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CRYSTALLOGRAPHIC STUDIES OF SOME
CYCLOAMYLOSE COMPLEXES
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Schardinger Dextrins and the enzyme from Bacillus Macerans which produce them will continue to serve, delight, teach and intrigue the carbohydrate chemist for many years to come.

Dexter French (1957)

## ABSTRACT

Cycloheptaamylose(C7A) and $p$-nitroacetanilide(PNA) were cocrystallised to form the $1: 1$ complex(C7APNA). Crystals of this complex were examined using X-ray diffraction. The cell dimensions are $a=15.20$
 with $Z=2$ and the space group is Pl. 5500 unique reflections were measured from Weissenberg photographs. The structure was solved by the trial and error rotation and translation of a model of the structure in the position and orientation that had been indicated by the Patterson map. Inspection of the difference Fourier map after initial refinement located both PNA molecules. The structure was further refined using blocked matrix refinement to an $R$-factor of 0.132 for all the data. THe PNA molecule is included in the cavity. The C7A molcules are hydrogen bonded together to form a dimer which has approximately twofold symmetry.

Attempts were made to solve both the C7A complex with water using trial and error methods and the C7A iodine complex using Patterson maps to locate the iodine and then conventional heavy atom techniques. Preliminary crystallographic investigations of the C7A complexes with $\underline{p}$-bromoacetanilide, which was isomorphous with C7APNA, and $\underline{m}$-nitroacetanilide are reported.

Structural data for C6A complexes were reviewed, and a new classification scheme for these complexes was proposed. The binding between the guest and the host molecule was examined, and a modified torsion angle index(MTAI) was defined to simplify the analysis of the distortion of the C6A molecule.

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Cycloamyloses, sometimes called Schardinger Dextrins or cyclodextrins are a homologous series of cyclic polymers of D-glucose, Figure 1.1 , in the Cl chair conformation with the bridging oxygens being $\alpha-1-4^{\prime}$ linked $\left[\mathrm{C}_{6} \mathrm{H}_{10} \mathrm{O}_{5}\right]_{n},(\mathrm{n}=6-9)$. These compounds are formed by the action of the Bacillus Macerans amylase on starch. This knowledge of the chemistry of the cycloamyloses took almost sixty years to establish conclusively. It was not until 1965 , almost seventy years after their discovery by Villiers $\{1\}$, that a structure determination by Hybl, Rundle, and Williams $\{2\}$ confirmed the above information.

Figure 1.1 THE GLUCOSE RESIDUE,


Villiers\{1\}, discovered and co-crystallised cycloheptaamylose (C7A) with $n$-propanol and water from a crude bacterial digest of starch. Schardinger in 1903 \{3\} discovered "crystallised dextrins A and B", probably cyclohexaamylose( C 6 A ), and C7A respectively. He found that in addition to the substances discovered by Villiers the crystalline dextrins could co-crystallise in an aqueous solution with iodine, ether and chloroform. In 1904 he isolated the bacterium, Bacillus Macerans, which formed the cycloamyloses\{4\}, and by 1910 , he had suggested an empirical formula for C6A\{5\}.

Pringsheim and his co-workers chemically analysed the cycloamyloses in order to elucidate their structure, but as their work was based on erroneous precepts the results were incorrect. Freudenberg partially corrected this and showed that cycloamyloses were cyclic polymers of glucose containing maltose type linkages\{6-10\}. It was not until work done by French that the correct number of glucose residues per cycloamylose for C6A and C7A \{11\} and cyclooctaamylose,(C8A), \{12\} was discovered using X-ray methods.

Freudenberg devised a scheme for separating cycloamyloses. He knew that different cycloamyloses have markedly different solubilities in aqueous solutions in the presence of small amounts of certain organic substances. By making up concentrated solutions of the crude starch digest he could selectively precipitate out specific cycloamyloses by co-crystallising them with specific organic species. French improved and refined this scheme, making the separation and purification much easier\{13\}.

French and his colleagues were responsible for the steady increase in the knowledge of these compounds, and demonstrated the existance of higher members of the homologous series\{14\}. He showed that above cyclononaamylose(C9A) the residues that resulted from the bacterial action probably contained branched cycloamyloses rather than cyclic polymers of glucose. C9A was especially difficult to separate as it did not form complexes\{15\}. These results were confirmed by Beadle using gas chromatographic methods\{16\}.

There was and still is no evidence for any lower members of the series than C6A, the generally accepted reason being that the structures would be too strained. Theoretical calculations done by Sundararajan and Rao\{17\} on the bonding energies of cycloamyloses
constructed from glucoses in the Cl chair conformation, showed that cyclopentaamylose could not be formed because the structure would be strained, and atoms on adjacent glucoses would collide. French\{18\}, however, pointed out that the cis(boat) form of glucose would allow the formation of a strainless set of rings which could be made up of any number of glucose residues from three to infinity. There have been no reports of the existance of cycloamyloses with the boat conformation formed by the Bacillus Macerans amylase.

