1962

Starch and Schardinger dextrins: I. Complexes with hydrophobic compounds; II. Higher Schardinger dextrin homologs

Arden Orion Pulley

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PULLEY, Arden Orion, 1934—
STARCH AND SCHARDINGER DEXTRINS. I. COMPLEXES WITH HYDROPHOBIC COMPOUNDS,
II. HIGHER SCHARDINGER DEXTRIN HOMOLOGS.

Iowa State University of Science and Technology
Ph.D., 1962
Chemistry, biological

University Microfilms, Inc., Ann Arbor, Michigan
STARCH AND SCHRADINGER DEXTRINS

I. COMPLEXES WITH HYDROPHOBIC COMPOUNDS

II. HIGHER SCHARDINGER DEXTRIN HOMOLOGS

by

Arden Orion Pulley

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Biochemistry

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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Head of Major Department

Signature was redacted for privacy.

Dean of Graduate/College

Iowa State University
Of Science and Technology
Ames, Iowa
1962
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PART I. COMPLEXES WITH HYDROPHOBIC COMPOUNDS
INTRODUCTION

An understanding of the nature of the complexes of amylose would be increased by a comparison with corresponding complexes of the Schardinger dextrins. There are several nonpolar organic compounds that form excellent crystalline complexes with the low molecular weight cyclic dextrins. Comparison of the amylose complex-forming tendencies of these various hydrophobic compounds would be expected to give some insight into the requirements for complex formation and the nature of the complexes formed.

Due to the helical nature of the crystalline amylose complexes and the fixed cyclic structure of the cyclo-amyloses, it is to be expected that certain correlations would be found with respect to complex formation. This has indeed been found to be true. However, not all agents which form good complexes with the cyclic dextrins form good amylose complexes, and similarly the classical complexing agents for amylose form relatively poor complexes with the cyclic Schardinger dextrins.
REVIEW OF THE LITERATURE

The precise mechanism whereby amylose forms complexes with many compounds is not completely known. The discovery by Schoch (1) that starch could be fractionated with butanol was particularly important for those interested in the study of starch. Many experiments performed on unfractionated starch needed re-examination when it was discovered that starch could be fractionated into 2 different components. The starch fraction which formed an insoluble precipitate with butanol was called the "A-fraction" by Schoch but amylose by others.

Several explanations as to the mechanism of the complex formation were offered. Schoch (2) first thought the selective precipitation might be explained as being due to some undefined optimum of molecular volume or "hydrophil balance." Several classes of compounds capable of association with starch were examined by Bear (3). In an effort to identify the essential feature of complexing agents, he concluded that all the compounds possess a permanent electric moment or a tendency to assume an induced moment (iodine). Virtually all workers now accept the explanation that a "polar molecule" is required.

Hydrogen bonding is widespread in nature and has been suggested by Whistler and Hilbert (4) as being responsible for the binding of the entities in the complex. They
investigated a few nitroalkanes and found them to be capable of fractionating starch by the formation of insoluble complexes with amylose. They reasoned that compounds of several different classes having donor, acceptor or both groups would be capable of fractionating starch. Compounds possessing nitro, ester, ketone, mercapto, carboxyl groups and cyclic nitrogen were given as examples of excellent fractionating agents for starch.

The crystallized complex of amylose with palmitic acid exhibits a "V" X-ray pattern (5) indicating a helical configuration. Deuterium exchange has been found to take place readily between D$_2$O and this crystallized complex by Taylor, Zobel, White and Senti (6). In contrast to the butanol-complex of amylose, the palmitic acid complex is insoluble in hot water but is nonetheless completely exchangeable. The reverse is true with polypeptides which if in the helical configuration, are resistant to exchange (7). In the case of the amylose complex, the complete exchangeability indicates that mechanisms other than hydrogen bonding are of major importance in stabilizing this helical structure. This is said with particular emphasis regarding the hydroxyl hydrogens of the amylose. The hydrocarbon-like character of the complexing agent seems to be the most likely stabilizing factor.

The crystallization of starch in the presence of nonpolar compounds was first reported by Wiegel (8). He observed "starch crystals," which would now be interpreted...
as complexes, when starch was treated with 1,2-dichloroethane and 1,4-dioxane, as well as several polar compounds such as aliphatic alcohols. The 2 nonpolar agents mentioned constituted a minor fraction of the compounds examined. Since other compounds which he studied formed better complexes and have been more completely studied by others, the significance of the nonpolar complexing agents of Wiegel has been neglected.
EXPERIMENTAL

Hydrophobic Complexes of Starch

A slurry of 5 g of potato starch in 100 ml of water was poured into 350 ml of hot water with rapid stirring. After dilution to 500 ml, it was autoclaved at 120 degrees for 1 hr. The viscosity was slightly reduced by brief treatment with a Waring blender followed by filtration through a coarse fritted glass filter. When soluble starch was used, enough starch to make a 3 per cent solution was gently heated with swirling until a clear solution resulted. Following dilution to 500 ml, the dispersion was autoclaved for 1 hr.

After removal from the autoclave, 10 ml of starch was placed in each of several test tubes and 1 ml or 1 g of the hydrocarbon or halocarbon was added. In every case this was sufficient to form a distinct layer. The hot tubes were then placed in a Dewar flask and slowly cooled over a period of several days. The tubes were checked from time to time to see when a precipitate formed. When the tubes were checked, the temperature was also noted. The temperature of 1 typical starch solution gradually dropped from about 85 degrees the first day to 53 degrees the next day, then to about 41 degrees the third, finally falling to about 25 degrees at the end of a week. In the presence of the complexing agents, some starch solutions first became cloudy and then a distinct precipitate.
was observed while others remained clear. The temperature of precipitation was observed to fall in a certain range for each effective complexing agent. Any observed precipitates were examined microscopically for crystallinity. The complex with 1,1,2,2-tetrabromoethane was separated by centrifugation, air dried slightly and then put in a capillary tube for an X-ray powder pattern. The blue value was measured in a 20 mm cell with a Klett-Summerson photoelectric colorimeter. The concentration of starch was 0.01 per cent, iodine was 0.002 per cent and potassium iodide was 0.02 per cent.

