34. *The Constitution of Laminarin. Part IV.*[1] *The Minor Component Sugars.*

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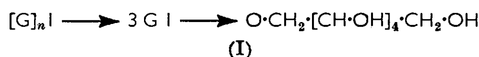
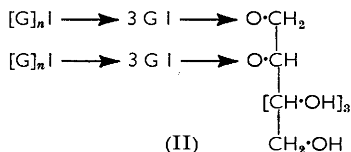
Various samples of laminarin have been examined by acid hydrolysis and periodate oxidation methods. The mannitol contents were in the range 2—3%, and the mannitol residues were monosubstituted. The mannose content of the samples was less than 0·2%, and was not structurally significant.

Mixtures of polyhydric alcohols can be separated by chromatography on a basic anion-exchange resin.

Previous studies by Peat and his co-workers,[2] and by ourselves,[1] have shown that laminarin from *Laminaria cloustoni* (hereafter referred to as *L. hyperborea* [3]) consists mainly of linear chains of β-1,3-linked D-glucose residues; in addition about half of the molecules are terminated at the reducing end by a monosubstituted mannitol residue (I). On the other hand, F. Smith and his co-workers [4-7] have examined various samples of laminarin, and have concluded that D-mannose is a constituent sugar, and that the mannitol is disubstituted (II). In view of these divergent claims, we now report the results of a re-examination of these aspects of laminarin structure; a preliminary account has been published elsewhere.[8]



(I)



(II)

[1] Part III, Anderson, Hirst, Manners, and Ross, *J.*, 1958, 3233.
[2] Peat, Whelan, and Lawley, *J.*, 1958, 729.
[3] Parke, *J. Marine Biol. Assocn.*, 1953, **32**, 497.
[4] Goldstein, Smith, and Unrau, *Chem. and Ind.*, 1959, 124.
[5] Smith and Unrau, *Chem. and Ind.*, 1959, 636.
[6] Smith and Unrau, *Chem. and Ind.*, 1959, 881.
[7] Unrau, Ph.D. Thesis, University of Minnesota, 1959.
[8] Annan, Hirst, and Manners, *Chem. and Ind.*, 1962, 984.

The alleged presence of D-mannose in laminarin was based on the isolation of a small quantity of methyl α-D-mannopyranoside after treatment of an acid hydrolysate with boiling methanolic hydrogen chloride.[5] The mannose content of various laminarin samples has been estimated at 2·4—5·4%.[7] Traces of mannose were also detected by Chesters and Bull [9] in certain enzymic hydrolysates of a commercial sample of laminarin, and these authors have kindly provided us with some of this preparation (sample 3).

The experimental procedure of Smith and Unrau involved acid hydrolysis of laminarin with N-hydrochloric acid at 100° for 12 hours, evaporation of the hydrolysate to dryness without neutralisation, and treatment of the residue with methanolic 2% hydrogen chloride under reflux for 20—24 hours. Methanolysis of an acid hydrolysate of our laminarin sample 3 did not give methyl α-D-mannoside. Application of the above procedure to the laminarin sample used previously [1] (sample 1) gave a small quantity of material with the R_G value of methyl α-D-mannoside in two solvents; however, chromatography in a third solvent showed it to be a mixture of two substances, neither of which had the R_G value of methyl α-D-mannoside. This material has not yet been identified, but is also produced when glucose is treated with hot aqueous and then with methanolic hydrogen chloride.

