lized from ethanol, m. p. 268–269°. Mixed with an authentic sample, m. p. 272–273°, it melted at 270–271°. The quinone was further characterized by preparing the derivative with phenylhydrazine, m. p. 180–181°.

Oxidation of II to the Ketone.—Six grams (0.023 mole) of II was dissolved in 75 cc. of glacial acetic acid and cooled to about 10°. The solution was stirred vigorously while 3 g. (0.031 mole) of chromic anhydride, dissolved in 10 cc. of water and 10 cc. of acetic acid, was added slowly. The cooling-bath was then removed and the reaction mixture stirred at room temperature for three-quarters of an hour. It was poured into ice-water (200 cc.) yielding an oil which was extracted with benzene. The benzene solution was washed with sodium bicarbonate solution followed by water. The solvent was evaporated leaving an oil (5.6 g., 90%). This crude ketone was purified by the use of betainehydrazide hydrochloride (Girard's reagent T). The oil was dissolved in 125 cc. of absolute ethanol, to which was added 8.4 g. (0.05 mole) of Girard's reagent T and 12 cc. of glacial acetic acid. The mixture was refluxed for one hour, cooled and poured into 900 cc. of ice-water containing enough sodium hydroxide to neutralize nine-tenths of the acetic acid. The solution was then acid to brom thymol blue indicator. The water solution was extracted three times with 125-cc. portions of benzene, then acidified with enough concentrated hydrochloric acid to make the entire solution 0.5 normal, and allowed to stand overnight. It was again extracted with benzene (250 cc. in three portions). The benzene solution was washed with sodium bicarbonate, followed by water. The benzene was evaporated, yielding 2 g. (35%) of pale, viscous oily ketone. From this material a 2,4-dinitrophenylhydrazone was prepared by refluxing in alcohol solution. Crystals began to separate in a few minutes; yield, 3.1 g. (95%). After crystallization from alcohol and ethyl acetate, the bright red crystals melted sharply at 215°.

Anal. Calcd. for $C_{24}H_{20}O_6N_4$: C, 62.60; H, 4.35; N, 12.18. Found: C, 62.72; H, 4.34; N, 12.32.

Reaction of Methyl Furoate and Chlorobenzene.—Seventy-five grams (0.6 mole) of methyl furoate dissolved in 500 cc. of chlorobenzene was cooled to 0° in an ice-bath. The solution was stirred and 162 g. (1.2 mole) of anhydrous aluminum chloride was added over a period of thirty minutes. The stirring was continued for thirty minutes at 0°, for one and one-half hours at room temperature and finally for twenty-eight hours at 90–100°. The reaction mixture was then poured into a mixture of ice and hydrochloric acid and stirred for three hours at room temperature. The mixture emulsified badly and one liter of ether was added to facilitate the separation. The ether layer was washed with dilute hydrochloric acid, with water, three times with saturated sodium bicarbonate, and finally with water. It was then dried over anhydrous magnesium sulfate and the ether removed by evaporation. The residue was placed in a modified Claisen flask and distilled; 20 g. (15%) of methyl 6-chloro-1-naphthoate was obtained, b. p. 165–170° (2 mm.).

The sodium bicarbonate extract was acidified with hydrochloric acid and 53 g. (44%) of crude 6-chloro-1-naphthoic acid was obtained. This was recrystallized once from 95% ethanol and once from benzene, yielding 46 g. (39%) of 6-chloro-1-naphthoic acid, m. p. 188–189°.

Summary

The aluminum chloride-catalyzed reaction of methyl furoate with benzene has been found to yield methyl α-naphthoate in 32–46% yield while chlorobenzene was converted to 6-chloro-1-naphthoic acid in good yield. Esterification of furoic acid thus appears to favor this condensation to a naphthalene derivative.

In addition to methyl α-naphthoate, the reaction with benzene yielded a higher boiling product in 11–20% yield. Evidence has been presented indicating that this compound is methyl 9-ethyl-9,10-dihydro-9-anthroate.