Hydrophobic Complexes of α-Dextrin

The solubility of α-dextrin in the presence of several hydrocarbons and halocarbons was determined at room temperature (about 25 degrees). Enough complexing agent was added to form a distinct layer above or below the solution of α-dextrin in each flask. The flasks were shaken from time to time over a period of 2 weeks. After this time, enough solution was pipetted from above the crystalline complex to measure the concentration by optical rotation. A Rudolph Polarimeter was used to measure the optical rotation of the solutions in a 4 dm tube.
Preparation of α-Dextrin

High yields of α-dextrin (cyclohexaamylose) were obtained by allowing *Bacillus macerans* amylase to act for 3 conversion periods (9) in the absence of a precipitating agent. At this point, the digest was clarified and a few small lumps of starch removed by filtration. An excess of 1,1,2,2-tetrachloroethane was added and the solution was stirred for 3 more conversion periods at room temperature. During this time, the crystalline α-dextrin tetrachloroethane complex formed and was then removed by suction filtration. The complex was dispersed in sufficient water to make about a 10 or 15 per cent solution and carefully boiled until the complexing agent was all volatilized. This solution was diluted to 2 per cent and allowed to react with *Bacillus subtilis* α-amylase for 1 or 2 days. A small amount of precipitate which formed was removed by filtration and then the solution was heated to inactivate the α-amylase. Cyclohexane was then used to form a crystalline complex with the α-dextrin. Reprecipitation with cyclohexane can be repeated followed by crystallization from water, or the dextrin can be crystallized from 60 per cent 1-propanol. The cyclohexane is easier to remove completely, and therefore, its use is preferred when the absence of alcohol is important.
RESULTS AND DISCUSSION

The temperatures at which any individual complex formed during the cooling of the mixture may be taken as an indication of the strength of the complex. The amount of the amylose removed by a complexing agent is also related to the strength of its complex. This was visually estimated by the amount of blue color produced in the supernatant with an iodine solution. The complexing agents which form a complex with starch at a high temperature also remove the amylose most completely from solution. Table 1 gives precipitation temperatures for various hydrocarbons and halogenated hydrocarbons which are nonpolar (at least in 1 molecular conformation).

Table 1. Nonpolar complexing agents of starch

<table>
<thead>
<tr>
<th>Compound</th>
<th>Temperature at which precipitation was observed</th>
<th>Compound</th>
<th>Temperature at which precipitation was observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,1,2,2-Tetrachloroethane</td>
<td>83°</td>
<td>Benzene</td>
<td>56°</td>
</tr>
<tr>
<td>Cycloheptane</td>
<td>81°</td>
<td>*Cyclooctane</td>
<td>53°</td>
</tr>
<tr>
<td>Hexachloroethane</td>
<td>78°</td>
<td>*1,2-Dichloroethane</td>
<td>50°</td>
</tr>
<tr>
<td>1,1,2,2-Tetrabromoethane</td>
<td>69°</td>
<td>Cyclopentane</td>
<td>41°</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>68°</td>
<td>2,3-Dibromobutane</td>
<td>27°</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>66°</td>
<td>2,3-Dimethylbutane</td>
<td>26°</td>
</tr>
</tbody>
</table>

*These compounds do not form a complex with 3 per cent aqueous soluble starch but do with 1 per cent potato starch under the conditions used. All others in Tables 1 and 2 complex both types of starch.
Table 2 lists precipitation temperatures for similar compounds which, though more or less polar, are very hydrophobic and incapable of extensive hydrogen bond formation.

Table 2. Polar complexing agents of starch

<table>
<thead>
<tr>
<th>Compound</th>
<th>Temperature at which precipitation was observed</th>
<th>Compound</th>
<th>Temperature at which precipitation was observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1.2.2] Bicycloheptene</td>
<td>70°</td>
<td>Chloroform</td>
<td>52°</td>
</tr>
<tr>
<td>Cyclohexene</td>
<td>65°</td>
<td>Fluorobenzene</td>
<td>52°</td>
</tr>
<tr>
<td>Cycloheptene</td>
<td>61°</td>
<td>1,1,2-Trichloroethane</td>
<td>50°</td>
</tr>
<tr>
<td>Cycloheptadiene</td>
<td>57°</td>
<td>Methylcyclohexane</td>
<td>41°</td>
</tr>
<tr>
<td>1,1,1-Trichloroethane</td>
<td>57°</td>
<td>Methylcyclopentane</td>
<td>40°</td>
</tr>
<tr>
<td>Bromocyclopentane</td>
<td>53°</td>
<td>*o-Xylene</td>
<td>32°</td>
</tr>
<tr>
<td>1,2-Dichloropropane</td>
<td>53°</td>
<td>*o-Bromotoluene</td>
<td>32°</td>
</tr>
</tbody>
</table>

*These compounds do not form a complex with 3 per cent aqueous soluble starch but do with 1 per cent potato starch under the conditions used. All others in Tables 1 and 2 complex both types of starch.

Compounds listed in Table 3 do not form visible precipitates with starch. Some of these compounds are nonpolar; some of them are at least as polar as the good complexing agents in Table 2. All of them are hydrophobic; thus, there must be an additional feature essential for facile complex formation with amylose. Perhaps it is the molecular shape or the ability of the complexing agent to form a good "fit" with the amylose helix.
Each of the precipitated complexes was examined with a microscope to see whether or not crystals could be detected. In almost every case, some crystalline form could be seen. Spherocrystals were the most common, but a few complexing agents gave needles. Only the crystals obtained with 1,1,2,2-tetrabromoethane were birefringent. Photographs of some of the spherocrystals are shown in Fig. 1.

The X-ray diffraction powder pattern of the starch-1,1,2,2-tetrabromoethane complex is essentially identical in line position and intensity with that of the amylose-phenol complex. The calculations of the packing from the powder pattern agree with the calculated measurements of an amylose coil with 7 glucose units per turn.* The complexes are crystalline, form at different temperatures, depend upon the size and shape of the complexing agent and include nonpolar compounds such as

---

Fig. 1. These are photomicrographs at 450X of the spherocrystals obtained with starch and (A) cycloheptane, (B) 1,1,2,2-tetrachloroethane, (C) cyclohexanol and (D) cyclohexane. The cyclohexanol is shown as an example of a complexing agent which has been used to fractionate starch.
cyclohexane. The 1,1,2,2-tetrachloroethane complex gave a positive Beilstein test after washing with alcohol and vacuum drying. This evidence clearly indicates that the precipitation is not due to a surface effect but that the complexing agent is within the helix and aids its formation. Similar examples are those of the starch-butanol and the starch-iodine complexes (10).