We therefore examined acid hydrolysates of laminarin directly, and found that the solvent ethyl methyl ketone–acetic acid–water, saturated with boric acid (9 : 1 : 1, by vol.) [10] gave a good separation of glucose, mannose, fucose, and mannitol; in synthetic mixtures, mannose contents as low as 1% could be detected. Acid hydrolysates of six different samples of laminarin (including one, sample 4, kindly provided by Professor F. Smith and stated to have a mannose content of 2·4%) were neutralised, and examined by paper chromatography. Mannose could not be detected in any hydrolysate, but small and varying amounts of fucose, presumably arising from contaminating fucoidin, were present. To increase the sensitivity of the detection of mannose, neutralised hydrolysates were fractionated on a Dowex resin column,[11] appropriate fractions combined, and examined by paper chromatography. The hydrolysate of sample 1 did not contain mannose, but those from samples 2, 3, and 4 contained traces of this sugar; qualitatively, the largest amount was in sample 3. In a quantitative experiment, *ca.* 1 g. of the latter gave, on hydrolysis, 915 mg. glucose and 5·4 mg. of a mixed fraction containing glucose, fucose, and a smaller quantity of carbohydrate with the R_G value of mannose. The apparent mannose content of this laminarin sample does not exceed 0·2%. We conclude that mannose is not a constituent sugar of our samples of laminarin, in agreement with the results of a previous unpublished study [12] of this problem, and the experiments of Beattie, Hirst, and Percival.[13] In all these experiments [12,13] it was not possible to detect mannose in a laminarin hydrolysate, or, in the present work, to isolate sufficient mannose to enable a crystalline derivative to be prepared. It is important, however, to note that acid hydrolysates of crude samples of laminarin contain traces of mannose and xylose, in addition to fucose, but on subsequent purification the polymeric forms of the first two sugars are removed.

The presence of mannitol in laminarin was first observed by Peat and his co-workers [2] who isolated mannitol, 1-*O*-β-D-glucosylmannitol and 1-*O*-β-laminaribiosylmannitol from a partial hydrolysate. Trace amounts of 1,6-di-*O*-β-glucosylmannitol (0·03%) and 1-*O*-β-isomaltosylmannitol (0·01%) were also isolated, but these sugars were believed to be formed by acid-reversion reactions. The mannitol contents of samples of insoluble and soluble laminarin were 1·7 and 2·7%, respectively,[2] and it was suggested that a proportion of the laminarin molecules were terminated by 1-substituted mannitol residues (as in I).

Since the latter molecules (M-chains) rapidly yield one mol. of formaldehyde on periodate oxidation at 2°, the mannitol content of laminarin can be measured, and a value

[9] Chesters and Bull, *Biochem. J.*, 1963, **86**, 31.
[10] Rees and Reynolds, *Nature*, 1958, **181**, 767.
[11] Jones and Wall, *Canad. J. Chem.*, 1960, **38**, 2290.
[12] Cunningham, Ph.D. Thesis, University of Edinburgh, 1961.
[13] Beattie, Hirst, and Percival, *Biochem. J.*, 1961, **79**, 531.

of 2% was found [1] for sample 1. [Laminarin molecules terminated by reducing glucose residues (*i.e.*, G-chains) do not yield formaldehyde under these conditions.]

Application of the reaction sequence periodate oxidation, reduction with borohydride, and acid hydrolysis, to M-chains of structure (I) should convert the mannitol residue into ethylene glycol.[4] This alcohol could not be detected by Smith and his co-workers,[4] who suggested that the M-chains were 1,2-disubstituted as in structure (II). This reaction sequence has therefore been examined.

The mannitol content of various samples of laminarin was measured by periodate oxidation (see Table); a control oxidation with 1-*O*-β-laminaribiosylmannitol gave the expected yield of formaldehyde. Since these results would be invalidated by the presence of 1,6- or 1,5-disubstituted mannitol residues, the mannitol content of two samples was also determined by paper-chromatographic separation of glucose and mannitol from an acid hydrolysate, followed by estimation with phenol–sulphuric acid [14] and periodate oxidation reagents respectively. The results for samples 1 and 6 were 2·1 and 2·5%, in good agreement with values of 2·4 and 2·7% by periodate oxidation, thereby indicating the absence of 1,6- or 1,5-disubstituted mannitol residues.

