substitution (excess of the order of 20–1 molar ratio). Such a mixture, however, was not practicable for our experimental set-up due to the relatively large bulk of such a reaction mixture. The results obtained from the 0.05–0.1 molar mixture show, apparently, a somewhat different reaction course from the other three runs. The amount of ethyl bromide collected corresponds very closely to the theoretical for complete disubstitution (*i. e.*, 7.25 cc.) according to reaction (2), while the distillation of the reaction mixture yielded approximately one-half of the triethyl phosphite used in the form of diethyl ethylphosphonate. It appears that the amount of the bromoethyl ester formed is very small, but due to its ready decomposition, on attempted distillation, and evolution of hydrogen bromide, the tetraethyl ester, which apparently forms by a normally expected reaction (2), suffers decomposition, either *per se* or influenced by the hydrogen bromide, with diethyl ethylphosphonate being one of the decomposition products. The mechanism of this decomposition is obscure and appears to require a form of disproportionation. The results with ethylene bromide are in general accord with the above cited work of Nylen, who was able to isolate only decomposition products. It is curious to note that the methane member of the series yields a halomethyl product of some stability, as well as the di-substitution product (in modified form), as shown by Arbuzov (see above) and Nylen (see above). It is now shown that the ethane derivative apparently undergoes the normal reaction course for reactions (2) and (3), with the attendant complication of poor stability of the bromoethyl compound (not unexpected, by analogy with β-haloethyl carboxylic acid derivatives), making it impossible to isolate pure reaction products.

Summary

The reaction between triethyl phosphite, on one hand, and *n*-butyl bromide, *n*-hexyl bromide and ethylene bromide, on the other hand, was studied by following the rate of evolution of ethyl bromide. The latter reaction apparently follows the normally expected mono- and di-substitution courses, controllable by proportion of the reactants.

DAYTON, OHIO RECEIVED AUGUST 12, 1943

[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

The Relation of Starch–Iodine Absorption Spectra to the Structure of Starch and Starch Components[1]

BY R. R. BALDWIN, R. S. BEAR[2] AND R. E. RUNDLE

The activity of iodine in solution in the presence of starch has been shown to be a function of the nature of the starch, *i. e.*, of the proportion of amylose (or unbranched) component, the chain length of the amylose and the length of the free (or unbranched) portions of the amylopectin component.[3] Changes in starch-iodine color have long been known to accompany starch fractionation and starch degradation. It is to be expected that a more quantitative study of the absorption spectra of solutions of starch and its components should yield information, perhaps more detailed than that provided by the potentiometric study, concerning the structure of starch and its components.

Simerl and Browning made a spectrophotometric study of the iodine complex of a number of starches, dextrins, and starch components,[4] and Lampitt, Fuller and Goldenberg examined the absorption spectra of the starch–iodine complexes given by starch ground in a ball mill.[5] Both examinations were handicapped by the lack, at that time, of adequate methods of fractionating starch and characterizing the resultant fractions. Recent fractionation procedures and a rapid method of analysis of starch fractions[3] have overcome many of the previous difficulties.

Apparatus and Procedure.—Absorption spectra of the various solutions have been examined by means of a Coleman Model 10S double monochromator spectrophotometer, with an accompanying vacuum tube electrometer (Coleman pH meter) serving as an intensity measuring device. A slit selecting a 15 millimicron band of light was used for all transmission data. The solution to be examined was placed in a square cuvette, 13 mm. thick, and compared with a control solution in a matched square cuvette. The control solution contained a concentration of iodine equal to that used in preparing the starch–iodine solution. This procedure overcorrects for the iodine not involved in the complex, but the error is slight because of the negligible absorption of the very dilute iodine solutions used. For highly absorbing solutions a glass prism with optically parallel sides was placed in the cuvette to reduce the solution thickness from 13 to 1.63 mm. The temperature in all cases was kept at 23.0 ± 0.5°.

The perfectly clear solutions required for spectrophotometric work are possible only for very dilute starch solu-

(1) Journal Paper No. J-1150 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 660. Supported in part by a grant from the Corn Industries Research Foundation. This paper is based on a thesis submitted by R. R. Baldwin to the Iowa State College.