URBANA, ILLINOIS RECEIVED APRIL 14, 1942

[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

A Study of the Products Obtained from Starch by the Action of the Amylase of *Bacillus macerans*

BY W. S. MCCLENAHAN, EVELYN B. TILDEN AND C. S. HUDSON

Preliminary experiments on the conversion of potato starch to the crystalline Schardinger dextrins by *Bacillus macerans* showed that the action was produced by a new type of amylase present in bacteria-free filtrates of the cultures.[1] In this paper we wish to report precise data on the nature and extent of the changes in optical rotation,[2] viscosity and reducing action occurring during digestion of various starch samples by purified concentrates[3] of the *macerans* amylase, and to record the yields of alpha and beta dextrins obtainable. Also, the crystalline alpha and beta dextrins have been purified, their constants determined, and their stability toward *macerans* amylase studied.

When *macerans* amylase was allowed to act for one month upon a 2% suspension of potato

(1) (a) Tilden and Hudson, THIS JOURNAL, **61**, 2900 (1939); (b) Tilden and Hudson, *J. Bact.*, **43**, 527 (1942).

(2) In the preliminary publication it was reported that there was no significant change in rotation during digestion, but we now find under more exact test that the change is of considerable magnitude, as shown in Fig. 3.

(3) Tilden, Adams and Hudson, THIS JOURNAL, **64**, 1432 (1942).

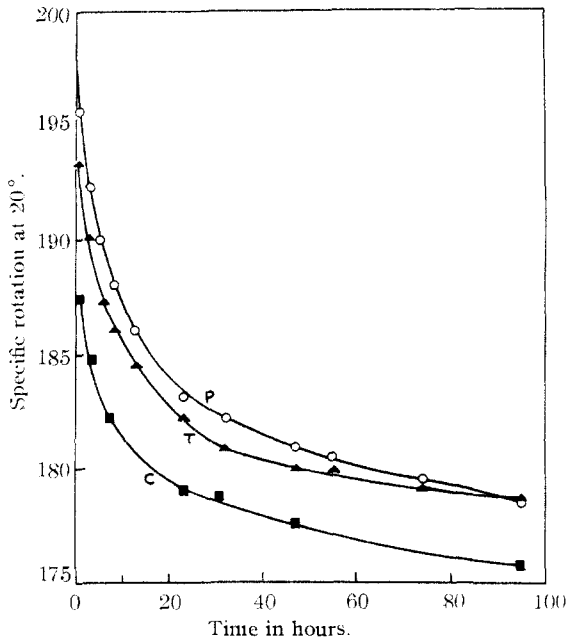

Fig. 1.—Rotatory changes in buffered digests at 20°; *p*H 6.2–6.5; enzyme concentration 10 units per gram of starch in 2% solution: P, potato starch; T, tapioca starch; C, oxidized cornstarch (hypochlorite).

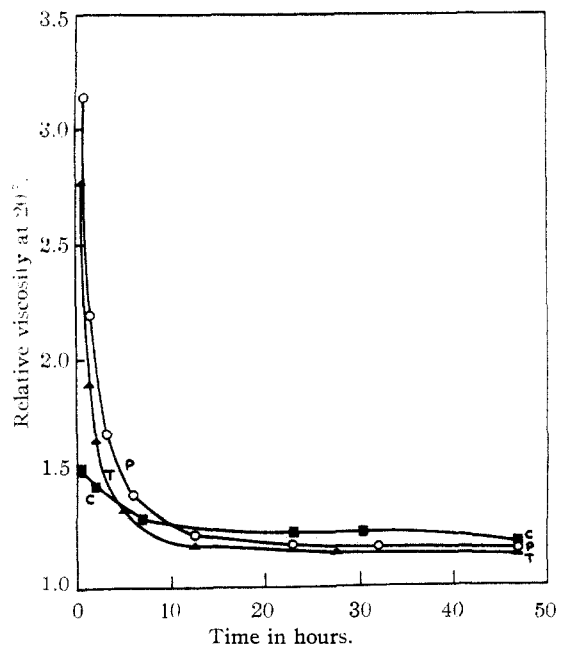

Fig. 2.—Viscosity changes in buffered digests at 20°; *p*H 6.2–6.5; enzyme concentration 10 units per gram of starch in 2% solution: P, potato starch; T, tapioca starch; C, oxidized cornstarch (hypochlorite).

starch, the reducing power[4] was only 1.6%, calculated as the percentage of the total amount of glucose available by complete acid hydrolysis of the starch; this result confirms the earlier work[1a] in showing that the *macerans* enzyme does not produce reducing sugars. Correspondingly low values for reducing power were obtained with digests of tapioca starch and with a commercial thin boiling cornstarch which had been made by the hypochlorite process.

