products. A purple color in the sulfuric acid wash bottle suggests that the iodide is being carried over.

Conclusions

1. A convenient method has been described for preparing mercury di-*iso*butyl by the method of Frankland and Duppa. Yields of 50–55% may be obtained.
2. Other esters may be used in place of ethyl acetate. The discovery has been made that carbonyl compounds other than esters can be used as catalysts in the reaction.
3. The principal by-product is di-*iso*butyl formed by the Wurtz reaction between sodium and *iso*butyl iodide.
4. Bromide may not replace iodide under the experimental conditions described.
5. Optimum yields are obtained when the following molar concentrations are used: one mole of *iso*butyl iodide, two moles of sodium, one half mole of ethyl acetate or one mole of acetone and forty six moles of mercury.

MOUNT VERNON, IOWA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY, No. 591]

THE DISRUPTION OF THE CORN STARCH GRANULE AND ITS RELATION TO THE CONSTITUENT AMYLOSES[1]

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In some earlier work in this Laboratory, a method for separating the amyloses in corn starch was devised[1a] and the amount of the insoluble or α-amylose and the soluble or β-amylose determined for corn and other starches. This separation, like all other separations, has as its first step the disruption of the granule in water in order to make available the constituent amyloses in the resulting paste. The completeness of the disruption in this case was gaged by the absence of the characteristic granules under the microscope. To obtain the proper type of paste it was necessary to pre-treat the starch with alcoholic hydrogen chloride and then use an effective swelling agent like ammonium thiocyanate to break open the granules when water was added subsequently.

The primary purpose of this paper is to show that the amount of the amyloses in a given starch, such as that of corn, is not dependent on any specific chemical action of the above reagents but only on their effective-

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[1] The work covered in this paper is taken from a part of a dissertation presented by C. O. Beckmann to the Faculty of Pure Science of Columbia University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[1a] Taylor and Iddles, *Ind. Eng. Chem.*, **18**, 713 (1926).

ness in causing complete rupture of the granule. Other swelling agents or mechanical abrasion give starch pastes from which the amyloses may be separated in the same proportion as found previously, whenever such pastes contain no unbroken granules. It is taken for granted that in all methods hydrolytic decomposition or dextrinization must be avoided.

Proof of correctness of this point of view comes from a detailed study of the constitution of a starch paste as a function of swollen granules and of constituent amyloses after their liberation through rupture of these granules. Discrepancies in the literature with regard to the amount of amyloses can be accounted for and avoidance of these difficulties made possible, it is felt, by the results of the investigation. As will be seen, a combination of microscopic examination, viscosity and amylose determination is used to fix the properties of the paste and give the picture we have drawn.

Before proceeding it might be well to set down briefly for purposes of reference two current interpretations of the mechanism of paste formation. It is believed by many[2] that the mechanism of paste formation consists of the imbibing of water by the granule, usually by heating, the swelling of the granule, its bursting, the leaching out of the inner substance and, upon cooling, the setting of this material to a viscous gel.

This gel is generally supposed to consist of two parts, separable because of their different solubility in water, but here is where the difficulty begins, for even with the same starch, for example, potato, the amount of insoluble material is reported as varying from 80% down to 2% (the soluble material by difference).[1a,3]

On the other hand, there are those[4] who apparently regard starch as a single chemical individual which in water becomes soluble to a greater or less extent due to hydration. According to this view a starch paste would consist of an equilibrium mixture of more or less hydrated forms of the one substance. The discrepancies in the ratio of insoluble to soluble portions can be explained as a shifting of the equilibrium point due to the imposed conditions.

That there are at least two fairly well defined constituents of the starch granule has been repeatedly demonstrated and is now generally accepted by most investigators.[5] While these amyloses probably do hydrate to a

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[2] See Haas and Hill, "An Introduction to the Chemistry of Plant Products," Longmans, Green and Co., London, **1921**, Vol. I, pp. 112–118.

[3] (a) Maquenne and Roux, *Compt. rend.*, **140,** 1303 (1905); (b) Gatin-Gruzowska, *ibid.*, **146,** 540 (1908); (c) Tanret, *Bull. soc. chim.*, [4] **17,** 83 (1915); (d) Sherman and Baker, THIS JOURNAL, **38,** 1885 (1916); (e) Ling and Nangi, *J. Chem. Soc.*, **124,** 2666 (1923).

[4] (a) Fouard, *Bull. soc. chim.*. **3,** 1170 (1908); (b) Harrison, *J. Soc. Dyers Colourists*, **32,** 40 (1916).

