693. *Physicochemical Studies on Starches. Part XX.* The Existence of an Anomalous Amylopectin in Starch.*

By W. Banks and C. T. Greenwood.

The polysaccharide from the supernatant liquor from the recrystallisation of the initial amylose-complex of potato starch has a significantly lower average chain length and β-amylolysis limit than amylopectin. A similar polysaccharide occurs in rubber-seed starch. This material, which represents 5—10% of the granule, is thought to be inherent in the granular structure. Its physical properties are discussed.

Although the dual-component nature of the starch granule is well established,[1] there are reports of the existence of material with properties different from those of amylose and amylopectin. From a comprehensive study of the iodine affinities of various fractions and subfractions of amylose, Schoch and his co-workers [2] have suggested that in maize starch there may be 5—7% of such a substance. This polysaccharide was apparently precipitated by " Pentasol " but not by butan-1-ol. On the basis of optical-density and β-amylolysis measurements on mixtures of pure potato amylose and amylopectin, Peat, Pirt, and Whelan [3] postulated that potato starch granules may contain material with a higher iodine affinity but a lower β-amylolysis limit than amylopectin. An anomalous " thymol-amylopectin " has been isolated from the supernatant liquors from the recrystallisation of the initial thymol-amylose complex of potato starch by Cowie and

* Part XIX, *Stärke*, in the press.

[1] See Greenwood, *Adv. Carbohydrate Chem.*, 1956, **11**, 335.
[2] Lansky, Kooi, and Schoch, *J. Amer. Chem. Soc.*, 1949, **71**, 4066.
[3] Peat, Pirt, and Whelan, *J.*, 1952, 705.

Greenwood.[4] Recently, Perlin [5] has isolated a similar fraction (termed "Amylopectin-C") from wheat starch, whilst a second component, of low molecular weight, in waxy maize starch has been reported from sedimentation studies by Erlander and French.[6] In this paper, we give the results of detailed investigations made on the "thymol-amylopectin" isolated from potato starch and rubber-seed starch.

Experimental

Methods of isolating potato starch (vars.: Epicure = potato 1; Redskin = potato 2; Golden wonder = potato 3) and fractionating it so as to obtain the "thymol-amylopectin" have been described previously.[4] The polysaccharide was isolated by freeze-drying the supernatant liquor which had been concentrated to small volume under reduced pressure (temp. $<35°$). For Epicure starch, fraction 1A was isolated in this way, whilst fraction 1B was obtained by freeze-drying the supernatant directly. All samples were exhaustively extracted with boiling methanol. The sample of polysaccharide from rubber-seed starch had been isolated in earlier work.[7] Samples were characterised by measurements of iodine affinity (I.A.), average length of unit-chain ($\overline{C.L.}$), limiting viscosity number $[\eta]$, and percentage conversion to maltose on β-amylolysis (see earlier papers in this series [7]). The percentage of phosphorus in some samples was determined by wet oxidation of the polysaccharide with perchloric acid,[8] followed by colorimetric estimation of the phosphomolybdate complex as described by Fogg and Wilkinson.[9] (Sample weights of *ca.* 250 mg. were taken, and results were reproducible to $\pm 5\%$.)

Subfractionation of "thymol-amylopectin." A 0·25% aqueous solution of the Epicure potato "thymol-amylopectin" (250 ml. containing 0·1% of sodium chloride) was kept at 30°. Ethanol was added slowly with vigorous mechanical stirring until a permanent turbidity was observed (some 20% by volume was required). The temperature was then raised until the turbidity disappeared (50—55°), and the flask was then allowed to cool to 30° during 6 hr. After a further 48 hr. at this temperature, the supernatant liquor was decanted from the precipitated gel. A second fraction was precipitated by raising the alcohol concentration to 40%. Both fractions were dispersed in water and freeze-dried to yield fractions 1C and 1D, respectively. (Each fraction contained *ca.* 50% of the original polysaccharide.)

Results and Discussion

The results shown in the Table indicate that about 5—10% of potato starch appears to be precipitated with the initial thymol-amylose complex, and can be isolated from the recrystallisation liquors. A similar result has been found with butan-1-ol as the initial precipitant, but other reagents have not been investigated. This polysaccharide has a low iodine affinity, but a significantly lower chain length and β-amylolysis limit than amylopectin. The material would normally be discarded during fractionation.

The values of chain length and β-amylolysis limit for the "thymol-amylopectin" from rubber-seed starch and the "Amylopectin-C" from wheat starch are comparable to those for the potato polysaccharide. It is therefore suggested that such a polysaccharide might be a universal constituent of starches.

Adsorption of this branched polysaccharide on the initial amylose complex is unlikely, for the iodine affinity of the complex was unaltered by repeated washing with thymol-saturated water, or varying the force-field used in the isolation, or using butan-1-ol instead of thymol. Further, on dispersion of a starch, which had been previously leached with water at 65° and had lost about 60% of its amylose, the resultant amylose–thymol complex was 45% pure. If it is assumed that the same amount of branched polysaccharide is co-precipitated, the calculated purity of the complex is *ca.* 50%. This agrees well with the

[4] Cowie and Greenwood, *J.*, 1957, 4640.
[5] Perlin, *Canad. J. Chem.*, 1958, **36**, 810.
[6] Erlander and French, *J. Amer. Chem. Soc.*, 1958, **80**, 4413.
[7] Greenwood and Robertson, *J.*, 1954, 3769; Cowie and Greenwood, *J.*, 1957, 2658; Bryce, Cowie, Greenwood, and Jones, *J.*, 1958, 3558.
[8] Smith, *Analyt. Chim. Acta*, 1953, **8**, 397.
[9] Fogg and Wilkinson, *Analyst*, 1958, **83**, 406.

observed value. It is not known why co-precipitation should occur. There was the possibility that the phosphate groups in potato starch were predominantly associated with this fraction, but the "thymol-amylopectin" from Epicure starch contained 0·07% of phosphorus, whilst there was a similar amount (0·09%) in the corresponding amylopectin.

