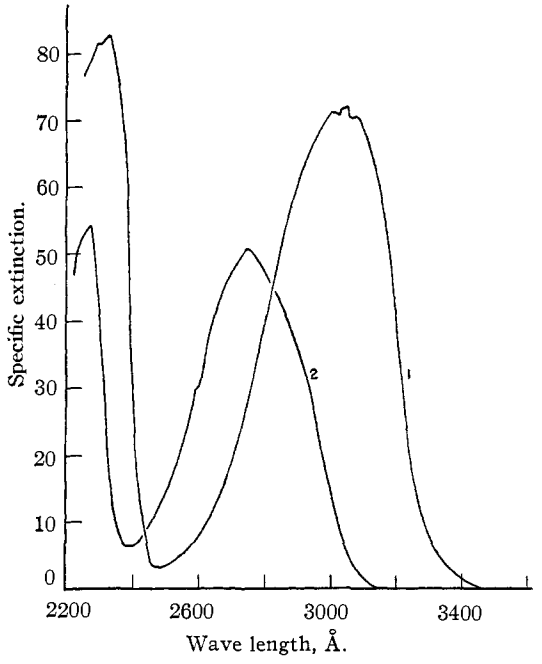

Fig. 2.—Ultraviolet absorption spectra for syringaldehyde (1) and syringic acid (2).

minima of the absorption spectra of the other esters are shown in Table II. Very little change in the fundamental protocatechuic acid absorption curve is effected by esterification with various alcohols or phenols.

The ultraviolet absorption spectra of the syringates were determined in 95% ethanol solution. The absorption spectrum for syringic acid is reproduced and compared with that of syringaldehyde in Fig. 2. As was the case in going from vanillic acid,[1] when syringaldehyde is oxidized to the acid, the absorption curve exhibits a hypsochromic shift. The principal absorption maxima for syringic acid and its derivatives are 2750 and 2280 Å. These compare with 3050 and 2325 Å., respectively, for syringaldehyde.[9] The maxima and minima of some of the esters are shown in Table III. Very little change in the fundamental syringic acid absorption curve is effected by esterification with various alcohols and phenols.

Acknowledgment.—The authors are indebted to the Microbiological and Analytical Departments of The Institute of Paper Chemistry for the toxicities and analyses reported in this paper.

Summary

A number of esters of syringic and protocatechuic acids have been prepared and tested for their toxicities toward representative microörganisms and for their ultraviolet absorption spectra.

(9) Pearl, THIS JOURNAL, **70**, 1747 (1948).

APPLETON, WISCONSIN RECEIVED MAY 21, 1949

[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

Studies on the Schardinger Dextrins. IV. The Action of Soy Bean Beta Amylase on Amyloheptaose[1]

BY DEXTER FRENCH, MELVIN L. LEVINE,[2] J. H. PAZUR[3] AND ETHELDA NORBERG[4]

In paper II of this series dealing with the preparation and properties of amyloheptaose[5] it was stated that this substrate was hydrolyzed by β-amylase to give two moles of maltose and one mole of a trisaccharide, thus indicating the heptasaccharide character of the initial hydrolytic product of Schardinger β-dextrin. Amyloheptaose is indeed an inviting test material for the examination of enzyme action, since it is a chemically defined oligosaccharide of the starch type; yet it is easily soluble in water, and it is free from the structural irregularities and inhomogeneities of natural starch. The present paper deals with the course and products of the action of soy bean β-amylase on amyloheptaose.

(1) Journal paper No. J-1709 of the Iowa Agricultural Experiment Station, Proj. 1116. This work was supported in part by the Corn Industries Research Foundation.

(2) Present address: Hawaiian Pineapple Co., Honolulu.

(3) Corn Industries Research Fellow 1948-1949.

(4) Present address: Biochemical Division, Medical School, University of California, Berkeley 4, California.

(5) French, Levine and Pazur, THIS JOURNAL, **71**, 356 (1949).

In order to follow the hydrolytic action of β-amylase on amyloheptaose, the authors used at first conventional analytical methods including optical rotations and reducing determinations. Later it was found advantageous to use methods giving both qualitative and quantitative information; in particular the techniques of quantitative elution chromatography (Fig. 1) and electrophoretic analysis of the oxidized oligosaccharides (as the potassium aldonates, Fig. 2) have been of especial help in arriving at direct answers on the qualitative and quantitative composition of enzyme digestion mixtures.

