688. *The Constitution of Laminarin. Part I. An Investigation on Laminarin isolated from* Laminaria cloustoni.

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Laminarin has been investigated with particular reference to the products of hydrolysis of the methylated polysaccharide, and the conclusion of Barry (*Sci. Proc. Roy. Dublin Soc.*, 1939, **22**, 59) that the main structure is built up from β-D-glucopyranose units linked through $C_{(1)}$ and $C_{(3)}$ is confirmed. The yield of tetramethyl glucopyranose set free on hydrolysis corresponds to a chain length of 20 glucose units, rather than of 16 units as deduced previously (Barry, *J.*, 1942, 578). Determinations of the average molecular weight of the methylated laminarin suggest that this chain may represent the physical molecule itself. The results of the oxidation of laminarin with potassium periodate give general support to this view although there are complications due to over-oxidation, and estimates of the reducing power by the hypoiodite method appear to indicate that a proportion of the potential aldehyde groups are modified in some way.

The polysaccharide laminarin, a reserve carbohydrate abundant in the autumn in the sub-littoral brown seaweeds, is readily isolated from *Laminaria cloustoni* fronds on their immersion in dilute aqueous solutions of mineral acids. In the first structural studies on this substance Barry (*Sci. Proc. Roy. Dublin Soc.*, 1939, **22**, 59) showed that the glucose building units of which

the molecule is constructed are mutually united through the 1 : 3-positions by β-linkages, since he isolated 2 : 4 : 6-trimethyl glucose by the hydrolysis of methylated laminarin and the specific rotations of laminarin and its derivatives are low. Further evidence was secured in the resistance to attack by periodate (with the exception of the terminal groups).

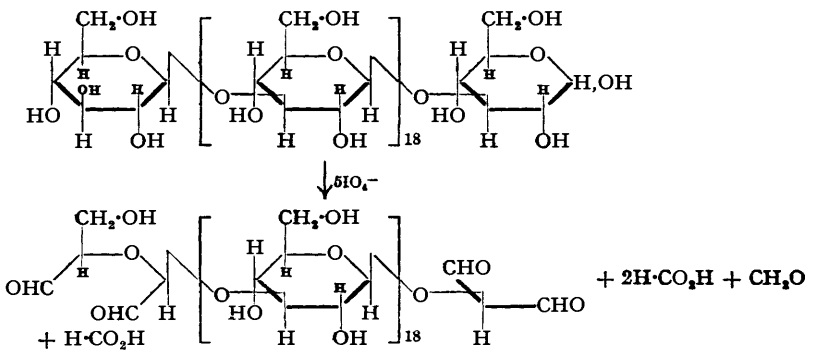
Barry (*J.*, 1942, 578) made an estimate of the apparent chain length of laminarin by oxidation, first with periodate and then with bromine, followed by determination of the equivalent weight of the derived acid, arriving at a value of sixteen glucose units for the chain length. This result was calculated on the assumption that no oxidation with periodate occurred at the reducing end of the chain. If the oxidation proceeded normally, however, for each free reducing group a second pair of carboxyl residues would be produced, to give a tetrabasic acid, and the chain length calculated on that basis would be greater than sixteen units, and probably about twenty units, although exact computation is impossible. By the hydrolysis of methylated laminarin and separation of the tetramethyl glucopyranose Barry (*loc. cit.*) reported a provisional value for the chain length of about seventy units, but since a relatively small quantity of starting material was used and the distillation method of separation was employed he placed little reliance on this result.

On taking up another field of chemistry, Dr. Barry suggested that this work might be continued in our laboratory, and we have been glad to do this in view of our interest in seaweed carbohydrates.

Laminarin from *Laminaria cloustoni* has been purified, acetylated, and methylated. The fully methylated substance, on hydrolysis and separation by filter-paper chromatography (Hirst, Hough, and Jones, *J.*, 1949, 928) and by the cellulose column (Hough, Jones, and Wadman, *J.*, 1949, 2511), gave tetramethyl glucopyranose amounting to 5%, which corresponds to an average chain length of about 20 glucose units. The main product of hydrolysis was identified as 2 : 4 : 6-trimethyl glucose (cf. Barry, *loc. cit.*), which was accompanied by a mixture of 2 : 6- and 4 : 6-dimethyl glucose (8%). It seems doubtful, however, if these last components of the hydrolysate can have any structural significance since 2 : 4 : 6-trimethyl glucose was found to be particularly susceptible to demethylation and, under the conditions employed for the hydrolysis of methylated laminarin, gave an 8% yield of a mixture of dimethyl glucoses almost identical in composition with that mentioned above.

