785. *Polysaccharides of Baker's Yeast. Part II.* Yeast Glucan.*

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Baker's yeast glucan has been partly hydrolysed with acid, and the products have been fractionated on charcoal–Celite. Eight mono-, di-, tri-, and tetra-saccharides, considered to be fragments of the glucan molecule, have been isolated and identified. These are D-glucose, gentiobiose, laminaribiose, gentiotriose, 6-*O*-β-laminaribiosylglucose, laminaritriose, 3-*O*-β-gentiobiosylglucose, and gentiotetraose. It is concluded that the glucan is a linear polymer of β-D-glucopyranose in which 1 : 3- and 1 : 6-linkages are arranged at random or in sequences such that a group of at least three 1 : 6-linkages is flanked on either side by 1 : 3-linkages. Periodate-oxidation results suggest that the combined proportion of non-reducing end groups and 1 : 6-links is 1 per 10 glucose residues.

Baker's yeast contains a cell-wall polysaccharide (yeast glucan) which " can be isolated free from other material, still retaining the shape of the whole cell and obviously constituting a complete membrane." [1] The original name " yeast cellulose," applied by Salkowski,[2] reflects the insolubility and inert character of this substance. The polysaccharide was distinguished from cellulose by Zechmeister and Toth,[3] who showed that it did not give the colour reactions of cellulose but that it was, nevertheless, a polyglucose since the major product of hydrolysis of the methylated glucan was 2 : 4 : 6-tri-*O*-methyl-D-glucose. The same result was obtained by Hassid, Joslyn, and McCready.[4] In neither case was tetra-*O*-methylglucose detected. The main polymeric linkage is therefore of the 1 : 3-type and the low specific optical rotations of the acetyl and the methyl derivatives of the glucan suggest that the glucose units are in the β-form. Barry and Dillon [5] found that the glucan was oxidised to only a small extent by periodate and isolated laminaribiose (as the osazone) from a hydrolysate of the oxidised polysaccharide, confirming the conception of β-1 : 3-linkages. From a study of yeast glucan by methylation and periodate oxidation Bell and Northcote [6] concluded that this polyglucose is a highly branched structure in which unit chains of about nine 1 : 3-linked β-glucose radicals are interconnected through 1 : 2-links. These workers report a substantial yield of tetra-*O*-methylglucose (about 10%, molar basis) from the methylated glucan.

We have investigated the glucan by partial hydrolysis and examination of the oligosaccharide fragments, as for yeast glycogen.[7] The glucan was isolated, following the method of Bell and Northcote,[6] by extracting baker's yeast with alkali to remove mannan and then with dilute acetic acid to remove glycogen. We found the latter treatment not to be fully effective, but complete extraction of glycogen was achieved by autoclaving. The vacuum-dried (60°) glucan contained polyglucose (90·3%), ash (0·4%), and nitrogen (0·6%). The tri-*O*-acetyl derivative had the specific optical rotation (−62·3°) recorded by Bell and Northcote.[6]

It proved impossible appreciably to hydrolyse the polysaccharide in hot 0·33N-sulphuric acid, and acetolysis proceeded only very slowly (9% degradation in 93 hr.). The glucan was, however, soluble in hot 90% formic acid and was submitted to hydrolysis in this solvent until it became soluble in dilute sulphuric acid, heating in which caused further hydrolysis and elimination of the formyl esters produced in the first stage.[8] A partial

* Part I, *J.*, 1955, 355.

[1] Northcote and Horne, *Biochem. J.*, 1952, **51**, 232.
[2] Salkowski, *Ber.*, 1894, **27**, 3325; *Z. physiol. Chem.*, 1914, **92**, 75.
[3] Zechmeister and Toth, *Biochem. Z.*, 1934, **270**, 309; 1936, **284**, 133.
[4] Hassid, Josyln, and McCready, *J. Amer. Chem. Soc.*, 1941, **63**, 295.
[5] Barry and Dillon, *Proc. Roy. Irish Acad.*, 1943, **49**, *B*, 177.
[6] Bell and Northcote, *J.*, 1950, 1944.
[7] Peat, Whelan, and Edwards, *J.*, 1955, 355.
[8] Andrews, Hough, and Jones, *J.*, 1953, 1186.

acid hydrolysate [40·6% apparent conversion of the polysaccharide (25·4 g.) into glucose] was then fractionated on charcoal–Celite.[9-11] The fractions were combined in batches and examined by paper chromatography and ionophoresis. When necessary, further fractionation was effected by preparative-scale paper chromatography. In most cases the sugars were characterised by measurement of specific optical rotation and by the preparations of the crystalline β-acetyl derivatives. The yields and properties of the mono- and oligo-saccharides isolated are shown in the Table.