In an attempt to decide between structures (I) and (II), laminarin was oxidised at 2° with a dilute solution of periodate (0·4 mM) using conditions similar to those devised by Clancy and Whelan [15] for the selective oxidation of the hexitol portion of disaccharide alcohols. G-Chains (as shown by the model compound laminaribiose) do not yield formic acid. M-Chains of structure (I) should produce 3 mol. of formic acid (a value observed with the model compound 1-*O*-β-laminaribiosylmannitol), whereas M-chains of structure (II) would give only 2 mol. of formic acid per mannitol residue. Under these conditions, three samples of laminarin gave 3·0—3·1 mol. of formic acid per mannitol residue (see Table), a result in accord with structure (I).

An acid hydrolysate of laminarin polyalcohol was examined for ethylene glycol. Although this alcohol could be detected by paper chromatography in a mixture with arabitol and glycerol (which would arise, respectively, from reducing glucose residues, and non-reducing terminal or 1,6-linked glucose residues in laminarin) it was completely masked by glycollic aldehyde which is also a hydrolysis product of laminarin polyalcohol. However, glycollic aldehyde could be removed by adsorption on Amberlite IRA 400 basic ion-exchange resin, without loss of ethylene glycol.

Laminarin sample 1 was converted into the polyalcohol, and a neutralised hydrolysate from 7·22 g. passed down a resin column. The eluate contained arabitol, glycerol, and ethylene glycol (paper chromatography). The mixture was separated by cellulose column chromatography to give arabitol (48 mg., characterised as the pentabenzoate), glycerol (227 mg., characterised as the tri-*p*-nitrobenzoate), and ethylene glycol (38 mg., identified as the di-*p*-nitrobenzoate). The observed yield of ethylene glycol is considered to be in good agreement with that calculated for structure (I) (*ca.* 54 mg.), in view of the several manipulations involved, and provides further evidence for structure (I). The yield of arabitol was low; the reason for this is discussed in the Experimental section.

Additional confirmation of structure (I) was obtained by the detection, by gas chromatography, of 2,3,4,5,6-penta-*O*-methylmannitol in a methanolysate of methylated laminarin.

We therefore conclude that our samples of laminarin do not contain mannose, have a mannitol content of 2·4—3·4%, and that this mannitol is 1-substituted. Laminarin sample 4 was originally examined by Smith and Unrau [7] who reported mannose and mannitol contents of 2·4 and 12·8%, respectively; the latter would imply a structure with one mannitol residue per 7 glucose residues, which is not consistent with any published data, or with the formic acid released on periodate oxidation (see Table). Our analysis of this sample gave a mannitol content of 3·4%, in general agreement with that of the other samples.

[14] Dubois, Gilles, Hamilton, Rebers, and Smith, *Analyt. Chem.*, 1956, **28**, 350.
[15] Clancy and Whelan, *Chem. and Ind.*, 1959, 673.

Since the completion of the above work, M. Fleming (unpublished work) has examined a further seven samples of laminarin; the mannitol contents were in the range 2·5—3·7%, and no sample gave mannose on acid hydrolysis.

Properties of laminarin samples.

Sample:	1	3	4	6
Hexose content (as glucose) (%)	94	90	90	91
Ash content (%)	0·6	1·5	—	0·8
Yield of HCHO on periodate oxidn. (mol. per glucose residue)	0·024	0·029	0·034	0·027
Mannitol content (%):				
(*a*) by periodate oxidation	2·4	2·9	3·4	2·7
(*b*) by direct analysis	2·1	—	—	2·5
Yield of HCO_2H on periodate oxidn. (mol. per mannitol residue)	3·0	3·1	3·1	—

Experimental

Analytical Methods.—(a) *Paper chromatography.* The following solvents were used: *A*, ethyl acetate–pyridine–water (5 : 2 : 7 v/v); *B*, butan-1-ol–pyridine–water (6 : 4 : 3 v/v); *C*, butan-1-ol–ethanol–water (4 : 1 : 5 v/v); *D*, ethyl methyl ketone–acetic acid–water, saturated with boric acid (9 : 1 : 1 v/v),[10] together with aniline phthalate, silver nitrate, and periodate–permanganate [16] spray reagents.

