129. *A Constitutional Synthesis of Cellobiose and Gentiobiose.*

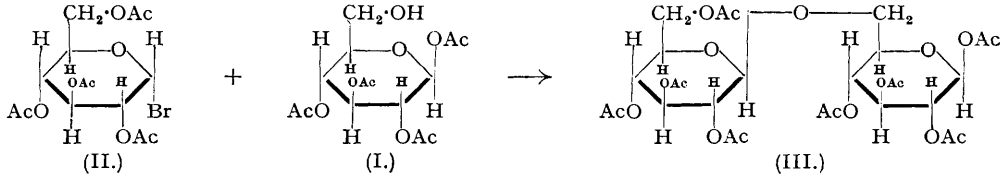
By Violet E. Gilbert, F. Smith, and M. Stacey.

The sodio derivative of 1 : 2 : 3 : 6-tetra-acetyl β-*d*-glucopyranose has been condensed in the molten state with acetobromoglucose to form directly octa-acetyl cellobiose, smoothly converted to cellobiose. Likewise 1 : 2 : 3 : 4-tetra-acetyl β-*d*-glucose was condensed with acetobromoglucose to give octa-acetyl gentiobiose and thence gentiobiose. When 3-sodio diacetone glucose was heated in a sealed tube in benzene with 6-tosyl diacetone galactose there resulted in moderate yield a *tetra-acetone disaccharide* of the true ether type. The separation of acetylated hexoses and acetylated disaccharides by chromatographic methods is described.

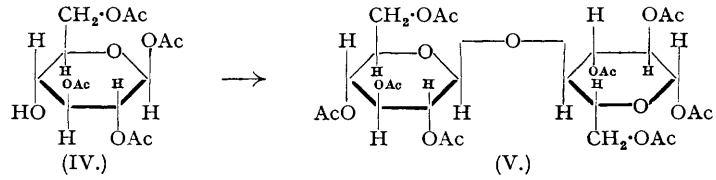
A synthesis of hepta-acetyl β-methylcellobioside in minute yield was claimed by Helferich and Bredereck (*Ber.*, 1931, **64**, 2411) who condensed acetobromoglucose with 2 : 3 : 6-triacetyl β-methylglucoside. In 1933 Freudenberg and Nagai (*Ber.*, 1933, **66**, 27) coupled 1 : 6-anhydro β-glucose with acetobromoglucose and from the syrupy reaction mixture, by means of a preferential hydrolysis to effect scission of the anhydro ring only, they obtained a product which on acetylation yielded cellobiose octa-acetate in 2% yield. This synthesis did not give any information on the structure of cellobiose because 1 : 6-anhydro β-glucose possesses three free secondary hydroxyl groups available for coupling.

Haskins, Hann, and Hudson (*J. Amer. Chem. Soc.*, 1942, **64**, 1289) have achieved in reasonable yield an ingenious total synthesis of cellobiose by a method which, although somewhat involved, gave complete support to the structure elucidated by the classical degradative methods (Haworth, Long, and Plant, *J.*, 1927, 2809). This work, which was the first constitutional synthesis of cellobiose, was carried out as follows: 2 : 3-Monoacetone 1 : 6-anhydro mannopyranose (which possesses a free hydroxyl group at C_4) was condensed with acetobromo glucose in the presence of silver carbonate to form the octa-acetyl epicellobiose (4-(β-*d*-glucopyranosido)-*d*-mannose) of Haworth, Long, and Plant (*loc. cit.*). With this as initial material the following steps were carried out:

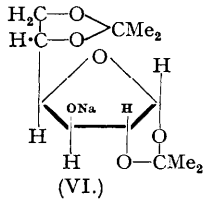
Octa-acetyl epicellobiose $\xrightarrow[\text{acetone}]{\text{HBr in}}$ acetobromo epicellobiose $\xrightarrow[\text{acetic acid}]{\text{Zinc in}}$ hexa-acetyl cellobial $\xrightarrow[\text{in ethyl acetate}]{\text{perbenzoic acid}}$ hexa-acetyl cellobiose $\xrightarrow[\text{and pyridine}]{\text{acetic anhydride}}$ octa-acetyl cellobiose $\xrightarrow[\text{methoxide}]{\text{barium}}$ cellobiose.



(II.) + (I.) → (III.)



(II) + (IV.) → (V.)