Properties of amylopectin-type polysaccharides in starches.

Starch	Component	Amount (%) [a]	Amylose (%) [b]	$\overline{C.L.}$	β-Limit [c]	External chain length [d]	[η]
Potato	Amylopectin 1	~75	0·5	23·1	57	15·7	190
	"Thymol-amylopectin" 1A	5—10	1·0	13·4	53	9·6	170
	,, ,, 1B	5—10	0·5	13·6	51	9·4	150
	,, ,, 1C	—	—	13·8	52	9·7	—
	,, ,, 1D	—	—	14·0	50	9·5	—
	Amylopectin 2	~75	0·5	23·3	57	15·9	200
	"Thymol-amylopectin" 2	5—10	5·0	14·7	53	9·3	220
	Amylopectin 3	~75	1·0	23·2 *	56	15·5	180
	"Thymol-amylopectin" 3	5—10	2·0	15·8 *	—	—	76
Rubber-seed	Amylopectin	~70	0·5	23·1 *	64	17·3	—
	"Thymol-amylopectin"	10	2	15·8 *	61	12·1	—
Wheat †	Amylopectin	~70	low	Not accurately measured	55—60	—	—
	"Amylopectin C"	5—10	low		48—53	—	—

[a] % of total starch. [b] Calc. from iodine affinity. [c] Expressed as percentage conversion into maltose. [d] Calc. from $\{[\overline{C.L.} \times (\beta\text{-limit})] + 2·5\}$.

* Results by courtesy of Mr. J. Thomson. † Results from ref. 5.

Phosphate was therefore unlikely to be causing co-precipitation. Further, rubber seed and wheat starch have little phosphate. Although the purity of the thymol-amylose complex was independent of the length of time of dispersion of the starch for up to 2 hr. (*i.e.* the usual conditions for fractionation), the polysaccharide could be residual undispersed granular particles. These might disperse in the more dilute conditions of the recrystallisation. This will be discussed further below.

Although a component with properties intermediate between those of amylose and amylopectin (*e.g.*, an amylopectin with external chains of sufficient length to form complexes with thymol and other reagents) might be expected,[1] "thymol-amylopectin" is not such a molecule. On the basis of β-amylolysis limits, the external chain length is only 9—11 anhydroglucose units compared with 15—16 in amylopectin. Erlander [10] has suggested that glycogen may be a precursor for the synthesis *in vivo* of amylopectin, but although the chain length of the polysaccharide approaches that for glycogen, its physical behaviour is inconsistent with this concept. (It is to be noted that on subfractionation of the polysaccharide, fractions 1C and 1D showed no variation in chain length and β-limit, which may indicate that the polysaccharide is not a mixture of amylopectin and glycogen.) The limiting viscosity number of *ca.* 180 is little different from that for amylopectin (whereas the value for glycogen [11] is *ca.* 10). The relatively low limiting viscosity number suggests that a molecule of "herring-bone" type of structure (*i.e.*, a main chain with side chains of *ca.* 15 glucose units) is unlikely. Rather would the polysaccharide appear to be degraded amylopectin. It is not thought that the difference in internal chain lengths of 5—6 glucose units for the polysaccharide compared with 7—8 units for amylopectin is significant.

The molecular weight of the "thymol-amylopectin" cannot be accurately determined. Ultracentrifugal examination showed that the properties of both the initial amylose-thymol complex and the corresponding "thymol-amylopectin" varied with the variety of potato starch. The initial thymol complex was not always homogeneous (cf. ref. 4).

[10] Erlander, *Enzymologia*, 1958, **19**, 273.
[11] Bryce, Greenwood, and Jones, *J.*, 1958, 3845.

The isolated " thymol-amylopectin " showed a small but variable amount of rapidly sedimenting material—probably amylopectin; estimates of the amount of the latter are made difficult by the Johnston–Ogston effect.[12] However, unpublished light-scattering experiments by Mr. I. G. Jones have indicated that the dissymmetry and the molecular weight (*ca.* 10^8) of " thymol-amylopectin " were of the same order as those of the parent amylopectin.

It is suggested that " thymol-amylopectin " could be amylopectin which has undergone enzymic modification—either by premature cessation of synthesis, or by degradation. As amylopectin is mainly on the outside of the granule,[13] any enzymically-modified material might be associated with residual " granular sacs." The material may be therefore an inherent part of the granule, but the amount and the molecular properties will depend entirely on the botanical environment of the source of the starch.

The authors thank Professor E. L. Hirst, C.B.E., F.R.S., for his interest and helpful comments, the Rockefeller Foundation for financial support, and the Department of Scientific and Industrial Research for a maintenance grant (to W. B.).

THE UNIVERSITY, EDINBURGH, 9. [*Received, April 30th,* 1959.]

[12] Johnston and Ogston, *Trans. Faraday Soc.*, 1946, **42**, 789.
[13] Cowie and Greenwood, *J.*, 1957, 2658.