Cycloamyloses have properties that differentiate them from any other carbohydrates with a comparable molecular weight $\{18\}$.
a)Due to their cyclic nature they have neither a reducing nor a non reducing end group and are not decomposed by alkali.
b) They are more resistant to acid hydrolysis, the common starch splitting alpha amylases(except microbial enzymes), and they are completely resistant to yeast fermentation and beta amylase.
c) They crystallise well from water and aqueous alcohols.
d) They form an abundance of crystalline complexes with hydrophobic organic substances, especially those with a low solubility in water $\{19\}$.
e) They form a variety of inorganic complexes with neutral salts, halogens, bases and inert gases.

From the late 1950's there has been a rapid expansion in the interest shown in the behaviour and properties of cycloamyloses. Many fascinating facets of their nature have been recognized, including their ability to catalyse reactions stereospecifically. In the presence of cycloamyloses, the rate of hydrolysis of phenyl esters can be up to 300 times faster in aqueous solution\{20\}, and up to 13000 times faster in a mixed solvent (Dimethyl sulphoxide, DMSO and water) $\mathcal{L}^{2}$. Observations of this type led to the proposal that they may behave as enzyme models\{21\}.

A Comparison Of The Solution And Crystallographic Structural Data.


#### Abstract

There have been a large number of C6A complexes solved crystallographically, and more recently the first C7A structures have been reported $\{22,23\}$. A detailed analysis of the X-ray and neutron diffraction studies will be presented in Chapter two. A large amount of data is available from a variety of solution techniques including n.m.r. o.r.d., and induced circular dichroism. A common criticism levelled against crystallographic studies is that the results need bear little or no relationship to the properties observed in solution. The aim of this section is to show that this is not the case for cycloamyloses and that the observed solution properties correspond well with the observed crystallographic properties. This is especially important when one considers that the catalytic properties of cycloamyloses are observen at low cycloamylose concentration, typically 0.001 to 0.01 M .


Figures 1.2 shows the general form of $C 6 A$ and $C 7 A$, the two lowest members of the homologous series of cycloamyloses. In order to simplify the diagrams, the hydrogen atoms have been omitted. Few of the crystallographic studies of cycloamylose complexes have managed to locate the hydrogen atom positions. There is a cavity in the cycloamylose molecule. When a cycloamylose complex is formed it is believed that the guest molecule is physically included into the cavity of the cycloamylose molecule. One end of the molecule has a ring of secondary hydroxyl groups surrounding the cavity, and at the other end there is a ring of primary hydroxyls. These hydroxyl groups account for the solubility of the cycloamyloses in water.

It has been chemically established that cycloamyloses are cyclic polymers of between six and nine glucose molecules, joined

$$
\text { Figure } 1.2 c \text { Gensrui view of the CEf molecule. }
$$



Figure $1.2 b$ General view of the CTA molecule.

together through an $\alpha-\left(1-4^{\prime}\right)$ linkage $\{6-10\}$. There are two conformational aspects of the cycloamylose molecule that are not defined by the above statement and may depend on the environment. These are the conformation of the glucose residue, (i.e. is it in the boat or chair form?), and the mode and interactions of any intramolecular hydrogen bonds.

Conformation of The Glucose molecule in cycloamylose.

Reeves studied the conformation of the glucose rings and concluded that they prefer to exist in the chair rather than the boat form\{24\}. The conformation of the glucose ring was investigated using ${ }^{1} \mathrm{H}$ n.m.r. and was found to have the $C 1$ chair arrangement $\{25,26\}$. This was confirmed for C8A by Rao and Foster\{27\}. Casu suggested that the energy barrier between the chair and boat or skew boat forms of glucose may be greater than was previously thought, and showed that the Cl chair conformation is inherently more stable than the boat forms, and that the glucose residue is not held in that conformation by the hydrogen bonding $\{28,29\}$. A comparison of the o.r.d. spectra of methyl(D)glucopyranosides with those of C6A and C7A shows that they have the Cl chair conformation \{30\}. In all of the crystallographic studies so far published the glucose residues are in the Cl chair conformation.

Intramolecular Hydrogen Bonds In The Cycloamylose Molecule.

Data about the nature and strength of the hydrogen bonding can be obtained from three different sources, namely from n.m.r., theoretical and crystallographic studies. The interaction between secondary hydroxyl groups on neighbouring residues of the cycloamylose
molecule is the most likely source of intra molecular hydrogen bonds, the bonds being formed between 02 of one residue and 03 of the adjacent one, in the manner shown in Figure 1.3.

