313. *The Polysaccharides of the Florideæ. Floridean Starch.*

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The polysaccharide designated Floridean starch, which is known to occur in many red algæ, has been isolated from the fronds of *Dilsea edulis*. It is shown to consist entirely of glucose residues. Investigation of its oxidation by the periodate ion shows that it is structurally different from normal starches or glycogens in that it contains a large proportion of 1 : 3-linked glucose residues. It is resistant to attack by crystalline β-amylase.

Granular material which gives a colour with iodine has been observed in various red algæ when sections of the latter are examined under the microscope [see, *e.g.*, Nägeli, "Die Stärkekorner," 1865, p. 533; Belzung, *Ann. Sci. Nat.*, (*a*) *Botanique*, 1887, (vii), **5**, 179; Kylin, *Z. physiol. Chem.*, 1913, **83**, 174; Colin, *Compt. rend.*, 1933, **197**, 423]. The colour with iodine is not the characteristic blue shown by normal starch, but varies from deep violet to brown. It was attributed to a polysaccharide designated Floridean starch which was said to have certain properties in common with starch and glycogen. For instance, hydrolysis of the polysaccharide with amylolytic enzymes resulted in the formation of degraded products which gave no colour with iodine. These observations were, however, not made on purified material, and the so-called Floridean starch was not actually isolated until Colin (*loc. cit.*) obtained from *Lemanea*, a red fresh-water alga, a glycogen-like substance which had $[\alpha]_D$ +105°, gave a violet colour with iodine, and yielded D-glucose on hydrolysis. This author demonstrated the presence, in some twenty red algæ, of a substance which gives a coloration with iodine. The brown algæ, on the other hand, contain laminarin as a reserve polysaccharide and are devoid of Floridean starch, whilst the green algæ, for example *Ulva lactuca*, contain particles which give a colour with iodine and potassium iodide very similar to that shown by starch.

The difficulties encountered in the isolation and purification of Floridean starch are not unexpected, since aqueous extracts of marine red algæ always contain a complex mixture of polysaccharides. It is possible, therefore, that this Floridean starch may be present also in other red algæ, thereby adding still another complication to structural studies in this group. *Chondrus crispus*, for example, undoubtedly contains some Floridean starch, and, in interpreting the results of experiments with carragheen mucilage, it must be ascertained that none of this material is present adventitiously before it can be concluded that the glucose which accompanies the galactose in the hydrolysis products of the mucilage does in fact constitute a part of the mucilage molecule.

In the present paper an account is given of the isolation, purification and preliminary examination of Floridean starch from *Dilsea edulis*. The fronds of this sea-weed, when extracted with cold, dilute hydrochloric acid, yield a viscid solution which contains the sulphate ester of a galactan. The disintegrated fronds are washed with cold distilled water and are then suspended in boiling water for about an hour. The filtered aqueous extract is poured into an excess of alcohol or acetone, giving a white colloidal precipitate which, after further purification, is finally obtained in the form of a cream-coloured powder. This swells in cold water and then dissolves giving an opalescent solution which displays a faint reducing power towards Fehling's solution. It gives with iodine in aqueous potassium iodide a colour varying from brownish-red to deep violet according to the concentration of the reagents. This colour disappears on heating and reappears on cooling. As ordinarily obtained, the product contains some 10% of ash which can, however, be much reduced by dialysis. The ash contains phosphate, and, apparently, combined phosphorus occurs in the molecule as it does in certain starches. The polysaccharide resembles glycogen in that it is not precipitated by half-saturation with ammonium sulphate, but is so by full saturation. It is composed entirely of glucose residues and gives on hydrolysis with mineral acid a yield of 96% of glucose, determined polarimetrically.

No sugar other than glucose can be detected when the products of hydrolysis are examined on the paper chromatogram using the method of Partridge (*Nature*, 1946, **158**, 270; *Biochem. J.*, 1948, **42**, 238). It has been claimed (Kylin, *Kgl. Fysiograf. Sällskap. Lund. Förh.*, 1943, **113**, 51) that Floridean starch gives maltose on hydrolysis with dialysed malt extract. This would point to the occurrence of maltose residues in the polysaccharide, but no crystalline derivative of the sugar has yet been reported from these hydrolyses. In the present paper it is shown that the yield of maltose obtainable by treatment with a solution of crystalline β-amylase is, at best, very small, an apparent conversion into maltose of no more than 3% being observed. This was not due to poisoning of the enzyme since normal hydrolysis of waxy-maize starch occurred in the presence of the Floridean polysaccharide.

