phene-5-dioxide. As expected from the known reactions of sulfones with Grignard reagents,[22] bicarbonate-soluble products were isolated in each case but only from methyl phenyl sulfone was the product identified. Here, *n*-butyllithium gave 47% of benzenesulfonylacetic acid and 45.5% of the sulfone was recovered. Ethyl phenyl sulfone reacted vigorously with ethylmagnesium bromide to yield on carbonation an acidic gum which was not identified. There was also separated a trace of an unidentified bicarbonate-insoluble but potassium hydroxide-soluble crystalline solid, m. p. 156°. The starting material was recovered to the extent of 61%. An unidentified acidic oil was also obtained from ethyl phenyl sulfone and *n*-butyllithium. This substance turned black on standing. Diphenyl sulfone reacted vigorously with *n*-butyllithium to give in low yield an acid melting at 200° after darkening from 120°. Attempts to purify this substance by recrystallization from a variety of solvents were unsuccessful.

(22) Ziegler and Connor, *ibid.*, **62**, 2596 (1940); Kohler and Tishler, *ibid.*, **57**, 217 (1935); Kohler and Potter, *ibid.*, **57**, 1316 (1935); **58**, 2166 (1936); Kohler and Larsen, *ibid.*, **57**, 1448 (1935); **58**, 1518 (1936).

An acidic gum was obtained from dibenzothiophene-5-dioxide and *n*-butyllithium following carbonation. All efforts to crystallize the product were unsuccessful.

Summary

1. Methyl aryl sulfides, in general, undergo lateral metalation with metalating agents of the Group I metals.

2. Methyl phenyl sulfide is metalated in the nucleus by *n*-butylmagnesium bromide.

3. Ethyl phenyl, *n*-propyl phenyl, isopropyl phenyl and *n*-butyl phenyl sulfides give *ortho*-nuclear metalation with *n*-butyllithium in diethyl ether.

4. Some cleavage reactions of alkyl phenyl sulfides with lithium and sodium metals and with *n*-butylmagnesium bromide and *n*-butyllithium have been examined.

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[CONTRIBUTION FROM THE GEORGE M. MOFFETT RESEARCH LABORATORIES, CORN PRODUCTS REFINING COMPANY]

Properties of the Fractions and Linear Subfractions from Various Starches

BY SYLVIA LANSKY, MARY KOOI AND THOMAS JOHN SCHOCH

There have been no critical standards by which to evaluate the purity and extent of degradation of the fractions from various starches. As a consequence, doubts frequently arise regarding the quality of the parent starch, the possibility of hydrolytic degradation during fractionation, and the purity of the final fractions. The present investigations were initiated primarily to isolate the linear and branched components of various common starches by optimum fractionation techniques, and to establish precise standards of purity and degree of degradation on the basis of iodine affinity and intrinsic viscosity. As this work progressed, the substantial differences between the linear fractions from the various starches required further elucidation from a molecular standpoint. This led to the development of a technique for separating the total linear material into a graded series of subfractions by partial precipitation methods. Various reducing value determinations for aldehydic end-groups have been investigated as a means for comparing the relative molecular size of the linear fractions and subfractions. These physical and chemical criteria have likewise had application in estimating the degree of hydrolysis of various modified starches and in elucidating the mechanism of β-amylase action on the starch fractions. Through the kindness of A. L. Potter and W. Z. Hassid, osmotic molecular weights and non-aldehydic end-group assays (by periodate oxidation) have been reported on many of the identical fractions here described.[1]

In the present paper, the linear and branched starch components are designated as the A-fraction and the B-fraction, respectively, in conformance with previously established definitions.[2]

Source of Starches.—Various samples of commercial corn starch were selected over a period of four years, choosing those lots which possessed the highest "hot paste viscosity." The wheat starch was an experimental batch produced at the Northern Regional Research Laboratory by sulfur dioxide steeping. Tapioca starches included samples from Brazil (kindly supplied by Mr. George Caesar of Stein, Hall and Company) and from the Dominican Republic, in each case selected by reason of high paste viscosity. Limited data are likewise available on the A-fraction from pre-war Javanese tapioca. Potato starches were of Maine and Idaho origin, from the New England Starch Company and Idaho Potato Starch Company, respectively. Data are also included on the B-fraction from pre-war German potato starch. Since commercial sago starch is frequently bleached with oxidizing agents, a sample of virgin unmodified sago flour was secured through the courtesy of Dr. C. G. Caldwell of the National Starch Company. This was suspended in methanol and screened through silk bolting cloth, then repeatedly sedimented in methanol and finally in distilled water. This treatment served to remove all foreign material and most of the pigment. Easter lily starch was furnished by Dr. R. M. Hixon of Iowa State College. The corn, wheat, sago and lily starches were defatted by five two-hour digestions under reflux with boiling 85% methanol, filtering and washing with 85% methanol after each digestion. In later studies, raw corn starch (non-defatted) was fractionated directly.

Fractionation Methods.—In all cases, primary separation was effected by selective precipitation with Pentasol (commercial mixture of amyl alcohols marketed by Sharples Solvents). Where the starch was dissolved by autoclaving, defatted starch was employed and the system was buffered at *p*H 6.2–6.3 prior to autoclaving by the addition of 40 ml. of 20% phosphate solution (16.4% with respect

(1) Potter and Hassid, THIS JOURNAL, **70**, 3488, 3774 (1948).

(2) Schoch in "Advances in Carbohydrate Chemistry," edited by Pigman and Wolfrom, Vol. I, Academic Press, New York, N. Y., 1945, pp. 247–277.

to anhydrous KH_2PO_4 and 3.6% to anhydrous K_2HPO_4) per 450-g. batch of starch in 15 l. of water. A number of starches were fractionated without autoclaving; in these cases, raw starch may be employed directly without preliminary defatting. In a 5-gal. Pyrex bottle equipped with mechanical stirrer and reflux condenser and heated in a suitable boiling water-bath, 300 g. of the starch was gelatinized in a boiling mixture of one liter of Pentasol and 15 l. of distilled water containing 50 ml. of the above phosphate buffer. The mixture was gently refluxed for three to four hours at 92° with continuous agitation, then cooled overnight with stirring and finally refrigerated for twenty-four hours. The A-fraction complex crystallized as rounded spherules; centrifugation at 50,000 r. p. m. in the Sharples continuous supercentrifuge gave a dense well-packed deposit, with no evidence of slimy material. This mode of fractionation is preferred over previous methods, since it avoids both the defatting operation and the possible hydrolytic degradation during autoclaving.

To isolate the B-fraction, the supernatant solution from the centrifuge was treated with one-third its volume of methanol, the mixture vigorously stirred for several minutes, then refrigerated overnight.[3] The B-fraction precipitated quantitatively as a soft curd which was removed and dehydrated by grinding for two or three minutes in a Waring Blendor with fresh methanol.

