42. *Physicochemical Studies on Starches. Part XXIV.* The Fractionation and Characterization of Starches of Various Plant Origins.*

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Granular starches from a wide variety of botanical sources have been isolated under carefully controlled conditions. The starches have been fractionated into their amylose and amylopectin components by procedures involving aqueous leaching and dispersion. The resultant components have been characterized, and their properties compared.

Earlier papers in this series have discussed the isolation and fractionation of starches from the potato [1] and some cereals.[2] In the present work, we have carried out comparable studies on starches from a wide variety of botanical sources. A preliminary account of some of this work has been given elsewhere.[3]

Experimental

Isolation and Purification of the Starches.—The plant material was extracted in a Blendor in the presence of 0·01M-mercuric chloride to inhibit enzyme activity. The extract was then filtered through muslin, and the starch granules were obtained by sedimentation. Protein impurities were removed by repeated extraction of a saline suspension of the starch product with toluene.[4] More specific details about the isolation of some of the starches shown in Table 1 (where the botanical sources are classified by Badenhuizen's system [5]) are given below.

The sample of high-amylose maize starch (amylomaize) was kindly provided by Dr. F. R. Senti of the Northern Regional Research Laboratory of the U.S. Department of Agriculture, Peoria, Illinois. Its isolation had involved steeping the maize for 24 hr. at 37° in water before a laboratory wet-milling process.

The starches from broad-beans and peas were extracted from freshly collected pods. Both the initial starch products were heavily contaminated with protein and a large number of toluene-extractions were required for their purification. (Starch was also readily extracted from dried seeds if the latter were soaked overnight in 0·01M-mercuric chloride at room temperature before extraction.)

Starch is present in any appreciable amount only in immature fruit, and hence mid-season apples and green bananas had to be used. The starch granules obtained from mango kernels were dark. As the presence of Fe^{3+} in mango kernels has been reported,[6] and as this would have interfered with later enzymic experiments, the granules were washed several times with 0·1M-hydrochloric acid and then very carefully with water. The treatment removed most

* Part XXIII, *J.*, 1961, 1534.

[1] (*a*) Cowie and Greenwood, *J.*, 1957, 2862, 4640; (*b*) Banks, Greenwood, and Thomson, *Makromol. Chem.*, 1959, **31**, 197.

[2] (*a*) Greenwood and Das Gupta, *J.*, 1958, 703; (*b*) Arbuckle and Greenwood, *J.*, 1958, 2626.

[3] (*a*) Greenwood, *Stärke*, 1960, **12**, 169; (*b*) Greenwood and Thomson, *Chem. and Ind.*, 1960, 1110.

[4] Greenwood and Robertson, *J.*, 1954, 3769.

[5] Badenhuizen in "Protoplasmatologia, Handbuch der Protoplasmaforschung," ed. Heilbrunn and Weber, Springer-Verlag, Wien, 1959, Vol. IIB, Section 2b8.

[6] Dhingra, Kapoor, and Chandra, *Proc. Ann. Convention Oil Technol. Assoc. India*, 1948, **3**, 39 (*Chem. Abs.*, 1951, **45**, 8273).

of the colour from the granules and qualitative tests confirmed the presence of Fe^{3+} in the acid-extract. Isolation of the starch from the cotyledons of the seeds of the rubber tree, *Hevea brasiliensis*, has been described elsewhere.[7] With the exception of the *Hevea* samples, the isolated fruit and seed starches contained up to 5% of fibrous material which was not removed by filtration through muslin or by differential centrifugation. The contaminant was only removed when a dispersion of the granules (obtained before fractionation) was passed through the Sharples supercentrifuge.

Fractionation.—Before fractionation, each starch was defatted by extraction in a Soxhlet thimble with boiling methanol for 2 hr.

(a) *Complete dispersion of granular starch.* The starch was pretreated with liquid ammonia [1b,8] and then dispersed to give a 0·5% aqueous solution at 98° under nitrogen. The initial amylose complex was formed with thymol, and the recrystallization was as the butan-1-ol complex.[1b] The amylopectin was obtained by freeze-drying.

