previously reported constituent of orange seeds. This substance occurs in the albedo, the center fibrovascular bundle, and in the section covering of the fruit, in non-bitter water-soluble form. When these tissues are ruptured it is extracted into the juice, where it is slowly converted to the intensely bitter lactone form. It is shown that this substance has a higher molecular weight than that previously reported, corresponding to the formula $C_{26}H_{30}O_8$. It is apparently an isomer of limonin, and of citrolimonin. The probable identity of these last named substances is discussed.

Limonin, which has heretofore only been reported in the seeds of citrus fruit has been isolated also from the pulp of the Valencia orange.

Both limonin and isolimonin form non-bitter water-soluble salts with alkali and alkaline earth metals, from which they are precipitated unchanged upon acidification.

Treatment of isolimonin with dilute hydrochloric acid in acetone solution gave fractions of limonin, and of hexahydrolimoninic acid. This latter substance has been obtained previously only by the catalytic hydrogenation of limonin. It appears that limonin results from a molecular rearrangement in the isolimonin, and that hexahydrolimoninic acid is produced by the splitting of a lactone ring, to form a carboxyl group.

ONTARIO, CALIF. RECEIVED OCTOBER 4, 1938

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF COLUMBIA UNIVERSITY]

The Action of Chlorine Water on Some β-Amyloses

BY HARRY H. FLETCHER[1] AND T. CLINTON TAYLOR[2]

Introduction

Industrial technologists have known for over ten years that if chlorine water is mixed with a starch dispersion, the viscosity of the dispersion drops after ten to twenty minutes of treatment.[3] The appearance of this chlorine-treated starch, the optical rotation of its dispersion, and the blue color with iodine remain unchanged. Nevertheless, the viscosity drop indicates that the chlorine water probably has begun to break the starch particles into smaller ones,[4,5] although this breakdown is still in too early a stage to be detected by other means. With two exceptions, the chemical and physical nature of the action of chlorine water on starch has not been investigated. Craik[6] found that the optical rotation of a potato starch dispersion gradually increased during several days' contact with hypochlorous acid at room temperature. However, the optical rotation is not so sensitive to slight changes in the structure of the starch as is the viscosity. Recently Rassow and Lobenstein measured the viscosity decrease during the reaction between potato starch and an alkaline hypochlorite solution.[7] They noticed also that the dispersion became more acidic during the course of the reaction. This they attributed to oxidation of aldehyde groups to carboxylic acids with concurrent reduction of the hypochlorous acid to hydrochloric acid.

Rassow and Lobenstein determined the reducing power of their starch dispersions after alkaline treatment only. The work described herein extends it into acid regions. As will be shown later, the results obtained in solutions of *p*H less than 7 were, so far as we are aware, entirely new and unexpected.

Outline of Procedure

Rassow and Lobenstein used Fehling's solution to measure the reducing power of their dispersions. As this reagent is not very satisfactory for rapid analysis,[8] the recently standardized alkali-labile procedure was used in these new determinations.[9] When the hot alkaline digestion is omitted, the iodine consumption of the carbohydrate, determined and calculated in the usual manner, will be

---

(1) Present address: U. S. Rubber Products, Inc., Gen. Labs., Passaic, N. J.

(2) The experimental work described herein was completed just before the death of Prof. Taylor in April, 1936. As the present author is no longer engaged in research on starch or related materials, he relinquishes this field with the hope that others will be interested in continuing this investigation.

(3) (a) Watson and Kent-Jones, U. S. Patent 1,519,014 (1924); (b) Neumann and Kalning, *Landw. Jahrb.*, **61**, 305 (1925); (c) Neumann and Kalmius, *Rass. intern. Agr.*, **444** (1926); (d) Staudt, Can. Patent 274,290 (1927); (e) Frey, U. S. Patent 1,897,251 (1933).

(4) Staudinger and Schweitzer, *Ber.*, **63B**, 2323 (1930).

(5) Hatschek, "Viscosity of Liquids," D. Van Nostrand Co., Inc., New York, 1928, pp. 196–201.

(6) Craik, *J. Soc. Chem. Ind.*, **43**, 171 (1924).

(7) Rassow and Lobenstein, *Kolloid Beihefte*, **33**, 179 (1931).

(8) Amick, *J. Phys. Chem.*, **31**, 1441 (1927).

(9) Taylor, Fletcher and Adams, *Ind. Eng. Chem., Anal. Ed.*, **7**, 321 (1935).

expressed in this paper as the "initial reducing value."

Three requirements must be met if the first stages of the reaction between β-amylose (used instead of starch because of its greater homogeneity) and any desired reagent are to be followed quantitatively. (1) The reagent must act slowly; chlorine water and alkaline hypochlorite were used at 30°. (2) A simple process for stopping the reaction quickly and completely must usually be devised; such a process will be given later in this paper. (3) Methods of magnifying and estimating slight physical or chemical changes in the carbohydrate must be employed; viscosity determinations were used for following physical changes, and the estimation of the "initial reducing value" and alkali-labile value was used for magnifying chemical changes in reducing power.