The fraction of soluble starch which precipitated with 1,1,2,2-tetrachloroethane had a blue value of 0.236, corresponded to 23 to 28 per cent of the total starch and had a tendency to retrograde. The supernatant fraction had a blue value of 0.028 and would not retrograde. An authentic sample of amylose (1 per cent solution) produced a very heavy precipitate upon addition of 1,1,2,2-tetrachloroethane. An authentic sample of amylopectin (1 per cent solution) gave no precipitate upon addition of 1,1,2,2-tetrachloroethane. An iodine and iodide solution gave a purple color with the supernatant from a 1,1,2,2-tetrachloroethane-starch mixture while the redissolved precipitate gave a deep blue color. This definitely proves that the insoluble complex is amylose and that amylopectin is the soluble fraction.

The solubility of α-dextrin (cyclohexaamylose) was measured in the presence of several hydrophobic organic compounds at 25 degrees and the results are summarized in Table 4.
Table 4. Apparent solubility of \(\alpha\)-dextrin in the presence of excess hydrophobic complexing agents

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solubility of (\alpha) in g/100 ml</th>
<th>Solubility of (\alpha) in g/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,1,2,2-Tetrachloroethane</td>
<td>.057</td>
<td>Cyclooctane</td>
</tr>
<tr>
<td>1,1,2,2-Tetrabromoethane</td>
<td>.061</td>
<td>Bromocyclopentane</td>
</tr>
<tr>
<td>Methylcyclopentane</td>
<td>.123</td>
<td>2,3-Dimethylbutane</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>.130</td>
<td>1,2-Dichloropropane</td>
</tr>
<tr>
<td>Methylcyclohexane</td>
<td>.136</td>
<td>Bromocyclopentane</td>
</tr>
<tr>
<td>2,3-Dibromobutane</td>
<td>.145</td>
<td>Cyclohexene</td>
</tr>
<tr>
<td>2,2-Dimethylbutane</td>
<td>.172</td>
<td>Toluene</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>.212</td>
<td>Cyclopentane</td>
</tr>
<tr>
<td>1,1,1-Trichloroethane</td>
<td>.221</td>
<td>Benzene</td>
</tr>
<tr>
<td>Cycloheptane</td>
<td>.231</td>
<td>Fluorobenzene</td>
</tr>
<tr>
<td>1,1,2-Trichloroethane</td>
<td>.275</td>
<td>Chloroform</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesitylene</td>
</tr>
</tbody>
</table>

The apparent solubilities were calculated from the observed optical rotations with the assumption that the specific rotation of the carbohydrate is not changed significantly by the complexing agent. Two hydrophobic complexing agents (benzene and toluene) were individually mixed with solutions of \(\alpha\)-dextrin but no change in optical rotation was observed.

As a point of comparison, the solubility of \(\alpha\)-dextrin and \(\beta\)-dextrin in the presence of several compounds which are reported in the literature (9) are compared below in Table 5.

Table 5. Comparison of solubility of \(\alpha\)- and \(\beta\)-dextrins in water with excess complexing agent

<table>
<thead>
<tr>
<th>Complexing agent</th>
<th>Solubility of (\alpha) in g/100 ml</th>
<th>Solubility of (\beta) in g/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrachloroethylene</td>
<td>0.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Bromobenzene</td>
<td>2.4</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table 5 (Continued).

<table>
<thead>
<tr>
<th>Complexing agent</th>
<th>Solubility of α in g/100 ml</th>
<th>Solubility of β in g/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Cymene</td>
<td>3.3</td>
<td>0.04</td>
</tr>
<tr>
<td>1,1,2,2-Tetrachloroethane</td>
<td>0.057*</td>
<td>0.12</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>0.13*</td>
<td>0.06</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>0.21*</td>
<td>0.1</td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*Values from Table 4.

It is noteworthy that the first 3 are very good complexing agents of β-dextrin, but poor complexing agents of α-dextrin. Moreover, it was shown earlier (Table 3) that they do not form insoluble complexes with amylose. The next 3 are very good complexing agents of α-dextrin, rather poor complexing agents of β-dextrin but readily form an insoluble complex with amylose. It is very striking that the best complexing agents of α-dextrin also complex amylose while the best complexing agents of β-dextrin do not form a precipitate with starch. Cyclohexane is said to be "good" for α-dextrin but "poor" for β-dextrin. This is relative to the solubility in water.

The amylose complex has 7 glucose units per turn while α-dextrin has 6 glucose units in a fixed ring. The apparent contradiction can be explained if the helix is slightly smaller than β-dextrin.

A consideration of the complexing agents indicates that molecules of the correct size, steric arrangement and shape...
will form a complex with amylose; for example, cycloheptane forms a complex with amylose, n-heptane does not. It appears that the greater number of possible conformations of the n-heptane prevents complexation.

Hydrogen bonding may strengthen certain complexes and thus make certain complexes possible. The inside of a starch helix contains glycosidic oxygens and carbon-hydrogen groups. The carbon-hydrogen groups could constitute the "hydrocarbon core" while the glycosidic oxygens serve as acceptor groups. Any compound of the correct size which has a donor group and a hydrophobic group would hydrogen bond to the glycosidic oxygen on one end with the other in the "hydrocarbon core." It is instructive to compare 1-butanol which complexes with amylose and 1-bromobutane which does not.

The action of B. macerans amylase on starch can give yields of α-dextrin as high as 30 per cent of the original starch in the presence of 1,1,2,2-tetrachloroethane. Since 1,1,2,2-tetrachloroethane is a good complexing agent of starch and α-dextrin, the addition of this complexing agent to a digest of B. macerans amylase and starch must be made after 3 conversion periods. This allows enough degradation of the amylose to prevent it from contaminating the α-dextrin. A small amount of β-dextrin is present but is removed after reprecipitation. The α-dextrin 1,1,2,2-tetrachloroethane complex appears to crystallize as double octahedra while the β-dextrin complex appears to be monoclinic.
SUMMARY

The previous explanation of the mechanism for the formation of a complex between starch and any other organic compound is that a "polar molecule" is required. In contradiction to this explanation, it has been found that several nonpolar hydrophobic compounds such as cyclohexane, 1,1,2,2-tetrachloroethane and carbon tetrachloride form well defined crystalline complexes with the amylose fraction of starch. Size, shape, hydrophobic nature and restriction of the number of conformations possible seem to be important features of the complexing agent. An X-ray powder pattern of the 1,1,2,2-tetrabromoethane complex looks very much like the amylose-phenol complex which is a 7 membered or expanded helix.

There is a marked correlation between the best complexing agents of cyclohexaamylose and the good hydrophobic complexing agents of amylose. A model of an amylose helix has an inside in which the hydrocarbon nature of the carbohydrate is manifested by carbon-hydrogen groups or a "hydrocarbon core." It seems reasonable that hydrocarbons and halocarbons would fit inside of the helix to form the complex.