(b) *Periodate oxidation.* All periodate oxidations were carried out in the dark. Formaldehyde was determined by use of a chromotropic acid reagent,[17,18] residual periodate being removed with sodium sulphite.[19] A portion (1 ml.) of the periodate-free sample was heated with the chromotropic acid reagent (9 ml.) at 100°, for 30 min., cooled, and 4·6% thiourea solution (2 ml.) added [18] before measurement of the optical density at 570 mμ. Laminarin samples were treated with ethanol, to cause precipitation of the polysaccharide so as to reduce interference caused by the interaction of polysaccharide and the acid reagent, before analysis.[20] Formic acid was determined by potentiometric titration to pH 6·25 using 0·00070N-barium hydroxide. Deionised water was used in the preparation of solutions, and a stream of nitrogen was passed through the solutions during titration. In some experiments where the hydrolysis of formyl esters had to be prevented, the titration vessel was immersed in an ice bath.

Laminarin Samples.—The following samples were used: 1, the sample of insoluble laminarin from *L. hyperborea* examined in Part III; [1] 2, a sample of soluble laminarin prepared from *L. digitata* by Dr. W. A. P. Black; 3, a sample of laminarin from the Liverpool Borax Company, provided by Professor C. G. C. Chesters and Dr. A. T. Bull; 4, a sample of laminarin from Professor F. Smith, reported to contain glucose (84·8%), mannose (2·4%), and mannitol (12·8%); 5, a sample of insoluble laminarin isolated from *L. hyperborea* by extraction with water at 70° and purified by re-deposition from aqueous solution; 6, a sample extracted from *L. saccharina* by hot water, and purified by passage down a mixed-bed ion exchange resin column (Amberlite IR 120 H^+ and IR 45 OH^-), and freed from mannitol by reprecipitation with ethanol.

Action of Methanolic Hydrogen Chloride on Laminarin and Glucose.—A neutralised acid hydrolysate of laminarin sample 3 (100 mg.) was treated with 3% methanolic hydrogen chloride (5 ml.) for 6 hr. at 60—70°. Methyl α-D-mannoside could not be detected. The same result was obtained when the polysaccharide was heated at *ca.* 70° for 48 hr. with 6% methanolic hydrogen chloride. Treatment of a mixture of glucose (97·5%) and mannose (2·5%) with 3% methanolic hydrogen chloride at 60—70° for 3 hr. gave detectable amounts of methyl α-D-mannoside (R_G 2·7 in solvent *A*; cf. R_G 1·9 for methyl α-D-glucoside), and this result was unaffected if the sugars were dissolved in dilute sulphuric acid, neutralised (barium carbonate), and evaporated to dryness before the treatment.

Laminarin sample 1 (150 mg.) was treated by Unrau's procedure,[5,7] and a small amount of material indistinguishable from methyl α-D-mannoside in solvents *B* and *C* was obtained. In

[16] Lemieux and Bauer, *Analyt. Chem.*, 1954, **26**, 920.
[17] MacFadyen, *J. Biol. Chem.*, 1945, **158**, 107.
[18] Frisell, Meech, and Mackenzie, *J. Biol. Chem.*, 1954, **207**, 709.
[19] Speck and Forist, *Analyt. Chem.*, 1954, **26**, 1942.
[20] Parrish, Ph.D. Thesis, University of London, 1958.

solvent *A*, the substance showed two components, R_G 3·1 and 3·8, but no component with R_G 2·7. The same products were formed on similar treatment of AnalaR D-glucose. The material from the laminarin experiment was isolated by thick paper chromatography (solvent *B*), and this was separated into two components on chromatography in solvent *A*. The latter have not yet been identified; on acid hydrolysis, they gave glucose and a trace of material R_G 1·49 in solvent *B*.