Outline of Experimental Results

By these means it was found that the reducing power and viscosity changed in two distinct ways depending on the *p*H of the halogen solution.

(1) In alkaline solutions (*p*H 10 or above) the reducing power of the β-amylose decreased gradually from that of the untreated β-amylose to substantially zero after several days' contact with the hypochlorite. The viscosity was not affected.

(2) In a neutral or acid solution the reducing power and viscosity changed in an entirely different and hitherto unsuspected way. During the first few minutes after mixing any one of four β-amylose dispersions with chlorine water almost nothing appeared to happen. The quiescent period lasted for three or four hours unless a phosphate buffer was added in order to keep the *p*H near 7. In the presence of the buffer this period was shortened to two minutes. This inactive phase of the reaction was terminated in every case by a sudden, large change in the reducing power and viscosity. The reducing power, measured both as "initial reducing value" and as alkali-labile value, rose sharply. Simultaneously, the viscosity dropped markedly. Hatschek[5] has pointed out that the viscosity of highly hydrated colloidal materials, such as starch or β-amylose,[10] varies directly with particle size. Consequently, the close inverse relationship found between reducing power and viscosity indicates strongly that the sensitivity of β-amylose to chemical attack is at least partly dependent on its particle size. This point will be developed further.

After this sudden reaction was complete, the reducing power and viscosity of the unbuffered dispersions remained relatively constant throughout thirty days' further contact with chlorine water. However, the reducing power of the buffered dispersions dropped gradually toward zero just as it did in the alkaline solutions mentioned above. The viscosity likewise was unaffected.

Theory of Reaction Mechanism

These changes in reducing power and viscosity, both in alkaline and in acid solution, can most easily be understood if we accept the micellar structure of starch proposed by Meyer and Mark[11] and elaborated by Taylor and Keresztesy.[12,13]

By using this concept of the starch micelle the action of chlorine water on β-amylose in both alkaline and acid solutions can be accounted for as the effect of one or more of three reactions—oxidation, disassociation and degradation.

In alkaline medium the major reaction is the oxidation of aldehyde groups to carboxylic groups. Hatschek's correlation[5] between viscosity and particle size, mentioned previously, leads us to the conclusion that there has been little disassociation of the micelle. Otherwise the viscosity would not have been found to remain constant throughout the reaction.

In a neutral or slightly acid medium the reaction possibly begins in the same manner as in an alkaline one: *viz.*, oxidation of aldehyde groups. However, it seems necessary to postulate that the chlorine water acts in a second, and more important, manner on the β-amylose micelle. This reagent seems to catalyze the working of the water molecules between the glucoside chains, thereby swelling the micelle and putting it under strain. As long as this strain is less than that necessary to break the coördinate linkages, the micelle will remain intact. Further swelling causes the micelle to be ruptured abruptly into its constituent chains, uncovering the shielded aldehyde groups. This disassociation should, and does, cause the re-

---

[10] Kolkmeijer and Favejee, *Z. Krist.*, **88**, 226 (1934).

[11] Meyer and Mark, "Der Aufbau der hochpolymeren organischen Naturstoffe," Akademische Verlag., Leipzig, 1930.

[12] Taylor and Keresztesy, *Ind. Eng. Chem.*, **28**, 502 (1936).

[13] Throughout this paper the terms "disassociation" and "hydrolytic scission" will be used as they are by Taylor and Keresztesy. The term "degradation" will be used to refer to a shortening of the glucoside chains when we do not wish to distinguish between hydrolytic carbon-oxygen scission and carbon-carbon scission of a type similar to that proposed by Evans [*Chem. Rev.*, **6**, 28 (1929)] for the action of dilute alkali on glucose.

ducing power to rise and the viscosity to drop.

Before disassociation the micelle is probably not subject to much degradation because the most sensitive portions of the chains, the aldehyde groups and the glucoside ether linkages, are shielded from attack. However, after the sudden separation of the particle into the individual chains, degradation should proceed much more easily. The extent of degradation can be found approximately by subjecting the dispersion, after treatment with chlorine water, to the standard procedures for hydrolyzing the carbohydrate to glucose[14] and estimating it as such.[15] Although it was found that the "initial reducing value" has no simple stoichiometric relation to the β-amylose, it can be used to estimate glucose quantitatively by means of the equation

$$C_5H_{11}O_5CHO + I_2 + 3NaOH \longrightarrow C_5H_{11}O_5COONa + 2NaI + 2H_2O$$

The calculated "initial reducing value" for the glucose obtained from $(C_6H_{10}O_5)_x$ is 163.3. If we assume with Hirst[16] that the average chain contains 25 glucose residues, and that every aldehyde group is oxidized to carboxylic acid before carrying out the hydrolysis to glucose, the "initial reducing value" would drop approximately 4% to 156.8. Any experimentally obtained value much lower than this figure can be explained only by assuming either that the chain length is less than that given by Hirst or that degradation has occurred. The former assumption is considered to be untenable because Hirst's exhaustive methylation experiments prove rather conclusively that 25 is a *minimal* value which may possibly be considerably less than the true one.