Oxidation of the polysaccharide with periodic acid results in a pronounced fall in the specific rotation. The final value ($[\alpha]_D$ +10°) is very close to that reported for oxidised maize starch (+8°) by Jackson and Hudson (*J. Amer. Chem. Soc.*, 1937, **59**, 2049; 1938, **60**, 989). In the course of this oxidation the polysaccharide consumes 0·6 mol. of periodate per glucose residue, 40% of the glucose residues remaining unattacked. It is probable, therefore, that some of the glucose residues are not linked through C_1 and C_4 as in amylose, but through C_1 and C_3 as in laminarin. The yield of formic acid after oxidation with potassium periodate corresponds to the production of 1 mol. of acid per 18 glucose residues, but, since the polysaccharide displayed some reducing power, it is possible that much of the formic acid came from reducing terminal groups, and no precise conclusions can be drawn at present regarding the presence or absence of branched chains in the Floridean polysaccharide. It is apparent, however, that the substance differs markedly in structure from starch and glycogen in that it contains, in addition to 1 : 4-linked glucose residues, other glucose units mutually linked through the 1 : 3-positions. In this connection it is of interest to compare the properties of the Floridean polysaccharide with those of lichenin which has been recently investigated by Meyer and Gürtler (*Helv. Chim. Acta*, 1947, **30**, 751; see also Boissonnas, *ibid.*, p. 1703), and it is possible that the Floridean polysaccharide bears a relation to starch similar in type to that which lichenin does to cellulose. The high specific rotation of Floridean starch points to the presence of α-glucosidic links, but the maximum value so far recorded ($[\alpha]_D$ +156° in water) is so much lower than the corresponding figures for starch and glycogen (*ca.* 200°) that it cannot be regarded as certain that all the links are of the α-type, even when possible differences in molecular structure are taken into account. In this connection it is significant that the acetate obtained from Floridean polysaccharide has a rotation much lower than that of starch acetate.

Experimental.

Isolation of Floridean Starch.—Sections of the fronds of *Dilsea edulis*, when treated with iodine in aqueous potassium iodide, show, under the microscope, granules stained reddish-brown. It has not been possible to devise a method for the isolation of these granules, but the material which is stained reddish-brown was obtained in an amorphous condition by the following procedure. The fronds were first extracted with cold dilute hydrochloric acid as described by Barry (*Proc. Roy. Irish Acad.*, 1945, **50**, *B*, 349) for the removal of a galactan sulphuric ester. The disintegrated fronds were washed exhaustively with distilled water by decantation, until the washings were free from sulphate. They were then suspended in boiling water for 1 hour. After filtration of the hot solution through cloth, it was poured into an excess of alcohol (or acetone), giving a finely-divided white precipitate. This was purified by dissolution in water, clarification on the centrifuge, and reprecipitation by alcohol. Alternatively, purification can be effected by dialysis in dilute hydrochloric acid against distilled water, until all chloride has been removed. If the fronds are treated with boiling water before the extraction with cold aqueous acid has been carried out, no Floridean starch is obtained, the action of the acid on the frond cells being apparently essential in rendering the polysaccharide extractable. It was observed on occasions that even strong aqueous solutions fail to give a precipitate with alcohol until a trace of electrolyte (*e.g.*, calcium chloride) has been added (compare glycogen). The product so obtained appears, after drying, as a cream-coloured powder which shows a very slight reducing action towards Fehling's solution. With iodine and aqueous potassium iodide it gave a colour varying from reddish-brown to violet according to the concentration of the reagents. The product had $[\alpha]_D^{15°}$ +156° in water (*c*, 0·6) [Found : (*a*) reducing power by the hypoiodite method; 5800 g. were equivalent to 1 mol. of hypoiodite; (*b*) N, 0·9%; (*c*) ash, as sulphate, 0·8%). The yield of Floridean starch from 200 g. of air-dried fronds of *Dilsea edulis*, gathered in the early autumn, was 5 g. There may be seasonal variations in the content, but this aspect of the problem has not been studied.

The acetate of Floridean starch was prepared by the method described for starch by Haworth, Hirst, and Webb (*J.*, 1929, 2479). It was obtained by pouring the reaction mixture into water and, after being washed and dried in a vacuum desiccator, it appeared as a heavy white powder resembling glycogen acetate and was insoluble in water and alcohol, but soluble in chloroform and acetone. It was ash free and had $[\alpha]_D^{15°}$ +107° in chloroform.

Hydrolysis of Floridean Starch.—The polysaccharide (101 mg.) was heated for 20 hours with hydrochloric acid (0·54N.; 10 ml.). Whilst being heated, the solution became brown. After hydrolysis

a small amount of the solution was neutralised with aqueous ammonia. Three drops of the neutralised solution were placed on the paper chromatogram, which was developed with the butanol layer resulting from a mixture of butanol (40%), ethanol (10%), and water (50%). Only glucose was detected in the developed chromatogram. An amylose estimation was carried out on the Floridean polysaccharide by means of the potentiometric iodine-titration method of Bates, Rundle, and French (*J. Amer. Chem. Soc.*, 1943, **65**, 142) as modified by Hudson, Schoch and Wilson (*ibid.*, p. 1380). No iodine was taken up, and no point of inflection occurred in the plot of the e.m.f. against volume of iodine added. The material, therefore, contained no amylose.