Figure 1.3 Hydragen bond is ind.cated by the hat ched


Casu et al. investigated the hydrogen bonds between secondary hydroxyl groups using ${ }^{1} H$ n.m.r. The retention of these bonds in dimethyl sulphoxide (DMSO), which is a very polar solvent that effectively competes for intramolecular hydrogen bonds\{28\}, shows that these hỳdrogen bonds are strong. The hydrogen bonding in glucose is broken down in DMSO \{28\}, showing that cycloamylose has stronger hydrogen bonds than glucose. Another indication of the strength of the hydrogen bonding is the readiness with which the secondary hydroxyl groups undergo deuterium exchange\{31\}. The stronger the hydrogen bond, the less firmly bound the hydrogen atom, and hence these hydrogen atoms are more easily deuterated. Furthermore it has been shown that the rate of exchange is greater for C7A than C6A, which suggests that the C7A molecule is more rigid, due to the stronger hydrogen bonds\{31\}.

Sundararajan and Rao\{17\} predicted from theoretical calculations the conformation that C6A, C7A, and C8A would adopt by minimising the steric interactions and maximising the energy derived from hydrogen bonding.

Two major assumptions were made. Firstly, that all of the glucoses are in the $C 1(D)$ chair conformation, which is valid in the light of the previous discussion, and secondly that all of the glycosidic linking oxygens are coplanar. The latter assumption, in light of crystallographic information, discussed later (Chapter 2), is almost but not exactly true. Their calculations showed that intramolecular hydrogen bonds occur at the expense of a small increase in the angle strain, while creating a very deep and narrow potential well for the minimum energy conformation. The result of this strong hydrogen bonding interaction is to confer a degree of rigidity on the C6A and C7A molecules.

From their calculations it appeared that C6A was more stable than C7A. Sundararajan\{17\}, however, agrees with Casu\{31\} that C7A is the most stable cycloamylose, and suggests that this apparent contradiction is probably due to the simplifying assumptions that were made.

The primary hydroxyl groups have a free rotation about the C5-C6 bond and this allows good hydrogen bonding to occur between the solvent and the cycloamylose molecule. There are three minimum energy conformations when the C5-C6 bond is rotated(see Figure 1.4).


Figure 1.4
MINIMUM ENERGY CONFORMATIONS FOR THE ROTATION OF THE PRIMARY HYDROXYL GROUP ABOUT C5-C6

The first letter of the conformational symbol indicates the relationship of 05 to 06 and the second letter refers to the relationship of 06 to $C 4$ (gauche or trans). In solution the proportion of the $g g$ form is higher in cycloamyloses than in glucose, probably due to the potential steric interaction between either of the gt or tg forms\{32\}. In the gg form the distance between hydroxyls on contiguous residues is greater than four ingstroms. There is no evidence in solution or in the solid state of hydrogen bonds between the primary hydroxyls\{2\}.

The rigidity of the cycloamyloses, which is the result of the hydrogen bonding was investigated by Rees\{33\}. Using o.r.d. techniques he observed the optical rotations and calculated a parameter called the "linkage rotation", which was correlated with the dihedral angle about the glycosidic oxygen. He found that in going from C6A through to C9A, the "linkage rotation" approached the value that is observed for the corresponding linear polysaccharide. This deviation in the "linkage rotation", which is greatest for C6A, is related to the conformational rigidity of the molecule which decreases as more glucose residues are incorporated into the cyclic structure.

In all of the crystal structures so far determined there is the clear potential for hydrogen bonding, with few exceptions, between oxygens 02 and 03 in adjacent glucose residues. Crystals of cycloamylose complexes normally contain water of crystallisation. There is the possibility that some of the structured water molecules surrounding the cycloamylose molecule in solution are included in the crystal lattice. This, together with the observation that the crystal usually disintegrates when it loses its water of crystallisation, indicates that the crystal structure has a dependance on water in the lattice,
and hence that the crystal structure has a relevance when discussing the solution properties of cycloamyloses.

```
    Structural information gleaned from n.m.r. studies showed a
time averaged view of the cycloamylose molecule. The crystal lattice
essentially "freezes" the molecule in to one particular conformation.
Taking this into account it is clear that there is no major conflict
between the structural information derived from crystallographic sources
and structural information derived from all other sources.
```

Complex Formation In Cycloamyloses.

A multiplicity of independant techniques all indicate that the guest molecule is included in the cavity. The limiting criterion of the inclusion of a molecule is that it should not be large. Before discussing the inclusion process, the nature of the cavity should be described.

