# SYNTHESIS OF

# MONOSUBSTITUTED CYCLOHEXAAMYLOSES

# AND MONOSUBSTITUTED MALTOSES

by

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ABSTRACT

This thesis reports the first systematic preparation of monosubstituted cyclohexaamyloses in which the position of substitution is known. On obtaining the monosubstituted cyclohexaamyloses, they were treated with Aspergillus oryzae amylase and this led to a novel method of preparing maltose derivatives substituted at the 6' position.

The key reaction for synthesizing monosubstituted cyclohexaamyloses is the mono-tosylation (O-p-toluenesulphonyl derivative) of cyclohexaamylose. To prevent formation of large amounts of multiply substituted derivatives, the tosylation was carried out only for a short time. The resulting mixture of starting material, monotosyl-cyclohexaamylose and more-highly substituted derivatives was separated on an activated charcoal column. Since tosylation occurs preferentially at primary, rather than secondary, hydroxyl groups, the monosubstituted product is specifically substituted at C-6 of one of the α-D-glucopyranosyl residues of cyclohexaamylose.

Having prepared monotosyl-cyclohexaamylose, the tosyl group was readily displaced, by nucleophilic substitution, with various nucleophiles to give the following compounds:- 6-azido-6-deoxy-, 6-chloro-6-deoxy-, 6-bromo-6-deoxy, and 6-deoxy-6-iodo-cyclohexaamylose.

The monoazido and monoiodo derivatives were reduced to 6-amino-6-deoxy-, and 6-deoxy-cyclohexaamylose respectively. These seven monosubstituted cyclohexaamyloses were obtained as crystalline products, none of which have been reported before.

The action of a crude preparation of Aspergillus oryzae amylase on monosubstituted cyclohexaamyloses results in a new and elegant method of preparing 6'-substituted maltoses in yields which are superior to previous methods. By this means 6'-O-p-toluenesulphonyl-, 6'-azido-6'-deoxy-, 6'-chloro-6'-deoxy-, 6'-bromo-6'-deoxy-, and 6'-deoxy-6'-iodo-maltose were produced from the corresponding cyclohexaamylose derivatives. The other major product of the enzyme action was D-glucose and there were also traces of monosaccharides from the autohydrolysis of the glycoproteinaceous enzyme. To obtain pure monosubstituted maltoses the mixtures were separated on activated charcoal columns. These five compounds are reported for the first time. The position of substitution on the maltose was proven by mass spectral analysis of the pertrimethylsilyl derivatives, and in the cases of 6'-chloro-6'-deoxy- and 6'-deoxy-6'-iodo-maltose this was confirmed by chemical means. The 6'-azido-6'-deoxy-maltose was readily reduced to 6'-amino-6'-deoxy-maltose. The possible mode of action of the amylase is discussed in terms of these results.

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# INTRODUCTION

## Purpose

The aim of this work was to prepare specifically modified cyclohexaamyloses for use in enzyme and model enzyme studies. The chemistry of enzyme action is one of the most important unsolved problems in biochemistry and by studying cycloamyloses, which act as model enzymes [1,2], the understanding of enzyme action should be increased. In un-modified cycloamyloses, used by previous workers in model enzyme studies, the position of the "active site" was unknown. However, if cyclohexaamyloses, substituted with reactive groups in known positions, were used as model enzymes the "active site" would be known and hence the stereochemistry and mechanism of "enzymic" catalysis would be better understood. In the present investigation the position chosen for modification of the molecule was the C-6 position of one of the D-glucopyranosyl residues. 6-Amino-6-deoxy-cyclohexaamylose would be especially useful as a model for binding involving a protonated centre ($RNH_3^+$) and as an improved nucleophilic centre ($RNH_2$) at higher pH. Furthermore the monosubstituted cyclohexaamyloses would be invaluable as substrates in studies on enzymes that act on cycloamyloses, viz *Bacillus macerans* cyclodextrinase[3] and amylases from *B. macerans*[4], *B. polymyxa*[5], *Penicillum africanum*[6] and *Aspergillus oryzae*[6,7]. Such studies would lead to an

improved understanding of the mode of action of glycolytic enzymes.

Specifically substituted cycloamyloses offer attractive intermediates for the synthesis of modified oligosaccharides, substances which are often available only by long and arduous synthetic routes. Thus mono-substituted cyclohexaamyloses were to be treated with A. oryzae amylase in order to obtain specifically substituted maltooligosaccharides. These chemically modified oligosaccharides could be useful in a number of ways such as substrates for studying amylases[8,9] and gluco-amylases[9,10]. The chemical modification of some of the C-6 positions of amylose[11,12,13] has been accomplished, although the positions of the substituted residues in the polymer are unknown. The distribution of the substituents in the modified amyloses could be found by enzymatic and partial acid hydrolysis. Oligosaccharides substituted at a C-6 position would provide reference compounds for such hydrolyses. They could be reacted with unmodified malto-oligosaccharides, by the action of B. macerans amylase, to give substituted cycloamyloses and substituted higher oligosaccharides. In addition, these reactions would provide information about the action and in particular the specificity of B. macerans amylase.

Background

The commonly occuring cycloamyloses consist of 6, 7 or 8 α-D-glucopyranosyl residues linked 1→4 to form cyclic molecules. Cyclohexaamylose (1), consisting of 6 residues, is shown in Fig. 1, and the conformation of crystalline cyclohexaamylose is shown in Appendix 1. Cycloamyloses are formed by the action of B. macerans amylase on starch[4].

The syntheses of cyclohexaamylose derivatives have been limited due to the difficulty in obtaining these products in a pure form. Preparations in which the degree of substitution was high (12 or 18), have been reviewed by French[4]. Subsequently, dodecamethylcyclohexaamylose has been prepared, but a controversy exists over the positions of the O-methyl groups. In one report the methyl groups were assigned to the C-2 and C-6 positions of the D-glucose residue[14], whereas in another they were assigned to the C-3 and C-6 positions[15].

Lautsch and coworkers[16] were the first to attempt to modify only one position specifically in each glucose residue. They attempted the preparation of hexa-(6-O-tosyl)- and hexa-(6-O-mesyl)-cyclohexaamyloses using one equivalent of sulphonyl chloride per glucose residue. Recently Cramer and coworkers[17], using this method with a 50% excess of tosyl chloride (9:1 molar ratio), claim to have prepared hexa-(6-O-tosyl)-cyclohexaamylose.

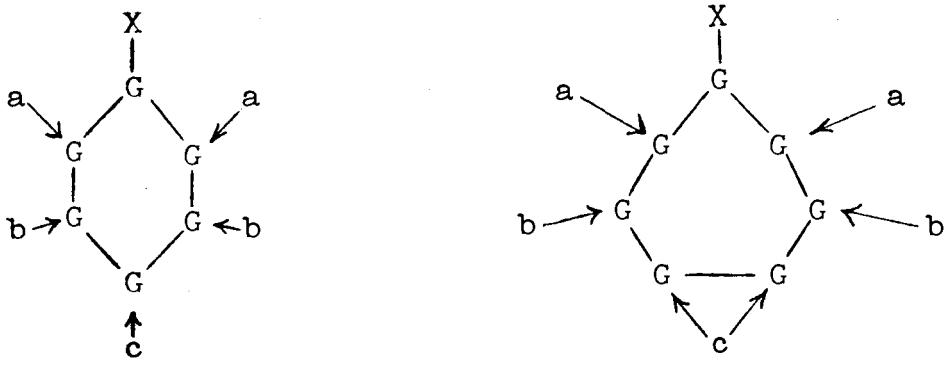
This material was subjected to nucleophilic displacements to give products that analyzed as penta-(6-amino-6-deoxy)-cyclohexaamylose and dodecaacetyl-penta-(6-deoxy-6-iodo)-mono-(6-O-tosyl)-cyclohexaamylose[17]. It is doubtful that tosylation did occur exclusively at the primary hydroxyl groups in all residues as claimed by these workers, as tosylation of α-D-glucose[18], methyl α-D-glucopyranoside[19] and amylose[20,21] under comparable conditions gives some additional tosylation at secondary hydroxyl groups. Similarly, mesylation of methyl α-D-glucopyranoside even with only one mole of mesyl chloride gives a mixture of products which includes methyl 2,6-di-O-mesyl-α-D-glucopyranoside and methyl 2-O-mesyl-α-D-glucopyranoside[22]. In an even more recent paper[23] Cramer and Mackensen claim to have obtained hexa-(6-O-benzenesulphonyl)-cyclohexaamylose using a 10:1 molar ratio of benzenesulphonyl chloride to cyclohexaamylose, but for reasons already given for other sulphonylations this claim is dubious. Umezawa and Tatsuta[24], using a 9:1 molar ratio found that a mixture of tosylated products resulted and that the pure hexa-(6-O-tosyl)-cyclohexaamylose had to be obtained by separation on a silica-gel column. Hexa-(6-azido-6-deoxy)-, hexa-(6-amino-6-deoxy)- and hexa-(6-acetamido-6-deoxy)-cyclohexaamyloses were prepared from this pure hexa-tosylate[24].

Cramer and Mackensen[23] have attached various imidazole groups to the C-6 positions of cyclohexa- and

cycloheptaamylose. The degree of substitution was two or three imidazole groups per cyclohexaamylose molecule and either two, three, four, or six per cycloheptaamylose molecule. It seems likely that these materials were mixtures containing not only different degrees of substitution but also (except for the hexasubstituted cycloheptaamylose) positional isomers* as well.

Other preparations of specifically modified cyclohexaamylose have resulted from kinetic studies of cycloamyloses as model enzymes. The "enzyme-substrate" intermediates, cycloheptaamylose monophosphate[25] and tri-(isopropyl methylphosphonyl)-cyclohexaamylose[26], as well as a spin-labelled cycloheptaamylose ester[27] have been identified and isolated. In a similar manner Bender and coworkers[28] have attempted to prepare cyclohexaamylose monobenzoate, but the ester was contaminated with cyclohexaamylose. One "enzyme-substrate", the cyclohexaamylose ester of pyridine-2,5-dicarboxylic acid[29],

* A second identical substituent at a C-6 position of cyclohexa- or cycloheptaamylose can be introduced at any one of 3 positions to produce 3 positional isomers:



has been obtained on a preparative scale. The position of ester attachment has not been unambiguously established in any of these derivatives.

There has been only one serious attempt[30] at the systematic preparation of monosubstituted cyclohexaamyloses. The use of chlorotriphenylmethane to alkylate specifically one C-6 hydroxyl position[30], is based on the fact that tritylation occurs preferentially at a primary hydroxyl group[31]. The formation of 6-O-trityl-cyclohexaamylose(2) (see Fig. 1) provided a means of obtaining monosubstituted derivatives. In this work[30] monotrityl-cyclohexaamylose was fully acetylated, detritylated, and monotosylated, and then nucleophilic displacement of the sulphonate group was effected by iodide. Deacetylation yielded 6-deoxy-6-iodo-cyclohexaamylose (8), which was finally reduced to 6-deoxy-cyclohexaamylose(9).

There have been relatively few preparations of maltose [4-O-(α-D-glucopyranosyl)-D-glucose] with a substituent at a C-6 position and the author has not found any reports of higher maltooligosaccharides with a substituent at one of the C-6 positions. 6-O-Benzoyl-maltose[32] has been prepared from octa-O-benzoyl-β-maltose in a moderate yield (32%). 6'-O-Trityl-maltose[33](20) has been synthesised from maltose in a moderate yield. Maltobiouronic acid [4-O-(α-D-glucopyranosyluronic acid)-D-glucose] has been prepared in low yields from benzyl

β-maltoside[34,35] and methyl β-maltoside[36]. Starting with 1,6-anhydro-β-maltose Dutton and Slessor[37] have synthesised 6'-deoxy-(19), 6'-deoxy-6'-thio- and 6'-amino-6'-deoxy-maltose (15), but the yields were very low. Hence, there is a need for a simple and efficient method of preparing maltooligosaccharides substituted at a C-6 position.

A possible way of producing monosubstituted maltooligosaccharides is by the action of Aspergillus oryzae amylase[8] on monosubstituted cyclohexaamyloses. In an early investigation[7] A. oryzae amylase was found to degrade cyclohexa-, cycloheptaamylose and amylose to maltose and D-glucose, but when maltose was the substrate, no hydrolysis occurred. Later workers[38] showed partially O-methylated amylose, on treatment with the amylase, gave 6'-O-methyl-maltose amongst other products, but no 6-O-methyl glucose was detected. However, the smallest fragment, aside from D-glucose and maltose, produced from the degradation of starch and amylopectin by the amylase is 6"-α-D-glucopyranosylmaltriose[39]:-

$$\begin{array}{l} \qquad\quad G \\ 1,6 \;\big|\; \alpha \\ G\ \underset{\alpha}{\overset{1,4}{}}\ G\ \underset{\alpha}{\overset{1,4}{}}\ G' \end{array}$$

where G is a α-D-glucopyranosyl residue, G' is a reducing D-glucose residue. In a recent investigation[40] using synthetic substrates, it was found that the following 6'-substituted phenyl α-maltosides were hydrolysed to the corresponding 6'-substituted maltose and phenol

(the rates of amylase action relative to phenyl α-maltoside are given in brackets):-deoxy(0.05), chloride(0.24), iodide(0.29) and O-methyl(2.10). Phenyl 3,6-anhydro-[41] and 3',6'-anhydro-α-maltoside[40] gave no reaction. The following 6-substituted phenyl α-maltosides were not hydrolysed by the amylase:- O-acetyl[42], chloride[40], iodide[40] and O-methyl[40]. Two exceptions are the 6-deoxy[40] and 6-deoxy-6-fluoro[41] derivatives of phenyl α-maltoside, which exhibit very slow hydrolysis to yield phenol.

Hence it may be concluded that:- (a) a change in ring conformation of a glucopyranosyl residues prevents amylase action, at least in 3,6-anhydro compounds; (b) a large substituent at a C-6 position of a substrate results in a 6"-derivative of maltotriose; (c) a medium sized substituent produces a 6'-substituted maltose; (d) no 6-substituted maltose is formed unless the substituent is very small. Therefore, it seems probable that the action of A. oryzae amylase on cyclohexaamylose derivatives substituted at one C-6 position, will result in 6'-substituted maltoses. It is expected that monodeoxy-cyclohexaamylose will be an exception. Other possible exceptions are monotrityl-(2) and monotosyl-cyclohexaamylose(3) which might be degraded to 6"-O-trityl- and 6"-O-tosyl-maltotriose respectively.

Approach

In the present work the approach adopted to prepare monosubstituted cyclohexaamyloses was to synthesize the 6-*O*-*p*-toluenesulphonyl (tosyl) derivative(3)(see Fig. 1) from cyclohexaamylose. Tosylation occurs preferentially at primary, rather than secondary, hydroxyl groups[43]. However, there are 6 primary hydroxyl groups in cyclohexaamylose, so to obtain a monotosyl derivative the reaction must be carried out only for a short time. Under these conditions, a mixture of products tosylated at the C-6 positions as well as starting material was to be expected. There appeared to be three ways of separating monotosyl-cyclohexaamylose from the mixture: by preferential complexation; by separation on a charcoal column; by nucleophilic displacement of the tosyl ester by azide, followed by reduction to amine and separation of the mixture of cyclohexaamylose, monoamino- and polyamino-cyclohexaamyloses on an ion-exchange resin column.

Since the tosyl group is a good leaving group, it may undergo displacement with a wide variety of nucleophiles[44] to produce a series of monosubstituted cyclohexaamyloses. By this means it was planned to prepare 6-azido-6-deoxy-(4), 6-deoxy-6-fluoro-(10), 6-chloro-6-deoxy-(6), 6-bromo-6-deoxy-(7), 6-deoxy-6-iodo-cyclohexaamylose(8)(see Fig. 1). The monoazido and monoiodo derivatives could be reduced to 6-amino-6-deoxy-(5)-

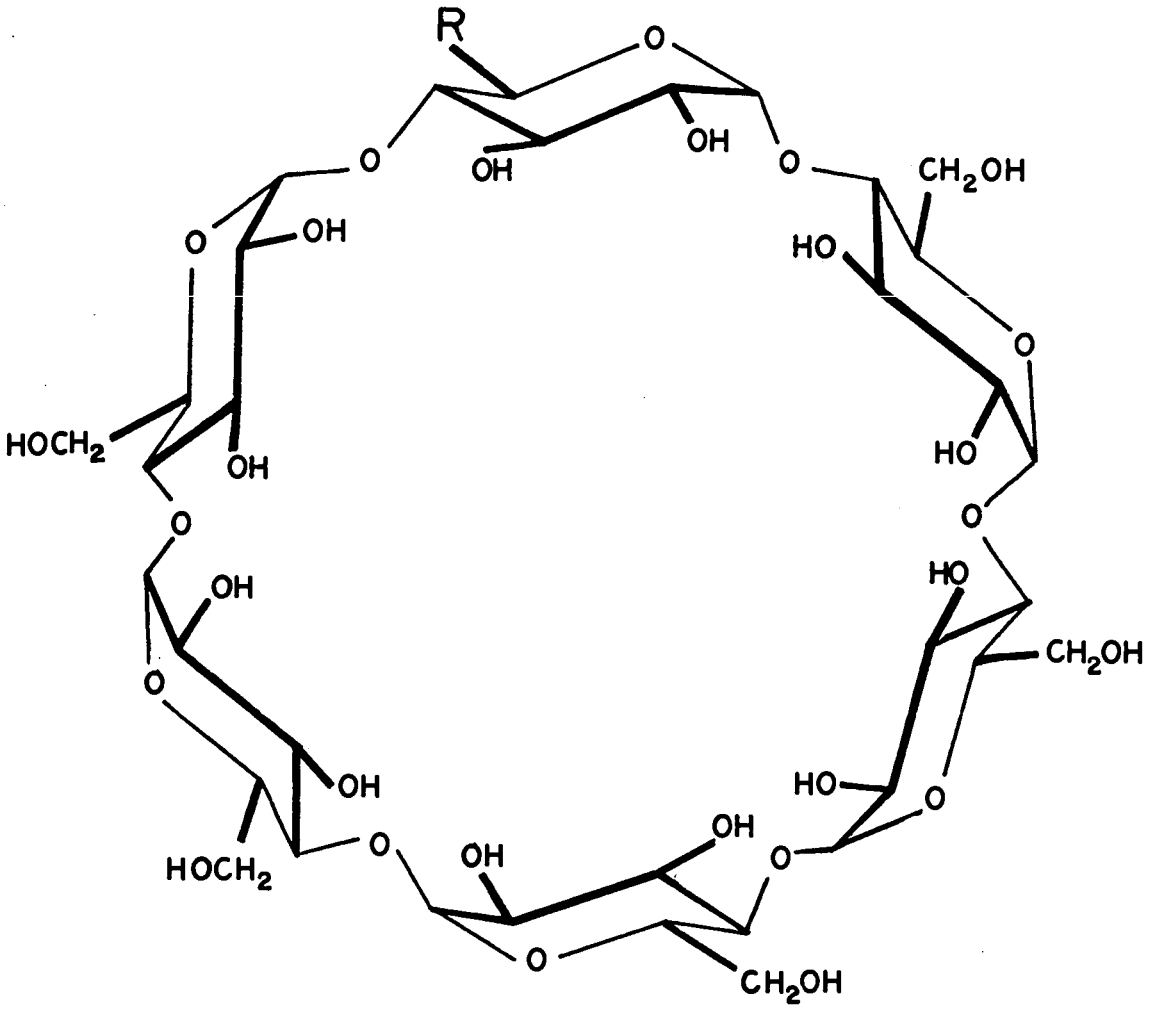



Fig. 1. A schematic representation of the cyclohexaamylose derivatives.

