Treatment of *cis*-Orthoester with Hydrogen Chloride in Acetic Acid.—Dry hydrogen chloride was absorbed in a solution of 4.3 g. (0.1 mole) lithium chloride and 15.0 ml. of 90–95% acetic anhydride in 100 ml. of acetic acid (m. p. 16.2°) until the gain in weight was 21 g. (0.58 mole). The solution was left for twelve hours and then 16.0 g. (0.086 mole) of orthoester was added. The container was sealed and the mixture was allowed to remain at room temperature for fourteen days. After neutralization of the reaction mixture with bicarbonate and extraction of the reaction products with ether, the ether extract was dried over potassium carbonate. Distillation through the Weston-type column yielded 7.0 g. of nearly pure 1-acetoxy-2-chlorocyclohexane, b. p. 98.5–100.5° (12 mm.), and 3.0 g. of nearly pure 1,2-diacetoxycyclohexane, b. p. 118–120° (12 mm.). An intermediate fraction of 3.5 g., b. p. 100.5–118° (12 mm.), $n^{25}\mathrm{D}$ 1.4592, was obtained. Counting the hold-up the total yield was 92% approximately; *trans*-acetoxy chloride, 63%; *cis*-diacetate, 29%.

Treatment of some of the acetoxy chloride fraction with aqueous–alcoholic sodium hydroxide and sublimation of the resulting glycol gave rise to a 36% yield of *trans*-glycol, m. p. 98–100°. This is essentially the same result obtained with authentic *trans*-1-acetoxy-2-chlorocyclohexane. Analogous treatment of *cis*-1-acetoxy-2-chlorocyclohexane yields no glycol.

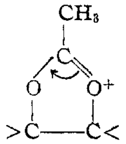
Saponification of the diacetate fraction yielded predominantly *cis*-glycol, m. p. 85–91°; recryst., m. p. 93–95°.

A control experiment showed that, under the conditions used in treating the orthoester with the hydrogen chloride–acetic acid solution, *cis*-diacetate is less than 5% converted to acetoxy chloride.

Summary

The *cis*- and *trans*-cyclohexene ethyl orthoacetates have been prepared and the behavior of the *cis*-isomer toward various conditions has been noted.

The *cis*-orthoester has been isolated in good yield from the solvolysis of *trans*-2-acetoxycyclohexyl *p*-toluenesulfonate in extremely dry alcohol. It yields almost pure *cis*-2-acetoxycyclohexanol on short treatment with water in alcohol. These experiments afford proof of the previously postulated occurrence of the orthoester as an intermediate in the conversion of the acetoxycyclohexyl tosylate to monoacetate. Also, they afford support for the idea that the ion I is an intermediate in such solvolyses.



I

It is believed that certain acid-catalyzed reactions of the *cis*-orthoester proceed largely by way of the ion I. The nature and configuration of the products of several reactions of the *cis*-orthoester in acetic acid as a solvent are those expected on this basis. Water gives rise to nearly pure *cis*-2-acetoxycyclohexanol; *p*-toluenesulfonic acid and acetic anhydride to *cis*-1,2-diacetoxycyclohexane; hydrogen chloride, lithium chloride and acetic anhydride to a mixture of *cis*-1,2-diacetoxycyclohexane and *trans*-1-acetoxy-2-chlorocyclohexane.

A surprising reaction of the *cis*-orthoester takes place with acetic anhydride at 130° to produce *trans*-1,2-diacetoxycyclohexane and a very high-boiling derivative of *trans*-1,2-cyclohexanediol. At room temperature a very high boiling derivative of *cis*-1,2-cyclohexanediol is produced.

LOS ANGELES, CALIF. RECEIVED NOVEMBER 27, 1942

[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

Solution Viscosities of the Amylose Components of Starch[1]

BY JOSEPH F. FOSTER AND R. M. HIXON

The development of a potentiometric iodine titration procedure has demonstrated the presence of an unbranched component in starch which differs markedly in character and in quality in the starches from various sources.[2] This component will be called "amylose" in conformity with the nomenclature suggested by Meyer.[3] The titration method indicates that the amylose components from various starches form the iodine complex at characteristic iodine activities which are postulated to be a function of the molecular

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(1) Journal Paper No. J-1076 of the Iowa Agricultural Experiment Station, Ames, Iowa; Project No. 817. Supported in part by a grant from the Corn Industries Research Foundation.