If this conclusion is valid, methylated laminarin should have a molecular weight of *ca.* 4000. Evidence that this value is of the correct order of magnitude was obtained by a comparison, in chloroform solution, with solutions of octa-acetyl sucrose by the modification of Barger's method (*J.*, 1904, 286) employed for high polymers by Caesar, Gruenhut, and Cushing (*J. Amer. Chem. Soc.*, 1947, **69**, 617); from this it was concluded that the molecular weight lay between 2600 and 3500. A provisional estimate by the osmotic pressure method gave a value between 3000 and 5000, and viscosity measurements in *m*-cresol also indicated a low value for the molecular weight, on the assumption that the Staudinger equation is obeyed for this polysaccharide.

Attempts to determine the size of the molecule of unsubstituted laminarin have given somewhat conflicting results. Oxidation with potassium periodate (Brown, Halsall, Hirst, and Jones, *J.*, 1948, 27) and titration of the formic acid released gave results which were not as easy to interpret as, for example, similar experiments with amylopectin; although the liberation of formic acid was fairly rapid for up to five days, a steady state was never reached. After about



five days, at which point 1 mole of formic acid had been liberated from 6·8—7 glucose units, acidity continued to develop, albeit more slowly, in an approximately linear fashion, and iodine

was liberated after 10—14 days. This is presumptive evidence of over-oxidation and it is significant that the derived disaccharide laminaribiose (Barry, *Sci. Proc. Roy. Dublin Soc.*, 1941, **22**, 423), which was isolated by the partial hydrolysis of laminarin and separation on the cellulose column, when used as a model substance also underwent oxidation with the liberation of iodine and more than the expected three molecules of formic acid, in a reaction which showed no sign of reaching equilibrium.

If the laminarin molecule were as depicted, oxidation with periodate would result in the release of 1 molecule of formic acid from 6·7 glucose units for the consumption of 5 equivalents of periodate. Both these conditions are fulfilled after oxidation for 5 days, although it cannot be claimed that the result is wholly reliable owing to the continuing oxidation mentioned above and the uncertainty mentioned below as to the " reducing " end of the chain. An estimate of the formaldehyde liberated during the oxidation gave 1 molecule from 27 glucose units, and, whilst this is a considerable deviation from the calculated figure of 1 molecule from 20 units, it can be taken as fairly satisfactory in view of the small proportion of formaldehyde produced.

The postulation of such a formula as that discussed above requires, however, that one potential aldehydic group should terminate the chain. From treatment with sodium hypoiodite under varying conditions however it is estimated that one aldehydic group is present in a structure containing 40 glucose units, and oxidation with bromine and determination of the equivalent weight of the derived acid give a result corresponding with 1 in 48. The colorimetric method of estimating the reducing power, in which 3 : 5-dinitrosalicylic acid is used (Meyer, Noelting, and Bernfeld, *Helv. Chim. Acta*, 1948, **31**, 103) and the intensity of colour is compared with that developed by laminaribiose solutions of known concentration, indicated the presence of one aldehyde group for 13—14 glucose residues, but since there is evidence that laminarin is degraded by hot alkali the validity of this result is in doubt.

Because of the apparently anomalous results for the chain length calculated from estimates of the reducing power we are not at this stage prepared to claim that a chain of about twenty β-D-glucopyranose units linked through positions 1 and 3 fully represents the structure of laminarin, although the balance of the evidence at present available is in favour of such a view. In any event a highly branched structure appears to be definitely excluded and it is proposed to investigate the molecular weight of laminarin and its derivatives by physical measurements more fully than has been possible hitherto, in order to attempt a final solution of the problems involved.

Experimental

Extraction of Laminarin.—Laminarin was extracted by Dr. W. A. P. Black, Institute of Seaweed Research, Musselburgh, Midlothian, as follows. Fresh *Laminaria cloustoni* fronds, collected at Oban (October—November, 1947), were chopped into small pieces and covered with 1% hydrochloric acid (1 l.) for 2 days. The mixture containing the finely suspended polysaccharide was agitated and then filtered through a coarse filter, and the residue re-extracted with fresh acid. During several days crude laminarin was deposited; the supernatant liquid was then siphoned off and the product dissolved in warm water (80°) and filtered to remove seaweed particles. The product which separated on cooling was collected by filtration, re-deposited from hot water, washed with ethanol and ether, and dried in a vacuum-desiccator. The fine white powder so obtained had $[\alpha]_D^{15}$ −14·4° (*c*, 0·9 in water) [Found: moisture, 10·0; ash (as sulphate), 0·4%].