[5] See R. P. Walton, "A Comprehensive Survey of Starch Chemistry," Chemical Catalog Co., New York, **1928.**

different degree, the following observations indicate that the explanations for the discrepancies in the ratio of the amyloses, at least in corn starch, come from a consideration of the physical condition of the paste from which they are separated rather than from differences in degree of hydration or the presence of a large number of amyloses mutually transferable one into the other. In this respect our results bear out quantitatively the observations and conclusions of Alsberg on the properties of a starch paste.[6]

In this Laboratory it has always been noticed that when amyloses were separated from a paste of high viscosity the amount of insoluble material (classed as α-amylose) was high and whenever pastes of the same concentration were made that were limpid (low viscosity) the insoluble portion was low. Since under the microscope the highly viscous pastes as well as the separated, insoluble material from them showed large quantities of unbroken granules, it was concluded that the amount of so-called α-amylose so determined was fictitious. Contrariwise, in the limpid pastes as used by Taylor and Iddles,[1a] no characteristic granules can be observed under the microscope and the amount of insoluble material is relatively low (amount of soluble or β-amylose is correspondingly high).

Apparently, high paste viscosity, non-availability of the soluble amylose and swollen granules are definitely interrelated. That this is so is borne out by some quantitative measurements given later. It will be shown that the viscosity of pastes made from separated amyloses in a given concentration is only slightly greater than that of water, while that of pastes of the same concentration made by gelatinization[7] is relatively high.

These observations lead logically to another picture[6] of a starch paste, namely, that in the gelatinization of whole starch by hot water some granules only swell while others rupture. The number swelling and the number rupturing depend on the previous treatment given the raw starch or on treatment of the paste. On this basis the viscosity would be due almost entirely to whole but swollen granules[8] which still contain their quota of constituent amyloses. As will be seen, complete rupturing or even partially complete rupturing of the granules is not easily accomplished.[9]

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[6] Alsberg, *Ind. Eng. Chem.*, **18**, 190 (1926).

[7] The term "gelatinization" should not be confused with disruption of the starch granule. Frequently the terms are used interchangeably. In the course of this research it was found that, although related, they are by no means the same thing. To differentiate between them, therefore, gelatinization will be defined simply as the swelling of the granules, while disruption will mean the actual breaking up of the granule.

[8] Harrison, *J. Soc. Dyers Colourists*, **27**, 84 (1911).

[9] Pastes made in the usual way by boiling the starch with water either at atmospheric pressure or in an autoclave at 15 pounds above atmospheric (to get the corresponding increased temperature) contain swollen granules in large quantities. Long treatment under these conditions causes the granules to swell to such an extent that their index of refraction becomes almost that of water and they can be observed only after staining with iodine or gentian violet.

Experimental Part

Effect of Swelling Agents.—Repetition of the work of Taylor and Iddles[1a] on corn starch but omitting the pre-treatment with alcoholic hydrogen chloride gave a stiff paste, instead of the limpid one they describe, on the addition of ammonium thiocyanate. The acid apparently contributes to the process of rupturing also. Substitution of potassium iodide[10] for the ammonium thiocyanate in the Taylor-Iddles[1a] method gave the same amount of the amyloses as found by them.

A good grade of alkali-washed corn starch was treated with an alcoholic-hydrochloric acid solution according to the method.[1a] Sixty grams of this sample was treated with a solution of 46 g. of potassium iodide, 46 g. of water and 37 cc. of 95% alcohol. The starch was added to this solution in small portions with constant stirring and rubbing until a homogeneous suspension was formed. Alcohol was added to precipitate the starch after microscopic observation showed that no more whole granules existed. The precipitated, gelatinous mass was ground in a mortar with portions of alcohol until it became granular. It was then washed repeatedly with hot alcohol to remove the potassium iodide, which gave the starch a slight yellow color. It was found impossible to remove the potassium iodide completely. Refluxing the sample in a Soxhlet extractor with alcohol for seventy-two hours failed to remove it. About 10–14% of potassium iodide was absorbed by the starch. The determinations of β-amylose by ultrafiltration made on these samples were corrected, therefore, for absorbed salt: β-amylose found, 85%.

Other swelling agents such as ammonium nitrate or zinc chloride, although effective, were disregarded either because they could not be removed or corrected for subsequently or else because they had a tendency toward the promotion of amylose hydrolysis. The latter reaction is the one that must be avoided. Even the pre-treatment with alcoholic hydrogen chloride gives some substances that reduce Fehling's solution[9] and causes the liberation of some of the associated fatty acids.[11] The amount of the starch so affected is very small and in the light of the subsequent work may generally be neglected.