(1) R = $CH_2OH$ (4) R = $CH_2N_3$ (7) R = $CH_2Br$ (10) R = $CH_2F$

(2) R = $CH_2OTr$ (5) R = $CH_2NH_2$ (8) R = $CH_2I$ (11) R = CHO

(3) R = $CH_2OTs$ (6) R = $CH_2Cl$ (9) R = $CH_3$ (12) R = COOH

and 6-deoxy-cyclohexaamylose (9) respectively. Another useful compound to prepare would be 6-aldehydo-cyclohexaamylose(11), which would require only mild oxidation to give 6-carboxyl-cyclohexaamylose(12). Ninhydrin is known to react with organic primary amines by oxidative-deamination, yielding the corresponding aldehyde[45]. This oxidative-deamination of monoamino-cyclohexaamylose was expected to yield 6-aldehydo-cyclohexaamylose. When the monosubstituted cyclohexaamyloses had been prepared, it would be a simple matter to treat them with Aspergillus oryzae amylase to produce corresponding monosubstituted maltooligosaccharides.

DISCUSSION

Monosubstituted Cyclohexaamyloses

Considering the difficulties experienced working with monosubstituted cyclohexaamyloses, the yields obtained were reasonable (20 - 46% from cyclohexaamylose).

6-O-p-Toluenesulphonyl-cyclohexaamylose (3) is the key intermediate in the synthesis of all of the cyclohexaamylose derivatives except the trityl (2). Although the tosylation of cyclohexaamylose was carried out for only a short time a mixture of tosylated products and starting material resulted. Column chromatography on activated charcoal resulted in the isolation of pure monotosyl-cyclohexaamylose in reasonable yield. The compound obtained from the charcoal column with 25% 1-propanol gave a characteristic reaction for a tosyl group with diphenylamine on t.l.c. and the analysis showed that there was only one tosyl group present. Tosylation is known to occur preferentially at primary, rather than secondary, hydroxyl groups[43] and the ease with which the sulphonate is displaced by nucleophiles proves that the compound is 6-O-tosyl-cyclohexaamylose (3).

The tosylate group was then readily replaced by nucleophilic substitution using a variety of standard nucleophiles. In this way, the monochloro (6), monobromo (7) and monoiodo (8) derivatives were obtained,

and the monofluoro (10) derivative was probably prepared but it could not be isolated.

Solvolysis of the monotosyl cyclohexaamylose (3) is found to compete with the desired nucleophilic substitution when the reaction is carried out in water, producing cyclohexaamylose. In the case of a good nucleophile, such as iodide, there is virtually no solvolysis, whereas azide and bromide give small amounts of cyclohexaamylose (approximately 5% and 10% respectively by t.l.c.). In order to avoid formation of any cyclohexaamylose, the procedure finally adopted to prepare the monobromide (7) was to use dry dimethylformamide When this procedure was used in the azide displacement less cyclohexaamylose was formed then in aqueous solution. With the even poorer nucleophile, chloride, it was essential to exclude water. An attempt to prepare the monochloro derivative (6) by heating monotosyl-cyclohexaamylose with 0.25% aqueous sodium chloride solution resulted in approximately 95% cyclohexaamylose and only 5% monochloro-cyclohexaamylose as indicated by t.l.c.. When tetramethylammonium chloride with dry N,N-dimethylformamide as solvent was used, monochloro-cyclohexaamylose was obtained exclusively. In attempts to prepare the monofluoride (10) even greater care had to be taken to exclude water from the reaction.

The reduction of monoazido- (4) and monoiodo-

cyclohexaamylose (8) to monoamino- (5) and monodeoxy-cyclohexaamylose (9) respectively, was easily accomplished using standard methods. However, the reduction of the monochloro derivative (6) to monodeoxy- cyclohexaamylose (9) presented more difficulty. A number of reductions were tried, including heating an aqueous solution of the sugar at 100° with palladium black and hydrogen at 1000 lb. $in^{-2}$ pressure. None were successful until the method of Jones and coworkers[46] was used. In this method reductive-dehalogenation is achieved by using a very active form of Raney nickel, W-4, and hydrogen at 45 lb. $in^{-2}$.

The proposed scheme of separating monoamino-cyclohexaamylose (5) from a mixture of cyclohexaamylose and its amino derivatives was not satisfactory for two reasons. The azide replacement of tosylate groups in the mixture of tosyl derivatives was not possible unless the mixture had been desalted. However, when the solution, containing the tosyl derivatives, was desalted with Dowex 50 ($H^+$) and Dowex 3 ($OH^-$), t.l.c. indicated that half of the monotosyl (3) and most of the ditosyl derivatives cound not be recovered from Dowex 3 ($OH^-$) resin. Furthermore, attempts to separate the tosyl derivatives of cyclohexaamylose from salts by complexation were not successful. If the tosylation reaction mixture, which had been desalted with ion exchange

resins, was treated with sodium azide and then reduced to the amines, there remained the problem of the separation of the amines. The mixture of amino derivatives and cyclohexaamylose was applied to a Dowex 50 ($H^+$) column and the following eluents were tried: - 0.3 N HCl, 25% methanolic ammonia, 0.2 M pyridinium acetate and 10% ammonia. In all cases the recovery of monoamino-cyclohexaamylose (5) was less than 50% (by t.l.c.) and it was contaminated with unknown compounds. Therefore, it was decided to attempt the more tedious procedure of separating the tosylated mixture on an activated charcoal column. Through elution of the charcoal column with aqueous alcohol eluents separation of monosubstituted from unsubstituted and polysubstituted cyclohexaamylose was achieved.

Oxidative-deamination of monoamino-cyclohexaamylose (5) by ninhydrin gave the monoaldehydo derivative (11). Although the reaction of ninhydrin with primary amines is well known[45], this is the first time it has been used on a preparative scale in carbohydrate chemistry. A mechanism for the reaction is given in Fig. 2. Ninhydrin reactions with a secondary amino group in sugars have been carried out previously in 4% aqueous pyridine[47-49]. In the present work, it was found that in 4% pyridine there was incomplete conversion of the amine to the aldehyde. Solutions ranging from pH 0.0 to 9.0 were tried and the best medium for complete reaction was

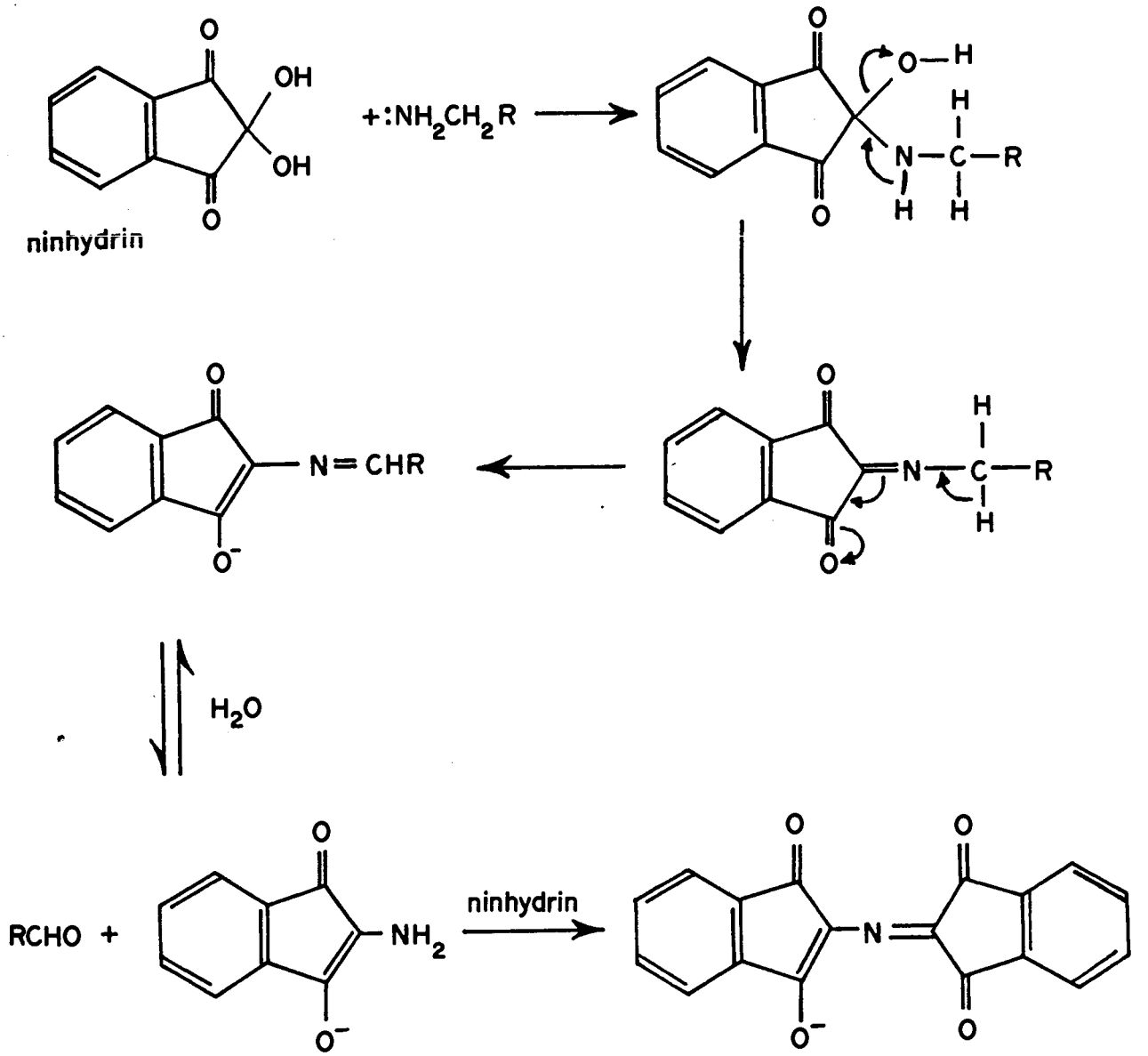



Fig. 2. Mechanism[45] for the reaction of ninhydrin with primary amines, which do not have an α-hydroxyl group.

found to be a carbonate buffer of pH 8.0. Work in our laboratory[50] has confirmed that carbohydrate primary amines are converted to aldehydo sugars by reaction with ninhydrin. Using the same reaction conditions the known 6-aldehydo-1,2;3,4-di-O-isopropylidene-α-D-galactose[51] was prepared from 6-amino-6-deoxy-1,2;3,4-di-O-isopropylidene-α-D-galactose. An alternative method of preparing a ω-aldehydo sugar is by photolysis of the corresponding azide [13,52]. Photolysis of monoazido-cyclohexaamylose (4) and 6-azido-6-deoxy-1,2;3,4-di-O-isopropylidene-α-D-galactose[50] were carried out and the expected aldehydes were produced. However, the photolysis products were not as pure as the aldehydes prepared by the ninhydrin reaction, nor were they as easy to purify.

The reaction of ninhydrin with amino-sugars, in which the amine is attached at the C-2 position, causes carbon-carbon bond cleavage between C-1 and C-2 with concomitant oxidation of the C-2 carbon to an aldehyde[47-49,53]. As a result ketoses cannot be obtained from 2-amino-2-deoxy-sugars by ninhydrin oxidation. Furthermore, it is probable that the ninhydrin reaction will result in carbon-carbon bond cleavage in primary amines if there is a free α-hydroxyl group present. Therefore, the oxidative-deamination without carbon-carbon bond cleavage is limited to amines where the

α-hydroxyl groups are blocked or replaced by an unreactive group such as hydrogen.

The monoaldehydo-cyclohexaamylose (11) readily reacted with modified Tollen's reagent to give a single product, which is thought to be the monocarboxyl derivative (12). Further work is required to characterise this oxidation product.

The method of Whelan and Slessor[30] for preparing monotrityl-cyclohexaamylose (2) gives the desired product in satisfactory yield, but it cannot be easily converted to other monosubstituted cyclohexaamyloses. The preparation of monoiodo (8) and monodeoxy (9) derivatives from the monotrityl compound (see introduction) involves more steps and the overall yields were low compared to the methods used in the present work.

Now that the monosubstituted cyclohexaamyloses are available, they have a number of potential uses. They could be used to prepare specifically substituted linear oligosaccharides. Another possible use is as substrates to study amylases that are capable of acting on them. Perhaps the most exciting potential use is as model enzymes, assuming that the "active site" is the end of the central cavity in the molecule, which is surrounded by primary hydroxyl groups.

The cyclohexaamylose derivatives have a number of interesting properties, which are worth mentioning. One interesting property of the monohalo

derivatives was their distinct gradation of water solubility. The iodide was the least soluble, being only partially soluble in boiling water. While the bromide was intermediate in solubility, the chloride was readily soluble in warm water but reluctant to dissolve in cold water.

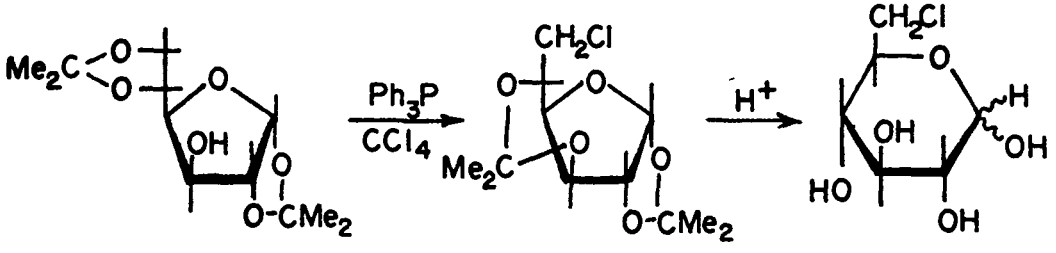
A well known characteristic of cycloamyloses is their ability to form complexes with widely different types of compounds[1,4,54]. This complexing property has been useful in several instances in the present investigations. Iodine has been known for a long time to give a coloured complex with cyclohexaamylose and it was found that all cyclohexaamylose derivatives complexed with iodine vapour on t.l.c. plates to give characteristic colours which aided in their identification. (Maltohexaose in high concentrations gave only a pale yellow colour with iodine on t.l.c. and its lower homologues gave even weaker reactions.) Organic liquids which form insoluble complexes have been used previously in the separation of cycloamyloses[4,55] and they were employed in the purification of some of the cyclohexaamylose derivatives. The monoazide derivative (4) was separated from a small amount of unidentified side product by precipitation with tetrachloroethane. The monotrityl derivative (2) was separated from a mixture of cyclohexaamylose and its ditrityl derivative with

with cyclohexane. Unfortunately, the separation of monotosyl-cyclohexaamylose (3) from cyclohexaamylose and polytosyl derivatives by complexation was unsuccessful. Tetrachloroethane, bromobenzene, toluene, cyclohexane, and carbon disulphide were tried as complexing reagents for the monotosyl derivative. Complexes with organic liquids and the soluble yellow complex formed with iodine during the preparation of monoiodo-cyclohexa-amylose could be decomposed by heating in boiling water until the complexing agent had evaporated, yielding the free sugar.

Water included in the central cavity of crystalline cycloamyloses is not easily removed[56,57], but it has been shown[56] that freeze drying can remove all the water. Therefore all samples for elemental analysis were freeze dried and quantitatively dried immediately before analysis.