(b) *Combination of aqueous leaching and dispersion.* A starch suspension (0·5%) was leached successively with water at 70°, 80°, and 90° for 1 hr. under nitrogen. The resultant sediment of gelatinized granules was pre-treated with liquid ammonia before the material was dispersed in water at 98° and fractionated as above.

(c) *Subfractionation of amylopectin.* The samples of pea and amylomaize amylopectin obtained from dispersions were subjected to differential ultracentrifugation; 0·2% aqueous solutions were centrifuged for two periods of 2 hr. each at 40,000 r.p.m. in the preparative rotor of the Spinco model E ultracentrifuge (*i.e.*, at 90,000 ***g***.). The supernatant liquors were then removed and any soluble material obtained by freeze-drying. The sediments were resuspended in water and also freeze-dried.

Characterization of the Granular Starches.—The average size of the granules was estimated from enlarged photomicrographs. The range of temperature over which gelatinization occurred was measured by Schoch and Maywald's method.[9] Potentiometric iodine titration was used to determine the iodine affinity of the starches.[10]

Characterization of the Amyloses.—(a) *Purity* (with W. Banks). The purity of the amylose samples was determined by potentiometric titration [10] and by enzymic assay with a mixture of Z-enzyme and β-amylase. As shown by Peat, Pirt, and Whelan,[11] the concurrent action of these enzymes hydrolyses amylose completely to maltose, whereas the β-amylolysis limit of amylopectin is unaffected by the presence of Z-enzyme. Hence, if in the presence of Z-enzyme the β-amylolysis limit for an amylose is not 100%, amylopectin must be present; and the purity of the amylose can be calculated easily if a value of 57% is assumed for the β-amylolysis limit of amylopectin. (When the β-amylolysis limit is 98—99%, it is difficult to decide on the evidence of reducing power alone whether amylopectin is present or whether experimental inaccuracy can account for the remaining 1—2%. In such a case, the addition of a few drops of 0·01M-iodine to the digest can distinguish between these possibilities; if the solution is achroic, the difference is due to experimental error, whereas a mauve stain indicates the presence of contaminating amylopectin.) Results for estimations of the purity of four samples of amylose by both iodine titration and enzymic assay were as shown (the preparation of the enzymes and the estimation of reducing power have been described elsewhere [1b]):

Amylose sample	1	2	3	4
Purity (%) by iodine titration	>98	>98	94	82
Purity (%) by enzymic assay	100	100	96	85

It can be seen that the results for the two methods show good agreement.

(b) *β-Amylolysis limit.* This was measured by using purified soya-bean β-amylase.[1b]

(c) *Limiting viscosity number.* The limiting viscosity number $[\eta]$ in M-potassium hydroxide at 22·5° was measured, and an estimate of the degree of polymerization ($\overline{D.P.}$) obtained from the relation [1b] $\overline{D.P.} = 7{\cdot}4[\eta]$.

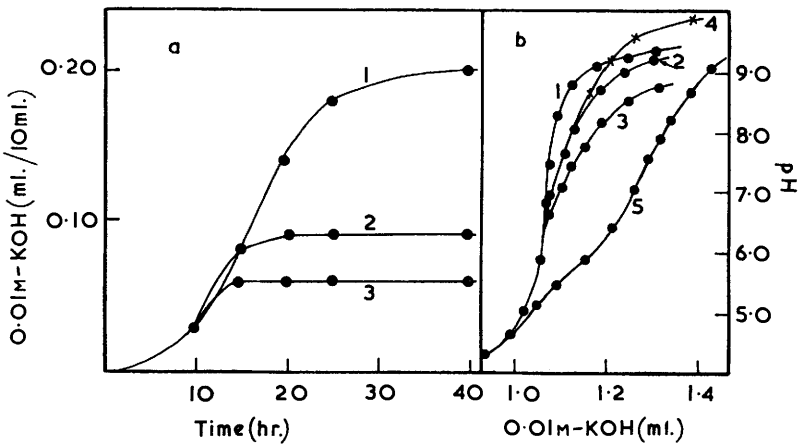
Characterization of the Amylopectins.—(a) *Purity and β-amylolysis limit.* These were determined as for amylose.