Purification.—The laminarin (60 g.) was dissolved in water (1 l.) at 50°, and deposition allowed to continue for 3 days. This process was repeated three times, to give laminarin (40 g.), $[\alpha]_D^{15}$ −14·8° (*c*, 0·8 in water) (Found: moisture, 24·0; ash, 0·2%). Hydrolysis at 95° in 0·85N-hydrochloric acid was complete in 2½ hours and gave glucose 96% (polarimetric estimation), 95% (hypoiodite). No other sugar could be detected on the paper chromatogram.

Acetylation of Laminarin.—Laminarin (0·75 g.), dissolved in warm water and precipitated by ethanol, was dispersed by warming it in pyridine (10 c.c.). Acetic anhydride (3 c.c.) was added slowly, most of the polysaccharide dissolving. After being kept at room temperature for 48 hours the mixture was poured into water (100 c.c.), and the precipitate filtered off, washed with water, and dried over phosphoric oxide in a vacuum desiccator. The product (1·26 g.) was dissolved in chloroform (20 c.c.) and precipitated by the addition of light petroleum (75 c.c.; b.p. 40—60°). It had $[\alpha]_D^{15}$ −60° (*c*, 0·96 in chloroform) (Found: $CH_3{\cdot}CO$, 44·0. Calc. for $C_{12}H_{16}O_8$: $CH_3{\cdot}CO$, 44·8%).

The reducing power (Bergmann and Machemer, *Ber.*, 1930, **63**, 316) corresponded to 1 reducing group per 35 $C_6H_{10}O_5$ units.

Benzoylation of Laminarin.—Dry laminarin gave unsatisfactory results, but a compound corresponding to a *monobenzoate* was obtained by using laminarin containing moisture (*ca.* 10%).

Laminarin (2 g.) was dispersed in pyridine (20 c.c.) by warming, and benzoyl chloride (6 c.c.) was added slowly with vigorous stirring, a gummy precipitate being deposited. After being kept for 12 days with daily stirring, the mixture was poured into water (250 c.c.), and the whole set aside for 3 days. The buff powder so obtained was filtered off, washed with hot water, dried, and purified as described for the acetate. The final product was a pale buff-coloured powder (2·66 g.), readily soluble in chloroform

and acetone, insoluble in ether or ethanol, having $[\alpha]_D^{14}$ −54·9° (*c*, 1·03 in chloroform) (Found : C, 58·7; H, 5·2; C_6H_5·CO, 40·4 $C_{13}H_{14}O_6$ requires C, 58·7; H, 5·3; C_6H_5·CO, 39·5%).

Methylation of Laminarin.—Laminarin (21 g.) was dissolved in sodium hydroxide solution (550 c.c.; 30%) at room temperature to give a slightly viscous solution which was methylated by the gradual addition, during 5 hours, of methyl sulphate (200 c.c.) with continuous stirring. Stirring was continued overnight, the reaction mixture was partly neutralised with sulphuric acid and evaporated almost to dryness (35°/15 mm.), and the solution methylated again as before. Dialysis in Cellophane bags against running tap-water for 8 days removed soluble sulphates, and the solution (7·5 l.) was then evaporated to a small bulk (diminished pressure). Acetone (150 c.c.) and sodium hydroxide solution (350 c.c.; 30%) were then added and the methylation was repeated by the slow addition of methyl sulphate (170 c.c.) in the usual way. After most of the acetone had been evaporated off, partly methylated laminarin separated which was collected; nine such treatments gave a product soluble in chloroform. Fractionation from chloroform solution by the addition of light petroleum (b.p. 60—80°) gave (*a*) 8·75 g. (OMe, 41·2%), (*b*) 6·25 g. (OMe, 43·8%), $[\alpha]_D^{15}$ −7·5° (*c*, 1·0 in chloroform), and, by evaporation, (*c*) 0·75 g. (OMe, 37·5%).

Fraction (*b*) (0·5 g.) was treated twice in boiling methyl iodide (5 c.c.) with silver oxide (1·5 g.) added in small portions during 30 hours, but the methoxyl content remained unchanged.

Fractions (*a*) and (*c*) were combined and remethylated thrice in dioxan (100 c.c.) with sodium hydroxide (150 c.c.; 30%) and methyl sulphate (75 c.c.).

Fractionation as above gave (*d*) 3·49 g. (OMe, 44·4%), and (*e*) 3·04 g. (OMe, 43·6%).