Two methods were employed for recrystallization of the A-fraction. By the earlier method,[2] the crude moist A-fraction was dissolved in boiling water in the presence of excess *n*-butyl alcohol to give a 1% solution (on dry substance basis). This was supercentrifuged to remove extraneous impurities, cooled and refrigerated to crystallize the A-fraction. Two such recrystallizations sufficed to raise the iodine affinity of the product to a maximum value. Since it became evident that this technique caused some subfractionation, a later method employed Pentasol as the recrystallizing agent. The crude moist A-fraction was dissolved in hot water (95–100°) with vigorous agitation, to give a solution of 0.2% concentration (dry substance basis). The hot solution was passed through the Sharples supercentrifuge, giving only a small amount of dark brown sediment in the lower part of the rotor. An excessive amount of white or light colored material indicates incomplete solution of the A-fraction, due to too low a temperature or to inadequate stirring during solution of the crude material. Any such undissolved residue can usually be recovered by dissolving in boiling water and recentrifuging. The total centrifugate was reheated to 90–95°, 5–6% by volume of Pentasol added, the mixture cooled overnight with continuous agitation, then refrigerated twenty-four hours and centrifuged. When crystallized at 0.2% concentration, the A-fraction separated as well-defined rosettes which gave a dense deposit in the centrifuge rotor. Attempts to recrystallize 1% solutions with Pentasol gave soft, bulky and even "sloppy" deposits. As many as seven recrystallizations with Pentasol were employed in an effort to attain maximum iodine affinity. While no attempt was made in these studies to control *p*H during recrystallization, it might be advisable to add a small amount of phosphate buffer.

The A-fraction was dehydrated by suspending in 10 volumes of *n*-butyl alcohol, stirring for several hours and filtering. This treatment was repeated several times and the product finally dried *in vacuo* at 70°. This method of drying avoids retrogradation, and the dried A-fraction is completely soluble in boiling water. Yields of A-fraction were not determined except in the case of certain modified starches.

Subfractionation of A-Fraction.—A number of methods have been investigated for the separation of A-fraction into a graded series of subfractions of different chain lengths. Selective retrogradation and partial precipitation with methanol were ineffectual. By far the best procedure involved partial precipitation with minimal amounts of the higher alcohols. While cyclohexanol has been used in some of these studies, *n*-octyl alcohol is preferred by reason of its lower solubility in water and more precise control of subfractionation. It should be noted that these agents precipitate the A-fraction at temperatures substantially above the levels of *n*-butyl alcohol or Pentasol precipitation.

The following procedure is cited as an example of optimum subfractionation technique (Run 17). One hundred g. of potato A-fraction (Batch P-7/9-A, twice recrystallized with Pentasol) was dissolved in 10 l. of boiling water and passed through the supercentrifuge or filtered through Pyrex glass wool to remove any slight trace of insoluble material. The clarified solution was reheated to 80–85°, 1.5 ml. of *n*-octyl alcohol added dropwise with agitation, and the mixture allowed to cool spontaneously with continuous stirring. Turbidity developed when the temperature dropped to 72°, and crystalline precipitation occurred in the range from 70 to 62°, as indicated by a "watered-silk" appearance on stirring. The mixture was allowed to cool overnight with stirring, then passed through the Sharples supercentrifuge at a rate of 200 ml. per minute, using the smallest injector nozzle. The first subfraction (designated as 17-a, to indicate Run 17, first subfraction) was removed from the rotor, dehydrated by successive digestions in *n*-butyl alcohol, filtered and dried to constant weight in the vacuum oven at 70°. The centrifugate was reheated to 80–85°, 1.0 ml. of octyl alcohol added and the mixture similarly cooled with stirring. After removal of the second subfraction (17-b), the third, fourth and fifth subfractions were similarly precipitated by the addition of 1.0-ml. portions of octyl alcohol. The sixth and last portion was precipitated with excess octyl alcohol. Mechanical losses of linear material during these successive centrifugings may total 10–15%. Consequently, weight distribution of the subfractions was calculated on the basis of total recovered material.

Hydrolysis Studies.—It was also of interest to determine the characteristics of linear and branched molecules smaller than those in the native starch. For this purpose, granular corn starch was hydrolyzed to various levels by treatment with dilute acid at a temperature below the gelatinization point, in much the same fashion as for the commercial production of "thin-boiling" starches. Suspensions of raw corn starch (40% on weight basis) in 0.075 *N* hydrochloric acid were digested in a constant temperature bath at 50° for five, sixteen, twenty-six and forty hours. The converted starches were neutralized to *p*H 6.0 with sodium carbonate, filtered, washed and air-dried. Similar conversions were run in 0.3 *N* hydrochloric acid for two, seven and sixteen hours at 50°. The acid-modified starches were subsequently fractionated by Pentasol precipitation and the linear fractions recrystallized twice with Pentasol.

Iodine Affinity.—The potentiometric titration method of Bates, French and Rundle[4] has been modified for routine evaluation of iodine affinity. A bright platinum electrode was employed in conjunction with a low resistance calomel electrode (Leeds and Northrop No. 1199–11 electrode, or Coleman No. 3-070 electrode with saturated potassium chloride junction). Potentials may be determined with an ordinary *p*H meter adaptable to millivolt readings, though use of a Leeds and Northrop Type K bridge and No. 2420-C galvanometer provides much simpler and more precise operation. To calibrate the electrode system, 373 mg. of potassium chloride and 830 mg. of potassium iodide were dissolved in 100 ml. of water and titrated with standard iodine solution between e. m. f. limits of 230–280 millivolts, the solution being stirred mechanically during the titration. The standard iodine solution was 0.05 *N* with respect to potassium iodide, 0.05 *N* to potassium chloride, and contained exactly 0.200 mg. of iodine per ml.; this was most conveniently prepared just prior to use by tenfold dilution of a stock iodine solution, the latter being standardized against arsenious oxide. Since the solution and iodine reagent were both 0.05 *N* with respect to potassium chloride and potassium iodide, there was no change in salt concentration during

(3) Schoch and French, *Cereal Chem.*, **24**, 231 (1947).

(4) Bates, French and Rundle, THIS JOURNAL, **65**, 142 (1943).

the titration. From the plotted curve of this titration, a calibration chart was prepared giving the total milligrams of free iodine in solution for each half-millivolt reading from 230–280 mv.

Before evaluation of iodine affinity, all starchy materials were Soxhlet-extracted for twenty-four hours with ethyl alcohol, to remove residual fractionating agents or natural lipids which repress the iodine affinity. The sample was then dried and pulverized to pass a 40-mesh screen. To prepare unmodified granular starches for analysis, the starch was gelatinized in boiling water to give a 1% paste, this was disintegrated by five minutes agitation in the Waring Blendor and the starch substance flocculated by the addition of excess methanol. Modified or so-called "thin-boiling" starches do not require pregelatinization. An appropriate sample (*i.e.*, approximately 40 mg. of A-fraction, 100 mg. of whole starch, or 200 mg. of B-fraction) was transferred to a dry 250-ml. beaker. Five ml. of 1 *N* potassium hydroxide solution was added by pipet and the sample dispersed in the alkali by trituration with a stirring rod, with care to avoid the formation of difficultly soluble lumps. The mixture was allowed to stand with occasional stirring for one hour, or until a perfectly clear solution was obtained. It was then neutralized to methyl orange with 0.5 *N* hydrochloric acid, 10 ml. of 0.5 *N* potassium iodide added and the solution diluted to 100 ml. (*i.e.*, by addition of 75 ml. of water). With some B-fractions, the sample did not dissolve to give the requisite crystal-clear solution in the alkali. In such cases, the mixture was neutralized and gently heated to assist solution.

Except for the presence of starchy substance, this solution was identical with that employed for the calibration. It was titrated potentiometrically in the same manner. After each addition of iodine, the solution was allowed to equilibrate for two minutes before determining the e. m. f.