## Monosubstituted D-Glucoses

D-Glucose derivatives substituted at the C-6 position were prepared as chromatography standards. The 6-chloro derivative was obtained in good yield by chlorination of 1,2;5,6-di-O-isopropylidene-α-D-glucose using carbon tetrachloride and triphenylphosphine. The chlorination involves rearrangement of the 5,6-O-isopropylidene group and chloride substitution at C-6:-




Hydrolysis of the acetal groups gave 6-chloro-6-deoxy-D-glucose (22). However, there is evidence that this is not the only reaction that occurs. P.m.r. spectroscopy showed that 6-chloro-6-deoxy-1,2;3,5-di-O-isopropylidene α-D-glucose (23) was impure. Furthermore, when compound (23) was reduced with lithium aluminium hydride[58] two products were seen on t.l.c. in the approximate ratio of 9:1. The minor component was identical in chromatographic mobility with 3-deoxy-1,2;5,6-di-O-isopropylidene-α-D-ribohexose. The most likely explanation is that during the chlorination of

1,2;5,6-di-O-isopropylidene-α-D-glucose, as well as acetal rearrangement and chloride attack at C-6, there is a small amount of nucleophilic displacement at C-3 to give 3-chloro-3-deoxy-1,2;5,6-di-O-isopropylidene-α-D-allose. This is not too surprising considering the recent report of sulphonate displacements at C-3 of 1,2;5,6-di-O-isopropylidene-3-O-tosyl-α-D-glucofuranose[59]. Therefore the impurity in 6-chloro-6-deoxy-D-glucose would be 3-chloro-3-deoxy-D-allose. It is concluded that, although chlorination of fully protected sugars with carbon tetrachloride and triphenylphosphine gives chloro sugars in excellent yields[58], it is not a satisfactory method when the blocking groups are capable of rearrangement.

6-O-Trityl-D-glucose has been prepared by tritylation of D-glucose[60], but the compound was impure[31]. In the present work the method of Reynolds and Evans[61] was used to prepare 1,2,3,4-tetra-O-acetyl-6-O-trityl-β-D-glucose (25) which can be readily purified. The pure compound (25) was deacetylated to give the required product in good yield.

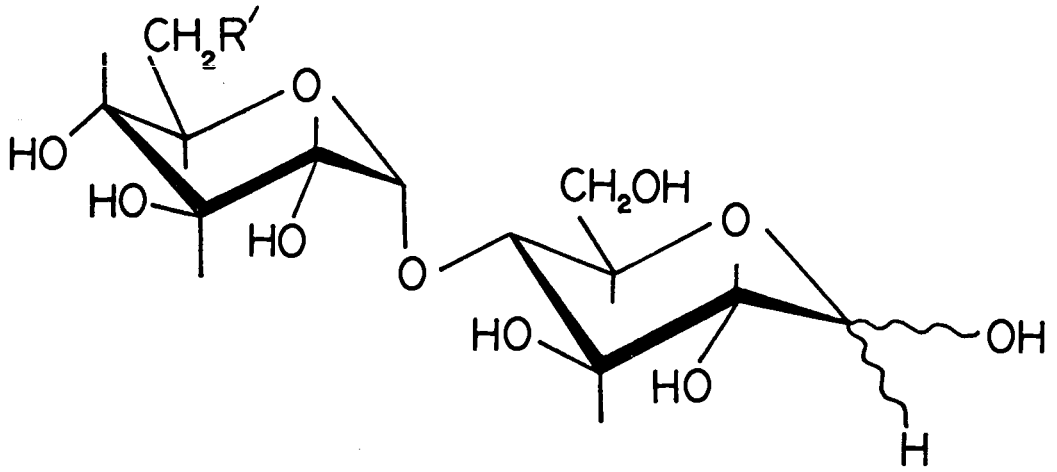
The other glucose derivatives with substituents at C-6, tosyl (26), azido (29), bromo (32), and iodo (33), were prepared by new methods, but the yields were low and the products contained small amounts of impurities. The formation of the tosyl (26), azido (29), and iodo (33)

derivatives was confirmed chromatographically by preparation of the compound by a second method. 6-Bromo-6-deoxy- (32) and 6-deoxy-6-iodo-D-glucose (33) have been prepared[62,63] by reacting 1,2:5,6-di-O-isopropylidene-α-D-glucose with triphenyl phosphite and bromine or iodine, followed by hydrolysis of the acetal groups. However, the compounds were difficult to purify and the yields were low. Neither compound (32) nor (33) could be obtained crystalline in these investigations [62,63]. A new method for selective bromination of primary alcohol groups, using N-bromosuccinimide and triphenylphosphine, has recently been published[64] and this would be the method of choice for future preparations of 6-bromo-6-deoxy-D-glucose.

Monosubstituted Maltoses

The action of a crude preparation of Aspergillus oryzae amylase on monosubstituted cyclohexaamyloses results in a new and elegant method of preparing 6'-substituted maltoses in yields (36 - 75%) which are superior to those obtained by previous workers. By this method 6'-O-tosyl-(13), 6'-azido-6'-deoxy-(14), 6'-chloro-6'-deoxy-(16), 6'-bromo-6'deoxy-(17), and 6'-deoxy-6'iodo-maltose (18) (see Fig. 3) were prepared for the first time. The 6'-deoxy-6'-iodo-maltose was obtained in a crystalline state. With a few exceptions this appears to be a general method for obtaining 6'-substituted maltoses. Thus any compounds that can be prepared from monosubstituted cyclohexaamyloses, especially from the monotosyl (3) derivatives, could be converted to the corresponding 6'-substituted maltoses. Two compounds worthy of study are monoaldehydo-(11) and monocarboxyl-cyclohexaamylose (12). These two compounds should give 6'-substituted maltoses, but it is possible that they will exhibit such decreased rates of hydrolysis that a mixture of maltose and maltotriose derivatives will result (see p41 for explanation).

A. oryzae amylase is glycoprotein, in which the carbohydrate moiety varies according to the source of the enzyme[65,66]. In the present work, the carbohydrate




(13) R' = OTs
(14) R' = $N_3$
(15) R' = $NH_2$
(16) R' = Cl
(17) R' = Br
(18) R' = I
(19) R' = H
(20) R' = OTr

Fig. 3. 6'-Substituted maltoses.

moiety of the amylase underwent hydrolysis during the course of the enzyme action. The four monosaccharides formed by the hydrolysis of the enzyme correspond to glucose, galactose, mannose and either xylose or N-acetylglucosamine on paper chromatography. The loss of the oligosaccharide side chain does not affect the activity of the amylase[67]. Activated charcoal columns were found to be suitable for separating monosaccharides and maltose derivatives formed by amylase action on monosubstituted cyclohexaamyloses.

During the action of the amylase on monotosyl-cyclohexaamylose a side product was observed, which slowly increased while the 6'-O-tosyl-maltose (13) decreased. Since the reaction was carried out in acetate buffer it seems probable that the acetyl group was displacing the tosyl group to give 6'-O-acetyl-maltose. Although there was no problem in separating the acetyl and tosyl derivatives on a charcoal column, in future the reaction time should be reduced to 48 h so as to achieve a maximum yield of monotosyl-maltose (13).

The enzyme hydrolysis of monoamine-cyclohexaamylose appears to be a poor method of preparing 6'-amino-6'-deoxy-maltose (15) because of the presence of a large amount of 6"-amino-6"-deoxy-maltotriose (21) even after 7 days. Indeed, it would be better to shorten the time of reaction and so increase the yield of the monoamino-

maltotriose, which has not been prepared before. In any case, a superior method of obtaining the monoamino-maltose is now available. Hydrogenation of 6'-azido-6'-deoxy-maltose gives 6'-amino-6'-deoxy-maltose in virtually quantitative yield and since the azide can be prepared in high yield (75%), this is obviously the method of choice. 6'-Amino-6'-deoxy-maltose has been prepared previously[37], but the yield was considerably lower than that obtained in the present work.

Another exception to the general reaction is monodeoxy-cyclohexaamylose which gave a mixture of 6'-deoxy-maltose (19) and 6-deoxy-D-glucose. However, a preliminary experiment showed the reductive-dehalogenation of 6'-deoxy-6'-iodo-maltose (18), with hydrogen and 5% palladium on charcoal, readily gave 6'-deoxy-maltose.

The preparation of 6'-O-trityl-maltose (20) by amylase action on monotrityl-cyclohexaamylose was unsuccessful. Further investigation is required, but from the available evidence there appears to be several competing reactions. Detritylation is known[31] to occur under acidic conditions, therefore it is expected that the trityl group on cyclohexaamylose and on a maltooligosaccharide would be slowly hydrolysed in a slightly acidic buffer (pH 5.5). The products of

detritylation, cyclohexaamylose and maltooligosaccharide are rapidly attacked by the crude enzyme to yield mainly D-glucose. In another reaction the amylase slowly hydrolyses the glucosidic bonds of monotrityl-cyclohexaamylose and a monotrityl-maltooligosaccharide is formed.

Crystalline maltose exists as a monohydrate[68], therefore it is not surprising that the tosyl (13), azido (14), amino (15) and chloro (16) derivatives of maltose were found on analysis to be monohydrates. The azido derivative (14) was hygroscopic. The analysis results for the iodo derivative (18) after a short drying period indicate that it too contains a molecule of water.

In order to determine the structures of monosubstituted maltoses and monoamino-maltotriose two assumptions were made. It was assumed that the $\alpha$, $1\rightarrow4$ linkage between the glucopyranosyl residues had not changed during the treatment of the monosubstituted cyclohexaamylose with the amylase preparation. It was also assumed that the position of the substituent on the glucopyranosyl unit had not changed during the preparation of the maltose derivatives. These assumptions are reasonable because there is no evidence that an amylase can bring about either change in structure. In addition there is no chemical reason for the substituent to migrate on the glucopyranosyl unit.

The structures of monosubstituted maltoses and monoamino-maltriose were determined by mass spectroscopy and the structures of 6'-chloro-6'-deoxy- and 6'-deoxy-maltose were established independently by chemical methods. The monochloro-maltose was reduced with sodium borohydride and then hydrolysed. T.l.c. showed 6-chloro-6-deoxy-D-glucose and D-glucitol were formed, but not 6-chloro-6-deoxy-D-glucitol and D-glucose.

The base "peeling" method of Painter[93] was used to establish the structure of monodeoxy-maltose. Chromatography showed the products of base "peeling" were 6-deoxy-D-glucose and a very slow running material. In addition no reaction intermediates were detected on thin-layer chromatography during the course of the reaction. The absence of intermediates indicates the compound is a disaccharide and the formation of 6-deoxy-D-glucose indicates the structure of the disaccharide is 6'-deoxy-maltose. Hence the structure of monoiodo-maltose, from which the 6'deoxy-maltose was prepared, is 6'-deoxy-6'-iodo-maltose. The base peeling method could not be used to determine the structure of the other monosubstituted maltoses, because when 6-chloro-6-deoxy- and 6-amino-6-deoxy-D-glucose were subjected to this procedure the substituents at C-6 reacted. Thin-layer chromatography showed that the 6-substituted glucose was still present but one or more

unknown products had been formed. No reaction occured with D-glucose and 6-deoxy-D-glucose. Furthermore, since tosyl groups are susceptible to base solvolysis[43], base "peeling" could not be used to establish the structure of monotosyl-maltose. Hence this method is applicable only to deoxy- and unsubstituted oligosaccharides.

Recent reports[69-71] have shown the sequence of sugar residues in oligosaccharides can be found by use of mass spectroscopy. The interpretation of the mass spectra in the present investigation was based principally on the work of Kochetkov and coworkers[69]. This interpretation enabled the sequence of the glucopyranosyl residues in the pertrimethylsilyl (TMS) derivatives of monosubstituted maltoses and monoamino-maltotriose to be established. The mass spectra of the permethylsilyl derivatives of maltose (sample temperatures, 150° and 190°) and maltotriose (sample temperature, 170°) were measured as reference compounds.

In the mass spectral analysis of maltose and maltotriose derivatives (Appendices 2 and 3) only the peaks, which give the molecular weight and help establish the sequence of residues, are shown. Naturally many other peaks were seen in the mass spectra. The mass spectral interpretation that the molecular-ion decomposes to fragment I (Appendix 2) which in turn decomposes to IV, is based on the fragmentation pattern observed[72] for

permethylated disaccharides containing a 1→4 linkage. The process involving the formation of fragment II and the subsequent loss of a methyl radical to give fragment III has been found in earlier work[73] with the pertrimethylsilyl derivatives of a 1→4 linked disaccharide. The formation of fragments I, II, III, and IV, which are characteristic of the non-reducing residue of a disaccharide, are in agreement with the results of Kochetkov and coworkers[69]. The decomposition of the molecular-ion to fragment V, which is characteristic of the reducing residue, has been demonstrated by the same workers[69]. This means each glucopyranosyl residue in pertrimethylsilylated maltose exhibits a distinctive fragmentation process. This is the key to obtaining the sequence of the sugar residues, because, when a TMSO group is replaced by a different substituent, the substituted residue will have a new mass, which will be reflected by new peaks in the mass spectrum for some fragmentation processes but not for others. Hence the sequence of the residues in a monosubstituted disaccharide can be established.

The mass spectra of the pertrimethylsilyl derivatives of monochloro- (16) and monobromo-maltose (17) are comparatively easy to interpret, because fragments containing chloride or bromide have a distinctive isotopic cluster due to the 37 and 81 isotope masses respectively.

In both cases there is ample evidence (fragments $\overline{I}$, $\overline{II}$, $\overline{III}$ and $\overline{IV}$ with X = Cl or Br) to demonstrate that the halide is attached to the non-reducing glucose residue. The presence of fragment $\overline{V}$ with X = OTMS, but the absence with X = Cl or Br indicates the reducing residue does not contain a halide. So the product is not a mixture of monohalo-maltoses. There were no mass peaks in the mass spectrum of pertrimethylsilylated maltose corresponding to the fragments containing either chloride or bromide found in mass spectra of the halide derivatives. Therefore the structures of the halo derivatives are 6'-chloro-6'-deoxy- and 6'-bromo-6'-deoxy-maltose. The mass spectra of the pertrimethylsilyl derivatives of the other monosubstituted maltoses were analyzed in a similar manner to prove the substituent was attached at the 6' position.

With the monoiodo-maltose (18), the mass spectrum of the pertrimethylsilyl derivative showed the required peaks for a substituent on the non-reducing residue, and no peaks for a substituent on the reducing residue. All the peaks for fragments containing the iodide group, except m/e 633, were not seen in the mass spectrum of pertrimethylsilylated maltose. The peak at m/e 633 is relatively low in intensity in the mass spectrum of pertrimethylsilylated maltose, whereas it has very high intensity in the mass spectrum of the monoiodo-maltose

derivative. Therefore it is probable that the peak at m/e 633 in the mass spectrum of the monoiodide derivative is due predominantly to the fragment containing an iodide group. Hence all the mass spectral data indicated the monoiodo-maltose is 6'-deoxy-6'-iodo-maltose.

In the mass spectrum of pertrimethylsilylated monotosyl-maltose the relatively weak peak at m/e 651 should be absent if the tosyl group is attached at the 6' position only. However, an intense peak was observed at m/e 651 in the mass spectrum of pertimethylsilylated maltose. Assuming the peak at m/e 651 is due to a fragment which does not contain a tosyl group, all the data indicates the structure of monotosyl-maltose is 6'-O-tosyl-maltose. Since the other monosubstituted maltoses have been proved to contain the substituent in the non-reducing residue, there is no reason to expect the monotosyl derivative to be different.

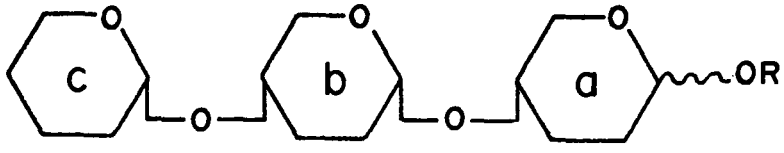
Pertrimethylsilylation of monoamino-maltose (15) resulted in a mixture of free amine and mono-N-trimethysilyl-amine in the approxiamte ratio of 2:1 according to the mass spectrum. After 10 h at room temperature the mass spectrum of the trimethylsilylated monoamino-maltose showed no change. These results seem to be contrary to those of Karkkainen and Vihko[74], who found that the amine substituted on a secondary carbon in hexosamines was not

trimethylsilylated under the conditions used in the present investigation. Furthermore, when the secondary amine was N-trimethylsilylated by another method[74] it was found that hydrolysis of the Si — N bond occured readily on standing at room temperature. However, the difference in results appears to depend on the position of the amine group. Primary amines are relatively easy to mono-N-silylate, whereas secondary amines are considerably more difficult to mono-N-silylate[75].

The molecular-ion peaks for the free amine and the N-silylated amine are not present in the mass spectrum, but the $M^+ - CH_3$ peaks are, which indicates the compound is a monoamino-maltose. The evidence for the 6'-amino-6'-deoxy-maltose structure in the mass spectrum of the permethylsilyl derivative is less convincing than that obtained for other 6'-substituted maltoses. A number of peaks that would prove the structure are obscured by isotopic clusters of more intense peaks. The m/e 595 peak (fragment $\overline{III}$ with X = $NH_2$) can also arise by a different fragment process (i.e. fragment $\overline{IV}$ with X = OTMS), so this peak is ambiguous. The only fragments that do indicate the structure is 6'-amino-6'deoxy-maltose are $\overline{I}$ and $\overline{II}$ with X = NHTMS, $\overline{V}$ with X = OTMS and the absence of $\overline{V}$ with X = $NH_2$ and X = NHTMS. The paucity of key peaks for sequence determination in the mass spectrum of silylated monoamino-maltose is unfortunate,

but the position of the substituent can still be obtained. The position of the substituent in monoazido-maltose has been clearly established by mass spectroscopy, hence reduction of the monoazide derivative gives 6'-amino-6'-deoxy-maltose.