Viscosity Measurements.—The viscosities of various derivatives, determined in *m*-cresol at 20° with an Ostwald viscometer, were as follows :

Derivative.	*c*.	Average time of flow (secs.). Solution.	Solvent.	$\eta_{sp.}$	$\eta_{sp.}/c$.
Acetyl laminarin	0·0693	581	459	0·266	3·84
Methyl laminarin (*b*)	0·0977	635	497	0·274	2·80
,, ,, (*d*)	0·0982	654	,,	0·316	3·22
,, ,, (*e*)	0·0980	649	,,	0·306	3·12

The concentration, in g.-mol., of repeating units per litre is represented by *c*. The fractions of methylated laminarin appear to be substantially identical. By use of Staudinger's equation $\eta_{sp.} = KmMc$ and $Km = 6·3 \times 10^{-4}$ for the acetate and 12×10^{-4} for the methylated laminarin (the values for the corresponding cellulose derivatives; Staudinger and Reinecke, *Annalen.*, 1938, **535**, 47) the apparent values for the molecular weight are *ca.* 6100 and *ca.* 2600 respectively, but since the true values for the constants are unknown these figures can be taken only as an indication of the order of magnitude of the molecular weights.

The Molecular Weight by Barger's Method.—The micro-molecular-weight method of Barger (*J.*, 1904, 286), as modified for high polymers (Caeser, Gruenhut, and Cushing, *J. Amer. Chem. Soc.*, 1947, **69**, 617), was applied to methylated laminarin (*e*) as follows. Droplets of a 1·05% solution of this derivative in chloroform were compared, in capillary tubes, with solutions of sucrose octa-acetate ($2—5 \times 10^{-3}$M.), a travelling microscope reading to 0·02 mm. being used. The isopiestic condition was found to lie between 3×10^{-3} and 4×10^{-3}M-sucrose octa-acetate solution, from which the molecular weight of (*e*) appears to lie between 3500 and 2600, corresponding to a chain length of 13—17 $C_9H_{16}O_5$ units.

Molecular Weight by Osmometry.—An osmotic pressure determination of the molecular weight of (*d*), carried out through the kindness of Professor H. W. Melville, F.R.S., by Mr. G. Forsyth, B.Sc., indicated the value to lie between 3500 and 5000. This result must be considered as provisional only, because of indications of diffusibility through the membrane.

Hydrolysis of Methylated Laminarin and Separation of Methylated Glucoses.—Fractions (*b*) (4 g.), (*d*) (2·5 g.), and (*e*) (2·5 g.) were combined and boiled under reflux in methanolic hydrogen chloride (300 c.c.; 2%) and chloroform (60 c.c.) for 54 hours. After neutralisation with silver carbonate, treatment with hydrogen sulphide, etc., and concentration a clear golden non-reducing syrup I (9·69 g.; n_D^{16} 1·4608) was obtained.

(a) *Hydrolysis for 7 hours.* Syrup I (4·19 g.) was heated under reflux at 95° with hydrochloric acid (120 c.c.; 2N.) to constant rotation. The solution was then neutralised with silver carbonate, a portion removed for paper chromatography experiments (see below), and the remainder evaporated and dried by the addition of alcohol–benzene and distillation. The crystalline mass (2·98 g.) was then separated on a column of cellulose (powdered Whatman No. 1 ashless filter tablets; 3·5 × 41 cm.) in the usual way (Chanda *et al.*, *J.*, 1950, 1289). The collected fractions were dissolved in water, warmed with charcoal, filtered through " Filter Cel," and, after evaporation of the solvent, dried over phosphoric oxide at 35° in a vacuum.

By elution with 60% light petroleum (b.p. 100—120°)—40% *n*-butanol, saturated with water, fractions (1) 309 mg.) and (2) (2259 mg.) were obtained and, by elution with water, fraction (3) (194 mg.) was isolated (total recovery 93%). From the results of analysis of the partly crystalline fraction (1) it was suspected of containing trimethyl methylglucoside and this was confirmed by hydrolysis with hydrochloric acid (10 c.c.; 2N.) for 4 hours at 95° and examination on the paper chromatogram, trimethyl glucose being shown to be present. This hydrolysate gave a crystalline mixture (291 mg.) which was separated on the cellulose column, to give fraction (1*a*) (160 mg.) and (1*b*) (100 mg.) (recovery 89%). Fraction (1*a*), $[\alpha]_D^{15}$ +66° (*c*, 1·3 in water) (OMe, 49·8%), contained 80·3% of tetramethyl glucopyranose (hypoiodite oxidation). Crystallisation from light petroleum (b.p. 40—60°) gave a substance, $[\alpha]_D^{15}$ +84° (*c*, 0·87 in water), m. p. 83—85° not depressed on admixture with tetramethyl D-glucopyranose (Found : C, 51·3; H, 8·5; OMe, 50·4. Calc. for $C_{10}H_{20}O_6$: C, 50·9; H, 8·5; OMe, 52·5%). The aniline derivative

had m. p. 134—136° and m. p. 135—136° when mixed with authentic tetramethyl D-glucopyranose anilide (Found: C, 61·3; H, 8·0; OMe, 39·2. Calc. for $C_{16}H_{25}O_5N$: C, 61·7; H, 8·0; OMe, 39·9%).

