two substituted glucose molecules, Walden inversion occurred on only one of the $C_{(1)}$ atoms, whereas in Fischer and Delbruch's synthesis (*loc. cit.*) inversion must have occurred on both $C_{(1)}$ atoms. Inspection of the literature and our experiences provide insufficient evidence to indicate the precise conditions under which trehalose-type syntheses are accompanied by Walden inversion on either or both of the reacting $C_{(1)}$ atoms.

EXPERIMENTAL.

(Ligroin and light petroleum refer to materials of b. p. 40—60° and 60—80°, respectively.)

α-Acetobromogalactose.—This was prepared by the method of Ohle, Maracek, and Bourjeau (*Ber.*, 1929, **62**, 848); the water-insoluble mass solidified on being stirred with ligroin. After recrystallisation from ether–ligroin the compound had m. p. 83°.

Synthesis of Galactobiose Octa-acetate.—Dry α-acetobromogalactose (1·5 g.) was fused in an oil-bath at 90°, in a dry test-tube open to the air; sodium (0·09 g.) was added gradually with stirring and pressing with a glass rod, and the mixture was then heated at 100—110° for 4 hours, during which the sodium dissolved. After acetylation of the reaction mixture for 45 minutes at 100° with sodium acetate and acetic anhydride, the product was left overnight under ice-water. The resulting insoluble syrup was dissolved in ether, and some discoloured material was precipitated by addition of light petroleum. The filtered solution was concentrated to a syrup which was again acetylated, poured into water, and extracted by chloroform. The dried extract was evaporated to a syrup which solidified and then was crystallised from acetone by addition of ligroin (yield 0·7 g., approx. 50%).

The acetate was very soluble in chloroform and acetone, less soluble in methanol, ethanol, or ether, almost insoluble in water or light petroleum. It had m. p. 85°, $[\alpha]_D^{20}$ +58° (*c*, 1·0 in chloroform) (Found : C, 49·2; H, 5·6; OAc, 50·0. Calc. for $C_{28}H_{38}O_{19}$: C, 49·6; H, 5·6; OAc, 50·7%). [The galactobiose octa-acetate of Vogel and Debowska-Kurnicka (*loc. cit.*) had m. p. 82—83°, $[\alpha]_D$ +51·7° (*c*, 2·1 in chloroform)]. Molecular weight (Rast) in the lactam of *cis-p*-aminohexahydrobenzoic acid (Wendt, *loc. cit.*) = 680; molecular weight of a disaccharide octa-acetate = 678.

The octa-acetate was non-reducing to Fehling's solution, but yielded a strongly reducing solution after being hydrolysed for 7 hours at 85—90° with 0·6% hydrochloric acid in 1 : 1 acetone–water. A sample of the octa-acetate (30 mg.) was directly oxidised for 1 hour at 95° with 1 : 3 concentrated nitric acid–water and yielded insoluble crystals (10 mg.) of galactosaccharic acid (mucic acid), m. p. and mixed m. p. 210° (decomp.).

Galactobiose.—(a) *Preparation and properties.* Deacetylation of the octa-acetate (0·25 g.) with sodium methoxide (Zemplen, *Ber.*, 1926, **59**, 1258) yielded a crystalline disaccharide (0·1 g.), m. p. 110° (decomp.), $[\alpha]_D^{20}$ +56° (*c*, 0·4 in water) {Vogel and Debowska-Kurnicka, *loc. cit.*, give m. p. 122°, $[\alpha]_D$ +67·8° (*c*, 0·99 in water), for αβ-galactobiose}. The disaccharide was water-soluble, and non-reducing to Fehling's solution. Its reduction value was equivalent to 0·8% of glucose (Schaffer and Hartmann, *J. Biol. Chem.*, 1920, **45**, 365). After being boiled for 5 minutes with 5% hydrochloric acid, and neutralised, the solution was strongly reducing to Fehling's solution and could readily be oxidised to give galactosaccharic acid.

(b) *Hydrolysis and osazone formation.* A sample of the disaccharide (0·05 g.) was boiled for 30 minutes with 5% hydrochloric acid (5 c.c.), and concentrated in a vacuum at 60°, with removal of most of the hydrogen chloride by distillation with several additions of water, and removal of water by distillation with alcohol. The solid residue was heated for 30 minutes at 100° with water (1 c.c.), phenylhydrazine (0·1 c.c.), and glacial acetic acid (0·15 c.c.). Yellow crystals (0·02 g.) were obtained, having m. p. 188°, alone or in admixture with authentic galactosazone (m. p. 188°).

Fusion in Nitrogen.—α-Acetobromogalactose (0·5 g.) was fused at 100—110° with sodium (0·03 g.) in an atmosphere of pure dry nitrogen, for 4½ hours, after which metallic sodium was still present. The mixture was poured off the sodium, acetylated, poured into water, and extracted with chloroform, and the extract concentrated under reduced pressure (60°) to a dry syrup. When stirred with ether, this yielded crystals (0·13 g.), m. p. 138°, alone or in admixture with β-galactose penta-acetate, m. p. 140°. The solution was evaporated to a syrup having *M*, 340 (Rast camphor method). No disaccharide octa-acetate could be isolated from the products of this reaction.