[7] Anderson, Greenwood, and Robertson, *J.*, 1957, 401.
[8] Hodge, Montgomery, and Hilbert, *Cereal Chem.*, 1948, **25**, 19.
[9] Schoch and Maywald, *Analyt. Chem.*, 1956, **28**, 382.
[10] Anderson and Greenwood, *J.*, 1955, 3016.
[11] Peat, Pirt, and Whelan, *J.*, 1955, 705.

(b) *Percentage of phosphorus.* Percentages of phosphorus were determined by wet oxidation of the polysaccharide (~100 mg.) with perchloric acid [12] followed by colorimetric estimation of the phosphomolybdate complex by Fogg and Wilkinson's method.[13] Results were reproducible to ±5%.

(c) *Average length of unit chain.* The average length of the unit chain for the amylopectins was determined by Potter and Hassid's method,[14] *i.e.*, by periodate oxidation with sodium metaperiodate at 2°. The oxidation of starch-type polysaccharides with this reagent has been studied in detail as the experimental conditions are not well defined (see ref. 15).

(i) Oxidation conditions. Although Potter and Hassid [14] carried out oxidation in the presence of 5% of sodium chloride, side-reactions involving periodate occur and the " reagent blank " for this system was found to increase steadily with time as shown in curve 1 in Fig. a. This makes corrections very ambiguous. The blank can be stabilized by the addition of IO_3^- (curve 2, Fig. a), and the presence of the latter is essential to obtain the correct value for the



(a) Variation of reagent blank with time for (1) $NaIO_4$–NaCl–H_2O, (2) $NaIO_4$–NaCl–IO_3^-–H_2O, and (3) $NaIO_4$–H_2O.

(b) Titration curves for: (1) $H{\cdot}CO_2H$–KOH. (2) ($H{\cdot}CO_2H$ + $NaIO_4$ + ethylene glycol)–KOH. (3) [As (2) + oxidized amylopectin]–KOH. (4) [As (2) + oxidized methyl-α-D-glucose]–KOH. (5) [As (2) + oxidized maltose]–KOH.

reagent blank in this system. Difficulties of this type were avoided when the sodium metaperiodate was used in aqueous solution without added salt. The reagent blank is then small and constant (Fig. a, curve 3).

Polysaccharide (*ca.* 200 mg.) was dissolved in water (80 ml.) and cooled to 2°. Sodium metaperiodate (20 ml., 0·58M) was added and the mixture was kept at 2°. 10 ml. portions were removed for analysis. The excess of periodate was destroyed by the addition of ethylene glycol (0·5 ml.), and the sample shaken in the dark at room temperature for 30 min. The liberated formic acid was then titrated in a nitrogen atmosphere with 0·01M-sodium hydroxide on a Pye mains-operated pH-meter.

(ii) Estimation of liberated formic acid. In earlier work [16] we analysed potentiometrically the titration curves for oxidation mixtures using *potassium* metaperiodate. Fig. b shows comparable results for the present system. The correct end-point was again at pH 6·25. This end-point was confirmed analytically by Gran's procedure.[17]

It was of interest to study the titration curves for various suggested model compounds.

[12] Smith, *Analyt. Chem. Acta*, 1953, **8**, 397.
[13] Fogg and Wilkinson, *Analyst*, 1958, **83**, 406.
[14] Potter and Hassid, *J. Amer. Chem. Soc.*, 1948, **70**, 3488.
[15] Bobbitt, *Adv. Carbohydrate Chem.*, 1956, **11**, 1.
[16] Anderson, Greenwood, and Hirst, *J.*, 1955, 225.
[17] Gran, *Analyst*, 1952, **77**, 661.

Methyl α-D-glucopyranoside and maltose were examined; we have already shown that sucrose is unsatisfactory.[16] The titration curve for methyl α-D-glucopyranoside was normal, but that for maltose was completely abnormal and there was no definite end-point (see Fig. 1b). The latter substance is clearly unsatisfactory as a model.

