[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

Studies on the Schardinger Dextrins. The Preparation and Solubility Characteristics of Alpha, Beta and Gamma Dextrins[1]

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A distinctive character of the Schardinger dextrins[3,4,5] which facilitates their separation from crude starch digests is their ability to form crystalline insoluble complexes with many liquids notably the hydrocarbons and halogenated hydrocarbons.[6] In a search for superior precipitants for the Schardinger dextrins, the authors have collected data on the precipitation efficiency of several organic liquids. The solubilities of the pure dextrins in water and aqueous propanol, and of the dextrin acetates in a few selected solvents have been measured. These data have been incorporated into a scheme for the separation and purification of the Schardinger dextrins which does not require the acetylation and saponification steps used by Freudenberg.[4] Aqueous propanol has been found doubly useful in the purification of the dextrins since it promotes sharp crystallization with a minimum of clouding or gum formation and the crystal forms of the individual dextrins are so characteristic that it is readily possible to identify the individual dextrin propanol complexes under the microscope (Figs. 1, 2, 3, 4).

That varying the enzymolysis conditions affects the total yield as well as the proportion of the Schardinger dextrins has been recorded by McClenahan, Tilden and Hudson.[5a] These observations have been corroborated and utilized so that the starch digestion is carried out under conditions most favorable for the individual compound sought. In particular, it has been possible to obtain the gamma dextrin which has hitherto been mentioned only by Freudenberg.[4] The modifications of enzymic and separative procedures are presented in detail in the experimental section.

Experimental

Preparation of the Schardinger Dextrins.—In the early part of this study, enzyme digestion mixtures[5] were separated by the method of Freudenberg and Jacobi.[4a] In this way crude dextrin stocks were acquired and the individual compounds were purified by repeated crystallization. In later preparations the enzymolysis conditions have been varied according to the individual dextrin which is desired. In order to allow for differences in amount of enzyme, concentration of substrate and temperature of the reaction, it is convenient to describe the duration of enzymolysis in terms of *conversion periods*. A conversion period may be defined as that time of reaction between enzyme and substrate which under the conditions would be just sufficient to convert an equal weight of starch to the Tilden and Hudson end-point.[5b]

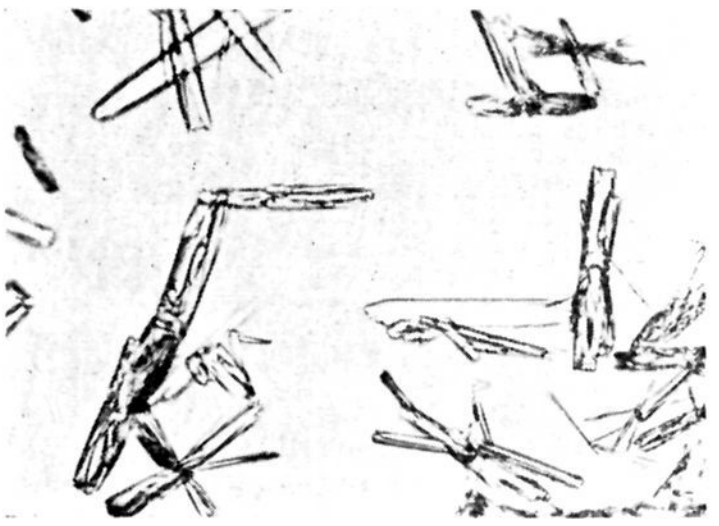

Fig. 1.—Alpha dextrin from 60% *n*-propyl alcohol, blade form.

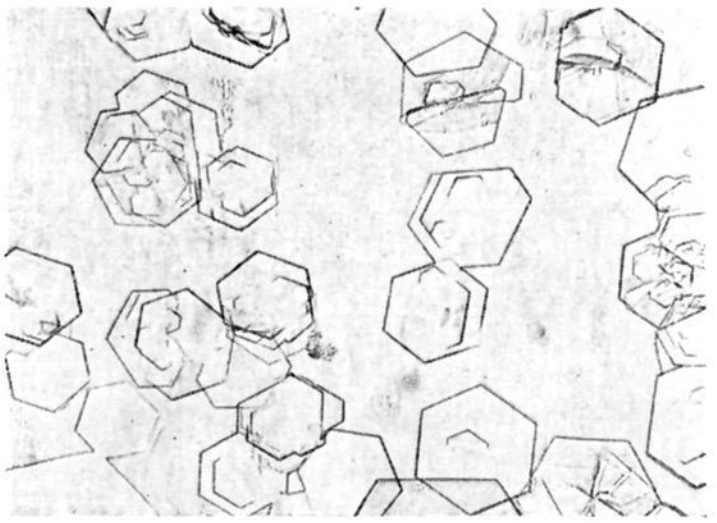

Fig. 2.—Alpha dextrin from 60% *n*-propyl alcohol, hexagonal form.