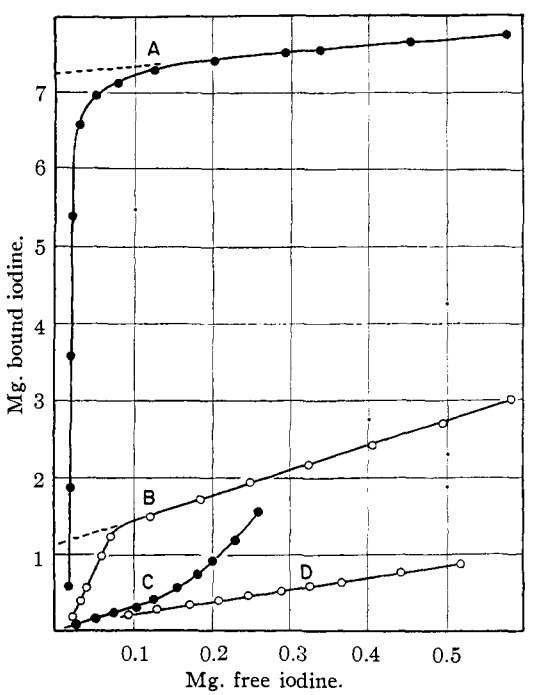

Fig. 1.—Method of plotting potentiometric evaluation of iodine affinity, showing titrations of (A) 38.4 mg. of corn A-fraction, (B) 182 mg. of corn B-fraction, (C) 193 mg. of tapioca B-fraction, and (D) 190 mg. of soluble "phytoglycogen" from Golden Bantam sweet corn. High quality waxy maize starch yields a curve identical with (C); the shape of the latter cannot be explained.

For each point of the titration, the free iodine in solution was calculated for the corresponding e. m. f. of the calibration chart, and this amount was deducted from the total iodine added at that point (*i.e.*, ml. of iodine added × 0.200 mg.) to give the bound iodine. Free iodine was then plotted against bound iodine as shown in Fig. 1. The upper linear portion of this curve was extrapolated to the zero axis (a mode of plotting suggested by Dr. Dexter French) and this amount of bound iodine taken as the iodine affinity of the sample. Results were converted to dry basis by separate moisture determination on the sample (*viz.*, four hours *in vacuo* at 70°). In the titration of A-fractions and normal starches, it is only necessary to obtain some 5 or 6 readings between 250–280 mv., omitting the lower ascending portion of the curve. With B-fractions or waxy starches, readings should be taken between 230–270 mv. Two or more determinations have been run on all samples here reported; average deviation from the mean is ±0.08% iodine affinity.

Intrinsic Viscosity.—The primary difficulty in evaluation of starch viscosity is the choice of solvent. The viscosity of solutions in such basic media as anhydrous ethylenediamine or aqueous potassium hydroxide shows a progressive decrease over a period of days, probably due to slow oxidative degradation of the polysaccharide. This is particularly objectionable when ethylenediamine is employed as solvent for the B-fraction, since one to three days are required to effect a homogeneous solution. Use of chloral hydrate or 30% ammonium thiocyanate solution avoids this drift in viscosity, but these agents do not dissolve the A-fraction, particularly when retrograded. One normal potassium hydroxide solution was finally selected because of its ease of preparation and standardization. Also, since its solubilizing action is much more rapid than ethylenediamine, degradation may be kept to a minimum. The intrinsic viscosity can be determined within two hours; hence no precautions are exercised to exclude oxygen or carbon dioxide.

The A-fraction (either retrograded or non-retrograded) dissolves readily and completely in 1 *N* potassium hydroxide solution. The B-fraction does not dissolve completely in either 1 or 5 *N* alkali, seemingly due to persistent aggregation effects.[3] However, the following procedure overcomes aggregation and is recommended for either fraction. An amount of the fraction equivalent to 2.00 g. on dry basis was quantitatively sifted into 200–300 ml. of cold water in a 600-ml. beaker, stirring vigorously with a glass propeller-type agitator to avoid formation of lumps. The suspension was heated in a boiling water-bath for thirty minutes, then allowed to cool to room temperature, stirring being continued throughout the heating and cooling periods. To prevent formation of insoluble skins by evaporation, the beaker was covered with a perforated watchglass during the heating and cooling operations. When cold, 100 ml. of 5 *N* potassium hydroxide solution was added by pipet and stirring continued for ten to fifteen minutes, usually producing a perfectly clear solution, entirely devoid of gel particles. The alkaline solution was rinsed into a 500-ml. volumetric flask and diluted to volume with distilled water, to give an 0.4% solution of the starch fraction in 1 *N* potassium hydroxide. This was filtered through a loose plug of Pyrex glass wool in a funnel stem, to remove any trace of fiber or insoluble material which might impede capillary flow. Accurate dilutions were made to some five or six concentrations between 0.1–0.4%, using 1 *N* potassium hydroxide solution for dilution. Viscosity was determined at each concentration with an Ostwald-Cannon-Fenske No. 100 pipet, maintained in a thermostated water-bath at 35.0°. Flow time at each concentration was determined to a precision of ±0.1 second or better (average deviation from mean), filling the viscometer at least twice and running at least three flow times for each filling. Specific viscosity was determined with reference to 1 *N* potassium hydroxide, and intrinsic viscosity was evaluated by plotting the function (η_{sp}/concn.) against concentration and extrapolating to zero concentration. Both the 1 and 5 *N* potassium hydroxide solutions must be adjusted within 1% of the

stipulated values (*i.e.*, 0.99–1.01, 4.95–5.05 *N*), since the flow time of the solvent varies markedly with concentration. Duplicate determinations on a large number of samples indicated a precision of ±0.02 in the intrinsic viscosity (average deviation from the mean).

This method overcomes any aggregation or retrogradation of the starch substance. For example, non-retrograded wheat A-fraction had an intrinsic viscosity of 1.54 ± 0.03. When this material was dissolved in boiling water at 0.2% concentration and retrograded by prolonged refrigeration, the intrinsic viscosity of the product was 1.50. In another test, corn B-fraction was precipitated from aqueous solution by three different methods: (a) by gross addition of methanol, (b) by slow addition to excess methanol, and (c) by addition of 10% methanol followed by freezing, thawing, filtering and spontaneous air-drying.[3] These three products had radically different solubility behavior, both in water and in 1 *N* potassium hydroxide, yet their intrinsic viscosities were 1.32, 1.28 and 1.30, respectively. However, the method has not been reliable for evaluating the viscosity of unmodified granular starches, apparently due to persistent granule structure which is not completely disaggregated either by pregelatinization in boiling water or by treatment with 1 or 5 *N* alkali.

The slope of the viscosity curve (η_{sp}/concn., against concentration) was determined for each sample, but this value appeared to have no significance. In general, the higher the viscosity, the greater was the slope.

The use of intrinsic viscosity does not entirely eliminate the energy characteristics of the viscometer. For example, the intrinsic viscosity of a commercial 75-fluidity corn starch was 0.53 with a No. 50 Cannon–Fenske pipet, 0.48 with a No. 100 pipet, and 0.33 with a No. 200 pipet. The No. 100 pipets employed in these studies gave flow times of approximately forty-five seconds for distilled water. Identical intrinsic viscosities were obtained with a regular Ostwald pipet of similar flow time.

Retrogradation Time.—It was of interest to compare the relative retrogradation tendencies of various linear starch substances, particularly to explain the low retrogradation of tapioca A-fraction. Retrogradation has been entirely a qualitative characteristic which is difficult to translate into quantitative terms. As an approximation, the retrogradation tendency is here expressed as the time required for development of initial turbidity in a 1% solution of the linear substance. Admittedly, the criterion and mode of testing are not good. It is often difficult to estimate the point at which turbidity first appears, particularly with linear substances which require more than fifteen to twenty hours for retrogradation. While flocculation normally occurs soon after the initial appearance of turbidity, a number of samples showed initial turbidity followed by a long period during which there was no further change. As a further criticism of the data, it was not feasible to run these tests at constant temperature, despite the known influence of temperature on retrogradation.

