641. *The Structure of the Mannan present in* Porphyra umbilicalis.

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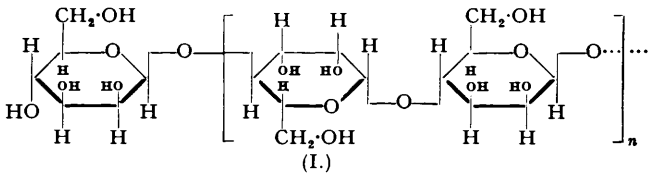
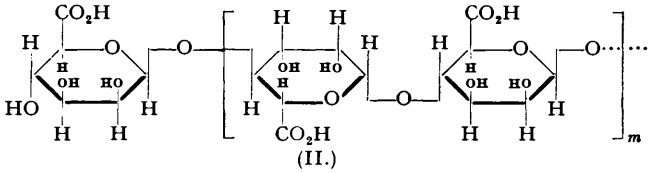
A mannan has been isolated from the seaweed, *Porphyra umbilicalis*. Hydrolysis of the methylated derivative yields 2 : 3 : 6-trimethyl D-mannose and *ca.* 8% of 2 : 3 : 4 : 6-tetramethyl D-mannose. The action of periodate solution on the mannan indicates that it is a polymer of β-linked D-mannopyranose residues, linked through $C_{(1)}$ and $C_{(4)}$.

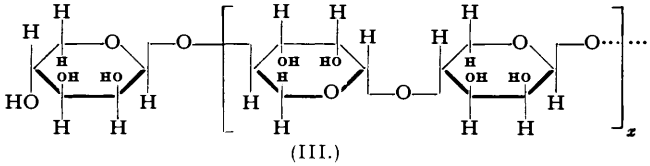
The seaweed *Porphyra umbilicalis* (syn. *laciniata*) has long been used as a foodstuff in South Wales and is sold as laver or laver bread. It is also used as a foodstuff in Japan under the name of Nori. The seaweed was investigated by Oshima and Tollens (*Ber.*, 1901, **34**, 1422) who showed that it gave galactose, fucose, and mannose on acidic hydrolysis; *Ascophyllum nodosum* is said to give a similar mixture of sugars on hydrolysis with acids. The possible occurrence of a mannan in seaweeds is of particular interest in connection with the origin of alginic acid (II), a polymannuronic acid derivative, which might arise from a mannan (I) by oxidation, and would



(I.)



(II.)



(III.)

yield a lyxosan (III) on decarboxylation. So far, a lyxosan has not been isolated from seaweed, and, in fact, lyxose has not been encountered in any natural product. *Porphyra umbilicalis*, known to contain a number of polysaccharides, some of which are soluble in hot water, was accordingly extracted exhaustively with this solvent, after having been dried; this served to remove one or more polysaccharides which on acidic hydrolysis gave fucose and galactose in approximately equal amounts. Sugar-alcohols, polypeptides, and a red colouring matter were

also extracted by the hot water. The insoluble residue was then extracted with dilute sodium hydroxide solution to remove protein material. Sulphur-containing compounds were also removed by the alkaline solution. Hot, strong sodium hydroxide solution was required to extract the mannan; at the same time, green colouring matter and other polysaccharide material, which gave mainly xylose on acidic hydrolysis, was liberated. No lyxose-containing material was detected. Fortunately, the alkaline solution of mannan gave, with Fehling's solution, an insoluble copper derivative which could be washed free from colouring matter and other carbohydrate material. The formation of insoluble copper complexes seems to be one of the characteristics of the mannans (cf. the mannans from ivory nut, yeast, and *orchidaceæ*). The copper complex was decomposed with alcoholic hydrogen chloride with the liberation of the polysaccharide, which on hydrolysis with anhydrous formic acid gave 88% recovery of mannose, no other sugar was detected on the paper chromatogram. This treatment with alcoholic hydrogen chloride renders the mannan insoluble in alkaline solution, 30% sodium hydroxide solution failing to dissolve it.

Sodium periodate solution oxidises the mannan with the formation of a small amount of formic acid and with the consumption of one mol. of periodate per sugar residue. These observations indicate that 1 : 3- and 1 : 6-linked mannose residues can be present to a small extent only in the polysaccharide. A 1 : 2- or 1 : 4-linked polymer of mannopyranose residues would, however, be oxidised by periodate, with the results described above. The pyranose form rather than the furanose form of the sugar is favoured because of the stability of the polysaccharide to hydrolysis with acids.