Physical properties of the partial hydrolysis products of yeast glucan.

Sugar *	Source †	Yield ‡ (g.)	$[\alpha]_D$ in water	β-Acetate M. p.	β-Acetate $[\alpha]_D$ in $CHCl_3$
Glucose	G	*ca.* 6·0	—	131°	—
	A	—	—	131	—
Gentiobiose	G	0·473	+11·5°	192—193	−5·1°
	A	—	+9·9	191—192	−5·3
Laminaribiose	G	3·17	+20·5	162—163	−28·1
	A	—	+18·6	160—161	−28·8
Gentiotriose	G	0·159	−10·5	212—213	−7·7
	A	—	−10·3	214—215	−9·4
6-*O*-β-Laminaribiosylglucose	G	0·137	—	215—216	−25·0
	A	—	—	217—218	−27·0
Laminaritriose	G	1·17	+2·2	121	−40·3
	A	—	+2·3	120—121	−40·4
3-*O*-β-Gentiobiosylglucose	G	0·100	—	—	—
Gentiotetraose	G	0·168	−15·9	—	—
	A	—	−18·4	—	—

* Di- and tri-saccharides are listed in the order in which they emerged from the charcoal–Celite column.

† G = glucan, A = authentic specimen.

‡ From 30 g. of air-dried glucan, containing 25·4 g. of polyglucose, hydrolysed to 40·6% apparent conversion into glucose.

Mono- and Di-saccharide Products of Hydrolysis.—The only monosaccharide detected was glucose. There was no evidence of the presence of mannitol (found in the β-1 : 3-linked glucose polymer, laminarin [12,13]). It was expected from the work of Bell and Northcote [6] that the disaccharide products would be laminaribiose and sophorose [*O*-β-D-glucopyranosyl-(1 ⟶ 2)-D-glucose]. Laminaribiose was obtained in quantity (3·17 g. from 30 g.) and a second disaccharide was present. The latter proved to be, not sophorose, but gentiobiose (473 mg.). Circumstantial evidence was obtained of the presence of a very small amount of isomaltose (21 mg.) (see p. 3867) but since a greater quantity (50 mg.) was isolated when glucose (21·3 g.) was similarly treated with formic and sulphuric acids [14] it was concluded that the isomaltose was a reversion product. The weight (52 mg.) of gentiobiose formed during acid reversion [14] was much less than was isolated from the glucan. The laminaribiose and gentiobiose were characterised as shown in the Table and the gentiobiose was also oxidised with sodium metaperiodate; 4·7 molecular proportions of formic acid were released (Calc. for gentiobiose: 5 mol. prop.).