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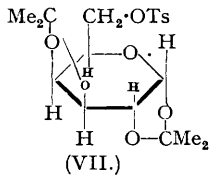

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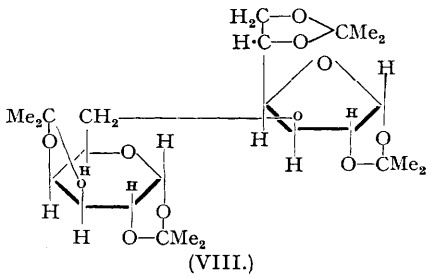
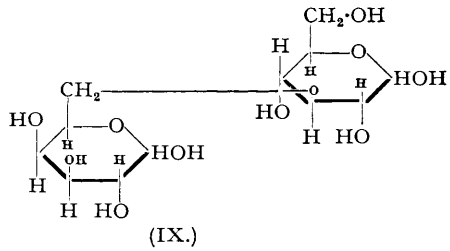


(VIII.)



(IX.)

There still appeared to be a need for a direct synthesis of cellobiose, and this achievement is now described. We discovered that some partly substituted sugars in the molten state would form sodio derivatives and, while still liquid, would react with aceto-bromohexoses with elimination of sodium bromide to form directly acetyl disaccharides.

The reaction was conveniently worked out with 1 : 2 : 3 : 4-tetra-acetyl β-*d*-glucopyranose (I) and aceto-bromoglucose (II). The former was recrystallised many times and then melted in a dry atmosphere to form a water-clear liquid in which one equivalent of dry sodium was cautiously dissolved. After addition of aceto-bromoglucose (1 mol.), and thorough mixing there was a ready separation of sodium bromide with only faint darkening of the reaction mixture. The product, extracted by chloroform, was mainly octa-acetyl gentiobiose (80% yield) (III) which was smoothly deacetylated to gentiobiose. It was noted that traces of impurity in any of the reagents rapidly caused decomposition during the reaction and the condensation mainly failed. Gentiobiose has previously been synthesised from (I) and (II) (Helferich and Klein, *Annalen*, 1926, **450**, 219) using silver oxide, or preferably silver oxide with " drierite " and iodine, as the condensing agents (Reynolds and Evans, *J. Amer. Chem. Soc.*, 1938, **60**, 2559).

For the synthesis of cellobiose, (I) was converted into 1 : 2 : 3 : 6-tetra-acetyl β-glucopyranose (IV) by the acyl migration method of Helferich and Müller (*Ber.*, 1930, **63**, 2142), and the sodio derivative of this was condensed in the molten state with (II). Since crystallisation of the disaccharide acetate was not readily achieved it was found convenient to resort to a chromatographic adsorption method of separation. For this the silica gel column of Gordon, Martin, and Synge (*Biochem. J.*, 1943, **37**, 80) was found to be eminently suitable, and using a differential solvent-extraction of the adsorbed material (including the use of a jacketed column to give elevated temperatures) it was possible, for example, to separate quantitatively 10 mg. of disaccharide acetate from 490 mg. of monosaccharide acetate.

A highly pure specimen of synthetic octa-acetyl cellobiose (V) in about 10% yield was produced from the fusion of (II) and (IV) together with approximately 30% of less pure material. The identity of the synthetic material was confirmed by comparison of its rotation, mixed melting point, and *X*-ray powder photograph with octa-acetyl cellobiose prepared from cellulose, and also by its deacetylation to authentic cellobiose.

A variation of the condensation method to give disaccharides of the true ether type has been investigated in a preliminary way. It involved the condensation of a suitable sodio derivative of a partially substituted sugar (made, for example, by the well-known method of sodio formation in liquid ammonia) with an appropriate monotosyl sugar derivative. The condensation could conveniently be carried out in a solvent at elevated temperatures in a sealed tube. 3-Sodio diacetone glucofuranose (VI) was condensed in this way with 6-tosyl diacetone galactose (VII). Sodium toluenesulphonate was separated together with unchanged diacetone glucose and diacetone galactose, and there remained a water-soluble glass having b. p. 220°/0·02 mm., $n_D^{20°}$ 1·4640. This substance was not crystallised but was considered to be a *tetra-acetone* derivative of a disaccharide having structure (VIII) for the following reasons. It had $[\alpha]_D$ −74° in chloroform whereas diacetone glucose has $[\alpha]_D$ −13° and diacetone galactose has $[\alpha]_D$ −53°. Its molecular weight was 475 (a tetra-acetone disaccharide has molecular weight 502), and it was shown to contain no free ⁻OH groups. On oxidation with nitric acid, galactosaccharic acid was formed in the expected yield, thus indicating the presence of one galactose constituent. Treatment of the tetra-acetone disaccharide with 0·5% methanolic hydrogen chloride simultaneously removed the acetone groups with loss of the furanose form of the (VI) moiety, and presumably formed the *dimethyl-glucoside* of (IX) having OMe 16·6% (theory 16·7%). Removal of the glycosidic groups with 0·25% hydrochloric acid gave (IX) as a glass having a molecular weight 340 (theory 342) and a reducing value 20% of that of glucose. The disaccharide was stable to prolonged boiling with 1% hydrochloric acid, an observation which supports the true ether type of linkage.