Unlike the other pertrimethylsilylated monosubstituted maltoses, the silylated monoamino-maltose and monoamino-maltriose exhibit thermal decomposition during mass spectroscopy. According to Kochetkov and coworkers[69] the fragmentation of a trisaccharide during mass spectroscopy




occurs in a characteristic manner. Fragmentation of ring "c" depends only on the structure of that residue (e.g. fragment XI in Appendix 3). While rupture of ring "b" gives the same ions as the fragmentation of the disaccharide "c→b" (fragments II and III). Fragmentation of ring "a" leads to ions having mass numbers differing from that of the corresponding ions of the disaccharide "b→a" by the mass of the ring "c" (378 mass unit for tetra-O-trimethylsilyl-glucopyranosyl unit or 305 mass unit for 6-amino-6-deoxy-tri-O-trimethylsilyl-glucopyranosyl unit) (fragments VI and V). In addition fragments V and VII arise from the rupture of rings "b" and "c" respectively. The only

molecular-ion detected in the mass spectrum was for the free amine, although, peaks of lower mass unit were seen for fragments arising from the mono-N-trimethylsilyl-amine derivative. The peaks containing the amine substituent were not observed in the mass spectrum of pertimethylsilylated maltotriose. The only structure which fits all the mass spectral data is 6"-amino-6"deoxy-maltotriose. The presence of peaks at m/e 845 and 917 (fragment VIII with Y = $NH_2$ and Y = NHTMS) and other peaks of lower mass unit indicate the monoamino-maltotriose is contaminated with 6'-amino-6'-deoxy-maltose.

Mode of Action of A. oryzae Amylase

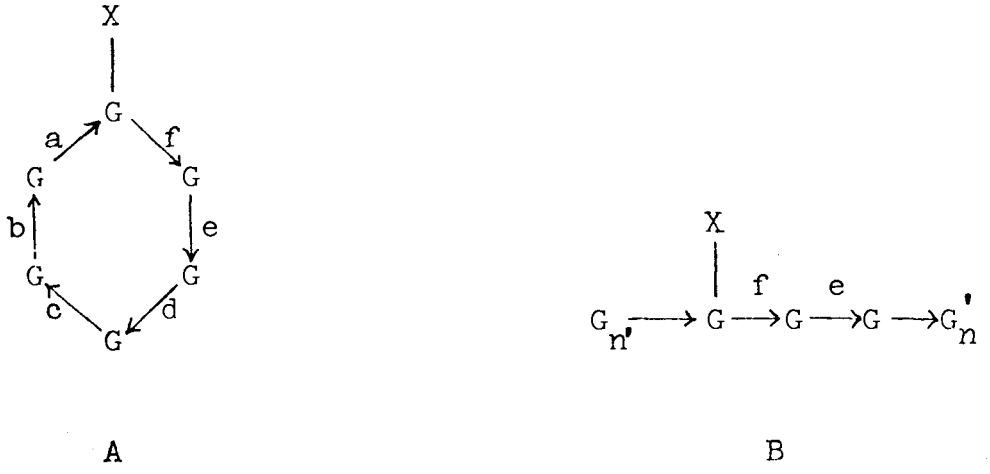
As well as using the amylase as part of a synthetic method, it is also interesting to consider its mode of action. In general, the action of the crude amylase preparation on a monosubstituted cyclohexaamylose produced a 6'-substituted maltose, as well as D-glucose. This result is in agreement with previous investigations[38,40] where "pure" crystalline A. oryzae amylase was used. The formation of 6-deoxy-D-glucose from monodeoxy-cyclohexaamylose is probably due to the additional action of the glucoamylase, which is known to be present in the amylase preparation. On commencing the present investigation, it was predicted that cyclohexaamyloses with bulky substituents such as tosyl and trityl groups might give 6"-O-tosyl- and 6"-O-trityl-maltotriose respectively. This was definitely not the case with the tosyl derivative, where the product was 6'-O-tosyl-maltose (13). Further work is required to establish the structure of the product from the trityl derivative, but preliminary experiments do indicate that the trityl group has a considerable influence on the rate of amylase action. Probably the most interesting result is the formation of a mixture of monoamino derivatives of maltose (15) and maltotriose (21) from monoamino-cyclo-hexaamylose (5). As no previous work had been done with the amylase and substrates containing an amine at C-6,

this result was not predicted. However, much earlier studies[76] on almond emulsin β-glucosidase have shown that when the hydroxyl on C-6 of phenyl β-D-glucopyranoside was replaced by an amine, there was a large decrease in the overall rate of the enzyme action. However, phenyl 6-acetamido-6-deoxy-β-D-glucopyranoside was hydrolysed faster than the free amino compound[76].

To understand the action of the crude A. oryzae amylase preparation, it is important to realize that even with pure amylase there are two processes involved. Firstly, the amylase breaks the cyclohexaamylose ring by hydrolyzing one glucosidic bond to give linear maltohexaose. Only A. oryzae amylase and three other amylases (B. macerans[4], B. polymyxa[5], and P. africanum[6]), which are assumed to be absent in the present investigation, can open the cyclohexaamylose ring. Secondly, the A. oryzae amylase acts like any α-amylase and degrades maltohexaose to maltose and D-glucose. With crude amylase preparation the situation is complicated, because a glucoamylase is present (which explains why the degradation of cyclohexaamylose gave almost entirely D-glucose instead of maltose and D-glucose) and furthermore, the Bacillus detected in the enzyme preparation may contain amylases which will degrade maltodextrins. In spite of the complications it is possible to gain some information on the mode of amylase action from the present investigation.

One other important point for interpreting the enzyme action is that the amylase does not recognize the aglycon. In fact the aglycon can be a phenyl group[40,41], or a substituted phenyl group[77], or a 6-O-methyl-glucopyranosidic residue[38], or the isomaltosyl residue in 6"-α-D-glucopyranosylmaltotriose[39].

It has been shown in this report that in general the products of degradation of a cyclohexaamylose derivative by the amylase preparation are 6'-substituted maltose and D-glucose. This means the glucosidic bond of the glucopyranosyl residue containing the substituent (bond "f" in Fig. 4) cannot be hydrolysed by any amylase or glucoamylase in the crude enzyme preparation. Assuming A. oryzae has the same specificity for cyclic and acyclic oligosaccharides, the presence of 6"-amino-6"-deoxy-maltotriose amongst the products from the reaction of crude amylase with monoamino-cyclohexaamylose (5) indicates that bond "e" is the last glucosidic bond to be broken in the formation of 6'-amino-6'-deoxy-maltose. Therefore "e" is not hydrolysed in the initial attack by A. oryzae amylase. Amylase degradation of starch and amylopectin to 6"-α-D-glucopyranosylmaltotriose[39] as the final product, supports the idea that "e" is the last bond to be hydrolysed. It is possible that this is true of all the monosubstituted cyclohexaamyloses. There is no evidence available at present to indicate




A B

Fig. 4. Labelled glucoside bonds in cyclohexaamylose monosubstituted at C-6 (A) and monosubstituted maltooligosaccharides (B), where n and n' can be 0, 1, 2 or 3 so that $n + n' \leqslant 3$.

that the initial attack by the amylase cannot occur at any of the other glucosidic bonds ("d", "c", "b" or "a" in Fig. 4A). Once the linear maltohexaose derivative (Fig. 4B, with $n + n' = 3$) has been formed any α-amylase, assisted by the glucoamylase, degrades it to the 6'-substituted maltose and D-glucose.

Another way of increasing the understanding of the mode of action of an enzyme is by studying the change in rates of reaction with different substrates. In the present work only preliminary experiments of this type were carried out, so the amount of information obtained was not great. Different reaction times are reported in the enzymatic preparations of monosubstituted maltoses. Some derivatives, particularly monoiodo- (18) and

monotosyl-maltose (13), were easily separated from higher substituted and non-substituted maltodextrins on a charcoal column, with the result that the reaction could be stopped before completion. Other substrates had to be left for long periods to ensure the higher maltodextrins were degraded, because they could not be readily separated from the monosubstituted maltose on a charcoal column. The other possible reason is that some enzyme reactions take longer because of inhibition caused by C-6 substituted maltooligosaccharides, especially monosubstituted maltoses. For example, it seems probable that the slow enzymatic hydrolysis of monoamino-maltotriose to monoamino-maltose is due to inhibition caused by the amino group which exists as a salt ( $R—NH_3^+$ $AcO^-$) in the acetate buffer. The inhibition may be due to the strong electrostatic attraction between $-NH_3^+$ and the carboxylic anion[8] found at the active sites of α-amylases. Alternatively, the inhibition may be due to stringent steric requirements for the substituent at the C-6 position of the substrate. In other words the salt ($R—NH_3^+$ $AcO^-$) and its solvation layer of water is too bulky to fit readily into the active site of the enzyme. If the monoaldehydo- (11) and monocarboxyl-cyclohexa-amylose (12) do show decreased rates of hydrolysis when they are used as substrates, the explanation is probably similar. An amine and an imidazole group are

thought to be at the active sites of α-amylases[8] and the decrease in rates of hydrolysis could be caused by the interaction of the amine with the aldehydo and carboxyl groups or by interaction of the imidazole with the carboxyl group. The alternative explanation is that the decrease in rates could be caused by steric effects. In the present work it is probable that the slow rate of initial hydrolysis of monoamino-cyclohexaamylose compared to the parent compound (see Table 2) is also due to either the electrostatic or steric interactions of the amino group. Further work using 6-acetamido-6-deoxy- and 6-diethylamino-6-deoxy-cyclohexaamylose should provide a greater understanding of the effect of the amino group on the mode of action of the amylase.

## CONCLUSIONS

The primary aim of this research, to prepare specifically modified cyclohexaamyloses, has been achieved. Seven crystalline cyclohexaamyloses with one substituent at a C-6 position have been prepared and fully characterised. Three other monosubstituted cyclohexaamyloses have been synthesised and partially characterised. These ten monosubstituted cyclohexaamyloses have not been prepared before. The yields are reasonable considering the difficulties inherent in working with these compounds.

The reaction of a crude A. oryzae amylase preparation with monosubstituted cyclohexaamyloses as substrates, led to a new method of preparing 6'-substituted maltoses. The yields are superior to those obtained by other methods. Five of these compounds have not been reported before. 6'-Amino-6'-deoxy-maltose, which could not be prepared by amylase action, was easily obtained by reduction of 6'-azido-6'-deoxy-maltose. The products obtained are in agreement with the known action pattern of A. oryzae amylase. Thus the enzymatic degradation of monosubstituted cyclohexaamyloses is a useful and general method for preparing 6'-substituted maltoses.

EXPERIMENTAL

General Methods

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Evaporations were carried out on a Buchi rotary evaporator at bath temperatures not exceeding 45°. Optical rotations were measured on a Perkin Elmer Model P22 spectropolarimeter. Freeze drying was done at -40°, 0.3 mm for approximately 20 h. N,N-Dimethylformamide was dried over calcium hydride, distilled and kept over barium oxide. All samples for elemental analysis were dried immediately before analysis. The monosubstituted maltoses and 6-O-trityl-D-glucose were dried in a drying pistol at ca. 60°, 0.01 mm pressure for 4 - 24 h. Analyses of cyclohexaamylose derivatives were performed by Alfred Bernhardt Ltd., West Germany, and the analyses of the glucose and maltose derivatives were done by M.K. Yang, Chemistry Dept., Simon Fraser University. 6-Deoxy-D-glucose was purchased from Koch-Light Laboratories Ltd., England. 6-Amino-6-deoxy-D-glucose hydrochloride was bought from Raylo Chemicals Ltd., Edmonton, Canada. Maltotriose and maltotetraose were purchased from K & K Laboratories Inc., Plainview, N.Y., U.S.A.. Tes-tape, glucose analysis paper, was bought from Eli Lilly & Co., Toronto, Canada.

Thin-layer chromatography (t.l.c.) was carried out on Silica gel G with the solvents shown in Table I and solvent G

Table 1. Thin-layer chromatography solvents (v/v).

| Solvent | butanone | methanol | water | 1.0 N acetic acid |
|---|---|---|---|---|
| A | 14 | 3 | 3 | |
| B | 145 | 28 | 27 | |
| C | 6 | 1 | 1 | |
| D | 12 | 3 | | 5 |
| E | 3 | 1 | | 1 |
| F | 9 | | 1 | |

which was ethyl ether-toluene, 2:1. Paper chromatography (p.c.) using Whatman 3MM paper was run by the descending technique with the following solvents: H, ethyl acetate-pyridine-water, 5:4:3; I, ethyl acetate-pyridine-water, 10:4:3. All sugars were detected on t.l.c. with 10% aqueous sulphuric acid spray followed by heating. Tosylate derivatives were seen on t.l.c. plates as fluorescent spots after spraying with 2% diphenylamine in ethanol and exposing to U.V. light for several minutes. When t.l.c. plates were sprayed with 0.3% 2,4-dinitrophenylhydrazine in 2N hydrochloric acid, ω-aldehydo derivatives and reducing sugars reacted to give bright yellow spots. Iodine vapour was used to detect cyclohexaamylose and its derivatives on t.l.c. and p.c.. Amines were seen on t.l.c. and p.c. when sprayed with 0.2% ninhydrin in ethanol. Paper chromatograms were stained by silver nitrate-sodium hydroxide method[78] to

detect reducing sugars and simple polyhydric alcohols. The $R_f$ values were measured relative to cyclohexaamylose ($R_c$) for cyclohexaamylose derivatives and relative to glucose ($R_G$) for all other sugars.

The method used for preparation of pertrimethylsilyl (TMS) derivatives of 6'-substituted maltoses for mass spectroscopy was essentially the method of Sweeley and coworkers[79]. The maltose derivative (3-5 mg) was dissolved in pyridine (1 ml) then hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) were added. The mixture was shaken vigorously and then kept at 40°. After 1 h the solution was evaporated to dryness and the solid extracted with hexane ( 4 ml). The extract was filtered and the filtrate evaporated to give a colourless syrup. The mass spectra of pertrimethylsilyl derivatives were measured within 3 h of preparation. The mass spectra were recorded with a Hitachi Perkin-Elmer RMU-7 mass spectrometer. The ionizing potential was 80ev and the source temperature 200°. The sample temperature was 170° unless otherwise stated. Spectra were calibrated by direct counting of spectra recorded at high recorder gain. The mass marker was calibrated on the m/e 481 peak of perfluorokerosene.

Monosubstituted Cyclohexaamyloses

Cyclohexaamylose(1)was prepared according to the method of French, Pulley and Whelan[55], on a smaller scale and without using Bacillus subtilis amylase to remove starchy residues. A culture of Bacillus macerans (American Type Culture Collection 7069) was the source of B. macerans amylase. An amylase activity of 0.5 to 1.5 Tilden and Hudson units[80] was used to prepare pure cyclohexaamylose (72 g) from potato starch (305 g).

6-O-Triphenylmethyl-cyclohexaamylose[30](2). - To cyclohexaamylose (8.3 g) dissolved in pyridine (1650 ml), triphenylmethyl chloride (10.0 g) was added. The solution was heated at 100° for 1 h and then poured into water (1650 ml). Barium carbonate (16.5 g) was added and the solution evaporated to about 100 ml. Ethanol (2 x 600 ml) was added and the mixture evaporated to dryness. The residue was extracted twice with boiling water (2 x 650 ml), cooling each extract before filtration. Cyclohexane (7.5 ml) was added to the combined extracts and the mixture shaken thoroughly before filtration. The precipitate was washed with cold water (250 ml) and added to boiling water (600 ml) which was kept boiling for exactly 20 min with continuous stirring. The mixture was cooled to room temperature and filtered. Cyclohexane (5 ml) was added to the filtrate and the above procedure repeated. The filtrate obtained was

freeze dried to yield 6-O-triphenylmethyl-cyclohexaamylose (3.42 g, ca. 95% pure: $R_c$=2.5, solvent A, yellow-brown with iodine). A small portion of this material was purified twice by cyclohexane precipatation as described above and crystallized from aqueous solution. Recrystallization from water gave crystals with m.p. 286-289°, $[\alpha]_D^{21}$ + 128° (c 0.7, ethanol).

Anal. Calc. for $C_{55}H_{74}O_{30}$: C, 54.36; H, 6.14. Found: C, 54.16; H, 6.23.

6-O-p-Toluenesulphonyl-cyclohexaamylose (3). - Freeze dried cyclohexaamylose (2.0 g) and p-toluenesulphonyl chloride (8.0 g) were dissolved in pyridine (400 ml). After 40 min at room temperature the reaction was stopped by addition of water. T.l.c. in solvent A showed the product contained about 2/3 monotosyl-cyclohexaamylose ($R_c$ = 2.1, yellow-brown with iodine), about 1/3 unreacted cyclohexaamylose (purple with iodine) and small amounts of higher tosylate derivatives. Pyridine was removed by evaporation until a syrup was obtained. To this material water (100 ml) was added and the evaporation continued. This process was repeated several times until no odor of pyridine could be detected. The aqueous solution (400 ml) was filtered and applied to a column (27 x 2.5 cm) of activated charcoal (50 g), Darco, 20-40 mesh (Matheson, Coleman and Bell). The column was eluted with water (1.5 liters), followed

by 30% ethanol (1 liter) and 25% 1-propanol (1 liter). All eluents were deaerated by evacuation with a water pump before use. Collection of the ethanol column eluate allowed recovery of unreacted cyclohexaamylose. The 1-propanol eluate was collected in 100 ml fractions. Concentrated ammonia (ca. 2 ml) was added to each fraction and the solutions were evaporated to dryness. (In all charcoal column separations, concentrated ammonia was added to each fraction eluted with 20% or more alcohol before evaporation). Purity of monotosyl-cyclohexaamylose was checked on t.l.c. (solvent A) and the appropriate fractions (dissolved in water) were combined, filtered through a Celite pad and freeze dried to yield the monotosylated product (3) (1.07 g). Part of this material was crystallized from 95% ethanol, m.p. 150-162°d., $[\alpha]_D^{22}$ + 111° (c 0.41, water).