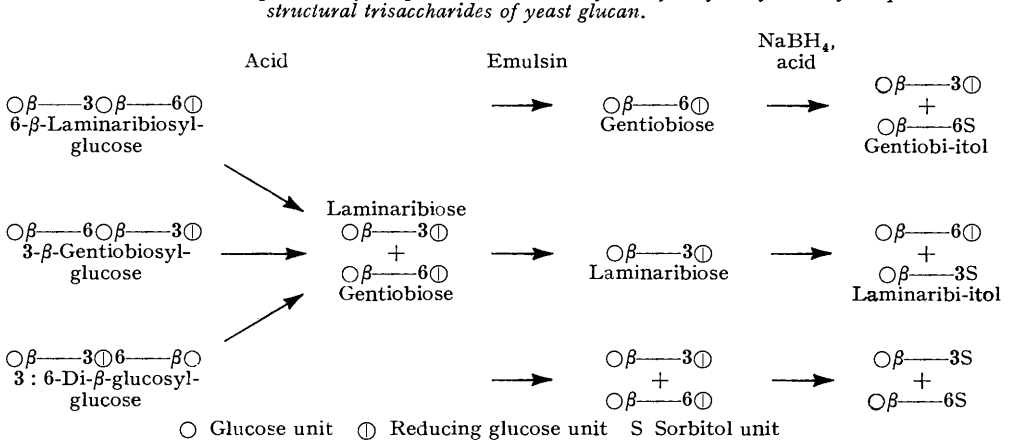
Tri- and Tetra-saccharide Products of Hydrolysis.—Four trisaccharides were isolated and three were identified as the crystalline acetates (see Table). In order of elution from the charcoal column, these were gentiotriose, 6-*O*-β-laminaribiosylglucose, laminaritriose, and 3-*O*-β-gentiobiosylglucose. The last three oligosaccharides had also been obtained from insoluble laminarin.[13] Gentiotriose and laminaritriose were identified by comparison with authentic specimens of these trisaccharides. In the case of the two " mixed " trisaccharides (those containing 1 : 3- and 1 : 6-linkages) the authentic compounds had

[9] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.
[10] Whelan, Bailey, and Roberts, *J.*, 1953, 1293.
[11] Alm, *Acta Chem. Scand.*, 1952, **6**, 1186.
[12] Peat, Whelan, and Lawley, *Chem. and Ind.*, 1955, 35.
[13] *Idem*, *J.*, 1958, **724**, 729.
[14] Peat, Whelan, Edwards, and Owen, *J.*, 1958, 586.

been prepared by chemical synthesis and were used to study the mode of action of the β-glucosidase of sweet almonds (emulsin).[15] 6-*O*-β-Laminaribiosylglucose, hydrolysed by this enzyme, yields gentiobiose, but not laminaribiose, as an intermediate product, whereas 3-*O*-β-gentiobiosylglucose gives laminaribiose, but not gentiobiose. It is clear that this β-glucosidase acts by removing the non-reducing end glucose unit and that it cannot hydrolyse the reducing-end linkage in either of these trisaccharides.

The evidence of identification of the two " mixed-linkage " trisaccharide is as follows. The first to be eluted gave gentiobiose and laminaribiose as the products of partial acid hydrolysis Emulsin released gentiobiose, but not laminaribiose. After reduction of the trisaccharide with borohydride and partial hydrolysis, the " disaccharide " products were laminaribiose and a non-reducing compound which had the R_F value of gentiobi-itol. These observations establish the structure of the trisaccharide as 6-*O*-β-laminaribiosylglucose (Fig. 1). Confirmatory evidence came from the comparison of the crystalline acetate with that of the chemically synthesised trisaccharide (see Table). Partial acid hydrolysis of the second trisaccharide also gave gentiobiose and laminaribiose; emulsin liberated laminaribiose, and the borohydride-reduced trisaccharide gave gentiobiose as the only reducing disaccharide in the partial acid hydrolysate. The trisaccharide is therefore

FIG. 1. *Disaccharide products of the partial acid and enzymic hydrolysis of some of the possible structural trisaccharides of yeast glucan.*



3-*O*-β-gentiobiosylglucose (Fig. 1). Its R_F value was identical with that of the authentic specimen. There was no evidence of the presence in any fraction of 3 : 6-di-*O*-β-glucosylglucose (see Fig. 1). Only one tetrasaccharide was separated in a form sufficiently pure to be identified. This was gentiotetraose.

