873. *The Chemical Synthesis of Polysaccharides. Part I. Synthesis of Gentiodextrins.*

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When treated with silver oxide in chloroform, 2 : 3 : 4-tri-*O*-acetyl-α-D-glucopyranosyl bromide polymerises with elimination of hydrogen bromide. The products are *O*-acetyl derivatives of levoglucosan and the series of gentiodextrins, *i.e.*, β-glucopyranose polymers based on the repeating 1 : 6-linkage. Molecules containing up to nine glucose units have been detected and the deacetylated products have been characterised by measurement of physical properties and formation of crystalline *O*-acetyl derivatives. Glucose and, probably, cellobiose and ββ-trehalose are also formed. The potentialities of this type of polymerisation are considered.

There are two main methods for the chemical synthesis of polysaccharides. First, a monosaccharide can be caused to undergo condensation polymerisation by being treated with an acid catalyst. For example, glucose heated with hot dilute aqueous acid yields a mixture consisting mainly of disaccharides.[1] Larger polymers can be formed by excluding water from the reaction medium,[2,3,4] thereby limiting the hydrolysis of the polymers. While the yields of the products are appreciable it is not possible to control the reaction

[1] Thompson, Anno, Wolfrom, and Inatome, *J. Amer. Chem. Soc.*, 1954, **76**, 1309.
[2] Ricketts, *J.*, 1954, 4031.
[3] Kent, *Biochem. J.*, 1953, **55**, 361.
[4] Pacsu and Mora, *J. Amer. Chem. Soc.*, 1950, **72**, 1045.

in such a way as to cause the formation of only one type of linkage. Thus Thompson, Anno, Wolfrom, and Inatome [1] obtained *iso*maltose (α-1 : 6-link), gentiobiose (β-1 : 6), maltose (α-1 : 4), cellobiose (β-1 : 4), sophorose (β-1 : 2), nigerose (α-1 : 3),[5, 6, 7] and ββ-trehalose on treatment of glucose with acid. There is, therefore, no control over the position of substitution of the sugar or of the configuration of the linkage, although the 1 : 6-linkage predominates with approximately equal amounts of the α- and β-isomers formed.

The second type of polymerisation leads to linkages of only one type. An example is the Koenigs–Knorr reaction, in which an aldose sugar substituted by halogen at position 1 and with all hydroxyl groups protected, *e.g.*, by esterification, is allowed to react with a second sugar molecule containing only one unprotected hydroxyl group. The elements of hydrogen halide are eliminated, being taken up by a suitable base, *e.g.*, silver oxide or carbonate, and union of the two sugar molecules is usually accompanied by inversion of configuration at $C_{(1)}$ of the halogeno-sugar.[8] Only one product is possible, and by using appropriate reactants di-, tri-, and tetra-saccharides have been synthesised.[8]

It occurred to us that if the halogen atom and the hydroxyl group were present in the same monosaccharide molecule (HO·M·Br) the Koenigs–Knorr reaction might provide a method for the controlled synthesis of a polysaccharide, thus :

$$\text{HO·M·Br} + \text{HO·M·Br} \longrightarrow \text{HO·M·O·M·Br} + \text{HBr, etc.} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

It is also possible that an intramolecular elimination of hydrogen halide might occur to form an anhydro-sugar, and this was expected to take place to some extent with the substance used in this investigation, namely, 2 : 3 : 4-tri-*O*-acetyl-α-D-glucopyranosyl bromide,[9] which is prepared by the action of titanium tetrabromide on 2 : 3 : 4-tri-*O*-acetyl-1 : 6-amhydro-α-D-glucopyranose (triacetyl-levoglucosan) and could, therefore, give rise to the parent levoglucosan derivative when treated with silver oxide. Being devoid of free hydroxyl groups, the triacetyl-levoglucosan could not itself be incorporated into an oligosaccharide.

In a preliminary communication [10] we reported the formation from the bromide of the acetates of levoglucosan and a polymeric series of sugars apparently based, as expected, on the β-1 : 6-linkage, *i.e.*, the gentiodextrins. Characterisation of the products was at first based on paper chromatographic evidence. We now report further, more rigid evidence for the nature of these substances.

