156. *The Constitution of Laminarin. Part II. The Soluble Laminarin of* Laminaria digitata.

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Soluble laminarin, in distinction from the normal laminarin of *Laminaria cloustoni* (Part I, *J.*, 1950, 3494), requires precipitation by ethanol for isolation from acidified extracts of *L. digitata*. When isolated in this way it contains a small proportion (*ca.* 3%) of fucoidin which is reduced (to *ca.* 1%) only by electrodialysis. Methylation and subsequent hydrolysis indicates that soluble laminarin is constructed of β-D-glucopyranose units linked through $C_{(1)}$ and $C_{(3)}$, and the chain length determined from the yield of tetramethyl glucopyranose is *ca.* 20 units. On *X*-ray examination, the acetyl and methyl derivatives of the two forms are also almost indistinguishable and the molecular weights of the methylated derivatives appear to be of the same order.

Slight differences between normal and soluble laminarin are detectable on treatment with periodate, but soluble laminarin is only about one-third as reducing as normal laminarin towards hypoiodite. The reducing power increases, however, with the removal of fucoidin, but no satisfactory explanation can be advanced either for this phenomenon or for the difference in physical properties between the two forms.

From the common seaweed *Laminaria cloustoni*, the polysaccharide laminarin is liberated as an insoluble precipitate when the fronds are kept in acidulated water (see Part I, *J.*, 1950, 3494). *L. digitata*, however, differs in that the addition of a precipitant such as ethanol is necessary before the laminarin, which is exuded when this species is kept in dilute acid, is precipitated. Kylin (*Z. physiol. Chem.*, 1915, **96**, 337), among others, noted and studied this phenomenon and suggested that laminarin was a mixture of polymers of differing molecular sizes. Barry (*Sci. Proc. Roy. Dublin Soc.*, 1939, **22**, 59) re-investigated the problem and also expressed the opinion that the only difference between the soluble and insoluble forms of laminarin lay in the size of the calloidal particles and that only one laminarin occurs in seaweeds. In addition

Barry has pointed out (personal communication) that, by the addition of glycerol, the spontaneous precipitation of normal laminarin can be prevented and has suggested that the extract from *L. digitata* contains a substance which interferes with the aggregation of the colloidal molecules.

We have attempted to determine whether structural variations were responsible for this difference between the two forms of laminarin.

A comparison of the two specimens by the *X*-ray powder photography technique showed the presence of a diffuse band corresponding to a spacing of 2·2 A., but there was no apparent difference between the samples. For the acetylated and methylated derivatives, no obvious differences were detectable in such properties as specific rotation or specific viscosity.

When the fully methylated soluble laminarin was hydrolysed and the fragments analysed by quantitative paper chromatography and by separation on the cellulose column (see Part I, *loc. cit.*) the yield of tetramethyl glucopyranose (5·1%) corresponded to a chain length of about twenty glucose units, the main product of hydrolysis being 2 : 4 : 6-trimethyl glucose. This result is virtually indistinguishable from that previously reported for the laminarin of *L. cloustoni* (Part I, *loc. cit.*). The accompanying mixture of dimethyl glucoses (4·1%), although smaller in quantity than that isolated in the former experiments, owing to a modification of the conditions of hydrolysis, could, as in the former case, be accounted for very largely by the accompanying demethylation (2·9%) of the 2 : 4 : 6-trimethyl glucose, and the mixture consisted as before of 2 : 6- and 4 : 6-dimethyl glucoses.

It has not been possible as yet to determine precisely the molecular weight of methylated soluble laminarin but, estimated by the method of Caesar, Gruenhut, and Cushing (*J. Amer. Chem. Soc.*, 1947, **69**, 617), the value appears to lie between 2800 and 3800. This is similar to the value found for normal laminarin and in no way explains the difference in behaviour.