Mechanical Rupture of Granules in Paste.—If the viscous paste made by heating 5 g. of dry, untreated corn starch with water in the usual way is shaken[4b] or ground in a pebble mill,[12] it becomes more limpid. This may be interpreted as a breaking of some of the swollen granules. Other and perhaps more strenuous methods ought to bring about rupture of all the granules. Accordingly, a similar paste was passed six times through an homogenizer[13] where at a pressure of 4000 lb. per square inch it was discharged through a small outlet orifice.

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[10] Reychler, *Bull. soc. chim. Belg.*, **29**, 118 (1920).

[11] Taylor and Nelson, THIS JOURNAL, **42**, 1726 (1920); Taylor and Lehrman, *ibid.*, **48**, 1739 (1926).

[12] Alsberg and Perry, *Proc. Soc. Exptl. Biol. Med.*, **22**, 60 (1924).

[13] A Colony homogenizer used for making emulsions was employed. The authors wish to thank Pease Laboratories, Inc., New York City, for the use of this machine and also Messrs. Knoll and Stone for their assistance.

Under the microscope even on stained samples no characteristic granules could be seen in this paste, but the striations so characteristic of the whole granule were very much in evidence. The insoluble or α-amylose as determined by the electrophoretic method of Taylor and Iddles[1a] was 62.5%, while they report 15%. A comparison of the viscosities of this paste with that of raw starch and of the two amyloses under identical conditions and at the same concentrations (see Table I) shows the homogenized paste to be nearer that of raw starch. Apparently this treatment distorts but does not rupture a large number of the already swollen granules, hence the striations, high viscosity and large amount of insoluble "α-amylose." Of course some of the swollen granules are broken and whatever soluble material (β-amylose) is in solution would come, according to our point of view, from these disintegrated starch particles. Further work corroborates this hypothesis.

Disintegration of Dry Granules by Grinding.—It was evident from the results of the previous attempts that breaking swollen starch granules in a paste is not so easily accomplished. Grinding dry starch with sand and subsequently leaching out the soluble material (β-amylose) has been practiced.[14] Alsberg and Perry[15] report that pastes made from dry wheat starch ground in a pebble mill are less viscous than those from unground starch. From these ground samples, moreover, much more soluble material was obtained than from the unground by extraction with water at room temperature. They note also that after grinding in a pebble mill for a certain period there are still grains which like the whole starch are birefringent and show the characteristic black cross under the polarizing microscope.[16]

This method of pre-treatment of corn starch seemed to offer great promise in the present problem for it would eliminate completely the necessity for using chemical swelling agents in making the pastes. Suspicion of possible chemical changes in the amyloses would then be removed and the significance of viscosity measurements made more sure. Therefore a series of experiments was made substituting grinding in a ball mill for the use of swelling or gelatinizing agents in the Taylor-Iddles[1a] method. Comparisons were made of the viscosities of pastes from whole starch, with those from samples after various periods of grinding. These results were correlated with the microscopic appearance and with the amount of β-amylose which could be extracted with cold water from the sample or separated from it by electrophoresis. Later the viscosity of the amyloses themselves which had been separated by the electrophoretic method of Taylor and Iddles[1a] from a paste made from a completely ground sample was determined.[17]

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[14] Delffs, *Pogg. Ann.*, **109**, 648 (1860).

[15] Alsberg and Perry, *Proc. Soc. Exptl. Biol. Med.*, **22**, 60 (1924).

[16] Reichert, Carnegie Publication No. 173, pt. 1.

[17] In a strict sense it is incorrect to speak of the viscosity of a starch paste because

The ground samples were prepared finally as follows. Three hundred g. of dry corn starch was put in a four-gallon ball mill with about the same volume of quartz balls averaging about $^3/_4$ inch in diameter. The mill was rotated at 45–55 r.p.m. for the periods of time indicated. Twelve-hundred-g. starch loads were used at first but the load was reduced to 300 g. and the grinding continued. If 300 g. is the initial load birefringence disappears and the value for β-amylose reaches 85% after about 172 hours of grinding.