FIGURE 1.5 CROSS SECTION OF THE CYCLOHEXAAMYLOSE MOLECULE


The diagram above shows the cross section of a C6A molecule. The cavity is roughly cylindrical, and tapered with the maximum width being level with the secondary hydroxyl groups and the minimum width level with the primary hydroxyls. The glycosidic oxygens have one of their lone pair of electrons(sp ${ }^{3}$ orbital) normal to the cavity axis. The two hydrogens, those attached to $C 5$ and $C 2$, act as a shield to reduce the effect on any substrate of these orbitals. The sum total of these interactions is to make the cavity hydrophobic $\{30\}$.

The solubilities of a series of aliphatic and aromatic carboxylic acids have been investigated in the presence and absence of cycloamyloses\{34\}. It was found that their solubilities were increased by factors of 1.2 to 30 in the presence of cycloamylose. Benzoic acid's solubility is iacreased by the presence of C 6 A , whereas the solubility of $2,3,5,6$-tetramethylbenzoic acid is unaffected by the presence of C 6 A . These observations together with the fact that glucose, methyl-(D) -glucoside, and maltose do not influence the solubilities of the substrates suggest that an inclusion complex is formed in solution \{34\}. Within a series of similar reagents the complexing tendencies towards different cycloamyloses can be correlated with the size of the reagent. Taking for example substituted benzenes, C6A forms the best complex with benzene, C7A with bromobenzene, and C8A with anthracene. Using this steric selectivity an effective separation scheme can be devised to isolate the various cycloamyloses $\{18,35\}$.

The second technique confirming that the substrates are included in the cavity is the spectroscopic investigation of cycloamylose complexes in the visible and ultraviolet regions of the electromagnetic spectrum. When an azo dye, congo red, is dissolved in a cycloamylose solution a Cotton effect is observed at the absorbtion band of the azo dye. This indicates that the non ultraviolet absorbing, but asymmetric cycloamylose and the UV absorbing but non asymmetric azo dye form a molecular entity\{36\}, which is probably an included complex \{37\}. Circular dichroism also confirms this, as there is the induction of optical activity due to an assymetric interaction with the formation of a complex $\{38\}$.

VanEtten studied the spectra of $p$-t-butylphenol in a variety of solvents, and concluded that the environment of the aromatic chromophore in the presence of cycloamylose is similar to its environment in dioxane. The only region with a similar chemical composition to dioxane in the cycloamylose molecule is the cavity, and this further substantiates the evidence that there is an inclusion complex formed $\{20\}$.
E.s.r. studies have shown that there is a definite association between a spin labelled guest and a cycloamylose host due to the loss of the rotational freedom of the guest $\{39\}$.

Crystallographic evidence also shows that the substrate, or a substantial portion of the substrate, is included in the cavity. This is direct evidence that, in the solid state at least, inclusion complexes are formed.

Equally direct evidence for the inclusion of the substrate in the cavity in solution has been derived from n.m.r. studies. Demarco and Thakkar\{40,41\}, complexed an aromatic moiety with C6A, realising that should the aromatic substrate bind inside the cavity the hydrogen atoms attached to carbons 3,5 and 6 on each glucose residue would be strongly shielded by the aromatic ring. This was verified for a series of substituted benzoic acids and phenols. In cases where the cycloamylose guest dissociation constants are known, the magnitude of the substrate induced change in the chemical shift correlates well with the strength of binding. Relaxation and dynamic correlation studies have been done, and the results can only be interpreted by the the substrate being included into the cavity\{42\}.

The final piece of evidence for the formation of inclusion complexes was the kinetics of cycloamylose catalysed reactions. It was found that cyclohexanol competitively inhibited rate accelerations in a manner that was analagous to the competitive inhibition characteristic of enzyme catalysis. This can only be the result of the inhibitor and the substrate competing actively for a discrete site on the cycloamylose molecule. The only such site is the central cavity of the cycloamylose molecule $\{20\}$. The kinetic scheme also indicates complex formation. This will be discussed later in this chapter.

Rate of Inclusion.

The binding rate constants have been measured $\{37\}$ for, amongst others, $\underline{p}^{-n i t r o p h e n o l, ~ a n d ~ a ~ s e r i e s ~ o f ~ a z o ~ d y e s ~ w i t h ~ C 6 A . ~ T h e ~ r a t e s ~ a r e ~}$ in the order of $10^{8} \mathrm{M}^{-1} \sec ^{-1}$, which is close to the diffusion limit. Within a series of dyes, however, the binding rates decrease as the steric bulk of the dye increases\{37\}. In most cases, the stoichometries of complexes have been shown to be l:l from spectophotometric titrations. More complex interactions have been found; for example methyl orange apparently forms complexes with two C6A molecules. This is consistent with the above data as the methyl orange molecule has two aromatic rings at the opposite ends of the molecule, each of which is capable of binding with a C6A molecule\{37\}.
N.m.r. studies on the interaction between the phenyl rings on p-hydroxybenzoic acid and C7A show that every time the phenyl ring enters the C7A molecule it does so in a different orientation about the ring axis or/and it is spinning rapidly. There is also reversible association between free and associated C7A on the n.m.r. timescale\{40\}.