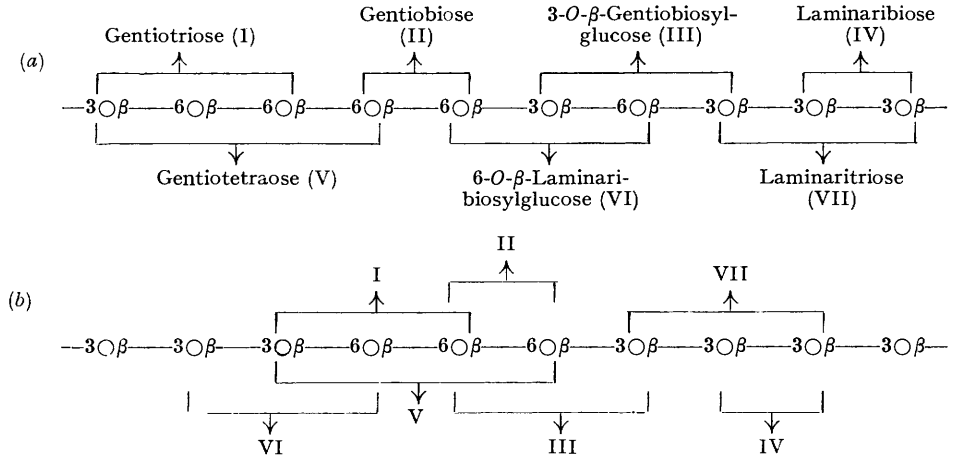
Structure of Yeast Glucan.—The foregoing evidence permits of the following conclusions. (i) Yeast glucan is constituted of β-glucopyranose units. (ii) While the majority of the glucose units are jointed by 1 : 3-linkages, there is a small proportion of 1 : 6-links. (iii) Having regard to the apparent absence of 3 : 6-di-*O*-β-glucosylglucose from the hydrolysis products, the glucan molecule is probably unbranched. (iv) The isolation of 3-*O*-β-gentiobiosylglucose and 6-*O*-β-laminaribiosylglucose shows that the two types of linkage occur in the same molecule. (v) The four trisaccharides isolated (see Table) are just those to be expected from a linear molecule containing a random arrangement of the linkages. This is shown in Fig. 2*a*. Alternatively, a " block " of 1 : 6-linked glucose units flanked on either side by 1 : 3-linked units would also satisfy the experimental findings (Fig. 2*b*): it is not possible to decide between the two types of structure on the evidence available.

The structure now proposed for yeast glucan is very different from that proposed by

[15] Peat, Whelan, and Evans, unpublished work.

Bell and Northcote,[6] both as to the nature of the minor linkage and the shape of the molecule. We have found no evidence for the presence of the 1 : 2-linkages inferred by Bell and Northcote from the isolation of 4 : 6-di-*O*-methylglucose from the methylated and hydrolysed polysaccharide. On the other hand, we have confirmed the presence of 1 : 6-links in yeast glucan by an alternative method which is described in the succeeding paper.[16]

FIG. 2. *Alternative structures of yeast glucan, showing the origin of the identified products of partial hydrolysis.*



Bell and Northcote " accounted for *ca.* 10% of the original polysaccharide " in the form of 2 : 3 : 4 : 6-tetra-, 2 : 4 : 6-tri-, and 4 : 6-di-*O*-methylglucose, in the molar ratio 1 : 7 : 1. Deductions as to structure based upon this ratio would be valid only if the polysaccharide were fully methylated. The actual methoxyl content, was, however, 41·7%, the calculated value for a tri-*O*-methylpolyglucose being 45·6%.

Bell and Northcote [6] found that oxidation of the glucan with metaperiodate liberated 1 molecular proportion of formic acid per 10·2 glucose residues. It was assumed that this acid was derived exclusively from the non-reducing end groups and the assumption seemed justified since the proportion of non-reducing end groups measured by methylation analysis was 1 in 9 glucose residues. However, a 1 : 6-linked glucose residue, such as we believe to be present in yeast glucan, contains the same 1 : 2 : 3-triol grouping, from which periodate liberates formic acid, as is present in a non-reducing end group. When oxidised with metaperiodate our specimen of glucan gave 1 molecule of acid per 10·3 glucose residues, but we regard this as a measure of the proportion of non-reducing end groups plus 1 : 6-linked glucose units, and not of end groups alone. It is assumed, as also by Bell and Northcote, that the reducing end groups of the glucan made a negligible contribution to the yield of acid, which was the amount present after the initial rapid oxidation had ceased. Reducing glucose end groups which are 3-linked are known not to yield acid at this stage, but 6-linked end glucose units yield 4 molecules of acid.[17] The initial oxidation was followed by a slower over-oxidation, characteristic of 3-linked but not of 6-linked end groups.[17] Some, if not all, of the reducing end groups were therefore 3-linked.