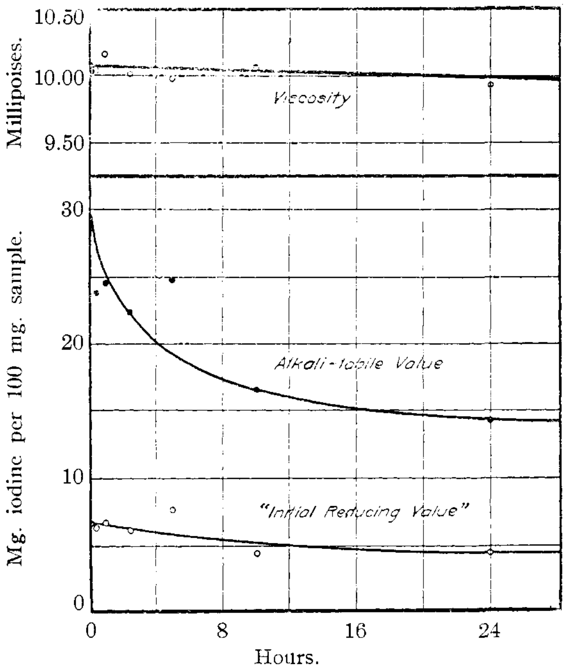

Fig. 1.—Action of alkaline hypochlorite solution on corn β-amylose ground one week.

Action of Alkaline Hypochlorite

A more detailed examination of our experimental results will now be made. In order to obtain these results it became necessary to devise a simple and rapid method of stopping all reaction at any desired time after mixing a water-dispersed β-amylose with a standardized sodium hypochlorite solution at 30°. Our method consisted in acidifying the solution with hydrochloric acid, followed by removal of most of the liberated chlorine as Cl_2 with nitrogen. The last traces of hypochlorous acid were removed from solution by reaction with ethylene, the excess of which was in its turn removed by a second addition of hydrochloric acid and nitrogen. After neutralization and dilution to standard volume the viscosity, "initial reducing value" and alkali-labile value were measured.[17]

The manner in which these three properties changed during the reaction between a sodium hypochlorite solution of pH greater than 13 and a dispersion of corn β-amylose ground for one week is easily seen from Fig. 1. Over thirty days' time the "initial reducing value" and the alkali-labile value dropped gradually and smoothly toward zero, while the viscosity remained constant within the limits of precision of the experiment.[18]

It should be observed that the alkali-labile value always was greater than the "initial reducing value" of the same solution. The importance of this observation will be apparent later.

On hydrolyzing[14] and estimating as glucose[15]

---

(14) (a) Schulz and Steinhoff, *Z. Spiritusind.*, **56**, 63 (1933); (b) Taylor and Salzmann, This Journal, **55**, 264 (1933).

(15) Klein and Acree, *Bur. Standards J. Research*, **5**, 1063 (1930).

(16) Hirst, Plant and Wilkinson, *J. Chem. Soc.*, 2375 (1932).

(17) So far as we are aware, the reaction between hypochlorous acid and ethylene has never before been used in quantitative analysis. The function of each reagent in this process will be given under "Experimental Details."

(18) The variations in the viscosity were caused by the differences in the amount of hydrochloric acid used to stop the hypochlorite reaction. As it seemed imperative that the freed chlorine and hypochlorous acid be removed from solution as rapidly as possible in order to eliminate the possibility of their reacting with the β-amylose, the second addition of hydrochloric acid—5 cc. concd. acid—was made by graduated cylinder with no attempt at high precision. The variations of the "initial reducing value" and alkali-labile value from the smoothed curves are of the expected order of magnitude.[9]

the material remaining after reaction for thirty days at room temperature between β-amylose and hypochlorite, the "initial reducing value" was found to be 90.2. This is only 55% of that calculated for hydrolyzed $(C_6H_{10}O_5)_x$ and only 58% of that calculated for hydrolyzed $C_6H_{11}O_6\cdot(C_6H_{10}O_5)_{23}\cdot C_5H_{10}O_4\cdot COOH$, the acid we have assumed would be formed from Hirst's glucoside chain containing 25 glucose residues.[16]

Another sodium hypochlorite solution of *p*H 10 instead of *p*H 13 was mixed with aliquots of the same corn β-amylose dispersion and tested in the same way in order to find out whether this oxidation–degradation reaction required a strongly alkaline medium. The "initial reducing value," alkali-labile value and viscosity of these aliquots changed in a manner almost identical with those shown in Fig. 1, and the hydrolytic treatment indicated that degradation took place to about the same extent.