Catalytic Properties Of Cycloamyloses.

When it was realised that cycloamyloses formed complexes in solution, it was proposed that the inclusion process may affect the course of a reaction. This was first studied by Cramer and Dietsche\{43\}, who discovered that the rates of hydrolysis of several mandeleic acid derivatives were accelerated in the presence of cycloamylose. The inclusion process, in addition to causing rate accelerations, could also cause a rate deceleration when the reaction site of the substrate was shielded from the reactant by the cycloamylose molecule\{44,45\}.

In order to exhibit the attributes of a really efficient catalytic system, namely selectivity and speed, there must be the formation of an intermediate complex\{46\}, which is a feature of the cycloamylose catalysis.

The system which has been most extensively studied is the hydrolysis of substituted aryl esters in aqueous solution. This reaction follows the normal Hammett trends (the reaction rate is dependant on the nature and position of the substituent on the phenyl ring), both in the presence and absence of methyl-D-glucoside. However, when cycloamyloses are added, the pseudo first order rates of reaction are in some cases dramatically increased $\{20\}$ (see Table 1.2 ). These rate increases are independent of the Hammett relationship, but are dependant on the position of the substituent.

The rate accelerations must be due to an interaction of the phenyl ester with the cycloamylose molecule, and in particular, to the prior complexation of the ester by the cycloamylose molecule. This
observation is supported by the fact that the rate acceleration is not linearly dependant on the concentration of added cycloamylose. The pseudo first order rate constant asymtotically reaches a maximum as the cycloamylose concentration is increased, which is a characteristic of reactions that undergo complexation prior to the rate determining step $\{47\}$. The reaction can be competitively inhibited by the addition of inert materials which form complexes with cycloamylose in preference to the reacting substrate, thus effectively reducing the concentration of the catalyst. This reinforces the theory that there is prior complexation of the substrate.

$$
\mathrm{CA}+\mathrm{S} \stackrel{\mathrm{~K}_{\text {dius }}}{\rightleftharpoons} \mathrm{CA} \cdot \mathrm{~S} \stackrel{\mathrm{~K}_{2}}{\rightleftharpoons} \mathrm{P} 1+\mathrm{CA} \cdot \mathrm{P} 2
$$

Table 1.1 The dependance of catalysis on the phenyl ring substituent position and its independance of the dissociation complex.
Acetate $\quad k_{2} / k_{u n} \quad K_{\text {diss }} . \quad k_{2} / k_{u n} \quad K_{\text {diss }}$

C6A

| p-nitrophenyl | 3.5 | $1.3(0.4)$ | 9.1 | $0.61(0.3)$ |
| :--- | :---: | :---: | :---: | :---: |
| m-nitrophenyl | 300 | $1.9(0.4)$ | 96 | $0.80(018)$ |
| p-t-butylphenyl | 1.1 | $0.65(0.4)$ | 2.2 | $/$ |
| m-t-butyl phenyl | 226 | $0.20(0.08)$ | 250 | $0.013(0.003)$ |
| p-chlorophenyl | 3.0 | $/$ | 10 | $/$ |
| m-chlorophenyl | 113 | $0.56(0.03)$ | 18 | $0.35(0.09)$ |

The pseudo first order rate of reaction is kun in the absence, and k 2 in the presence of cycloamylose, measured at pH 10.60 at $25 \mathrm{C}^{\circ}$. The error limits for the dissociation constant measurements are shown in parentheses after the measurement. $k_{2}$ is the maximal rate acceleration, which is the rate of reaction in an large excess of cycloamylose.

It is immediately apparent from the above table that the rate enhancement is considerably greater for meta than for para substituted phenyl esters. The independance of the binding constant and rate constant may be explained on the basis that the binding constant reflects the strength of binding, whereas the catalytic rate constant depends on the stereochemistry of binding\{46\}.

In order to find out which functional group in the cycloamylose molecule is responsible for the catalytic effect, VanEtten\{47\} selectively blocked off first the primary and then the secondary hydroxyl groups. The results showed that the catalytic properties of cycloamyloses in the hydrolysis of esters were due to the secondary hydroxyl groups\{47\}. When one examines the molecular disposition of the p- and m-nitrophenols $\{49,50\}$ complexed with C6A it is apparent that the m-nitrophenol molecule is considerably further out of the cavity than the p-nitrophenol guest(Figures 1.6), and that the hydroxyl group on the $\underline{m}$-nitrophenol is in much closer proximity to the secondary hydroxyl groups on the cycloamylose molecule. The substituted phenols have a marked resemblance to the corresponding aryl acetates and it is likely that the disposition of the aryl acetates within the cavity will be similar, although the effect that the acyl groupwill have on the complex cannot be predicted.