Oxidation with potassium periodate (Halsall, Hirst, and Jones, *J.*, 1947, 1399) liberated from soluble laminarin 1 mole of formic acid from 5·7 glucose residues, which is a slightly higher proportion than from the normal form, but the result cannot be evaluated in terms of structure because of the uncertainty as to the nature of the " reducing " terminal group of the chain. The yield of formaldehyde (1 mole/30 glucose units) was slightly lower than from normal laminarin (Part I, *loc. cit.*).

More marked, however, were the differences between the apparent chain lengths calculated from the reducing action on hypoiodite, the figures being 112 for soluble and 45 for normal laminarin. When the reducing power was estimated by the alkaline 3 : 5-dinitrosalicylate technique of Meyer *et al.* (*Helv. Chim. Acta*, 1948, **31**, 103) the value of 27 units for soluble laminarin was double that for normal laminarin. The results of this experiment, which involves heating with moderately concentrated alkali, must be taken with reserve since a progressive increase of reducing action on heating with alkali was found to take place with both forms. Thus the apparent chain length of normal laminarin estimated by the hypoiodite method fell to 17 after contact with sodium hydroxide (2N.) at 40° for 5 minutes and to 8 after 30 minutes at 65°.

Since, apart from the difference in reducing power, unexplained as yet, no obvious structural differences had been revealed to account for the physical differences exhibited by the two types of laminarin, another explanation was sought. The ash content of the spontaneously deposited laminarin is invariably low (*ca.* 0·2%) whereas that in the soluble variety is with difficulty reduced below *ca.* 1·5% even by prolonged dialysis. This ash was found to contain a high proportion of sulphate which amounted to about half that (*ca.* 1·25%) released on the hydrolysis of the polysaccharide itself, so that the presence of ethereal sulphate residues was apparent. Furthermore, treatment of the soluble laminarin with " Amberlite IR-100 (H) " to remove cations, dialysis, and titration with potassium hydroxide solution, both potentiometrically and to phenolphthalein, gave a product with an acid equivalent of *ca.* 8100 which would agree with the presence of *ca.* 1·2% of SO_4 in the polysaccharide. Autohydrolysis of this acid followed by examination on the paper chromatogram revealed the presence of fucose, although earlier experiments on the hydrolysis of soluble laminarin had seemed to show that glucose was the sole product of hydrolysis; the detection of fucose was facilitated by the fact that only a small quantity of free glucose was produced under the mild hydrolytic conditions employed. On re-examination of the hydrolysates of the soluble laminarin produced in the usual way, but with larger quantities on the paper chromatogram, it became clear that fucose was indeed produced on hydrolysis, a quantitative determination indicating the presence of 1·4%. The most obvious explanation is that the soluble laminarin examined contains a proportion (*ca.* 3·2%) of fucoidin (Percival and Ross, *J.*, 1950, 720). Attempts to lower the sulphate content by precipitation

methods failed but by the regeneration of the polysaccharide from the acetate the sulphate content fell to 0·83%. A similar fall was observed on electrodialysis, and when this process was repeated a product was obtained containing 0·38% of SO_4. Hydrolysis of the solution in the anode compartment and examination on the paper chromatogram showed the presence of more fucose than glucose, which is evidence that some separation had occurred. A determination of the reducing power of the electrodialysed polysaccharide by the hypoiodite method showed the value to be about double that recorded for the original material and to correspond to a chain length of *ca.* 57 glucose units. It might be concluded from this result that the fucoidin component, which is certainly difficult to remove, is associated in some way with the potential reducing group of the laminarin chain.

It is difficult to decide if it is the presence of the small amount of fucoidin which enhances the solubility of laminarin by acting as a protective colloid in accordance with Barry's suggestion (*loc. cit.*). Direct addition of isolated fucoidin to " normal " laminarin showed that although precipitation proceeded more slowly it was not inhibited, but it must be remembered that some modification of the fucoidin molecule may have taken place during the lengthy process of isolation (Percival and Ross, *loc. cit.*). Although a final answer to this question must, therefore, be deferred, it seems certain that there are no gross chemical differences between the two forms of laminarin examined.

Experimental.