From this experiment the actual amount of tetramethyl glucopyranose in (1*a*) is calculated to be 128 mg., assuming 80% purity whence, taking into account the recoveries from the various separations, it is estimated that 144 mg., *i.e.* 4·9%, were present in a total of 2·762 g. of the mixed sugars isolated from the column.

Fractions (1*b*) and (2) crystallised completely and had m.p. 115—117°, raised to 124—126° by two recrystallisations from dry ether. The R_G value on a filter-paper chromatogram was identical with that of 2 : 4 : 6-trimethyl glucose. The substance had $[\alpha]_D^{17}$ +91·3° (initial), +75·5° (12 hours, constant; *c*, 2·2 in water) (Found: C, 49·3; H, 8·3; OMe, 41·3. Calc. for $C_9H_{18}O_6$: C, 49·1; H, 8·1; OMe, 41·8%). The derived lactone had n_D^{16} 1·4662, $[\alpha]_D^{17}$ +95° (initial), +42·7° (6 hours, constant; *c*, 2·2 in water). The aniline derivative, after three recrystallisations from ethyl acetate, had m.p. 163—165°, $[\alpha]_D^{15}$ −81° (20 hours, constant; *c*, 0·6 in methanol) (Found: C, 59·9; H, 7·6; N, 4·9. Calc. for $C_{14}H_{23}O_5N$: C, 60·2; H, 7·7; N, 4·7%). Fraction (3) was a colourless glass consisting mainly of dimethyl glucoses, small quantities of monomethyl glucoses, and traces of glucose; it was not examined in detail.

(b) *Hydrolysis for* 11 *hours.* Syrup I (3·56 g.) was hydrolysed with hydrochloric acid (100 c.c.; 2N.) for 11 hours at 95° and worked up, to give a crystalline mixture (3·213 g.; dried over phosphoric oxide at 35° in a vacuum for 8 hours). Separation as before on the cellulose column gave fraction (4) (250 mg.; eluted with 70% light petroleum–30% butanol), fraction (5) (2481 mg.; 50% light petroleum–50% butanol), fraction (6) (220 mg.; same mixture), and fraction (7) (47 mg.; eluted with water) (recovery, 93%).

Partly crystalline fraction (4) was hydrolysed with hydrochloric acid (10 c.c.; 2N.) for 4 hours at 95° and, after isolation in the usual way, the completely crystalline solid (230 mg.) was separated on the column, to give fractions (4*a*) (148 mg.) and (4*b*) (38 mg.; eluted with water). Fraction (4*a*) which was completely crystalline was chromatographically pure tetramethyl glucopyranose, $[\alpha]_D^{15}$ +77·0° (*c*, 1·0 in water) (OMe, 49·0%), both figures corresponding to 93% purity. Corrected for losses in this second separation the yield of tetramethyl glucopyranose is 170 mg., *i.e.* 5·7% on the total weight (2·998 g.) of mixed sugars isolated from the column. This figure is probably too high since the total recovery of sugars is usually *ca.* 90% which would give an overall yield of 5·1%. Fractions (4*b*) and (5) were chromatographically pure 2 : 4 : 6-trimethyl glucose. Fraction (6) was spread over 600 tubes but no difference between the R_G values (*ca.* 0·55) of the first and last fractional cuts could be detected. It had the properties of a dimethyl hexose and was isolated as a deliquescent glass which crystallised slowly (Found: OMe, 27·7. Calc. for $C_8H_{16}O_6$: OMe, 29·8%), $[\alpha]_D^{16}$ +77·2° (20 mins.), +68·6° (260 mins., constant; *c*, 1·4 in water). When the glass was examined on a paper chromatogram (butanol–ethanol–water) run for a sufficient time to give a flow of over 30 cm. of the leading spot, it was observed that the single spot obtained on spraying with aniline oxalate appeared to be composite, having a pink " body " and a brown " tail " towards the upper portion of the oval. It was found that 2 : 6- and 4 : 6-dimethyl glucoses gave pink and brown spots respectively with aniline oxalate and had practically the same R_G values as fraction (6), whereas the R_G value for 2 : 4-dimethyl glucose was considerably higher.

After two recrystallisations from dry ethyl acetate a portion of fraction (6) gave fine needles, m.p. 159—162°, and m.p. 155—159° on admixture with authentic 4 : 6-dimethyl glucose (m.p. 154—159°).

2 : 6-Dimethyl glucose (a glass) has $[\alpha]_D$ +58·3° (in water) (Bell and Synge, *J.*, 1938, 1711), $[\alpha]_D$ +63·3° (Freudenberg and Hüll, *Ber.*, 1941, **74**, 237), and 4 : 6-dimethyl glucose has $[\alpha]_D$ +65·7° (Bell and Lorber, *J.*, 1940, 453).