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(3) F. Bates, D. French and R. Rundle, THIS JOURNAL, **65**, 142 (1943).

(4) L. Simerl and B. Browning, *Ind. Eng. Chem., Anal. Ed.*, 11, 125 (1939).

(5) L. Lampitt, C. Fuller and N. Goldenberg, *J. Soc. Chem. Ind.*, **60**, 99 (1941).

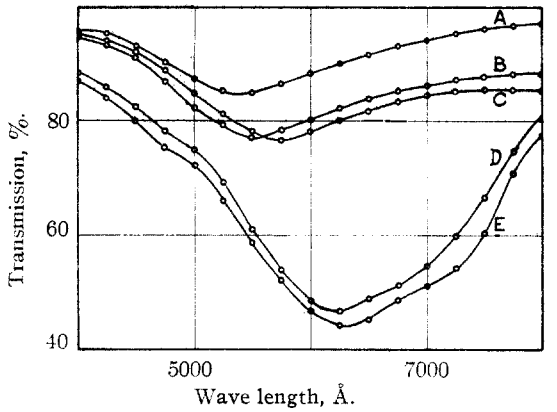


Fig. 1. Transmission curves of the iodine complex of amylose and amylopectin materials: A, waxy maize; B, potato amylopectin; C, corn amylopectin; D, corn amylose; E, potato amylose; cell depth, 1.63 mm.; iodine concn., 0.00005 *M*; starch concn., 0.01%.

tions. The starch or polysaccharide concentrations used in this investigation were generally 0.01%. In this concentration molecularly dispersed solutions can be approached, if not actually obtained, by the following procedures:

Pastes of native, granular starches were poured into boiling water, boiled for five minutes, and autoclaved for an hour at 18 lb. pressure. In strictly neutral solutions, degradation during this interval did not appear to be serious.

Amylose preparations are more difficult to disperse. A weighed amount of dried amylose was allowed to stand in dilute potassium hydroxide until a perfectly clear solution was obtained. It was found that 5 ml. of 0.5 *N* potassium hydroxide would readily disperse 50 mg. of amylose. The time required depended on the plant source of the amylose, but was generally less than an hour. The amylose solution was neutralized with aqueous hydriodic acid. If the solution was neutralized immediately before use retrogradation was not significant during the time required for the spectrophotometric observations.

Materials.—The whole starches used were generally milled in this Laboratory. The "crystalline amylose" was furnished by Kerr. Its preparation and properties have been described by Kerr and Severson,[6] and in papers from this Laboratory.[3,7] The term amylose is used to designate the unbranched component of starch. The amyloses discussed here were prepared by the fractionation procedure of Schoch.[8] Although this procedure proved the most successful of the methods tried for the separation of starch components, the amyloses thus prepared were generally contaminated by a small amount of amylopectin, or the branched component. Analyses of these fractions have been reported.[3] The amylopectins were likewise obtained by Schoch's fractionation, and the last traces of amylose were removed by cotton adsorption columns where necessary.

The amylodextrin used in this investigation was a Nageli dextrin[9] further fractionated by butanol-methanol precipitation, and is identical with the amylodextrin fraction examined by Foster and Hixon[10] and the fraction examined by Bates, French and Rundle.[3] It digests to 90% with β-amylose, retrogrades readily to give a crystalline diffraction diagram and in its change of viscosity with concentration it resembles the amyloses rather than the amylopectins.[10] All these properties indicate that it possesses unbranched chains. Its molecular weight is small, however, in the neighborhood of 44 glucose residues, or only about one-tenth as great as for the amyloses from whole starches.

Absorption Spectra of Amylose- and Amylopectin–Iodine Solutions.—The difference in ability of amylose and amylopectin to form complexes with iodine and the difference in color of the complexes[3] makes it necessary to regard starch as a complex substance in the study of iodine absorption spectra. These two components of starch were, therefore, studied separately. In Fig. 1 typical transmission curves of amylose and amylopectin are shown. The wave length of minimum transmission is greater for the amylose, and the % transmission under similar conditions is always far less. These factors make possible the colorimetric analysis of starch for amylose and amylopectin, and such an analysis has been described for potato starch.[11] It will be clear from what follows concerning specific amylose and amylopectin materials that such an analysis cannot be applied generally to starch without a preliminary investigation of the properties of the specific amylose and amylopectin making up the particular starch to be analyzed. This is, of course, a serious limitation on the method.