(iii) Oxidation time. Typical results of measurements of chain length (from formic acid release) and periodate uptake (measured by the Fleury–Lange method [18] and expressed as moles/mole of glucose) for amylopectin and glycogen were as shown:

		Time (hr.)								
		2	4	5	6	10	15	20	25	40
Potato amylopectin *var.* Epicure	Chain length	—	—	—	—	33·4	28·2	25·6	24·2	24·0
	HIO_4 uptake	0·75	0·89	—	0·92	0·95	0·97	0·99	1·01	—
Brewer's yeast glycogen	Chain length	—	—	—	—	17·5	14·6	13·5	12·8	12·8
	HIO_4 uptake	0·82	—	0·94	—	0·98	1·02	1·05	1·06	—

It can be seen that under these conditions periodate uptake and formic acid release were constant after 25 hr., and no overoxidation occurred.

Results and Discussion

Table 1 shows the general properties of the granular starches isolated in this work. The starches from the broad bean, the iris rhizome, the parsnip, and the mango kernel do not appear to have been studied in detail before. In an earlier part of this Series,[4] we

Table 1.

Properties of whole starches.

Starch	Granule shape	Av. granule size (μ)	Protein [a] (%)	Iodine [b] affinity	Amylose [c] (%)	Gelatinization temp. range
Algae						
Floridean [19]	Round	15	0·44	<0·1	<1	45—47°
Grasses						
Amylomaize	Round	25	0·27	9·9	52	85—87
Barley [20]	,,	20	0·11	4·3	22	59—64
Oat [2b]	,,	25 *	0·24	5·1	27	—
Wheat [2b]	,,	30 *	0·33	5·0	26	65—67 *
Zea mays [2a]	,,	30 *	0·83	5·5	28	—
Leguminosae						
Broad bean	Oval	30	0·16	4·5	24	64—67
Pea (smooth-seeded)	,,	30	0·19	6·6	35	>98
Pea (wrinkled-seeded)	Probably compound	40	0·23	12·5	66	>98
Underground storage organs						
Iris rhizome	Oval	30	0·08	5·0	26	64—67
Parsnip	Round	15	0·27	2·1	11	53—55
Potato	Oval	40	0·05	4·3	23	62—65
Miscellaneous seeds and fruits						
Apple	Round	10	0·10	3·6	19	55—57
Banana	Oval	35	0·32	3·0	16	68—70
Mango kernel	,,	25	0·25	4·5	24	74—77
Hevea (endosperm) [4]	Round	5	0·30	3·7	19	67—69 *
,, (cotyledon)	,,	5	0·15	4·2	22	67—69
Waxy types						
Maize [21]	Round	15	0·10	<0·1	<1	66—69 *

[a] Calc. from (% N × 6·25). [b] Expressed as mg. of iodine bound per 100 mg. of starch. [c] Calc. from [(Iodine affinity of starch/iodine affinity of amylose) × 100].
* Additional results obtained in this work.

described the properties of the starch from the endosperm of the seed of the rubber tree, *Hevea brasiliensis*, and here the properties of the comparable starch in the cotyledons of the seed have been examined. These two starches are of interest as relatively few studies

[18] Fleury and Lange, *J. Pharm. Chim.*, 1933, **17**, 107.

have been reported of starches from different portions of the same plant material. For comparative purposes, our results for Floridean starch (from the algæ *Dilsea edulis*) [19] and some other starches from grasses [2,20,21] are included.

It can be seen that the extraction method described in the Experimental section provides a general method for the isolation and purification of granular starches, almost completely free from contaminating protein, without the use of any chemical treatment. As has been stressed elsewhere,[3a] this purely physical method cannot remove all the protein and some form of acid- or alkali-treatment is necessary to achieve this. (Consequently, commercial samples of starches may contain less protein.) The reason for this behaviour is not known, but the residual protein may well be bound chemically to the granule and might arise from residues of the enzyme-protein responsible for biosynthesis. Further, if the protein exists at the surface, the extent of contamination may well affect the behaviour of the granule towards swelling agents. Notwithstanding these small traces of residual protein, laboratory starches are preferred for fundamental studies as our earlier work on potato [1] and wheat [2b] starches indicated that the amylose component is considerably degraded in commercial samples.