The action of B. macerans amylase upon starch in the presence of 1,1,2,2-tetrachloroethane produces α-dextrin in yields as high as 30 per cent of the original starch with only trace contamination of dextrin. The 1,1,2,2-tetrachloroethane must be added 3 conversion
periods or else the amylose complex precipitates and contaminates the desired dextrin.
PART II. HIGHER SCHARDINGER DEXTRIN HOMOLOGS
INTRODUCTION

The group of cyclic oligosaccharides which are known as the Schardinger dextrins constitute one of the most intriguing and interesting series of homologous carbohydrates that is known. The formation of these cyclic dextrins by the action of the amylase from Bacillus macerans on starch and the degradation products of starch is anomalous when compared with the vast number of enzymes capable of acting upon starch.

Since the mechanism of action of B. macerans is that of a transferase, it seems reasonable that a few enzymes of this class would form cyclic products by coupling the transferred portion onto itself as well as other substrate molecules; however, the above mentioned enzyme is the only one which has been found to produce macro-cyclic carbohydrates.

The ratio of the rate of cyclization reaction compared to the coupling reaction is reflected by the equilibrium concentration for each of the products. On the basis of the relative amounts of the first 3 Schardinger dextrins, it would be predicted that higher members may be present but in reduced amounts perhaps falling off exponentially.

The development of high temperature column chromatography capable of separating the individual homologous compounds of many series has permitted the isolation of several new Schardinger dextrins. The amounts produced are indeed reduced but do not follow an exponential decline. The
compounds are relatively free from neighboring members of the series and do not contain any of the corresponding linear maltosaccharides.

The major portion of this investigation is concerned with the structural determination, characterization of these new Schardinger dextrins and demonstrating that they form an uninterrupted series of cyclic carbohydrates containing the amylose type of linkage between glucosyl residues. The resistance or susceptibility to attack by certain enzymes, molecular weight determination, relative membrane permeation rates and optical rotations constitute the backbone of the information obtained in answer to the problem.

A comparison of the properties of the Schardinger dextrin series with the maltosaccharides is interesting and informative. The isolation of the new Schardinger dextrins extends this series and makes possible a better comparison between the 2 series.
A complete coverage of the publications dealing with the Schardinger dextrins can be found in the reviews of Pringsheim (11, p. 280; 12, p. 35) up to 1932, Samec (13; 14, p. 239) through 1940 and French (15) up to late 1956. The excellent review by French is recommended because of its coverage and because it includes some important previously unpublished work. The references included in this review will be those which are considered to have a direct bearing upon the work.

A heat resistant organism was found by Schardinger (16) to produce crystalline dextrins from starch. He gave the name of Bacillus 
\textit{macerans} to the organism and described the properties of the $\alpha$- and $\beta$-dextrins. In recognition of the work that Schardinger did with these dextrins, they were named after him. After the work of Schardinger, there was much confusion and little progress for many years with respect to the molecular structure of these dextrins.

The work of Freudenberg and his group after 1935 represents the greater part of the contributions concerning the Schardinger dextrins for about 15 years. Much of the confusion that had previously existed was due in large part to impure preparations and failure to recognize the fantastic complexing ability of the cyclic dextrins with such diverse agents as salts, alcohols, fatty acids and water. It had been found that the crystalline dextrins were precipitated
from an aqueous solution by alcohol, ether, chloroform or iodine solutions but this information had been little expanded and poorly exploited.

Freudenberg (17) developed a much needed fractionation scheme capable of separating not only the previously known α- and β-dextrins but also the hitherto unknown γ-dextrin through the use of solubility differences of the dextrins themselves as well as the differences of solubilities and rates of crystallization of the dextrin acetates. Two other fractions were designated S- and ε-dextrins; however, the claim of isolating S- and ε-dextrins was later withdrawn (18) and the less specific names of r- and s-dextrins were substituted.

Circumvention of the tedious acetylation procedure was made possible by French, Levine, Pazur and Norberg (9) who measured the solubility of the 3 dextrins in the presence of several complexing agents and 60 per cent propanol. There is a reduction in solubility of β-dextrin by bromobenzene without a similar change in the solubility of α-dextrin. This use of selective complexing agents makes feasible a simple procedure for isolating higher yields of dextrins with high purity. The more recent fractionation scheme of Cramer and Henglein (19) relies entirely upon the use of specific organic precipitants.

The following table presents the reported optical rotations of the dextrins in water.
The first concrete evidence for higher Schardinger
dextrins came from the work of French and Effenberger with
radioactive glycogen after prolonged action of \textit{B. macerans}
amylase (15). The resultant digest after treatment with
\(\beta\)-amylase was separated by paper chromatography with a water
pyridine butanol solvent. Radioautography located radio-
active maltose, maltotriose, \(\alpha\)-, \(\beta\)- and \(\gamma\)-dextrins with
a definite spot which was undoubtedly \(\delta\)-dextrin. A
densitometer tracing of the radioautograph produced peaks
for \(\alpha\)-, \(\beta\)-, \(\gamma\)- and \(\delta\)-dextrins with a shoulder which was
tentatively designated \(\varepsilon\)-dextrin. The densitometer tracing
reveals peak heights for the last 4 mentioned dextrins which
seem to fall off in amount exponentially.

Brintzinger and Brintzinger (22) developed a method of
determining molecular weights of low molecular weight
molecules by determining their dialysis constants. A good
correlation of dialysis constant compared to molecular weight was found for arabinose, rhamnose, glucose, galactose, fructose, lactose, sucrose and raffinose. Freudenberg (23) used the Brintzinger method for molecular weight determination for the Schardinger dextrins and came to the conclusion that α-dextrin is a mixture of straight-chain molecules with 4 and 5 glucose units per molecule. Unfortunately, Freudenberg did not appreciate the effect of the cyclic nature and the consequent result on the dialysis constant. French and Wild (15) determined the chromatographic mobility of the Schardinger dextrins and compared them with the corresponding maltosaccharides. They found the mobility of α-dextrin to fall between that of maltotetraose and maltopentaose, thus corresponding very well with the dialysis constant obtained by Freudenberg. It was not until later that Freudenberg (24) considered the possibility of large rings. The major evidence for the cyclic nature was: first, the formation of glucose as the sole product of hydrolysis; second, nonreducing character; third, resistance to enzymatic degradation; and fourth, the production of only 2,3,6-tri-O-methyl-D-glucose by methylation analysis. Molecular weight determinations (17) by cryoscopic methods of the acetates in camphor and camphenilone gave a value of 5 glucose units for α- and 6 glucose units for β- and γ-dextrins. Many early workers did not realize the concentration dependence of the observed molecular weight when the cryoscopic method was used.
The first accurate molecular weight determinations were made by French and Rundle (20). Through the correct interpretation of single crystal X-ray analysis, unambiguous molecular weights were obtained which indicated that $\alpha$-dextrin contains 6 glucose units and $\beta$-dextrin contains 7 glucose units. The systematic names of cyclohexaamylose and cycloheptaamylose were proposed. The necessary information obtained included crystal density, per cent solvent of crystallization, the unit cell dimensions and space group. A similar treatment of $\gamma$-dextrin (25) indicated that $\gamma$-dextrin is the cyclic octasaccharide which was named cyclooctaamylose.