Oxidation by Periodate.—Floridean polysaccharide (158·4 mg.) was dissolved in water (40 ml.) containing potassium chloride (5 g.), and sodium metaperiodate (0·27M.; 10 ml.) was added as oxidant. The oxidation, and the determination of the resulting formic acid, were carried out according to the method described for glycogen by Halsall, Hirst, and Jones (*J.*, 1947, 1399). The amount of formic acid produced after 150 hours (2·5 mg.) corresponded to the production of 1 mol. of acid per 18 glucose residues. Floridean starch (89·1 mg.) was oxidised by potassium periodate as above, and after 8 days the uptake of periodate was determined by the arsenite–bicarbonate method. One mole of periodate was taken up by 263 g. of Floridean polysaccharide. Thus, 56% of the glucose residues are oxidised.

Floridean starch (577 mg.) was dissolved in water (20 ml.) containing potassium periodate (1·5 g.). The resulting solution was shaken for 8 days, and then filtered to remove excess of potassium periodate. To the filtrate, ethylene glycol (1 ml.) was added, and then barium formate until no further precipitation of barium iodiate occurred. This precipitate was filtered off. Ethyl alcohol was then added to the filtrate until no further precipitation occurred. The precipitate was allowed to settle, and the supernatant liquid decanted. The solid was isolated and dried (yield, *ca.* 250 mg.). The solid proved to be mainly inorganic matter and, after treatment with N-sulphuric acid at 100°, glucose was found to be absent (as shown by tests by the paper-chromatogram method).

The decanted liquor was dialysed against running water for 4 days. It was then evaporated under reduced pressure to a small volume. A large excess of alcohol was added, whereupon a dark coloured precipitate was formed. This was isolated by centrifuging, washed with alcohol and ether, and dried (yield, *ca.* 15 mg.). This solid (11·5 mg.) was hydrolysed in a sealed tube with N-sulphuric acid (0·4 ml.) by heating it in a water-bath for 6 hours. The tube was then opened and the contents neutralised with barium carbonate. The liquid was centrifuged, and the clear hydrolysate examined on a paper chromatogram. One sugar only was present, the position of which corresponded exactly to that of glucose (glucose, galactose, and mannose were used as reference sugars). It is evident therefore, that intact glucose residues are present in the polysaccharide after oxidation of the latter by periodate.

Treatment of Floridean Starch with a Solution of Crystalline β-Amylase.—Through the kindness of Dr. R. K. Balls of the Enzyme Research Laboratory of the United States Department of Agriculture, we were able to obtain a sample of crystalline β-amylase prepared from the sweet potato. Floridean polysaccharide (518·7 mg.) was dissolved in water (43 ml.), and acetate buffer solution (pH 4·8; 5 ml.) and a solution of crystalline β-amylase (2 ml. of 0·04%) were added. The resulting solution was covered with a thin layer of toluene to maintain aseptic conditions and kept in an incubator maintained at 37°. After 42 hours, the reducing power of a 5-ml. portion of the Floridean solution was determined by the hypoiodite method. After allowing for the initial reducing power of the polysaccharide, the apparent conversion into maltose was 3%. In order to determine whether this low figure was due to poisoning of the enzyme by the polysaccharide, two solutions of waxy-maize starch were prepared. One (*A*) contained 69·2 mg. in water (44·5 ml.), and the other (*B*) 54·4 mg. in water (44·5 ml.) to which had been added the Floridean polysaccharide (31·8 mg.). To each of these were added acetate buffer solution (pH 4·8; 5 ml.) and β-amylase solution (0·04%; 0·5 ml.). The solutions were then covered with a thin layer of toluene and kept in an incubator at 37° for 24 hours. After this time the reducing powers of the two solutions were determined by the hypoiodite method. The reducing power of the solution (*A*) corresponded to a 53% conversion into maltose. After allowing for the initial reducing power of the "Floridean starch" in the case of the solution (*B*), the reducing power corresponded to an apparent conversion of 57% of the waxy-maize starch into maltose provided that no production of reducing substances from the Floridean starch had occurred. This indicates that the hydrolysis of waxy-maize starch in the presence of Floridean polysaccharide proceeded to about the same extent as the hydrolysis of the waxy-maize starch in the absence of Floridean polysaccharide. If the slight increase in reducing power is entirely due to hydrolysis of the Floridean polysaccharide, it indicates an apparent conversion into maltose of 8%.

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