The iodine affinity of the starches varied enormously and corresponded to values of amylose-content ranging from $<1\%$ in Floridean and waxy maize starch to 66% in the starch from wrinkled-seeded peas. This high percentage in wrinkled-seeded peas confirms the results of earlier workers.[22] There is also a wide range of values between these extremes, but there is no fixed value for each of the different botanical groups. Parsnip starch is unusual as it contains only 11% of amylose.

The percentage of amylose must be controlled by genetic factors as work on the development of amylomaize has shown.[23] It has to be stressed, however, that the amylose percentages are only approximate; differences may arise when different varieties are studied but, more significantly, the apparent percentage of amylose depends on the maturity of the plant source.[3a] This work will be described elsewhere.[24]

The factors which influence the gelatinization temperatures are complex. All the granules in a given sample do not gelatinize at the same time; the small ones are more resistant than the large ones and the whole process takes place over a range of 5—10°. The temperatures in the last column of Table 1 correspond to the range when 25—75% of the granules have gelatinized.[9] No simple relation was found between gelatinization temperature and either the granule size or the percentage of amylose. Floridean starch requires an abnormally low temperature, whilst the converse applies to the two pea starches. The organization of the granule itself must be of prime importance, and these differences in gelatinization temperature are not understood.

Many starches, particularly those from grasses and *Leguminosae*, do not readily disperse into aqueous solution without some form of pretreatment. We find that pretreatment with liquid ammonia is the most satisfactory of the several that have been suggested. This method completely destroys granular structure, ensuring complete dispersion and efficient fractionation without altering in any way the properties of the amylose.[1b] The polar ammonia molecule must be able to penetrate into the granular structure and disrupt the hydrogen-bonding. (In contrast, when potato starch granules were treated with liquid nitrogen for 4 hr., they were microscopically unaltered in appearance, and did not gelatinize at 50°—after liquid-ammonia treatment, potato starch will disperse at room temperature.)

The properties of the amylose components obtained from the fractionation of a dispersion are shown in Table 2. All the samples were free from amylopectin, as shown by

[19] Greenwood and Thomson, *J.*, 1961, 1534.
[20] Greenwood and Thomson, *J. Inst. Brewing*, 1959, 346.
[21] Arbuckle and Greenwood, unpublished experiments.
[22] Potter, Silveira, McCready, and Owens, *J. Amer. Chem. Soc.*, 1953, **75**, 1335.
[23] Senti, *Chemurgic Digest*, 1958, **17**, 7 and references therein.
[24] Greenwood and Thomson, *Biochem. J.*, 1962, **82**, 156.

TABLE 2.

Properties of the amylose components.

Starch	Iodine affinity [a]	β-Amylolysis limits [b] (i)	(ii)	[η]	Approx. D.P.
Grasses					
Amylomaize	19·2	77	101	180	1300
Barley [20]	19·0	73	100	250	1850
Oat [2b]	19·2	77	—	180	1300
Wheat [1b]	19·1	68	—	280	2100
Zea mays [2a]	18·8	78	100	150	1100
Leguminosae					
Broad bean	19·2	82	99	240	1800
Pea (smooth-seeded)	19·2	81	100	180	1300
Pea (wrinkled-seeded)	19·2	82	101	140	1000
Underground storage organs					
Iris rhizome	19·1	84	100	240	1800
Parsnip	19·4	72	99	590	4400
Potato	19·5	76	100	410	3000
Miscellaneous fruits and seeds					
Apple	19·0	84	99	200	1500
Banana	19·9	82	100	240	1300
Mango kernel	19·2	77	100	240	1800
Hevea (endosperm) [4]	19·2	79	101	220	1600
,, (cotyledon)	19·0	74	100	200	1500

[a] As in Table 1. [b] Percentage conversion into maltose with (i) pure β-amylase and (ii) a mixture of β-amylase and Z-enzyme.