VanEtten et al.\{47\} also investigated the mechanism of the reaction using substituted aryl benzoates. The inclusion of an aromatic group in the acyl fragment allowed them to determine the reaction pathway. The rate of appearance of the substituted phenol followed similar trends to that observed in the catalysis of aryl acetates, but the rate of appearance of the benzoate ion was almost the same in each case. The only explanation for the observations summarised in Table 1.2 is the

Figure 1.6a Cross section of the p-nitrophenol complex


Figure 1.6b Crose sect on of the m-nit rophenal IGR complex.


```
formation of a covalently bound post reaction adduct, namely, a benzoyl C6A adduct. This has been isolated, and has the same rate constant for hydrolysis as the benzoate C6A derivative in Table l.1. This is consistent with the n.m.r. evidence which shows that the substrate undergoes rapid association/dissociation \(\{40\}\). The rates \(k_{2}\) and \(k_{3}\) are defined in Figure 1.7.
```

Table 1.2. Rate of hydrolysis of $\underline{m}$-substituted aryl benzoates, and the rate of release of the benzoate moiety, in the presence and absence of cycloamylose.
benzoate

$$
\begin{array}{rcc}
k_{\text {un }} * 1000 & k_{2} *_{100} & k_{3} *_{1000} \\
\text { phenol formation } & \text { CA benzoate hydrolysis } \\
\operatorname{Sec}^{-1} & \operatorname{Sec}^{-1} & \operatorname{Sec}^{-1}
\end{array}
$$

| m-nitrophenyl | 15.4 | 1400 | 4.6 |
| :--- | ---: | ---: | ---: |
| m-chlorophenyl | 5.5 | 390 | 4.6 |
| m-t-butylphenyl | 1.2 | 140 | 4.4 |

The reaction pathway proposed by VanEtten, is illustrated schematically in Figure 1.7. E.s.r. evidence confirmed the reaction scheme in Figure 1.7 by showing the presence of the pre-reaction complexes(A in Figure 1.8) $\{51\}$.


REACTION SCHEME FOR CYCLOAMYLOSE CATALYSE HYDROLYSIS OF ESTERS
Figure 1.7



(B)

Illustration Of The Physical Mechanism of Catalysis

Figure 1.8

VanEtten showed that the catalytically active secondary hydroxyl groups on the cycloamylose molecule have a $\mathrm{pK}_{\alpha}$ of 12.1 , which he noted to be low for an aliphatic alcohol\{42\}. There are precedents for this. Rao and Foster studied starch solutions at varying pHs using the optical rotation method and concluded that the pH dependence of the optical rotation was consistent with an hydroxyl group with a $\mathrm{pK}_{a}$ of 12 \{52\}. Other studies showed that the secondary hydroxyl groups had a $\mathrm{pK}_{Q}$ of 12.1 $\{53\}$. Similar studies showed that the $\mathrm{pK}_{a}$ value of the two adjacent secondary hydroxyl groups of the ribose moiety in adenosine was 12.35 \{54\}. The enhanced acidity of these hydroxyl groups may be due to the combined inductive effects of the relatively electronegative oxygen atoms, and also to the stabilisation of the alkoxide ion by means of an inter molecular hydrogen bond with a neighbouring secondary hydroxyl group $\{46\}$.

Enantiomeric Specificity In Cycloamylose Catalysed Reactions.

Cycloamyloses can induce an assymetric interaction when they form complexes with substrates\{37,38\}. The solubility of the substrate host complex is usually less than that of the host alone, thus in a concentrated solution of cycloamylose the addition of a potential guest has the effect of precipitating the complex.

This has been used to separate a racemic mixture where one of the isomers complexes preferentially with the cycloamylose molecule and is thus precipitated out $\{55\}$. This technique has been used to resolve a racemic mixture, firstly by Cramer and Dietsche\{56\}, who obtained optical purities of 2 and $12 \%$ for a series of carboxylic acid esters. Subsequent opical purities, reported by other workers, are $66 \%$ for isopropyl methyl phosphinates\{57\} and $71.5 \%$ for chiral sulphoxides\{58\}.

The optical purities reported in the foregoing papers are the result of repeated complexation, followed by separation of the complex and the release of the substrate. This has subsequently been improved to a $68 \%$ optical purity after one inclusion process for isopropyl methane sulphinate\{59\}. It has also been suggested that the inclusion process may fix the conformation of the phenyl group in cinnamic acid into the $\mathrm{R}-(-)$ - configuration in the cavity $\{60\}$. These phenomena are the result of specificity in the binding of the substrate; i.e. they are dependant on the $K_{\text {diss }}$ of the guest.