Anal. Calc. for $C_{43}H_{66}O_{32}S$: C, 45.82; H, 5.90; S, 2.84. Found: C, 45.64; H, 5.69; S, 2.72.

6-Azido-6-deoxy-cyclohexaamylose (4). - Freeze dried monotosyl-cyclohexaamylose (3) (0.62g) and sodium azide (0.30 g) in N,N-dimethylformamide (65 ml) were heated at 100° for 2 h with frequent stirring. Dimethylformamide was removed by evaporation at reduced pressure (0.005 mm), water (30 ml) was added and the evaporation repeated to remove the last traces of dimethylformamide. T.l.c. (solvent D) showed that as well as monoazido-cyclohexaamylose (ca. 95%), 2 side products (ca. 5%) were

found, one of which had the same $R_f$ value as cyclohexaamylose. Two methods are available for further purification of the monoazido-cyclohexaamylose. In the first method 1,1,2,2-tetrachloroethane (ca. 0.5 ml) was added to a small volume (3 ml) of aqueous solution. The tetrachloroethane complex was separated from the solution by centrifugation. The monoazide-cyclohexaamylose was recovered by heating the complex in boiling water. Evaporation of the aqueous solution yielded the product(4). T.l.c. indicated that the product was pure except for cyclohexaamylose (ca. 5%). This procedure required 0.90 g of starting material(3) to give 0.51 g of product(4). In the second method the products were dissolved in water (100 ml) and passed through Dowex 50($H^+$)(20 ml) and Dowex 3($OH^-$)(20 ml). The eluate was evaporated to dryness to give a white amorphous material (0.52 g, from 0.62 g of starting material(3)), which still contained both side products according to t.l.c. To obtain pure monoazido-cyclohexaamylose a small amount of this material was recrystallized three times from water, m.p. 217°d., $[\alpha]_D^{22}$ + 128° (c 0.40, water). T.l.c. indicated the recrystallized material ($R_c$ = 1.7, solvent A, dark purple with iodine) was pure.

Anal. Calc. for $C_{36}H_{59}N_3O_{29}$: C, 43.33; H, 5.96; N, 4.21. Found: C, 43.12; H, 5.85; N, 4.07.

6-Amino-6-deoxy-cyclohexaamylose (5). - 95% Pure 6-azido-6-deoxy-cyclohexaamylose (0.51 g) was dissolved

in water (100 ml) and reduced with palladium black (150 mg) at 14 p.s.i. of hydrogen on a Parr hydrogenator for 10 h. After removal of the catalyst by filtration, the solution was evaporated to dryness (0.51 g). A small amount of the product (5) was obtained chromatographically pure (solvent D, $R_c$= 0.8, brown with iodine) by recrystallization 3 times from water, m.p. 200°d., $[\alpha]_D^{22}$+ 117° (c 0.40, water).

Anal. Calc. for $C_{36}H_{61}N_{29}$: C, 44.49; H, 6.33; N, 1.44. Found: C, 44.38; H, 6.39; N, 1.64.

6-Chloro-6-deoxy-cyclohexaamylose(6). - Freeze dried monotosyl-cyclohexaamylose(3) (0.50 g) which was dried in a drying pistol under vacuum (0.005 mm pressure) and tetramethylammonium chloride (1.25 g), which had been kept in a vacuum desiccator over potassium hydroxide pellets, were added to dry N,N-dimethylformamide (250 ml). The suspension was stirred magnetically and kept at 100° for 90 min. T.l.c. (solvent A) showed that the starting material was converted entirely to one product. The solvent was kept at 5° overnight and a white solid formed which was removed by filtration. The dimethylformamide was removed by codistillation with 1-butanol. An aqueous solution (50 ml) of the product was then passed through Dowex 50($H^+$)(25 ml) ion exchange resin followed by Dowex 3($OH^-$)(25 ml). The aqueous eluate was freeze dried to give (6) (0.32 g). Part of freeze dried product was

crystallized from water. The crystals had m.p. 205° d., $[\alpha]_D^{22}$+ 128° (c 0.38, pyridine) and t.l.c. ($R_c$ = 1.8, solvent A, purple with iodine) showed they were pure.

Anal. Calc. for $C_{36}H_{59}ClO_{29}$: C, 43.61; H. 6.00; Cl, 3.58. Found c, 43.44; H, 6.11; Cl, 3.47.

Monochloro-cyclohexaamylose was subjected to reductive dehalogenation using the method of Jones and coworkers[46]. The chloro derivative(6) (50 mg) was dissolved in water (20 ml) containing triethylamine (3 drops) and W-4 Raney nickel (50 mg) was added. The reduction was carried out at 45 lb. $in^{-2}$ of hydrogen for 10 h. T.l.c. (solventA) showed the product had the same $R_f$value and colour reaction with iodine as the monodeoxy-cyclohexaamylose(9) prepared from monoiodo-cyclohexaamylose (8).

6-Bromo-6-deoxy-cyclohexaamylose (7). - Freeze dried monotosyl-cyclohexaamylose(3) (0.71 g) and lithium bromide* (2.84 g) in dry N,N-dimethylformamide were heated at 100° for 2 h. T.l.c. (solvent A) indicated that the reaction was complete in 1 h, giving only one product. N,N-Dimethylformamide was removed by vacuum distillation at 50° leaving ca. 3 ml of solution to which water was added and the vacuum distillation repeated to yield a syrup. The syrup was dissolved in water (150 ml) and passed through Dowex 50 ($H^+$) (100 ml). Concentration of the eluate gave a syrup which was dissolved in a small volume of water from which colour-

* Oven dried at 150°.

less crystals of (7) were formed with m.p. 174°d., $[\alpha]_D^{22}$ + 122° (c 0.40, pyridine). T.l.c. (solvents A and D) showed the crystals were pure ($R_c$ = 1.9, solvent A; $R_c$ = 1.1, solvent D, purple with iodine). The mother liquor was freeze dried to give a total yield of 0.58 g of (7).

Anal. Calc. for $C_{36}H_{59}BrO_{29}$; C, 41.74; H,5.74; Br, 7.71. Found: C, 41.72; H, 5.67; Br, 7.85.

6-Deoxy-6-iodo-cyclohexaamylose (8).- An aqueous solution (100 ml) of monotosyl-cyclohexaamylose (3) (0.54 g) and sodium iodide (2.16 g) was heated on a boiling bath for 1 h. T.l.c. (solvent A) indicated that the tosylate had reacted to give mainly one new compound and a small amount (< 5%) of material with the same $R_f$ value as cyclohexaamylose. The reaction solution was concentrated to 2 ml and on cooling pale yellow crystals (0.36 g) were formed, which were shown to be pure on t.l.c. (solvent A). The yellow colour was removed by dissolving the crystals in boiling water and heating until the solution was colourless. On concentration to 1 ml, colourless crystals (0.27 g) were readily obtained ($R_c$ = 1.9, purple with iodine). Part of the product (8) was recrystallized twice from water, to give colourless crystals with m.p. 175°d., $[\alpha]_D^{22}$ + 106° (c 0.42, pyridine).

Anal. Calc. for $C_{36}H_{59}IO_{29}$: C, 39.93; H, 5.49; I, 11.72. Found: C, 39.75; H, 5.33; I, 11.57.

6-Deoxy-cyclohexaamylose (9). - Monoiodo-cyclohexaamylose (8) (450 mg) was dissolved in pyridine:water (1:9) solution (100 ml) and W-2 Raney nickel (ca. 0.5 g) was added. The reduction was carried out at 14 lb. $in^{-2}$ of hydrogen for 6 h. T.l.c. (solvent A) showed the reaction was complete. The catalyst was filtered off using a Celite pad and the filtrate was evaporated to 20 ml, water (80 ml) was added and the evaporation repeated. The solution was then made up to 100 ml with water and passed through Dowex 3 ($OH^-$) (5 ml). On evaporation of the eluate to dryness, a colourless glass (400 mg) was obtained. T.l.c. (solvent A) showed this material was pure except for a trace (< 5%) of very slow running material, which is possibly a pyridinium salt of cyclohexaamylose. In order to obtain pure 6-deoxy-cyclohexaamylose, some of this material (230 mg) in water (50 ml) was applied to a charcoal column (10 g, 16.0 x 1.5 cm; see preparation of monotosyl-cyclohexaamylose (3)). The column was washed successively with water (500 ml), 10% ethanol (100 ml) and 25% ethanol (300 ml). Fractions (100 ml) of the 25% ethanol eluate were collected. T.l.c. (solvents A and D) showed that the first two fractions contained pure deoxy-cyclohexaamylose. On evaporation to dryness colourless material (190 mg) was obtained ($R_c$ = 1.3, solventA; $R_c$ = 1.1, solvent D, purple with iodine). This material was crystal-

lized from water by adding ethanol dropwise until the critical concentration was reached. Colourless crystals (150 mg) were obtained which could be recrystallized from water to give the product (9) with m.p. 287-289°, $[\alpha]_D^{22}$ + 119° (c 0.40, water).

Anal. Calc. for $C_{36}H_{60}O_{29}$: C, 45.19; H, 6.32. Found: c, 44.81; H, 6.42.

Attempted preparation of 6-deoxy-6-fluoro-cyclohexaamylose (10). - Tetraethylammonium fluoride, dried in a desiccator over phosphorus pentoxide at 0.025 mm pressure, and anhydrous magnesium sulphate were added to dimethylformamide* (300 ml) and the suspension was stirred magnetically for 20 minutes at 60°. After stirring at room temperature for a further 4 h, freeze dried monotosylcyclohexaamylose(3) (0.52 g) was added and the suspension was heated at 90° for 1 h with stirring. T.l.c. (solvent A) showed only one product ($R_c$ = 1.7) which was purple with iodine. Dimethylformamide was removed by vacuum distillation at 0.025 mm pressure. An aqueous solution of the product was passed through Dowex 50 ($H^+$) (150 ml) followed by Dowex 3($OH^-$) (150 ml). Attempts to obtain crystals from a concentrated aqueous solution were frustrated by the precipitation of unidentified white amorphous material.

* Dried by distillation as a benzene azeotrope and kept over anhydrous magnesium sulphate.

6-Aldehydo-cyclohexaamylose (11). - Monoamino-cyclohexamylose(5) (0.51 g) and ninhydrin (2,2-dihydroxy-1,3-indandione) (1.0 g) were dissolved in sodium carbonate buffer of pH 8.0 (50 ml) and the solution was heated on a boiling water bath for 10 min. T.l.c. (solvent D) showed the reaction was complete in 5 minutes to give only one carbohydrate product ($R_c$ = 0.95, brown with iodine and yellow with 2,4-dinitrophenylhydrazine spray). During the reaction an intermediate was seen on t.l.c. ($R_c$ = 1.1, yellow with iodine). On standing overnight at room temperature a brown precipitate formed, which was filtered off and the filtrate passed through Dowex 50($H^+$) (40 ml) followed by Dowex 3($OH^-$) (180 ml). Evaporation to dryness gave a yellow glass (0.48 g). The glass was dissolved in water (2 ml) and tetrachloroethane (5 drops) was added. The complex was separated from the solution by centrifugation and the monoaldehydo-cyclohexaamylose was recovered by heating the complex in boiling water. Evaporation to dryness yielded a pale yellow glass (0.30 g), part of which was dissolved in a small volume of water (ca.1 ml) and ethanol added dropwise until the critical concentration was reached. A few colourless crystals resulted, m.p. 220°d.. This material gave a positive test with Fehling's solution, while a blank test with cyclohexaamylose was negative. In later work[50] the monoaldehydo-cyclohexa-

amylose was purified by passage through a charcoal column and the freeze dried material gave $[\alpha]_D^{24}$ + 136° (c 1.0, water).

An alternative route to the monoaldehyde(11) is by photolysis of the monoazide(4) using the method of Horton and Clode[13]. Monoazido-cyclohexaamylose (160 mg) dissolved in water (95 ml) under nitrogen, was irradiated for 6 h. The source of radiation, a 250v. mercury lamp (unfiltered), was placed in a water-cooled quartz immersion well. T.l.c. (solvent D) showed the reaction was over in 3 h giving one major product and at least one side product. The major component of the mixture of products exhibited characteristics on the t.l.c. (solvents A and D) identical to the monoaldehydo derivative from the ninhydrin oxidation (in solvent A, $R_c$ = 1.0, brown with iodine, yellow with 2,4-dinitrophenylhydrazine). No attempt was made to isolate the aldehyde made by photolysis.

Reactions with monoaldehydo-cyclohexaamylose: - The monoaldehydo derivative(11), (10 mg) was dissolved in water (1 ml) and reduced with sodium borohydride (5 mg), overnight at room temperature. The excess borohydride was destroyed by adding 1 N acetic acid to pH 4.5. The solution was passed through Dowex 50 ($H^+$) and then evaporated five times on the rotary evaporator with methanol (2 ml), with 2 drops of 1 N hydrochloric acid added to the first

three evaporations. P.c. (solvent H) showed the aldehyde(11), ($R_c$ = 0.7, purple with iodine) had been converted to a substance with the same $R_f$ and iodine reaction as cyclohexaamylose.

Tollen's reagent[81] was adapted in order to oxidise the aldehyde group of (11). The modified Tollen's reagent was prepared by adding silver oxide (2.0 g) to water (40 ml) and then slowly adding 1.3 N ammonia (60 ml) until the oxide just dissolved. The modified Tollen's reagent (5 ml) was added to an aqueous solution (1 ml) containing monoaldehydo-cyclohexaamylose (5 mg). The mixture was shaken thoroughly, kept at room temperature for 30 minutes and then heated on a boiling water bath for 10 minutes. The black precipitate was removed by filtration through Celite. The filtrate was concentrated to a small volume, by which stage more precipitate had formed and this was removed by filtration. The filtrate was passed through Dowex 50 ($H^+$) (10 ml) and concentrated to approximately 2 ml. The concentrated solution was very strongly acidic according to pH paper. T.l.c. (solvent D) showed that the monoaldehyde(11) had been converted entirely to one substance ($R_c$ = 0.8) which was considered to be 6-carboxyl-cyclohexaamylose (12).

Monosubstituted Maltoses

Aspergillus oryzae α- amylase (EC 3.2.1.1; α-1,4-glucan-4-glucanohydrolase) was purchased from Sigma Chemical Co., lot #37B-1370-1; Crude; Type IV-A. Application of the crude enzyme preparation to malt-agar plates containing penicillin G and streptomycin sulphate, indicated the absence of fungus. However, rod shaped bacteria with a tendency to form filaments were seen, when the crude enzyme was streaked on malt-agar plates. After treatment of cyclohexaamylose with the amylase preparation (see 6'-tosyl-maltose(13) for experimental details) under sterile conditions for 100 h, glucose but no maltose was detected on p.c. (solvent I). When the sugar substrate was omitted, the crude enzyme preparation in acetate buffer formed four monosaccharides in 8.5 h according to p.c. (solvent I). Three of the monosaccharides had the same $R_f$ values as glucose, galactose and mannose and the fourth corresponded to xylose and N-acetylglucosamine in chromatographic mobility.

6'-O-p-Toluenesulphonyl-maltose(13). - Monotosyl-cyclohexaamylose(3) (360 mg), which contained cyclohexa-amylose (ca.5%), was dissolved in 0.2 N acetate buffer, pH 5.5, containing 0.01M calcium chloride, so that the concentration of sugar was 10 mg/ml. Crude A. oryzae amylase (10 mg/ml) was added and the solution was kept

at 40°. After 5 days t.l.c. (solvent B) showed the solution contained mainly glucose and a tosylated compound ($R_G$ = 1.4) which was not 6-O-tosyl-D-glucose ($R_G$ = 1.6) or monotosyl-cyclohexaamylose ($R_G$ = 0.7). There was also a side product ($R_G$ = 1.3) and some very slow running material on t.l.c.. The amount of side product ($R_G$= 1.3) was one tenth that of the tosyl compound ($R_G$ = 1.4). The enzyme action was stopped by addition of 3 N ammonia (0.4 ml for each ml of acetate buffer). The precipitated protein was removed by centrifugation, and the supernatant liquid was evaporated to remove excess ammonia. The material was dissolved in water (60 ml) and applied to an activated charcoal column (20x 1.7 cm) (see preparation of monotosyl-cyclohexaamylose(3) for experimental details). The column was eluted with water (1200 ml), followed by 10% ethanol (600 ml), 20% ethanol (600 ml), 25% ethanol (600 ml), 20% 1-propanol (8 x 50 ml) and 25% 1-propanol (10 x 50 ml). T.l.c. (solvent B) indicated that monosaccharides were eluted with water and 10% ethanol, and most of the side product ($R_G$ = 1.3), which was possibly 6'-O-acetyl-maltose, was eluted with 20% and 25% ethanol. 6'-O-Tosyl-maltose ($R_G$ = 1.4) and decreasing amounts of the side product were eluted with 20% 1-propanol. Pure 6'-O-tosyl-maltose, except for a trace of material seen near the origin on t.l.c., was eluted with 25% 1-propanol.