Preparation of Soluble Laminarin.—Dried ground fronds of *Laminaria digitata* (500 g.) were extracted with dilute hydrochloric acid by counter-current extraction. A sample (50 g.) was stirred with dilute hydrochloric acid (0·088N.; 5 l.) for 24 hours and the weed residue removed by centrifugation. A second 50-g. sample was then added to the same solution and extracted in the same way. This process was repeated until the whole 500 g. had been extracted. To the extract was added lead acetate solution till precipitation was complete, followed by aqueous barium hydroxide to alkalinity to phenolphthalein. The combined precipitates were removed at the centrifuge, leaving a cloudy solution which was clarified by filtration through " Filter Cel." The solution (4350 c.c.) was then diluted with ethanol (25 l., to give 85%), and the laminarin which was precipitated was allowed to settle, washed by decantation with ethanol, and centrifuged off. After dialysis against running water for one week, the solution was evaporated to small bulk *in vacuo* and poured into ethanol (4 l.). The laminarin was filtered off, washed with alcohol and ether, and dried *in vacuo* over phosphoric oxide [yield, 38 g., containing 2·31% of ash and having $[\alpha]_D^{15}$ −12° (*c*, 4·37 in water)]. This specimen was used for the acetylations and methylations described below.

Hydrolysis.—Soluble laminarin by hydrolysis at 95° with N-sulphuric acid reached a constant rotation after 4½ hours and the solution after neutralisation, filtration, and evaporation to a syrup was analysed by paper chromatography which gave no indication of the presence of any reducing sugar other than glucose (Found : glucose, 95·3%).

Attempted Purification of Soluble Laminarin.—The presence of sulphur having been demonstrated by sodium fusion, determinations of the sulphate content after hydrolysis at 100° with hydrochloric acid (5%) for 3 hours gave SO_4 1·25%, SO_4 in ash 0·62%. Free sulphate was absent. Normal laminarin contained only a trace of sulphate.

An examination of hydrolysed soluble laminarin by filter-paper chromatography, using highly concentrated solutions, showed the presence of a small quantity of fucose. Determination (Hirst and Jones, *J.*, 1949, 1659) gave fucose 1·4%.

Since it was considered probable that fucoidin was present as an impurity the following experiments were carried out.

(1) Mercuric sulphate solution was added to a solution of soluble laminarin (5%), the small quantity of precipitate removed at the centrifuge, and the solution dialysed to remove inorganic salts, concentrated under diminished pressure, and precipitated in ethanol (Found : SO_4, 1·5%).

(2) To a solution of laminarin (5%) lead acetate solution (10 c.c.; saturated) was added, followed by barium hydroxide (saturated) until alkaline to phenolphthalein. After 18 hours the solution was filtered, and dialysed, and the laminarin recovered as before (Found : SO_4, 1·36%). In a control experiment with a synthetic mixture of normal laminarin (2 g.) and fucoidin (0·1 g.), 83% of the original sulphate content was removed by the above process.

(3) A solution of soluble laminarin (2 g. in 150 c.c. of water) was submitted to electrodialysis in a three-compartment cell for the use of which we are indebted to Dr. H. T. Macpherson, the cathode compartment being separated from the solution by a parchment membrane (8 × 12 cm.) and the anode compartment by a cloth membrane covered with gelatin. The electrodes were of platinum (3 × 2 cm.) and the centre compartment had a capacity of 250 c.c., and its contents were stirred mechanically throughout. The apparatus was operated at 300 v., the current rising to 600 milliamps. after 1 hour and falling to 120 milliamps. after 4 hours. The laminarin was isolated in the usual way (Found : SO_4, 0·82%). The contents of the cathode compartment were found to contain calcium ions. The contents of the anode compartment after evaporation, hydrolysis, and examination on the paper chromatogram gave spots on development with aniline oxalate corresponding to fucose with a smaller amount of glucose.

The product was submitted to a second electrodialysis (1 g. in 150 c.c. of water) in the same manner, the current rising to 200 milliamps. in 1·5 hours, and falling to 60 milliamps. in 5·5 hours. The isolated

laminarin on hydrolysis and examination on the paper chromatogram showed the presence of a trace of fucose only (Found : SO_4, 0·38%).