Sodium Hydroxide Fusion.—α-Acetobromogalactose (0·8 g.) and solid sodium hydroxide (0·08 g.) were fused in the presence of air at 110° for 3 hours, considerable darkening occurring. After acetylation, pouring into water and extraction with chloroform, the extract was concentrated under reduced pressure (60°) to a syrup. Addition of ether gave β-galactose penta-acetate (0·25 g.), m. p. 139° alone and when mixed with an authentic specimen having m. p. 140°. The solution was evaporated to a syrup having *M*, 400. Hence this experiment also yielded no disaccharide acetate.

Attempted Synthesis of Lactose Octa-acetate.—β-D-Glucose 1 : 2 : 3 : 6-tetra-acetate (3·0 g.) (Helferich and Müller, *Ber.*, 1930, **63**, 2142) and sodium (0·16 g.) were fused in the presence of air, at 120—140° for one hour; α-acetobromogalactose (3·0 g.) was added and the mixture heated to 100° for 1¾ hours and then acetylated with sodium acetate and acetic anhydride. An elaborate systematic chromatographic fractionation of the mixture on a silica column (Gilbert, Smith, and Stacey, *loc. cit.*) yielded no lactose octa-acetate but a sample (1·3 g.) of a non-reducing disaccharide octa-acetate, m. p. 83°, $[\alpha]_D^{20}$ +59° (*c*, 1·0 in chloroform), identical with the αβ-galactobiose octa-acetate described above. It was deacetylated to yield α-galactopyranosyl-β-galactoside, m. p. 110°, $[\alpha]_D^{20}$ +56° (*c*, 1·0 in water).

β-Acetofluoroglucose.—This compound was prepared from α-acetobromoglucose by the method of Helferich and Goatz (*Ber.*, 1929, **62**, 2505); it had m. p. 86°, $[\alpha]_D^{18}$ +21° (*c*, 1·2 in chloroform); combined bromine was absent, combined fluorine (cerous nitrate test) present. The compound was stable for several weeks in solution in dry ether at 0° or when kept in a vacuum over phoshoric oxide.

αβ-Trehalose Octa-acetate.—*Method* (*A*). The action of sodium on molten β-acetofluoroglucose gave non-reducing crystalline products of molecular weight of the order of 600, but the method was not persevered with since the following reaction was successful.

The general technique was similar to that of McCloskey, Pyle, and Coleman (*loc. cit.*). Solid reagents, except " Anhydrone," were dried under reduced pressure over phosphoric oxide; chloroform was five times distilled over phosphoric oxide.

β-Acetofluoroglucose (5·0 g.), silver carbonate (5·0 g.), " Anhydrone " (5·0 g.), and iodine (1·0 g.) were shaken together in the dark in chloroform (30 c.c.) for 48 hours. The solution was filtered, the residue washed with hot chloroform, and the solution freed from iodine by washing it with dilute aqueous sodium thiosulphate. The dried chloroform solution was evaporated to a syrup which was acetylated with sodium acetate and acetic anhydride at 110° for one hour, then poured into water, and extracted with chloroform. The chloroform solution was washed with aqueous sodium hydrogen carbonate, dried, and evaporated to a glassy syrup (3·9 g.) which was twice chromatographed on silica (4 × 25 cm.) by the method of Gilbert, Smith, and Stacey (*loc. cit.*). The various fractions had molecular weights (Rast camphor method) corresponding to mixtures of monosaccharide and disaccharide acetates and hardly any separation was achieved. The bulked product was then chromatographed on B.D.H. adsorption alumina (4 × 25 cm.), yielding fractions (1*a*) 0·88 g., *M*, 560, (1*b*) 0·22 g., *M*, 680, and (1*c*) 0·02 g., *M*, 520. Fractions (1*a*) and (1*c*) were bulked and again chromatographed on alumina, yielding fractions (2*a*) 0·27 g., *M*, 510, (2*b*) 0·19 g., *M*, 700, (2*c*) 0·23 g., *M*, 700, (2*d*) 0·14 g., *M*, 660, and (2*e*) 0·07 g., *M*, 480.

Fractions (1*b*), (2*b*), (2*c*), and (2*d*) had molecular weights approximately corresponding to a fully acetylated hexose disaccharide (678) and were combined (0·78 g.; yield approx. 40%) and recrystallised several times from ether–ligroin.

The acetate, which did not reduce Fehling's solution until after acid hydrolysis, had m. p. 120°, $[\alpha]_D$ +67° (*c*, 1·0 in chloroform) (Found : C, 49·6; H, 5·6%; *M*, 690. Calc. for $C_{28}H_{38}O_{19}$: C, 49·6; H, 5·6%).

