641. *Marine Algal Cellulose.*

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Algal cellulose, isolated from various species of marine algæ, has been shown to be essentially the same as cotton cellulose. Hydrolysis with 72% sulphuric acid gave only D-glucose. Cellobiose octa-acetate has been prepared by acetolysis, indicating the presence of 1 : 4-β-linkages, a fact confirmed by oxidation with sodium periodate. Determinations by potassium periodate oxidation indicate a chain length of 160 units but the original cellulose may have been degraded during isolation. *X*-Ray diagrams of algal cellulose show the characteristic pattern of normal cellulose.

The insoluble residue after the removal of alginic acid from seaweeds was called " algic cellulose " by Stanford (*J. Soc. Chem. Ind.*, 1885, **4**, 518), and it was assumed by many workers to be identical with the cellulose of land plants. Kylin (*Z. physiol. Chem.*, 1915, **94**, 337) showed that it gave the colour reaction with iodine and sulphuric acid, and Russell-Wells (*Nature*, 1934, **133**, 651) demonstrated its solubility in cuprammonium hydroxide and prepared an acetate. Dillon and O'Tuama (*Sci. Proc. Roy. Dublin Soc.*, 1935, **21**, 147) obtained a sugar by hydrolysis which yielded glucosazone and also showed the absence of mannose. They were also able to prepare a viscose and chloroform-soluble acetyl and methyl derivatives.

These results are indicative of the similarity of algal and land-plant cellulose but no evidence has been brought forward showing that the substance is composed entirely of glucose units or that the fundamental 1 : 4-β-linkages are present.

The present investigation has shown that algal cellulose contains no sugar other than D-glucose. Celluloses were prepared from *Laminaria cloustoni*, *L. digitata*, and *Fucus vesiculosus* by a modification of the A.O.A.C. (" Methods of Analysis," Assoc. Off. Agr. Chem., Washington, 1935) method for the determination of crude fibre, by successive extractions of the seaweed with 1·25% solutions of sulphuric acid and sodium hydroxide whereby soluble polysaccharides and alginic acid were removed, followed by bleaching with chlorine water and treatment with N/10-sodium hydroxide. The material thus obtained contained a small quantity of ash which was reduced by dissolution in, and reprecipitation from, cuprammonium hydroxide.

The product gave the characteristic blue colour with iodine and zinc chloride and dissolved readily in Schweizer's reagent, the solution thus obtained being used for the determination of fluidity (Clibbens and Little, *J. Text. Inst.*, 1936, **27**, 285); the value obtained was *ca.* 40 reciprocal poises as compared with cotton cellulose (2 reciprocal poises). This difference was shown to be due, in part, to degradation during isolation, since cotton cellulose, treated as in the extraction, gave a fluidity of 28 reciprocal poises. The extraction of cellulose from the cell wall of the pear is also presumed to involve considerable degradation, the chain length of pear-cell-wall cellulose as isolated being given as *ca.* 240 units as against a value of 1030 for cotton cellulose (Hirst, Isherwood, Jermyn, and Jones, this vol., p. S 182).

Hydrolysis to glucose by the method of Monier-Williams (*J.*, 1921, **119**, 803) gave 80% of the theoretical yield (cf. 88% from cotton cellulose), the glucose being determined both polarimetrically and by Somogyi's method (*J. Biol. Chem.*, 1933, **100**, 695) before and after incubation of the solution with yeast at 37° (Harding and Selby, *Biochem. J.*, 1931, **25**, 1815). The presence of glucose was confirmed by the isolation of glucosazone, and no other reducing sugars could be detected either in the latter estimation or by the method of paper chromatography.

Attempts to prepare cellobiose octa-acetate by Haworth and Hirst's method (*J.*, 1921, **119**, 197) were only partly successful owing to the physical condition of the material, but the more recent method of Hibbert and Barsha (*Canadian J. Res.*, 1934, **10**, 178) gave good results. A yield of 31% was obtained, giving strong presumptive evidence of the presence of 1 : 4-β-linkages as in cotton cellulose. Oxidation of the cellulose with sodium periodate confirmed this result, since 1·02 mols. of periodate were consumed per $C_6H_{10}O_5$ unit (constant after 7 days). The possibility of the existence of 1 : 3- and 1 : 6-linkages is thus negatived since, in the former case none, and in the latter, two molecules of periodate per $C_6H_{10}O_5$ unit would be required, apart from the requirements of the terminal groups.

Oxidation with potassium periodate (Brown, Dunstan, Halsall, Hirst, and Jones, *Nature*, 1945, **156**, 785) and titration of the formic acid produced gave a result corresponding to a chain of 160 glucose units. The estimated chain length of cotton cellulose fell from *ca.* 1000 to 360 units on treatment as in the original extraction. The possibility that any significant numbers of glucose residues are united in the polysaccharide by 1 : 6-linkages is also negatived.

Through the kindness of Professor W. T. Astbury, the *X*-ray diagrams of an algal cellulose prepared from *Laminaria cloustoni* and of the material regenerated from it after dissolution in cuprammonium hydroxide have been obtained. The former shows the characteristic pattern of normal cellulose, and the latter that of " hydrate " cellulose (Percival and Ross, *Nature*, 1948, **162**, 895).

Experimental.