their high iodine affinity and the high conversion into maltose under the concurrent action of β-amylase and Z-enzyme. However, all the samples are incompletely converted into maltose under the action of pure β-amylase. As the action of the enzyme is very specific, this suggests that there is some structural anomaly in amylose. Evidence regarding the nature of this has been discussed elsewhere.[3a] Although the structural anomaly could be an artefact, the most conclusive proof that it is native to amylose is that when starch is isolated from plants at various stages of maturity, the β-amylolysis limit for the resultant amyloses decreases with increase in maturity; essentiallly linear amylose can be isolated from an immature source.[3a,24]

The molecular size of the amyloses has not been determined directly, but an estimate of the degree of polymerization has been obtained from the viscosity measurements. The calculated values are not absolute but should give the correct order of magnitude. There are large differences in apparent size, but the size of all the amyloses in the miscellaneous fruits and seeds group appears to be comparable. The amyloses from potato and parsnip are very much larger than those from the other samples. However, differences in size between amyloses in the same botanical group are not surprising, as our experiments on starches from plants at various stages of growth have shown that the molecular size of the amylose component increases with increase in maturity of the plant. However, notwithstanding this fact the properties of the two *Hevea* samples are comparable.

Our previous experiments [3a] have shown that amylose is heterogeneous and can be separated very easily into fractions of varying size and β-amylolysis limit by successive aqueous leaching of the granules at varying temperatures. Table 3 shows the properties of the amyloses obtained from leaching one or two starches from each of the botanical groups. For all the starches, leaching at 70° yielded an amylose which was essentially linear, as shown by its high β-amylolysis limit, and had a low limiting viscosity number compared with those of other fractions. As the temperature of extraction increased, the resultant amylose had a *lower* β-amylolysis limit and a higher limiting viscosity number. (All the leached products were pure amyloses, as shown by their complete conversion into maltose under the concurrent action of β-amylase and Z-enzyme.) The amount of

TABLE 3.

Properties of the amylose components obtained on successive aqueous leaching of the granules.

Starch	Procedure	Amylose extracted (%) [a]	β-Amylolysis limit [b]	$[\eta]$
Amylomaize	80° leach	15	98	140
	90° leach	12	79	170
	Dispersion of residue [c]	73	74	200
Barley [20]	70° leach	26	96	90
	80° leach	15	74	150
	90° leach	20	65	250
	Dispersion of residue	39	62	270
Broad bean	70° leach	16	98	130
	Dispersion of residue	84	77	280
Pea (smooth-seeded)	70° leach	2	100	110
	80° leach	3	88	125
	90° leach	4	83	170
	Dispersion of residue [c]	91	80	190
Iris (rhizome)	70° leach	19	98	190
	80° leach	25	89	230
	90° leach	25	76	260
	Dispersion of residue [c]	31	72	280
Mango kernel	70° leach	9	98	190
	80° leach	25	89	200
	90° leach	25	72	230
	Dispersion of residue [c]	41	68	260

[a] Calc. from iodine-affinity measurements. [b] Percentage conversion into maltose with β-amylase. [c] Followed by addition of thymol.

extractable linear amylose varied from starch to starch. It was found that a smaller amount of amylose was extracted from the high-amylose amylomaize than from barley starch which had a normal amylose content. However, the most unusual behaviour was that shown by the samples of pea starch where only 2—3% of the total amylose was extracted.

Some form of pretreatment (which must affect the granular structure) appears to be necessary before aqueous leaching results in the extraction of linear amylose. Here, boiling methanol was satisfactory for all but potato starch. In the case of this starch, pretreatment with boiling aqueous methanol (80%) appears to be essential [1b] (this phenomenon is being investigated in more detail).

Table 4 shows the properties of the amylopectin components isolated from a dispersion. All the samples were obtained reasonably free from contaminating amylose except those from the amylomaize and wrinkled-seeded pea. The percentage of phosphorus in the amylopectins from underground storage organs was significantly higher than that for the other groups. The β-amylolysis limits for the amylopectins varied between 56% and 59%; they are not significantly different. However, the values for the average length of unit chain vary from 18 to 28 glucose units, and it is thought that these differences are significant. (Oxidation of these branched glucans by sodium metaperiodate at 2° is best carried out in aqueous solution in the absence of salt. The reaction is then complete in 24 hr. and there is no overoxidation.) It is of interest that the two *Hevea* samples have again comparable properties.