Each of the methods mentioned above indicates that soy bean β-amylase hydrolyzes amyloheptaose to give two molecules of maltose and one molecule of amylotriose ("maltotriose"). The amylotriose has been isolated and characterized by its optical rotation, reducing value, and digestibility by salivary amylase (thus indicating "amylo" character). Amylotriose appears to be completely resistant to the action of β-amylase; under no cir-

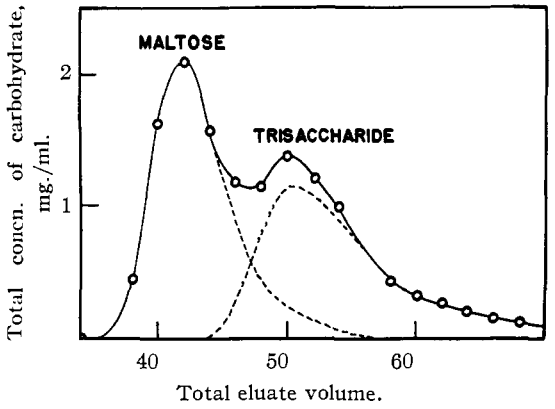

Fig. 1.—Elution chromatogram of amyloheptaose β-amylase digest: the dotted curves have been sketched in to estimate the areas corresponding to the individual components.

cumstances, even after greatly prolonged enzyme action, has glucose been observed in the digests.[6]

The rate of formation of maltose by the action of β-amylase on amyloheptaose was found to be as rapid as from starch, indicating that the low molecular weight of amyloheptaose is not a rate-limiting factor, at least under ordinary conditions. From this it appears that the slowing-down often observed in the latter stages of the action of β-amylase on starch must be due either to steric factors such as the partial association of starch chains and the proximity of neighboring chains or branches in amylopectin molecules, or perhaps to the inhibition of the amylase by accumulating maltose. In any event such slowing-down cannot be due to a slower action of this enzyme on short chains, such as is commonly considered to take place with the α-type amylases.

Since complete hydrolysis of amyloheptaose liberates two molecules of maltose, one might expect to find appreciable quantities of amylopentaose at intermediate stages in the digestion; however, electrophoretic analysis of a 55% completed digest did not reveal the presence of any components except maltose, amylotriose and amyloheptaose (see Fig. 2). From this we conclude that even with such a low molecular weight substrate, β-amylase completely hydrolyzes each molecular chain before attacking the next.[7]

When potassium amyloheptaonate[5] was used as a substrate for β-amylase, complete enzymolysis yielded two molecules of maltose and one molecule of amylotrionate; the maltose was determined quantitatively by the measurement of reducing power and the amylotrionate was identified by its characteristic electrophoretic mobility.

(6) On the basis of an ingenious but less direct method of approach Myrbäck and Ahlborg have concluded that amylotriose is not attacked by barley β-amylase: *Biochem. Z.*, **311**, 213 (1942). For a resumé of Myrbäck's views on the enzymic decomposition of starch see *Adv. Carbohydrate Chem.*, **3**, 251 (1948).

(7) Swanson, *J. Biol. Chem.*, **172**, 805 (1948).

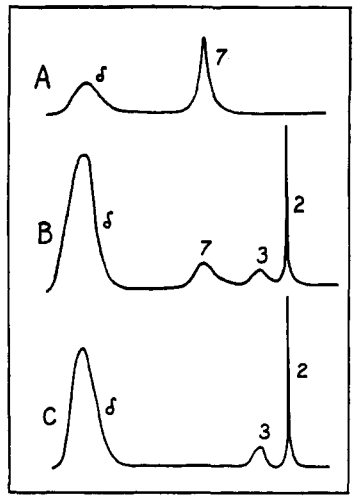

Fig 2.—Electrophoretic analysis of amyloheptaose β-amylase digests: A, before digestion; B, at 55% digestion; C, at 100% digestion; δ, boundary anomaly; 7, 3, 2, amyloheptaose, amylotriose and maltose, respectively.