(4) Soluble laminarin acetate (2 g.) in chloroform (50 c.c.) was treated with sodium (0·1 g.) in methanol (10 c.c.) at 0° for 2 hours. The precipitate was then removed by filtration, and washed with chloroform, hot ethanol, and ether. The product had $[\alpha]_D^{15}$ −12° (*c*, 2·8 in water) (Found : SO_4, 0·83%).

Acetylation.—Samples of soluble and normal laminarin (3 g. each) were gelatinised by gentle warming with dry pyridine (30 c.c.), to give almost clear solutions to which was added acetic anhydride (9 c.c.). The solutions were kept in the dark for 3 days, during which slight precipitation occurred in both cases, and then poured into water (300 c.c.). After being kept overnight, the product was filtered off on a hardened filter paper, washed with water (2 l.) till free from acid, and dried in a vacuum-desiccator over phosphoric oxide. Yields and acetyl contents were 4·49 g. and 4·29 g., 44·0% and 44·9% for the soluble and normal forms respectively. $[\alpha]_D^{11}$ were −65° and −62° (*c*, 1·0 in chloroform).

Viscosity Measurements.—The viscosities of the two foregoing acetyl derivatives in *m*-cresol at 20° and in chloroform at 20° were determined by means of an Ostwald viscometer (see Table).

		Average time of flow in secs.			
	c.	Solution.	Solvent.	$\eta_{sp.}$	$\eta_{sp.}/c$.
In chloroform.					
Normal	0·0351	84·5	71	0·190	5·41
Soluble	0·0351	87·5	71	0·232	6·61
In m-*cresol.*					
Normal	0·0702	521	413	0·255	3·66
Soluble	0·0702	519	413	0·257	3·73

c represents the concn. of $C_6H_{10}O_5$ units per l.

The results obtained with chloroform solutions indicate a slight difference in the two samples but this is not confirmed by the results with *m*-cresol. In the latter case, by use of Staudinger's equation, $\eta_{sp.} = K_m Mc$ and taking K_m as $6·3 \times 10^{-4}$ (the value for the corresponding cellulose derivatives, Staudinger and Reinecke, *Annalen*, 1938, **535**, 47) the apparent value for the molecular weights of both samples is found to be *ca.* 5800 but, since the true value for the constant is unknown, this figure only gives an indication of the order of magnitude of the molecular weights.

Methylation of Laminarin.—Laminarin (15 g.) was dissolved in sodium hydroxide solution (400 c.c.; 30%) at room temperature and the solution methylated by the gradual addition during 5 hours, with stirring, of methyl sulphate (140 c.c.). Stirring was continued overnight, the reaction mixture was neutralised with sulphuric acid and evaporated almost to dryness (40°/15 mm.), and the solution methylated as before. Dialysis in Cellophane bags against running water removed soluble sulphates, and the solution was then evaporated to small bulk. Two further methylations were carried out in the same manner. After dialysis and evaporation to dryness, a sample of the product was dissolved in chloroform and dried (Na_2SO_4), and the chloroform evaporated off to small bulk. The methylated compound was then precipitated with light petroleum (b. p. 60—80°), filtered off, and dried *in vacuo* (Found : OMe, 41·0%; ash, nil). The bulk of the material was then suspended in acetone (100 c.c.), and sodium hydroxide solution (200 c.c.; 30%) added. Methyl sulphate (70 c.c.) was then added with stirring during 4 hours, and stirring continued overnight. When the mixture was warmed to remove acetone, the methylated compound coagulated and was filtered off through muslin and remethylated as before. The coagulated product of the second methylation was dissolved in chloroform, and the residual liquor extracted with chloroform. The combined chloroform solutions were dried (Na_2SO_4) and evaporated to 50 c.c., and the methylated laminarin precipitated with light petroleum (b. p. 60—80°: 4 l.), separated off, and dried (Found : OMe, 43·3%; ash, 0·49%). In an attempt to raise the methoxyl content still further, two more methylations in acetone as described were carried out, yielding finally 9·8 g. of methylated laminarin (OMe, 44·1; ash, 0·31%).