Partial acid hydrolysis of the cyclic dextrins followed by removal of the unchanged dextrin with an organic complexing agent gives a series of maltosaccharides (25). This series terminates abruptly with the maltosaccharide containing the same number glucose residues as the parent dextrin. The molecular weight determination can thus be reduced to a counting process.

Paper chromatography is capable of separating oligosaccharides in the range of 1 to 12 monosaccharide residues including those obtained from hydrolyzed polysaccharides. Thoma and French (26) developed a procedure for selection of solvent proportions of ternary systems capable of the highest mobility concomitant with complete separation on chromatograms. For best results, the solvents should have similar volatilities (for example, water, ethanol and nitromethane).
An extension of this technique (27) to high temperature column chromatography has produced useful results. Megasaccharides (D.P. > 10) were isolated up to a D.P. of 18 with little contamination up to D.P. 12 as determined by paper chromatography and detected by a silver dip method (28).

Mixtures of products from polypeptide hydrolysis were partially separated through the use of dialysis by Craig and King (29). It was soon found that a pure material permeated a cellophane membrane according to a first order rate process under certain conditions. The time required for half of the substance to pass through the membrane (the half escape time, t₁/₂ ET) increases rapidly with molecular size. If the log of per cent of the solute remaining is plotted against time, a straight line is obtained for a pure nonassociated solute. A marked positive curvature indicates heterogeneity and thus the method can be used to determine purity (30).

The pore size and shape or the permeability of the membrane is of considerable importance with respect to molecular weight. A membrane with very large pore sizes is practically useless in separating small and medium range molecules. The ideal membrane is one which just passes the solute of interest in a reasonable length of time and thus effectively prevents the passage of anything with a much larger diffusion constant. Craig and Konigsberg (31) have presented methods for modification of cellophane membranes which can be used for
compounds with a molecular weight of 200 on one hand or up to 100,000 on the other.

Previous determination of sedimentation and diffusion constants for the Schardinger dextrins (32,33,34) allows the calculation of the molecular weight. The values deviate by an amount greater than 1/2 the equivalent weight of a glucose unit for the worst determinations. The results are as accurate as would be expected considering the method and the small size of the dextrins. Much more accurate results can be obtained by centrifugation until equilibrium is reached. Yphantis (35) has been able to reach equilibrium quickly using short column heights and has obtained good values for the molecular weight of sucrose.

The method of approach to equilibrium proposed by Archibald (36) is very useful and is now widely used. The method involves calculation according to the equation

$$\frac{1}{x_c} \cdot \left(\frac{dc}{dx}\right) = \frac{M(1-V_\rho)\omega^2}{RT}$$

Equation 1

where \(c\) is concentration, \(\frac{dc}{dx}\) is the concentration gradient at distance \(x\) from the center of rotation, \(M\) is the molecular weight, \(V\) is the partial specific volume of the solute, \(\rho\) is the density of the solution, \(R\) is the gas constant, \(T\) is the absolute temperature and \(\omega^2\) is the angular velocity. For a pure substance, the molecular weights calculated from \(\frac{dc}{dx}\) at the base and at the meniscus should be identical and
time-independent. At true equilibrium, the calculation of the molecular weight from \( \frac{dc}{dx} \) is the same throughout the depth of the solution as well as at the meniscus and base.

Mathematical expressions given by Ginsburg, Appel and Schachman (37) require a measurement of \( c_0 \) (the initial concentration) before calculating \( c_m \) (the concentration at the meniscus). The following equation is used in making the calculations:

\[
c_m = c_0 + \frac{1}{x_b - x_m^2} \left[ \int_{x_m}^{x_b} x^2 \left( \frac{dc}{dx} \right) dx - x_b \int_{x_m}^{x_b} \left( \frac{dc}{dx} \right) dx \right]
\]

Equation 2

The calculation of \( c_b \) (the concentration at the base) can be made as follows:

\[
c_b = c_m + \int_{x_m}^{x_b} \left( \frac{dc}{dx} \right) dx
\]

Equation 3

or

\[
c_b = c_0 + \frac{1}{x_b - x_m^2} \left[ \int_{x_m}^{x_b} x^2 \left( \frac{dc}{dx} \right) dx - x_b \int_{x_m}^{x_b} \left( \frac{dc}{dx} \right) dx \right]
\]

Equation 4

Equations 2 and 4 can be combined to give:

\[
M = \frac{RT}{(1 - \frac{\rho}{\rho_0})^2} \cdot \frac{1}{x_b} \left( \frac{dc}{dx} \right)_{b} - \frac{1}{x_m} \left( \frac{dc}{dx} \right)_{m}
\]

\[
M = \frac{c_b - c_m}{c_b - c_m}
\]

which does not require determination of \( c_0 \).
Equation 5 holds true for a pure substance only (where \( M_m = M_b \)). Rewriting equation 4 for conditions at the meniscus gives:

\[
M_m = \frac{RT}{(1 - \overline{V_p})\omega} \cdot \frac{(\frac{d\alpha}{dx})_m}{x_m c_m} \quad \text{Equation 6}
\]

while the conditions at the base give:

\[
M_b = \frac{RT}{(1 - \overline{V_p})\omega} \cdot \frac{(\frac{d\alpha}{dx})_b}{x_b c_b} \quad \text{Equation 7}
\]
EXPERIMENTAL

Enzymatic Preparation

The amylase was obtained from a culture of \textit{B. macerans}\textsuperscript{1} grown on the usual medium of 10 per cent potatoes and 2 per cent calcium carbonate. The organism was grown for about 3 or 4 weeks and then allowed to stand in the sterile flasks until just prior to filtration. Following filtration, the enzyme was used without further purification.