Experimental.

Chromatography of Mono- and Di-saccharide Acetates.—Silica gel, prepared by essentially the method of Gordon, Martin, and Synge (*loc. cit.*) but without methyl-orange treatment, was supported on a filter disc in a glass column narrowed at the base and held in a filter flask. The dimensions of the column and quantities of solvents depended upon the amount and composition of the mixture to be separated. The column was filled by suspending the gel in a 50 : 50 mixture of chloroform and light petroleum and pouring the suspension through the column under gentle suction. The liquid level was always maintained above the top of the gel. The sugar acetate mixture, dissolved in about twenty times its weight of the 1 : 1 chloroform–light petroleum solute, was drained under gentle suction (or nitrogen pressure) through the column at the rate of one drop per second.

The column was then eluted fractionally by successive extraction with small amounts of a 1 : 1 chloroform–light petroleum solute, then with chloroform alone at 20°, and finally with chloroform at 50° using a jacketed column. Each extract was worked up separately, the solvent being evaporated under reduced pressure from a weighed flask.

The separation was improved with increasing column length, but this was limited by ease of liquid flow through the gel and by the difficulty of removing the last traces of disaccharide acetate adsorbed on a large column. In those cases where partial separation only was achieved the mixed fraction could be refractionated on a fresh column.

Examples of the Separation of Artificial Mixtures.—(1) β-Glucose penta-acetate (0·05 g.) and β-cellobiose octa-acetate (0·05 g.) dissolved in a 1 : 1 chloroform–light petroleum (b. p. 40—60°) mixture (10 c.c.) were adsorbed on a column 20 cm. × 1 cm. and eluted fractionally with 10 portions (5 c.c.) of the same mixture. A complete quantitative separation was achieved, the glucose derivative being recovered in the first few extractions, and the cellobiose derivative in the remainder.

(2) β-Glucose penta-acetate (4·99 g.) and β-cellobiose octa-acetate (0·01 g.) dissolved in the 1 : 1 chloroform–light petroleum mixture (100 c.c.) were adsorbed on a column 30 cm. × 1·8 cm. The elution was carried out with five 30 c.c. fractions of the mixture, three 50 c.c. fractions of chloroform at room temperature, and two 50 c.c. fractions of chloroform

at 50°. The glucose derivative was recovered quantitatively in the first five fractions and the cellobiose derivative was extracted quantitatively by the chloroform.

(3) Using the method of example (2), β-gentiobiose octa-acetate (0·01 g.) was separated quantitatively from β-glucose penta-acetate (4·99 g.).

(4) A mixture of β-glucose penta-acetate (4 g.) and β-cellobiose octa-acetate (1·0 g.) was partially separated by the method of example (2) into β-glucose penta-acetate (2·4 g.), β-cellobiose octa-acetate (0·2 g.), and a mixture (2·4 g.) with one treatment. With two fresh columns the intermediate fractions were completely separated.

