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 ± 0.5° (*c* 1, chloroform); beta, $[\alpha]_D$ + 122.0 ± 0.5° (*c* 1, chloroform); gamma, $[\alpha]_D$ + 138.5 ± 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 ± 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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[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

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

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

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)

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

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.

The rate of destruction of β and the net rate of increase of H and R may then be expressed by the differential equations

$$d\beta/dt = -k_1\beta \quad (2)$$

$$dH/dt = k_1\beta - 6k_2H \quad (3)$$

$$dR/dt = k_1\beta + k_2(7\beta_0 - 7\beta - R) \quad (4)$$

with the corresponding solutions

$$\beta = \beta_0 e^{-k_1 t} \quad (5)$$

$$H = \beta_0 \frac{k_1}{(6k_2 - k_1)} [e^{-k_1 t} - e^{-6k_2 t}] \quad (6)$$

$$R = \beta_0 \left[7 - e^{-k_1 t} + \frac{6}{(k_2 - k_1)} (k_1 e^{-k_2 t} - k_2 e^{-k_1 t})\right] \quad (7)$$

The degree of purity of the amyloheptaose, assuming complete removal of unchanged beta dextrin and inorganic material, is $H/(\beta_0 - \beta)$.

Evaluation of k_1 was carried out by determining the initial rate of appearance of reducing groups (eqn. 4) as well as by recovery of unchanged beta dextrin after varying periods of hydrolysis; k_2 was approximated by plotting R (eqn. 7) against k_1t for trials values of k_2/k_1 and comparing with the observed reducing power.

Using k_2/k_1 thus determined, an extent of hydrolysis of 2% leads to an amyloheptaose of 95% purity. This limit was arbitrarily selected as a satisfactory compromise between purity of product and single-step yield.

Properties of Amyloheptaose

A carefully purified batch of beta dextrin was hydrolyzed, using the principles outlined above, and 13.3 g. of amyloheptaose obtained. The product was a white glassy substance which has not yet crystallized. It is very soluble in water and insoluble in alcohol, forming a sirup when alcohol is added to an aqueous solution. It is only slightly sweet and the iodine coloration is scarcely noticeable. The observed rotation $[\alpha]_D$ +176° is somewhat lower than that calculated using Freudenberg's equation[9] and the customary constants for starch[10]: $[\alpha]_D$ +179.6°. The reducing power indicates a molecular weight of 1200 (alkaline ferricyanide method) or 1206 (iodine titration of aldoses) as compared with a calculated value of 1154. These discrepancies may be harmonized if one assumes that the glassy sample holds a small amount of difficultly removable water or other inert impurity (*i. e.*, about 3%). Chromatography of the amyloheptaose preparation failed to detect any glucose, maltose or trisaccharide although these substances are easily observed after more extensive hydrolysis of cycloheptaamylose.

The phenylhydrazone and potassium aldonate of amyloheptaose were prepared using standard procedures, and the nitrogen content of the phenylhydrazone and the potassium content of the aldonate were in good agreement with the values calculated for corresponding heptasaccharide derivatives.

(9) Freudenberg, Friedrich and Bumann, *Ann.*, **494**, 41 (1932).
(10) Levine, Foster and Hixon, THIS JOURNAL, **64**, 2331 (1942).

In studies to be reported subsequently it has been found that beta amylase degrades amyloheptaose to give a mixture of maltose and trisaccharide in the mole ratio of two to one. This indicates that the original oligosaccharide contains an odd number of glucose units, as beta amylase converts even-numbered amylose chains completely to maltose.[11] The mole ratio of maltose to trisaccharide as well as the increase in reducing power may be determined with sufficient precision to eliminate the possibility that the enzyme substrate was either a pentaose or a nonaose.

The chemical evidence concerning the identity and purity of the initial hydrolysis product of cycloheptaamylose, *i. e.*, amyloheptaose, is in harmony with the theoretical calculations within the experimental error of the methods used.

Experimental

Cycloheptaamylose.—This substance was prepared using methods previously outlined[4,7] and had the following constants: water, 13.4%; $[\alpha]_D$ + 163.2 ± 0.5°, dry basis; ash by ignition, 0; ash by conductivity as sodium chloride, 0.005%.