A 50 g sample of potato starch was dispersed in about 1,000 ml of water by autoclaving for 1 hr. When cooled, 500 ml of enzyme solution containing 4 Tilden and Hudson units per ml was added and allowed to incubate for 400 conversion periods after dilution with water to 3 per cent starch. Some cloudiness had resulted which now was removed by filtration, then brief autoclaving inactivated the enzyme. After concentrating the solution, cyclohexane was added to form a complex with and remove most of the $\alpha$- and $\beta$-dextrins. The high molecular weight limit dextrins were precipitated in 2 stages—first, with 60 per cent ethanol and then, after removal of the ethanol, with 60 per cent propanol. The volume of the solution at this point was about 1500 ml. The solution was concentrated and the alcohol removed under reduced pressure in a cyclone evaporator. A mixture of 1,1,2,2-tetrachloroethane and tetrachloroethane was stirred with the solution of about 200 ml to
reduce the $\alpha$, $\beta$- and $\gamma$-dextrins as much as possible.
Extensive treatment with crystalline sweet potato $\beta$-amylase
hydrolyzed contaminating linear maltosaccharides.

Chromatographic Separation

The sample was applied as a 20 ml solution to the
cellulose column described by Thoma (27). Solutions of
water, ethanol and 1-butanol used to develop the column con­tained a slightly lower ratio of water than that used by
Thoma and are given in Fig. 2. Every fifth tube of the eluate
was analyzed by evaporating 0.5 ml to dryness, adding 2 ml of
water and 4 ml of 0.2 per cent anthrone in concentrated
sulfuric acid. After mixing in an ice bath, the samples were
heated in a boiling water bath for 10 min. The absorbancies
were measured in a Klett-Summerson photoelectric colorimeter
with a red (K66) filter. The carbohydrate fractions were
obtained by pooling eluate fractions corresponding to the
central portions of each peak and evaporating to dryness
in vacuo. Acetone was used to extract acetone-soluble
organic contaminants. Reducing values of zero for $\delta$-, $\epsilon$-, $\zeta$- and $\eta$-dextrins with 1 to 2 mg when tested with the 3,5-
dinitrosalicylic acid method are compared to expected values
of 40 to 100 Klett units for reducing open chain oligo-
saccharides in this chromatographic range.
Fig. 2. Elution diagram of the selected fraction 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 water: ethanol: butanol. Peaks labelled G₂, G₃, G₄ have been identified as maltose, maltotriose, maltotetraose. Peaks labelled α, β, γ have been identified by the characteristic shapes of crystals from 60 per cent 1-propanol. In addition, α with I₂-KI shows characteristic dichroic needles and blue hexagons.
The approximate yields of the new Schardinger dextrins with respect to the per cent of original starch was as follows: \( \delta \)-dextrin, 0.39 per cent; \( \epsilon \)-dextrin, 0.22 per cent; \( \zeta \)-dextrin, 0.21 per cent; and \( \eta \)-dextrin, 0.21 per cent.

**Membrane Permeation Rates**

The cell (31) used in the experiment with dialysis or permeation rates provided approximately 50 sq cm of area. The volume of the initial solution or retentate was about 0.6 ml while the diffusate varied from 4 ml to 7 ml. The amount of sample was usually about 5 mg and the solute concentrations were determined by weight or with the use of anthrone.

Membrane No. 3 was Visking 18/32 acetylated 2 hr while membrane No. 5 was Visking 20/32 acetylated 2 hr. The preparation of different porosities of membranes was achieved by proper selection of the initial cellophane tubing, linear stretching to the limit and acetylation in anhydrous pyridine at 65 degrees. Permeation rates were measured at 25 degrees, 40 degrees and 60 degrees controlled by the use of a small constant-temperature water bath.

**X-ray Analysis**

Crystalline samples of \( \delta \)- and \( \zeta \)-dextrins were prepared by letting a concentrated solution of the carbohydrate and
l-propanol diffuse together. A concentrated solution of 0.5 ml of carbohydrate was put in the bottom of a small test tube. Over this was layered 0.3 ml water and then a layer of 0.3 ml of 60 per cent propanol and then on top of that 1.2 ml of l-propanol to make a final concentration of 60 per cent propanol. The tube was then placed in the refrigerator and the contents allowed to diffuse together. Crystals were obtained only for $\delta$- and $\zeta$-dextrins. In the case of $\epsilon$- and $\eta$-dextrins, small droplets of syrup were deposited on the walls of the test tube above the initial carbohydrate solution. Single crystals of $\delta$- and $\zeta$-dextrins were subjected to X-ray analysis by Dr. A. Hybl.

**Ultracentrifugation Molecular Weights**

The ultracentrifuge (Spinco model E) used had a calibrated automatic temperature control unit. A double sector cell was filled with 100 $\lambda$ of approximately 1 per cent dextrin and 50$\lambda$ of Fluorocarbon FC-43 (perfluorotributyl amine). Water was used to establish a base line. The cell was run at 42,040 rpm for 4 or 5 hrs to obtain the concentration gradient. The regular schlieren optical system was used. A synthetic boundary cell was used to determine $c_0$. The plates were measured with a microcomparator.
Paper Chromatographic Analysis

Whatman No. 1 paper was used with water, ethanol, nitromethane (25:40:35) solvent to purify and separate the partial acid hydrolysis products of the Schardinger dextrins. Each dextrin was hydrolyzed in 0.01N HCl heated with a boiling water bath and samples were withdrawn at 400 and 500 min. Excess pyridine was used to neutralize the acid in the sample. The sample was then applied to the corner of a sheet of Whatman No. 1 paper and 4 to 6 ascents were made in 1 direction. After spraying with β-amylase, 2 ascents were made in the other direction and then the chromatograms were developed by a silver dip method (28). The method was modified by dipping in methanolic sodium hydroxide and then Kodak Fixer F-24 instead of ammonium hydroxide to remove excess silver oxide.

Several good complexing agents of γ-dextrin were tested by putting a layer of the organic phase in contact with a 2 per cent solution of δ-dextrin in small tubes. After several days at room temperature, these were put in a refrigerator.

The optical rotations \([\alpha]_D\) of the new dextrins were measured in solutions of about 1 per cent in a 1 dm tube by using a Rudolph Polarimeter. Due to the lack of enough crystalline material, 8 to 12 mg of δ-, ε- and γ-dextrins were weighed and solutions prepared. The optical rotations
of these solutions were measured and the carbohydrate concentration determined with the phenol-sulfuric acid test (38) and the anthrone test (39). A crystalline sample of \(\zeta\)-dextrin was carefully dried at 100 degrees in a vacuum oven and from this a solution was prepared. The phenol-sulfuric acid test gave a value for the carbohydrate concentration in agreement with the weight.
RESULTS AND DISCUSSION

Starch was the substrate used in the preparation of the new Schardinger dextrins. In forming the $\alpha$-, $\beta$- and $\gamma$-dextrins, the amylase from B. macerans acts by cleavage of an $\alpha1\rightarrow4$ linkage as it is in starch with the consequent formation of another $\alpha1\rightarrow4$ linkage. No one has ever reported any enzyme from B. macerans which acted as a transferase in a manner different from this. It is therefore most logical to expect that any products formed from starch will contain only the types of linkages found in starch in the first place. The first 3 members of the Schardinger dextrins fit this pattern and have only the maltose type linkage, and therefore, it is expected that the higher members would also be the same in this respect.