The changes in viscosity and rotation that were observed were very similar for digests of the three starches, as shown in Figs. 1 and 2. Unoxidized defatted cornstarch has been found to give similar results.

The data obtained from digestion of several lots of potato starch, on which the enzyme was allowed to act for periods varying from eight hours to fifty days, are shown in Table I. The presence of the dextrins was demonstrated by the iodine test[1] and confirmed by precipitation of the crystalline products with trichloroethylene. Under the conditions employed the beta dextrin appeared to be formed more gradually than the alpha dextrin, the yield of which appeared to reach a maximum of 20% in the early stages of the conversion. Longer digestion resulted in a higher total yield of dextrins, but there seemed to be a decrease in yield of the crude alpha compound and a definite increase in its rotation, indicating a possible secondary action of the enzyme. This hypothesis was confirmed by experiments in which the enzyme was added to sterile solutions of pure alpha dextrin; the rotation gradually rose from $+150.5°$[5] to about $+169°$, while a slight reducing action became evident (Fig. 3 and Table II). No beta dextrin could be detected in the solution at the end of these experiments, and the new dextrin or mixture of dextrins behaved in the iodine test in a manner similar to that of the alpha compound. No effect of the enzyme on sterile solutions of pure β-dextrin was detected.

In an attempt (Digest 4, Table I) to increase the yield of crystalline dextrins, the products were continuously precipitated from solution by adding trichloroethylene soon after the addition of the enzyme, and by allowing the digest to stand for several weeks. Surprisingly, the yield of beta dextrin was thereby increased from 22 to 54%,[6]

(4) Values for reducing power were determined by the Shaffer-Hartmann micro-technique (*J. Biol. Chem.*, **45**, 365 (1921)).

(5) All rotations here reported are specific rotations at 20° for sodium light; c is the concentration in grams per 100 ml. of solution, and l is the tube length in decimeters. The crystalline dextrins contain water of crystallization, but the rotations are expressed on the anhydrous basis corresponding to their general formula $(C_6H_{10}O_5)_x$.

(6) Beta dextrin has been obtained in a yield of 46% from the action of whole cultures of *Bacillus macerans*.

TABLE I

PREPARATION OF CRYSTALLINE DEXTRINS FROM POTATO STARCH AT 20°[a]

Digest	Time, days	$[\alpha]^{20}D$	Relative viscosity	Reducing power,[b] %	Yield of dextrin, % crude	beta	alpha[c]	$[\alpha]^{20}D$ crude alpha dextrin
1	0.67	+189.8	1.49	0.05	33	9	19	+152
2	3	180.5	1.16	0.25	45	12	20	156
3A	20	177.4	1.09	1.35	50	22	17	160
3B	32	177.4	1.09	1.64	50	22	16	161
4[d]	50	...	..	..	61	54[e]	1	...

[a] The starch concentration was 2%, and 8 units of enzyme was used for each gram of starch. No buffer was added; the *p*H was 6.5. [b] Percentage of the theoretical quantity of glucose available. [c] Includes all material of apparently crystalline nature from aqueous methanol solution. [d] Trichloroethylene was present during the digestion. [e] Crude material rotated +164°.