One hundred mg. (on dry basis) of the linear substance was dissolved in 2.0 ml. of 1 *N* potassium hydroxide solution. The mixture was stirred occasionally for one to two hours to effect a perfectly clear solution, 5.5 ml. of water added and the solution neutralized to phenolphthalein with 1 *N* hydrochloric acid. Since *p*H has a marked influence on retrogradation, 0.5 ml. of *p*H 6.3 phosphate buffer solution was added (16.4% with respect to anhydrous KH_2PO_4 and 3.6% to anhydrous K_2HPO_4). The solution was transferred to a stoppered 5-inch test-tube which was placed in a device designed to rotate some fifteen tubes end-over-end at a rate of about 20 r. p. m. Incidence of the characteristic blue-white haze as viewed against a black background was taken as the time of retrogradation. In some cases, shreds of insoluble material were formed during the neutralization; however, this did not significantly influence the time of retrogradation. Two to six tests were run on each sample here reported; precision was of the order of ±15%.

Reducing Value.—A variety of oxidizing agents and different conditions of oxidation have been investigated for aldehydic end-group assay of the A-fraction. Hypoiodite methods (variations of the Harris[5], Kline–Acree,[6] and Willstätter–Schudel techniques) were completely useless, since a large and indeterminable amount of iodine was consumed in oxidation of hydroxyl groups along the starch chain. This over-oxidation increased progressively (a) with increasing *p*H levels from 8.7 to 11.9, (b) with increasing time and temperature of reaction, and (c) with increasing concentration of iodine. No method could be devised to limit the oxidation to terminal aldehyde (*e.g.*, by use of borate buffer) or to correct for over-oxidation (by plotting iodine consumption against reaction time and extrapolating to zero time). The same criticisms applied to oxidation with bromine–potassium bromide mixtures at buffered *p*H levels between 4.5–7.0. While ferricyanide,[7] alkaline copper[8] and alkaline 3,5-dinitrosalicylate reagents appeared somewhat more selective toward terminal aldehyde, the reducing values by these methods were influenced to a major degree by concentration of oxidizing agent, the *p*H of the medium, and the time and temperature of the reaction. As a further complication, these last three oxidizing agents do not yield a stoichiometric relationship between glucose and maltose; hence, any extension to the higher polysaccharides is entirely arbitrary. It is therefore concluded that absolute evaluation of terminal aldehyde is not possible with any of the above reagents.

However, some relative index of reducing value was required for comparison of various linear subfractions. While alkali numbers have been determined on most of the samples here reported, this value is primarily a test for hydrolytic degradation, not a relative index of molecular weight. The colorimetric 3,5-dinitrosalicylate method of K. H. Meyer and his associates[9,10] was finally employed because of its simplicity and because it gave more reproducible results than the copper, ferricyanide or hypoiodite method. Furthermore, the intensity of color produced with either maltose or A-fraction followed Beer's law; the reducing value was therefore independent of the size of sample. Meyer's technique was modified in several respects. When the dinitrosalicylate reducing value was plotted against time of digestion at 65°, it was found that the initial primary oxidation was not always complete in thirty minutes; oxidations were therefore conducted for one hour to bring the reducing value into the more uniform over-oxidation range. An amount of the linear starch substance equivalent to 2–4 mg. of maltose was transferred to a clean dry 6 × 0.75 inch test-tube. Fifteen ml. of alkaline dinitrosalicylate reagent (666 mg. of twice-recrystallized 3,5-dinitrosalicylic acid dissolved in 200 ml. of 1 *N* potassium hydroxide) was added by pipet, and the sample dispersed by thorough mixing with a stirring rod. The mixture was allowed to stand with occasional stirring for an hour, or until the sample completely dissolved. The solution was then heated for one hour in a thermostated bath at 65°, cooled, quantitatively rinsed into a 50-ml. volumetric flask and diluted to mark. Blanks were simultaneously run on 15-ml. portions of the dinitrosalicylate reagent. Light transmission at 5000 Å. (predetermined as the point of maximum color development) was compared against the blanks, using 1-cm. cuvettes in a Beckman quartz spectrophotometer. In order to standardize the method, 1–5-mg. samples of maltose (analyzed by the Kline–Acree technique) were digested in similar fashion and transmission at 5000 Å. determined against comparable blanks. Percentage transmission was plotted against mg. of maltose hydrate to give a calibration curve.

(5) Martin, Smith, Whistler and Harris, *J. Research Nat. Bur. Standards*, **27**, 449 (1941).

(6) Kline and Acree, *Bur. Standards J. Research*, **5**, 1063 (1930).

(7) Gore and Steele, *Ind. Eng. Chem., Anal. Ed.*, **7**, 324 (1935); Farley and Hixon, *ibid.*, **13**, 616 (1941).

(8) Richardson, Higginbotham and Farrow, *J. Textile Inst.*, **27**, 131 (1936).

(9) Meyer, Noelting and Bernfeld, *Helv. Chim. Acta*, **31**, 10 (1948).

(10) Noelting and Bernfeld, *ibid.*, **31**, 286 (1948).

TABLE I

PROPERTIES OF THE PENTASOL-SEPARATED FRACTIONS ISOLATED FROM AUTOCLAVED STARCH SOLUTIONS; A-FRACTIONS RECRYSTALLIZED TO CONSTANT IODINE AFFINITY WITH *n*-BUTYL ALCOHOL

Parent starch		A-Fraction					B-Fraction			
Source	% Iodine affinity	Batch[a]	% Iodine affinity	[η]	Alkali number	D. P.[b]	Batch[a]	% Iodine affinity	[η]	Alkali number
Corn	5.30[c]	C-71/73-A	19.05	..	..		C-1/9-B	..	1.35	...
		C-85/89-A	18.90	1.19	20.2		C-10/12-B	..	1.30	4.6
		C-107/111-A	19.07	1.23	20.4	800	C-81/89-B	0.6	1.17	5.0
		C-112/134-A	...	..	20.7		C-90-B	..	1.22	...
		C-141-A[d]	...	1.45	18.7		C-107-B	.7	1.21	...
		C-146-A	19.35	1.13	23.5		C-109-B	.9	1.24	4.3
							C-137-B	..	..	4.1
							C-141-B[d]	..	1.35	4.6
Wheat	5.21	Wh-1/2-A	19.90	1.54	20.6	860	Wh-1-B	.56	1.14	4.6
							Wh-2-B	.44	1.22	4.3
Sago	5.10	S-1/2-A	18.52	1.13	17.4	740	S-1-B	.2	0.82	5.1
							S-2-B	.2	0.80	5.5
Easter lily	6.5[e]	L-3-A	20.03	1.06	19.4	620	L-3-B	.35	1.26	4.0
Potato	4.13	P-3/4-A	19.84	1.95	10.3	930	P-3/4-B	.4	1.45	6.4
(Maine)		P-5/6-A	19.96	1.75	12.1					
Potato	..	.....	...	..	..		P-1-B	.4	1.53	4.9
(German)							P-2-B	.4	1.50	5.2
Tapioca	3.27	T-3/4-A	18.55	2.25	12.8	1300	T-3-B	.0	1.27	3.8
(Dominican)							T-4-B	.0	1.23	3.7
Tapioca (Java)	3.30	T-1/2-A	18.6							

[a] Batch designations are given to facilitate cross reference and to identify samples investigated by other laboratories. Thus C-71/73-A indicates a composite sample of the corn A-fractions from Runs 71 to 73, inclusive. [b] Degree of polymerization, as determined from osmotic pressure by Potter and Hassid.[4] [c] This represents an average value for commercial dent corn starch. [d] Corn starch was solubilized by pregelatinization in liquid ammonia according to the method of Hodge, Montgomery and Hilbert.[13] Product was then dissolved in hot water, fractionated with Pentasol and the A-fraction thrice recrystallized with *n*-butyl alcohol. [e] High iodine affinity is not necessarily a characteristic of Easter lily bulb starch. Defatted starches from Croft, Ace, Estate and Creole varieties of Easter lily bulbs (from Vaughn Seed Co.) analyzed 5.16, 5.88, 5.43 and 5.36% iodine affinity, respectively.