2 : 3 : 4-Tri-*O*-acetyl-α-D-glucopyranosyl bromide (35 g.) was treated with silver oxide in chloroform at 2—4° for 9 days. Calcium sulphate was added to take up moisture, and iodine was added as a catalyst.[8] The deacetylated products were fractionated on charcoal–Celite and, where necessary, were further purified by paper chromatography and paper ionophoresis. Levoglucosan and oligosaccharides containing up to nine glucose units per molecule were detected and the yields of the di- and tri-saccharides were substantial. The identification of the polymers as gentiodextrins is based on the formation of crystalline acetyl derivatives of the di-, tri-, and tetra-saccharides with the physical properties reported for gentio-biose, -triose, and -tetraose, respectively (see Table); the identification extended to $[\alpha]_D$, molecular weights, and R_F and R_M values of the free sugars. Further, the disaccharide had the same M_G value [11] as gentiobiose, and its hydrolysis by almond emulsin indicated a β-linkage.

From the identification of the di-, tri-, and tetra-saccharides it is assumed that the higher saccharides also belong to the gentiodextrin series. For the penta- and the hexa-saccharide this was confirmed by linear relations between the degree of polymerisation and the following properties of the di- to hexa-saccharides : molecular rotations ($[M]_D$) of the free

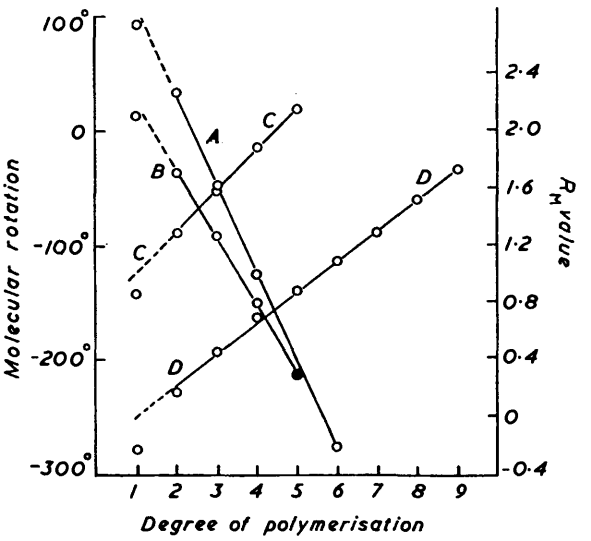
[5] Barker, Bourne, and Stacey, *J.*, 1953, 3084; Barker, Bourne, and O'Mant, *Chem. and Ind.*, 1955, 425.
[6] Peat, Whelan, and Hinson, *Chem. and Ind.*, 1955, 385.
[7] Matsuda and Aso, *Tohoku J. Agric. Res.*, 1954, **5**, 123.
[8] Evans, Reynolds, and Talley, *Adv. Carbohydrate Chem.*, 1951, **6**, 27.
[9] Zemplen and Gerecs, *Ber.*, 1931, **64**, 1545.
[10] Whelan and Haq, *Chem. and Ind.*, 1955, 600.
[11] Foster, *J.*, 1953, 982.

Products of the polymerisation of 2 : 3 : 4-*tri*-O-*acetyl*-α-D-*glucopyranosyl bromide* (35 g.).

Sugar *	Yield † (g.)	$[\alpha]_D$ in H_2O ‡	Mol. wt. Calc.	Found	β-Acetate ‡ M. p.	$[\alpha]_D$ in $CHCl_3$
Glucose	1·41	—	—	—	—	—
Levoglucosan...	1·64	—	—	—	109—110° (110°)	—
Gentiobiose ...	2·10	9·9° (9·5°)	—	—	191—192 (191—192)	− 5·3° (−5·3°)
Gentiotriose ...	3·30	−10·3 (−6·5, −10·6)	504	504	214—215 (211—211·5)	− 9·4 (−7·0)
Gentiotetraose	0·74	−18·4 (−14·5)	666	655	134—135 (134—135)	−12·0 (−10·9)
Gentiopentaose	0·34	−25·9	828	828	230—231	−13·2
Gentiohexaose	0·18	−28·0	—	—	—	—

* Listed in order of emergence from the charcoal–Celite column.
† Calc. from the optical rotations of the fractions eluted from charcoal.
‡ Values from the literature in parentheses. All except that for levoglucosan acetate [9] and the second value for gentiotriose [29] refer to products obtained from pustulan by Lindberg and McPherson.[13]

sugars, $[M]_D$ values of the crystalline acetates (except the hexasaccharide, the acetate of which was not prepared), and R_M values of the sugars (see Figure). These are criteria frequently used for depicting the interrelations of sugars in the same polymeric series.[12, 13, 14]



