136. *Physicochemical Studies on Starches. Part X.* The Molecular Weight of the Water-soluble Polysaccharides of Sweet Corn,* Zea mays.

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Detailed results are given of physical studies on samples of the water-soluble glucosans of sweet corn, *Zea mays*, isolated by Peat, Whelan, and Turvey.[1] Another sample of the total water-soluble polysaccharide has also been prepared. The glucosan-product had a very high molecular weight, and, further, on dissolution and fractionation with 67% acetic acid gave only 2% of the total as " soluble " material. It is therefore suggested that in the sample of seed examined the water-soluble glucosan is essentially *homogeneous*, and has a molecular weight of the order of 30×10^6, and an average length of unit chain of **14**. The structure of the glucosan is discussed in relation to that of glycosan and amylopectin.

Recently, Peat, Whelan, and Turvey [1] carried out a comprehensive study of the water-soluble amylaceous polysaccharides of sweet corn (*Zea mays*), in which they established the conditions necessary to avoid extensive enzymic degradation of polysaccharide during isolation. A full review of the previous literature was also given. In this paper, we report details of the physical measurements carried out on the samples kindly placed at our disposal by Professor S. Peat, together with the results of our own investigations on the isolation and fractionation of *Zea mays* glucosans.

Experimental

All samples were dried at 80° *in vacuo* before analysis. The carbohydrate content of the polysaccharide was determined by acid hydrolysis (0·5N-sulphuric acid for 7 hr. at 98°), followed by the estimation of the amount of liberated glucose by alkaline ferricyanide.[2] Nitrogen estimations were by the semimicro Kjeldahl method.

Isolation of the Water-soluble Polysaccharides of Zea mays.—Mature *Zea mays* (*var.* Golden Bantam; 400 g.) was ground and extracted with 0·01N-mercuric chloride as described by Peat *et al.*[1] Extracts were filtered through muslin, and the starch allowed to settle before the supernatant liquors were passed through the Sharples supercentrifuge and concentrated to $\frac{1}{10}$ vol. (under reduced pressure at 30°). Coagulated protein and any other insoluble material were then removed by careful filtration, and the soluble polysaccharide precipitated by the addition of ethanol (1·5 vol.) at 0°. The precipitate was removed on the centrifuge, washed with acetone and ether and dried (yield 79 g.) (Found: N, 0·32; carbohydrate, 88·8%.) The sugar-containing supernatant liquid was treated as by Peat *et al.*,[1] to yield approximately 13 g. of material, which contained no polysaccharide disclosed by chromatography.

Fractionation of the Water-soluble Polysaccharide.—Water-soluble polysaccharide (22 g.) was dissolved in water (300 ml.) and cooled to 0°, and glacial acetic acid (600 ml.) was added slowly with stirring, the temperature being kept *at* 0° *during the whole period*. After the mixture had been then kept at −2° for 18 hr., the precipitated phytoglycogen-A was removed on a refrigerated centrifuge (at 0°), and washed well with acetone to remove the acid. (Isolation of the material by centrifugation at room temperature caused slightly more degradation, as shown by a decrease in sedimentation constant from 226*s* to 214*s*.) After dissolution in water, the pH was adjusted to 6·5 with sodium hydroxide before reprecipitation of the polysaccharide by the addition of ethanol (1·5 vol.). The product was washed with ethanol and with ether and dried (yield 20·5 g.) (Found: N, 0·2; carbohydrate, 92%). The phytoglycogen-B was isolated from the original supernatant liquor by the addition (at 0°) of ethanol (0·5 vol.) (yield 0·83 g.) (Found: N, 1·0; carbohydrate, 47%).

Attempted Removal of Protein and Fractionation of the Water-soluble Polysaccharide.—Polysaccharide was dissolved in 0·1M-sodium chloride (8% solution) and shaken overnight with toluene ($\frac{1}{10}$ vol.). The toluene layer was then allowed to separate and was removed. The

* Part IX, preceding paper.

[1] Peat, Whelan, and Turvey, *J.*, 1956, 2317.

[2] Lampitt, Fuller, and Coton, *J. Sci. Food Agric.*, 1955, **6**, 656.

process was repeated until the amount of coagulated material in the toluene layer was negligible. The solution was then cooled to 0°, divided into 3 portions, and treated as follows: (i) the polysaccharide was precipitated with ethanol (1·5 vol.), washed with ethanol and with ether, and dried (Found: N, 0·09%); (ii) phytin was removed by acidifying the solution with hydrochloric acid (1%) and precipitating the polysaccharide immediately; [1] the polysaccharide was then isolated after adjustment of the pH of the solution to 6·5 as above; (iii) phytoglycogen-A and -B was obtained by the addition of glacial acetic acid (2 vol.) as above; phytoglycogen-A was purified from phytin and adhering acid; again the yield of phytoglycogen-B was small, and it was difficult to isolate this material by precipitation.