Examination of Laminarin Hydrolysates for Mannose.—In solvent *D*, mannose, fucose, and mannitol had R_G values of 1·5, 3·0, and 3·3. On chromatographing a mixture of glucose (82%), mannitol (15%), and mannose (3%) (cf. sample 4), the presence of mannose was readily revealed by aniline phthalate reagent. It was also detectable in a mixture of glucose (99%) and mannose (1%).

The laminarin samples (50 mg.) were hydrolysed with 1·5N-sulphuric acid (10 ml.) at 100° for 3 hr., neutralised (barium carbonate), and examined under similar conditions. Mannose could not be detected in any hydrolysate; the hydrolysate from sample 1 contained a trace of fucose and those from the other samples contained readily detectable amounts.

Fractionation of Monosaccharides on Ion-exchange Resin.—The general procedure of Jones and Wall [11] was used, with Dowex 50W × 8 (Ba^{++}) resin. In a control experiment, a mixture of glucose (200 mg.) and mannose (25 mg.) was placed on a column (70 × 1·8 cm.) and on elution with water, fractions (2·5 ml.) were collected at 25 min. intervals. Glucose was present in fractions 30—41, and mannose was the main component of fractions 42—55; there was only slight overlapping of the two sugars. In later runs with this column, the maximum amounts of glucose, mannose, and mannitol were collected in fractions 30, 39, and 43, respectively, the hexitol being detected with the periodate spray reagent.

Laminarin samples 1, 2 and 3 (*ca.* 1 g.), and 4 (0·15 g.) were hydrolysed with 1·5N-sulphuric acid (100 ml.) at 100° for 3 hr., neutralised, concentrated, and fractionated on the column. Examination of fractions 37—41 showed the presence of traces of mannose in the hydrolysates from samples 2, 3, and 4. For sample 3, fractions 25—36 and 37—41 were diluted with water to 1000 and 50 ml., respectively, and aliquot portions (1 and 3 ml.) analysed, in triplicate, by cuprimetric titration.

Determination of the Mannitol Content of Laminarin by Periodate Oxidation.—Laminarin (sample 1, 45·7 mg.; sample 3, 36·8 mg.; sample 4, 26·5 mg.; sample 6, 36·7 mg.) in water (10 ml.) was oxidised with 0·3M-sodium metaperiodate (0·5 ml.) at 2°. Samples (1 ml.) were removed at intervals, and mixed with 0·5M-sodium sulphite (0·5 ml.) and ethanol (4 ml.) and stored at 2° for 1—2 days. After centrifugation, a portion (1 ml.) of the supernatant solution was treated with the chromotropic acid reagent and thiourea solution. Control mixtures containing either water instead of laminarin, or unoxidised polysaccharide were also analysed. After oxidation for 1 and 6 hr., the release of formaldehyde (mol. per hexose residue) was: sample 1, 0·024 and 0·023; sample 3, 0·028 and 0·030; sample 4, 0·034 and 0·034; sample 6, 0·027 and 0·026.

When 1-*O*-β-laminaribiosylmannitol (6·4 mg.) was oxidised under similar conditions, the yield of formaldehyde after oxidation at 2° for 1, 6, 24, and 54 hr. was constant at 0·95 molecular proportions.

Determination of the Mannitol Content of Laminarin by Quantitative Paper Chromatography.—A synthetic mixture of glucose and mannitol (molar ratio 40 to 1) was analysed by quantitative paper chromatography on Whatman 3MM paper using solvent *D* and periodate–permanganate reagent to locate the position of mannitol in the guide strips. Glucose eluted from the paper was estimated by the phenol–sulphuric acid method,[14] and mannitol by measurement of the release of formaldehyde on periodate oxidation in bicarbonate buffer. The amounts of glucose and mannitol from one chromatogram were 6·6 mg. and 0·176 mg. respectively, equivalent to a molar ratio of 38 to 1. Control experiments showed that traces of borate from solvent *D* did not interfere with the phenol–sulphuric reagent (cf. ref. 21), and that small amounts of fucose, which was not completely separable from mannitol in this solvent, did not interfere with the estimation of the hexitol.