Outstanding is the difference in the behavior of amylose and amylopectin with different concentrations of iodine. In this respect the absorption curves are similar to the iodine titration curves, and are to be expected from that work.[3] In Fig. 2 are shown the spectrophotometric titrations characteristic of amylose and amylopectin materials. For both substances log *T* was chosen for the wave length of minimum transmission for that substance. The wave length of minimum transmission does not change materially with iodine concentration. The potentiometric titration of amylopectin with iodine indicates that not much of the iodine combines with the amylopec-

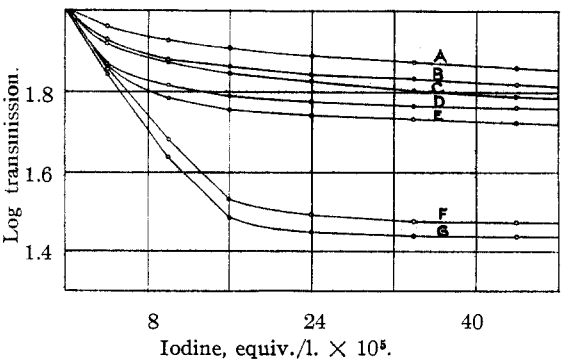


Fig. 2.—Spectrophotometric titration of various starchy materials: A, waxy maize; B, corn amylopectin; C, potato amylopectin; D, corn starch; E, potato starch; F, corn amylose; G, potato amylose; cell depth, 1.63 mm.; iodide concn., 0.005 *M*; starch concn., 0.01%.

(6) R. Kerr and G. Severson, THIS JOURNAL, **65**, 193 (1943).

(7) (a) R. Rundle and R. Baldwin, *ibid.*, **65**, 554 (1943); (b) R. Rundle and D. French, *ibid.*, **65**, 558 (1943); (c) R. Rundle and D. French, *ibid.*, **65**, 1707 (1943).

(8) T. Schoch, *ibid.*, **64**, 2957 (1942).

(9) C. Nageli, *Ann.*, **173**, 218 (1874).

(10) J. Foster and R. Hixon, THIS JOURNAL, **65**, 618 (1943).

(11) R. McCready and W. Hassid, *ibid.*, **65**, 1154 (1943).

tin, and as indicated in Fig. 2, the transmission of the iodine solution is affected far less by the presence of amylopectin than by the presence of amylose. Both the potentiometric and spectrophotometric titrations indicate that a complex of a definite composition is formed between amylose and iodine. The spectrophotometric titration could be made the basis of an analysis of starch for amylose, but it appears to have no advantages over and is more laborious than the potentiometric titration. Apparently the spectrophotometric titration would not suffer from the limitations of the colorimetric analysis[11] if the end-point were determined by the break in the titration curve. Both the potentiometric and the spectrophotometric titrations indicate a discontinuous difference between amylose and amylopectin. Differences in degree of branching of amylose and amylopectin cannot then be continuous.

Iodine–Iodide Relationships in the Amylose–Iodine Complex.—In the potentiometric titration it was found that the % iodine in the amylose–iodine complex at the end-point decreased as the iodide concentration was increased.[8] This is confirmed by the spectrophotometric titration. In Fig. 3 are titration curves for "crystalline amylose" at various iodide concentrations. In Fig. 4 the number of glucose residues per iodine molecule is plotted against a function of the iodide concentration. It is seen that the curve extrapolates to approximately 6 glucose residues per iodine molecule in infinitely dilute iodide solutions.

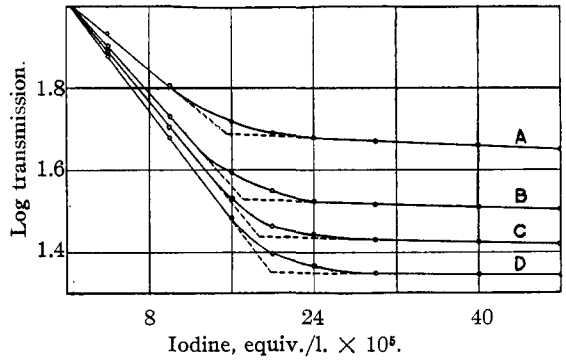


Fig. 3.—Spectrophotometric titration of crystalline amylose at various iodide concentrations: iodide concn. of A, 0.5 *M*; B, 0.05 *M*; C, 0.005 *M*; D, 0.001 *M*; cell depth, 1.63 mm.; amylose concn., 0.01%.