TABLE II

ACTION OF *macerans* ENZYME UPON STERILIZED 2% SOLUTIONS OF THE SCHARDINGER DEXTRINS AT 20°

Dextrin	Time, days	Units of enzyme per gram of dry dextrin	$[\alpha]^{20}D$	Reducing power,[a] %
alpha	23	0	+150.6	0.0
alpha	10[c]	8	166.4	0.8
alpha	24[c]	8	169.7	1.2
alpha	9	16	169.2	1.4
alpha	10	16	167.3[b]	1.4
alpha	13	16	168.6[b]	1.6
beta	6	0	162.0	...
beta	6[d]	8	162.0	...
beta	14	16	163.2	0.0

[a] As percentage of the theoretical quantity of glucose. [b] Rotation determined after diluting solution to 1%. [c] Not sterile at time of observation. [d] Beta dextrin recovered quantitatively.

while the yield of alpha dextrin was reduced to 1%.[7] No increase in yield resulted from the addition of fresh enzyme to the mother liquor, hence the limit of the conversion of whole starch appears to be about 55%. No explanation can be advanced as yet for the difference in the relative proportions of the two dextrins obtained under different conditions.

The preparations of beta dextrin, obtained from starch by the action of *macerans* enzyme, were readily purified to constant rotation by recrystallization from water. The specific rotation was always +162.5 ± 0.5° instead of +158°, the value which has previously been accepted.[8] After acetylation and careful purification of the crystalline acetate, deacetylation was found to give rise to beta dextrin of the same high rotation. This was the case irrespective of whether pyridine or zinc chloride was used as the acetylation catalyst. We conclude, therefore, that the higher value is the correct one. In other respects (appearance, solubility, decomposition point, formation of orange-brown needles or prisms with iodine solution, and content of water of hydration) the dextrin was identical with preparations previously described. Alpha dextrin rotated +150.5 ± 0.5°, which is somewhat higher than the value (+148°) recorded by other workers.[9]

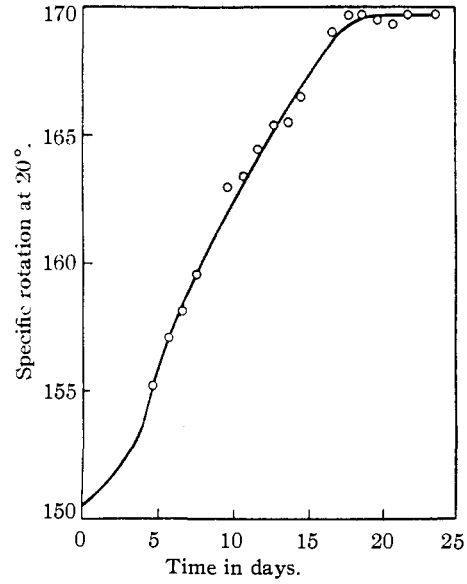

Fig. 3.—Rotatory change in a 2% solution of alpha dextrin at 20°: enzyme concentration 8 units per gram of dextrin (dry basis).

Beta dextrin acetate rotated +125.5° (chloroform); the values given by earlier workers are +121°[8h] and +142°.[9a] Its melting point was 196–196.5° (cor.), and it appears that this constant is a far better criterion of purity than is its rotation, since on several occasions a crystalline

(7) Dr. R. E. Rundle of Iowa State College has reported to us similar results, obtained through the precipitating action of benzene or toluene when used in the digests as preservatives.

(8) (a) Schardinger, *Zentr. Bakt. Parasitenk.*, Abt. II, **22**, 98 (1908); **29**, 188 (1911); (b) Pringsheim and Langhans, *Ber.*, **45**, 2533 (1912); (c) Pringsheim and Eissler, *ibid.*, **47**, 2565 (1914); (d) Pringsheim and Dernikos, *ibid.*, **55**, 1433 (1922); (e) Karrer and Bürklin, *Helv. Chim. Acta*, **5**, 181 (1922); (f) Leibowitz and Silmann, *Ber.*, **58**, 1889 (1925); (g) Pringsheim, Weidinger and Ohlmeyer, *ibid.*, **64**, 2125 (1931); (h) Freudenberg and Jacobi, *Ann.*, **518**, 102 (1935).