Molecular rotations and R_M *values of the gentiodextrins plotted against degree of polymerisation.*

A, $[M]_D$ *values of gentiodextrins.*
B, $[M]_D$ *values of gentiodextrin β-acetates.*
C, R_M *values of gentiodextrins in butan-1-ol–acetic acid–water.*
D, R_M *values in propan-1-ol–ethyl acetate–water.*

The curves are extended by broken lines towards the corresponding values for glucose, which is not, however, to be regarded as the lowest member of the polymeric series; this designation belongs to the disaccharide.[12]

Inclusion of the hepta- to nona-saccharides as members of the gentiodextrin series is based only on R_M values (see Figure) since these substances were present only in small quantity and were not separately isolated.

Thus it is proved that this polymerisation is feasible and, in the case examined, gives the products of lower molecular weight in substantial yields.

In our preliminary communication [10] we mentioned that the formation of a second series of sugars from 2 : 3 : 4-tri-*O*-acetyl-α-D-glucopyranosyl bromide was also detected when the saponified reaction products were fractionated by paper chromatography. The substances constituting the second series were detected on the paper as very faint zones lying between the intense zones corresponding to the gentiodextrins. The R_M values of the second series indicated that the fastest-moving component could have been cellobiose, but the line joining the R_M values was parallel to that for the gentiodextrins, indicating that the main polymeric linkage was probably of the β-1 : 6-type. Fractionation of a large-scale reaction mixture showed that the " impurities " were present only in minute amount. No anomalous product other than the disaccharide was isolated and this only in a yield of 13 mg. (which was probably not all cellobiose; see below), compared with 2·1 g. of gentiobiose. We believe

[12] Whelan, Bailey, and Roberts, *J.*, 1953, 1293.
[13] Lindberg and McPherson, *Acta Chem. Scand.*, 1954, **8**, 985.
[14] French and Wild, *J. Amer. Chem. Soc.*, 1953, **75**, 2612.

that the formation of the cellobiose(?) was probably due to acyl migration in the starting material. It is known that the alkalinity of soda-glass is sufficient to convert 1 : 2 : 3 : 4-tetra-*O*-acetyl-β-D-glucopyranose into the 1 : 2 : 3 : 6-isomers.[15] It seems likely that a similar migration could occur in our triacetyl-bromide, in which case an acetobromo-cellobiose could be formed. This would then react with the starting material, to give acetobromo-derivatives of two trisaccharides, *viz.*, *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-D-glucopyranose and *O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose. These in turn would react with starting material to give tetrasaccharides containing on β-1 : 4- and two β-1 : 6-linkages, but, since the R_M values of the isomers would probably be very similar, paper chromatography of the free sugars would reveal only a single zone in each molecular-weight group. The production of cellotriose and higher cellodextrins is also a possibility, but the amounts would be negligible.

If the polymerisation as defined in reaction (1) were to proceed unhindered then, in theory, the ultimate products would be levoglucosan acetate and a single acetylated molecule of macroscopic dimensions. However, the reaction was observed to have terminated when the average degree of polymerisation was not much greater than three glucose units, and the saponified reaction mixture contained an appreciable quantity of free glucose. That the sugar halides were no longer present was shown by adding methanol to the mixture : no methyl glycosides were detected when the deacetylated products were fractionated.

Termination of the polymerisation appears to be a consequence of the use of silver oxide. Its purpose is to remove the hydrogen bromide formed in the condensation, but this also results in the formation of water (or hydroxyl ions) in such quantity that for every polymeric or anhydro-linkage formed, one hydroxyl group is available to replace a bromo-group and only one-half of the bromo-groups can therefore participate in polymer or anhydro-sugar formation. In addition hydroxyl groups appear at $C_{(1)}$ capable of reacting in the formation of unwanted ββ-trehalose-type polymers. Anhydrous calcium sulphate is, of course, added to take up the liberated water and upon its efficiency in rendering the water unreactive will depend the degree of polymerisation of the oligosaccharides and the proportion of unwanted products. When the synthetic polymers were fractionated on charcoal one of the fractions immediately preceding the gentiobiose had a small negative optical rotation. ββ-Trehalose has a negative rotation ($[\alpha]_D$ −38·2° in H_2O),[16] and its emergence from a charcoal column just before gentiobiose has been noted previously.[16,17] The fraction was not examined separately but was combined with the two succeeding fractions, suspected of containing cellobiose (see above). The mixture was optically inactive, which is consistent with its containing roughly equal quantities of ββ-trehalose and cellobiose ($[\alpha]_D$ +35·2°).[16] The total weight of 13 mg. of disaccharide in these three fractions indicates that although bromo-groups are undoubtedly lost by replacement with hydroxyl, such hydroxyl groups do not offer serious competition to primary hydroxyl groups as acceptors in the polymerisation reaction. We are, nevertheless, seeking means to avoid the production of hydroxyl ions because they are undesirable in that they induce a chain-terminating reaction.