Soluble β-amylose was determined as follows: 2–3 g. of dry starch is shaken with water in a graduated flask and the volume made up to 500 cc. A small amount of toluene is added to prevent the growth of molds. The insoluble part settles out. When a clear supernatant liquid is obtained, an aliquot portion is removed with a pipet to a weighed evaporating dish almost filled with dry sand. This is put into an oven at 105° and evaporated to constant weight, which usually takes three hours.

After grinding the 1200 g. of starch for 130 hours, microscopic examination showed whole granules in large quantities and extraction gave 51.3% β-amylose. After 225 hours' total grinding, no characteristically shaped granules were apparent but birefringence, as evidenced by black crosses in the polarizing microscope, were still plainly visible. The β-amylose (soluble material by extraction) had risen to 63.1%. To cut down the time of treatment, 300 g. of this starch and the same ball load were ground an additional 325 hours, making the total period of 600 hours. In this sample there were no birefringent granules noticeable and the soluble β-amylose (fatty acid free) was 84.1%.

It is interesting to note that when birefringence disappears the amount of soluble or β-amylose is the same as that found by Taylor and Iddles.[1a] Before that point the granules, although no longer characteristically shaped (due no doubt to the effects of the beating in the mill), still hold in a coherent form from their quota of amyloses.[4b] By an electrophoretic separation about 11% of the α-amylose was found in the paste of the 600 hour sample by Taylor-Iddles.[1a] Continued grinding led to the formation of highly opalescent solutions which contained nearly 2% of silica from abrasion of the balls. While not significant in this connection the effect of long grinding on the insoluble α-amylose is being studied.

TABLE I

RELATION BETWEEN PRE-TREATMENT, AVAILABLE β-AMYLOSE AND VISCOSITY

Pre-treatment of corn starch	β-Amylose, %	Relative viscosity at 35°; 1% paste
(a) None	...	2.09
(b) Alc.-HCl and NH_4CNS according to ref. 1 a	85.0	1.05
(c) Orig. ground 130 hours	51.3	1.39
(d) Orig. ground 225 hrs.	63.1	1.32
(e) Orig. ground 600 hrs.	84.1	1.09
(f) β-Amylose from (b) by electrophoresis	100.0	1.07
(g) β-Amylose from (e) by electrophoresis	100.0	1.08

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of its heterogeneity, but it will be shown that settling has no appreciable effect on the values of viscosity relative to water in the concentration used.

Passing to a study of the viscosities of these pastes the results shown in Table I were obtained.

One-tenth of a gram (for a 1% paste or solution) of dry powdered sample was put into an ordinary test-tube and 10 cc. of distilled water added. The tube was then immersed in a beaker of boiling water. After one and one-half minutes the tubes were removed, shaken for ten seconds and re-immersed. After three minutes this was repeated and at the end of five minutes the tube was removed to a thermostat and allowed to come to temperature at 35°. After having attained the temperature of the thermostat, 5 cc. of the paste was removed to an Ostwald viscometer (of the ordinary type with a capillary 10 cm. long and a time value for water at 35° of about sixty seconds). Five to seven readings were taken on each sample. The time for a complete determination is from twenty to thirty minutes from the time of heating. Settling, which would obviously change the time of flow through the capillary, is negligible, since it can be noticed only after fifteen to twenty hours of standing. Moreover, deviations in a set of readings seem to be entirely independent of time.

Precision of Viscosity Measurements

Each value for relative viscosity is the average of those obtained on at least two samples. Each sample was run through the Ostwald viscometer 5 to 6 times. The average deviation for these 5 to 6 runs was 1–2%. The average deviation between two samples prepared (for viscosity measurements) independently was 3–4%.

This accuracy was not obtainable for relative viscosities above 7 or 8. The average deviation in these cases was about 6–7%.

The temperature at which the viscosities were run was $35 \pm 0.05°$. The change of viscosity due to temperature over this small range was found to be negligible.

In every case the α-amylose which carried the associated fatty acids is equal to $(100 - \beta)$. When extraction is used the β- is determined directly but in (b) and (d) it was easier to determine the α- directly. In (e) a direct current potential of 1200 volts was used to hasten the migration. This gave a clear, supernatant liquid which contained all of the β-amylose (g). A layer of toluene on the surface of the liquid prevented mold formation. Incidentally, a similar sample in water under toluene showed no signs of settling after standing for three months. This illustrates how the application of the direct current potential facilitates separation.