Laminarin samples 1 and 6 (*ca.* 50 mg. each) were hydrolysed and the neutralised hydrolysates chromatographed as above. The recovery of glucose from one chromatogram of each hydrolysate was 10·8 and 10·6 mg. respectively, and that of mannitol 0·234 and 0·273 mg., indicating mannitol contents of 2·1 and 2·5% for the laminarin samples.

Periodate Oxidation of Laminarin.—Laminarin [sample 1 (79·1 mg.), sample 3 (88·0 mg.),

[21] Briggs, Garner, Montgomery, and Smith, *Analyt. Chem.*, 1956, **28**, 1333.

sample 4 (51·8 mg.)] in water (*ca.* 200 ml.) was oxidised with 4mM-sodium metaperiodate (25 ml.) in a total volume of 250 ml. at 2°. Samples (25 ml.) were analysed at intervals for formic acid, after reduction of periodate by the addition of 1% ethylene glycol solution (2·5 ml.). The samples were kept for *ca.* 1 hr. at 2° before titration, also at 2°. The release of formic acid (mol. per mannitol residue) was:

Time of oxidation (hr.)	0·25	0·50	0·75	1·0	1·5	2·0	3·0
Sample 1	2·7	2·9	3·0	3·0	2·9	2·9	3·0
,, 3	—	3·1	3·0	3·1	2·9	3·2	3·0
,, 4	2·8	—	3·1	3·1	3·1	3·1	—

A sample of 1-*O*-β-laminaribiosyl mannitol (6·95 mg.) was oxidised under similar conditions. The production of formic acid, after 0·3, 0·7, 1·0, 1·5, and 2·0 hr. was 2·8, 2·9, 2·9, 3·0, and 2·9 mol. per mannitol residue. On oxidation of laminaribiose (4·6 mg.) under these conditions, formic acid could not be detected until after oxidation for 5 hr., and after 11, 30 and 57 hr. the yield was only 0·08, 0·18, and 0·18 molecular proportions.

Separation of Polyhydric Alcohols.—On paper chromatography in solvent *C*, ethylene glycol, glycerol, erythritol, and arabitol had R_G values of 3·7, 2·7, 1·9, and 1·4.

Preliminary experiments showed that columns of Amberlite IRA 400 OH^- would adsorb glycollic aldehyde and glucose from mixtures with ethylene glycol during elution with water. In a quantitative experiment 56·4 mg. of ethylene glycol was applied to a column (40 × 6·6 cm.) and eluted with 2·6 bed vols. of water. A recovery of 54 mg. (96%) was observed.

During these and other experiments, substantial losses of ethylene glycol could occur during concentration of column fractions owing to its volatility. To avoid this, a rotary film evaporator at 25° was used, and evaporation stopped before dryness; for example, 18·8 mg. of ethylene glycol in 10 ml. solution was concentrated to *ca.* 50 mg. of solution. This contained 18·1 mg. of glycol (96% recovery).

Preparation and Hydrolysis of Laminarin Polyalcohol.—Laminarin sample 1 (8·97 g.) was oxidised with sodium metaperiodate (5 g.) in a total volume of 500 ml., at 2° for 7 days. The oxopolysaccharide was recovered by addition of 6N-hydrochloric acid (44 ml.) and sodium iodide solution (27 g.; 20 ml.), the mixture then being added to cold ethanol (6 l.).[22] The precipitated oxopolysaccharide was collected, washed with methylated spirits, and dried; yield, 8·85 g. The oxopolysaccharide was dissolved in warm water (350 ml.), cooled to 20°, and potassium borohydride solution (1·5 g.; 15 ml.) added. After 26 hr. a further addition of borohydride (0·75 g.; 7·5 ml.) was made. After 42 hr., the excess of borohydride was decomposed by the addition of acetic acid, and the polyalcohol isolated by precipitation with ethanol; yield, 8·58 g.; moisture content, 6·3% indicating an overall yield of 90%.