Characterization of the Polysaccharide Fractions.—Measurements of (i) average length of unit chain by periodate oxidation, (ii) iodine affinity, and (iii) sedimentation velocity in 0·2M-sodium chloride were carried out as described in previous papers in this Series. The sedimentation constant (S_{20}) for the polysaccharides was found to be the same in water and in 0·2M-aqueous sodium chloride, and measurements have been made in both solvents. S_{20} was a linear function of c; its dependence on concentration is expressed here by values of k in the equation $S_{20} = (S_{20})_0(1 - kc)$, where $(S_{20})_0$ is the sedimentation constant at infinite dilution.

Determination of Diffusion Coefficient (with W. A. J. Bryce).—The diffusion coefficient of the polysaccharides dissolved in 0·2M-sodium chloride was measured in a new type of diffusion cell designed for the Antweiler microelectrophoresis and diffusion apparatus. In this instrument, the refractive-index gradient curve is measured by means of a Jamin interferometer. Diffusion coefficients (D_m) were calculated by the moment method. Measurements were carried out at 20°. Details of the apparatus and method will be presented elsewhere.

Light-scattering Measurements (with I. G. Jones).—Measurements were made with a Brice–Phoenix Photometer (Model 1000D), with cylindrical cells and the narrow diaphragm system.[3] Turbidities (τ) were determined by the "working standard" method. The dissymmetry ratio ($I_{45°}/I_{135°}$; corrected for back-reflection) was also measured. Solvent (0·1M-sodium chloride) was filtered through sintered glass (G5) directly into the scattering cell. Polysaccharide solutions (approx. 0·1%) were filtered through sintered glass (G4), and successive aliquot parts of the clarified solution were added to a weighed amount of solvent in the scattering cell to form a concentration series in the range (1—20) × 10^{-5} g./ml. [Concentrations (c in g./ml.) were estimated by hydrolysing a portion of the original filtered solution, and estimating the amount of liberated glucose by the alkaline ferricyanide–ceric sulphate method.[2]] This

Table 1. *The properties of Peat, Whelan, and Turvey's polysaccharides.*[1]

Polysaccharide	Extraction [a]	Carbohydrate (%) [b]	Unit chain length	Slope of iodine titration [c]	k [d]	$10^{13}(S_{20})_0$ [e]	$10^7 D_m$	$10^{-6}M$
Phytoglycogen A	+	94·2	13·2	0·017	—	(185)	—	(20)
,, ,, ...	—	95·1	9·7	0·007	—	(55)	—	(6)
,, B	+	94·7	7·3	0·007	—	(48)	—	(4)
,, ,, ...	—	93·8	5·8	0·000	—	(2·3)	—	—
Fractions								
55—60% AcOH ppt.	+	—	10·3	—	0·07	146	0·6	15
60—65% ,,	+	—	11·0	—	0·09	131	—	(14)
65—70% ,,	+	—	9·7	—	0·15	78	—	(8)
>70% ,,	+	—	7·8	—	0·75	9	3·5	0·2

[a] Extraction with (+) or without (—) $HgCl_2$. [b] Values from ref. 1. [c] Slope of linear portion of iodine titration curve. Values from Part III.[6] [d] Value of k in the equation $S_{20} = (S_{20})_0(1 - kc)$, where c = g./100 ml. [e] Values in parentheses were determined at a concentration of 0·5 g. per 100 ml. Calc. from $M = \boldsymbol{R}T(S_{20})_0/(1 - \overline{V}\rho)D_m$, where $\overline{V}$ = partial specific volume = 0·62, the value found for glycogen (unpublished results). For the values in parentheses, D_m has been assumed to be $0·6 \times 10^{-7}$.

procedure gave solutions which showed reproducible turbidities, and, on the whole, reproducible low dissymmetries. (Large dissymmetries occasionally observed were thought to be due to anomalous aggregates which were not removed by filtration.) Molecular weights (M) were calculated from the equation:

$$Hc/\tau = 1/M(P_{90°}) + 2B(P_{90°})c/\boldsymbol{R}T \text{ where } H = 32\pi^2n^2(\mathrm{d}n/\mathrm{d}c)^2/3\lambda^4N;$$

[3] Brice, Halwer, and Speiser, *J. Opt. Soc. Amer.*, 1950, **40**, 768.

($P_{90°}$) is the particle scattering factor, and was calculated from the dissymmetry on the assumption that the molecules are spherical; [4] B is a constant. The refractive index increment (dn/dc) was measured at 546 mμ on a Brice–Phoenix differential refractometer,[5] and a value of 0·146 was found. Within experimental error, (Hc/τ) was independent of c for the range of concentrations studied, and hence the term $2B(P_{90°})c/RT$ was negligible. The reproducibility of results for a given sample is indicated in Table 3.