(9) (a) Pringsheim, Weidinger and Sallentien, *Ber.*, **64**, 2117 (1931); (b) Miekeley, *ibid.*, **63**, 1957 (1930).

product with approximately the correct rotation had a low acetyl content and softened at 140 to 150°. According to Pringsheim and his co-workers,[10] the use of zinc chloride as an acetylation catalyst brings about the depolymerization of both alpha and beta dextrins. This finding has not been confirmed by other investigators,[8e,8h,9b] and we have found no evidence of such behavior in studying beta dextrin and its acetate.[11] Our rotatory data also suggest that many of the conflicting observations with regard to the nature and behavior of these compounds may have been the result of insufficiently purified (though crystalline) materials.[12]

Experimental Part

Purification of the Enzyme.—The enzyme solution which was used had been concentrated and purified by precipitation with acetone, as previously described.[3] It contained one unit of enzyme in 0.05 ml. (1 unit = amount required to convert 30 mg. of starch in thirty minutes at 40°, as indicated by the iodine test).[1b] It had no rotation or reducing power and was free from maltase, as shown by its failure to influence the rotation of a maltose solution.

Comparative Digestions of Potato, Tapioca and Oxidized Cornstarches (Figs. 1 and 2).—The quantity of starch (commercial grade) equivalent to 2.00 g. of dry material was placed in a 100-ml. volumetric flask, and 50 ml. of water and 1 ml. of 0.2 *M* Sørensen phosphate buffer of *p*H 6.2 was added. A smooth paste was produced by swirling the flask in a boiling water-bath; the wall of the flask was then washed down with a little hot water, and the heating continued for thirty minutes. A layer of hot water was allowed to flow over the surface of the starch paste, after which the flask was plugged with cotton and the solution autoclaved for one hour at 125° (the glass stopper was sterilized at the same time). The solution was diluted with hot sterile water, and cooled to 20° overnight. There was then added sufficient enzyme (1 ml. or 20 units) to convert the starch to the brown-violet stage with iodine in about seven hours at 20°, and after dilution to 100 ml. the flask was closed with the sterile stopper and the contents thoroughly mixed. Viscosity measurements were made as described previously.[1b] Part of the digest was centrifuged to remove any traces of material which might clog the viscosity pipet; the centrifuged material was also used for measurements of rotation, while tests for reducing power[4] were made on 5-ml. aliquots. The entire experiment was carried out at 20–21°.

Preparation of Crystalline Dextrins from Potato Starch. (See Table I.)—Substrates containing 20.0 g. (dry basis) of potato starch were prepared in one-liter volumetric flasks in the above manner, except that the buffer solution was omitted. Eighty units (4 ml.) of enzyme was added to Digest 1 and 160 units to each of the others. The changes at 20° in rotation, viscosity, reducing power and appearance with iodine solution were similar to those obtained in the case of the smaller buffered digests. After sixteen hours and three days, respectively, Digests 1 and 2 were boiled for a few minutes to inactivate the enzyme, and the dextrins were precipitated with trichloroethylene. After Digest 3 had stood overnight, half of it was filtered through a Berkefeld *N* filter into a sterile flask and left undisturbed for thirty-two days. The remainder was kept in the stoppered volumetric flask, and after twenty days a portion of the clear solution gave no evidence of contamination when tested on glucose agar. The details of Digest 4 are given in the next section.

About 15 ml. of trichloroethylene was found sufficient to precipitate the crystalline dextrins in these experiments. The mixture was kept for a day at room temperature and was occasionally shaken; the precipitate was filtered on a Büchner funnel, washed with a little water, and suction applied until the precipitate was almost dry.[13] The aqueous layer of the filtrate was then concentrated *in vacuo* to about 100 ml. and treated again with trichloroethylene. A second precipitate was allowed to form at room temperature, and a third was obtained by stirring the mixture in an ice-bath and filtering after it had stood for a day in the refrigerator. Small quantities of dextrin continued to separate over a period of several weeks. The moisture content of the combined products from each experiment was determined by drying at 100° and 12 mm. to constant weight over calcium chloride. The air-dried material was then dissolved in two parts of water and the solution boiled to remove the trichloroethylene. A very small quantity of difficultly soluble material (Schardinger's "Schlamm") was removed by filtering the hot solution through carbon; crystalline beta dextrin separated on cooling the filtrate. A second crop was obtained by concentrating the mother liquor to a thin sirup, seeding, and allowing it to stand several days in the refrigerator.