Determination of k_1.—A ratio of one gram, dry weight, of cycloheptaamylose to 4 ml. of 0.001 *N* hydrochloric acid was uniformly used throughout all experiments and preparations in this paper. Tubes containing *ca.* 0.5 g. of cycloheptaamylose in 2 ml. of 0.001 *N* hydrochloric acid were stoppered and heated in a boiling water-bath for varying periods of time. The tubes were swirled occasionally to insure homogeneity of the reaction mixture. After a definite period of time a tube was removed, the contents transferred to a 300-ml. flask and the reducing power determined by the alkaline ferricyanide method.[10] The rate constant was calculated using the first term of eqn. 4 in integral form. After 180, 360, 540 min., $k_1 \times 10^5$ = 4.7, 6.5, 6.9.

Ten-gram samples of cycloheptaamylose dissolved in 40 ml. of 0.001 *N* hydrochloric acid were heated at 99° and after various periods of time the acid was neutralized and the mixtures were refrigerated. The crystals of cycloheptaamylose were collected quantitatively in weighed alundum filtering crucibles. The filtrates were treated with toluene by shaking twenty-four hours followed by refrigerating twenty-hour hours and the resulting precipitates collected in tared alundum crucibles. From the total dry weight of recovered cycloheptaamylose from each sample the k_1 values were calculated using eqn. 5: after 4,280, 7,130, 28,560 min., $k_1 \times 10^5$ = 4.0, 5.0, 4.6.

Determination of k_2/k_1.—The filtrate from the 28,560-minute sample above was collected quantitatively and the average molecular weight of the solids determined by iodine titration and specific rotation. From these figures the average number of reducing groups per mole of starting material was calculated to be 2.38. This value corresponded most closely to a value for k_2/k_1 of 0.64 on a plot of various values for k_2/k_1 in eqn. 7. Using $k_1 = 4.6 \times 10^{-5}$, $k_2 = 2.9 \times 10^{-5}$.

Preparation of Amyloheptaose.—A 100-g. sample of pure cycloheptaamylose was dissolved in 400 ml. of 0.001 *N* hydrochloric acid in a 1000-ml. flask fitted with a condenser. The flask was immersed in a boiling water-bath, 99°, and shaken frequently. After seven hours the flask was removed, the acid immediately neutralized with the calculated amount of lithium carbonate and the unhydrolyzed cycloheptaamylose allowed to crystallize on cooling to room temperature and refrigerating twenty-four hour. The crystals were removed by filtration and the remaining cycloheptaamylose removed by saturating the filtrate with *p*-xylene, shaking for twenty-four hours,

(11) Myrbäck and Leissner, *Arkiv Kemi, Mineral. Geol.*, **17A**, No. 18 (1943).

refrigerating twenty-four hours, and filtering off the precipitates. This final filtrate was then concentrated to one-fifth its original volume *in vacuo*. Six additional hydrolyses were carried to this stage and the concentrated filtrates combined. The resulting solution was again covered with *p*-xylene, shaken and refrigerated for twenty-four hour each, filtered, and the filtrate concentrated to a thin sirup *in vacuo*. In order to remove any lower saccharides and lithium chloride the amyloheptaose was precipitated by adding four volumes of absolute ethanol. The sirupy precipitate was taken up in a small amount of water and reprecipitated in like manner five times. Finally the precipitate was dehydrated with absolute ethanol to which a small amount of absolute butanol was added, filtered, and washed with dry butanol.[12] The material was finally dried to constant weight in the vacuum oven at 70°, $[\alpha]_D$ + 175.5° (c = 2, water); another preparation, + 176.5°; theoretical, + 179.6°.[9,10] Molecular weight by alkaline ferricyanide,[10] 1200; by the Kline and Acree hypoiodite method,[13] 1206; theoretical, 1152.

Potassium Amyloheptaonate.—The method of Levine, Foster and Hixon[10] for the preparation of dextrinic acids was applied to 0.5 g. of amyloheptaose. After the final grinding and acetone extraction, the potassium salt was dried to constant weight and a weighed sample ashed with ammonium sulfate. The molecular weight was calculated from the potassium content: observed, K 3.34%, m. wt. 1167; calcd., m. wt. 1206.

Amyloheptaose Phenylhydrazone.—The phenylhydrazone was prepared by a modification of the method of Bergmann and Machemer[14] previously described.[10] Nitrogen was determined by the micro-Dumas method and the molecular weight of the derivative calculated assuming two atoms of nitrogen per molecule: observed, N 2.19%, 2.27%, m. wt. 1275, 1235; calcd., m. wt. 1242.