Macerans Amylase.—The enzyme used was prepared according to the directions of Tilden and Hudson.[5b] In order to prepare the gamma dextrin in good yield it is especially important that the enzyme be free from hydrolytic amylases. This can be determined by incubating a sample of starch with the enzyme for 50 conversion periods (using the conditions of the Tilden test, for example). At the end of this time the digest should show no more reducing power than the starch originally used, as indicated by copper or iron methods. Preparations with slight hydrolytic activity may be used for short conversions, as in the preparation of alpha dextrin.

Alpha Dextrin.—Enzymolysis of a 3–5% potato starch paste for 2–6 conversion periods in the absence of a dextrin

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(3) Schardinger, *Zentr. Bakt. Parasitenk*, II **29**, 188 (1911).

(4) (a) Freudenberg and Jacobi, *Ann.*, **518**, 102 (1935); (b) Freudenberg, Plankenhorn and Knauber, *Chemistry & Industry*, 731 (1947). Substantially the same information is included in *Ann.*, **558**, 1 (1947), and FIAT Report No. 1096.

(5) (a) McClenahan, Tilden and Hudson, THIS JOURNAL, **64**, 2139 (1942); (b) Tilden and Hudson, *J. Bact.*, **43**, 527 (1942).

(6) Pringsheim, "Bacterial Degradation and Constitution of Starch," in "A Comprehensive Survey of Starch Chemistry," ed. Walton, The Chemical Catalog Co., Reinhold Publishing Corp., New York, N. Y., 1928, p. 35.

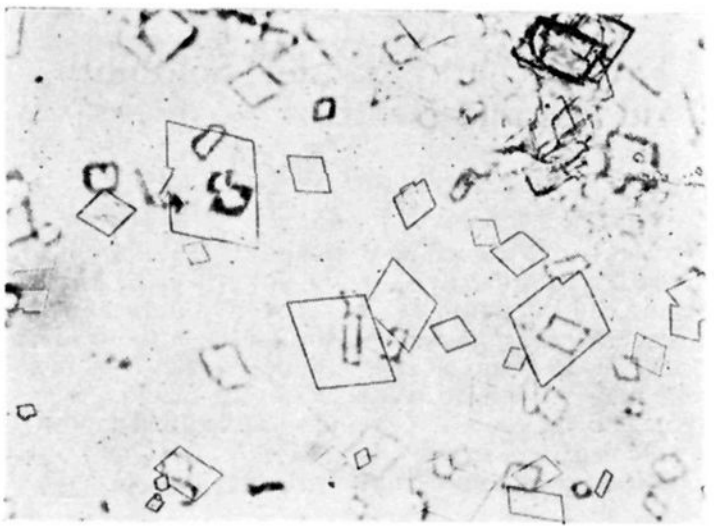

Fig. 3.—Beta dextrin from 60% *n*-propyl alcohol.

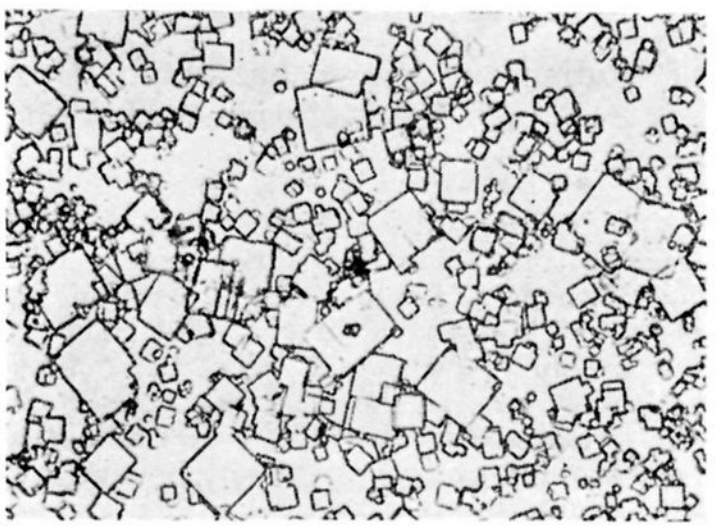

Fig. 4.—Gamma dextrin from 60% *n*-propyl alcohol.