Action of Chlorine Water on β-Amyloses

However, as indicated previously, when the reaction between hypochlorite and β-amylose was carried out in a medium more acid than *p*H 7 the viscosity and reducing power changed in a very different manner. In order to study these changes in slightly acid solutions the experimental procedure was modified by bubbling chlorine gas through the dispersions for a few minutes each day until the reaction was to be quenched. The rest of the procedure was identical with that described above.

The marked discontinuity of the viscosity, "initial reducing value" and alkali-labile value of a dispersion of corn β-amylose (ground for two weeks) is strikingly shown in Fig. 2. It will be observed that a high "initial reducing value" is always associated with a high alkali-labile value and a low viscosity, showing that there is a close correlation between reducing power and particle size. It is impossible to show in Fig. 2 the course of the reaction after the first seven hours' contact between chlorine water and carbohydrate. The viscosity and "initial reducing value" were found to remain constant within the limits of the precision of the measurement up to thirty days' reaction time, the final values being, respectively, 38.6 mg. of iodine per 100 mg. of sample and 8.97 millipoises. The alkali-labile value dropped gradually from its peak of 59.5 to a final value of 38.7.

As it might be thought that the absence of sodium ion during this reaction of β-amylose with chlorine water was responsible for the great differences between Figs. 1 and 2 this experiment in acid medium was repeated in the presence of the same amount of sodium ion, as sodium chloride, which was used in the solutions of *p*H 13. The viscosity and reducing power curves so obtained were similar to those of Fig. 2. The salt raised the viscosity of all the solutions to the same extent.

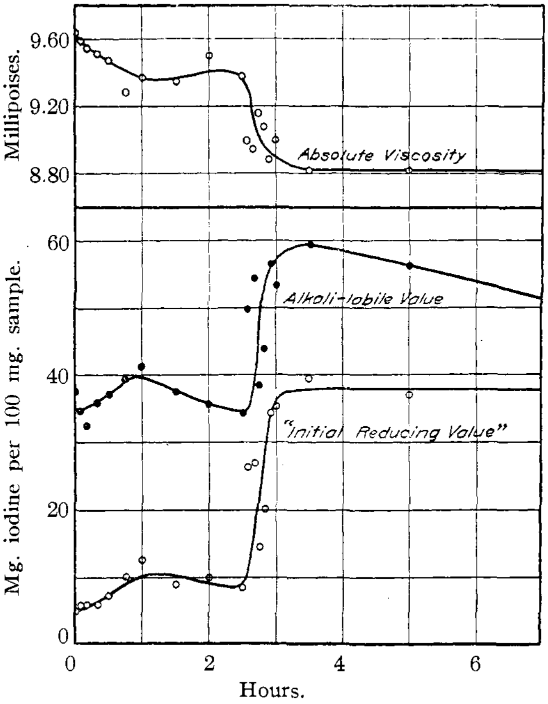

Fig. 2.—Action of chlorine water on corn β-amylose ground two weeks.

A dispersion of β-amylose from corn starch ground for one week behaved in a manner almost identical with that of the dispersion ground for two weeks except that the sudden change in magnitude of the viscosity and reducing power occurred one hour later—between two hundred and ten and two hundred and forty minutes after beginning to bubble chlorine into the dispersion. This agrees with the observation[12] that grinding of starch for a time longer than one week continues to break it down in some way not yet well understood.

Dispersions of β-amylose from tapioca and potato starches ground two weeks were also subjected to the action of chlorine water under the same conditions. The tapioca β-amylose behaved in the same manner as the corn β-amylose shown in Fig. 2. The potato β-amylose had a somewhat higher "initial reducing value" and alkali-labile value, and lower viscosity before treatment with chlorine water than the corn or tapioca. Furthermore, during the course of the reaction of chlorine water on potato β-amylose these three properties changed less abruptly and less in magnitude.

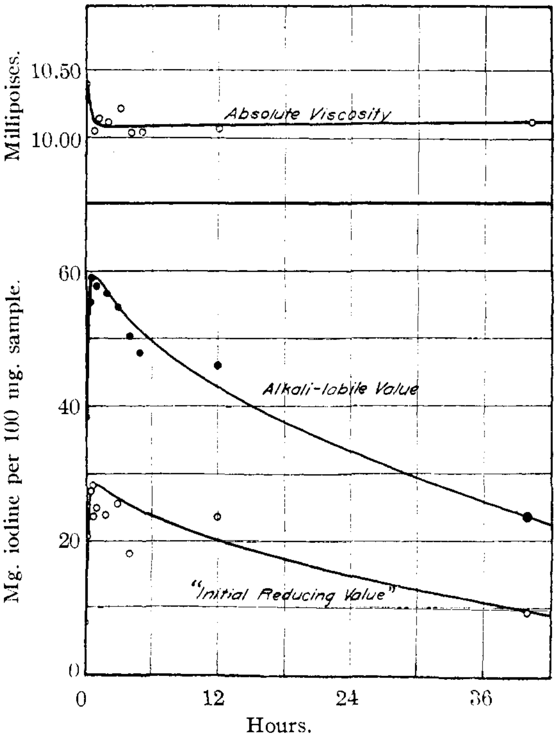

Fig. 3.—Action of chlorine water buffered by potassium phosphate on corn β-amylose ground one week.