Further experiments on the composition of fraction (6) are described below in conjunction with the work on demethylation.

Fraction (7) consisted of monomethyl glucoses with a trace of glucose and was not analysed in detail.

(c) *Separation by paper chromatography.* Syrup I was hydrolysed (95°) with hydrochloric acid (7 hours). The neutralised hydrolysate was passed through small columns of " Zeo-Karb HI " and " De Acidite B," and the ion-free solution evaporated (35°/15 mm.) to a thin syrup which was analysed as described by Hirst, Hough, and Jones (*J.*, 1949, 928); the more convenient phosphate buffer was used in the analysis (Chanda *et. al.*, *loc. cit.*).

In a typical analysis the following titres (0·01N-sodium thiosulphate) were obtained: tetramethyl glucopyranose (0·58 c.c.), trimethyl glucose (9·36 c.c.), and dimethyl glucoses (0·95 c.c.), corresponding to 5·3, 86·0 and 8·7% (molar).

The Partial Demethylation of 2 : 4 : 6-*Trimethyl Glucose.*—(a) *Treatment with* 2N-*hydrochloric acid for* 7 *hours.* Pure 2 : 4 : 6-trimethyl glucose (0·2 g.) was boiled under reflux (5·4 hours) with 2% methanolic hydrogen chloride (7 c.c.) and chloroform (1·3 c.c.). After being worked up with silver carbonate the syrupy methylglucosides were hydrolysed with hydrochloric acid (6 c.c.; 2N.) for 7 hours (95°), and the mixture of free sugars, after removal of inorganic ions, was isolated as a thin syrup. Paper chromatography revealed the presence of dimethyl glucoses and traces of monomethyl glucoses and glucose. The appearance and R_G value of the composite spot representing the dimethyl glucoses were indistinguishable from those of the product obtained directly from methylated laminarin. Separation on the paper chromatogram and estimation with buffered hypoiodite gave the following titres (0·01N-thiosulphate): trimethyl glucose (9·19 c.c.; 6·28 c.c.) and dimethyl glucoses (0·44 c.c.; 0·31 c.c.), corresponding to 4·6% and 4·7% (molar) of the dimethyl sugars.

(b) *Treatment with* 2N-*hydrochloric acid for* 11 *hours.* Chromatographically pure 2 : 4 : 6-trimethyl glucose (2·6 g.) was boiled with methanolic hydrogen chloride (84 c.c.; 2%) for 52 hours, together with chloroform (17 c.c.), as in the experimental procedure for the hydrolysis of methylated laminarin. The

syrupy methylglucosides were then hydrolysed for 11 hours at 95° with hydrochloric acid (2N.). The crystalline mixture of reducing sugars (2·596 g.) so obtained was separated on a cellulose column (3·5 × 40 cm.), giving 2 : 4 : 6-trimethyl glucose (2164 mg.) (elution with 50% light petroleum–50% butanol), dimethyl glucoses (202 mg.) (same solvent), monomethyl glucoses, glucose, and unidentified material (119 mg.) (eluted with water) (recovery, 95·7%). The weight of dimethyl glucoses represents 8·7% (molar) of the total recovered product. This experiment was repeated; 2 : 4 : 6-trimethyl glucose (1·922 g.) gave pure 2 : 4 : 6-trimethyl glucose (1705 mg.) dimethyl glucoses (139 mg.), and monomethyl glucoses (35 mg.) (recovery 97·6%, the dimethyl glucoses corresponding to 7·9% of the total).

The dimethyl fraction isolated from the first experiment was a glass which crystallised slowly, had $[\alpha]_D^{16}$ +67·8° (*c*, 1·4 in water), and resembled closely the product obtained from methylated laminarin hydrolysates when examined on the paper chromatogram.

Oxidation of the Dimethyl Glucose Fractions with Periodate.—(a) 4 : 6-*Dimethyl glucose.* Authentic 4 : 6-dimethyl glucose (38·4 mg.) in aqueous sodium metaperiodate (1·036 g.; 0·5M.) underwent the following changes in rotation: α_D^{15} +0·64° (15 mins.); 0·58° (45 mins.); 0·54° (90 mins.); 0·49° (150 mins.); 0·49° (19 hours); 0·44° (44 hours); 0·44° (96 hours, constant). The derived 2 : 4-dimethyl-D-erythrose showed, therefore, $[\alpha]_D^{13}$ +33·4° (*c*, 2·6 in 0·5M-sodium periodate).

(b) *Fraction* (6). Treatment with periodate as described by Bell (*J.*, 1948, 992) gave no formaldehyde.