In the absence of knowledge of the molecular size of the glucan the periodate oxidation data cannot be used to estimate the proportion of 1 : 6-links but an approximation is given by the proportion of gentiobiose in the disaccharide fraction of the partial hydrolysate (13%; see Table). That at least three contiguous 1 : 6-links can occur in a yeast glucan chain is proved by the isolation of gentiotetraose from the partial hydrolysate but it is not

[16] Peat, Turvey, and Evans, succeeding paper.
[17] Lawley, Ph.D. Thesis, University of Wales, 1955.

known whether all the molecules are alike in this respect or whether the proportion and/or the arrangement of 1 : 6-links varies between individual molecules.

Experimental

General Methods.—These are described by Peat, Whelan, and Roberts [18] and in Part I of this series.[7] Solutions for optical rotations were in a 4 dm. tube. Small quantities (10 mg.) of sugar were reduced by treating them with an equal weight of sodium borohydride in about 1 ml. of solution for 2 hr. at room temperature. Excess of borohydride was then destroyed by the dropwise addition of 6N-sulphuric acid until evolution of hydrogen ceased. All paper-chromatographic fractionations were made in butan-1-ol–acetic acid–water (4 : 1 : 5, by vol.).

Periodate oxidation of the glucan was carried out exactly as by Bell and Northcote [6] except that the formic acid was measured by iodometric titration.[13] After 49, 96, 144, and 216 hr. the amounts of formic acid produced per glucose residue were 0·0875, 0·101, 0·106, and 0·112 mol., respectively. These results were plotted graphically and tangents were drawn to the curves representing the initial and secondary (over-oxidation) stages of oxidation, in order to determine the point of inflexion. This corresponded to an acid yield of 0·0972 mol. per glucose residue, or 10·3 glucose residues per molecule of acid. The measured carbohydrate content, and not the weight of the glucan, was used in calculating the results.

Isolation of Yeast Glucan.—Fresh baker's yeast (6 kg.; Distillers Co. Ltd.) was extracted with alkali (to remove mannan) and then with acetic acid (to remove glycogen).[6,7] The residue was washed with water in a centrifuge, then suspended in 0·02M-sodium acetate (pH 7·0; 2 l.) and heated for 1 hr. at 135° in an autoclave. After cooling, water (2 l.) was added and the residue separated in a centrifuge. The supernatant liquid gave an intense red colour with iodine, indicating the extraction of glycogen. After six washings of the residue with water (1·5 l. each) the wash-liquid was achroic but further autoclaving at 135° in 3 l. of water extracted more glycogen. The residue was therefore centrifuged, washed three times with water (2 l. each), and again autoclaved. This time the supernatant liquid was achroic. The gelatinous solid was dehydrated with ethanol (3 vol.), centrifuged, and washed successively with ethanol, ether, and light petroleum (b. p. 60—80°). The product (39 g.) was a light-buff-coloured powder. After being dried in a vacuum for 36 hr. at 60° over phosphoric oxide the glucan contained 0·4% of non-volatile matter and 0·6% of nitrogen. The carbohydrate content was measured by heating the dried material (75·2 mg.) with " AnalaR " 90% formic acid (5 ml.) in a boiling-water bath for 2 hr. After addition of 3N-sulphuric acid (20 ml.), the mixture was heated for 3 hr., then cooled, neutralised, and diluted to 50 ml. and the reducing power was measured, as for glucose.[19] When " AnalaR " glucose (55·3 mg.) was treated in the same way the recovery was 94·8%. After correction for this loss, the polyglucose content of the specimen was calculated to be 90·3%.

Acetylation of Yeast Glucan.—The method was that of Bell and Northcote.[6] The final product (451 mg. from 500 mg. of glucan) was dissolved in chloroform (300 mg. in 5 ml.) and precipitated with light petroleum (5 ml.; b. p. 40—60°). The precipitate had $[\alpha]_D$ −62·3° (*c* 0·58 in $CHCl_3$) (Found: $CH_3{\cdot}CO$, 45·3. Calc. for the tri-*O*-acetyl derivative: 44·8%).