The fractions containing the required product (13) were combined and evaporated to yield a colourless glass (75 mg). Chromatographically pure 6'O-tosyl-maltose (60 mg) was obtained by extracting the glass three times with 100% ethanol (0.2 ml per mg of material). The mass spectrum (see Appendix 2) of the pertrimethylsilyl derivative (sample temperature 190°) was consistent with the assignment of the structure 6'-O-tosyl-maltose to (13). Freeze dried 6'-O-tosyl-maltose had $[\alpha]_D^{22}$ + 87° (c 1.1, water) and after being dried for 4 h, analyzed as a monohydrate.

Anal. Calc. for $C_{19}H_{28}O_{13}S.H_2O$ : C, 44.36; H, 5.88. Found: C, 44.27; H, 5.69.

6'-Azido-6'-deoxy-maltose (14). - Monoazido-cyclohexaamylose (4) (0.35 g) containing <5% cyclohexaamylose, was treated with A. oryzae amylase for 7 days and the product worked up according to the procedure for compound (13). On t.l.c. (solvent B) the solution was found to contain two major components: glucose and a substance ($R_G$ = 1.2) which was slower than 6-azido-6-deoxy-D-glucose ($R_G$ = 1.4). Glucose oxidase Tes-Tape confirmed the presence of D-glucose. The charcoal column (20 x 1.7 cm) was washed successively with water (1500 ml), 5% ethanol (1250 ml), 10% ethanol (24 x 100 ml) and 15% ethanol (7 x 100 ml). According to t.l.c. the aqueous eluate contained glucose and the 5% ethanol

eluate contained small quantities of monosaccharides and maltose. In the initial fractions of 10% ethanol, 6'-azido-6'-deoxy-maltose was detected as well as 5 - 10% of another compound, possibly monoazido-maltotriose, that ran immediately behind it on t.l.c. In subsequent 10% ethanol fractions the amounts of "monoazido-maltotriose" decreased until the 6'-azido-6'-deoxy-maltose was pure except for a small amount of unidentified material seen near the origin on t.l.c. The 15% ethanol fractions were found to contain diminishing amounts of monoazido-maltose. The appropriate fractions were combined and on evaporation the crude product (112 mg) was obtained as a colourless glass which was extracted with ethanol to give chromatographically pure 6'-azido-6'-deoxy-maltose (101 mg) with $[\alpha]_D^{22}$ + 103° (c 1.1, water). The mass spectrum of pertrimethylsilyl derivative is consistent with the structure 6'-azido-6'-deoxy-maltose (see Appendix 2). The compound was hygroscopic and a freeze dried sample could not be readily obtained.

Anal. Calc. for $C_{12}H_{21}N_3O_{10} \cdot H_2O$: C, 37.41; H, 6.02; N, 10.91. Found: C,37.59; H,5.69; N, 10.59.

6'-Amino-6'-deoxy-maltose (15). - 6'-Azido-6'-deoxy- maltose (14) (15 mg) was dissolved in water (4 ml) and reduced with hydrogen at 14 lb. $in^{-2}$ and palladium black (5 mg) for 3 h. D-Glucose was not

reduced to D-glucitol ($R_G$ = 1.2, t.l.c. solvent A) under same conditions. The catalyst was removed by filtration and the solution containing monoamino-maltose was evaporated to dryness, giving a colourless glass (13 mg). The compound (15) was chromatographically pure (solvent E, $R_G$ = 0.4) and had $[\alpha]_D^{22}$ + 111° (c 0.64, water), lit[37]. $[\alpha]_D$ + 88° (c 0.3, water). The mass spectrum of the pertrimethylsilyl derivative (see Appendix 2) is consistent with the structure 6'-amino-6'-deoxy-maltose. To prepare the pertrimethylsilyl derivative, compound (15) (3 mg) was dissolved in pyridine (2 ml) at 80°. The solution was cooled to 40° and twice the usual volumes of silylating reagents were added. From this stage the standard procedure was followed. Thermal decomposition occured during the mass spectroscopy. Freeze drying resulted in the formation of a glass.

Anal. Calc. for $C_{12}H_{23}NO_{10} \cdot H_2O$: C, 40.11; H, 7.01; N, 3.90. Found: C, 39.92; H, 6.67: N, 3.57.

6'-Chloro-6'deoxy-maltose (16). - Freeze dried monochloro-cyclohexaamylose (6) (150 mg), which contained approximately 5% cyclohexaamylose, was incubated with A. oryzae amylase for 7 days (see preparation of compound (13) for experimental details). T.l.c. (solvent A) showed the two major products to be glucose and a substance that was slower ($R_G$ = 1.2) than 6-chloro-

6-deoxy-D-glucose ($R_G$ = 1.5). The reaction solution was applied to a charcoal column (20 x 0.9 cm) which was eluded with water (450 ml), 10% ethanol (8 x 50 ml), 15% ethanol (7 x 50 ml) and 20% ethanol (3 x 50 ml). Most of the monochloro-maltose was eluated in the 10% and 15% ethanol fractions. After ethanol extraction a colourless glass (32 mgm) was obtained by evaporation. Freeze dried 6'-chloro-6'-deoxy-maltose had $[\alpha]_D^{22}$ + 116° (c 1.1, water). The mass spectral analysis of the per-trimethylsilyl derivative showed that the material was 6'-chloro-6'-deoxy-maltose (see Appendix 2).

Monochloro-maltose (1.5 mg) in an aqueous solution (1 ml) was reduced with sodium borohydride (1.5 mg) for 1 h. The reduction was stopped by adding 1 N acetic acid until the solution was at pH 4.5. After passage through Dowex 50 ($H^+$) (1 ml) the solution was evaporated three times with methanol (2 ml) which contained 2 drops of 1 N HCl and then twice with methanol alone. T.l.c. (solvent A) showed there was only one product. Under the same conditions 6-chloro-6-deoxy-D-glucose was reduced to one compound, presumably 6-chloro-6-deoxy-D-glucitol. The material from the reduction of monochloro-maltose was heated on a steam bath in 1 N HCl (0.3 ml) for 2 h. The solution was neutralised on a Dowex 3 ($OH^-$) column (4 ml). Glucose oxidase Tes-Tape gave a negative test on the neutral solution (0.2 ml). T.l.c. (solvent A,

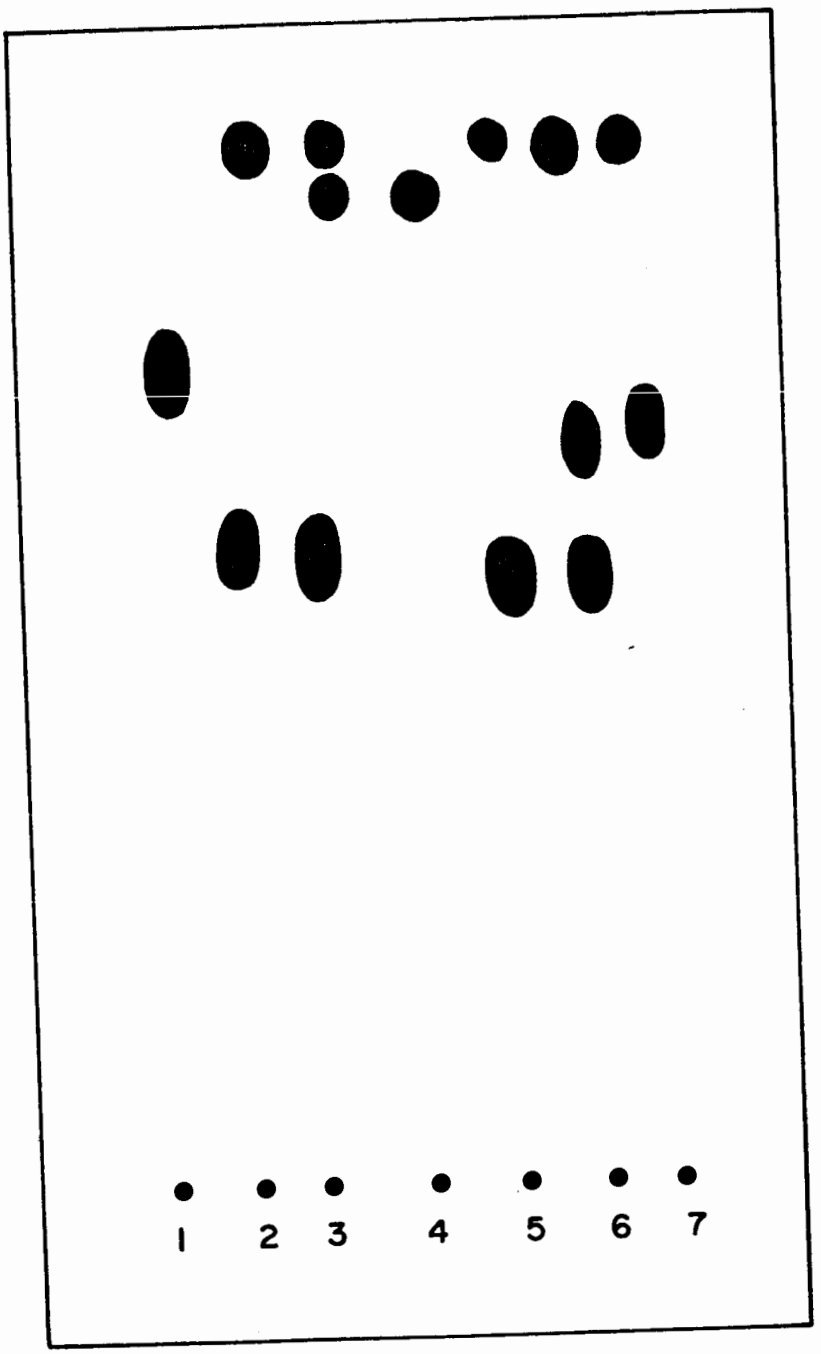



Fig. 5. - see following page for legend.

Fig. 5. Thin-layer chromatogram (solvent A) showing the chemical elucidation of the structure of 6'-chloro-6'-deoxy-maltose: 1,6'-chloro-6'-deoxy-maltitol; 2, D-glucitol and 6-chloro-6-deoxy-D-glucose; 3, Acid hydrolysate of 6'-chloro-6'-deoxy-maltitol plus D-glucitol plus 6-chloro-6-deoxy-D-glucitol; 4, 6-chloro-6-deoxy-D-glucitol; 5, Acid hydrolysate of 6'-chloro-6'-deoxy-maltitol; 6, Acid hydrolysate of 6'-chloro-6'-deoxy-maltitol plus D-glucose plus 6-chloro-6-deoxy-D-glucose; 7, D-glucose and 6-chloro-6-deoxy-D-glucose.

see Fig. 5) showed the hydrolysis was complete in 1 h and only D-glucitol and 6-chloro-6-deoxy-D-glucose were formed. This confirmed that the structure of the product of amylase action was 6'-chloro-6'-deoxy-maltose.

After 21 h of drying, freeze dried 6'-chloro-6'-deoxy-maltose analyzed as a monohydrate.

Anal. Calc. for $C_{12}H_{21}ClO_{10} \cdot H_2O$: C, 38.05; H, 6.12. Found: C, 38.22; H, 5.81.

6'-Bromo-6'-deoxy-maltose (17). - Freeze dried monobromo-cyclohexaamylose (7) (350 mg), which contained approximately 5% cyclohexaamylose, was treated with A. oryzae amylase for 7 days in the usual manner (see compound 13). Glucose and another compound with $R_G$ = 1.3 were the major products on t.l.c. (solvent C), ($R_G$ = 1.6 for 6-bromo-6-deoxy-D-glucose). The charcoal column (20 x 1.7 cm) used to separate the products, was washed with water (1500 ml), 5% ethanol (700 ml), 10% ethanol (10 x 100 ml) and 20 % ethanol (8 x 50 ml). T.l.c. indicated the 5th to 10th 10% ethanol and the 1st 20% ethanol fractions contained monobromo-maltose and decreasing amounts of maltose. The remaining 20% ethanol fractions contained no maltose and the latter were combined and evaporated to give a colourless glass (61 mg). After extraction of the glass with ethanol and evaporation, chromatographically pure 6'-bromo-6'-deoxy-maltose (50 mg) was obtained. The mass spectrum of the per-

trimethylsilyl derivative (sample temperature 150°) confirmed the structure (see Appendix 2). A freeze dried sample of (16) gave $[\alpha]_D^{24}$ + 105° (c 1.0, water). The analysis was done on freeze dried material.

Anal. Calc. for $C_{12}H_{21}BrO_{10}$: C, 35.57; H, 5.22. Found C, 35.27; H, 5.15.

6'-Deoxy-6'-iodo-maltose (18). - Freeze dried monoiodo-cyclohexaamylose (8) (310 mg) containing approximately 5% cyclohexaamylose was incubated with A. oryzae amylase for 40 h (see compound (13) for general procedure). T.l.c. (solvent C) showed two prominent spots, one of which corresponded to D-glucose and the other ($R_G$ = 1.3) ran slower than 6-deoxy-6-iodo-D-glucose ($R_G$ = 1.4). The purification of monoiodo-maltose was done on a charcoal column (20 x 1.7 cm), which was eluted with water (300 ml), 10% ethanol (600 ml), 1% 1-butanol (7 x 100 ml) and 4% 1-butanol (7 x 50 ml). Except for a small amount of material near the origin, t.l.c. indicated that the 4% 1-butanol fractions contained pure monoiodo-maltose. Before evaporation, concentrated ammonia (1 ml per 50 ml of eluate) was added to the 4% 1-butanol fractions. Evaporation followed by extraction with ethanol yielded the pure product (18) (61 mg) as a colourless glass. Mass spectral analysis of the pertrimethylsilyl derivative (Appendix 2) showed that 6'-deoxy-6'-iodo-maltose had

indeed been formed. Crystals (47 mg) were obtained by freeze drying a concentrated aqueous solution, m.p. 110-112°. A freeze dried sample of (18) had $[\alpha]_D^{22}$ + 89° (c 1.1, water). After drying for 4 h the analysis figures for freeze dried (18) were intermediate between those of a monohydrate and a non-hydrate. When drying was carried out for 23 h, (18) analyzed as an anhydrous compound.

Anal. Calc. for $C_{12}H_{21}IO_{10}$: C, 31.87; H, 4.68. Found: C, 31.69; H, 4.66.

Reductive-dehalogenation of 6'-deoxy-6'-iodo-maltose. - A preliminary experiment was carried out in an attempt to convert 6'-deoxy-6'-iodo-maltose to 6'-deoxy-maltose (19). A slightly modified method of Dutton and Slessor[37] was used. An aqueous solution (0.5 ml) of the iodo compound (18) (3 mg) containing 5% palladium on charcoal catalyst (3 mg) and a drop of pyridine was subjected to a stream of hydrogen introduced through a capillary. After 5 h the catalyst was removed by filtration and the solution chromatographed. T.l.c. (solvent A) showed the starting material ($R_G$ = 1.3) had reacted completely to give one product which had the same $R_G$ value (1.1) as the supposed 6'-deoxy-maltose produced by a amylase action (see later). Under such conditions D-glucose was not reduced to D-glucitol, according to t.l.c.. To determine the structure of monodeoxy-maltose the base "peeling"

method of Painter[93] was used. The reaction was followed on t.l.c., which showed that after 2 h monodeoxy-maltose had been converted completely to 6-deoxy-D-glucose and some very slow running material. This result was confirmed by p.c. (solvent I). No glucose or reaction intermediates were seen on t.l.c.. When 6-deoxy-D-glucose was treated under the same conditions no reaction was detected on t.l.c.. Hence base "peeling" indicated the structure of the monodeoxy-maltose was 6'-deoxy-maltose and consequently the monoiodo-maltose was 6'-deoxy-6'iodo-maltose.

Action of A. oryzae amylase on monoamino-cyclohexaamylose. - Monoamino-cyclohexaamylose (5) (25 mg) was treated in the usual way (see compound (13) with A. oryzae amylase for 7 days. After removal of the protein the reaction solution was adjusted to pH9 with 1.0 N potassium carbonate. The solution was filtered and applied to a charcoal column (10 x 1.0 cm) which was eluted with water (500 ml), 2.5% ethanol (10 x 50 ml) and 10% ethanol (3 x 50 ml). The following fractions were combined: - 1st and 2nd 2.5% ethanol (fraction A); 3rd to 8th 2.5% ethanol (fraction B); 9th and 10th 2.5% ethanol and 1st to 3rd 10% ethanol (fraction C). T.l.c. (solvent E) suggested that fraction A consisted of monoamino-maltose ($R_G$ = 0.4), fraction C of monoamino-maltotriose ($R_G$ = 0.3) and fraction B was a mixture of both (6-amino-6-deoxy-D-glucose had $R_G$ = 0.6). From t.l.c.

it was estimated that the two amino derivatives were produced in approximately equal amounts. The pertrimethylsilyl derivative of the crude material in fraction C was prepared by dissolving the material (4 mgm) in pyridine (3 ml) heated at 80 - 90°. The solution was cooled to 40°, hexamethyldisilazane (0.6 ml) and trimethylchlorosilane (0.3 ml) were added and the mixture was kept at 40° for 3 h. The work up was carried out in the usual way. The mass spectrum of the pertrimethylsilyl derivative (Appendix 3) indicated that 6"-amino-6"-deoxy-maltotriose (21) and some 6'-amino-6'-deoxy maltose (15) were present in fraction C. Thermal decomposition occurred during mass spectroscopy of this material.