precipitant (*i.e.*, the reaction mixture should be sterile or protected against microörganisms by a microbial inhibitor such as thymol) yields a mixture from which alpha dextrin can be readily obtained by concentration to about 15% solids, precipitation with trichloroethylene and centrifugation of the crystalline complex.[7] The crude precipitate is suspended in water, boiled to remove trichloroethylene, treated with carbon and filtered. After cooling and diluting to about 2% solids concentration, bromobenzene is added to effect precipitation of any beta or gamma dextrin which may be present. The mixture is best stirred with a mechanical stirrer or shaken overnight to insure complete equilibration with the bromobenzene. The resulting suspension is filtered with suction, the filtrate boiled down to about 40% of the original volume and the bromobenzene precipitate, which is usually small, worked up with beta-gamma crudes. The solution of crude alpha dextrin is now treated with trichloroethylene or tetrachloroethane by stirring or shaking overnight and the crystalline complex separated by suction filtration. The precipitate is air-dried, boiled with about 5 parts of water until the vapors are free from precipitant, clarified by filtration with carbon if necessary, treated with 1.5 volumes of *n*-propyl alcohol, and allowed to cool. The crystalline propyl alcohol complex is filtered off after several days and recrystallized by dissolving the air dry material in four parts of hot water and adding 1.5 volumes of propyl alcohol. The final crystallization may be carried out by dissolving the pure propanol complex in water, boiling off the propanol, concentrating the solution to about 35% solids and allowing to crystallize by evaporation at room temperature for a day or two. (Alcohol-free solutions of the dextrins should be guarded against mold growth by covering while hot.) The crystals are filtered and allowed to air dry. On drying in the vacuum oven the crystals lose about 10% moisture; the specific rotation of the anhydrous material is $[\alpha]_D + 150.5° \pm 0.5°$ (*c* 1, water).[8]

Beta Dextrin.—Enzymolysis of 3–5% paste of potato or waxy corn starch is carried out without a precipitant for 2–3 conversion periods. The digest, which is now very fluid, is clarified by filtration through diatomaceous earth, fresh enzyme added, layered over with toluene and enzymolysis allowed to proceed for 30–50 conversion periods. Beta dextrin–toluene complex separates during the digestion and to secure a maximum yield of dextrin the digestion vessel should be agitated from time to time to promote contact between the toluene and the enzymolysis mixture. After the conversion the precipitate is most conveniently separated by the supercentrifuge. The crude material is suspended in boiling water, toluene removed by boiling until there is no odor of toluene in the vapor, treated with carbon, and the boiling hot solution filtered with pressure through a clarifying filter.[9] The concentration at this point should not exceed 20–25% solids; higher concentrations give trouble through crystallization of the beta dextrin during filtration. If it is necessary to use suction filtration, this may be carried out at a concentration of 10% solids, and the filtrate concentrated by boiling to the 20–25% level. The filtrate should be perfectly clear; if not, it is filtered hot through successively finer filters, with carbon and filter aid if necessary. The resulting solution is allowed to cool, whereupon large crystals of beta dextrin are formed. After standing overnight in the cold, the crystals are filtered, washed, and purified by repeated crystallization from 4–5 parts of boiling water. In this way is obtained a product which is ash-free, completely soluble in water to give a clear solution, and which contains about 14% water of crystallization. After drying to constant weight at 70° in the vacuum oven, the rotation is $[\alpha]_D +162.5° \pm 0.5°$ (*c* 1, water)[8].