Polyalcohol (100 mg.) was hydrolysed with 1·5N-sulphuric acid (25 ml.) for 3 hr. at 100°. The neutralised hydrolysate was concentrated to 3 ml., applied to a column of Amberlite IRA 400 OH^- resin, and the aqueous eluate concentrated. Paper chromatography showed the presence of arabitol, glycerol, and ethylene glycol.

Polyalcohol (7·22 g.) was hydrolysed with 1·5N-sulphuric acid (500 ml.) for 3 hr. at 100°, neutralised, concentrated to 200 ml., and passed through a column (40 × 6·6 cm.) of Amberlite IRA 400 OH^- resin. The aqueous eluate (4 l.) was concentrated to *ca.* 2 ml. and applied to a column (53 × 3 cm.) of Whatman cellulose powder which had been packed dry and washed successively with water and then solvent *C*. Fractions (2·5 ml.) were collected and examined by paper chromatography.

Fractions 132—149 contained ethylene glycol, and were combined. The yield, based on the amount of formaldehyde produced on periodate oxidation of a suitable aliquot portion (and with a 5·5% correction for the effect of butanol and ethanol on the formaldehyde analysis) was 38 mg. Treatment with pyridine and *p*-nitrobenzoyl chloride at 100°, followed by appropriate purification, gave the corresponding ethylene glycol di-*p*-nitrobenzoate, with m. p. and mixed m. p. 144—145°.

Fractions 150—184 contained glycerol, the yield, based on periodate oxidation analysis, being 227 mg. The derived tri-*p*-nitrobenzoate had m. p. 188—192°, and with an authentic sample (m. p. 194—196°) gave a mixed m. p. 193—196°.

Fractions 185—188 contained a trace of a substance with the R_G value of erythritol.

Fractions 189—250 contained arabitol (48 mg.) characterised as the pentabenzoate, m. p.

[22] Sloan, Alexander, Lohmar, Wolff, and Rist, *J. Amer. Chem. Soc.*, 1954, **76**, 4429.

and mixed m. p. 150—152°. The latter appears to be a new derivate, and was prepared as follows: D-arabinose (5 g.) in water (100 ml.) was reduced with potassium borohydride (1 g.) in water (50 ml.). After a constant rotation had been observed (*ca.* 1 hr.) the borohydride was neutralised with acetic acid, and the solution deionised by passage through columns of Amberlite IR 120 H^+ (12 × 1·7 cm.) and Amberlite IRA 400 OH^- (10 × 3·6 cm.). The product (2·3 g. 45%) was chromatographically pure, and on crystallisation from ethanol had m. p. 103—104° (lit., m. p. 103°). Arabitol (300 mg.) was benzoylated; the pentabenzoate, yield 760 mg. (57%), had m. p. 151—152° and $[\alpha]_D$ +28° (*c* 1·06 in chloroform) (Found: C, 70·8; H, 4·58. $C_{40}H_{32}O_{10}$ requires C, 71·4; H, 4·76%).