The polymerisation was also studied with the chloro-analogue [9] of our bromide. The formation of levoglucosan, gentiobiose, and gentiotriose was detected by paper chromatography, as for the bromo-compound, but, as expected, reaction was much slower.

Also of interest are the paper-chromatographic observations made on samples of the bromide which had been suspended and warmed gently in saturated barium hydroxide until dissolved. Levoglucosan and gentiobiose were formed in detectable amounts during the few minutes occupied by this treatment.

The polymerisation outlined above may have useful applications. For example, the synthesis is now in progress of what may be called the sophorodextrins, *i.e.*, the β-glucopyranose polymers based on the repeating 1 : 2-linkage. These will provide reference

[15] Helferich and Klein, *Annalen*, 1926, **450**, 219; 1927, **455**, 173.
[16] Peat, Whelan, and Hinson, *Nature*, 1952, **170**, 1056.
[17] Hinson, Ph.D. Thesis, University of Wales, 1953.

compounds for oligosaccharides expected to arise from the crown-gall polysaccharide [18] during linkage analysis.[19] If the final average degree of polymerisation can be increased by avoiding the loss of sugar halide arising as explained above, it should be possible to synthesise polysaccharides for comparison with naturally occurring material, as a test of the validity of the structural analysis of the natural polymers. There is no reason, in theory, why branched polysaccharides should not be synthesised. For this one would require 1-halogeno-sugars containing two or more unsubstituted hydroxyl groups. The degree of branching of such a polymer could be regulated by varying the proportions of halogeno-sugars containing one and more than one free hydroxyl group. Branching in such polymers should be completely random.

Experimental

Preparation of Tri-O-acetyl-levoglucosan.—This was prepared as by Zemplen and Gerecs [9] except that levoglucosan was not isolated in crystalline form, the syrupy distillate (139 g.) from potato starch (300 g.) being acetylated as for the crystalline substance and the product crystallised three times from ethanol and once from hot water: our product (72 g., 24%) had m. p. 110°, $[\alpha]_D$ −50·4° ± 0·6° in EtOH (*c* 0·38; mean of six determinations). Zemplen and Gerecs [9] give m. p. 110°, $[\alpha]_D$ −45·5°. When the compound was left overnight at room temperature in 0·12N-sodium hydroxide, $[\alpha]_D$ changed to −66·3° (neutralised solution). Levoglucosan [9] has $[\alpha]_D$ −66·2°.

Titanium Tetrabromide.—Of the two preparations tried, that utilising the reaction between titanium tetrachloride and hydrogen bromide [20] gave very poor yields. The more satisfactory method employed the reaction between bromine and titanium dioxide.[21] Three 20 g. batches of oxide gave a total of 177 g. of twice-distilled titanium tetrabromide, b. p. 228—230°.

2 : 3 : 4-*Tri*-O-*acetyl*-α-D-*glucopyranosyl Bromide and Chloride.*—Prepared from 25 g. of tri-*O*-acetyl-levoglucosan as by Zemplen and Gerecs,[9] the bromo-compound (18·4 g., 57%) had m. p. 123°, $[\alpha]_D$ +218° in $CHCl_3$ (*c* 0·45; lit.,[9] m. p. 126—127°, $[\alpha]_D$ +217°). The chloro-analogue had m. p. 126—127° (lit.,[9] m. p. 123—124°).

Polymerisation of the Bromo-compound.—(a) *Small scale.* In a typical experiment the bromide (5 g.) was dissolved in alcohol-free chloroform (30 ml.), and anhydrous calcium sulphate (15 g.), silver oxide (3 g.), and iodine (1 g.) were added. There was slight evolution of heat. (When the solids were mixed before adding the solvent the mixture became hot on contact with chloroform.) The flask was fitted with a calcium chloride tube and shaken in the dark at 2—4°. Samples (0·5 ml. each) were removed at daily intervals and centrifuged, and the chloroform was evaporated from the supernatant layer. 0·4N-Barium hydroxide (1 ml.) and 1 drop of phenolphthalein were added and the mixture gently warmed in a water-bath for 1 min. Thereafter the alkali was added dropwise until the pink colour persisted for 5 min. Barium ions were then removed as barium sulphate, and the supernatant solution was applied to paper chromatograms which were irrigated in one or both of the solvent systems butan-1-ol–acetic acid–water (4 : 1 : 5, by vol.) and propan-1-ol–ethyl acetate–water (6 : 1 : 3, by vol.).