The filtrate contained the more soluble alpha dextrin, together with a considerable amount of amorphous material. Methanol was added to the concentrated solution at room temperature until turbidity developed. The solution was clarified by filtering with carbon, and crystallization induced by scratching or seeding. By adding more methanol, filtering and allowing to stand, first at room temperature and then in the refrigerator, the yield could be increased; however, in order to obtain the maximum quantity of crude alpha dextrin, it was usually necessary to concentrate the mother liquor to a sirup, add methanol in the manner described, and repeat the process several times. Whenever considerable amorphous material separated, it was removed by filtration with carbon; it was then dissolved in water and the solution tested for the presence of alpha dextrin with iodine. The yields of crude dextrins are given in Table I.

(10) Pringsheim, "Chemistry of the Monosaccharides and of the Polysaccharides," McGraw-Hill Book Co., Inc., New York, N. Y., 1932, p. 280.

(11) The depolymerization theory was based to a great extent upon cryoscopic molecular weight determinations. This method has been shown in a number of instances to lead to erroneous conceptions of the molecular size of polysaccharides and their derivatives [Hanes, *New Phytologist*, **36**, 101 (1937); Klages, *Ann.*, **520**, 71 (1935); Haworth, Hirst and Ant-Wuorinen, *J. Chem. Soc.*, 2368 (1932); Freudenberg and Bruch, *Ber.*, **63**, 535 (1930); Kratky and Mark, *Fortschr. Chem. org. Naturstoffe*, **1**, 255 (1938)].

(12) Samples of alpha and beta dextrin prepared by Dr. Thomas J. Schoch of the Corn Products Refining Company showed the same rotations as our preparations.

(13) When bulky precipitates are obtained in large scale work, it is advantageous to remove the trichloroethylene by washing the filter cake with small portions of methanol.

Precipitation with Trichloroethylene during Digestion (Digest 4, Table I).—After an hour's digestion at 20°, Digest 4 was filtered through filtercel; 50 ml. of trichloroethylene was added to the filtrate, and the digestion was allowed to continue at 20°. The crystalline dextrins were removed by filtration after two, three, five and six weeks, the final crop being obtained after concentrating at low temperature to 100 ml. Three milliliters of enzyme was added to the mother liquor, but no further precipitation occurred after the solution had stood at 20° for several weeks.

Purification of the Dextrins.—The beta dextrin was recrystallized four times from four parts of water. The air-dried product rotated +139.4° (*c*, 1.0; *l*, 4). Its weight became constant after drying at 66° and 12 mm. for ninety minutes over calcium chloride; loss, 14.13%. The specific rotation was therefore +162.4°; other samples showed +163.0° and +162.8°.

Crude alpha dextrin, which contained some amorphous material, was recrystallized by adding methanol to its concentrated aqueous solution, according to the procedure given in the preceding section. Further recrystallizations were made from 70% methanol by dissolving the dextrin in one part of hot water and adding the alcohol to the warm solution. After two such recrystallizations the air-dried material rotated +136.5° (*c*, 1.1; *l*, 4). Its weight became constant after drying five hours at 100° and 12 mm.; loss, which may have been partly methanol, 9.48%. The specific rotation was therefore +150.8°; after two further recrystallizations it rotated +150.4° and +150.3°, respectively, while an entirely different preparation showed +150.8°.

Acetylation of Beta Dextrin.—Five grams of pure, anhydrous, powdered beta dextrin was dissolved in 15 ml. of pyridine, the solution becoming slightly warm. Since cooling the solution caused it to set to a gel, 15 ml. of acetic anhydride was added while it was still warm. The stoppered flask was then shaken and cooled occasionally, its contents becoming homogeneous after half an hour. After standing at room temperature for three days, the solution was poured into 400 ml. of ice water, and the product crystallized immediately in nearly theoretical yield. The acetate was recrystallized four times from seven parts of methanol, from which it separated in the form of elongated, hexagonal plates. It could not be recrystallized satisfactorily from absolute ethyl alcohol, possibly because a partial deacetylation occurred. It melted at 196–196.5° (cor.). The rotation and analytical data were obtained from a portion which had been dried *in vacuo* to remove the methanol and then allowed to stand overnight in the air. This sample rotated +122.3° (*c*, 1.0, chloroform; *l*, 4); its loss in weight after forty-five minutes at 66° and 12 mm. was 2.20%, and hence the specific rotation was +125.0°. A second preparation rotated +125.4°. The dry acetate was extremely hygroscopic, and in order to determine its moisture content accurately it was necessary to employ a modified Abderhalden drying apparatus.[14]