Synthesis of β-Gentiobiose Octa-acetate.—1 : 2 : 3 : 4-Tetra-acetyl β-*d*-glucose (3·5 g.), which had been repeatedly recrystallised, was put into a boiling-tube and cautiously melted in an oil-bath kept at 140°. To the colourless liquid, freshly cut sodium (0·23 g.) was added in small amounts under an atmosphere of dry nitrogen. The bath temperature was lowered to 110° and in about an hour most of the sodium had dissolved. 2 : 3 : 4 : 6-Tetra-acetyl α-glucosyl 1-bromide (4·1 g.), freshly prepared and many times recrystallised, was then added to the melt which formed a highly mobile liquid. The few residual bright globules of sodium gently dissolved and in 10—15 minutes no further visible reaction took place. The solution then gradually became opalescent owing to the separation of sodium bromide. (In the most successful reactions little or no darkening occurred, but when impure initial material was used there was extensive decomposition.) After being kept for a further hour at 110—120° the melt was cooled and extracted with chloroform (30 c.c.). The sodium bromide was thoroughly extracted with chloroform. Ether (20 c.c.) was added to the chloroform solution in order to throw out a small amount of brown tarry material and a little sodium bromide. On removal of the solvents there remained a pale yellow syrup which was dissolved in ethyl alcohol and separated into three fractions by addition of light petroleum. On being kept overnight the first two fractions crystallised (3·2 g.) and were recrystallised from alcohol–light petroleum; m. p. 190° alone or in admixture with β-gentiobiose octa-acetate, $[\alpha]_D^{20°}$ −4° in chloroform (*c*, 1·1) (Found : C, 49·6; H, 5·6; *O*-Ac, 50·3. Calc. for $C_{28}H_{38}O_{19}$: C, 49·6; H, 5·6; *O*-Ac, 50·7%). The residue from the crystallisation and the third fraction were combined (3·0 g.) and methylated, using sodium hydroxide and methyl sulphate at 30°. The product, a syrup, was distilled at 0·02 mm. giving F_I (0·5 g.), b. p. 120—130°, $n_D^{17°}$ 1·4448; F_{II} (0·5 g.), b. p. 130—180°; F_{III} (1·2 g.), b. p. 180—200°; and F_{IV}, a dark residue (0·7 g.). Fractions II—IV rapidly crystallised and after recrystallisation from light petroleum (2·1 g.) had OMe, 54·0%, $[\alpha]_D^{20°}$ −34° in water (*c*, 1·1), m. p. 106° alone or in admixture with octamethyl gentiobiose. Fraction I was shown to consist of 2 : 3 : 4 : 6-tetramethyl methylglucoside.

It was convenient after crystallising out a portion of octa-acetyl gentiobiose to acetylate the residual syrup and to separate the rest of the synthetic disaccharide acetate by fractional adsorption on a column in the manner previously described. The yields in the most successful experiments were about 80%. If the synthetic disaccharide was kept in the moist syrupy state for some days extensive decomposition with loss of acetyl residues occurred.

Octa-acetyl β-Cellobiose.—A typical synthesis was as follows. 1 : 2 : 3 : 6-Tetra-acetyl β-*d*-glucose (1·75 g.) (Helferich and Müller, *loc. cit.*) was converted mainly into the 4-sodio derivative and then fused with 2 : 3 : 4 : 6-tetra-acetyl glucosyl bromide (2·05 g.) by the method described above. (A most rigid purity was necessary for all the reactants, otherwise there was considerable decomposition and the condensation failed.) The product, a pale yellow syrup, was extracted by chloroform and the sodium bromide filtered off. The chloroform was distilled off under diminished pressure leaving a pale yellow syrup which was immediately acetylated with anhydrous sodium acetate and acetic anhydride. After removal of the acetylating reagents by distillation, the syrup was dissolved in a 1 : 1 mixture of chloroform–light petroleum and filtered from sodium acetate. It was then fractionally separated on the chromatogram in the manner described in example (4) above. There were separated penta-acetyl β-glucose (0·12 g.), various syrupy fractions, and octa-acetyl β-cellobiose (0·4 g.), m. p. 221° alone or in admixture with an authentic specimen (Found : C, 49·5; H, 5·4. Calc. for $C_{28}H_{38}O_{19}$: C, 49·6; H, 5·6%). Its identity was clearly confirmed by its *X*-ray powder photograph and by its smooth deacetylation to cellobiose, m. p. 218° alone and in admixture with a specimen from cellobiose.

Inasmuch as further small yields of the octa-acetyl β-cellobiose could be obtained after treatment of the syrupy fractions with pyridine and acetic anhydride and as low yields only of penta-acetyl glucose were obtained, it was considered that the failure of the main part of the synthetic octa-acetyl disaccharide to crystallise was due to the presence of α- and β-forms.

3-*Sodio Diacetone Glucose.*—Diacetone glucose (10 g.) was dissolved in liquid ammonia and treated with sodium (1 mol.; 0·88 g.). The ammonia was allowed to evaporate and the solid residue (10·85 g.) dried in a vacuum over phosphorus pentoxide and concentrated sulphuric acid.