The chromatograms were developed with alkaline silver nitrate [22] to detect reducing and non-reducing sugars and benzidine–trichloroacetic acid [23] for reducing sugars only. After 24 hours' reaction the deacetylated mixture contained sugars corresponding, in order of decreasing R_F value, to levoglucosan, glucose, and gentio-biose, -triose, and -tetraose. Gentio-pentaose became detectable after 48 hr. and the hexaose after 72 hr. Thereafter, up to 11 days, no products of higher molecular weight were detected. However, increasing quantities of the chloroform-soluble material became very difficultly soluble in aqueous barium hydroxide, even when warmed. The insoluble residue, when deacetylated in 50% aqueous-ethanolic sodium hydroxide, proved to be a mixture of the three- to six-membered gentiodextrins.

(b) *Large scale.* The bromide (35 g.) in dry ethanol-free chloroform (150 ml.) was treated with anhydrous calcium sulphate (50 g.), silver oxide (18 g.), and iodine (1 g.) for 9 days at 2—4° as above. Silver oxide (10 g.) and dry methanol (20 ml.) were added and after being shaken overnight the filtered solution was evaporated under reduced pressure, leaving a syrup (27·6 g.). This was dissolved in methanol (600 ml.), and 0·6N-barium methoxide (50 ml.) was

[18] Putman, Potter, Hodgson, and Hassid, *J. Amer. Chem. Soc.*, 1950, **72**, 5024.
[19] Peat, Whelan, and Edwards, *J.*, 1955, 355.
[20] Olsen and Ryan, *J. Amer. Chem. Soc.*, 1932, **54**, 2215.
[21] Baxter and Butler, *J. Amer. Chem. Soc.*, 1928, **50**, 408.
[22] Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444.
[23] Bacon and Edelman, *Biochem. J.*, 1951, **48**, 114.

added. After 48 hr. at 0° the solution was neutralised with aqueous 3N-hydrochloric acid and evaporated. The residue, dissolved in a minimum of water, was added to a charcoal–Celite column [12] (1 : 1 by wt.; 160 × 5 cm.) and fractionated by gradient elution.[24] A reservoir of water (20 l.), some 8 ft. above, led to the top of the column and the level in the reservoir was maintained by a constant-head device feeding in 50% aqueous ethanol. Fractions (approx. 500 ml. each) were collected automatically and the rotation of each was measured in a 4 dm. tube after filtration. The curves relating optical rotation to fraction no. were plotted and the fractions constituting each node of optical activity were combined. The optical rotation returned to zero between all nodes from glucose to gentiopentaose, except between levoglucosan and gentiobiose, which were separated by three fractions (nos. 15—17) containing cellobiose(?) and ββ-trehalose(?) (see below). Glucose was found in fractions 5—7, levoglucosan in 10—16, and gentio-biose in 18—21, -triose in 27—33, -tetraose in 39—47, -pentaose in 49—57, and -hexaose in 58—75. After fraction 75 had been collected 50% aqueous ethanol was applied to the column and a further 25 fractions were collected and combined. The eluates were evaporated to dryness under reduced pressure. The solutions containing the di- to hexa-saccharides were examined by paper chromatography to determine the carbohydrate purity of the fractions. The gentiobiose contained a trace of cellobiose and the triose-to-hexaose fractions were each contaminated with traces of the next lower member of the series. Some or all of each fraction except the tetrasaccharide was purified by partition chromatography on Whatman No. 3 paper (80 mg. of sugar per sheet 18¼ × 22½ in.) in butan-ol–acetic acid–water. The products eluted from the papers by water were evaporated to dryness under reduced pressure, dissolved in known volumes of water, and filtered to remove cellulose fibre. Sufficient tetrasaccharide occurring as impurity in the pentasaccharide fraction was obtained to carry out the measurements of $[\alpha]_D$ and molecular weight of the free sugar (see below). The main tetrasaccharide fraction was reserved for acetylation (see below). As a precaution against the contamination of the gentiotriose by trisaccharides containing 1 : 4- or trehalose-type linkages, formed in side reactions, which might not be separable by partition chromatography but should be separable by ionophoresis, the gentiotriose was placed on sheets of Whatman No. 3 paper (200 mg. of sugar per sheet 18¼ × 22½ in.) and subjected to ionophoresis for 24 hr. as described below. The recovered sugar was freed from borate by addition of the acidified aqueous solution (hydrochloric acid to pH 5) to a small charcoal–Celite column (1 : 1, w/w), elution of the borate with water and of the trisaccharide with 50% aqueous ethanol, then evaporation to dryness, and dissolution in water.