Fractionation of Methyl Laminarin.—By addition of increasing amounts of light petroleum to a chloroform solution of methyl laminarin, various fractions were obtained but, as no analytical differences could be observed, these were later recombined.

Qualitative Paper-chromatographic Analysis of Methyl Laminarin.—Methyl laminarin (50 mg.) was treated with methanolic hydrogen chloride (1 c.c.; 4%) in a sealed tube at 95° for 7 hours. The tube was then opened and hydrolysis continued by heating with additional aqueous hydrochloric acid (5 c.c.; 4%) for 7 hours at 95° under reflux. The solution was neutralised with silver carbonate and treated with hydrogen sulphide to remove excess of silver, and the filtered solution finally treated with Amberlite ion-exchange resins to remove salts. After evaporation to small bulk, the solution was used for qualitative paper-chromatographic analysis with the following results. The paper strips on development with aniline oxalate solution indicated (1) tetramethyl glucopyranose, R_G, 1·00, weak, (2) 2 : 4 : 6-trimethyl glucose, R_G, 0·81, very strong, (3) 2 : 6-dimethyl glucose R_G, 0·54, weak pink spot, and (4) 4 : 6-dimethyl glucose, R_G, 0·49, very weak brown spot.

Separation of Methylated Glucose.—Methylated laminarin (2·71 g.) was treated in a sealed tube with methanolic hydrogen chloride (60 c.c.; 4%) at 100° for 7 hours. The acid was then neutralised with silver carbonate, the silver chloride filtered off, and the solution evaporated to dryness (40°/15 mm.). Sulphuric acid solution (400 c.c.; N.) was added to the residue, and the hydrolysis continued under reflux at 95° for 10 hours. After neutralisation with barium carbonate, filtration, and washing of the precipitate, the combined solutions were evaporated to a syrup (40°/15 mm.). The syrup was redissolved

and filtered through " Filter Cel " to remove trace of barium salts, and the syrup (2·89 g.) recovered by evaporation.

The syrup which partly crystallised was then separated on a column of powdered cellulose by the method of Chanda *et al.* (*J.*, 1950, 1289), using as solvent a mixture of 70% light petroleum (b. p. 100—120°) and 30% butanol saturated with water. The collected fractions were dissolved in water, warmed with charcoal, and filtered through " Filter Cel," and the solution and washings were evaporated to syrups which were dissolved in acetone, refiltered, and evaporated, and the syrup dried over phosphoric oxide *in vacuo*.

By elution with solvent, fraction (I) 0·756 g., and (II) 1·747 g., and by elution with water fraction (III) 0·180 g. were obtained. The fractions were shown by qualitative paper-chromatography to comprise (I) tetramethyl glucopyranose, (II) trimethyl glucose, (III) dimethyl glucoses (2) as far as free reducing sugars were concerned. Hypoiodite estimations of the free sugars on fractions (I) and (III) indicated that neither consisted solely of free sugars, fraction (I) containing 19·2% of free reducing sugar as tetramethyl glucose or 5·04% on the original syrup, and fraction (III) 65·8% as dimethyl glucose or 4·10% on original syrup.

Fraction (I) (0·747) was rehydrolysed with hydrochloric acid (2N.) for 5 hours at 95°, neutralised with silver carbonate, treated with hydrogen sulphide, filtered, and evaporated to a syrup (0·718 g.). This syrup was chromatographed as before, yielding two fractions (I*a*) and (II*a*) consisting of tetramethyl glucopyranose (0·242 g.) and trimethyl glucose (0·445 g.) respectively, showing that incomplete hydrolysis had taken place in the first experiment. Hypoiodite estimation showed that the tetramethyl glucose fraction contained 61·6% of free sugar, equivalent to 5·15% on the original hydrolysed syrup, which corresponds to a chain length of 20—21 glucose units in the polysaccharide.