Gamma Dextrin.—The enzymolysis with potato or waxy maize starch is carried out in the same manner as described above for beta dextrin, except that after the initial brief conversion and clarification, the reaction is allowed to proceed in the absence of a precipitant for 30–50 conversion periods. Gamma dextrin is scarcely detectable in the early phases of the enzymolysis when the alpha dextrin concentration is at a maximum. If the reaction mixture is not sterile, thymol may be used to inhibit molds. When the conversion is complete, the mixture is concentrated to about one-fourth the original volume, stirred with trichloroethylene, and the precipitate separated by centrifugation (see ref. 7). The precipitate is dissolved in boiling water, clarified, concentrated to about 25% solids, and allowed to stand at room temperature a day or two. Seeding with beta dextrin may be advisable to promote fairly complete removal of this component. The precipitate is removed and the clear filtrate is diluted to about 3% solids. This solution is stirred overnight with bromobenzene and the resulting suspension filtered with suction. The precipitate is washed, dissolved in boiling water and boiled to remove the bromobenzene, concentrated to about 20% solids and treated with 1.5 volumes of *n*-propyl alcohol. After standing for a few days at room temperature, the crystals of gamma dextrin–propanol complex are removed by suction filtration and allowed to air dry. Gamma dextrin is best purified by dissolving the air-dry complex in 4 parts of boiling water, filtering with carbon if the solution is colored or turbid, and treating the resulting solution with 1.5 volumes of *n*-propyl alcohol. Recrystallization should be repeated until there is no evidence for alpha or beta dextrins in the 60% propanol mother liquors. Generally two or three recrystal-

(7) The Sharples Supercentrifuge is very useful for this type of separation.

(8) Specific rotations previously reported are: ref. 4a, alpha +148°, beta +158°, gamma +160°; ref. 4b, alpha 148 ± 2°, beta 158 ± 2°, gamma +169 ± 2°; ref. 5a, alpha +150.5 ± 0.5°, beta 162.5 ± 0.5°.

(9) Horm laboratory pressure filter, F. J. Horman and Co., Brooklyn, N. Y.

lizations are required. The propanol cannot be removed from this complex by either air or vacuum drying, but is readily removed by boiling in water. By concentrating a boiled aqueous solution to 40–50% solids and standing at room temperatures a few days, large clear crystals of gamma dextrin hydrate are formed. The crystals effloresce in air; the air dry material loses 8.3% of its weight on drying at 70° in the vacuum oven. The specific rotation of the anhydrous material is $[\alpha]_D + 177.4 \pm 0.5°$ (c 1, water).[8]

Identification of the Schardinger Dextrins.—The dextrins form characteristic crystalline complexes with iodine and potassium iodide which permit the positive identification of alpha dextrin. If alpha dextrin is present to the extent of only 2–5% of the carbohydrate mixture, it forms blue-black hexagonal crystals when a solution containing a little 0.1 N iodine in 0.1 M potassium iodide is allowed to evaporate on a microscope slide.[3,5b] If the proportion of alpha dextrin is higher, needles exhibiting a colorless–blue-black dichroism are formed. These may be most readily identified by removing the polarizer of a microscope and rotating the analyzer, whereupon the crystals will be seen to alternate between blue-black and colorless. The hexagonal prisms described above exhibit a similar dichroism when turned over on a prism face. With the iodine–potassium iodide test the beta and gamma dextrins form brown to yellow complexes which are considerably less dichroic. No characteristics have been noted which enable the positive identification of the beta or gamma dextrin iodine complexes.

When the dextrins in 60% propyl alcohol are crystallized on a microscope slide, crystals of each complex can be readily identified. The crystallization is best carried out by allowing a drop of the 60% alcoholic solution to evaporate to dryness. A second drop is then placed on the slide and covered with a cover glass. Within a few minutes well-formed crystals will be observed. Alpha dextrin crystallizes as blade-shaped needles or hexagonal plates, beta dextrin as parallelograms, and gamma dextrin as square plates or rectangular rods. If the mixture contains some of each dextrin, it is usually possible to see each type of crystal. Particularly in the course of purification of the alpha and gamma dextrins it is worthwhile to examine the 60% propanol mother liquors for traces of other members of the dextrin series. Photographs of the dextrins as crystallized from 60% propanol are given in Figs. 1, 2, 3 and 4.

Solubilities of the Dextrins in Water.—Solutions containing 20% alpha dextrin, 5% beta dextrin or 35% gamma dextrin in water deposit crystals on standing at room temperature. Such solutions were allowed to equilibrate several days at room temperature with occasional shaking. The crystals were filtered off and the concentration of carbohydrate in the mother liquors was determined by measuring the rotations: alpha, 14.5 g./100 ml.; beta, 1.85 g./100 ml.; gamma, 23.2 g./100 ml.