Preferential catalysis of one enantiomorph was first reported by Cramer and Dietsche\{57\}, whilst investigating the hydrolysis of
esters of substituted mandeleic acids. More recently, however, the release of the fluoride ion from Sarin (isopropyl methyl phosphonofluoridate), has been shown to exhibit a dramatic enantiomeric specificity (Table 1.4) $\left\{60^{\circ}\right\}$. An interesting point to note is that although the rate of reaction( $\mathrm{k}_{2}$ ) of $\mathrm{R}-(-)$-Sarin is much faster than S-(+)- Sarin the latter forms a more stable complex. The dissociation constant for R -Sarin is less than that for S-Sarin, although the rate acceleration is greater for the R -isomer. In this case there is a specificity in catalysis ( $k_{2}$ ).

Table 1.3. Kinetic data for the hydrolysis of R - and S -sarin in the presence of cycloamylose.

Sarin

$$
k_{2} / k_{u n}
$$

$K_{\text {diss }} * 100$ (M)

R-(-)-
157
4.0 (0.60)

S-(+)-
4.4
0.60 (0.04)

Flohr et al. showed that one of the critical factors in enantiomerically specific catalysis is the size of the cavity. If the fit of the substrate in the cycloamylose is tight then there can be enantiomeric specificity, but this specificity will be lost as the fit of the substrate becomes more loose\{62\}.

Similarities Between Cycloamylose And Enzyme Catalysed Reactions.


#### Abstract

The catalytic effects due to cycloamyloses have been likened to enzyme systems, as both form physical complexes, and exhibit speed and selectivity. It was proposed at a very early stage that cycloamyloses could act as enzyme models $\{56,61\}$. As the conditions of the cavity and its environs are made more similar to an enzyme's active site, its catalytic powers improve \{21\}.


Both form 1:1 complexes prior to the reaction\{64\}. This has been shown by e.s.r., which directly indicates the existance of a complex prior to the reaction $\{64\}$. As has already been discussed the scheme shown below is thought to be the mechanism by which cycloamyloses catalyse reactions.


Figure 1.9

[^0]increased there is a diminishing rate increase, in a manner which has been shown to satisfy the Michaelis-Menten kinetic scheme\{20\}.


#### Abstract

The degree of substrate specificity of enzyme catalysed reactions is well known, and the substrate specificity of cycloamyloses has already been discussed. Rate accelerations caused by cycloamyloses are competitively inhibited by the addition of inert reagents such as cyclohexanol. This phenomenon is frequently observed in enzymatic catalysis. Stereospecific complexation in cycloamyloses is similar in mode to the complex formation as proposed by Fischer in his 'lock and key' theory of enzymatic catalysis\{64\}.


Cycloamylose As A Model For The Chymotrypsin System.

Cramer initially noted the possibility of using cycloamyloses as models for chymotrypsin\{66\}. VanEtten summed up the similarities between cycloamylose catalysed and chymotrypsin catalysed phenyl ester hydrolysis as follows 447,67$\}$. Both catalyses occur by similar mechanisms which involves rapid association to form a complex. The ester substrate then reacts with the catalyst to form an acylated intermediate, which subsequently undergoes hydrolysis in a slow step. The dissociation constants of the two systems are similar and favour apolar substrates. Increasing the ionic strength of the solution tends to favour complex formation, and making the solvent more apolar tends to decrease the stability of the system. Both systems are subject to competitive inhibition with the addition of small organic molecules of a comparable size to the substrate. The maximal rate enhancements are unrelated to the stabilities of the complexes, but depend on their stereochemistry.

The secondary hydroxyl groups, which have a $\mathrm{pK}_{a}$ of 12 , act as nucleophiles in the acylation step in cycloamylose catalysed reactions. The acylation step in chymotrypsin is dependant on a catalytic group of $\mathrm{pK}_{\mathrm{a}}$ 7, probably an imidazole, which acts as a general acid base catalyst\{47\}.

The deacylation step in the cycloamylose catalysed reaction is much slower than the corresponding step in the chymotrypsin reaction. A detailed examination of the enantiomeric specificity of both systems has been carried out, and it was found that for similar substrates the enantiomeric specificity of the enzyme is only marginally better than that for cycloamyloses\{39\}. The principal difference between chymotrypsin and cycloamylose is the pH at which the catalysis takes place, pH 8 and pH 12 respectively. If it were possible to make cycloamyloses react at pH 8 , without the loss of any of their other properties, then they would compare well with enzyme systems.

Cycloamyloses As A Model for Penicillinase.