The polysaccharide was methylated by the thallium procedure. Hydrolysis of the methylated product with anhydrous formic acid furnished a mixture of two sugars only, identified on the chromatogram as 2 : 3 : 4 : 6-tetramethyl mannose and a trimethyl mannose (*A*). The evidence obtained from the periodate oxidation had indicated a structure built up of 1 : 2- or 1 : 4- linked mannopyranose residues. The former would yield, after methylation and hydrolysis, 3 : 4 : 6-trimethyl mannose; the latter the 2 : 3 : 6-trimethyl derivative. These two sugars are readily differentiated on the paper chromatogram and the trimethyl sugar (*A*) was identified as the 2 : 3 : 6-isomer. This was confirmed by converting (*A*) into the crystalline anilide and by oxidising (*A*) to the furanolactone of 2 : 3 : 6-trimethyl mannonic acid, identified as its crystalline phenylhydrazide. For comparison the phenylhydrazide of 2 : 3 : 4-trimethyl mannonic acid was also prepared. The isolation of 2 : 3 : 6-trimethylmannose shows that the mannopyranose residues are linked through $C_{(1)}$ and $C_{(4)}$.

The optical rotation of the mannan in formic acid ($-41° \longrightarrow -22°$) and of the derived acetate in acetone ($-30°$) indicated that the majority of the linkages in the polysaccharide are of the β-form. The linkage which joins the mannuronic acid residues in alginic acid are also of this type.

On oxidation of the mannan with periodate, a yield of formic acid corresponding to the presence of some 6% of end group was obtained. This value is in close agreement with the amount of end group (8%) determined as tetramethyl mannose by the method described by Hough, Hirst, and Jones (*J.*, 1949, 928), as modified by Chanda, Hirst, Jones, and Percival (*J.*, 1950, 1289). These observations indicate that the polysaccharide is of the branched-chain type with, on the average, one point of branching per *ca.* 12 mannose residues. At this stage, the possibility that the polysaccharide is a mixture cannot be excluded. Despite the similarity in structure, it is considered unlikely that the polysaccharide is the precursor of alginic acid (cf. Hirst, *J.*, 1941, 71).

Experimental.

Isolation of Mannan.—The seaweed, *Porphyra umbilicalis* (syn. *laciniata*), was collected in the north of Scotland in August 1947 by Dr. A. P. Orr. It was dried at 100° and powdered, and a portion of the resultant olive-green powder hydrolysed with N-sulphuric acid on the boiling-water bath for seven hours. The cooled solution was neutralised with barium carbonate, and filtered, and the filtrate concentrated to a syrup. Examination on the paper chromatogram showed the presence of reducing sugars, corresponding to fucose, xylose, mannose, glucose, and galactose, fucose and galactose predominating.

A portion of the dried seaweed (500 g.) was extracted with hot water and the viscous extract filtered through cloth. This extraction was repeated until a sample of the filtrate no longer gave a precipitate on being poured into six volumes of ethanol. Six extractions sufficed. A sample of the filtrate was hydrolysed with N-sulphuric acid for seven hours in a sealed tube, immersed in a boiling-water bath. The tube was cooled and opened, and the contents were neutralised with barium carbonate and filtered. On concentration, the filtrate yielded a syrup in which fucose and galactose were detected on the chromatogram. Amino-acids were also present.

The bright-green material remaining after extraction of the water-soluble polysaccharides was next extracted with cold N-sodium hydroxide. A dark green extract was obtained which contained most of

the protein and sulphur-containing compounds originally present in the seaweed. The protein was precipitated on acidification of the solution with acetic acid. On hydrolysis with concentrated hydrochloric acid or formic acid, it furnished a mixture of at least ten amino-acids; no carbohydrate material could be detected.

The seaweed residues were next extracted with hot 20% sodium hydroxide solution and filtered. The filtrate contained green colouring matter, polypeptides, and mannan, which was separated as the insoluble copper complex by addition of Fehling's solution. This complex was filtered off and washed with water until the washings were colourless. It was decomposed by grinding it with methanol containing hydrochloric acid, until the complex had been converted into a suspension of white powder. The mannan was filtered off and washed, first with methanol and then with acetone, until the washings were colourless. Finally, the polysaccharide was washed with a little methanol containing ammonia to remove traces of hydrochloric acid and dried under reduced pressure (yield, 19 g.). This treatment caused the mannan, initially soluble in sodium hydroxide solution, to become insoluble in this reagent (Found : C, 44·6; H, 6·2. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·1%). Nitrogenous, methoxyl, and uronic acid residues were absent. A sample of the polysaccharide was hydrolysed by being dissolved in formic acid (98—100%) and the solution heated in a sealed tube immersed in a boiling-water bath for seven hours. The tube was then cooled, the contents poured into an equal volume of water, and the solution evaporated to dryness. The residue was suspended in N-sulphuric acid and the mixture heated on the boiling-water bath for six hours. Acid was removed from the cooled solution with Amberlite resin IR4B, and the filtered solution then evaporated to a syrup. Examination of the syrup on the chromatogram showed the presence of mannose only, the identity of which was confirmed by the preparation of the phenylhydrazone, m. p. and mixed m. p. 180° (decomp.).