The separation of the Schardinger dextrins by high temperature column chromatography follows the same general conditions used by Thoma (27) for the separation of a linear starch hydrolyzate. The sequential elution of the linear compounds occurs in order from the smallest to the largest in this series. The sequential elution of the known Schardinger dextrins indicates that the 4 additional anthrone positive peaks after the 3 well known cyclic-dextrins are indeed sequential homologs. An extension of the previous nomenclature allows these to be called $\delta$-, $\varepsilon$-, $\zeta$- and $\eta$-dextrins.
The compounds did not reduce 3,5-dinitrosalicylic acid and were unaffected by extensive treatment with \( \beta \)-amylase (maltoheptaonic acid is an example of a linear nonreducing maltosaccharide in this molecular weight range that is degraded by \( \beta \)-amylase).

The elution diagram in Fig. 2 shows a peak for maltose (\( \beta_2 \)), another for maltotriose (\( \beta_3 \)) and another smaller peak between maltotriose and \( \alpha \)-dextrin. This has been identified as impure maltotetraose by paper chromatography and degradation by \( \beta \)-amylase to maltose. The crystals that formed in the eluate in the same place as the appearance of the maltotetraose are anthrone negative and are most likely not carbohydrate. The next 3 peaks have been identified in sequence as \( \alpha \)-, \( \beta \)- and \( \gamma \)-dextrins by their typical crystalline form from 60 per cent 1-propanol. Further evidence for the \( \alpha \)-dextrin is the formation of characteristic dichroic needles and blue hexagons produced upon addition of \( \text{I}_2 \). Hexagonal and spindle shaped crystals were obtained from \( \delta \)-dextrin in 60 per cent 1-propanol under a cover slip on a slide. The small rods previously reported (40) for \( \epsilon \)-dextrin in 60 per cent 1-propanol have not been obtained again and it is an open question whether or not they represented crystals of \( \epsilon \)-dextrin. Enough pure material was not available to measure the solubility of the new dextrins in water. However, due to their failure to crystallize from water, it seems that they would have a very high solubility.
The cyclic Schardinger dextrins are uncharged carbohydrates and for this reason are of interest in comparison with peptides and proteins as far as their membrane permeation rates are concerned. The half escape time of an ideal solute in water should increase 1.44 times as the permeation temperature is raised from 25 degrees to 40 degrees and about the same amount from 40 degrees to 60 degrees. The ratios noted in Fig. 3 can be seen to be higher than this. An explanation might be that there is a reduction in water of hydration of the dextrins and/or the cellophane membrane since the diffusional volume includes the water of hydration.

Membrane No. 3 had a half escape time of 6 hr for α-dextrin at 40 degrees. The half escape time for β-dextrin was 12 hr, 23 hr for γ-dextrin and 30 hr for δ-dextrin. This membrane had a sufficiently large difference in the permeation rates from 1 member of the series to the next that the last 3 were not run due to the length of time which would have been required. Every member of the series could be run, however, with membrane No. 5 with half escape times varying from 0.42 hr for α-dextrin up to 2.0 hr for γ-dextrin. The sigmoid shape of the curve obtained with membrane No. 5 is somewhat unexpected if all of the dextrins are large symmetrical rings. A deviation from the symmetrical configuration is expected to be likely due to the greater flexibility of the higher members. This deviation would produce a smaller diffusional volume than expected and the
Fig. 3. The relative permeation rates are indicated by the half escape time in hrs. The half escape time at 40 degrees of α-dextrin in membrane No. 5 was 0.42 hrs but in membrane No. 3 it was 6 hrs.
consequent increase in permeation rate would also be lower.

The half escape times of the series of the Schardinger dextrins fall on a smooth continuously increasing curve which provides further evidence that each member is a distinct entity and falls into a regular homologous series.

Well formed crystals of $\delta$- and $\zeta$-dextrins were prepared which were large enough for single-crystal X-ray analysis. However, both crystals slowly lost solvent of crystallization on exposure to air with a rather poor pattern being obtained with $\delta$-dextrin and no usable pattern with $\zeta$-dextrin. A crystal of $\zeta$-dextrin was put in a capillary tube in the presence of the solution from which it was crystallized and a very clear pattern was obtained except that the crystal shifted. The unit cell dimensions for $\delta$-dextrin are:

$a = 32.59$ Å, $c = 30.80$ Å, $d(100) = 28.21$ Å. The crystal is hexagonal or pseudohexagonal and gives the correct molecular weight with 12 molecules per unit cell if the carbohydrate density is about 1.03.

The molecular weight determinations made with the ultracentrifuge depended upon the accuracy with which $(dc/dx)$ can be measured at the meniscus and the base. The ultracentrifuge was run for a period of 4 to 5 hrs and with the cell depth of slightly less than 3 mm. Equilibrium was not completely attained but was sufficiently close enough that a plot of the log of $(dc/dx)$ against $x^2$ gives less subjective extrapolations at the meniscus and base. This method is not quite
as reliable as plotting \( \frac{1}{x^C} (dc/dx) \) against \( x \) for several centrifugation times during the run but it is less time consuming and in this case has given satisfactory results. The extrapolation to the base is more reproducible than that to the meniscus due in part to fringe interference and in part due to the higher value of \( (dc/dx)_b \) compared to \( (dc/dx)_m \). The molecular weights calculated from \( (dc/dx)_b \) can be regarded to be more reliable than those calculated from \( (dc/dx)_m \). The molecular weights obtained are given in Table 7.