(12) The butanol was used to prevent condensation of water and consequent gumming of the amyloheptaose due to the evaporation of the ethanol during the filtration and drying.

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

(14) Bergmann and Machemer, *Ber.*, **63**, 322 (1930).

Chromatographic Test for Lower Saccharides.—A 20 mg. sample of the sugar was dissolved in 0.3 ml. of water and 4.8 ml. of absolute ethanol added. The resulting mixture was placed on a chromatographic column consisting of 12 g. of a 3:1 mixture of Florex XXX and Celite 535 as described by Lew, Wolfrom and Goepp.[15] The sugars were eluted with 90% ethanol and the concentration of sugar in the eluate determined colorimetrically by the diphenylamine method.[16] The rate of flow of the individual sugars was found to be sufficiently characteristic and the diphenylamine test sufficiently sensitive that an amount of glucose, maltose or trisaccharide as low as 0.01 mg./ml. could be readily detected.

On testing 20 mg. of amyloheptaose in this manner, there was no evidence whatsoever for contamination by glucose, maltose or trisaccharide. The column was then extruded, cut into 2-cm. portions and the individual portions extracted and tested for carbohydrate. The top 2-cm. portion was carbohydrate free, the next two 2-cm. portions contained carbohydrate, and the remainder of the column was carbohydrate free.

A cycloheptaamylose sample which was hydrolyzed for *ca.* 7000 minutes (k_2t = 0.35) was similarly chromatographed, and glucose, maltose and trisaccharide were found to be present in sizable amounts (109 mg., 81 mg. and 62 mg.).

Summary

Amyloheptaose has been prepared by controlled acid hydrolysis of cycloheptaamylose. The analytical constants of amyloheptaose, its potassium aldonate and its phenylhydrazone agree with those calculated for a heptasaccharide.

(15) Lew, Wolfrom and Goepp, THIS JOURNAL, **68**, 1449 (1946).

(16) See Oppel, *Biochem. Z.*, **229**, 85 (1930), and Davidson, Kermack Mowat and Stewart, *Biochem. Z.*, **30**, 433 (1936), for two modifications of the quantitative diphenylamine method.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, RENSSELAER POLYTECHNIC INSTITUTE]

The Reaction of Diethanolamine with Nitriles and Potassium Cyanide

BY WILLIAM H. RAUSCHER, IRVING S. GOLDSTEIN AND MAXWELL BRESLAU

Since ethanolamines have been used as agents for the reduction of organic compounds,[1] especially nitro compounds, it was though possible they might convert nitriles to amines. However, benzonitrile and benzyl cyanide, when refluxed with substantially dry diethanolamine, undergo hydrolysis rather than reduction to yield benzoic and phenylacetic acid, respectively, with concomitant evolution of ammonia. Maximum yields are obtained with a nitrile to diethanolamine ratio of 1:5 and a reflux period of forty-eight hours.

From larger scale runs, conducted in an attempt to isolate intermediate products, it was possible to obtain substantial quantities of water and piperazino-1,4-bis-(β-ethanol). It appears therefore that diethanolamine undergoes cyclization to the piperazine and water which effects hydrolysis of the nitrile. No other details of the mechanism have been established.

Under similar conditions, potassium cyanide yields ammonia and a mixture of potassium formate and succinate. The latter salt, the formation of which was unexpected, was always obtained in much larger amount than the formate. It was established that only a small amount of ammonia is evolved by refluxing diethanolamine alone and that the presence of a small amount of potassium cyanide in refluxing diethanolamine does not catalyze the evolution of ammonia from the amine or cause its cyclic dehydration to the piperazine. Potassium succinate was not formed by refluxing potassium formate with diethanolamine. The mechanism of the conversion of potassium cyanide to potassium succinate has not been determined.

(1) (a) M. Meltsner, C. Wohlberg and M. J. Kleiner, THIS JOURNAL, **57**, 2554 (1935); (b) C. B. Kremer, *ibid.*, **59**, 1681 (1937); (c) M. Meltsner, L. Greenstein, G. Gross and M. Cohen, *ibid.*, **59**, 2660 (1937); (d) C. B. Kremer and B. Kress, *ibid.*, **60**, 1031 (1938); (e) C. B. Kremer and A. Bendich, *ibid.*, **62**, 1279 (1940).