Anal. Calcd. for $(C_{12}H_{16}O_8)_x$: C, 49.99; H, 5.60; CH_3CO, 44.79. Found: C, 50.04; H, 5.86; CH_3CO, 45.85.

(14) Milner and Sherman, *Ind. Eng. Chem., Anal. Ed.*, **8**, 427 (1936).

Four grams of the acetate in 50 ml. of methanol was deacetylated in the cold with 4 g. of potassium hydroxide dissolved in 25 ml. of methanol. The resulting precipitate was dissolved in water, and, after neutralization of the solution with dilute acetic acid, the beta dextrin began to crystallize. The recovery was 92% of the theoretical amount. After two recrystallizations from water, the dextrin rotated +162.9°.

Four grams of dry powdered beta dextrin and 0.5 g. of pulverized zinc chloride were added to 20 ml. of acetic anhydride, and the mixture was heated on the steam-bath for one hour. The hot, faintly yellow solution was poured into a liter of ice-water, and the acetate began to solidify after a few minutes of stirring. The acid was neutralized with sodium bicarbonate, and the product obtained in nearly theoretical yield. After recrystallization the acetate melted at 196–196.5° (cor.). The air-dried sample contained 2.06% moisture and rotated +123.8° (*c*, 1.0; *l*, 4), or +126.3° calculated to the dry basis.

Anal. Calcd. for $(C_{12}H_{16}O_8)_x$: C, 49.99; H, 5.60; CH_3CO, 44.79. Found: C, 50.10; H. 5.96; CH_3CO, 45.62.

A three-gram portion of the acetate was deacetylated. The dextrin was recovered quantitatively, and after two recrystallizations from water it rotated +163.0°.

Action of *macerans* Enzyme on the Crystalline Dextrins.—One-gram samples of the pure, air-dried dextrins were dissolved in hot water in 50 or 100 ml. volumetric flasks. Alpha dextrin solutions were heated fifteen minutes on the steam-bath to remove possible traces of methanol before autoclaving for thirty minutes at 124°; beta dextrin solutions were autoclaved directly. After the flasks had been cooled to 20°, the enzyme was added, and the solutions were diluted to 50 ml. with sterile water. The small amount of beta dextrin which separated after a few days was redissolved by warming to 40° before the flasks were opened. Sterility tests were made on glucose agar at the conclusion of each experiment. The data obtained in several experiments with both dextrins are presented in Table II, while the changes in rotation observed in one non-sterile experiment are shown in Fig. 3.

During the conversion of alpha dextrin the solutions gave iodine tests similar to that of the original substance. The product could be precipitated with trichloroethylene, but no beta dextrin was obtained when the solution was concentrated *in vacuo* to a sirup. Addition of methanol to the solution caused it to cloud, and a mixture of amorphous and crystalline material gradually separated. Methods for fractionating the mixture are being investigated.

The authors wish to express their appreciation to Dr. Thomas J. Schoch of the Corn Products Refining Company for samples of oxidized starch and Schardinger dextrins, and to Dr. E. Justin Wilson, Jr., of this Laboratory for helpful assistance.

Summary

The action of the purified enzyme of *Bacillus macerans* on starch substrates proceeds with a

rapid decrease in viscosity and a gradual decrease in optical rotation. Unlike other amylases, the *macerans* amylase does not increase the reducing power of whole starch to any noteworthy extent.