Identification of 2,3,4,5,6-*Penta-O-methylmannitol.*—A sample of 1-*O*-β-glucosylmannitol, isolated from a partial acid hydrolysate of laminarin, was kindly provided by Dr. D. H. Hutson. On periodate oxidation, it gave 0·96 mol. of formaldehyde and 3·80 mol. of formic acid (theory 1·0 and 4·0 mol. respectively). 1-*O*-β-Glucosylmannitol (50 mg.) was dissolved in *NN*-dimethylformamide (2·5 ml.) and dimethyl sulphoxide (2·5 ml.), cooled to 0°, barium oxide (1 g.) and barium hydroxide (0·5 g.) added,[23] and then treated with dimethyl sulphate (1·5 ml.). After neutralisation with ammonia, the mixture was extracted with chloroform and the extracts washed with water, dried (sodium sulphate), and evaporated to dryness and to constant weight; yield 33 mg. (48%). A portion (*ca.* 5 mg.) was heated in a sealed tube with methanolic 3% hydrogen chloride for 6 hr., neutralised (silver carbonate), concentrated, and the residue dissolved in chloroform. Gas–liquid partition chromatography on a column using polyphenyl ether as the stationary phase gave only three peaks, two of which corresponded to the anomeric glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose. The third peak had a retention time of 1·94. Using a butane diol succinate polyester column, the relative retention time of this peak was 3·67. A portion of methylated laminarin sample 1, prepared by Dr. A. G. Ross,[1] was methanolysed and the products examined by gas–liquid chromatography. A small " shoulder " was observed on a peak arising from a main component (2,4,6-tri-*O*-methyl-D-glucose) which had relative retention times of 1·97 and 3·66 respectively, on the two columns.

Improved Separation of Polyhydric Alcohols.—The yield of arabitol isolated from the hydrolysate of laminarin polyalcohol (48 mg.) was low compared to the theoretical yield (124 mg.) calculated on the basis that laminarin sample 1 had a degree of polymerisation of 24, and contained 57% of M-chains. It has since been found that polyhydric alcohols are partly retained by the Amberlite IRA 400 resin. On application of a mixture of ethylene glycol (48·6 μM) and arabitol (23·5 μM) to a column (15 × 1·7 cm.) and elution with 3 bed vols. of water, all the glycol but only 9·1 μM of the arabitol was recovered. Elution with a further 6 bed vols. of water was required to complete the recovery of the arabitol.

Since the ionisation constants of the polyhydric alcohols increase steadily from ethylene glycol ($5·7 \times 10^{-15}$) to mannitol ($3·4 \times 10^{-14}$),[24] Dr. J. C. P. Schwarz suggested that the observed retention arose from the increase in acidity shown by the higher alcohols. A mixture (5 ml.) containing ethylene glycol, glycerol, erythritol, arabitol, and mannitol (each *ca.* 30 mg.) was applied to a column (42 × 2·5 cm.) of Amberlite CG 400 OH^- Type I resin, and eluted with a slight linear concentration gradient of ammonium carbonate. This was supplied at 125 ml./hr. from a beaker equipped with a magnetic stirrer containing 1·25 l. of water to which a reservoir consisting of ammonium carbonate solution (99 mg., 1·25 l.) was connected by a syphon. Fractions (25 ml.) were collected, concentrated, and examined by paper chromatography. Fractions 7, 10, 15, 20, and 40 contained pure samples of the alcohols ranging from ethylene glycol to mannitol, the latter being completely recovered between fractions 30 and 49.

Since the completion of this work, Austin, Hardy, Buchanan, and Baddiley [25] have reported the fractionation of glycosides on strongly basic anion-exchange resin, during the removal of reducing carbohydrates.

The authors are grateful to Dr. J. R. Turvey for the sample of 1-*O*-β-laminaribiosylmannitol, and to Dr. G. O. Aspinall for the gas chromatography. This work was sponsored by the Institute of Seaweed Research.

Department of Chemistry, University of Edinburgh. [*Received, February 28th,* 1964.]

[23] Kuhn and Trischmann, *Chem. Ber.*, 1963, **96**, 284.
[24] Michaelis, *Ber.*, 1913, **46**, 3683.
[25] Austin, Hardy, Buchanan, and Baddiley, *J.*, 1963, 5350.