Experimental

Materials.—Amyloheptaose and potassium amyloheptaonate were prepared as previously[5] except that 500-g. batches of cycloheptaamylose and correspondingly larger quantities of reagents were used. A sample of soy bean β-amylase prepared by Dr. J. M. Newton in 1938 and labeled "saccharogenic activity 1072" was found to have a saccharogenic activity of over 800 when tested early in 1949 by the method used by Newton and Naylor.[8] For use a weighed quantity of the dry preparation was shaken in water or dilute acetate buffer for about thirty minutes and filtered. Except for rate studies, a large excess of the enzyme was used; in fact, the hydrolytic reaction was usually completed so rapidly that by the time the digests were made up to volume and placed in polarimeter tubes the only observable change in rotation was the upward mutarotation of the β-maltose formed during the enzymolysis.

Chromatography of Amyloheptaose Digests.—Elution chromatographic analysis as previously used[5] was standardized by pure glucose, maltose, mixtures of the two, and Schardinger dextrin hydrolysates; the concentration of carbohydrate in each 1-ml. portion of the eluate was measured by the quantitative diphenylamine method. On plotting the carbohydrate concentration against the eluate volume, glucose, maltose and amylotriose fell into well-defined peaks with maxima at 34, 42 and 50 ml., respectively. The areas under each peak were proportional to the amount of that component taken in the calibration runs with glucose and maltose, although in the presence of higher saccharides the recoveries were never 100% complete. The data for a typical digest are graphed in Fig. 1, from which the weight proportions of maltose and amylotriose are found by graphical integration to be 55 and 45%, respectively; calculated for two moles maltose to one mole amylotriose, 57.2 and 42.8% ($C_6H_{10}O_5$ basis).

Electrophoretic Analysis of Enzyme Digests.—Using oxidative and electrophoretic procedures previously indicated[9] β amylase–amyloheptaose digests were examined at 0, 55 and 100% of the total possible hydrolysis. The ascending electrophoretic patterns (tracings) are reproduced in Fig. 2.

Digestion of Amyloheptaonic Acid.—Potassium amyloheptaonate, 0.0728 g., was dissolved in a small amount of water, a filtered solution of 5 mg. of β-amylase added and

(8) Newton and Naylor, *Cereal Chem.*, **16**, 71 (1939).

(9) Norberg and French, THIS JOURNAL, **72**, 1202 (1950).

the volume made up to 10 ml. After standing overnight the maltose produced was determined by the alkaline ferricyanide method[10]; observed, 57.5% maltose; calcd. for two moles of maltose per mole of amyloheptaonate, 56.7%. A similar digest was subjected to electrophoretic analysis and the amylotrionate produced in the enzymolysis identified by its characteristic electrophoretic mobility.

Amylotriose.—A 4-g. sample of amyloheptaose was treated with an excess of β-amylase solution and allowed to stand overnight. The products of hydrolysis were worked up by fractional precipitation with 95% ethanol, retaining and reprecipitating the least soluble fraction. After four precipitations there was obtained 1.0 g. (58%) of an amorphous saccharide with the properties: $[\alpha]_D$ +158.0 (*c*, 1 in H_2O); mol. wt. by hypoiodite[11] 501.9, by alkaline ferricyanide[10] 477; calcd. for amylotriose[12]: $[\alpha]_D$ +158.1,[10] mol. wt. 504.

Rate of Action of β-Amylase on Amyloheptaose.—Parallel digests of amyloheptaose and soluble starch were set up as follows: 0.25 g. of substrate was treated with an amount of enzyme solution corresponding to 0.25 mg. of the dry preparation, diluted to 25 ml. and incubated in a water-bath at 40°. From time to time samples were withdrawn and the increase in reducing value determined. In the initial phase of the digest (0–30% maltose formed) the rate of hydrolysis was constant and the same with both starch and amyloheptaose (7 mg. maltose/min. under these conditions). After complete hydrolysis of the amyloheptaose sample, the increase in reducing power corresponded to the formation of 62.5% maltose; calcd. for two moles of maltose, 57.5%.

(10) Levine, Foster and Hixon, THIS JOURNAL, **64**, 2331 (1942).