The Schardinger dextrins have been prepared from potato starch by means of the *macerans* amylase in a maximum yield of 55%. The relative proportions of alpha and beta dextrins in the product have been shown to vary greatly with different digestion conditions; the factors involved in this behavior are receiving further study. Beta dextrin is stable toward *macerans* amylase at 20°, whereas the alpha dextrin is converted, at least in part, to higher rotating material which exhibits slight reducing properties and contains no beta dextrin.

The rotations of carefully purified alpha and beta dextrins have been found to be +150.5 ± 0.5° and +162.5 ± 0.5°, respectively, instead of +148° and +158°, as previously reported. The new value for beta dextrin was confirmed by preparing its acetate (rotation +125.5° in chloroform; m. p. 196–196.5° (cor.)), from which the original high rotating beta dextrin was regenerated. The same beta dextrin acetate was produced when either pyridine or zinc chloride was used as the acetylation catalyst.

BETHESDA, MARYLAND RECEIVED MAY 28, 1942

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE CITY COLLEGE OF THE COLLEGE OF THE CITY OF NEW YORK]

The Nature of the Fatty Acids Associated with Starch. The Adsorption of Palmitic Acid by Potato and Defatted Corn and Rice Starches

BY LEO LEHRMAN

It is well known that starches, with the exception of potato, have associated with them small amounts of fatty material which is not extracted by the usual fat solvents, such as ether or carbon tetrachloride.[1] Acid hydrolysis is usually employed to liberate this fatty material, which is therefore termed "fat by hydrolysis" to distinguish it from the fatty material which can be extracted by ether or carbon tetrachloride. The fatty material that can be extracted from raw starches by ether or carbon tetrachloride is usually referred to as extraneous extractable fatty matter. Recently Schoch reported that fat solvents having hydrophilic groups, particularly methanol, the cellosolves and 80% dioxane, extract practically all the fatty acids in three cereal starches.[2] He further reported that the defatted starches retain a number of the usual properties of the original starches. In addition, fatty acid can be introduced into the defatted starches and cannot be removed by extraction with the usual fat solvents, such as ether or carbon tetrachloride. On the basis of these results, Schoch concluded that free fatty acid is present in starch as an extraneous impurity.

This author submitted a comment which stated his reasons for disagreeing with this conclusion and suggested the possibility of the fatty acids being adsorbed by starch.[3] Lately, it has been shown that the amount of fatty material extracted by methanol from corn starch ground in a rod mill is the same as that extracted from the unground corn starch. From this observation the conclusion has been drawn that the fatty acids are not present extraneously.[4] Adsorption has been mentioned in connection with the occurrence of fatty acids in starch but the evidence is meager.[5]

Potato starch has been shown not to contain any "fat by hydrolysis"[6] and, therefore, could be used like a defatted starch. Oleic acid was introduced into potato starch, though only in a small percentage, by refluxing with a methanol solution of the fatty acid.[7] In order to determine how a fatty acid combines with starch, varying concentrations of palmitic acid in a hydrophilic solvent (methanol) were refluxed with potato starch. Palmitic acid was chosen because it occurs in the "fat by hydrolysis" of all starches; it is saturated and, therefore, no special precautions are neces-

(1) (a) Sostegni, *Gazz. chim. ital.*, **15**, 376 (1885); (b) Taylor and Nelson, THIS JOURNAL, **42**, 1726 1920); (c) Taylor and Lehrman, *ibid.*, **48**, 1739 (1926); (d) Lehrman, *ibid.*, **51**, 2185 (1929); **52**, 808 (1930); **54**, 2527 (1932); **55**, 850 (1933); **59**, 1050 (1937).

(2) Schoch, *ibid.*, **60**, 2824 (1938).

(3) Lehrman, *ibid.*, **61**, 212 (1939).

(4) Evans and Briggs, *Cereal Chem.*, **18**, 447 (1941).

(5) (a) Schoch, *ibid.*, **18**, 124 (1941); (b) ref. 4, p. 453; (c) Evans and Briggs, *ibid.*, **18**, 467 (1941).

(6) Lehrman and Kabat, THIS JOURNAL, **55**, 850 (1933).

(7) Schoch, private communication.