TABLE 2. *The properties of the polysaccharides isolated in this work.*

	Total polysaccharide *		Phytoglycogen A *		Phytoglycogen B *
	(*a*)	(*b*)	(*a*)	(*b*)	(*b*)
Carbohydrate (%)	92	100	92	97	83
Length of unit chain	14·0	14·0	13·0	13·0	11·3
k †	0·025	0·025	0·025	—	0·75
$10^{13}(S_{20})_0$	252	242	226	—	8
$10^7 D_m$	—	0·53	—	—	—
$10^{-6}M$	(30) ‡	29	(27) ‡	—	(0·14) ‡

* (*a*) Purified free from protein; (*b*) purified free from protein and phytin. † As in Table 1.
‡ Calc. by assuming $D_m = 0·53 \times 10^{-7}$ for total polysaccharide and phytoglycogen A, and $D_m = 3·5 \times 10^{-7}$ for phytoglycogen B.

TABLE 3. *Results of light-scattering measurements.*

Sample	Ref.	Uncorrected $M(\times 10^{-6})$	Dissymmetry	$1/(P_{90°})$	Corrected $M(\times 10^{-6})$
Phytoglycogen A	1	18	3·0	2·0	(21) *
,, B	1	4	1·18	1·12	4·5
		3·9	1·16	1·11	4·3
55—60% AcOH fraction ...	1	18	3·0	2·0	(21) *
Total polysaccharide	This work	22	1·26	1·17	26
		22	1·45	1·28	28

* Calc. by assuming a particle-scattering factor of 1·17.

Results.—The properties of the polysaccharide samples supplied by Professor Peat [1] are shown in Table 1. Table 2 shows the corresponding properties of our own samples. Table 3 summarizes the light-scattering data.

The results of typical sedimentation experiments are shown in the Figure.

DISCUSSION

Our lower yield of water-soluble polysaccharide (cf. ref. 1) is not thought to be due to degradation, since the amount of simple sugars was low, and the molecular weight of the product was high; it is more probable that the amount of this polysaccharide present in the seed varies.

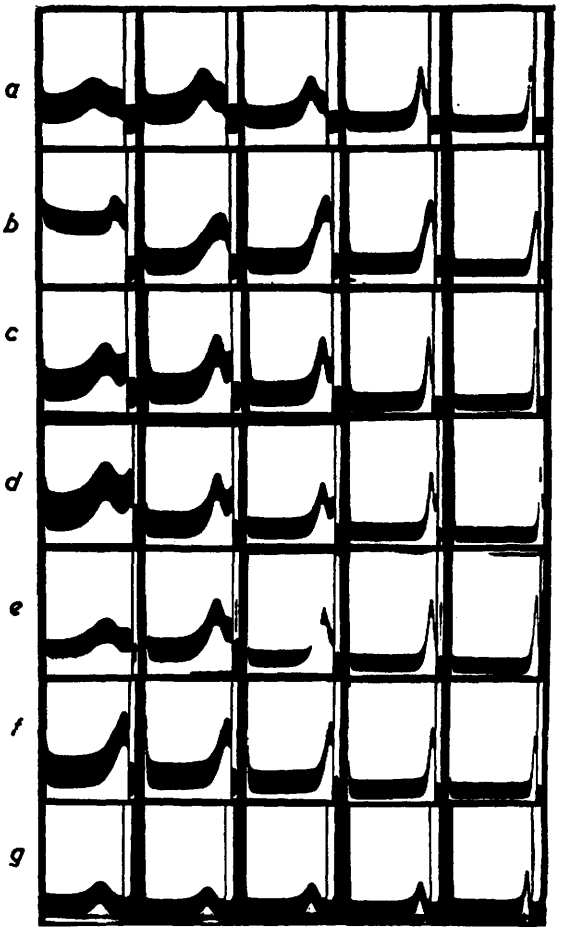
Previous workers (see ref. 1) have suggested that 67% acetic acid separates the water-soluble polysaccharide into two fractions of different properties. Peat, Whelan, and Turvey [1] have shown that these fractions are similar and that, by minimizing enzymic degradation, the percentage of insoluble fraction (phytoglycogen A) is increased. They concluded that the acetic acid was causing a simple molecular-weight fractionation. However, from the batch of seed used we have isolated a glucosan with a larger molecular weight than the phytoglycogen A of these workers, and our yield of soluble polysaccharide (phytoglycogen B) was less than 2% of the total. On this basis, we suggest that this sample of water-soluble polysaccharide might well be essentially *homogeneous* [see the sedimentation diagrams (*g*) in the Figure].