Action of A. oryzae amylase on monodeoxy-cyclohexaamylose. - Monodeoxy-cyclohexaamylose (9), (16 mg) was treated for 12 h with A. oryzae amylase according to the standard procedure (see compound (13)). P.c. (solvent I) of samples taken at 4 and 10 h showed that 6-deoxy-D-glucose ($R_G$ = 1.6) and an unknown substance ($R_G$ = 1.4) were present, as well as glucose, mannose ($R_G$ = 1.2) and a slow running substance ($R_G$ = 0.4). The unknown material ($R_G$ = 1.4) did not correspond to xylose ($R_G$ = 1.5) or galactose ($R_G$ = 0.9). When the 4 h and 10 h samples were compared, there was an obvious increase in 6-deoxy-D-glucose and a decrease in the unknown. The enzyme hydrolysate was applied to a charcoal column (10 x 0.9 cm)

which was washed with water (200 ml), 0.5% 1-butanol (6 x 50 ml), 1% 1-butanol (5 x 50 ml) and 4% 1-butanol (2 x 50 ml) (Before evaporation concentrated ammonia was added to 4% 1-butanol fractions). P.c. indicated that 6-deoxy-D-glucose was eluted with 0.5% and 1% 1-butanol and the majority of it was in the first two 0.5% 1-butanol fractions, which also contained large ammounts of other monosaccharides. The first 4% 1-butanol fraction contained the unknown compound ($R_G$ = 1.4) and glucose in the approximate ratio of 9:1. The unknown has the same $R_f$ value ($R_G$ = 1.1, solvent A) as the compound produced by reductive-dehalogenation of 6'-deoxy-6'-iodo-maltose. Therefore the unknown is considered to be 6'-deoxy-maltose.

Action of A. oryzae amylase on monotrityl-cyclohexaamylose. - Monotrityl-cyclohexaamylose (2) (25 mg) was acted on by A. oryzae amylase under the usual conditions (see compound (13)). Samples taken at 2, 4, 7, and 22 h were examined on t.l.c. (solvent A) and p.c. (solvent H). At 2 h a very small amount of glucose was seen and this increased slowly. After 22 h (approximately 90%) of the monotrityl-cyclohexaamylose ($R_G$ = 1.2 on t.l.c.; $R_G$ = 1.3 on p.c.) remained unreacted. No substance was seen with the same $R_f$ value as 6-O-trityl-D-glucose ($R_G$= 1.6 on t.l.c.; $R_G$ = 1.5 on p.c.), but there was a very small amount of slightly slower material ($R_G$ = 1.4 on t.l.c.; $R_G$ = 1.3 on p.c.) which increased slightly

during the 22 h. This material might be 6'-O-trityl-maltose (20) or the monotrityl derivative of a higher homologue. A very small quantity of triphenyl carbinol ($R_G$ = 1.8) was seen on t.l.c.. No cyclohexaamylose was seen on t.l.c. or on p.c.

Relative rates of the initial hydrolysis of monosubstituted cyclohexaamyloses by A. oryzae amylase. - Small samples (15 - 25 mg) of the monosubstituted cyclohexaamyloses were treated with A.oryzae amylase according to the standard procedure (see compound (13)). Aliquots (0.25 ml) were taken from the solution at 3, 3.5, 4, 4.5, 6 and 8 h. The enzyme reaction was stopped by the immediate addition of 3 N ammonia (0.1 ml) to each aliquot. The same amount of sample was applied to each t.l.c. plate (solvents used are given in the appropriate preparations above; solvent C was used for the monodeoxy-cyclohexaamylose experiment). The time of disappearance of the substrate was estimated from the t.l.c. plates. The relative rates of hydrolysis are given in Table 2. Under these conditions the parent compound, cyclohexa-amylose, had disappeared in 3.5 h. Monobromo-cyclohexa-amylose was not completely separated on t.l.c. from oligosaccharides produced in the course of the enzyme action, so no reliable value cound be obtained for it. As indicated earlier, the rate of amylase action on monotrityl-cyclohexaamylose appears to be very much

slower than that of the other monosubstituted cyclohexaamyloses.

Table 2. Relative rates of the initial hydrolysis of monosubstituted cyclohexaamyloses by *A. oryzae* amylase.

| Cyclohexaamylose derivative | Relative rate |
|---|---|
| cyclohexaamylose | 1.0 |
| monodeoxy | 1.0 |
| monotosyl | 0.8 |
| monochloro | 0.8 |
| monoiodo | 0.8 |
| monoazido | 0.4 |
| monoamino | $<0.4$, but $>0.2$ |

Monosubstituted D-Glucoses

6-Chloro-6-deoxy-D-glucose (22). - The chlorination method of Lee and Nolan[82,83] was used to prepare 6-chloro-6-deoxy-1,2,3,5-di-O-isopropylidene-α-D-glucose (23): 1,2;5,6-Di-O-isopropylidene α-D-glucose (20 g) was dissolved in carbon tetrachloride (2 liters) in which triphenylphosphine (45 g) had been dissolved. After 31 h of refluxing, the solution was cooled and diluted with petroleum ether (1 liter). The resulting mixture was kept at -20° overnight, filtered and evaporated to give a syrup, which was dissolved in petroleum ether (1 liter) and the process repeated. The resulting syrup was vacuum distilled through a 10 cm Vigreaux column, b.p. $_{0.01}$ 95 - 96°, to give material (17.0 g) which was homogeneous on t.l.c. (toluene-ethyl ether, 5:1), but which showed an impurity by p.m.r. spectroscopy. $[\alpha]_D^{22}$ 35.9° (c 1.07, $CHCl_3$). Lit.[84] b.p.$_{0.05}$ 84 - 85°, $[\alpha]_D$ 36° (c 2.64, $CHCl_3$). Hydrolysis of this chloro sugar derivative (5g) in 50% aqueous methanol (50 ml) with Dowex 50 ($H^+$) (30 ml) at reflux for 3 hours with stirring yielded 6-chloro-6-deoxy-D-glucose (1.7 g), m.p. 137 - 139° after recrystallization from ethanol-ethyl ether. $[\alpha]_D^{22}$ 98.8→51.5° (c 1.6, water, 18 h). Lit.[84] m.p. 135 - 136° $[\alpha]_D$ 95.8→51.1° (water, 18 h).

6-O-Trityl-D-glucose (24). - 1,2,3,4-Tetra-O-acetyl-6-O-trityl-β-D-glucose (25) was prepared by the method of

Reynolds and Evans[61]. The deacetylation method of Thompson and Wolfrom[85] was applied to this compound. Sodium methoxide (0.1 N) was added dropwise to compound (25) (1.0 g) in methanol (50 ml) and the suspension was stirred vigorously until all the sugar had dissolved. T.l.c. (solvent F) showed the deacetylation was complete within one minute of the starting material dissolving. The solution was stirred with Dowex 50 ($H^+$) until it was neutral, filtered, and evaporated to give a syrup. The syrup was dissolved in a small volume of water, treated with charcoal, filtered and evaporated to a syrup. Attempted crystallization from methanol gave a white amorphous material, 6-O-trityl-D-glucose (0.26 g) which was pure on t.l.c. (solvents A and F) and p.c. (solvent H). After drying to a constant weight, the product (24) softened to form a liquid at 95° and had $[\alpha]_D^{22}$ + 48.8° ⟶ +45.4° (c 1.0, pyridine, 90 h). Impure material previously prepared[31,60] had m.p. 60 - 100° $[\alpha]_D^{22}$ + 59.6° ⟶ +38.0° (pyridine, 90 h).

Anal. Calc. for $C_{25}H_{26}O_6$: C, 71.08; H, 6.20. Found: C, 71.11; H, 6.49.

6-O-p-Toluenesulphonyl-D-glucose (26). - The removal of the trityl group from 1,2,3,4-tetra-O-acetyl-6-O-trityl-β-D-glucose (25) (30 g) was accomplished by heating in 50% acetic acid (200 ml), on a steam bath for 100 min. The reaction mixture was added to water (1800 ml), filtered, and the filtrate was evaporated to a syrup. To

remove the last traces of acetic acid two aliquots of water (200 ml) were added and the solutions were evaporated. p-Toluenesulphonyl chloride (28.6 g) was dissolved in pyridine (200 ml) and the crude 1,2,3,4-tetra-O-β-D-glucose was added. After 7 h at 0° the solution was filtered and the filtrate was evaporated to yield a syrup. The syrup was dissolved in 100% ethanol (100 ml) and the solution was evaporated. The evaporation with ethanol was repeated. Recrystallization three times from 100% ethanol gave 1,2,3,4-tetra-O-acetyl-6-O-tosyl-β-D-glucose (27) (4.7 g), m.p. 181 - 184°. Lit., m.p. 185 - 190°[86], 194°[87] and 200°[88]. However, the product (27) appeared to be pure at t.l.c. (solvent G) and the p.m.r. was consistent with the assigned structure. 1,2,3,4-Tetra-O-acetyl-6-O-tosyl-β-D-glucose was deacetylated by the procedure[85] used for the corresponding trityl derivative. The acetyl derivative (27) (0.67 g) was stirred vigorously in methanol (67 ml) and 0.1 N sodium methoxide (7 ml) added. The solution was neutralized with solid carbon dioxide and evaporated to a syrup. The purification was continued according to the procedure used for the trityl derivative (24). The syrup obtained was extracted with dry ethyl acetate and the solution was concentrated by evaporation. Crystallization of the 6-O-tosyl-D-glucose, by adding petroleum ether to the concentrated solution, was not successful. T.l.c. (solvent F) indicated that the

6-O-tosyl-D-glucose was approximately 95% pure.

6-O-Tosyl-D-glucose was also prepared from 1,2-O-isopropylidene-6-O-tosyl-α-D-glucofuranose (28) by the method of Compton[89]. The 6-O-tosyl-D-glucose contained approximately 5% side product according to t.l.c. (solvent F). The major products from the two preparations were identical on t.l.c. (solvents B and F).

6-Azido-6-deoxy-D-glucose (29). - 1,2-O-Isopropylidene-6-O-tosyl-α-D-glucofuranose (28) (250 mg) was dissolved in acetone (5 ml) and sodium azide (185 mg) was dissolved in water (2.5 ml). The two solutions were mixed and heated on a steam bath for 5 h. T.l.c. (solvent F) indicated that two products were formed in the approximate ratio of 5:1. The solvent was evaporated and the resulting syrup extracted with acetone (100 ml). The acetone solution was evaporated to a small volume and chloroform (100 ml) was added. The chloroform solution was extracted three times with water (10 ml), dried with magnesium sulphate, filtered and the chloroform was removed by evaporation. Crystallization from petroleum ether gave 6-azido-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (30) (60 mg), m.p. 103° (Lit.[90], m.p. 104°) which was chromatographically pure. The crystalline material exhibited infrared absorptions at 2120 $cm^{-1}$ (strong) and 2190 $cm^{-1}$ (weak) which are indicative of an azido group in a sugar[91]. The isopropylidene group

was removed from 6-azido-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose by application of the method of Compton[89]: the compound (30) (73 mg) was dissolved in 70% acetic acid (2 ml) and left at room temperature for 9 days. The solution was evaporated to dryness, the material dissolved in water and evaporated twice more. T.l.c. (solvent F) showed that in addition to the expected product there was a side product (< 10%) present.

Another method used to synthesise 6-azido-6-deoxy-D-glucose was by the displacement of the chloride in 6-chloro-6-deoxy-1,2;3,5-di-O-isopropylidene-α-D-glucose (23) by an azide group and the subsequent hydrolysis of the isopropylidene groups. The chloro sugar (23) (0.5 g) and sodium azide (0.4 g) were dissolved in N,N-dimethylformamide and heated at 90 - 95° for 32 h. The reaction mixture was filtered and the filtrate evaporated by codistillation with 1-butanol to yield a brown syrup. The syrup was dissolved in chloroform (10 ml) which was extracted three times with water (10 ml). The chloroform solution was dried with magnesium sulphate and filtered. The filtrate was evaporated to give a pale yellow syrup (0.35 g) which was approximately 95% pure on t.l.c. (solvent F) and which absorbed at 2090 $cm^{-1}$ (strong) in the i.r.. The crude 6-azido-6-deoxy-1,2;3,5-di-O-isopropylidene α-D-glucose (31) was treated with Dowex 50 ($H^+$) (see compound (23)) to give 6-azido-6-deoxy-D-glucose in the form of a pale yellow syrup. According

to t.l.c. the syrup contained about 90% of one compound and three side products. Attempts at crystallization from ethanol-ethyl acetate solution were unsuccessful. The two different preparations of 6-azido-6-deoxy-D-glucose had the same $R_f$ values on t.l.c. (solvents B. and F).

6-Bromo-6-deoxy-D-glucose (32). - 6-Chloro-6-deoxy-1,2;3,5-di-O-isopropylidene-α-D-glucose (23) (1.5 g) and lithium bromide (1.5 g) were heated at 120° in N,N-dimethylformamide for 30 min. The solution was cooled to 5° to precipitate salts which were subsequently removed by filtration. On evaporation of dimethylformamide by codistillation with 1-butanol a brown syrup was obtained. The syrup was extracted with water (80 ml), and the aqueous solution was extracted twice with chloroform (40 ml). The aqueous solution was found to contain approximately 90% of one compound as well as two side products according to t.l.c. (solvent F). The isopropylidene groups were removed from the crude 6-bromo-6-deoxy-1,2;3,4-di-O-isopropylidene-α-D-glucose by the procedure given for the chloro compound (23). A yellow glass (0.27 g) was obtained. Further purification was carried out by applying an aqueous solution of the crude 6-bromo-6-deoxy-D-glucose to a charcoal column (10.0 x 1.4 cm) (see preparation of (3), which was eluted successively with water (150 ml) and

2% ethanol (200 ml). T.l.c. (solvent F) showed the aqueous eluate contained glucose and the ethanolic eluate contained mainly 6-bromo-6-deoxy-D-glucose (ca. 95%) and what is presumed to be 3-bromo-3-deoxy-D-glucose. The major product (32) has approximately the same $R_f$ value as the fully characterised 6-chloro-6-deoxy-D-glucose on t.l.c. (solvents A and F). On evaporation to dryness the ethanol eluates gave a colourless glass (0.12 g).

6-Deoxy-6-iodo-D-glucose (33). - 6-Chloro-6-deoxy-1,2;3,5-di-O-isopropylidene-α-D-glucose (23) (0.5 g) and sodium iodide (2.4 g) were dissolved in dry N,N-dimethylformamide (5 ml) and the solution was heated for 5 h at 90° followed by 45 min at 150°. The reaction mixture was filtered and the solvent removed by evaporation. The resulting brown syrup was dissolved in chloroform (10 ml) and the chloroform solution was extracted three times with water (10 ml). The combined aqueous extracts were evaporated to produce a mixture of syrup and colourless crystals. The syrup was taken up in acetone (10 ml) and separated from the insoluble crystals by filtration. Evaporation of acetone gave a brown syrup containing mostly one compound (80%) and at least two side products according to t.l.c. (solvent F). The isopropylidene groups were removed from the crude 6-deoxy-6-iodo-1,2;3,5-di-O-isopropylidene-α-D-glucose (34)

in the usual manner (see compound (23)). Purification was carried out on a charcoal column (7.5 x 1.4 cm) as with compound (32). The ethanol eluate on evaporation gave a colourless glass (50 mg) which was indicated to be 90% pure 6-deoxy-6-iodo-D-glucose by t.l.c.. No further purification was done. However, hydrolysis of methyl 6-deoxy-6-iodo-α-D-glucopyranoside[92] (30 mg), by heating on a steam bath for 30 h with an aqueous suspension of Dowex 50 ($H^+$) (2 ml) gave a compound which had the same $R_f$ value on t.l.c. (solvents C and F) as the product obtained from 6-chloro-6-deoxy-1,2;3,5-di-O-isopropylidene-α-D-glucose.

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APPENDIX 1. The conformation of crystalline cyclohexaamylose. The diagrams were drawn using the ORTEP program. The program "ORTEP, A Fortran Thermal-Ellipsoid Plot Program For Crystal Structure Illustrations" was written by C.K. Johnson. The atomic coordinates were obtained from the work of Hybl, Rundle and Williams[94]. In the diagrams the oxygen atoms are of greater diameter than the carbon atoms and the hydrogen atoms are not shown.