As all four of the β-amylose dispersions mentioned above had considerable reducing power even after thirty days of contact with chlorine water—both the "initial reducing value" and alkali-labile value were between 30 and 40 in every case—it was thought probable that one or more of the products had stopped all reaction. Bunzel and Mathews[19] have shown that hydrogen ion has exactly this effect on the reaction between glucose and chlorine water. Therefore, a potassium phosphate buffer of pH 7 was mixed with a β-amylose dispersion from corn starch ground for one week. Table I shows to what extent the buffer held the pH constant. Solubility limitations forbade the use of more buffer.

TABLE I

pH OF β-AMYLOSE AND BUFFER SOLUTIONS AFTER MIXING WITH CHLORINE

Material	No chlorine	Chlorine for 1 hour	Chlorine for 5 hours	Chlorine for 40 hours
β-Amylose dispersion[a]	7.2	1.50	1.06	..
β-Amylose dispersion + buffer[b]	...	2.49	..	2.08

[a] From cornstarch ground for one week, 4.000% by weight. [b] Another 25.00-cc. aliquot of the same β-amylose to which had been added 4.36 g. of K_2HPO_4 and 1.22 g. of KH_2PO_4. A solution of the same quantities of these two salts in 25.00 cc. of distilled water had a pH of 6.92.

However, even this relatively minor change in pH between the buffered and unbuffered solutions of corn β-amylose was sufficient to accelerate the chlorine water reaction tremendously and to drive it practically to completion in so far as reducing power is a criterion.[20] A comparison of Figs. 3 and 2 indicates this difference in a striking manner. (The change of grinding time of the corn starch from two weeks to one is insignificant as regards the contrast between Figs. 2 and 3.) The "initial reducing value" and alkali-labile value continued to drop, although less rapidly because the hydrogen ion concentration was rising, during reaction periods longer than forty hours, the final point shown in Fig. 3. The viscosity remained almost constant. The three values after six hundred seventy-two hours of reaction time were, respectively, 3.0 and 9.7 mg. of iodine per 100 mg. sample and 9.95 millipoises. Treatment of a portion of this sample with hot hydrochloric acid did not change the reducing values, indicating that degradation had been so complete that all the chains had been broken into fragments of less than six carbons.

It is not possible to say whether this degradation is hydrolytic or not. However, we tend toward the view that it proceeds largely by scission of carbon-carbon bonds, perhaps by some mechanism related to the "enolization" breakdown of glucose in the presence of alkali as postulated by Evans.[21] We feel that the observation that all the reactions between glucose[19] or β-amylose and chlorine water are decreased in velocity by the presence of acid lends weight to this viewpoint. Further-

---

[19] (a) Bunzel and Mathews, THIS JOURNAL, **31**, 464 (1909); (b) Bunzel, *J. Biol. Chem.*, **7**, 157 (1909).

[20] There is no indication that these changes in reaction rate and degree of conversion to material without reducing power are due *solely* to the action of the phosphate *as a buffer*. Phosphate *ion* greatly catalyzes the breakdown of glucose by chlorine water or hypochlorite to products containing less than six carbons at the expense of the formation of gluconic acid [Bleyer and Braun, *Biochem. Z.*, **180**, 105 (1927); **183**, 310 (1927); **199**, 186 (1928); Bernhauer and Schön, *ibid.*, **202**, 159 (1928)]. Apparently no one has heretofore considered the possibility of an analogous action of phosphate *ion* on starch or β-amylose.

[21] Evans, *Chem. Rev.*, **6**, 28 (1929).

more, Table II shows that hydrochloric acid had almost no effect on the "initial reducing value" and alkali-labile value of a dispersion of corn β-amylose ground for two weeks. This observation confirms that of Daish.[22]

TABLE II

ACTION OF HYDROCHLORIC ACID ON CORN β-AMYLOSE GROUND TWO WEEKS

(Reaction time 43 hours at room temperature)

Material	"Initial reducing value," mg. iodine/100 mg. sample	Alkali-labile value, mg. iodine/100 mg. sample
25.00 cc. 4.000% β-amylose	7.1	39.9
Same + 1.00 cc. 0.1 *M* HCl	6.0	37.8
Same + 1.00 cc. 1.0 *M* HCl	6.0	38.6
Same + 1.00 cc. 13 *M* HCl	5.7	37.3
Same + 5.00 cc. 13 *M* HCl	9.6	43.0