This value is, we feel, very significant. It has been found that the starch–iodine complex consists of helical starch chains with iodine molecules arranged along the helix axis.[7] The periodicity along the helix is probably 6 glucose residues. Apparently in the complex, free of iodide, one iodine molecule occupies one turn in the helix. It was found that dry amylose in the helical configuration takes up iodine vapor to the extent of one iodine molecule per 6 glucose residues.[7]

The reason for the decrease in iodine in the complex with increasing iodide concentration appears clear from the structure of the complex. With increasing iodide concentrations triiodide ions must enter the helix, probably accompanied by positive ions. A turn in the starch helix is 8 Å. long.[7] The space required for an iodine molecule is about 6.6 Å. along the helix, and the space required by a triiodide ion plus a positive ion must be considerably larger than 8 Å. For lack of space the amount of iodine in the helix must decrease as iodide enters. A more quantitative study based on the analysis of the complex for iodide is under way.

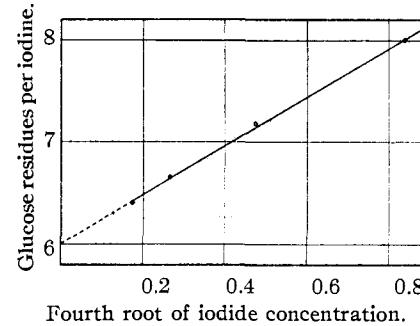


Fig. 4.—Glucose residues per iodine molecule at the end point of the spectrophotometric titration of "crystalline amylose" as a function of iodide concentration.

There is an interesting difference in the amount of iodine found to be bound in complex formation at the end-points of the spectrophotometric and potentiometric titrations (Table I). In all cases the spectrophotometric value is the higher. We believe that the difference can be explained in terms of the structure of the complex and the choice of the two end-points. The iodine activity of an amylose–iodine solution is fairly constant as long as the main body of the helices is being filled. From the ends of the helices, however, it would be expected that the iodine molecules could be removed more readily. An increase in the iodine activity then occurs before the last possible iodine molecules are put in the helices. The end-point of the potentiometric titration is taken at the first rapid increase in iodine activity and hence before complex formation is fully completed. In the spectrophotometric titration all iodine is counted as present in complex formation if it contributes to the increase in the absorption of the solution over that of an iodine–water solution.

TABLE I

GLUCOSE RESIDUES PER IODINE MOLECULE IN THE AMYLOSE–IODINE COMPLEX PRODUCED AT VARIOUS IODIDE CONCENTRATIONS AS DETERMINED BY SPECTROPHOTOMETRIC AND POTENTIOMETRIC METHODS

Iodide concn., moles/liter	Glucose residues per iodine molecule	
	Spectrophotometric	Potentiometric
0.5	8.0	9.9
.05	7.2	8.4
.005	6.7	7.8

Since amylopectin is quite different from amylose in its complex-forming ability with iodine, and

since the % iodine in the complex varies with iodide concentration it is clear why previous attempts to determine a formula for the starch–iodine complex by using whole starch, or poorly fractionated starch, and various iodide concentrations have failed.[12]

In Fig. 3 it is seen that the transmission of amylose–iodine solutions increases with added iodide. This effect is probably due to a change in the nature of the complex; more triiodide and/or iodide ions enter the helices at higher iodide concentrations.

The Effect of Chain Length on the Spectra.—In Fig. 5 absorption curves of amylose–iodine solutions are plotted against wave length. It can be seen that the wave length for maximum absorption differs for amyloses from different plant sources. These differences are quite reproducible, and are characteristic of the source of the amylose.