By use of the latter, color development in the starch sample was calculated in terms of mg. of maltose hydrate per gram of starch substance. Two or more determinations were run on each sample; average deviation from the mean was ±0.04 mg. of maltose hydrate. It should be stressed that these values cannot be translated into molecular weight. Much higher reducing values are obtained by digesting for five minutes at 100°. The use of Rochelle salt[10] in the digestion mixture merely depresses the reducing value without decreasing the rate of over-oxidation.

Discussion

There has been a tendency to consider that hypothetically "pure" linear material has an iodine-binding capacity of 20.0%. This value is frequently employed for calculation of the percentage of linear material in various starch preparations. The present work shows that no such criterion is possible, since the iodine affinity depends on the source of the A-fraction and on the methods of fractionation and recrystallization. The A-fractions listed in Table I were all recrystallized to constant maximum iodine affinity by identical methods of precipitation with *n*-butyl alcohol. They may surely be considered as "pure" substances in the sense that the B-fraction has been completely removed, yet their iodine affinities range from 18.5 to 20.0%. In several unrecorded instances, corn A-fraction was recrystallized by cooling only to 35–40° and centrifuged without refrigeration. The resulting products had iodine affinities in the range of 20.5–20.8%, apparently due to subfractionation and loss of linear material of somewhat lower iodine affinity. When Pentasol was employed as recrystallizing agent (Table II), the iodine affinities were consistently and substantially lower. While one or two recrystallizations with *n*-butyl alcohol sufficed to give a constant maximum iodine affinity, successive recrystallizations with Pentasol gave a very slow but progressive increase in iodine affinity, with no evidence of a constant maximum even after seven recrystallizations of corn A-fraction. Similarly, the A-fractions from thin-boiling corn starches have iodine affinities lower than that of unmodified corn A-fraction (Table III). As will be shown more definitely by subfractionation studies, the total A-fraction represents a graded spectrum of molecular types, certainly differing with respect to chain length and perhaps even containing irregularities of linkage. Since these factors must necessarily influence the iodine affinity, no standard linear material can possibly be established which will apply equally to all A-fractions, regardless of source, hydrolytic level or manner of purification. Hence, it is strongly recommended that the linear characteristic of starches be expressed merely in terms of iodine affinity, with no reference to percentage of linear substance.

TABLE II

PROPERTIES OF THE FRACTIONS ISOLATED FROM BOILED STARCH SOLUTIONS BY PENTASOL SEPARATION; A-FRACTIONS RECRYSTALLIZED WITH PENTASOL

	A-Fraction					B-Fraction			
Starch	Batch	No. of recrystns.	% Iodine affinity	[η]	Alkali number	Batch	% Iodine affinity	[η]	Alkali number
Corn	C-148/150-A	3	16.06	1.35	..	C-148-B	0.31	1.35	...
		5	17.86	1.27	..	C-149-B	.44	1.37	6.2
		7	18.10	1.26	21.6				
Brazilian tapioca	T-7/9-A	2	17.15	2.75	..	T-7-B	.14	1.21	3.5
		4	17.75	2.22	12.8	T-8-B	.15	1.25	3.9
						T-9-B	.11	1.35	3.4
Idaho potato	P-7/9-A	2	17.03	2.33	..	P-7-B	.17	1.58	3.7
		4	19.14	2.31	13.3	P-8-B	.14	1.49	3.7
						P-9-B	.15	1.49	4.0

TABLE III

PROPERTIES OF THE PENTASOL-SEPARATED FRACTIONS FROM ACID-MODIFIED CORN STARCHES; A-FRACTIONS TWICE RECRYSTALLIZED WITH PENTASOL

Parent starch				A-Fraction						B-Fraction		
Hours conversion	% Iodine affinity	[η]	Alkali number	Yield, %[a]	% Iodine affinity	[η]	Alkali number	Reducing value[b]	Retrogradn. time, hr.	% Iodine affinity	[η]	Alkali number
					0.075 *N* HCl at 50°							
5	4.81	1.06	13.0	28	15.38	1.01	23.6	5.95	5	0.37	0.85	6.3
16	4.77	0.66	18.0	22	14.25	0.71	27.7	7.41	4	.31	.56	10.9
26	4.75	.47	21.6	25	14.85	.59	32.7	10.45	2	.26	.42	15.1
40	4.69	.37	27.2	30	15.10	.57	37.6	10.70	1	.23	.34	19.4
					0.3 *N* HCl at 50°							
2	4.68	.89	13.9	29	15.29	.91				.39	.75	
7	4.74	.44	22.6	30	14.85	.54				.25	.40	
16	4.40	.24	34.8	27	15.55	.37				.19	.23	

[a] Yields of A-fraction are based on once-recrystallized product. [b] Reducing value toward dinitrosalicylate, expressed as mg. of maltose hydrate per gram of starch substance (dry basis).

The iodine affinities of the B-fractions likewise show a variation, from 0.0% for Dominican tapioca to 0.6–0.7% for corn starch. It has not been positively established whether this represents a residual trace of unremoved linear material, or whether certain B-fractions possess a small but definite iodine-binding capacity. The latter explanation seems more plausible. Otherwise, it would be difficult to explain why Pentasol consistently gives B-fractions of very low or negligible iodine affinity from potato and tapioca starches, while the B-fractions from the cereal starches are substantially higher in iodine-binding capacity. It has been claimed that the iodine affinity of the B-fraction can be reduced to zero by treatment with cotton. However, this is apparently due to the introduction of fatty substances from the cotton, thereby concealing the presence of residual iodine-binding material. If alcohol-defatted cotton is employed, there is no reduction of residual iodine affinity. In accord with theories suggested by Kerr[11] and more recently elaborated by K. H. Meyer,[12] a portion of the total B-fraction may have a few outer branches of sufficient unobstructed length to bind a small amount of iodine, though too short or too infrequent to precipitate with Pentasol.

Intrinsic viscosities also reveal substantial differences between the various A-fractions and likewise between the various B-fractions. In general, the fractions isolated from boiled starch pastes have somewhat higher intrinsic viscosities than those from autoclaved pastes. Despite careful buffering, autoclaving probably causes a slight degradation of the starch substance. For this reason, prolonged boiling in the presence of excess Pentasol is now preferred as a means of solubilizing starch prior to fractionation. To avoid autoclaving, use of the Waring Blendor has been suggested to disintegrate swollen starch pastes for fractionation. However, the B-fraction undergoes degradation under the violent shearing action of the Blendor, seemingly by a "mechanical hydrolysis" of the molecule. For example, a hot 3% corn starch paste (buffered at *p*H 6.3) was disintegrated in the Blendor for fifteen minutes, then fractionated with Pentasol and the A-fraction thrice recrystallized with *n*-butyl alcohol. Intrinsic viscosities of the A and B fractions were 1.39 and 1.07, respectively. The A-fraction was not degraded, perhaps because of its smaller molecular size together with the possibility that it can align itself along the flow lines and thus escape mechanical rupture.