Solubilities of the Dextrins in 60% Propanol.—Solutions of the pure alpha, beta and gamma dextrins were prepared by dissolving the air dry samples in four parts of hot water and adding six parts of anhydrous n-propyl alcohol. The solutions were shaken overnight and allowed to stand several days at room temperature. The suspensions were filtered and the concentration of carbohydrate in the filtrates determined by measuring the rotation. With alpha dextrin, at least three distinct crystalline forms, with different solubilities, were obtained. The solubilities ranged from 0.4 to 1.2 g./100 ml., with most frequent readings at about 0.8 and 1.1. With beta and gamma dextrins, no such variation was observed: beta, 0.54 g./100 ml.; gamma, 0.27 g./100 ml.

Solubilities of the Dextrins in the Presence of Precipitants.—Aqueous solutions of pure alpha, beta and gamma dextrins were treated with sufficient amounts of precipitants to form a distinct layer. Generally about 5 ml. of precipitant with 40 ml. of 0.3–0.5% dextrin was used. In some cases in which no precipitate was formed at this dextrin concentration, increasing amounts of dextrin were used. After equilibration by shaking overnight and standing for a few days at room temperature (*ca.* 27°) the precipitates were removed by filtration. The concentration of dextrin in the filtrate was determined by measuring the rotation. The results are presented in Table I.

TABLE I

SOLUBILITIES OF THE SCHARDINGER DEXTRINS IN THE PRESENCE OF PRECIPITANTS

Precipitant	Alpha g./100 ml.	Beta g./100 ml.	Gamma g./100 ml.
Petroleum ether		0.12	
Mineral oil		.03	
Cyclohexane	0.15	.06	
Decalin		.08	
Benzene	.8	.07	
Toluene	.9	.06	0.04
p-Xylene	.9	.02	.06
Ethylbenzene		.03	
p-Cymene	3.3	.04	.17
Naphthalene		.03	
Methyl iodide		.06	
Chloroform	0.8	.07	
Carbon tetrachloride		.10	
Nitromethane		Over .3	
Carbon disulfide	.08	.07	
Ethylene dichloride		.12	
Ethylene dibromide		.12	
Trichloroethylene	.26	.03	.03
Tetrachloroethane	.08	.12	.03
Tetrachloroethylene	.7	.004	.01
Tetrabromoethane	.10	.02	
Isoamyl iodide		.10	
Bromobenzene	2.4	.03	.01
Iodobenzene		.03	.03
p-Chlorotoluene		.02	.05
o-Bromotoluene		.02	.02
p-Dichlorobenzene		.18	
α-Bromonaphthalene		.02	
Benzyl chloride	Over 1		
Nitrobenzene	Over 1	.03	.04
Aniline		Over .3	.4
Azobenzene		.20	
Butanol		Over .3	
Ethyl ether		.66	
Ethyl acetate		Over .3	
Benzyl alcohol		Over .3	
Cyclohexanol	0.68		
Phenol	Over 1		
β-Phenylethanol		1.0	
Thymol		0.14	

Dextrin Acetates.—The crude alpha dextrin, oven dry, was added in four equal parts at intervals to five parts of boiling acetic anhydride containing one-half part anhydrous sodium acetate. After the final addition the mixture was refluxed for thirty minutes, allowed to cool to room temperature, and poured with stirring into cracked ice and water. As the dextrin acetate hardened, the water was replaced. When all the acetic anhydride was destroyed, the dextrin acetate was broken and suction filtered. Pure beta and gamma dextrins were conveniently acetylated in the same manner. The crude alpha and beta acetates were crystallized from 10–15 parts of boiling toluene by cooling to room temperature. Gamma dextrin acetate was crystallized from 2–3 parts of hot butyl acetate. The dextrin acetates appear to crystallize with

solvent of crystallization which is lost on exposure to air, giving in some cases glasses which retain the exterior form of the original crystals. The specific rotations of the pure acetates were: alpha, $[\alpha]_D + 105.5 \pm 0.5°$ (c 1, chloroform); beta, $[\alpha]_D + 122.0 \pm 0.5°$ (c 1, chloroform); gamma, $[\alpha]_D + 138.5 \pm 0.5°$ (c 1, chloroform).