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

(3) Excellent summaries of the present information regarding the heterogeneity of starch are given by K. H. Meyer in the following texts: "Natural and Synthetic High Polymers," Interscience Publishers, Inc., New York, N. Y., 1942, pp. 391–403; "Advances in Colloid Science," Interscience Publishers, Inc., New York, N. Y., 1942, pp. 143–165.

size, longer free chains having a greater tendency to bind iodine.

In order to verify this explanation of the characteristic potentials an investigation of the molecular sizes of the amyloses through their solution viscosities has been undertaken. Although there has been much criticism of the Staudinger equation relating molecular weight to specific viscosity,[4] it has recently been shown that in the case of the normal paraffins[5] the Staudinger function, η_{sp}/C, varies linearly with the molecular weight, but is not proportional to it. Fordyce and Hibbert[6] have proposed the equation

$$\eta_{sp}/C = K'_m M + \beta \qquad \text{(I)}$$

where β is a small additive constant.

Huggins[7] has recently given a theoretical derivation of equation I assuming random kinking of the molecules, thereby eliminating one serious theoretical objection to the Staudinger equation, namely, the assumption of rigid molecules which would not be expected in solution.

A further experimental verification for this method of determining molecular weights of linear polymers has been given by Baker, Fuller and Heiss,[8] who found that in the case of a series of synthetic linear polyesters the equation

$$[\ln \eta_R/C]_{C=0} = K_w M_w + B \qquad \text{(II)}$$

holds down to molecular weights as low as 5000.

The solvent chosen for this study, anhydrous ethylenediamine, gives clear solutions, which are stable so long as protected from the atmosphere. The anhydrous solvent was chosen rather than the hydrate, recommended by Meyer,[3] in order to decrease the complexity of the systems involved.

Experimental

Amyloses.—The butanol precipitated amyloses from corn, potato, tapioca and lily bulb starch, the "crystalline amylose" from corn and the synthetic starch used in this study are identical with the materials used in the development of the potentiometric titration.[2] Corn amylodextrin was fractionated by addition of butanol and methanol to the aqueous solution, fraction three used herein precipitating from a solution containing about 15% of methanol and saturated with butanol. Amylose was prepared from corn starch by hot water extraction of the granular starch according to the procedure of Meyer.[3] The yield was only 2% of the starch used.

Solvent.—Eastman Kodak Co. ethylenediamine (95–100%) was dried by refluxing over sodium until practically no reaction with the latter could be observed in the cold. The solvent was then distilled from sodium in an all-glass apparatus, completely enclosed and protected from the atmosphere by means of a calcium chloride tube. The dehydration was carried out in quantities just large enough to prepare a series of solutions (usually four) with enough left over for the determination of the time of flow of the solvent (t_0). The solvent was stored in a desiccator over phosphorus pentoxide.

Protection of both solvent and solutions from the atmosphere is essential at all times due to the extreme affinity of anhydrous ethylenediamine for both water and carbon dioxide. In spite of the above precautions, the viscosity of the pure solvent varied appreciably from sample to sample, hence the necessity for running the t_0 each time.

Preparation of Solutions.—The carbohydrate samples were dried for twelve to eighteen hours *in vacuo* at 60° and weighed directly into glass-stoppered volumetric flasks, usually of 10-ml. capacity. The solvent was added to volume, working in a transfer box in an atmosphere of dry nitrogen. After stoppering, the flasks were placed in the phosphorus pentoxide desiccator and permitted to stand until solution was complete; the potato amylose required several days, the others somewhat less time.

Filtration.—The solutions were filtered by gentle suction through a sintered-glass filter immediately before determining the viscosity. This filtration was at first carried out in the nitrogen chamber but later this was found unnecessary if the filter was protected from the atmosphere with a calcium chloride–soda-lime tube. By using a large 4 × 24-cm. test-tube as filtering flask the filtrate could be caught directly in the pipet.