Molecular Weights of the Water-soluble Polysaccharide and its Fractions.—For most of the samples, the agreement between molecular weight as measured by sedimentation and diffusion and by light-scattering is good. This suggests that true molecular weights are being measured. The general conclusions drawn by Peat and his co-workers [1] regarding

[4] Doty and Steiner, *J. Chem. Phys.*, 1950, **18**, 1211.
[5] Brice and Halwer, *J. Opt. Soc. Amer.*, 1951, **41**, 1033.
[6] Part III, *J.*, 1955, 3016.

the drastic effect of enzymic degradation during isolation—unless precautions are taken to avoid this—are completely substantiated from a comparison of the molecular weights quoted in Table 1. These values are larger than the previous relative ones since the diffusion constant was smaller than estimated. Nearly all the samples are somewhat polydisperse on ultracentrifugation [see diagrams (*a*)—(*f*) in the Figure]. This suggests that material of low molecular weight may be present, or that some degradation had

Typical sedimentation diagrams. All samples from Peat, Whelan, and Turvey[1] *[except (g)]. The concentration is* 1·0 g./100 ml. *except for (g) where it is* 0·5 g./100 ml. *Sedimentation is from right to left. The figures in parentheses after the times indicate the angle of the Schlieren bar.*



Phytoglycogen A: *speed* = 20,000 *r.p.m.*; 2(65°), 4·5(50°), 9·5(40°), 13·5(30°), *and* 18·5(30°) *min. after reaching full speed.*

Phytoglycogen B: *speed* = 20,000 *r.p.m.*; 2·5(70°), 6(60°), 10(45°), 16·5(30°), *and* 24(30°) *min. after reaching full speed.*

55—60% AcOH *fraction*: *speed* = 20,000 *r.p.m.*; 1(65°), 4(50°), 7(35°), 12(25°), *and* 17(25°) *min. after reaching full speed.*

60—65% AcOH *fraction*: *speed* = 20,000 *r.p.m.*; 2(65°), 5(50°), 10(40°), 14(30°), *and* 22(20°) *min. after reaching full speed.*

65—70% AcOH *fraction*: *speed* = 20,000 *r.p.m.*; 5(60°), 8·5(50°), 20(35°), 25(30°), *and* 36(30°) *min. after reaching full speed.*

>70% AcOH *fraction*: *speed* = 20,000 *r.p.m.*; 10(60°), 20(45°), 32(35°), 48(30°), *and* 65(25°) *min. after reaching full speed.*

Whole water-soluble polysaccharide: *speed* = 15,000 *r.p.m.* 5(70°), 9(60°), 12(55°), 15(55°), *and* 17(45°) *min. after reaching full speed.*

occurred during both isolation and subfractionation. The values for the subfractions (Table 1) may suggest that a good separation of products of different molecular weight was not obtained.

The molecular weight of the total water-soluble polysaccharide from the sample of seed examined is about 30×10^6 (*i.e.*, $\overline{\text{D.P.}} \approx 180{,}000$). This value is larger than that reported for glycogens,[7] with the exception of the recent work by Stetten, Katzen, and Stetten.[8] The frictional ratio ($f/f_0 = 2$; calculated from the sedimentation and diffusion results) indicated that in solution the polysaccharide was heavily hydrated or was not spherical.

[7] For review see Greenwood, *Adv. Carbohydrate Chem.*, 1952, **7**, 289; 1956, **11**, 385.
[8] Stetten, Katzen, and Stetten, *J. Biol. Chem.*, 1956, **22**, 587.

Structure of the Water-soluble Polysaccharide.—Our periodate oxidation results (chain-length = **14**) are in general agreement with those of Peat and his co-workers.[1] These authors suggested that the polysaccharide must possess a glycogen-type structure. It is certainly true that the sedimentation behaviour is similar to that of glycogen rather than of amylopectin. (We have shown recently [9] that, whereas S_{20} is virtually independent of c for glycogens, for amylopectin S_{20} is very strongly dependent for c. This must be related to differences in shape of the two molecules.) However, a considerable difference occurs between the iodine-binding power of this type of polysaccharide and of glycogen. We suggested previously [6] that, since the uptake was **3**—**4** times greater than for a glycogen of corresponding chain-length, the structure of the two polysaccharides differs in that the degree of multiple-branching of the water-soluble material is intermediate between those for glycogen and for amylopectin. However, in view of unit-chain-length, enzymic degradation experiments,[1] and sedimentation behaviour, the polysaccharide appears to be nearer in structure to a glycogen than an amylopectin.

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[9] Bryce, Cowie, and Greenwood, *J. Polymer Sci.*, 1957, **25**, 251.