The fraction (43·2 mg.), in aqueous sodium metaperiodate (1·031 g.; 0·5M.) showed α_D^{15} +0·86° (25 mins.); 0·84° (60 mins.); 0·32° (23 hours); 0·27° (26 hours); 0·24° (28 hours, constant). Since 2 : 6-dimethyl glucose must give an optically inactive product on oxidation with periodate, fraction (6) contains 48·5% of 4 : 6-dimethyl glucose.

(c) *Dimethyl glucoses from partial demethylation.* The fraction (41·6 mg.) in aqueous sodium metaperiodate (1·040 g.; 0·5M.) gave α_D^{15} +0·79° (20 mins.); 0·77° (90 mins.); 0·74° (2 hours); 0·55° (19 hours); 0·39° (43 hours); 0·26° (67 hours, constant), whence the mixture contained 53·5% of 4 : 6-dimethyl glucose.

Partial Hydrolysis of Laminarin: Isolation of Laminaribiose.—Laminarin was hydrolysed with oxalic acid (N.) at 95° for various periods of time. The filter-paper chromatogram (*n*-butanol–ethanol–water) showed two distinct spots (in addition to that due to glucose), having R_G values of 0·40 and 0·25, respectively. The former was established as arising from laminaribiose. With a faster-running solvent [benzene–butanol–pyridine–water (1 : 5 : 3 : 3)], run for 2 days, the laminaribiose showed R_G value of *ca.* 0·78 compared with glucose, and two other spots (R_G 0·50 and 0·32) due to other oligosaccharides were also observed. Under the above conditions the hydrolysis of laminarin was found to be complete in 12 hours, and the proportion of laminaribiose was highest after 6—8 hours. Laminarin (25 g.) was heated with oxalic acid (750 c.c.; N.) for 7 hours at 95°, and the solution cooled and neutralised with calcium carbonate, filtered, heated to 90° (30 minutes), filtered, and evaporated to 150 c.c. at 40°/15 mm. After treatment with charcoal the solution was evaporated to a thick syrup. A portion (3·4 g.) was added to the top of a column of powdered cellulose (3·5 × 41 cm.) previously prepared by percolation with a 1 : 1 mixture of butanol and butanol saturated with water. Elution was commenced with the same solvent, the eluate being collected in 500-c.c. portions. Until 4 l. had passed through the column the eluate still contained glucose, thereafter the next 1·5 l. contained no sugars, and the succeeding 4·5 l. contained laminaribiose only. Evaporation of this extract and treatment of the residue with charcoal gave a colourless deliquescent glass (0·59 g.), of which 5·01 mg. on treatment with alkaline hypoiodite consumed 2·71 c.c. of 0·01N-iodine, corresponding to 92·8% of $C_{12}H_{22}O_{11}$; the hydrolysate contained, therefore, *ca.* 15% of laminaribiose. Treatment of the glass with ethanol gave a white microcrystalline powder, m.p. 160—163° (with previous loss of water at 80°), $[\alpha]_D^{11}$ +23·4° (15 mins.), +19·0° (5 hours, constant; *c*, 2·7 in water) (Found: C, 38·3; H, 6·8. Calc. for $C_{12}H_{22}O_{11},2H_2O$: C, 38·1; H, 6·9%). The derived osazone (50% yield) on recrystallisation from water was obtained as long yellow needles, $[\alpha]_D^{14}$ −71·5° (*c*, 0·5 in ethanol), m. p. 200—202° (cf. Barry, *Sci. Proc. Roy. Dublin Soc.*, 1941, **22**, 423) (Found: C, 52·3; H, 6·2; N, 9·6. Calc. for $C_{24}H_{33}O_9N_4,2H_2O$; C, 52·0; H, 6·15; N, 10·1%).

Reducing Power of Laminarin.—(1) *Oxidation with alkaline hypoiodite.* Laminarin (0·2—1·0 g.) was dissolved in water (10 c.c.) by warming. After this solution had been cooled, iodine solution (10 c.c.; 0·1N.) and sodium hydroxide (8 c.c.; 0·2N.) were added. After 35—45 minutes the solution was acidified and the excess of iodine determined by titration. In six experiments laminarin (1·0 g.) consumed 3·11 ± 0·09 c.c. of 0·1N-iodine, corresponding to one aldehyde group in 40 ± 1 glucose residues. In similar experiments with a disodium phosphate–sodium hydroxide buffer (pH 11·4) consumptions of iodine corresponding to 47 (30—100 minutes) and 41 (18 hours) $C_6H_{10}O_5$ units per reducing group were recorded.