(11) Kerr, "Chemistry and Industry of Starch," Academic Press, New York, N. Y., 1944, p. 154.

(12) Meyer, Bernfeld, Rathgeb and Gurtler, *Helv. Chim. Acta*, **31**, 1536 (1948).

In another instance, a buffered 3% solution of corn B-fraction was agitated for one hour in the Blendor, then precipitated with methanol and dried. The intrinsic viscosity was lowered from 1.17 to 0.95 by this treatment. Pregelatinization in liquid ammonia (as recommended by Hodge, Montgomery and Hilbert[13]) provides an excellent but somewhat inconvenient method for dissolving granule structure without degradation (*cf.* Batch C-141 in Table I).

During acid-modification of granular corn starch (Table III), both fractions appear to be hydrolyzed at approximately the same rate, as indicated by increase in alkali number. In comparison with these laboratory-modified starches, the intrinsic viscosities of commercial thin-boiling corn starches (sulfuric acid modification) were as follows: 15-fluidity, 1.09; 40-fluidity, 0.90; 50-fluidity, 0.73; 60-fluidity, 0.62; 75-fluidity, 0.46; 90-fluidity, 0.38.[14] These values have had practical application in determining the hydrolytic level of various starch derivatives and of pregelatinized starches. Thus, when a 75-fluidity corn starch was gelatinized and dried on heated rolls, the product had an intrinsic viscosity of 0.45. Similarly, a granular starch ether of low degree of substitution prepared from 40-fluidity starch had an intrinsic viscosity of 0.92.

The intrinsic viscosity may have theoretical application in indicating the mechanism of enzyme action on the starch fractions. For example, the β-amylase limit dextrin from waxy maize starch had an intrinsic viscosity of 1.25 and an alkali number of 5.5 (values for the parent starch substance were 1.21 and 3.6, respectively). Since intrinsic viscosity is essentially a measure of molecular shape (*i. e.*, the "axial ratio" of length of molecule to diameter), the similar values for

TABLE IV

CHARACTERISTICS OF THE LINEAR SUBFRACTIONS FROM CORN, POTATO AND TAPIOCA STARCHES

Source	Sub-fraction	Yield, %	Iodine affinity, %	[η]	Alkali number	Reducing value[a]	Retrogradn. time, hr.
Corn	1-a	32	18.72	1.36	..	..	5
C-146-A[b]	1-b	63	18.53	0.94	24.3	6.11	4
	1-c	5	...	0.51	..	..	..
Same[b]	3-a	18	19.15	1.26	22.9	..	3
	3-b	25	19.08	1.28	20.7	4.57	6
	3-c	19	18.71	1.03	23.5	..	..
	3-d	38	17.86	0.82	28.0	5.99	2
Same	11-a	26	19.48	1.29	21.0	4.60	3
	11-b	41	19.69	1.19	22.3	5.06	5
	11-c	33	19.07	0.80	28.1	9.69	1
Corn	13-a	34	17.83	1.65	14.4	4.17	6
C-148/150-A	13-b	39	18.57	1.12	19.1	5.66	4
3 × recryst.	13-c	19	18.54	0.87	27.6	6.63	3
	13-d	8	11.53	0.82	23.4	..	..
Same	14-a	30	17.20	1.60	18.0	4.12	5
	14-b	25	17.88	1.26	21.2	3.75	4
	14-c	34	19.37	1.06	26.3	7.34	3
	14-d	11	13.30	1.02	20.7	7.37	2
Potato	2-a	46	20.70	1.78	14.5	3.38	8
P-5/6-A[b]	2-b	35	20.23	1.55	19.7	..	4
	2-c	19	17.60	1.12	18.4	..	4
Same[b]	4-a	19	20.22	2.18	14.1	2.77	18
	4-b	59	20.00	1.86	9.7	2.59	15
	4-c	21	18.31	1.25	14.9	4.28	7
Same[b]	5-a	21	20.12	2.19	10.7	..	13
	5-b	31	19.98	1.90	10.3	2.53	13
	5-c	33	...	..	..	..	..
	5-d	15	16.38	0.97	13.9	..	4
Same	6-a	67	20.54	1.88	9.9	2.52	15
	6-b	27	19.63	1.35	13.6	3.80	4
	6-c	6	18.45	..	..	...	2
Same	7-a	33	20.60	1.91	13.8	3.01	14
	7-b	42	20.64	1.78	13.8	2.86	6
	7-c	25	18.75	1.01	19.0	..	2
Same	8-a	60	20.48	2.01	9.3	2.54	18
	8-b	40	20.13	1.37	13.4	3.60	6
Same	9-a	49	20.49	2.05	9.2	2.64	22
	9-b	34	20.39	1.44	11.8	4.22	7
	9-c	17	18.29	1.23	18.6	..	4
Same	10-a	34	20.32	2.09	10.3	2.97	24
	10-b	14	20.39	1.60	13.7	..	..
	10-c	28	20.63	1.43	12.3	4.33	4
	10-d	23	19.07	1.30	18.1	..	..
Same	12-a	17	20.35	2.16	14.5	..	13
	12-b	39	20.66	1.83	10.0	2.79	11
	12-c	24	20.62	1.37	16.8	3.28	3
	12-d	21	20.11	1.34	17.0	3.71	6
Potato	17-a	18	19.73	2.63	11.4	2.32	50
P-7/9-A	17-b	17	19.77	2.03	13.4	2.77	15
2 × recryst.	17-c	14	19.98	1.64	15.5	3.51	8
	17-d	14	20.12	1.49	18.9	3.89	5
	17-e	14	19.86	1.45	18.2	4.02	6
	17-f	23	19.05	1.32	19.5	4.45	6
Tapioca	15-a	11	19.34	3.34	11.5	..	..
T-7/9-A	15-b	23	19.34	2.98	13.3	1.76	..
2 × recryst.	15-c	32	19.69	2.31	14.5	2.29	20
	15-d	20	19.90	1.96	13.9	2.66	10
	15-e	14	18.36	1.73	15.5	..	..
Same[c]	16-a	13	19.51	2.95	13.0	..	20
	16-b	33	19.26	2.21	15.0	2.35	15
	16-c	23	19.39	1.57	16.3	3.39	10
	16-d	22	19.33	1.32	18.9	4.02	7
	16-e	9	19.88	1.32	17.4	..	10

[a] Reducing value toward dinitrosalicylate, expressed as mg. of maltose hydrate per gram of starch substance (dry basis). [b] Runs 1–5 inclusive employed cyclohexanol as fractional precipitant. Due to its higher solubility in water, considerably larger quantities of this agent were employed than with octyl alcohol. Runs 6–17 inclusive were conducted with octyl alcohol. [c] Run 16 may have been slightly degraded during subfractionation.

(13) Hodge, Montgomery and Hilbert, *Cereal Chem.*, **25**, 19 (1948).