Action of Chlorine Water on Glucose

The conclusions already drawn concerning the mechanism of the reaction between β-amylose and chlorine water from the data presented above were deduced in part from a similar investigation of the reaction between glucose and chlorine water. It has been well established[23] that glucose is attacked in two distinct ways by chlorine or bromine water, dependent on the presence or absence of other substances. In the presence of a weakly alkaline buffer, such as barium carbonate, glucose is oxidized chiefly to gluconic acid without much carbon-carbon scission.[24] In the presence of a strong base or phosphate ion, and in the absence of any reagent other than the chlorine water itself, glucose is degraded completely into one- and two-carbon acids.[19,23]

From Fig. 4 it will be observed that glucose is converted gradually to products without reducing power, as indicated by the drop in the "initial reducing value" and alkali-labile value. The viscosity remained almost constant. Superficially these curves are similar to those of Fig. 1, but there is one fundamental difference. The "initial reducing value" of the glucose solutions is always *higher* than the corresponding alkali-labile value, whereas the "initial reducing value" of each β-amylose solution is always *lower* than the corresponding alkali-labile value. This agrees with previous work[14b] and indicates that the material present after treatment of β-amylose with alkaline hypochlorite or chlorine water cannot be glucose. Due to lack of space, Fig. 4 does not show the course of the reaction beyond the first seven hours with chlorine water. The "initial reducing value" and alkali-labile value continued to drop, although more slowly, because, as already mentioned, the rising concentration of hydrogen ion tends to stop all reaction. The viscosity showed no trend. The final values after thirty days of treatment were: "initial reducing value" 20.9 mg. iodine per 100 mg. sample, alkali-labile value 21.6, and viscosity 8.73 millipoises. The possibly fortuitous identity of the "initial reducing value" and the alkali-labile value suggests that there may be some close connection between the mechanism of the chlorine water reaction on glucose (or possibly β-amylose) and the mechanism of the alkali-labile digestion. This possibility was not investigated.

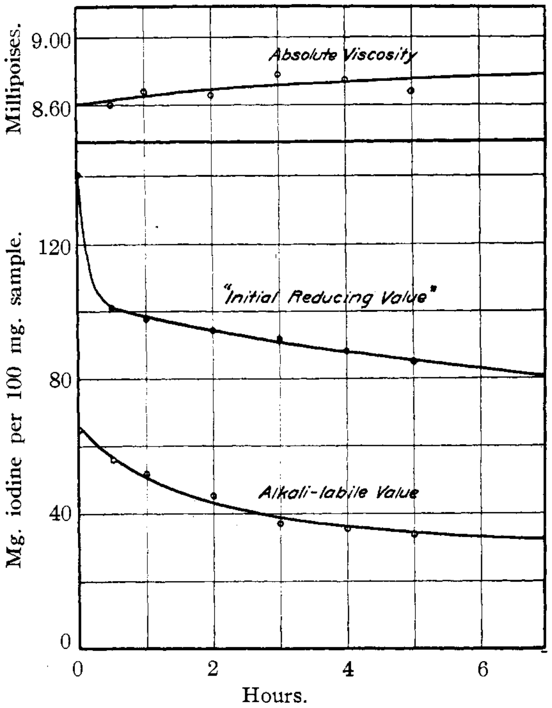

Fig. 4.—Action of chlorine water on glucose.

It was pointed out above that other workers have found that gluconic acid is not formed to any extent from glucose by chlorine water in the absence of other reagents. Table III substantiates this observation to the extent that it indicates that gluconic acid, if formed at all, is attacked in its turn. No attempt was made to follow this investigation further.

---

(22) Daish, *J. Chem. Soc.*, **105**, 2053 (1914).

(23) (a) Bleyer and Braun, *Biochem. Z.*, **180**, 105 (1927); **183**, 310 (1927); **199**, 186 (1928); (b) Bernhauer and Schön, *ibid.*, **202**, 159 (1928).

(24) (a) French Patent 635,603 (1927); British Patent 289,280 (1927); British Patent 293,322 (1928); (b) Honig and Ruziczka, *Ber.* **63**, 1648 (1930); (c) Isbell and co-workers, *Bur. Standards J. Research*, **8**, 327, 614 (1932); **10**, 337 (1933).

TABLE III

ACTION OF CHLORINE WATER ON *d*-GLUCONIC ACID[a]

Treatment	"Initial reducing value" mg. iodine per 100 mg. sample	Alkali-labile value mg. iodine per 100 mg. sample
None	1.5	1.8
2.5 hours chlorine water at 30°	29.1	19.6
25.0 hours chlorine water at room temperature	25.9	20.8

[a] Prepared according to the directions of Isbell and Frush [*Bur. Standards J. Research*, 11, 649 (1933)], seeding with crystals furnished by Dr. Isbell; m. p. 125.7°, corr. It was dissolved in distilled water in a concentration of 4.000 g. per 100 cc. of solution just before use.