(11) Kline and Acree, *Ind. Eng. Chem., Anal. Ed.*, **2**, 413 (1930).

(12) Sugihara and Wolfrom, THIS JOURNAL, **71**, 3357 (1949), report $[\alpha]^{23}_D$ +160°.

Summary

1. Soy bean β-amylase hydrolyzes amyloheptaose or amyloheptaonate to give two moles of maltose and one mole of amylotriose or amylotrionate, respectively.

2. The preparation and analytical values for amorphous amylotriose are reported.

3. Amylotriose and amylotrionate are stable to the action of β-amylase.

4. β-Amylase produces maltose from amyloheptaose and starch at essentially the same rate.

5. Amylopentaose does not accumulate in significant amount during the enzyme digests.

AMES, IOWA RECEIVED OCTOBER 24, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION, AND THE DEPARTMENT OF PHYSICS, PURDUE UNIVERSITY]

Behavior of Low Molecular Weight Amylose with Complexing Agents[1]

BY WILLIAM DVONCH, H. J. YEARIAN[2] AND ROY L. WHISTLER

A number of fractionating agents have been discovered which selectively precipitate amylose from a starch–water dispersion. Schoch initially discovered the action of *n*-butyl alcohol[3] and later[4] the similar action of other monohydroxy alcohols and of oleic acid.[5] The number of compounds with a selective precipitation action for amylose was further extended by Whistler and Hilbert[6] to include those containing nitro, ester, ketone, mercapto groups and cyclic nitrogen as in pyridine. Most recently, Haworth, *et al.*,[7] have recommended thymol.

All these complexing agents give approximately the same yield of amylose from the same source, about 22–29% from corn starch, and the purity as determined by iodine titration does not vary significantly. This uniformity of behavior among a group of compounds of such widely different nature is rather surprising. The present work was undertaken to see if this similarity exists with amylose of low molecular weight. The agents chosen for investigation were *n*-butyl alcohol, 2-nitropropane, *n*-amyl acetate, *n*-amyl methyl ketone and nitrobenzene[8] as being representative of the various types. Amylose was degraded by acid hydrolysis until only a portion of it would precipitate in the presence of each agent after two to four days of slow cooling in the manner in which amylose is usually isolated. Unlike the behavior of high molecular weight amylose, precipitation was not complete at this point, but continued for several weeks. These precipitates were also collected. The fractions so obtained were analyzed for molecular weight by the spectrophotometric method of Swanson.[9] Iodine sorption[10] and X-ray diffraction patterns were also obtained.

Experimental

Hydrolysis and Fractionation Procedure.—Approximately 240 g. of once-recrystallized butanol–amylose paste prepared by butanol precipitation from potato or

(1) Journal Paper No. 367 of the Purdue University Agricultural Experiment Station.

(2) Department of Physics, Purdue University.

(3) T. J. Schoch, THIS JOURNAL, **64**, 2957 (1942).

(4) T. J. Schoch, "The Fractionation of Starch" in *Advances in Carbohydrate Chemistry*, Academic Press, Inc., New York, N. Y., 1945, Vol. 1, p. 247.

(5) T. J. Schoch, THIS JOURNAL, **66**, 1232 (1944).

(6) R. L. Whistler and G. E. Hilbert, *ibid.*, **67**, 1161 (1945).

(7) W. N. Haworth, S. Peat and P. E. Sagrott, *Nature*, **157**, 19 (1946).

(8) Nitrobenzene is recommended as a fractionating agent in unpublished work of Whistler, Johnson and Hilbert. As the amount of a complexing agent needed is roughly determined by its solubility in water, nitrobenzene is useful in large-scale fractionations as only limited quantities are required in contrast to the large amounts required for other agents. With nitrobenzene the yields of amylose are dependent on *p*H. A maximum occurs in the range *p*H 7.5 to 9.0.

(9) M. A. Swanson, *J. Biol. Chem.*, **172**, 825 (1948).

(10) F. L. Bates, D. French and R. E. Rundle, THIS JOURNAL, **65**, 142 (1943); E. J. Wilson, T. J. Schoch and C. S. Hudson, *ibid.*, 1380.