(14) Industrial fluidity values represent the ml. of 5% starch paste in 1% sodium hydroxide solution passed by a standard fluidity funnel in seventy seconds (Buel, 8th Intern. Congr. Pure Applied Chem., *Orig. Com.*, **13**, 63 (1912)). It will be noted that these values show good correlation with intrinsic viscosity.

waxy maize and its limit dextrin are in accord with the concept of a highly branched globular molecule; the limit dextrin would be smaller in size but similar in shape. In another case, corn A-fraction was treated with β-amylase[15] and samples withdrawn at several stages during the conversion. From intrinsic viscosities and alkali numbers of the residues, it appears that the enzyme attacks and destroys the shorter molecules first, leaving a residue of longer average chain length than the parent A-fraction:

Conversion, hr.	Degradation, %	Alkali no. of residue	[η] of residue
0	0	20.4	1.23
1.75	41	17.4	1.39
6.0	61	16.3	1.38

The subfractions obtained by partial precipitation with cyclohexanol or octyl alcohol show a progressive gradation in intrinsic viscosity, alkali number, dinitrosalicylate reducing value and retrogradation time (Table IV). The subfraction first precipitated has the highest intrinsic viscosity and the lowest terminal aldehyde content, apparently consisting of those molecules of longest chain length. When the cumulative percentage yield of the successive subfractions is plotted against intrinsic viscosity as shown in Fig. 2, a frequency distribution curve is obtained indicative of a continuous series of homologous linear chains. There is no evidence of two or more separate and sharply defined component substances. Intrinsic viscosities of the various

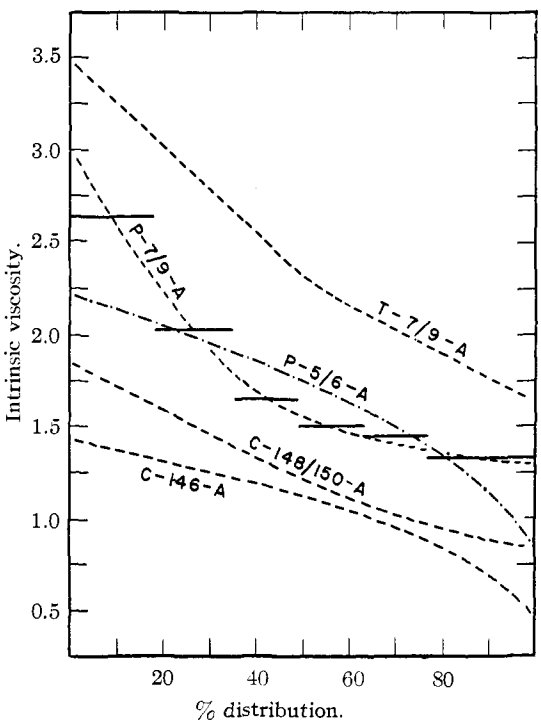

Fig. 2.—Frequency distribution of linear subfractions from various A-fractions plotted against their respective intrinsic viscosities. Method of plotting is shown for subfractionation No. 17 (twice-recrystallized potato A-fraction P-7/9-A); curves for other A-fractions are similarly derived.

(15) The enzyme used in these studies was furnished through the kindness of Miss Edna M. Montgomery, Northern Regional Research Laboratory, Peoria, Illinois.

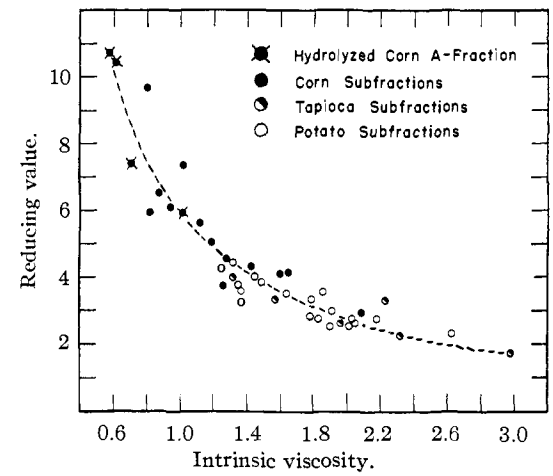

Fig. 3.—Relationship between intrinsic viscosity and dinitrosalicylate reducing value of various linear starch substances.

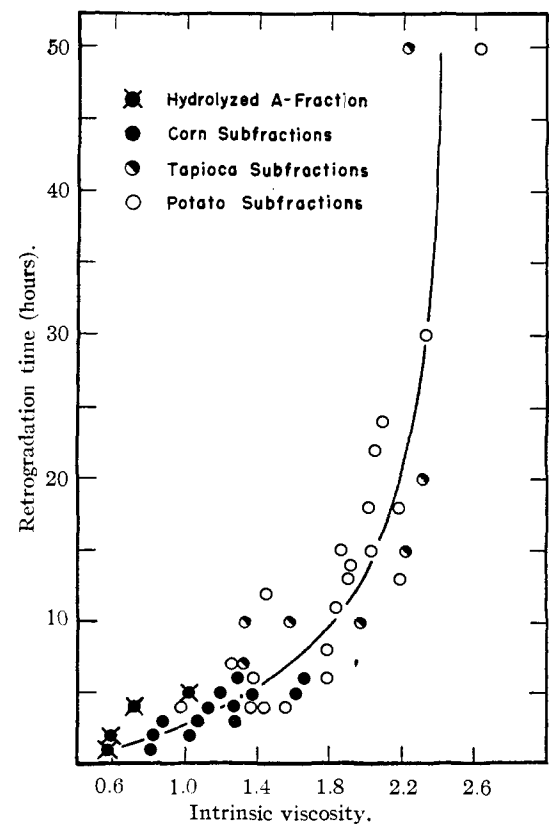

Fig. 4.—Relationship between intrinsic viscosity and retrogradation time of various linear starch substances.

subfractions show excellent correlation with dinitrosalicylate reducing value (Fig. 3) and with retrogradation time (Fig. 4), independent of the source of the subfraction. In addition, the intrinsic viscosities of the various A-fractions are arranged in the same order as their osmotic molecular weights, as determined by Potter and Hassid (Table I). Hence, the primary difference between tapioca, potato and corn A-fractions appears to be merely a matter of relative chain length. It does not seem probable that the A-fraction of one starch is strictly linear while that of another starch is slightly branched; such a situation would radically alter the relationship between intrinsic viscosity and reducing value. Since the shorter subfractions of potato A-fraction and the longer subfractions of corn A-fraction have the same intrinsic viscosity, dinitrosalicylate reducing value and retrogradation time, it must be assumed that they are molecularly identical. It has been claimed that tapioca A-fraction does not retrograde because its chain molecule is too long to permit ready orientation to an aggregated state.[16] Present evidence supports this theory, since the retrogradation tendency of the subfractions is inversely related to intrinsic viscosity. The theory is no longer tenable that the low-retrogradation tendency of tapioca starch is due to a slight branching peculiar to its A-fraction molecule.[16] Maximum retrogradation is observed with the hydrolyzed corn A-fractions down to an intrinsic viscosity of 0.57 (Table III). Somewhere below this level, a reversal must occur as the linear fragments become too short to retrograde.

Iodine affinities of the various fractions and subfractions are somewhat at variance with the foregoing concept of homologous series of linear chains. The following irregularities may be noted:

1. The B-fractions originally isolated from corn and potato starches by primary separation with *n*-butyl alcohol had iodine affinities of 1.5–1.7%,[2] as compared with present values of 0.0–0.6% by Pentasol precipitation. Since *n*-butyl alcohol gave only 22–23% yield of A-fraction from corn starch, while Pentasol gave 28–29%, it was previously assumed that Pentasol was a "more effective" fractionating agent.