(2) *Oxidation with bromine.* Laminarin (1·5 g.) in water (50 c.c.) and bromine (2·3 c.c.) was kept in the dark with occasional shaking for 15 days. After aëration to remove bromine the white suspension was filtered off, washed with water, and dried (1·14 g.). After dialysis for 6 days, which results in a loss in weight of 40%, possibly indicating that some degradation to particles of small molecular size had taken place, the product was isolated and dried. 0·3 G. of this material required 4·02 c.c. of sodium hydroxide (0·0095N.) for neutralisation to phenolphthalein, corresponding to the presence of 1 carboxyl group in 48 $C_6H_{10}O_5$ units.

(3) *Colorimetric method.* Meyer's method (*Helv. Chim. Acta*, 1948, **31**, 103) was used, a standard curve being constructed for laminaribiose (Table) by treatment of the sugar (0·3—2 mg.) in water (3 c.c.) with 3 : 5-dinitrosalicylic acid (1 c.c.; 1·5%) and sodium hydroxide (1 c.c.; 6N.) at 65° for 30 minutes, cooling, dilution to 25 c.c., and comparison with a blank in a "Spekker" absorptiometer (4-cm. cell; filter 604).

Laminaribiose (mg.)	0·38	0·50	0·74	0·96	1·12	1·49	1·86
Log I/I_0	0·048	0·118	0·195	0·360	0·434	0·740	1·01

Aqueous solutions of laminarin (2 c.c.; 0·238% and 0·508%) were heated with water (1 c.c.) and with the reagents described above, giving values for log I/I_0 of 0·21 and 0·70 respectively, corresponding to chain lengths of 12·9 and 13·9 $C_6H_{10}O_5$ units respectively.

Periodate Oxidation of Laminarin.—(1) *Determination of formic acid released.* Laminarin (1·014 g.) was shaken in the dark with sodium metaperiodate (0·5 g.), potassium chloride (2 g.), and water (100 c.c.). At intervals portions (10 c.c.) were removed after centrifuging, and titrated to methyl-red with sodium hydroxide solution (0·01N.) after destruction of periodate by the addition of ethylene glycol (0·5 c.c.). A blank experiment was carried out at the same time. After application of the necessary corrections (Halsall, Hirst, and Jones, *J.*, 1947, 1399, 1427) the following titres were obtained: 7·04 c.c. (1 day); 8·26 c.c. (3 days); 8·95 c.c. (5 days); 9·27 c.c. (6 days); 9·40 c.c. (8 days); 9·77 c.c. (11 days); 10·13 c.c. (15 days). There is a fairly rapid rise in titre for up to 5 days, after which the liberation of acid becomes slower and practically linear. The value after 5 days corresponds with the liberation of 1 mole of formic acid from 6·8 $C_6H_{10}O_5$ units. In similar experiments carried out in diffused light 1 mole of formic acid was liberated from 7·0 $C_6H_{10}O_5$ units on two occasions. It was observed that iodine was released after 10—14 days, a fact always indicating over-oxidation.

Laminaribiose (47·7 mg., $1·4 \times 10^{-4}$ mole) in water (25 c.c.) was treated with potassium metaperiodate (0·3 g.) and potassium chloride (0·6 g.) at pH 8. A blank experiment was also carried out. Portions (5 c.c.) were removed at intervals, ethylene glycol (0·3 c.c.) being added to them. After 5 minutes, titration against sodium hydroxide (0·01N.) to methyl-red gave 4·28 c.c. (1·53 moles of formic acid), 2 days; 6·57 c.c. (2·35 moles), 4 days; 7·97 c.c. (2·85 moles), 6 days; 9·27 c.c. (3·31 moles), 9 days. Iodine was liberated after the fifth day and there was a small unbroken rise in the acid content of the reaction mixture.

(2) *Uptake of periodate.* Laminarin (0·1725 g.) was shaken in diffused light for 5 days with sodium metaperiodate (5 c.c.; 0·25M.), potassium chloride (1 g.), and water (5 c.c.). The difference in the periodate content in comparison with a blank amounts to 5·90 c.c. of 0·1N-sodium arsenite and corresponds to the consumption of 1 mole by 3·9 $C_6H_{10}O_5$ residues.

(3) *Formaldehyde production.* Laminarin (2·294 g.) in water (20 c.c.) was shaken for 4 days with potassium metaperiodate (1·2 g.). After separation at the centrifuge formaldehyde was determined in the clear solution with dimedone (Bell, *J.*, 1948, 992) (Found: 35·9 mg. of CH_2O–dimedone complex). A control experiment with a solution of formaldehyde gave 27·6 mg. of the complex before shaking with periodate, and 25·65 mg. after treatment as described above, corresponding to a recovery of 93%. After application of this correction 1 mole of formaldehyde is found to be liberated from 27 $C_6H_{10}O_5$ residues.

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