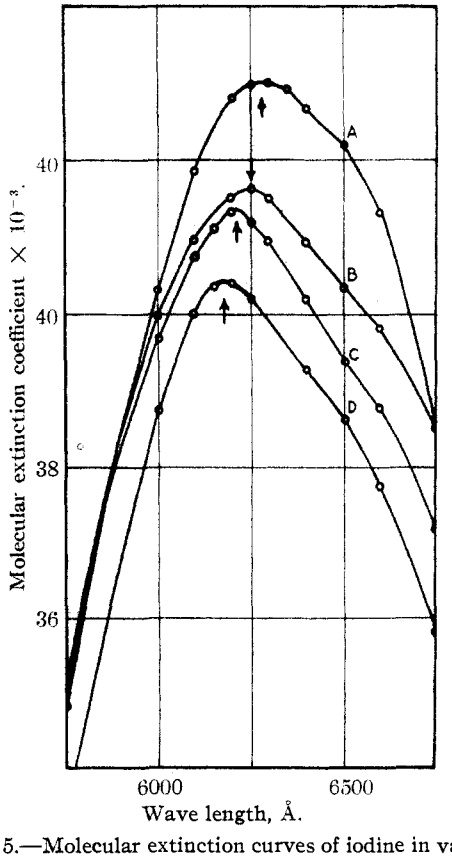


Fig. 5.—Molecular extinction curves of iodine in various amyloses: A, potato amylose; B, tapioca amylose; C, lily amylose; D, corn amylose; cell depth, 1.63 m.; iodine concn., 0.00005 *M*; amylose concn., 0.01%.

Even more variation is found in the molecular extinction coefficients of amylose–iodine solutions from different plant sources. For comparison purposes amylose must be kept in excess. The absorption is then a function of added iodine. In their spectrophotometric study of starch–iodine Lampitt, Fuller and Goldenberg found that an increased iodine concentration caused an increased value of the extinction coefficient.[5] If, however, the absorption is calculated as molecular extinction coefficient, the greater values are obtained with lower iodine concentrations. In Table II the molecular extinction coefficients are calculated for solutions 0.0001 *N* with respect to iodine and 0.01% with respect to starch.

It is to be noted that low iodine concentrations are particularly important in this comparison, for near the end-point of complex formation the molecular extinction coefficient is influenced profoundly by the per cent. amylopectin impurity which was never negligible in our work. At low iodine concentrations, however, the iodine first forms a complex with amylose, and the molecular extinction coefficient is nearly independent of the amylopectin impurity.

The amylose–iodine complexes listed have been found to differ in the activity of iodine in water solution of the complexes.[3] This difference was interpreted as due to the difference in chain lengths of the amyloses, and this point has been confirmed by the viscosity studies of Foster and Hixon.[10] In Table II are listed observed absorption maxima, molecular extinction coefficients, characteristic potentials for complex formation of an iodine half cell measured against a normal calomel half cell,[3] and estimates of chain length in glucose residues from the viscosity studies of Foster and Hixon.[10] Both the absorption maximum and the molecular extinction coefficient change in a regular fashion with chain length. Though the differences are beyond the experimental error, the change in the absorption maximum is too insensitive to be particularly valuable as a measure of chain length except for very short chains, such as encountered in the amylodextrins. The change in the molecular extinction coefficient with chain length is sufficient to permit its use as a rough, relative test of the chain length of an amylose, or unbranched material. It, too, is most sensitive to change in chain length for short chains. It is also a secondary method whose use depends upon the establishment of the molecular weights of a series of amyloses by other methods. It would appear advisable to await the further

TABLE II

Amylose	Molecular size	Wave length of absorption maximum	Molecular extinction coefficient	Characteristic potential
Potato	500	6280	43,000	0.197
Tapioca	450	6250	41,600	.200
Lily	310	6220	41,400	.202
Corn	250	6180	40,400	.203
Crystalline	175	6050	40,100	.205
Synthetic	85	5900	32,900	.204
Amylodextrin	44	5800	25,400	.218

(12) For a review of the literature on this point see G. Barger, 'Some Applications of Organic Chemistry to Biology and Medicine," McGraw Hill Book Co., New York, N. Y., 1930.

development of such standards before use of the molecular extinction coefficient for the determination of anything but the relative molecular weights of the amyloses.

