301. *Physicochemical Studies on Starches. Part XXIII.* Some Physical Properties of Floridean Starch and the Characterization of Structure-type of Branched α-1,4-Glucans.*

By C. T. Greenwood and J. Thomson.

Floridean starch has been isolated in granular and non-granular form from *Dilsea edulis*. Measurements were made on the polysaccharide of (i) the interaction with iodine, (ii) the variation of the sedimentation coefficient with concentration, and (iii) the limiting viscosity number. Comparable measurements are reported for rabbit-liver glycogen, the water-soluble polysaccharide from *Zea mays*, and the amylopectins from potato and malted-barley starch. A comparison of the results suggests that these physical measurements enable an unambiguous distinction to be made between amylopectin- and glycogen-type structures. On this basis, this sample of Floridean starch had a structure which was of the amylopectin-type. The weight-average molecular weight of the starch sample from light-scattering measurements was 7×10^8.

Although Floridean starch has been extensively studied,[1,2] it has not been established conclusively whether the polysaccharide is structurally more closely related to plant amylopectins or to animal glycogens. In this work, a sample of pure Floridean starch has been isolated in both granular and non-granular form, and some of its physical properties have been examined. These properties have been compared with those of other related branched α-1,4-glucans in order to investigate the relation between physical properties and structure-type.

Experimental

Isolation and Purification of the Floridean Starch.—(a) *Granular material.* A quantity of granular material (*ca.* 50 mg.) was isolated from the fronds of *Dilsea edulis* by extraction with 0·01M-mercuric chloride in a Blendor.[3] The extract was filtered through muslin, and the granular material obtained by centrifugation. The starch-product was deproteinized by repeated extraction of a saline suspension with toluene,[4] and then consisted of spherical, birefringent granules (diameter 5—10 μ), which stained violet with iodine (Found, N = 0·07%). The gelatinization temperature [3] was 45—47°. This procedure, normally used in starch extraction,[3] was not efficient, and other methods had to be used.

(b) *Non-granular starch.* Macerated fronds (*ca.* 250 g. wet-weight) were steeped in water (under toluene and at 4°) for 4 days to remove mucilaginous material. The fronds were then well washed with ethanol before they were covered with liquid ammonia in which they were left immersed for ½ hr. to disrupt cell walls. (This treatment has been shown to have no effect on the properties of the components of starch.[5]) The treated fronds were then extracted with water (1½ l.) at 98° for 1 hr. under nitrogen. Insoluble material was removed by centrifugation, calcium chloride (1 vol. of saturated solution) was added, and the mixture left at 2° for 24 hr. Precipitated calcium salts were removed by centrifugation, the solution was dialyzed, and the Floridean starch isolated by freeze-drying. The resultant product contained galactan (as shown by chromatographic examination of the hydrolysate). This was removed by three successive differential ultracentrifugations of an aqueous solution (0·5%) at 90,000 ***g*** (*i.e.*, at 40,000 r.p.m. in the preparative rotor of the Spinco model E ultracentrifuge). The sedimented product (0·75 g.) on hydrolysis gave only glucose on chromatographic examination, contained 98·5% of reducing sugar by alkaline ferricyanide titration, and had $[\alpha]_D^{18}$ +190° (*c* 0·1 in 0·1M-NaOH).

Characterization of the Floridean Starch.—Measurements were made of (i) the iodine-binding

* Part XXII, *Stärke*, 1960, **12**, 169.

[1] Fleming, Hirst, and Manners, *J.*, 1956, 2831, and references therein.
[2] Peat, Turvey, and Evans, *J.*, 1959, 3223, 3341.
[3] Banks and Greenwood, *Biochem. J.*, 1959, **73**, 237.
[4] Greenwood and Robertson, *J.*, 1954, 3769.
[5] Banks, Greenwood, and Thomson, *Makromol. Chem.*, 1959, **31**, 197.

power,[6] (ii) the optical density of the glucan–iodine complex at various wavelengths,[3] (iii) the percentage conversion into maltose under the action of purified soya-bean β-amylase,[5] (iv) the average length of unit-chain by periodate oxidation in aqueous solution with sodium meta-periodate,[7] (v) the sedimentation coefficient of the glucan in 0·2M-potassium hydroxide,[8] (vi) the limiting viscosity number $[\eta]$ of the glucan in M-potassium hydroxide,[5] and (vii) the molecular weight of the glucan in 10^{-2}M-sodium chloride from light-scattering measurements[9] (these scattering measurements were kindly carried out by Dr. W. Banks and details of the results will be given elsewhere).