Determination of Viscosity.—All measurements were made at atmospheric pressure in a standard Ostwald viscosity pipet having the approximate capillary dimensions 0.05 × 9 cm. and total capacity 5 ml. The time of flow for pure ethylenediamine varied from about 2.960 to 3.000 minutes for different samples. Measurements were made in a water-bath at approximately 30°, maintained to ±0.04° by means of a mercury thermoregulator. The usual precautions were observed. In addition, the pipet was protected from the atmosphere on both sides by means of tubes containing calcium chloride and soda-lime. (These tubes did not affect the time of flow appreciably.) The time of flow was measured by means of a stop-watch graduated in hundredths of a minute, and the values were estimated to thousandths. In general, results were discarded unless at least three checks within 0.1% were obtained, and in many cases the checks were better.

Discussion

The experimental results are presented in Table I and in Figs. 1, 2 and 3.

The concentration dependence of both the Staudinger function and the logarithmic function, Figs. 1 and 2, show a striking similarity to the re-

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(4) For a review see H. Mark, "Physical Chemistry of High-Polymeric Systems," Interscience Publishers, Inc., New York, N. Y., 1940. pp. 258–295.

(5) Meyer and Van der Wyk, *Helv. Chim. Acta*, **18**, 1067 (1935).

(6) Fordyce and Hibbert, THIS JOURNAL, **61**, 1912 (1939).

(7) Huggins, *J. Phys. Chem.*, **42**, 911 (1938); **43**, 439 (1939). The procedure is that used earlier by Kuhn. *Z. physik. Chem.*, **A161**, 1 (1932); *Kolloid-Z.*, **68**, 2 (1934).

(8) Baker, Fuller and Heiss, THIS JOURNAL, **63**, 3316 (1941).

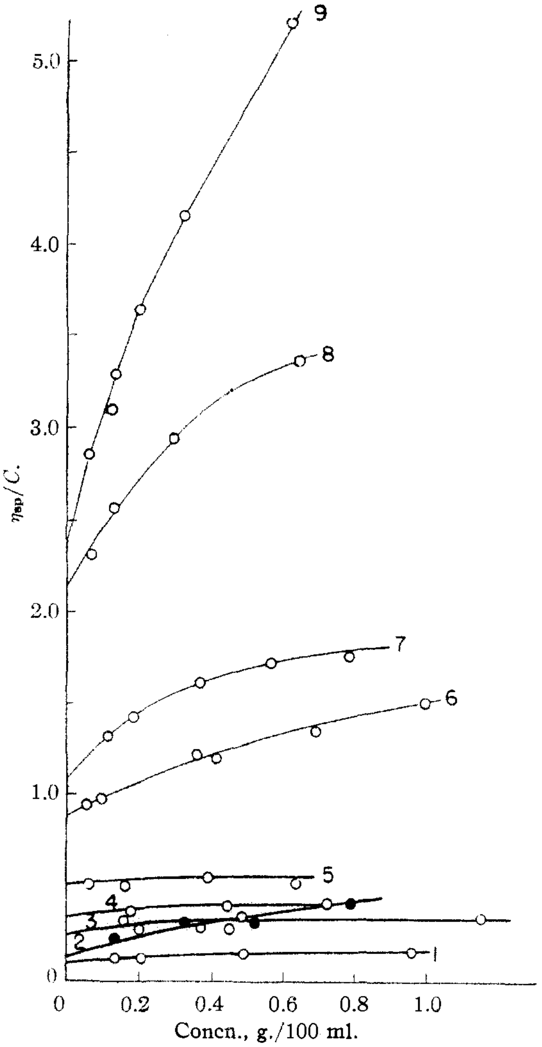

Fig. 1.—Concentration dependence of the Staudinger function, η_{sp}/C. The curves are numbered as follows: 1, amylodextrin fraction; 2, synthetic starch: 3, corn amylose by Meyer's method; 4, mixture of 62.5% crystalline amylose and 37.5% amylodextrin fraction; 5, corn "crystalline amylose" by Kerr's method; 6, corn butanol precipitate; 7, lily butanol precipitate; 8, tapioca butanol precipitate; and 9, potato butanol precipitate. The solid circles are for synthetic starch, curve 2.

sults of Baker, Fuller and Heiss.[8] Figure 1 shows an approximately linear relationship up to a concentration of about 0.2 g. per 100 ml., followed by a negative deviation from linearity. The logarithmic curve, Fig. 2, shows the characteristic maximum at a concentration of about 0.2 to 0.3.