Table 7. Ultracentrifuge molecular weights

<table>
<thead>
<tr>
<th>Molecular weight</th>
<th>( \beta )</th>
<th>( \delta )</th>
<th>( \epsilon )</th>
<th>( \xi )</th>
<th>( \eta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>At the meniscus</td>
<td>1161</td>
<td>1432</td>
<td>1626</td>
<td>1833</td>
<td>1867</td>
</tr>
<tr>
<td>At the base</td>
<td>1141</td>
<td>1470</td>
<td>1661</td>
<td>1748</td>
<td>1908</td>
</tr>
<tr>
<td>Combined meniscus and base</td>
<td>1117</td>
<td>1498</td>
<td>1684</td>
<td>1698</td>
<td>1930</td>
</tr>
<tr>
<td>Calculated</td>
<td>1135</td>
<td>1459</td>
<td>1621</td>
<td>1784</td>
<td>1946</td>
</tr>
</tbody>
</table>

The partial specific volume of \( \gamma \)-dextrin is 0.621 and was assumed to be near that of the new Schardinger dextrins. A difference of 0.4 per cent between the actual value and the assumed value would cause a 1 per cent error in the molecular weight. The reproducibility of certain measurements was as follows:
The optical rotations were measured and found to be 
+191° +3 for β-dextrin, +197° +3 for ε-dextrin, +200° +2 for ζ-dextrin and +201° +3 for γ-dextrin. These values are plotted in Fig. 5 along with the literature values for the optical rotation of α-, β- and γ-dextrins. The values fall on a smooth curve that levels off near +200° (the reported optical rotation for amylose). The increased flexibility of the macro-cyclic rings as the size increases would seem to permit these dextrins to rapidly assume some of the qualities like an infinitely long amylose chain.

None of the good complexing agents for the other dextrins were good complexing agents for the new higher dextrins. Since after partial acid hydrolysis the unhydrolyzed Scharding-er dextrins under consideration could not be removed with organic precipitants, the procedure previously used (25) was modified.

The partial acid hydrolysis and chromatographic separation revealed some interesting results. An amylodextrin hydrolyzate containing 1 mg of carbohydrate was applied to paper and 6 ascents were made in 1 direction. After the
The molecular weights were calculated by the use of equations 2 and 6 for those designated m, 3 and 7 for those designated b and 5 for those designated m,b. These calculations give the same value within experimental error for only a pure homogeneous ideal solute.
Theoretical Deviation by 1 glucose unit

Deviation by 1/2 glucose unit

ULTRACENTRIFUGE MOLECULAR WEIGHT

CALCULATED MOLECULAR WEIGHT
Fig. 5. The optical rotations of the previously known Schardinger dextrins are plotted with the optical rotations of the new Schardinger dextrins. The smooth, continuous increase is indicative that the new dextrins are higher homologs of the first 3 members.
paper was sprayed with a solution containing 1 $\lambda$ of suspended crystals of $\alpha$-amylase per ml until damp, the maltosaccharides were acted upon during the 15 min drying period at room temperature. Two ascents at 90 degrees from the original ascents were made and the paper was developed with the silver dip method. Distinct spots were observed for glucose, maltose and maltotriose (in some cases the glucose is obscured) in a diagonal line and then a very weak spot for maltotetraose and a stronger spot for maltose formed from the hydrolysis of maltotetraose and this in line with the maltose formed from the hydrolysis. Maltose was formed from the hydrolysis of the whole series of maltosaccharides and each maltose spot was on the same line. Maltotriose is formed from the odd-numbered maltosaccharides such as malto-pentaose and maltoheptaose but is absent from the even-numbered maltosaccharides such as maltohexaose and maltooctaose. In the cases where a large amount of each maltosaccharide is present, all of the degradation products can be observed and form a distinct pattern. This terminates abruptly with the maltosaccharide formed from the cleavage of 1 glycosidic bond of the dextrin in the case of the Schardinger dextrins. The results for $\delta$-dextrin and $\eta$-dextrin definitely show that the former contains 9 glucose units and the latter contains 12 glucose units. The degradation of the hydrolysis products of the dextrins by
β-amylase definitely proves that these new Scharligher 
dextrins are composed of molecules with the same type of 
linkage as that found in maltose and amylose. The results 
for ɛ- and ζ-dextrins indicate that the $R_f$ of the highest 
molecular weight hydrolysis product would correspond to 
10 glucose units for ɛ-dextrin and 11 glucose units for 
ζ-dextrin. The names of cyclononaamylose, cyclodecaamylose, 
cycloundecaamylose and cyclododecaamylose are now proposed.
SUMMARY

After the action of B. macerans on starch, preliminary solvent fractionation, treatment with $\beta$-amylase and separation by high temperature column chromatography, the 3 well known Schardinger dextrins ($\alpha, \beta, \gamma$) have been isolated in sequence followed by 4 new anthrone positive components. These components have no reducing power and are resistant to extensive attack by $\beta$-amylase. They have been named $\delta$-, $\epsilon$-, $\zeta$- and $\eta$-dextrins as an extension of the common nomenclature.

Permeation rates give half escape times that fall on a smooth continuous curve from $\alpha$-dextrin to the last component obtained in the eluate. The molecular weights obtained by ultracentrifugation show $\delta$-, $\epsilon$-, $\zeta$- and $\eta$-dextrins contain 9, 10, 11 and 12 glucose units, respectively. Partial acid hydrolysis of the dextrins produces degradation products which are degraded by $\beta$-amylase and do not contain more glucose units than the original dextrin. This substantiates size, cleavage of the cyclic structure and the presence of the maltose type linkage. The optical rotations of the new dextrins are: $\delta$, $+191^\circ$; $\epsilon$, $+197^\circ$; $\zeta$, $+200^\circ$; and $\eta$, $+201^\circ$. Hexagonal crystals have been obtained with $\delta$-dextrin and monoclinic crystals with $\zeta$-dextrin.

The evidence clearly shows that the new dextrins are structurally homologous with the previously recognized
Schardinger dextrins. The systematic names of \textit{cyclononaamylose}, \textit{cyclodecaamylose}, \textit{cycloundecaamylose} and \textit{cyclododecaamylose} are proposed.
LITERATURE CITED


ACKNOWLEDGMENTS

The author wishes to thank Dr. Dexter French for his profitable suggestions and encouragement of clear thinking. The freedom of approach permitted and use only of the "whip from within" has aided in the development of those qualities so necessary to scientific research.

The help of Dr. Malcolm A. Rougvie with the ultracentrifuge in the molecular weight determination was both necessary and appreciated.

X-ray analysis of the 2 crystalline dextrins in the new Schardinger dextrins was done by Dr. Albert Hybl while the powder pattern and the analysis of the 1,1,2,2-tetra-bromoethane of amylose was done by Dr. Bertram Zaslow.

Particular mention should be made of the hospitality of Dr. Lyman C. Craig for inviting the author into his laboratory to study his technique of dialysis or permeation rates.

The author would like to thank the National Science Foundation for the fellowships.