2. In the present studies, maximum iodine affinity is rapidly attained by one or two recrystallizations with *n*-butyl alcohol, while repeated recrystallizations with Pentasol give a very slow increase in iodine affinity, without reaching the maximum value of the product recrystallized with *n*-butyl alcohol. Yet the lower iodine affinity of the Pentasol-recrystallized product cannot possibly be attributed to contamination by B-fraction.

3. There is no correlation between iodine affinity and intrinsic viscosity or reducing value of the various linear subfractions. Likewise, there are substantial differences between the iodine affinities of the acid-modified corn A-fractions and of the linear subfractions of comparable intrinsic viscosity.

4. In the subfractionation of Pentasol-recrystallized A-fractions, the composite iodine affinity of the subfractions is substantially higher than that of the parent A-fraction. There is less discrepancy with the A-fractions recrystallized by *n*-butyl alcohol. In all cases, the final subfractions have the lowest iodine affinity.

To explain these anomalies, it is suggested that corn, potato and tapioca starches contain a minor proportion of material intermediate between strictly linear and highly branched molecules.[17] These transition types may conceivably range from branched molecules with long exterior branches to predominantly linear molecules containing a relatively small number of branches. This intermediate material is apparently precipitated by Pentasol but not by *n*-butyl alcohol; on this basis, its presence in corn starch is estimated at 5–7% of the total starch substance. In collaborative studies, W. Z. Hassid is endeavoring to isolate and identify this material. From present concepts of the dual enzymatic synthesis of starch, it seems entirely reasonable that such an intermediate range of molecular types might be formed. Except for the somewhat questionable criterion of β-amylase conversion, it is not possible at this time to distinguish between strictly linear and slightly branched starch substances. To prepare fractions with minimum inclusion of such intermediate types, it would seem advisable to conduct the primary separation with Pentasol and to recrystallize the A-fraction with *n*-butyl alcohol.

Summary

As a preferred mode of fractionation, starch is gelatinized in a buffered Pentasol–water mixture, gently boiled under reflux for several hours, then cooled and refrigerated to precipitate the linear A-fraction. This technique avoids the slight hydrolytic degradation occasioned by autoclaving and likewise permits direct fractionation of non-defatted cereal starches.

Improved methods have been developed for characterizing the starch fractions in terms of (a) intrinsic viscosity in 1 *N* potassium hydroxide solution, (b) iodine affinity by potentiometric titration, (c) reducing value toward alkaline 3,5-dinitrosalicylate reagent, and (d) retrogradation tendency of the linear component. These methods have been employed to describe and differentiate the linear A-fractions and branched B-fractions from corn, wheat, sago, Easter lily, potato and tapioca starches. In addition, these criteria have had useful application in detecting minor hydrolytic changes in the starch substance,

(16) Kerr, *Paper Trade J.*, **115**, no. 22, 30 (1942).

(17) The existence of an intermediate fraction has been suggested by Kerr and Trubell, *Paper Trade J.*, **117**, no. 15, 25 (1943).

in evaluating various modified starches, and in elucidating the action of β-amylase on the starch fractions. A study of the reducing values of the A-fraction toward hypoiodite, bromine, alkaline copper, ferricyanide and alkaline dinitrosalicylate indicates that none of these reagents is specific for terminal aldehyde groups.

A technique has been devised for subfractionating the A-fraction by successive partial precipitations with *n*-octyl alcohol. The A-fraction appears to consist of a continuous series of homologous linear polymers, rather than a limited number of discrete components. The linear material of longest chain length (as indicated by intrinsic viscosity and reducing value) is precipitated first by octyl alcohol, followed successively by subfractions of progressively shorter chain length. The ease of retrogradation of a linear starch substance is inversely related to its chain length. Linear subfractions of equal intrinsic viscosity and reducing value have the same retrogradation tendency, irrespective of their source. Thus the lower retrogradation of tapioca and potato starches must be attributed to the longer chain length of their A-fractions and not to anomalous branching.

However, indirect evidence from iodine affinities suggests the presence of a material intermediate between the strictly linear and the highly branched fractions, possibly amounting to 5–7% of the total starch substance in the case of corn starch. This material is precipitated by Pentasol but not by *n*-butyl alcohol. To minimize contamination of the fractions by this intermediate material, it is recommended that Pentasol be used for the primary separation and *n*-butyl alcohol for recrystallization.

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[CONTRIBUTION FROM THE DIVISION OF PLANT NUTRITION AND FOOD TECHNOLOGY, COLLEGE OF AGRICULTURE, UNIVERSITY OF CALIFORNIA]

Starch. III. Structure of Apple Starch

BY A. L. POTTER, W. Z. HASSID AND M. A. JOSLYN

While all starches possess certain fundamental common structural features, it is now recognized that starches from different sources vary in the following respects: (1) proportion of the two constituents, amylose and amylopectin, (2) average length of the amylose chains, (3) average chain length of the amylopectin branches in the molecule, (4) molecular size of amylose and of amylopectin.

Practically all the available information regarding starch structure has been derived from work on cereal and tuber starches, chiefly corn and potato. It was therefore of interest to examine a fruit starch in order to ascertain whether or not its structure differs from that of the cereal and tuber starches previously studied.

The starch was isolated from apples and separated by Schoch's "Pentasol" precipitation method[1] into amylose and amylopectin. The yield of amylose was 24.8% of the total starch. Analysis of the whole starch by Schoch's[2] modification of Bates and collaborators'[3] potentiometric iodine titration method showed an amylose content of 26.5%. The intrinsic viscosity of the amylose was 0.99 and that of the amylopectin was 0.96.

Treatment with crystalline β-amylase hydrolyzed the amylose to the extent of 90% maltose. With amylopectin the hydrolysis ceased when 63.5% was degraded to maltose.

Upon acetylation of the two fractions with acetic anhydride at room temperature and the determination of their osmotic pressures, a number-average molecular weight of 160,000 (560 glucose residues) was obtained for the acetylated amylose and 1,200,000 (4200 glucose residues) for the acetylated amylopectin.

In a previous study[4] the number-average molecular weights of six acetylated amylose components from starches of six different plant sources ranged from 180,000 to 370,000. The acetylated amylopectins from the same sources ranged from 2,000,000 to 10,000,000.

The same osmotic pressure–concentration relationship was used as previously reported for the other acetylated amyloses and amylopectins.[4] Using the values $n = 1.39$ and 2.25 for acetylated apple amylose and acetylated amylopectin, respectively, and plotting π/C against C^n, straight lines were obtained. Employing this method, the intercept of the coördinate could be determined with greater accuracy, thus resulting in more reliable molecular weight determinations.

End-group determination by the periodate oxidation method showed an average of 24 glucose residues per end-group for the amylopectin. For the amylose an average chain-length of 530 glucose residues was obtained. The latter value is in fair agreement with 560 obtained by osmotic pressure measurements, indicating that, like po-

(1) T. J. Schoch, "Advances in Carbohydrate Chemistry," edited by Pigman and Wolfrom, Academic Press, Inc., New York, N. Y., Vol. I, 1945, pp. 258–261.

(2) T. J. Schoch, THIS JOURNAL, **71**, 4066 (1949).

(3) F. L. Bates, D. French and R. E. Rundle, *ibid.*, **65**, 142 (1943).

(4) A. L. Potter and W. Z. Hassid, *ibid.*, **70**, 3774 (1948).