When the amylomaize and wrinkled-seeded pea amylopectins were examined directly after fractionation from a dispersion, values of the average length of unit chain of 36 glucose units, and β-amylolysis limits of 64—65% conversion into maltose, were obtained, in agreement with earlier workers.[22,25] The potentiometric iodine titration curves for these samples were abnormal and the linear portion of the iodine absorption curve was unusually steep (cf. ref. 10). It was difficult to estimate the percentage of linear component present, and hence to apply suitable corrections to the periodate oxidation results. When the two

[25] Wolff, Hofreiter, Watson, Deatherage, and MacMasters, *J. Amer. Chem. Soc.*, 1955, **77**, 1654.

TABLE 4.

Properties of the amylopectin components.

Starch	Purity (%)	Phosphorus (%)	β-Amylolysis limit [b]	Average length of unit chain [c]	[η]
Algae					
Floridean [19]	>99	—	49	18	160
Grasses					
Amylomaize *	98	0·03	58	28	130
Barley [20]	98	0·03 †	58	26	190
Oat [25]	99	—	57 †	20	—
Wheat [25]	96	—	57 †	19	140 †
Zea mays [2a]	98	—	58 †	23	100
Leguminosae					
Broad bean	98	0·02	57	23	125
Pea (smooth-seeded)	95	0·04	58	26	150
Pea (wrinkled-seeded) *	97	0·03	58	27	150
Underground storage organs					
Iris rhizome	99	0·05	57	22	100
Parsnip	99	0·05	58	21	175
Potato	99	0·04	56	24	160
Miscellaneous seeds and fruit					
Apple	99	0·03	58	22	170
Banana	98	0·02	59	21	120
Mango kernel	98	0·02	56	21	150
Hevea (endosperm) [4]	98	0·02 †	57 †	23	102
,, (cotyledon)	99	0·02	58	21	140
Waxy types					
Maize [21]	>99	—	58	20	145

[a] Calc. from iodine affinity. [b] Percentage conversion into maltose with pure β-amylase. [c] From the results of periodate oxidation.

* After differential ultracentrifugation (see Text). † Additional results obtained in this work.

amylopectins were studied in alkaline solution in the Spinco model E ultracentrifuge,[26] they were found to be heterogeneous; there was a fast-moving component (S_{20} ~100 × 10^{-13} c.g.s. units) and appreciable amounts of a slow-moving component (S_{20} ~1 × 10^{-13} c.g.s. unit). Differential ultracentrifugation (see Experimental section) enabled these two components to be separated. The properties of the sedimented materials shown in Table 4 were comparable with those of normal amylopectins, whilst the material in the supernatant liquors was similar to degraded amylose.[3b] The implications of these results have been discussed elsewhere.[3b]

The determination of the molecular size of amylopectin is difficult (see refs. 3*a* and 27). The differences between the limiting viscosity numbers for the samples (shown in Table 4) are significant and probably indicate differences in size (*e.g.*, compare the two *Hevea* samples). Dr. W. Banks kindly determined the weight average molecular weight of some samples in aqueous solution by light-scattering measurements. Typical values were: broad-bean amylopectin, 4·5 × 10^8; iris-rhizome amylopectin, 9 × 10^7; and banana amylopectin, 2·2 × 10^8. These values suggest that the amylopectin molecule must be one of the largest in Nature. (A detailed discussion of these results will appear elsewhere.)

The authors thank Professor E. L. Hirst, C.B.E., F.R.S., for his interest in this work and the Department of Scientific and Industrial Research for a maintenance grant (to J. T.).

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[26] Bryce, Cowie, and Greenwood, *J. Polymer Sci.*, 1957, **25**, 251.
[27] Greenwood, *Adv. Carbohydrate Chem.*, 1956, **11**, 335.