STUDIES ON THE SCHARDINGER DEXTRINS. XI.

THE ISOLATION OF NEW SCHARDINGER DEXTRINS[1]

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Three members of the Schardinger dextrin series have previously been isolated and studied. Schardinger (1903) described properties of the α- and β-dextrins, while Freudenberg and Jacobi (1935) were the first to obtain γ-dextrin. French *et al.* (1942, 1950) have proven these cyclic compounds to contain 6, 7, and 8 glucose units, respectively, and have named them cyclohexaamylose, cycloheptaamylose and cyclooctaamylose. For a comprehensive review of the Schardinger dextrins see French (1957).

Previous evidence for the existence of δ-dextrin[3] and possibly ε-dextrin[3] consists of preliminary unpublished radiotracer experiments by French and Effenberger. Extensive action of *Bacillus macerans* amylase on radioactive glycogen followed by treatment with β-amylase produced Schardinger dextrins which were separated and located by paper chromatography and radioautography. A densitometer tracing gave a distinct maximum for δ-dextrin, and a small definite shoulder which was thought to be ε-dextrin. (see p. 206-8 of French, 1957).

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[3] The terms "δ-dextrin" and "ε-dextrin" have been previously used by Freudenberg and Jacobi (1935) to describe certain fractions which differ substantially from the δ- and ε-dextrins mentioned herein.

This general approach has now been followed, using non-radioactive starch on a macroscopic scale. After B. macerans enzymolysis, preliminary solvent fractionation, and treatment with β-amylase, the fraction expected to contain the higher members of the Schardinger dextrin series was separated by high temperature column chromatography. The elution diagram (Fig. 1) shows well-defined maxima of anthrone-positive material (carbohydrate). Four components have been isolated in sequence after the three well known Schardinger dextrins (α, β, and γ). These components have no detectable reducing value and have survived extensive attack by β-amylase, in harmony with a presumed cyclic structure. Extending the previously used nomenclature these would be δ-, ϵ-, ζ- and η-dextrins (cyclononaamylose, cyclodecaamylose, cycloundecaamylose and cyclododecaamylose).

The peak between maltotriose and α-dextrin is a fraction that has appreciable reducing power, but less than that of an equal weight of maltotetraose. Under the conditions used, some maltotetraose escapes the action of β-amylase. This fraction might therefore be a mixture of maltotetraose and cyclopentaamylose. Crystals formed on standing in the column eluate fractions at the places corresponding to each peak after maltotetraose to γ-dextrin. The typical shapes of α-, β-, and γ-dextrin crystals formed in 60% 1-propanol were the same as those formed in the eluate.

Hexagonal and spindle shaped crystals have been obtained from δ-dextrin in 60% propanol. Small rods have been formed from ϵ-dextrin in 60% propanol, but with some difficulty. As the molecular size increases the ease of crystallization decreases. This may be an intrinsic property or it could be caused by contaminating branched cyclic molecules or other substances. No branched cyclic molecules have been proven to be formed but there is at present no evidence which would eliminate them.

EXPERIMENTAL

A 50 g sample of potato starch was dispersed in about 1000 ml of water, cooled, and 500 ml of enzyme solution containing 4 Tilden & Hudson units per