Results and Discussion

The isolation of granular material exhibiting birefringent properties strongly suggests that this amylaceous polysaccharide is, in fact, a starch. However, the crystallinity and degree of order in the granule must be radically different from that of a normal starch, as the gelatinization temperature (45—47°) is very much lower than is usual (60—98°, dependent on the source of starch [10]). This might be related to the different conditions of botanical environment.

The method of purification of the non-granular sample of Floridean starch by differential ultracentrifugation would remove any traces of short-chain amylose (cf. ref. 11). However, the supernatant liquor after ultracentrifugation did not give an iodine-stain, and it is not thought likely that any appreciable amount of linear material is present in the glucan.

The Table shows the properties of the non-granular sample of Floridean starch compared with those of a typical animal glycogen (from rabbit liver [9]) and a typical amylopectin (from potato starch [5]). Comparative results are also included for (i) the water-

Properties of Floridean starch and some other branched glucans.

Polysaccharide	Glycogen [9]	*Zea mays* polysaccharide [12]	Floridean starch	Malted barley amylopectin [13]	Potato amylopectin [5]
β-Amylolysis limit [a]	45 *	49	49	48 *	56
Average length of unit-chain.....	13·6	13 *	18·6	18·4 *	24
Average internal chain-length [b]	5	4·1	7	7·1 *	8·1
Limiting viscosity no. (with c in g./ml.)	7	7	160	146 *	200
$\lambda_{max.}$ of iodine-glucan complex (Å) ...	480	500	550	550	550

[a] Expressed as percentage conversion into maltose. [b] Calc. from {chain-length − [(chain-length × β-limit) + 2·5]}.

* Data taken from the reference at the head of the column.

soluble polysaccharide from sweet corn, *Zea mays*, which was thought to have a structure approximating to that of glycogen,[12] and (ii) the amylopectin from malted-barley starch, which differs from barley amylopectin only in the average length of external chain.[13]

The β-amylolysis results (49% conversion into maltose) are essentially similar to values of 46% and 42% reported earlier.[1,2] Our value of 18·6 anhydroglucose units for the average length of unit-chain of the *pure* glucan is similar to that of 15 reported by Peat and his co-workers.[2] A direct comparison with the results of Hirst and his co-workers [1] is not possible in view of the protein- and galactan-impurities in their samples; the protein-impurity in particular will interfere with the periodate oxidation (cf. ref. 14). Other comments on this estimation have been made by Peat and his co-workers.[2] The results of β-amylolysis experiments and chain-length estimations are not in themselves completely

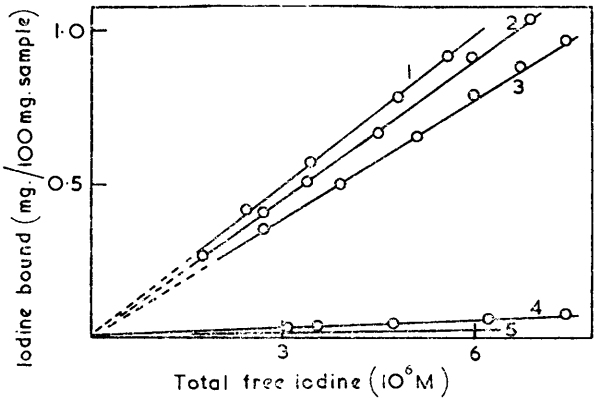
[6] Anderson and Greenwood, *J.*, 1955, 3016.
[7] Potter and Hassid, *J. Amer. Chem. Soc.*, 1948, **70**, 3488.
[8] Bryce, Cowie, and Greenwood, *J. Polymer Sci.*, 1957, **25**, 251.
[9] Bryce, Greenwood, and Jones, *J.*, 1958, 3845.
[10] Greenwood and Thomson, unpublished experiments.
[11] Greenwood and Thomson, *Chem. and Ind.*, 1960, 1110.
[12] Greenwood and Das Gupta, *J.*, 1958, 703.
[13] Greenwood and Thomson, *J. Inst. Brewing*, 1959, 346.
[14] Anderson and Greenwood, *J. Sci. Food Agric.*, 1955, **6**, 587.

indicative of type-structure as quite wide variations can occur in any series of samples. It is to be noted, however, that Floridean starch and the malted-barley amylopectin have similar properties. When calculations are made of the average length of *internal* chains (on the assumption that β-amylase action ceases 2 or 3 glucose units away from a branch-point [15]), the glycogen and *Zea mays* polysaccharide examined appear to have internal chain-lengths of 4—5 units, whilst the other samples have values of 7—8. The significance of these differences is not yet known.