The wave lengths of maximum absorption of the amylopectin–iodine solutions are all at much shorter wave lengths than those found for the amylose–iodine solutions. The wave lengths are again a reproducible function of the source of the amylopectin. There is a great deal of similarity in the wave length, molecular extinction coefficient, and general character of the absorption spectra of the amylopectins and the low molecular weight amylodextrin. The latter appears to have all the characteristics of an unbranched, but short chain material. The amylopectin, on the other hand, is of high molecular weight. The similarity in the absorption spectra seems to result from a similarity in the length of the free, unbranched portions of the chains in the amylopectin molecules and the length of the amylodextrin chains. It is believed that the wave length of maximum absorption and the molecular extinction coefficient of the amylopectin–iodine solutions are measures of the average length of the unbranched portions of the amylopectin chains. On this basis it appears that glycogen is most highly branched, and following in order, waxy rice, waxy maize, waxy barley, potato- and corn-amylopectin. Where tetramethyl end-group assay is known it is in agreement with this assignment. This order is also in agreement with the results of the potentiometric titration.[3]

Summary

Study of absorption spectra confirms the great difference in behavior of amylose and amylopectin with iodine found by the potentiometric iodine titration,[3] and provides another means of analyzing for the two components in whole starch. The differences in individual amyloses and amylopectins from different starches make a simple colorimetric analysis for the two components unreliable however.

The amount of iodine bound in complex formation with amylose increases as the concentration of iodide decreases, becoming one iodine molecule for six glucose residues for infinitely dilute iodide solutions.

The wave length of maximum absorption of an amylose solution shifts toward the red as the chain length of the amylose is increased. The shift is in the same direction when the lengths of the unbranched portions of an amylopectin are increased. An increase in the molecular extinction coefficient accompanies an increase in the length of an amylose or an increase in the lengths of the unbranched portions of an amylopectin. Both these properties permit the relative evaluation of molecular weight of an amylose and degree of branching of an amylopectin. The change in the molecular extinction coefficient is the more sensitive.

The relative molecular weights of a few amyloses and the degree of branching of some amylopectins have been examined. Results are in agreement with other determinations.

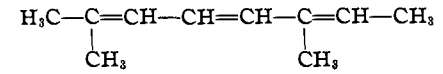
AMES, IOWA RECEIVED SEPTEMBER 28, 1943

[CONTRIBUTION FROM NAVAL STORES RESEARCH DIVISION, BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY, AGRICULTURAL RESEARCH ADMINISTRATION, U. S. DEPARTMENT OF AGRICULTURE]

Raman Spectra of Two Forms of *allo*-Ocimene

BY J. J. HOPFIELD,[1] S. A. HALL[2] AND L. A. GOLDBLATT

Among the products obtained in the pyrolysis of α-pinene[3] at about 375°, *allo*-ocimene



(2,6-dimethyl-2,4,6-octatriene), is of the greatest interest, both from a practical and theoretical viewpoint.

On careful re-fractionation of the portion of α-pinene pyrolysate boiling in the *allo*-ocimene range, two components were obtained, a major component boiling at 89.0° at 20 mm. pressure and a minor component boiling at 91°. Both components yield with maleic anhydride the same adduct (m. p. 83–84°) but the two components exhibit differences in their freezing points, densities and Raman spectra.

It may be reasonably assumed that these are two forms of *allo*-ocimene, of which there are four possible geometric isomers, arising from the double bonds at the 4 and 6 positions. Models (Fisher–Herschfelder) of the four geometrical isomers show that the *cis* form arising from the double bond at the C_4 carbon atom (regardless of whether the configuration at C_6 is *cis* or *trans*) is one of very limited rotation. The probability of its formation and continued existence during the high temperature pyrolysis of α-pinene would appear to be slight.[4] Such a configuration would be more likely to form a stable ring compound, such as one of the pyronenes which are also ob-

(1) Transferred to National Bureau of Standards, Washington, D. C., 10/18/42.

(2) Transferred to Bureau of Entomology and Plant Quarantine, 5/5/43.

(3) Goldblatt and Palkin, THIS JOURNAL, **63**, 3517 (1941).

(4) In the case of pentadiene-1,3 (piperylene), however, Rohey, *Science*, **96**, 470 (1942), reports that the *cis* isomer, which is similarly more hindered than the *trans* isomer, is much more prominent in mixtures from high temperature processes.