αβ-Trehalose.—Method (*A*). The above disaccharide acetate (0·2 g.) in cold chloroform (2 c.c.) was mixed with cold dry methyl alcohol (2 c.c.) containing sodium (0·02 g.). After 5 minutes, water (10 ml.) was added and the solution neutralised to Congo-red with dilute acetic acid. The aqueous layer was evaporated under reduced pressure (50°), giving a sugar admixed with sodium acetate. Attempted crystallisation from water and alcohol being unsuccessful, the residue was dissolved in water (20 c.c.) and passed through a column of mixed Zeo-Karb and De-Acidite resins (40 g.) which was then washed with water (50 c.c.). The aqueous eluate was evaporated in a vacuum to a white ash-free residue of free sugar (0·1 g.). This recrystallised with difficulty from aqueous alcohol and then had m. p. 80° (decomp.), $[\alpha]_D^{20}$ +64° (*c*, 0·2 in water). The disaccharide did not reduce Fehling's solution or form a phenylosazone until after acid hydrolysis.

α-D-*Glucose* 2 : 3 : 4 : 6-*Tetra-acetate.*—Prepared by Georg's method (*Helv. Chim. Acta*, 1932, **15**, 932), from α-acetobromoglucose, using silver nitrate and water in ethereal solution, this had m. p. 99°, $[\alpha]_D^{20}$ +139° (*c*, 1·0 in chloroform), after several recrystallisations.

αβ-Trehalose Octa-acetate.—Method (*B*). Pure dry 2 : 3 : 4 : 6-tetra-acetyl-α-D-glucose (1·0 g.) was shaken with phosphoric oxide (1·0 g.) in dry chloroform (3 c.c.) for 68 hours in the dark at room temperature. The dark sticky residue was extracted with chloroform, and the solution concentrated to a brown syrup, which was dissolved in chloroform (15 c.c.) and ligroin (b. p. 40—60°; 15 c.c.), and chromatographed on a column of B.D.H. adsorption alumina (30 × 1·8 cm.). Two fractions so obtained had molecular weights (Rast camphor method) corresponding to that of an acetylated disaccharide : fraction (1) 0·27 g., *M*, 710; fraction (2) 0·20 g., *M*, 750. Fractions (1) and (2) were bulked and crystallised by being kept under water. The crystals were recrystallised from alcohol-water and then had $[\alpha]_D^{18}$ +64° (*c*, 0·5 in chloroform) and m. p. 118°, not depressed on admixture with the non-reducing disaccharide of m. p. 120° described above.

αβ-Trehalose.—Method (*B*). Deacetylation of the acetate with sodium methoxide, followed by crystallisation from aqueous alcohol, yielded the free sugar, $[\alpha]_D^{18}$ +70° (*c*, 0·04 in water), m. p. 80° (decomp.). The disaccharide did not reduce Fehling's solution until after hydrolysis by heating with dilute hydrochloric acid.

Attempted Synthesis of Maltose Octa-acetate.—β-D-Glucose 1 : 2 : 3 : 6-tetra-acetate (0·8 g.), silver carbonate (1·0 g.), iodine (0·2 g.), " Anhydrone " (2 g.), and β-acetofluoroglucose (1·0 g.) were shaken together in dry chloroform (10 c.c.) in the dark for 24 hours. The filtered solution was washed with sodium thiosulphate solution and water, dried, and concentrated under reduced pressure to a syrup (1·05 g.), which was chromatographed on silica (1·8 × 35 cm.), as previously described, yielding the following fractions : (1) 0·80 g., *M*, 460; (2) 0·09 g., *M*, 500; (3) 0·15 g., *M*, 900. After recrystallisation, fraction (3) had m. p. 118—120°, and m. p. mixed with αβ-trehalose octa-acetate (*A*) (m. p. 120°), 118—120°. Fraction (3) was deacetylated with sodium methoxide to a water-soluble free sugar, m. p. 80°, $[\alpha]_D^{20}$ +70° (*c*, 1·0 in water), which did not reduce Fehling's solution until after hydrolysis by hot dilute hydrochloric acid.

Fusion of α-Acetobromoglucose with Sodium.—The method used was analogous to that employed to synthesise αβ-galactobiose octa-acetate. Pure dry α-acetobromoglucose (3·0 g.) was fused in a boiling-tube at 90—100°, open to the air, and sodium pieces (0·18 g.) were added gradually. The mixture was heated at this temperature for 4½ hours, with gentle stirring, and there was no extensive decomposition. After reacetylation during one hour at 100° with sodium acetate and acetic anhydride, the solution was poured into water, and after storage extracted with chloroform. The extract was washed with water, dried, and concentrated under reduced pressure (50°) to a brown syrup (2·5 g.). Stirring this with ether gave crystals of β-glucose penta-acetate, m. p. 128° alone or mixed with authentic β-glucose penta-acetate (m. p. 130°). The ethereal solution was concentrated to a syrup (2·0 g.), *M*, 420 (Rast camphor method). Hence no disaccharide acetate was isolated from the products of this reaction.

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