Small-scale Hydrolysis of Glucan.—Yeast glucan (309 mg., containing 279 mg. of polyglucose) was heated at 100° with 90% formic acid (3·42 ml.) for 25 min. and then with a second portion of acid (1·14 ml.) for a further 15 min. Hot 0·44N-sulphuric acid (45·6 ml.) was then added and the resulting white suspension filtered through sintered glass. The filtrate was diluted to 50 ml. and heated in a boiling-water bath. Samples (2 ml.) were withdrawn at 30 min. intervals between 1 and 2·5 hr. These were cooled quickly and a 1 ml. portion neutralised, diluted to 25 ml. and used for measurement of reducing power (as glucose). After 1, 1·5, 2, and 2·5 hr. the percentage apparent conversions into glucose were 38·1, 59·3, 66·3, and 77·2, respectively.

Large-scale Hydrolysis and Fractionation of Glucan.—Air-dried glucan (*ca.* 30 g. containing 25·4 g. of polyglucose) was heated on a boiling-water bath with 90% formic acid (342 ml.) for 25 min. and for a further 15 min. with added acid (114 ml.). 0·44N-Sulphuric acid (4·56 l.) was added and heating continued for 1 hr. The reducing power of the partial hydrolysate corresponded to an apparent conversion into glucose of 40·6%. The neutralised hydrolysate was

[1] Peat, Whelan, and Roberts, *J.*, 1957, 3916.
[19] Somogyi, *J. Biol. Chem.*, 1945, **160**, 61.

filtered through sintered glass. The brown residue was washed with water, ethanol, and ether and dried to give a dark-brown solid (1·39 g.) having the elementary composition: C, 45·6; H, 7·08; N, 3·33; S, 1·00%. The filtrate was concentrated until the salts began to crystallise and then applied to a charcoal–Celite column [9, 10] (1 : 1 by wt.; 123 × 5·5 cm.). A 2 m. head of water was applied to the column and fractions (*ca.* 400 ml. each) were collected automatically. When the bulk of glucose had emerged (8·75 l. of eluate) an ethanol gradient [11] was applied to the column by feeding 30% ethanol through a constant-head device into 10 l. of water in a reservoir connected to the column. A further 19 l. of eluate were collected. The fractions were combined in batches of 7—10, evaporated, and examined by paper chromatography and ionophoresis.[17] All the oligosaccharide components had M_G values of 0·67.

Mono- and Di-saccharide Components of the Hydrolysate.—A portion of combined fractions No. 17—33 (585 mg.) was acetylated with sodium acetate–acetic anhydride and a chloroform-soluble syrup was obtained (792 mg., 63%) which yielded crystals from ethanol of glucose β-acetate (see Table).

Fractions No. 43—58 contained a single component which had the same R_F and M_G values as gentiobiose and isomaltose. The solid was dissolved in water (20 ml.), the mixture clarified with Somogyi's deproteinising reagents,[10, 20] and the supernatant liquid and washings were diluted to 50 ml. The amount of disaccharide, measured by acid hydrolysis to glucose,[21] was 319·4 mg. The sugar had $[\alpha]_D$ +17·2°. This value was consistent with the fraction's being a mixture of isomaltose ($[\alpha]_D$ +122°) and gentiobiose ($[\alpha]_D$ +9·6°) in the ratio 6·7 : 93·3. When a portion of the solution (5 ml.) was oxidised with 0·37M-sodium metaperiodate (5 ml.) at room temperature, production of formic acid ceased after 70 hr. and after 140 hr. corresponded to 4·7 mol. The remainder of the sugar solution was evaporated and acetylated, yielding a chloroform-soluble syrup (536 mg. from 280 mg. of sugar; 92%) from which β-gentiobiose octa-*O*-acetate was crystallised (see Table).