These results may be summarised thus: tetramethyl glucopyranose 5·15%, trimethyl glucose (allowing for glucoside in fraction I) 80·2%, dimethyl glucose 4·10%, unaccounted portion of dimethyl fraction 2·10%, total recovery 92%.

Purification of Tetramethyl Glucose (*Fraction I*a).—This fraction (0·242 g.) was recrystallised from dry light petroleum (b. p. 40—60°), yielding 0·113 g. of purer crystalline material which was recrystallised, giving crystals (0·071 g.), m. p. 82—86°, and $[\alpha]_D^{15}$ +82° (equilibrium) (*c*, 0·71 in water) representing 96% purity. A second crop of crystals was obtained on partial evaporation of the solvent (0·014 g.). The m. p. of neither sample was depressed on admixture with authentic tetramethyl glucopyranose. The residual tetramethyl glucose was determined by hypoiodite oxidation on the combined mother-liquors, showing a further 0·060 g. to be present. The total amount of tetramethyl glucopyranose was, therefore, 0·145 g. or 5·02% on the original hydrolysate which is in good agreement with the 5·15% obtained in the original hypoiodite determination.

Partial Demethylation of 2 : 4 : 6-*Trimethyl Glucose.*—Pure 2 : 4 : 6-trimethyl glucose (0·257 g.) obtained by recrystallisation from fraction (II) above was treated with methanolic hydrogen chloride (6 c.c.; 4%) in a sealed tube at 95° for 7 hours, then neutralised with silver carbonate, the solution filtered, and the precipitate washed with dry methanol. The filtrate and washings were evaporated to a syrup (40°/15 mm.), and hydrolysis continued at 95° with sulphuric acid (2 c.c.; N.) for 10 hours. After neutralisation, filtration, and evaporation a syrup was obtained which showed on a qualitative paper-chromatogram, in addition to trimethyl glucose, a small quantity of dimethyl glucose. Quantitative determination by the same method gave dimethyl glucoses 2·86%. By use of larger amounts of syrup on a qualitative chromatogram, two dimethyl glucoses were observed having the same R_G values as those obtained from the methyl laminarin hydrolysate and in approximately the same relative amounts. Unfortunately the R_G values of the sugars occur too close together for separate quantitative determination, but these results indicate the origin of a large proportion of the dimethyl sugars separated on the cellulose column.

The Reducing Power of Soluble Laminarin.—(1) *Oxidation with alkaline hypoiodite.* To specimens of normal and soluble laminarin (0·3 g. each), iodine solution (10 c.c.; 0·1N.) and sodium hydroxide (15 c.c.; 0·1N.) were added. After 1 hour the solution was acidified and the excess of iodine determined by titration. The consumptions of iodine corresponded to one aldehyde group in 45 glucose units for normal laminarin and in 112 glucose units for soluble laminarin. Later experiments, the results of which are given in the Table below, indicated that warming of the laminarin with sodium hydroxide

Treatment.	Apparent no. of glucose units/reducing group.
Soluble laminarin.	
(1) Direct treatment with I–NaOH	112
(2) Warmed to 40° (1 min.) in NaOH (15 c.c.; 0·1N.), cooled, + iodine	87
(3) Electrodialysed product (ash, 0·38%); as (1)	57
Normal laminarin.	
(4) As (1)	45
(5) As (2)	32
(6) Warmed to dissolve in water (10 c.c.), cooled, +NaOH (5 c.c.; 6N.), + iodine	45
(7) Warmed to 40° (1 min.) in water (7·5 c.c.), cooled, +NaOH (7·5 c.c.; 0·2N.), + iodine	43
(8) As (6), but left for 3 days before addition of iodine	29
(9) Warmed to 40° (5 mins.) in NaOH (15 c.c.; 0·1N.), cooled, + iodine	17
(10) As (4), but with 2N-NaOH	29
(11) Warmed to 65° (30 mins.) in NaOH (15 c.c.; 2N.), cooled, + iodine	8

before the addition of the iodine caused an apparent increase in the reducing power and therefore a reduction of the apparent chain length. In the case of normal laminarin, on which most of the determinations were made owing to its availability, the apparent reducing power was increased to the equivalent of one reducing group in 8 glucose units by treatment for ½ hour at 65° with 2N-sodium hydroxide.