The seaweed residue, after extraction with hot 20% sodium hydroxide, still contained mannan, together with yellow acetone-soluble materials.

Hydrolysis of Alkali-insoluble Seaweed Material.—The seaweed residue (2·03 g.) was boiled under reflux with methanol (100 c.c.) containing concentrated hydrochloric acid (10 c.c.) for 24 hours. The resultant clear solution was concentrated to a small volume, and 2N-hydrochloric acid (10 c.c.) added. The solution was boiled under reflux for five hours, cooled, neutralised with silver carbonate, and filtered. Silver ions were removed from the filtrate by passage of hydrogen sulphide followed by filtration. The filtrate was concentrated to 5 c.c. and diluted with methanol (5 c.c.). The solution was run on to the top of a column of cellulose (2″ × 13″) and the sugars separated in the usual way; pure specimens of D-xylose (0·8 g.), m. p. 149°, $[\alpha]_D$ +13° in water, and D-mannose (0·116 g.) (isolated as the phenylhydrazone, m. p. 186°) were obtained. Fractions containing arabinose(?) and glucose were also isolated.

The mannan was insoluble in water, sodium hydroxide solution, and dilute acids. Hydrolysis with N-sulphuric acid was unsatisfactory; the polysaccharide did not dissolve and much decomposition occurred. For example, the mannan (302 mg.) gave mannose (182 mg.; determined as the phenylhydrazone) after being heated for 60 hours with N-sulphuric acid. The mannan showed $[\alpha]_D$ −41° falling to −22° in 60 hours (constant value; *c*, 1·64, in anhydrous formic acid).

Quantitative Analysis of Sugars Produced on Hydrolysis of Mannan.—Mannan (101·2 mg.) was hydrolysed with anhydrous formic acid and a solution of mannose obtained as described above. The solution was concentrated to a syrup, and the mannose content determined by the method described by Hirst, Jones, and Woods (*J.*, 1947, 1048). The yield of mannose phenylhydrazone was 101 mg.; m. p. 182° (decomp.); this corresponds to a yield of 98 mg. of mannose, *i.e.*, 88% recovery.

Acetylation.—Mannan (0·5 g.) was suspended in pyridine (10 c.c.) containing acetic anhydride (5 c.c.) and heated on the steam-bath for 48 hours. The dark-brown solution was poured on ice, and the precipitated acetate collected, washed with water, and dried. The product was purified by dissolving it in acetone, filtering it, and pouring the filtrate into water. The acetate (0·6 g.), $[\alpha]$ −30° ±5° (*c*, 1·7 in acetone) (Found : Ac, 43·0. Calc. for $C_{12}H_{16}O_8$: 44·4%), formed a brittle film from chloroform solution.

Oxidation of Mannan with Periodate.—The mannan (685·5 mg.) was oxidised with sodium periodate (0·29M.; 25 c.c.) at 0° in the dark. Although initially insoluble, it gradually dissolved, and after 40 hours solution was complete. A portion (5 c.c.) of the solution was withdrawn, ethylene glycol added, and the formic acid titrated. 0·51 C.c. of 0·1N-sodium hydroxide (constant value) was required; this corresponds to the formation of one mol. of formic acid per 16 mannose residues.

The amount of periodate consumed was also determined. 5 C.c. of the solution required 0·83 c.c. of M-periodate solution; this corresponds to the consumption of one mol. of periodate by 164 g. of polysaccharide.

Hydrolysis of the Periodate-oxidised Mannan.—A portion of the solution of mannan in periodate (10 c.c.) was added to ethylene glycol, and the solution dialysed to remove inorganic material, formaldehyde, and glycol. The oxidised mannan (232 mg.) was recovered by evaporation of the solution under reduced pressure. The product was hydrolysed with formic acid, and the carbohydrate material isolated as described above. Examination of the residual syrup on the paper chromatogram showed the presence of a trace of mannose only.