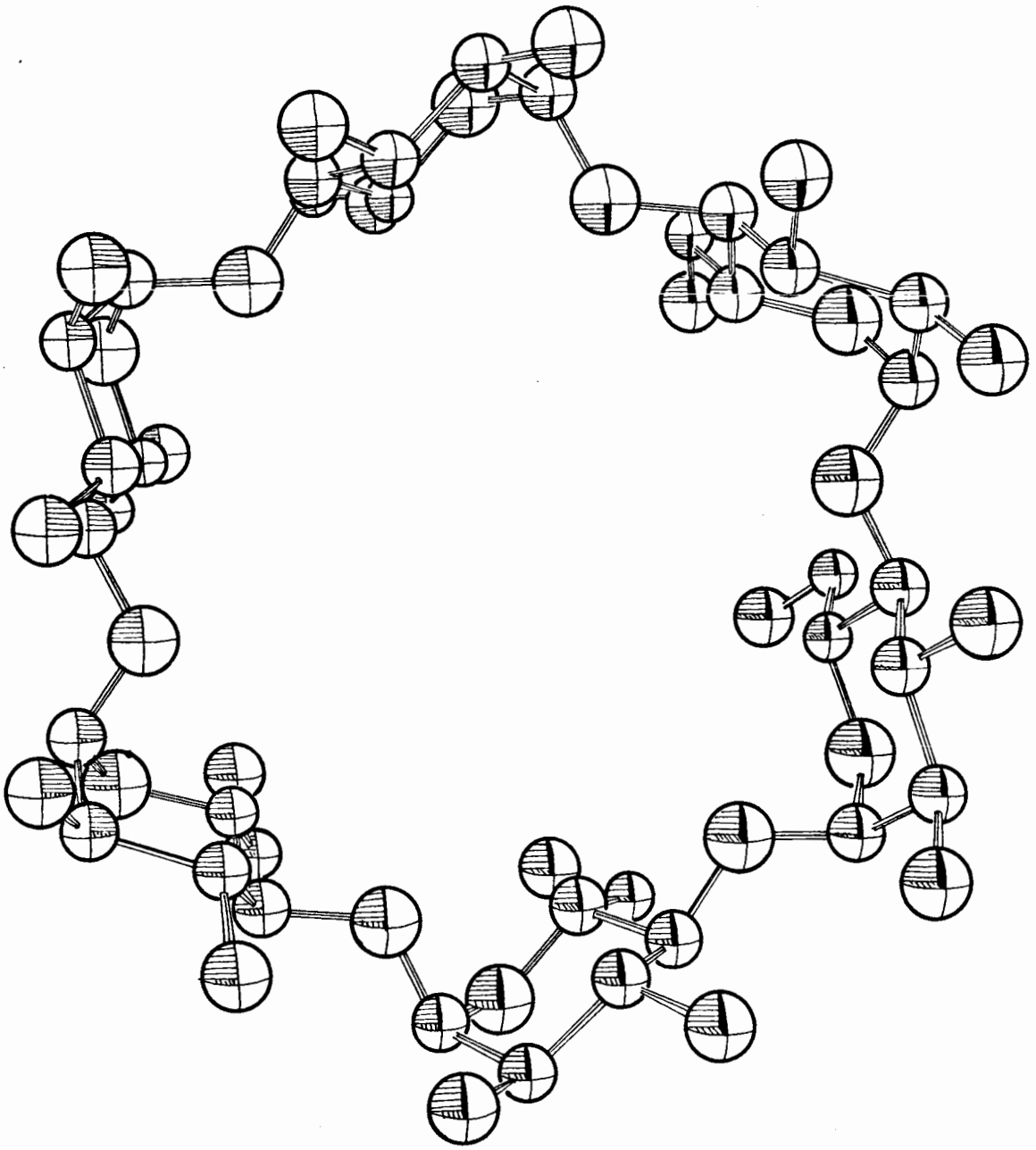


Fig. 6. The conformation of crystalline cyclohexaamylose; a view from the "bottom" of the molecule.

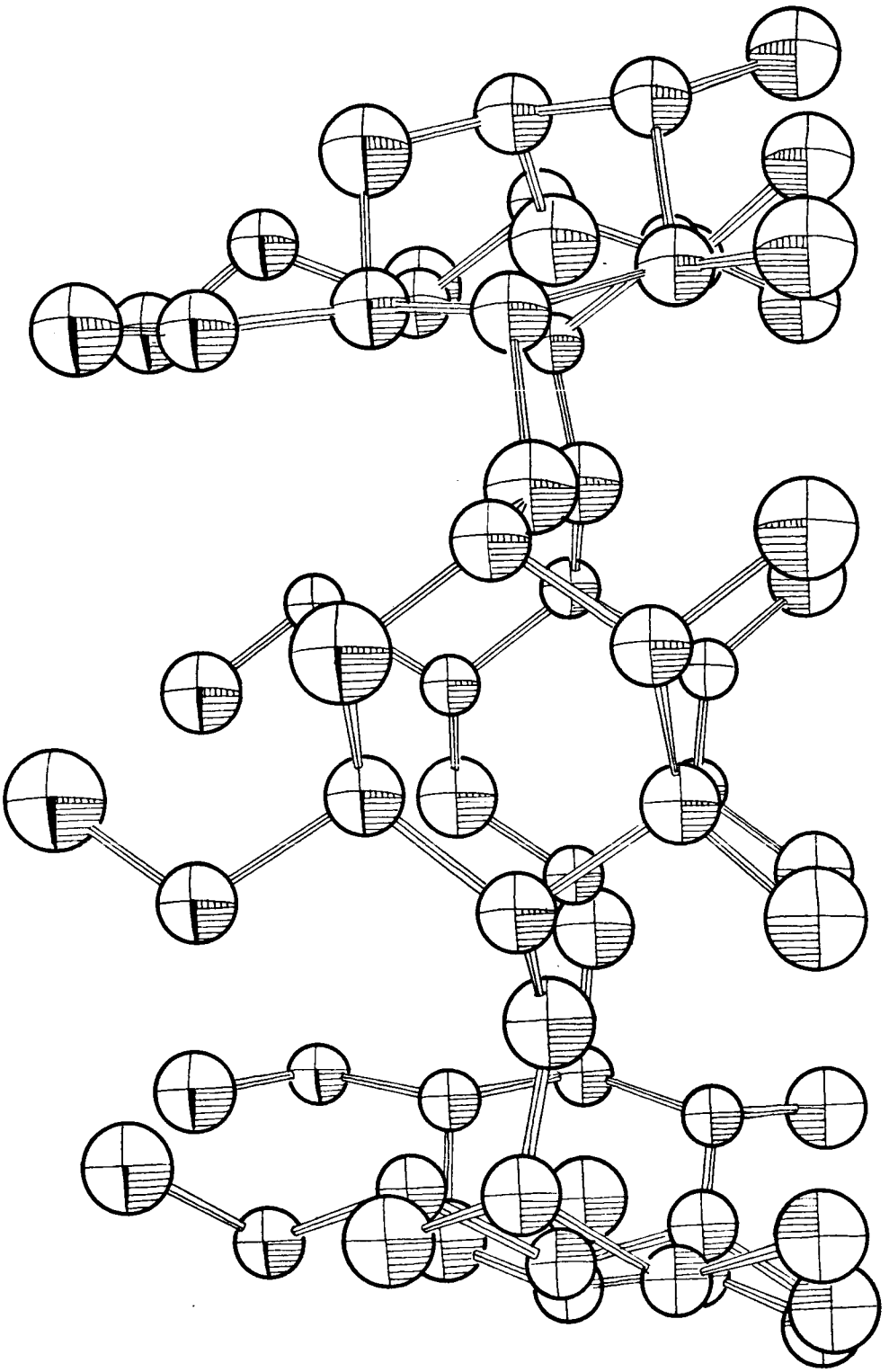


Fig. 7. The conformation of crystalline cyclohexaamylose; a "side" view.

Appendix 2: Analysis of mass spectra of pertrimethylsilylated 6'-substituted maltoses.

The first two columns of each table, where X = substituent, show the mass and relative abundance of fragments containing the substituent. The last two columns, where X = OT, show the mass and relative abundance of fragments I to IV which would be present, only if the 6' position were substituted by a trimethylsilyl ether instead of the substituent.

Symbols used in Appendices 2 and 3:

% ; relative intensity (% of base peak).

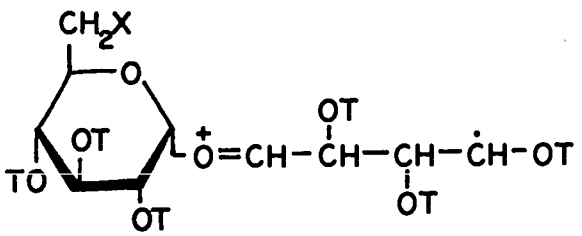
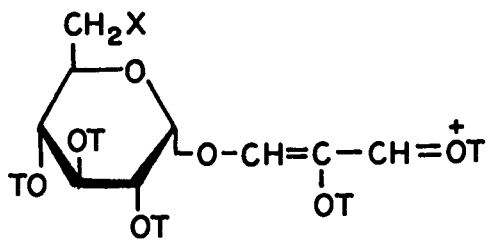
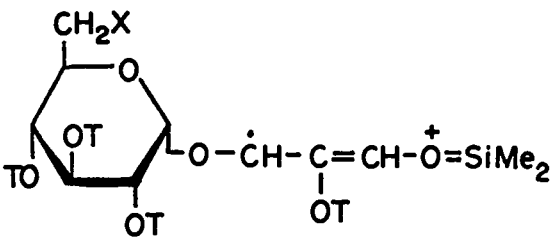
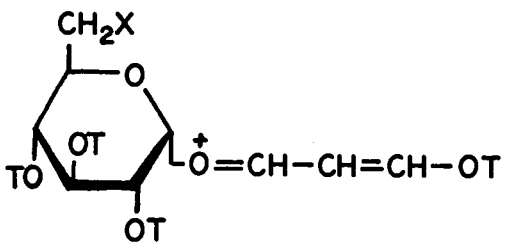
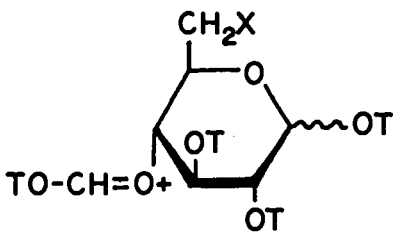
$M^{\bullet+}$ ; radical cation of intact oligosaccharide molecule

T ; trimethylsilyl group (TMS)

? ; peak is part of the isotopic cluster of a more intense peak.

B ; base (most intense) peak.

APPENDIX 2: Analysis of mass spectra of pertrimethylsilylated 6'-substituted maltoses.

| Assignment | | Tosyl (190°) X = OTs m/e | % | X = OT m/e | % |
|---|---|---|---|---|---|
| $M^{\cdot+}$ | | 1000 | 0.009 | | |
| $M^{\cdot+} - \cdot CH_3$ | | 985 | 0.01 | | |
|  | I | 868 | 0.0 | 786 | 0.0 |
|  | II | 765 | 0.002 | 683 | 0.03 |
|  | III | 750 | 0.50 | 668 | 0.0 |
|  | IV | 677 | 0.39 | 595 | 0.03 |
|  | V | 651 | 0.02 | 569 | 0.03 |
| B | | | | 73 | 100 |

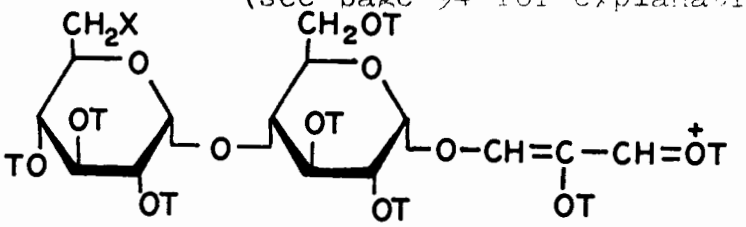
| Assignment | Azide (170°) X = $N_3$ m/e | Azide (170°) X = $N_3$ % | Azide (170°) X = OT m/e | Azide (170°) X = OT % | Chloride (170°) X = Cl m/e | Chloride (170°) X = Cl % | Chloride (170°) X = OT m/e | Chloride (170°) X = OT % |
|---|---|---|---|---|---|---|---|---|
| M· | 871 | 0.003 | | | 864 | 0.01 | | |
| $M\cdot^+ - \cdot CH_3$ | 856 | 0.007 | | | 849 | 0.02 | | |
| I | 739 | ? | 786 | 0.0 | 732 | 0.005 | 786 | 0.0 |
| II | 636 | 0.007 | 683 | 0.005 | 629 | 0.009 | 683 | 0.03 |
| III | 621 | 0.007 | 668 | 0.0 | 614 | 0.07 | 668 | 0.007 |
| IV | 548 | 0.08 | 595 | ? | 541 | 0.30 | 595 | 0.0 |
| V | 522 | 0.0 | 569 | 0.02 | 515 | 0.0 | 569 | 0.15 |
| B | | | 204 | 100 | | | 73 | 100 |

| Assignment | Bromide (150°) X = Br m/e | Bromide (150°) X = Br % | Bromide (150°) X = OT m/e | Bromide (150°) X = OT % | Iodide (170°) X = I m/e | Iodide (170°) X = I % | Iodide (170°) X = OT m/e | Iodide (170°) X = OT % |
|---|---|---|---|---|---|---|---|---|
| $M^{\cdot+}$ | 908 | 0.02 | | | 956 | 0.004 | | |
| $M^{\cdot+} - \cdot CH_3$ | 893 | 0.03 | | | 941 | 0.006 | | |
| I | 776 | 0.006 | 786 | 0.0 | 824 | 0.005 | 786 | 0.0 |
| II | 673 | 0.0 | 683 | 0.06 | 721 | 0.009 | 683 | 0.12 |
| III | 658 | 0.01 | 668 | 0.02 | 706 | 0.07 | 668 | 0.0 |
| IV | 585 | 0.10 | 595 | 0.01 | 633 | 0.37 | 595 | 0.0 |
| V | 559 | 0.0 | 569 | 0.07 | 607 | 0.0 | 569 | 0.21 |
| B | | | 204 | 100 | | | 204 | 100 |

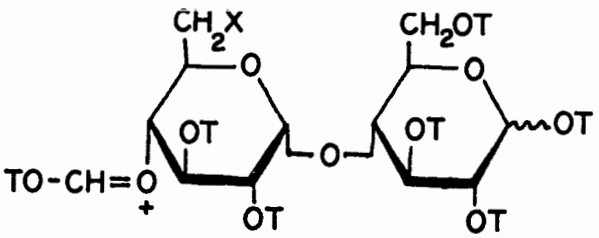
| Assignment | Amine (170°) X = $NH_2$ m/e | % | X = NHT m/e | % | X = OT m/e | % |
|---|---|---|---|---|---|---|
| $M^{\cdot+}$ | 845 | 0.0 | 917 | 0.0 | | |
| $M^{\cdot+} - \cdot CH_3$ | 830 | 0.007 | 902 | 0.003 | | |
| I | 713 | ? | 785 | 0.02 | 786 | ? |
| II | 610 | ? | 682 | 0.008 | 683 | 0.02 |
| III | 595 | 0.17 | 667 | ? | 668 | 0.03 |
| IV | 522 | ? | 594 | ? | 595 | 0.17 |
| V | 496 | 0.0 | 568 | 0.0 | 569 | 0.01 |
| B | | | | | 147 | 100 |

APPENDIX 3. Analysis of mass spectrum of pertrimethylsilylated 6"-amino-6"-deoxy-maltose (170°),

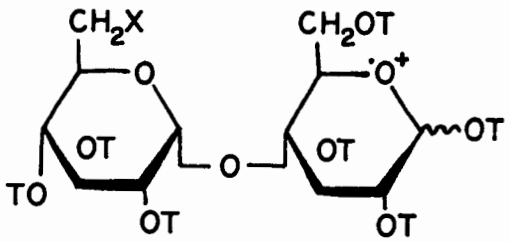
(see page 94 for explanation of symbols).




VI




VII

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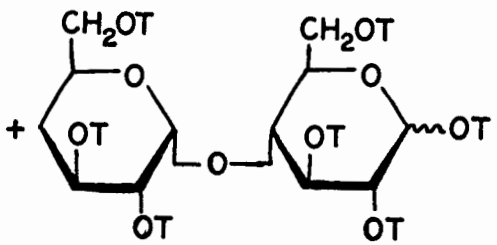
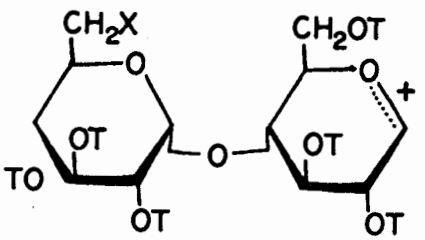
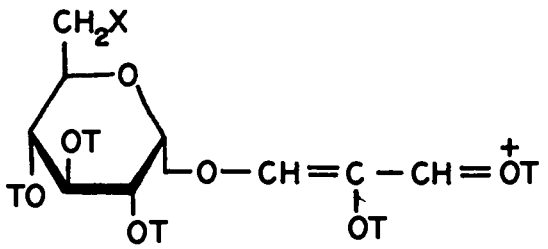


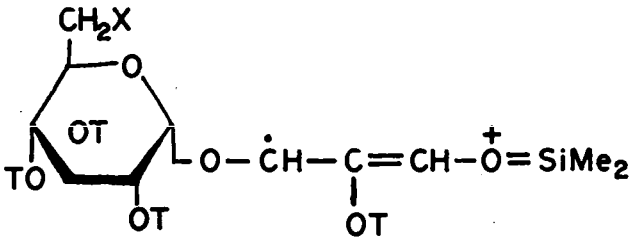

IX



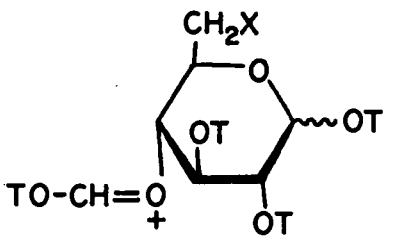

X



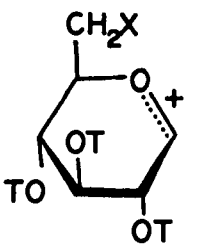
II



III



V



XI

| Assignment | X = $NH_2$ | | X = NHT | | X = OT | |
|---|---|---|---|---|---|---|
| | m/e | % | m/e | % | m/e | % |
| $M^{\cdot+}$ | 1223 | 0.21 | 1295 | 0.0 | | |
| $M^{\cdot+} - {\cdot}CH_3 - Me_3SiOH$ | 1118 | 0.10 | 1190 | 0.0 | | |
| VI | 988 | 0.04 | 1060 | 0.0 | | |
| VII | 874 | 0.0 | 946 | 0.0 | 947 | 0.04 |
| VIII | 845 | 0.36 | 917 | 0.12 | | |
| IX | | | | | 829 | 0.16 |
| X | 756 | 0.42 | 828 | 0.17 | | |
| II | 610 | 0.21 | 682 | 0.16 | | |
| III | 595 | 0.25 | 667 | ? | | |
| V | 496 | ? | 568 | 0.0 | 569 | 0.09 |
| XI | 378 | 16 | 450 | 3.2 | 451 | ? |
| B | | | | | 73 | 100 |