Experimental Details

Commercial whole starches were ground in a ball mill[25] for the designated periods of time and then dispersed in hot distilled water. The α-amylose was removed by electrophoretic separation,[26] using platinum electrodes. Each dispersion was made up to 4.000 ± 0.005 g. per 100 cc. of solution (determined by evaporating 50-cc. samples to dryness at 80°, followed by three hours at 100° or to constant weight). Toluene was added to prevent bacterial action.

For each determination 25.00 ± 0.02 cc. of the dispersion, free from toluene droplets, was pipetted into a 250-cc. Drexel tall form gas wash bottle modified by sealing a tube containing glass beads onto the outlet in place of the regular exit tube to reduce loss by spattering to a negligible amount. All reactions lasting less than eight hours were carried out in a water-bath kept at 30.00 ± 0.03°. Reactions of longer duration were done at room temperature in the dark. Chlorine was bubbled through the dispersion for forty minutes[27] at 0.75 liter per minute (N. T. P.). More chlorine was added from time to time to be sure that an excess was always present. The reaction[27] was stopped by the following procedure:

Operation	Rate of bubbling in liters per minute (N. T. P.)	Operation time, min.
Nitrogen cylinder connected	...	0.25
Nitrogen (A. R. Co.) bubbled	2.0	2.0
Cylinders interchanged	...	0.25
Ethylene (Ohio Chem. Co. anaesthesia) bubbled	1.6	6.0
Cylinders interchanged and 5.0 cc. concd. HCl added	...	0.5
Nitrogen bubbled	2.0	30.0
Total quenching time 39.0 minutes (stop watch)		

The dispersion was then neutralized to phenolphthalein (0.05% in 50% synthetic methanol) as quickly as possible with almost saturated sodium hydroxide solution, nitrazine yellow[28] added and neutralization completed with 0.1 *M* hydrochloric acid.[29] The solution was then made up to 100.0 cc., protected against bacteria with two drops of toluene, and kept in glass-stoppered bottles for analysis.

The alkaline hypochlorite solutions were made as follows: A. Fifty grams of sodium hydroxide in 250 cc. of distilled water was treated with excess chlorine with chilling, and then 20 g. more sodium hydroxide was added. The solution was diluted to 600 cc. and kept in a "Nonsol" bottle in the ice-box until used. Available chlorine—4.33 g. Cl/100 cc. solution[30]; *p*H—above 13 (with LaMotte Purple indicator).

B. Eighteen grams of sodium hydroxide and 40 g. of anhydrous sodium carbonate in 400 cc. of distilled water were partly neutralized by 20 g. of chlorine with chilling. The solution was made up to 500 cc. and handled like "A." Available chlorine—3.20 g. Cl/100 cc. solution. *p*H—9.7 (corrected for the effect of the potassium ion on the glass electrode).

5.00 cc. of one of these two hypochlorite solutions was added to the β-amylose dispersion by pipet. To stop the reaction 2.0 cc. of concentrated hydrochloric acid was added just before beginning the nitrogen–ethylene procedure.

The viscosity was measured with an Ostwald viscometer having a time of outflow for distilled water of sixty to sixty-five seconds at 30.00 ± 0.03°. Each viscosity figure is the average of those obtained on at least two aliquots from the same sample. Each aliquot was run through the viscometer 5 or 6 times, a. d. 0.5–1.0%. The average readings of the two aliquots were within 2% of each other. The density determinations were made as directed by Findlay,[31] using a pycnometer containing about 7 cc. of solution. Weighings were made to the nearest milligram, corrected for the buoyancy of air.

The alkali-labile determinations[32] were made by the standard method[9] except for two minor changes. (1) 5.00-cc. aliquots of the carbohydrate solution (1.000 g. carbohydrate per 100 cc. solution) were pipetted into test-tubes with 5.00 cc. of standardized 0.2 *N* sodium hydroxide. (2) During the first ten minutes of the alkaline digestion in boiling water the corks were removed from the test-tubes to allow the remaining ethylene or other volatile reducing material to steam off. This procedure gave a smaller and more reproducible alkali-labile value for the blank determinations, and had no effect on the value for untreated β-amylose.

---

(25) Taylor and Beckmann, THIS JOURNAL, **51**, 294 (1929).

(26) Taylor and Iddles, *Ind. Eng. Chem.*, **18**, 713 (1926).

(27) Reaction time is the period from the beginning of chlorine or hypochlorite addition to the beginning of the quenching process. In some cases it is less than forty minutes. Nitrogen alone will not remove all of the chlorine (hypochlorite), giving low reducing values. If the hydrochloric acid is not added the ethylene will not be completely removed by the nitrogen in any reasonable length of time, giving high and less reproducible reducing values.

(28) Wenker, *Ind. Eng. Chem.*, **26**, 350 (1934).

(29) If the latter indicator is added earlier it will be inactivated at the point where the concentrated alkali strikes the solution. Even without an indicator the solution can be approximately neutralized because it turns darker yellow rather suddenly when made alkaline. This color change is reversible in acid.

(30) Treadwell and Hall, "Analytical Chemistry," John Wiley and Sons, Inc., New York, Vol. II, 1924, p. 568.