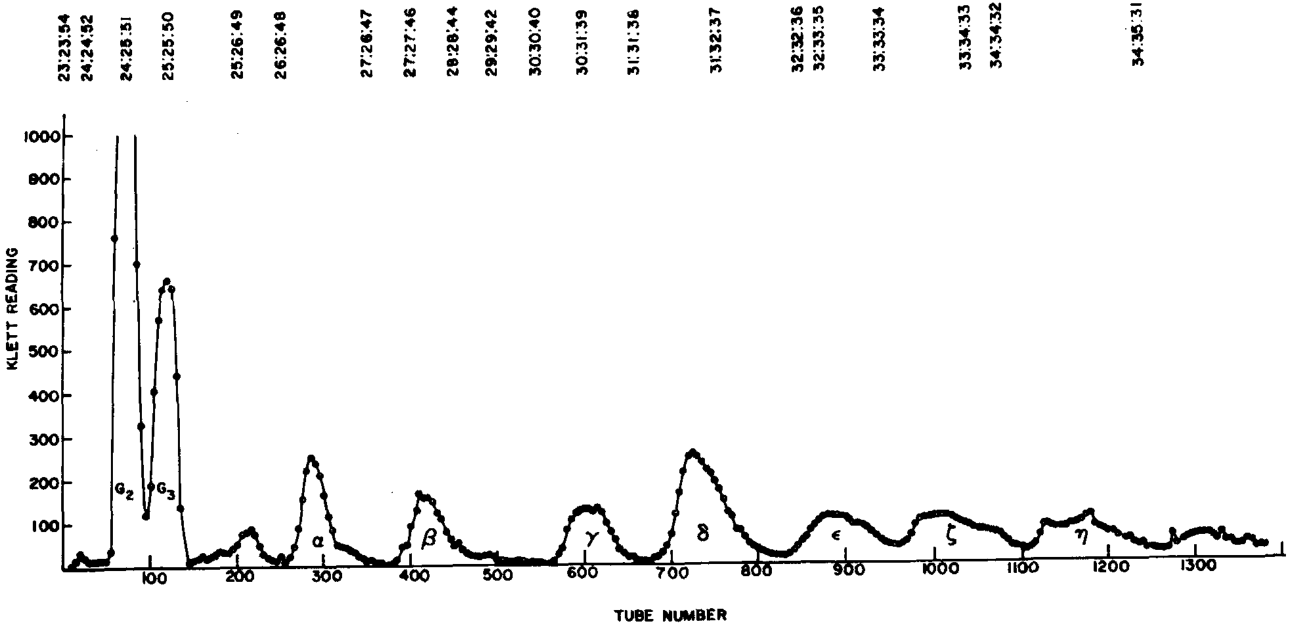

Fig. 1. Elution diagram of B. macerans digest of potato starch. The composition of the irrigating solvent is indicated at the top of the figure as volume ratios of H_2O: ethanol: butanol. Peaks labelled G_2, G_3 have been identified as maltose, maltotriose. Peaks labelled α, β, γ have been identified by the characteristic shapes of crystals from 60% propanol. In addition, α with I_2 -KI shows characteristic dichroic needles and blue hexagons.

ml was added. The solution was diluted to 3% starch with water and allowed to incubate at 40° for 400 conversion periods. After inactivation of the enzyme by heating, the solution was concentrated and stirred with cyclohexane to remove most of the α- and β-dextrins. The high molecular weight limit dextrins were precipitated with 60% 1-propanol (with ethanol or methanol a higher alcohol concentration is required). After the alcohol was removed under reduced pressure, the α-, β- and γ-dextrins were precipitated insofar as possible by treatment with a mixture of 1,1,2,2-tetrachloroethane and tetrachloroethene. Extensive treatment with crystalline sweet potato β-amylase removed small amounts of contaminating linear oligosaccharides.

The sample was applied to the cellulose column described by Thoma *et al.* (1959). The concentrations of water, ethanol and 1-butanol used to develop the column are given in Fig. 1. Every fifth tube of the eluate was analyzed by evaporating 0.5 ml to dryness, adding 2 ml of water and 4 ml of the anthrone reagent. After mixing in an ice bath the samples were heated for ten minutes in a boiling water bath. The absorbancies were measured in a Klett-Summerson photoelectric colorimeter with a red filter. The carbohydrate fractions were obtained by pooling eluate fractions corresponding to each peak and evaporating to dryness *in vacuo*. Organic contaminants were extracted with acetone.

Using the 3,5 dinitrosalicyclic acid method (Schwimmer, 1950), 1-2 mg samples of δ, ϵ, ζ, and η-dextrins gave reducing values of zero, whereas reducing oligosaccharides in this chromatographic range would be expected to give values of 40-100 Klett units.

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