TABLE I

SUMMARY OF RESULTS

Fraction	Concn.	η_R	ln η_R/C	η_{sp}/C
Potato amylose (butanol method)	0.0576	1.164	2.63	2.85
	.110	1.341	2.67	3.10
	.134	1.441	2.73	3.29
	.200	1.728	2.74	3.64
	.326	2.353	2.63	4.15
	.626	4.254	2.31	5.20
Tapioca amylose (butanol method)	.0648	1.150	2.16	2.32
	.126	1.324	2.23	2.57
	.291	1.857	2.13	2.94
	.640	3.161	1.80	3.38
Lily amylose (butanol method)	.117	1.155	1.23	1.33
	.183	1.263	1.27	1.44
	.373	1.599	1.26	1.61
	.565	1.969	1.20	1.72
	.785	2.382	1.11	1.76
Corn amylose (butanol method)	.0560	1.053	0.922	0.947
	.0968	1.095	0.941	0.982
	.356	1.432	1.01	1.21
	.398	1.479	0.983	1.20
	.696	1.944	.956	1.36
	.996	2.504	.923	1.51
Corn "Crystalline" amylose (Kerr's method)	.0568	1.030	.521	0.528
	.161	1.084	.501	.522
	.388	1.215	.501	.555
	.638	1.343	.463	.538
Corn amylose (Meyer's method)	.152	1.050	.321	.329
	.207	1.058	.272	.280
	.370	1.109	.279	.295
	.450	1.129	.270	.287
	.486	1.172	.327	.354
	1.155	1.398	.290	.345
Fraction 3 of amylodextrin	0.137	1.017	.121	.124
	.216	1.029	.131	.134
	.492	1.072	.142	.146
	.960	1.143	.139	.149
Mixture of 62.5% cryst. amylose and 37.5% amylodextrin, Fraction 3	.175	1.067	.371	.382
	.449	1.181	.371	.403
	.664	1.275	.366	.414
Synthetic starch	.130	1.029	.218	.223
	.258	1.064	.241	.248
	.332	1.111	.317	.334
	.516	1.164	.295	.318
	.723	1.298	.361	.412

TABLE II

COMPARISON OF LIMITING VISCOSITY AND CHARACTERISTIC IODINE POTENTIAL OF THE AMYLOSES[a]

Fraction	Lim. $\frac{\eta_{sp}}{C}$ (Fig. 3)	E
Potato butanol ppt.	2.58	0.197
Tapioca butanol ppt.	2.11	.200
Lily butanol ppt.	1.24	.202
Corn butanol ppt.	0.91	.203
Corn cryst. amylose	.53	.205
Synthetic starch	.22	.204
Amylodextrin fraction 3	.12	.218

[a] These values are taken from the data of Bates, French and Rundle, and are the potentials at the midpoint of the titrations, corrected for slight variations in iodide concentration.

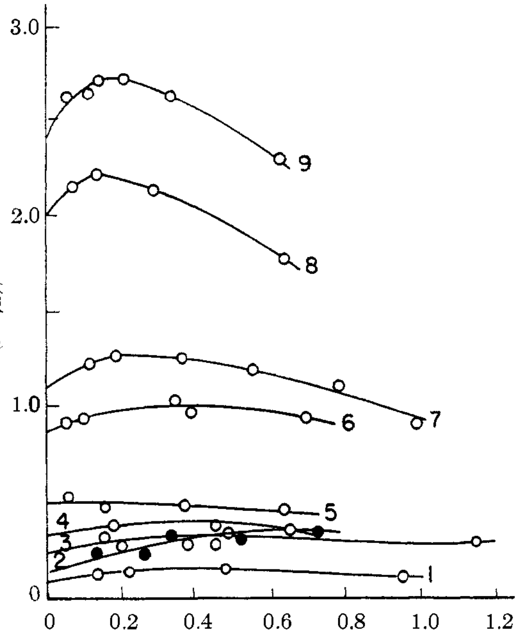

Fig. 2.—Concentration dependence of the Arrhenius function, ln η_R/C. The numbering of the curves corresponds to the numbering in Fig. 1. The solid circles are for synthetic starch, curve 2.