(31) Findlay, "Practical Physical Chemistry," 6th ed., Longmans, Green and Co., New York, 1935, p. 72.

(32) As the ordinary determination of the alkali-labile value, in which 50-mg. samples of the starch or β-amylose are weighed into the test-tubes, does not take into account the water content of 8–10% in most starches the values for untreated β-amyloses reported in this paper are approximately 10% higher than comparable ones reported earlier.[9]

The following blank values were subtracted from the observed reducing values to correct for the non-removable reducing material introduced by the nitrogen–ethylene quenching process.

Solution	"Initial reducing value," mg. iodine/100 mg. sample	Alkali-labile value, mg. iodine/100 mg. sample
Unbuffered chlorine water	0.02 ± 0.01	0.08 ± 0.02
Chlorine water buffered by phosphate	0.04 ± 0.01	0.05 ± 0.01
Alkaline hypochlorite solution	0.02 ± 0.01	0.08 ± 0.01

The hydrolysis of β-amylose dispersions was carried out as follows: 5.00 cc. of the chlorine-treated solution containing 1.000 g. of carbohydrate per 100 cc. of solution of *p*H 7 were pipetted into a Pyrex test-tube and mixed with 5 cc. of 2 *M* hydrochloric acid. The mixture was heated in a boiling water-bath for thirty-five minutes, cooled, almost neutralized with almost saturated sodium hydroxide solution and accurately neutralized with 0.1 *N* alkali to nitrazine yellow. The "initial reducing value" was then determined as usual.

Acknowledgment.—The authors wish to thank Prof. J. M. Nelson, Dr. A. F. Knoll and Dr. C. O. Beckmann for their valuable assistance in preparing this paper. They are grateful also to Dr. H. B. Hardt for carrying out the *p*H measurements, using the glass electrode circuit of Ellis and Kiehl.[33]

Summary

1. The action of chlorine water and of alkaline hypochlorite on β-amylose has been followed quantitatively throughout its course at room temperature by measurement of the reducing power and viscosity of the β-amylose.
2. In alkaline medium the reaction is primarily an oxidation of the aldehyde groups at the ends of the glucoside chains, but is accompanied by some disintegration of the carbohydrate.
3. In acid medium the reaction proceeds differently. After a short time during which no apparent reaction occurs there is a sudden rise in reducing power and simultaneous drop in viscosity. If the acid generated during the reaction is neutralized this abrupt reaction is followed gradually by a complete breakdown of the carbohydrate to products without reducing action toward alkaline hypoiodite reagent.
4. These changes in the reducing power and viscosity can be explained easily by assuming that β-amylose exists in the form of the micelle postulated by Meyer and Mark.
5. A rapid method for the quantitative removal of hypochlorous acid from aqueous solutions has been described.

NEW YORK, N. Y. RECEIVED APRIL 5, 1938

---

[33] Ellis and Kiehl, *Rev. Sci. Instruments*, **4**, 131 (1933).

[CONTRIBUTION NO. 132 FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, THE UNIVERSITY OF TEXAS]

Nitrogen Compounds from Petroleum Distillates. XII. Fractional Sulfiting of Bases and Fractional Degassing of their Acid Sulfites[1,2]

BY STILES M. ROBERTS AND J. R. BAILEY

Introduction

It is well known that acid sulfites of organic nitrogen bases dissociate on heating their aqueous solutions into base, sulfur dioxide and water.

$$\text{Base } H_2SO_3 \longrightarrow \text{Base} + SO_2 + H_2O$$

In this paper, there is described how, by employing the velocity of dissociation of acid sulfites at variable temperature levels, a process of "Fractional Degassing" has been developed which can be used to advantage in resolution or organic nitrogen base mixtures into component types. This procedure is applicable especially to petroleum and coal tar base fractions.

As an experimental background for the process, a crude fraction of kero bases (kerosene bases) in the 270–280° boiling range was selected. Here have been isolated previously 2,3- and 2,4-dimethylquinoline,[3] 2,3,8-trimethylquinoline,[4] 2,4,-8-trimethylquinoline,[5] and a $C_{16}H_{25}N$ naphthenic base.[6]

The 270–280° fraction of kero bases may be arranged in three different types.

---

[1] Presented before the Petroleum Division of the American Chemical Society, Dallas, Texas, April 19, 1938.

[2] "Sulfiting" implies conversion of bases to acid sulfites and "degassing" implies dissociation of the acid sulfites with the liberation of free bases and sulfur dioxide.

[3] Burnard S. Biggs and J. R. Bailey, THIS JOURNAL, **55**, 4141 (1933).

[4] W. A. King and J. R. Bailey, *ibid.*, **52**, 1245 (1930).

[5] T. S. Perrin and J. R. Bailey, *ibid.*, **55**, 4136 (1933).

[6] W. C. Thompson and J. R. Bailey, *ibid.*, **53**, 1002 (1931).