Characterisation of the Products of Polymerisation.—The oligosaccharide solutions were treated with Somogyi's deproteinising reagents [25] to remove substances interfering with reductometric measurements [12] and were then examined for the following properties.

(a) *Specific optical rotation.* Portions of the solutions were hydrolysed in 1·5N-sulphuric acid for 4 hr. at 100° and the oligosaccharide concentrations determined by measurement of the glucose liberated.[26] The optical rotations were measured in a 4 dm. tube and the $[\alpha]_D$ values calculated (see Table). Values for the molecular rotation ($[M_D]$) were calculated from molecular weights assumed from the formula $(C_6H_{10}O_5)_n - (n-1)H_2O$, where n is the degree of polymerisation (see Figure).

(b) *Molecular weight.* A preliminary experiment showed that gentiopentaose developed maximum reducing power during 30 minutes' heating with Somogyi's copper reagent,[27] the same time as required for gentiobiose. This behaviour parallels that of the *iso*maltodextrins [28] and contrasts with that of the maltodextrins [12] in which series the time of heating increases progressively with molecular weight. The reducing equivalent of each of the oligosaccharides from the biose to the pentaose was determined and, a molecular weight of 342 being assumed for the biose, that of each of the higher saccharides was calculated on the assumption of one reducing group per molecule (see Table).

(c) R_F *value.* The R_F values of glucose and the di- to hexa-saccharides were determined in the two solvents mentioned above. The values for the hepta- to nona-saccharides were measured on the material eluted from the charcoal column by 50% ethanol. R_F values were converted into R_M values [12] and are plotted in the Figure.

[24] Alm, Williams, and Tiselius, *Acta Chem. Scand.*, 1952, **6**, 826; Alm, *ibid.*, p. 1186.
[25] Somogyi, *J. Biol. Chem.*, 1945, **160**, 69.
[26] Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224.
[27] Somogyi, *J. Biol. Chem.*, 1945, **160**, 61.
[28] Turvey and Whelan, *Biochem. J.*, in the press.
[29] Edwards, Ph.D. Thesis, University of Wales, 1955.

(d) M_G *values.* These were measured on samples of sugars subjected to ionophoresis on Whatman No. 54 paper in 0·2M-borate buffer (pH 8·7) at 9 v/cm. for 18 hr.

(e) *Sugar acetates.* Dried samples of levoglucosan and the di- to penta-saccharides were separately acetylated with sodium acetate–acetic anhydride, and the products worked up in the usual way and crystallised to constant m. p. $[M]_D$ values were calculated from the measured $[\alpha]_D$ value and the assumed molecular weight, as for the free sugar (see above). Elementary analyses of the acetates were done for the disaccharide (Found : C, 49·5; H, 5·8. Calc. for $C_{28}H_{38}O_{19}$: C, 49·6; H, 5·6%), trisaccharide (Found : C, 49·8; H, 5·8. Calc. for $C_{40}H_{54}O_{27}$: C, 49·7; H, 5·6%), tetrasaccharide (Found : C, 49·1; H, 5·7. Calc. for $C_{52}H_{70}O_{33}$: C, 49·8; H, 5·6%), and for *gentiopentaose heptadeca*-O-*acetate* (Found : C, 50·3; H, 5·3. $C_{64}H_{86}O_{43}$ requires C, 49·8; H, 5·6%).

Minor Products of the Polymerisation Reaction.—When examined by paper chromatography, fractions 15—17 from the large-scale polymerisation (see above) migrated as a single spot occupying the position of cellobiose and reducing the benzidine reagent (N.B. ββ-trehalose has almost the same R_F value as cellobiose [17]). The M_G value of the material was that of cellobiose. The carbohydrate content of the fractions was measured as for the gentiodextrins (see above).

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