(2) *Colorimetric method.* As in Meyer's method (*Helv. Chim. Acta*, 1948, **31**, 103), a solution of the polysaccharide (2 c.c.; 0·5%) was heated with water (1 c.c.), 3 : 5-dinitrosalicylic acid solution (1 c.c.; 1·5%), and sodium hydroxide (1 c.c.; 6N.) at 65° for 30 minutes, the solution being then cooled and diluted to 25 c.c. The colour was then compared with a blank solution in a Spekker absorptiometer (4-cm. cell; filter 604). The readings were then compared with a graph obtained by treating laminaribiose in the same way at various concentrations. The readings thus obtained gave values for the chain lengths of soluble and normal laminarins of 27 and 13 $C_6H_{10}O_5$ units respectively. However, in view of the results obtained above for the effect of heating the polysaccharide with alkali, the interpretation of these results is open to doubt.

Oxidation of Laminarin with Potassium Periodate.—(a) *Determination of formic acid liberated.* Samples (0·5 g.) of soluble and normal laminarin were shaken in the dark with sodium metaperiodate (15 c.c.; 0·3M.), potassium chloride (5 g.), and water (105 c.c.) for 14 days, a blank experiment being run at the same time. At intervals samples (10 c.c.) were withdrawn and titrated to methyl-red with 0·01N-sodium hydroxide after the addition of ethylene glycol. For the normal laminarin it was necessary to separate the insoluble material at the centrifuge. After application of the necessary corrections (Halsall, Hirst, and Jones, *J.*, 1947, 1399, 1427) the following results (in c.c. of 0·01N-NaOH) were obtained: Soluble laminarin, 3·71 c.c. (3 days); 4·42 c.c. (7 days); 4·66 c.c. (14 days). Normal laminarin, 2·38 c.c. (3 days); 3·65 c.c. (7 days); 3·70 c.c. (14 days). The values after 7 days correspond to the release of 1 mol. of formic acid from 5·6 and 6·7 $C_6H_{10}O_5$ units for soluble and normal laminarin respectively.

(b) *Formaldehyde production* [With W. E. A. MITCHELL]. Soluble laminarin (0·5 g.), potassium periodate (1 g.), and water (20 c.c.) were shaken in the dark for 4 days. The contents were then separated at the centrifuge, a sample (15 c.c.) of the liquid was removed and acidified with dilute hydrochloric acid (2N.), and the iodine liberated removed by the addition of sodium arsenite solution (1·4N.). The solution was then buffered with acetate buffer to pH 4·6, dimedone solution [160 mg. in ethanol (2 c.c.)] added, and the mixture heated on the steam-bath for 20 minutes. After being set aside overnight the dimedone–formaldehyde compound was removed on a sintered filter-stick (porosity 4) and dried to constant weight in a drying block. The yield was 0·0280 g., and the m. p. 185—186° corresponding to the liberation of 1 mol. of formaldehyde from 30 $C_6H_{10}O_5$ units.

Treatment of Laminarin with "Amberlite 1R-100 (H)."—Samples of soluble laminarin (ash, 2·0%) and normal laminarin (ash, 0·28%) were dialysed against distilled water for 3 days to remove simple salts, and the polysaccharides recovered by concentration and the addition of ethanol. Ash (1·81%) was still present in the soluble laminarin, of which 0·23% only was due to the presence of siliceous matter as determined by the loss in weight on treatment with sulphuric and hydrofluoric acids. The purified specimens of soluble laminarin (0·326 g.) and normal laminarin (0·919 g.) in water (10 c.c.) were shaken with "Amberlite 1R-100 (H)" resin overnight. After dialysis against distilled water for 2 days to remove any inorganic acid, the solution which was still acid was filtered, the resin was washed, and the filtrate and washings were titrated against potassium hydroxide (0·01N.) potentiometrically in the presence of phenolphthalein using antimony and calomel/saturated potassium chloride electrodes. The end-points to phenolphthalein were noted during the course of the titrations. The results are given in the Table below. In the case of soluble laminarin the solution after titration was dialysed to remove