Fractions No. 59—73 (3·48 g., undried wt.) were shown by paper chromatography to contain gentiobiose and laminaribiose. A portion (600 mg.) was fractionated on 6 sheets of Whatman No. 3 paper and the two sugars were recovered quantitatively.[14] The amount of each sugar was determined by acid hydrolysis to glucose,[21] and the specific optical rotations were also measured (see Table). Analysis of the batch for sugar content showed it to contain 88·1% of disaccharide. These data were used to calculate the amounts of the two disaccharides in the batch. A portion of the laminaribiose (360 mg.) was acetylated and the chloroform-soluble syrup (750 mg., 100%) yielded a crystalline acetate (see Table). Fractions No. 74—81 (256 mg.) also gave the same acetate (m. p. 161°, $[\alpha]_D$ −29·3° in $CHCl_3$).

Identification of Gentiotriose.—Fractions No. 82—89 and 90—97 contained mainly gentiotriose and were combined and purified by chromatography on thick filter paper. Partial acid-hydrolysis followed by paper chromatographic fractionation yielded glucose, gentiobiose, and unchanged trisaccharide. After determination of $[\alpha]_D$ of the trisaccharide, the remaining material (159 mg.) was acetylated and the chloroform-soluble syrup (262 mg., 86%) deposited crystals of β-gentiotriose undeca-acetate from ethanol (see Table).

Identification of Gentiotetraose.—Fractions No. 98—107 (256 mg.) were fractionated on thick filter paper and three fractions were obtained: *A* (81·5 mg.), *B*, and *C* (51·4 mg.), R_F value increasing in that order. *B* had the R_F value of gentiotriose and was not examined further. Partial acid-hydrolysis of *A* liberated sugars having the same R_F values as glucose, gentiobiose, and gentiotriose. The R_M values of these last two sugars and that of *A* showed the linear relation between R_M and degree of polymerisation characteristic of a polymer-homologous series of sugars.[10, 22, 23] The tetrasaccharide *A* had $[\alpha]_D$ −15·9° in water (see Table).

Identification of 6-O-β-*Laminaribiosylglucose.*—A portion (1·36 g.) of fractions No. 108—116 (1·708 g.) was separated into three fractions by chromatography on thick filter paper. These were termed *D*, *E*, and *F*, in order of increasing R_F value. *E* had the same R_F value as *C*. When treated with emulsin both fractions *C* and *E* yielded gentiobiose and glucose. Partial acid-hydrolysis of *C* gave gentiobiose and laminaribiose as the disaccharide products. Fractions *C* and *E* were combined and a portion (8·4 mg.) was reduced with borohydride and thereafter partly hydrolysed with acid. The " disaccharide " components were laminaribiose

[20] Somogyi, *J. Biol. Chem.*, 1945, **160**, 69.
[21] Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224.
[22] Martin, Biochem. Soc. Symposium, 1950, No. 3, 4.
[23] French and Wild, *J. Amer. Chem. Soc.*, 1953, **75**, 2612.

and a non-reducing sugar having the same R_F value as gentiobi-itol (see Fig. 1). Acetylation of the mixed fraction *C* and *E* (50 mg.) gave a chloroform-soluble syrup (100 mg., 94%) from which crystals of β-6-*O*-β-laminaribiosylglucose undeca-acetate were obtained (see Table).

Identification of Laminaritriose.—Fraction *F* (see above) had the same R_F value as laminaritriose. The $[\alpha]_D$ of the sugar was measured and the crystalline β-acetate (1·03 g., 100%) was obtained from 512 mg. of sugar (see Table).

Identification of 3-O-β-*Gentiobiosylglucose.*—Fractions No. 124—131 (735 mg.) and No. 132—138 (170 mg.) contained laminaritriose and a sugar (*G*) migrating on paper between laminaritriose and -tetraose, and in the same position as authentic 3-*O*-β-gentiobiosylglucose.[15] The whole of fractions No. 132—138 and 600 mg. of fractions No. 124—131 were fractionated on thick filter paper to yield 137 mg. of *G*, still containing a little laminaritriose. Pure material (100 mg.) was obtained by repeating the fractionation. The disaccharide products of partial acid-hydrolysis of *G* were gentiobiose and laminaribiose; that of partial emulsin hydrolysis was laminaribiose only. The reduced trisaccharide gave rise to gentiobiose and laminaribi-itol when partly hydrolysed with acid (see Fig. 1).

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