It is apparent, however, that there is a great difference between the value of the limiting viscosity numbers for the glycogen and *Zea mays* polysaccharide ($[\eta] = 7$) and those for the other samples ($[\eta] = 150$—200).

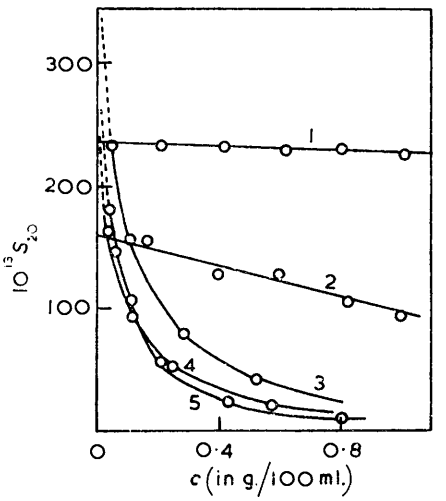
This difference is emphasized by the behaviour of the glucans towards iodine. Fig. 1 shows the potentiometric iodine-binding curves. (The curves for the amylopectins have been corrected for any preferential uptake of iodine by linear material.[6]) The glycogen and *Zea mays* polysaccharide both have a similar, low iodine-binding power, whilst all the other glucans have a pronounced higher affinity for iodine. The Table shows that there is also a corresponding difference in the wavelength of maximum absorption of the iodine complex, although the difference is not so pronounced.

FIG. 1. *The iodine-binding power of branched α-1,4-glucans.*



1, *Potato amylopectin.* 2, *Malted-barley amylopectin.* 3, *Floridean starch.* 4, Zea mays *water-soluble polysaccharide.* 5, *Rabbit-liver glycogen.*[6]

FIG. 2. *Variation of sedimentation coefficient (S_{20}) with concentration (c) for branched α-1,4-glucans.*



1, Zea mays *water-soluble polysaccharide.* 2, *Rabbit-liver glycogen (extracted with trichloroacetic acid, see ref. 9).* 3, *Floridean starch.* 4, *Malted-barley amylopectin.* 5, *Potato amylopectin.*[8]

A further essential difference between branched-glucan structures is their behaviour on ultracentrifugation in alkaline solution; the sedimentation coefficient for glycogen-type materials is linear and relatively independent of concentration, whilst amylopectins are highly concentration-dependent.[8] Fig. 2 shows the results for the different glucans studied here, and again whilst the glycogen and *Zea mays* polysaccharide behave similarly, all the other samples are very concentration dependent.

The above evidence suggests that this sample of pure " Floridean starch " behaves as though it had an amylopectin-type rather than a glycogen-type structure. This conclusion is supported indirectly by the enzymic degradation experiments of Peat, Turvey,

[15] Peat, Whelan, and Thomas, *J.*, 1952, 4546.

and Evans [2] who showed that their Floridean starch sample was degraded by R-enzyme. Further, its properties are remarkably similar to those of the amylopectin isolated from malted-barley starch.[13]

It is suggested that the results of comparative measurements of iodine-binding power, limiting viscosity number, and the concentration-dependence of the sedimentation coefficient can be used in conjunction unambiguously to classify the structure-type of a branched α-1,4-glucan. (The above results confirm our earlier suggestion [12] that the *Zea mays* polysaccharide has a glycogen-type structure.)

The weight-average molecular weight of this sample of Floridean starch was obtained from light-scattering experiments. Data for the intensity of the angular distribution of scattered light were evaluated by Zimm's method.[16] In 10^{-2}M-sodium chloride, the second virial coefficient for the sample was effectively zero. A molecular weight of 7×10^8 was found. This value is comparable to our results [17] for amylopectins. A detailed discussion of these results will be presented elsewhere.

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[16] Zimm, *J. Phys. Chem.*, 1948, **16**, 1093.
[17] Banks and Greenwood, unpublished results.