Preparation of Algal Cellulose.—Dried, ground seaweed (7 lb. of *Laminaria cloustoni*) was soaked overnight in sulphuric acid solution (15 l.; 0·1N.), and the liquid removed by centrifuging. The residue was then extracted in the cold for 3 days with sodium carbonate solution (20 l.; 3%), and the crude cellulosic residue centrifuged off and washed with water. The very dark green product was then suspended in water (3 l.), and chlorine passed in until the material was a dull white, whereupon it was separated and washed at the centrifuge. After this product had been warmed on a water-bath for 2 hours with sodium sulphite solution (3 l.; 3%), sodium hydroxide was added to give a 1% solution, and heating was continued for 10 minutes. The product when separated was of a greyish colour, which was not improved by rechlorination. Attempts to remove this colour with calcium hypochlorite solution (4·7% at 50°) and sodium chlorite solution (0·4% at pH 4 at 60°) were unsuccessful The product was dialysed against running water for 2 weeks to remove inorganic material, separated, washed with alcohol, and dried in a vacuum. The product which was hard and lumpy contained 15·1% of ash, which was reduced to 7·22% by treatment with cold dilute hydrochloric acid, dialysis, and separation as before. The product was difficult to break down to a powder but was soluble in Schweizer's reagent and gave the characteristic blue colour with iodine after being allowed to swell in zinc chloride solution. Samples were prepared in the same way from *L. digitata* and *Fucus vesiculosus*.

Fluidity in Cuprammonium Solution.—Fluidity measurements by the standard method (Clibbens and Little, *loc. cit.*), and with allowance for the presence of ash, gave *ca.* 40 reciprocal poises. A control experiment with cotton cellulose gave values of 2 and 28 reciprocal poises before and after treament as in the extraction of the algal cellulose.

Purification of Cellulose.—The crude material was dissolved in cuprammonium solution by shaking the mixture overnight, reprecipitated by acidification with hydrochloric acid, separated at the centrifuge, and made into a slurry with alcohol; this was evaporated to small bulk; benzene was then added, and the evaporation continued to dryness, giving an almost white product which could be ground to a fine powder, containing 4·32% of ash.

Chemical Properties of Cellulose.—All data given are calculated on an ash-free basis. Hydrolysis with 72% sulphuric acid according to Monier-Williams gave 80% of the theoretical yield of D-glucose (cf. cotton cellulose under the same conditions, 88%). The glucose was determined by weighing, by polarimetric measurements, and by determination of reducing power by the Somogyi micro-copper method using reagent 50, before and after incubation of the solution with yeast at 37° for 10 minutes. Paper-

chromatographic analysis showed the presence of no reducing sugar other than glucose, which confirmed the observation that no reducing sugar remained after treatment with yeast, and glucosazone was isolated in the usual way.

Attempts were made to prepare cellobiose octa-acetate by Haworth and Hirst's method (*loc. cit.*) from the original lumpy product but, owing to the difficulty of dissolving the hard lumps, only a very small yield was obtained. On the purified cellulose, Hibbert and Barsha's method (*loc. cit.*) gave good results. Cellulose (0·238 g.) was treated with a mixture of acetic anhydride (1 c.c.) and concentrated sulphuric acid (0·025 c.c.) at 50° for 19 days, the solution being then diluted with glacial acetic acid (1·5 c.c.), mixed, and poured into water (70 c.c.). The insoluble product was filtered off, and recrystallised from alcohol (charcoal); yield, 0·83 g. (34%); m. p. 221—222°. A second recrystallisation yielded 0·0576 g. (24%), m. p. 223°. Cotton cellulose (0·24 g.) under the same conditions gave a yield of 0·074 g. (31%), m. p. 222—223°, mixed m. p. 220°. The algal product had $[\alpha]_D^{20}$ +40° (*c*, 0·34 in chloroform) (Found: C, 49·6; H, 5·7. Calc. for $C_{28}H_{38}O_{19}$: C, 49·6; H, 5·6%).

Algal cellulose (1·293 g. on ash-free basis) was treated with sodium periodate solution (0·25 M.; 20 c.c.) and shaken for 9 days, samples (1 c.c.) being withdrawn after being allowed to settle at intervals of 3, 5, 7, and 9 days, to which were added saturated sodium hydrogen carbonate solution (5 ml.) and potassium iodide (2 c.c.; 2·5%). After 5 minutes, the solutions were titrated with 0·1N-sodium arsenite solution. After 9 days the uptake of periodate was constant and equivalent to 1·02 mols. per $C_6H_{10}O_5$ unit.

Potassium periodate being used as oxidising agent (*loc. cit.*), algal cellulose (0·485 g.) was shaken for 14 days with water (30 c.c.), potassium periodate solution (10 c.c.; 0·25M.) and potassium chloride (2 g.), samples (5 c.c.) being withdrawn as before at intervals of 4, 7, 10, and 14 days. The samples were titrated to methyl-red with sodium hydroxide solution (0·01N.) after excess of periodate had been destroyed with ethylene glycol. A blank determination was also carried out. The titration results were as follows:

Time, days	4	7	10	14
0·01N-NaOH, c.c.	0·51	0·61	0·67	0·72

The blank was negligible; therefore, after allowance for an indicator blank and formic acid removed in sampling, the final titration is equivalent to a chain length of 160 glucose units.

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