This similarity would seem to leave little room for doubt that the amyloses are essentially linear polymers as proposed by Meyer.[3]

This contention is further strengthened by the linear relation manifested in Fig. 3. Huggins, in a recent extension[9] of his theoretical derivation mentioned above, predicts a linear relationship between the functions η_{sp}/C and η_{sp} according to the equation

$$\eta_{sp}/C = [\eta_{sp}/C]_{C=0}\,(1 + k'\,\eta_{sp}) \qquad \text{(III)}$$

where k' is a constant. In Fig. 3, k' is reasonably constant for the materials of higher viscosity, but shows considerable variation for the lower materials, probably, however, within the experimental error.

The figures emphasize the importance of extrapolation to infinite dilution in any attempt to determine relative or absolute molecular weights. This fact has been overlooked in the bulk of the viscosity literature, since both the Staudinger and the Arrhenius (logarithmic) functions were originally reputed to be concentration-independent. Inasmuch as the two functions approach the same limiting value, either may be used equally well in any expression relating viscosity and molecular weight. If in equation (I) η_{sp}/C is replaced by $[\eta_{sp}/C]_{C=0}$, it becomes equivalent to equation (II). The sole basis for choosing between the functions would seem to be in the facility and accuracy with which the extrapolation can be carried out. In this respect the type of plot shown in Fig. 3 would seem to possess a distinct advantage.

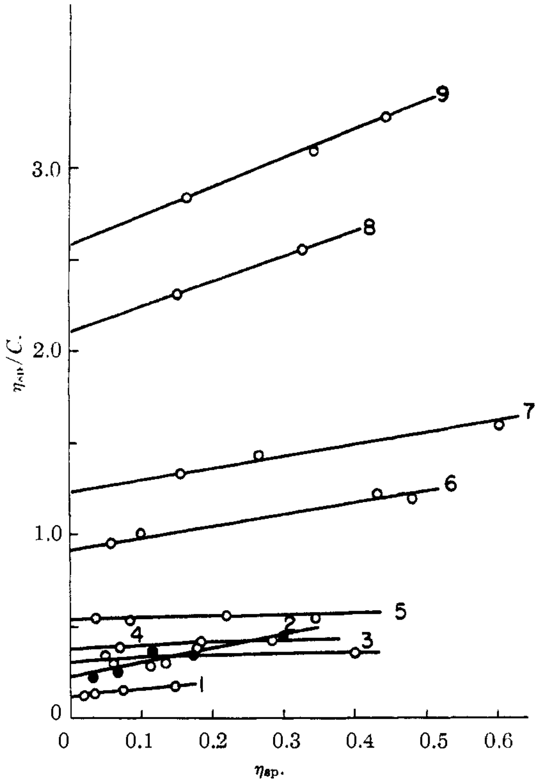

Fig. 3.—Relationship between the Staudinger function, η_{sp}/C, and η_{sp}. The numbering of the curves corresponds to the numbering in Fig. 1. The solid circles are for synthetic starch, curve 2.

In Table II the limiting values of the Staudinger function taken from Fig. 3 are compared with the characteristic iodine potentials. The viscosity limits undoubtedly represent the order of the molecular weights, and the fact that they fall in an inverse order to the iodine potentials (the one exception is synthetic starch) leaves little doubt that the potential at which the amyloses take up iodine is a function of the chain length.