Solubility of Dextrin Acetates.—The pure dextrin acetates were dissolved in about 15 parts of toluene, methanol, ethyl acetate and butyl acetate. If crystallization occurred on standing at room temperature, the mixtures were equilibrated for several days with frequent agitation and the concentration of material in the liquid phase determined by measuring the rotation as above. If crystallization did not occur, the solutions were evaporated until crystals separated or a glass was formed. After thorough equilibration the solubilities were determined as before. The values obtained are presented in Table II.

TABLE II

SOLUBILITIES OF THE SCHARDINGER DEXTRIN ACETATES

Solvent	Alpha g./100 ml.	Beta g./100 ml.	Gamma g./100 ml.
Toluene	0.22	0.24	Very sol.
Methanol	1.39	2.54	Very sol.
Ethyl acetate	8.84	Very sol.	Very sol.
Butyl acetate	0.67	17.8	11.0

Summary

The preparation of pure Schardinger dextrins using differential precipitants and differential solubility in water and 60% propanol is described. For anhydrous gamma dextrin, $[\alpha]_D +177.4 \pm 0.5°$ (c 1, water).[8] Characteristic crystal forms for the dextrins from 60% propanol are noted. The solubility behavior of the individual dextrins in water, 60% propanol and water saturated with various precipitants is reported.

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Studies on the Schardinger Dextrins. II. Preparation and Properties of Amyloheptaose[1]

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In studying the behavior of starch it is clearly desirable to have available model compounds which may be expected to exhibit characteristic starch reactions without the difficulties and uncertainties associated with native starch. Lack of homogeneous amyloöligosaccharide of chain length intermediate between maltose and amylose has led to conflicting opinions on the mode of action of the amylolytic enzymes, the relationship between chain length and iodine coloration, etc. This paper describes the preparation and properties of a maltose homolog containing seven glucose residues per molecule: amyloheptaose. The biochemical properties of amyloheptaose will be reported subsequently.

Amyloheptaose may be conveniently prepared by the controlled acid hydrolysis of Schardinger's beta dextrin, cycloheptaamylose. Since this is a cyclic molecule containing seven[4] glucose units linked together by maltose bonds,[5] rupture of any one bond per cyclic molecule leads directly to the linear heptasaccharide of the amylose series. The experimental difficulty in confining hydrolysis to one glycosidic linkage per molecule is accentuated by the fact that the beta dextrin is considerably more resistant to acid hydrolysis than corresponding linear compounds.[6] In order to avoid extensive degradation of the amyloheptaose it was necessary to establish an experimental procedure based upon the rate constants for the decyclization reaction and the subsequent heptasaccharide breakdown. The separation of the heptasaccharide from large amounts of unchanged beta dextrin was accomplished by allowing the latter to crystallize in the cold and removing the last trace as the insoluble p-xylene complex.[7] The dilute hydrochloric acid used as hydrolysis catalyst was exactly neutralized with lithium carbonate and the resulting lithium chloride removed by repeated precipitation of the aqueous heptasaccharide solution with absolute ethanol. The complete details of the preparation are given in the experimental section.

Reaction Kinetics Involved

A complete mathematical analysis of the kinetics of the hydrolysis of cycloheptaamylose (β)

$$\beta \longrightarrow \text{heptasaccharide} \longrightarrow \begin{matrix}\text{hexasaccharide}\\ \text{pentasaccharide}\\ \text{tetrasaccharide}\\ \text{trisaccharide}\\ \text{maltose and glucose}\end{matrix} \quad (1)$$

is complicated and unnecessary for the purpose at hand. It was found expedient, however, to derive expressions for the amount of heptasaccharide (H) and the number of reducing hemiacetal groups (R) as a function of time. In setting up this system k_1 was defined as the rate constant for the destruction of β and k_2 as the *average* rate constant for the destruction of the glycosidic bonds in all the open-chain molecules.[8]

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(4) French and Rundle, THIS JOURNAL, **64**, 1651 (1942).

(5) Freudenberg and Meyer-Delius, *Ber.*, **71**, 1596 (1938).

(6) (a) Cori, Swanson and Cori, *Federation Proc.*, **4**, 234 (1945). (b) Swanson and Cori, *J. Biol. Chem.*, **172**, 797 (1948), have come to similar conclusions using wholly different experimental and mathematical methods.

(7) French, Levine, Pazur and Norberg, THIS JOURNAL, **71**, 353 (1949).

(8) It is recognized that the rate of hydrolysis of the glycosidic bonds depends on such factors as the size of the molecule and the position of the bond within the molecule.