Dry samples of β-amylose were obtained by precipitation, on the addition of alcohol to the clear water solutions. The α-amylose was removed from the bottom of the electrophoretic cell[1a] and partially dehydrated by the addition of alcohol. In both cases, to remove the remains of water and obtain a granular mass, a mixture of benzene and alcohol was added in successive portions with mechanical stirring after each portion, forming the ternary mixture (water–benzene–alcohol), boiled off at 60°. The final treatment leaves the white, amorphous material behind in absolute alcohol, from which it is recovered by filtration and evaporation of the adhering alcohol at room temperature.

It should be noted that only when the viscosity has dropped to almost that of the amyloses themselves does the total β-amylose become available for separation from the paste. Of course the rather slimy, insoluble α-amylose contributes something to the viscosity of more concentrated pastes, but it is present in corn starch only to the amount of 15%. In mixtures of α- and β-amyloses in the proportion in which it exists in corn starch in pastes containing 1% total solids, the viscosity was practically that of β- alone and did not approach that of a heat-gelatinized whole starch of the same concentration. These results support the contention that the viscosity of an ordinary starch paste is due to the undisintegrated granule. More significant for the present purpose, however, is the fact that a paste made from a completely ground starch when subjected to the Taylor-Iddles[1a] method gives the same ratio of amyloses with the same general properties as that obtained from their ammonium thiocyanate treatment.

In these measurements it must be remembered that corn β-amylose has a solubility of 7 g. per 100 cc. at 25° and 10 g. per 100 cc. at 35°. These solutions are perfectly clear, but above the concentrations at the respective temperatures there is cloudiness and settling. This, of course, makes viscosity determinations of little quantitative use in this range. In the following table there are summarized the results of the observation.

TABLE II

VARIATION OF VISCOSITY WITH CONCENTRATION AT 35°

Concn. %	Raw starch	Ground starch (63.1% sol.)	β-amylose (from ground starch)
0.25	1.14	..	...
0.50	1.35	..	...
1.00	2.09	1.31	1.078
2.00	7.24	1.71	1.182
3.00	38.95	2.43	1.277
4.00	paste	3.39	1.396
5.00	too	4.77	1.512
6.00	stiff	6.41	1.667
7.00	..	..	1.807
8.00	..	..	1.986
9.00	..	..	2.117
10.00	..	..	2.308

From the above table it is evident that it takes about ten times as much β-amylose (in solution) as it does gelatinized raw starch to obtain the same viscosity. Because of the insolubility and rapid settling of the α-amylose, there is no way by this method of measurement, to tell how much this substance contributes to the viscosity of a partially ruptured paste (as in third column). For comparison in a qualitative way, however, these results are interesting.

Summary and Conclusions

1. It has been shown that the α- to β-amylose ratio in corn starch as determined previously[1a] is not dependent upon the method of treatment.

2. Complete rupture of the starch granule may be accomplished also by grinding. The product of this method yields amyloses of high purity.

3. Additional proof is brought forward that the viscosity of a starch paste is directly connected with the number of swollen granules (gelatinized) in that paste.

4. The structure of starch pastes has been discussed and it has been shown that its characteristic properties in the case of corn are functions more of the physical nature of the starch granules than of the chemical nature of the amyloses.

5. The results indicate that before a separation of the constituent amyloses in corn starch can be made, all of the granules must be ruptured. This is evidenced by a drop in the viscosity of starch pastes. Any attempts at separation before this point result in fictitiously high results in insoluble material. Although a few granules rupture easily, the great bulk do so only with great difficulty when a paste is made in the ordinary way.

NEW YORK CITY

[COMMUNICATION FROM THE DEPARTMENT OF INDUSTRIAL AND CELLULOSE CHEMISTRY, McGILL UNIVERSITY]

STUDIES ON THE REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES. XX. PREPARATION, PROPERTIES AND IDENTIFICATION OF GLYCEROL BETA-METHYL ETHER

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The important role played by glycerol esters and ethers in animal metabolism, industrial processes and organic research has led various investigators to point out the necessity for a reliable characterization of a series of readily identifiable, simple derivatives of glycerol containing substituents definitely located in the α-, β- and α'-positions.

For reasons defined later in this paper, the glycerol ethers are probably the most suitable for the preparation of such a series. Thus Irvine, Macdonald and Soutar[3] proposed the use of glycerol α,β- and α,α'-dimethyl ethers and α- and β-monomethyl ethers for the identification of mixed glycerides. Controlled hydrolysis of glycerides followed by methylation and further hydrolysis, with the aid of the above reference types, would serve to identify the configuration of the original glyceride substituents.

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[3] Irvine, Macdonald and Soutar, *J. Chem. Soc.*, **107**, 342 (1915).