Titres with 0·01N-KOH *and corresponding potentiometric readings for the various acids.*

Soluble laminarin (0·326 g.).				Normal laminarin (0·919 g.).			
KOH, c.c.	mv.	KOH, c.c.	mv.	KOH, c.c.	mv.	KOH, c.c.	mv.
—	191	3·53	262	—	198	3·00	264
0·50	191	4·01	300	0·31	198	3·50	304
1·00	194	4·63	370	0·81	202	4·00	346
1·50	198	4·87 *	404	1·20	207	4·51 *	388
2·00	204	5·40	455	1·50	212	5·00	454
2·50	212	5·94	485	1·91	220	5·50	486
3·00	226	7·05	570	2·49	238	6·00	502
						6·50	514

* End-point to phenolphthalein.

excess of alkali, then evaporated (40°/15 mm.), and the laminarin precipitated in alcohol. The ash content of the product was 1·99%. A blank titration after shaking of the resin with water overnight and dialysis gave a titre of 0·90 c.c. of potassium hydroxide (0·01N.).

Graphs were drawn of titre against millivolts. From the curves and the phenolphthalein end-points, the alkali used after allowance for the blank is equivalent in the case of soluble laminarin to 1 equivalent of acid in 8100 g., and for normal laminarin in 25,000. These figures correspond to SO_4 1·2% and 0·4% respectively, the acidity being assumed to be due to an ethereal sulphuric acid residue.

Autohydrolysis.—A solution of soluble laminarin, after overnight shaking with "Amberlite 1R-100 (H)" and removal of the resin, was heated at 95° for 1·5 hours. Barium carbonate was added to the hot solution, which after filtration was poured into ethanol. Filtration, evaporation, and examination on the filter-paper chromatogram revealed the presence of fucose as the principal sugar component together with a smaller quantity of glucose.

Uronic Acid in Laminarin.—By McCready, Swenson, and Maclay's method (*loc. cit.*) uronic acid determinations on both types of laminarin gave results of 0·84% for soluble and 0·70% for normal laminarin, but these figures are probably within the experimental error of the determination.

Molecular Weight by Barger's Method.—The micro-molecular weight method of Barger (*J.*, 1904, 286) as modified for high polymers (Caesar, Gruenhut, and Cushing, *J. Amer. Chem. Soc.*, 1947, **69**, 617) was applied to methylated soluble laminarin as follows. Droplets of a 1·13% solution of this derivative in chloroform were compared in capillary tubes, with solutions of sucrose octa-acetate ($2·5 \times 10^{-3}$M.), using a travelling microscope reading to 0·02 mm. The isopiestic condition was found to be between 3×10^{-3} and 4×10^{-3}M-sucrose octa-acetate solution, from which the molecular weight appears to lie between 3800 and 2800, corresponding to a chain length of 14—18 $C_9H_{16}O_5$ units.

Effect of Added Fucoidin on the Precipitation of Laminarin.—Known amounts (2—5 g.) of normal laminarin were dissolved in hot water (100 c.c.) and allowed to cool to room temperature. Sufficient fucoidin to make up 2·5% of the total was added to two of the solutions, together with sufficient hydrochloric acid to simulate the conditions of extraction. The deposited laminarin was removed after standing. Precipitation: Normal laminarin, 1 day 33; 2 days 66%. Laminarin + 2·5% fucoidin, 1 day 12; 2 days 61%.

X-*Ray Powder Photographs of Laminarin.*—Through the kindess of Dr. C. A. Beevers specimens of laminarin were examined, using a copper target and 1·5 hours' exposure (25 mA.; 50 kv.). The photographs given by specimens of normal and soluble laminarin were identical. They both showed a vague halo rather sharper on its low-order side than on its high-order side, corresponding to a spacing of 2·2 A.

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