Methylation of the Mannan.—Attempted methylation of the mannan with methyl sulphate and sodium hydroxide or by the liquid-ammonia procedure was unsatisfactory, owing to the insolubility of the polysaccharide in these reagents. Accordingly, the mannan was methylated as follows (cf. Hirst and Jones, *J.*, 1938, 502) : the mannan (2 g.) was suspended in water (10 c.c.) and thallium hydroxide (150 c.c.; 0·34 N.) was added. The solution was evaporated to dryness under reduced pressure (exclusion of carbon dioxide), and the finely powdered residue boiled under reflux with methyl iodide until it no longer showed an alkaline reaction to litmus paper. Extraction of the residue left a solid (2·38 g.) (Found : OMe, 36·0%). This material contained thallium iodide. It was dissolved in methyl iodide and

boiled under reflux with silver oxide for six hours. This operation removed traces of thallium salts and completed the methylation of the product, which was isolated by extraction with chloroform. Owing to slight cloudiness of its solution, it was not possible to observe the optical roatation of this derivative (1·5 g.) (Found : OMe, 44·1%).

A portion (0·75 g.) of the methylated polysaccharide was hydrolysed with anhydrous formic acid (see above). Examination of the resultant sugar syrup on the chromatogram indicated that two sugars were present, corresponding to tetramethyl and trimethyl mannose, the latter predominating. Accordingly, the mixture of sugars (0·79 g.) was separated on a column of cellulose, *n*-butanol–light petroleum (b. p. 80—100°) (1 : 1) being used as the eluent. Complete separation was not achieved. Fraction 1 (0·20 g.), $[\alpha]_D^{20}$ +3° (*c*, 4·0, in water) (Found : OMe, 45%), with alcoholic aniline yielded a small quantity of tetramethyl mannose anilide, m. p. and mixed m. p. 141°, and some 2 : 3 : 6-trimethyl mannose anilide, m. p. and mixed m. p. 127°, separated on recrystallisation from alcohol. The tetramethyl mannose anilide was dissolved in dilute hydrochloric acid and the solution examined on the chromatogram. The liberated sugar behaved exactly as tetramethyl mannose prepared from the authentic anilide. Fraction 2 (0·52 g.) consisted of trimethyl mannose only.

A portion (0·23 g.) of Fraction 2, $[\alpha]_D^{20}$ +4° (*c*, 6·6 in water) (Found : OMe, 42·0%), was oxidised with bromine water, and the mannonic acid derivative isolated. The product (0·18 g.) crystallised and after recrystallisation from ether had m. p. 88°, $[\alpha]_D$ +72° ⟶ +66° (in water; 4 days) (Found : OMe, 42·0. $C_9H_{16}O_6$ requires OMe, 42·3%). Its identity as 2 : 3 : 6-*trimethyl mannolactone* was confirmed by formation of its phenylhydrazide, m. p. and mixed m. p. 141° with sintering at 131° (Found : N, 8·6; OMe, 27·4. Calc. for $C_{15}H_{24}O_6N_2$: N, 8·5; OMe, 28·4%).

2 : 3 : 4-*Trimethyl mannonic acid phenylhydrazide*, prepared for comparison from authentic 2 : 3 : 4-trimethyl mannolactone, had m. p. 166° (Found : N, 8·5; OMe, 28·0%).

Examination of both Fractions 1 and 2 on the paper chromatogram showed that neither contained 3 : 4 : 6-trimethyl mannose, which moves to a different position from 2 : 3 : 6-trimethyl and 2 : 3 : 4 : 6-tetramethyl mannose on the chromatogram.

Quantitative Analysis of Methylated Mannan.—Methylated mannan (20 mg.) was hydrolysed by being heated with anhydrous formic acid (1 c.c.) in a sealed tube at 100° for 14 hours. The tube and contents were then cooled, the tube opened, and the formic acid distilled off. The brown residue was heated with N-sulphuric acid (1 c.c.) for 3 hours to hydrolyse any formic ester, and the cooled solution exactly neutralised with baryta. The filtered solution was concentrated to a syrup, and the mixture of sugars separated on the paper chromatogram. The sugars were determined by Chanda, Hirst, Jones, and Percival's procedure (*loc. cit.*) except that the oxidation was allowed to proceed for 15 hours (Found : in duplicate experiments, expressed as c.c. of 0·01N-thiosulphate : tetramethyl mannose, 0·44, 0·35; trimethyl mannose, 5·53; 4·65). These figures correspond to the presence of one tetramethyl mannose unit per 13 mannose residues.

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