Condensation of 3-*Sodio Diacetone Glucose with* 6-*Tosyl Diacetone Galactose.*—3-Sodio diacetone glucose from diacetone glucose (5 g.) was mixed with 6-tosyl diacetone galactose (7·9 g.) in benzene (50 c.c.). The condensation was effected by heating in a sealed tube at 100° for 12 hours and at 128° for a further 36 hours. During the first 12 hours the liquid turned brown and there was deposited an amorphous brown precipitate which increased in amount during the later heating stages but without any deepening in the colour of the liquid. The tube was cooled to 0° before it was opened. The liquid was filtered to remove the solid sodium *p*-toluenesulphonate (4·8 g.) (containing some sodium carbonate) which was washed well with acetone. The solvent was removed and the residue dissolved in a mixture of alcohol (10 c.c.) and water (80 c.c.). The solution, neutralised with carbon dioxide, was evaporated until all the alcohol was driven off and was then extracted with ether (80 c.c.). The ether extract gave a brown syrup *A* while the aqueous solution gave a syrup *B*. From *A* there was obtained by aqueous extraction diacetone glucose, m. p. 112°. The syrupy mother liquors from *A* were combined with *B* and the mixture distilled in a high vacuum giving Fraction I (5·4 g.), b. p. 155—160°/0·2 mm., $n_D^{18°}$ 1·4650, which was mainly a mixture of diacetone glucose and diacetone galactose, and Fraction II (1·1 g.), b. p. 220°/0·2 mm., $n_D^{20°}$ 1·4640. This *tetra-acetone* derivative was a hard glass-like solid insoluble in water but soluble in ether, acetone, and alcohol, $[\alpha]_D^{18°}$ −74° in chloroform (*c*, 2·0) (diacetone glucose had $[\alpha]_D^{18°}$ −14° in chloroform and diacetone galactose had $[\alpha]_D^{18°}$ −55° in chloroform). Attempts to methylate it by means of silver oxide and methyl iodide gave the initial material essentially unchanged (Found : OMe, nil.; *M* (Rast, using *K* = 47, a value found for diacetone galactose), 475. A tetra-acetone dihexose $C_{24}H_{38}O_{11}$ requires *M*, 502).

Oxidation of the Tetra-acetone Disaccharide with Nitric Acid.—The disaccharide material (120 mg.) was dissolved in nitric acid (2 c.c.; *d*, 1·2) and the solution heated at 95° for 4 hours. It was cooled and diluted with distilled water (5 c.c.). After some days a white crystalline deposit separated. This was thoroughly washed with water and the crystals were then dried (8 mg.), m. p. 220° alone or in admixture with a specimen of galactosaccharic acid (mucic acid) prepared from diacetone galactose. Under the same oxidation conditions 120 mg. of the latter yielded approximately 30 mg. of galactosaccharic acid.

Hydrolysis of the Tetra-acetone Disaccharide with Methanolic Hydrogen Chloride.—The material (0·422 g.) was boiled for 20 hours with 0·5% methanolic hydrogen chloride ($[\alpha]_D^{18°}$ −75° ⟶ + 73°) and the *dimethylglucoside* isolated in the usual manner. It was a water-soluble hygroscopic glass (0·24 g.) and was non-reducing to Fehling's solution (Found : OMe, 16·6%. $C_{14}H_{26}O_{11}$ (a dimethyl disaccharide) requires OMe, 16·7%).

Hydrolysis of the Dimethyl Disaccharide with Aqueous Hydrochloric Acid.—This material (0·16 g.) was heated for 4 hours with 0·25% aqueous hydrochloric acid ($[\alpha]_D^{18°}$ +28° ⟶ + 25°) and the product worked up as usual. It was a pale yellow water-soluble hygroscopic glass (0·06 g.), reduced Fehling's solution strongly, R.V. (Shaffer and Hartmann, *J.*

Biol. Chem., 1920, **45**, 365) 20% that of glucose, *M* (Barger's method, *J.*, 1904, **85**, 286) 340. A dihexose $C_{12}H_{22}O_{11}$ requires *M*, 342. No change could be observed in the rotation of the disaccharide after heating it with 1% aqueous hydrochloric acid for 4—5 hours.

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A. E. Hills Laboratories,
The University, Edgbaston, Birmingham, 15. [*Received, December 20th*, 1945.]