Since the viscosity of a solution of a high-polymeric substance depends on a weight-average molecular weight,[10] a consideration of homogeneity is important before comparing results obtained by this method with other properties. The results of the iodine titrations[2] indicate a reasonable degree of homogeneity for the amyloses, at least in comparison with the magnitude

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(9) Huggins, THIS JOURNAL, **64**, 2716 (1942).

(10) Kraemer and Lansing, *J. Phys. Chem.*, **39**, 153 (1935).

of the differences between them. The one exception to this statement, of the materials under consideration here, is synthetic starch which shows a much more pronounced slope in its iodine titration curve, interpreted as indicating considerable heterogeneity.

The fact that the characteristic iodine potential and viscosity of the synthetic starch do not correlate with those of the amyloses might be attributed to the manner in which the iodine potential is chosen. In the case of the amyloses it makes little difference what portion of the titration curve is used to compute the potential since they are fairly homogeneous. However, in the case of a heterogeneous material a point on the titration curve should be chosen which corresponds to the titration of material having the weight average molecular weight, before comparison with viscosity results is made.

The heterogeneity of the sample does not seem to offer a logical explanation for the anomalous viscosity–concentration relationship exhibited by synthetic starch (Figs. 1, 2 and 3), since both the slope and intercept of the curves are apparently functions of the weight average molecular weight. This was tested experimentally by preparing a heterogeneous amylose sample consisting of 62.5% of crystalline amylose and 37.5% of the amylodextrin fraction. This mixture gave a viscosity–concentration curve of the expected slope (Curve 4 of Figs. 1, 2 and 3). The greater polymer–polymer interaction shown by the synthetic starch as contrasted to the amyloses of similar size might be explained by the presence of polar groups. The method of synthesis would lead one to suspect phosphate groups; however, the low value for phosphorus reported for this material (0.09%)[11] might eliminate this explanation. It should be emphasized that the viscosity–concentration relationship does not eliminate the possibility of heterogeneity indicated by the iodine titration curves. It does indicate that some other structural factor must be involved.

A point of considerable interest in the fractionation of starch is the fact that the viscosity of the butanol precipitate from corn is much higher than that of the corn amylose prepared by Meyer's method. This is to be expected since hot water extraction of the granules would tend to remove the smaller amylose molecules, whereas butanol would undoubtedly tend to precipitate the longer chains first. It is also of interest that the amylose prepared by Kerr using a combination of the hot water extraction and butanol precipitation shows an intermediate viscosity, as would be expected.

The amylodextrin fraction used in this work appears to have a molecular size somewhat greater than 60 glucose units based on reducing value. On the basis of viscosities the amyloses would thus appear to have molecular weights somewhat above the range of 10,000 to 60,000 reported by Meyer.[3] Work is now in progress in this Laboratory toward the determination of independent molecular weights by means of osmotic pressure in the hope of testing further the validity of equations I and II and evaluating the constants.

Acknowledgments.—The writers are indebted to Dr. T. J. Schoch and Dr. R. W. Kerr of the Corn Products Refining Company, and Dr. W. Z. Hassid of the University of California for their generous donation of materials used in this work, and to Mr. F. L. Bates of this Laboratory who prepared the butanol precipitate from lily bulb starch and also fractionated the amylodextrin.

Summary

1. Viscosity data for ethylenediamine solutions of the amylose components from various starches are presented.

2. The viscosity–concentration relationships give added evidence that the amyloses are linear polymers.

3. The viscosity limits fall in an inverse order to the potentials at which the amyloses take up iodine from solution, confirming the postulate that this potential is a function of the molecular weight of the amylose.

4. Synthetic starch is anomalous both with respect to its iodine titration curve and the viscosity–concentration relationship of its solutions. The former anomaly can perhaps be best explained on the basis of heterogeneity. The latter indicates increased polymer–polymer interaction which cannot be explained by heterogeneity but may be due to the presence of polar groups in the molecule, perhaps phosphate groups.

5. The viscosity limits of the corn amyloses prepared by different methods are in the order which would be expected from the methods of preparation.

AMES, IOWA RECEIVED JANUARY 4, 1943

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[11] Hassid and McCready, THIS JOURNAL, 63, 2171 (1941).