The effect of C7A on a series of penicillins was studied by Tutt and Schwarz\{48,65\}, who found that the rate accelerations for penicillins followed the saturation kinetics that are observed for the hydrolysis of phenyl esters.

The alkaline hydrolysis of penicillins is first order in substrate and hydroxide ion, with the cleavage of the beta lactam ring yielding penicillinoic acid as shown below.

hydrolysis of the beta lactam ring of penicillin
Figure 1.10.

This suggests that the reaction proceeds with the prior complexation of the $R$ group with the C7A molecule before the nucleophilic attack by a C7A alkoxide. The rate of disappearance of penicillin is greater than the rate of appearance of the acid product, which suggests a mechanism similar to that found in the hydrolysis of phenyl esters, as does the presence of a covalently bound guest/host intermediate in the reaction pathway.

There is not the same degree of specificity with respect to the $R$ group as is observed with the cycloamylose catalysed hydrolysis of phenyi esters. The catalytic rate observed for the hydrolysis of the penicillin is in the region of 20 to 90 .

The carbonyl carbon of the phenyl ester is only two atoms removed from the phenyl ring, which is included into the cavity, whereas the reactive centre in penicillins is four atoms away from the directing group, which is the side chain. This suggests that the inclusion process does not aid the reaction so effectively, as there is more possibility for the penicillin to rotate in a manner which will reduce the interaction between the cycloamylose and the substrate.

Relatively little is known about the mechanism, action or nature of the active site in penicillinase and the way in which the beta lactam of resistant penicillins are rendered inactive to penicillinase. This model system comes closer to the enzymatic specificity\{48\}, and is the first model to show strong and specific binding of the side chain, and can be used in the developement of new penicillin derivatives.

Industrial And Pharmaceutical uses of cycloamyloses.

There are now a profusion of uses for cycloamylose throughout the whole spectrum of technological developement. This section will catalogue some of the more important uses of cycloamyloses.

The pharmaceutical industry has been interested by the behaviour of cycloamylose complexes. The initial research was carried out by Lach and co-workers $\{68,69,70\}$. The benefits that accrue from the use of cycloamyloses are all the result of complex formation. A cycloamylose complex usually has a solubility intermediate between that of the free host and the solubility of the free guest. This can be important if a drug has a low solubility, as drugs with low solubilities are not easily absorbed through the stomach wall(e.g. Barbiturate derivatives\{71\}). Cycloamyloses, in effect, act as carriers for the drug, due to their high solubility. When the cycloamylose complex enters the blood. stream the host molecule is degraded enzymatically to release the drug. Stability is also conferred on the drug by the cycloamylose which protects it\{72\}. This is very important as a wide variety of biologically active species are readily broken down by the environment of the stomach. Other advantages include the formation of a solid preparation from a liquid drug, and the improvement of a drug which is a stimulant or irritant to the stomach\{73\}. Finally there is a decrease in the volatility of a drug, which is the result of the formation of a complex. The pharmaceutical use of cycloamyloses is extensively covered by patent.

Cycloamyloses have also been used as n.m.r. shift reagents. If the species can be included into the cavity, then quite large spectral changes can be observed\{70\}.

It has been claimed that cycloamylose can protect delicate flavours from heat and light $\{75\}$, and can remove unwanted flavours from tea and coffee. The C7A complex of carbon dioxide can be used as a baking powder substitute. Cycloamyloses also make egg whites more beatable!!

The nitroglycerine complex of C7A can be used as an explosive \{76\}. The chloropicrin derivative is effective as a bactericide and insecticide\{77\}. The complex of methyl parathion with C7A has useful and persistent activity against cotton insects\{78\}, whereas clatharates of various pyrethroids prove more effective than the guest compound in its free state as an insecticide. The cyclohexylamine complex of C7A is useful in rust prevention $\{79\}$.

Some Japanese vorkers have discovered a one step formation of vitamins K1 and K2 using cycloamyloses as catalysts. This reaction has advantages over other methods, which all suffer from side reactions which yield undesirable coproducts. Furthermore the reaction has a very high yield\{80\}.

The Aim Of The Project

The introductory chapter has mentioned that cycloamyloses can induce stereospecific hydrolysis in the hydrolysis of phenyl acetates. There was, at the inception of this project, no crystallographic data on the disposition of the meta- and para- substituted phenyl acetates in the cycloamylose cavity. The aim of this project was to investigate the geometry of the complexes, and their relationship to the catalytic properties that are exhibited by cycloamyloses.

The reaction half lives for the hydrolysis of phenyl acetates in the presence of cycloamyloses is usually measured in minutes\{20\}. For this reason they are not suitable substrates for crystallographic studies, the formation of crystals requiring elevated temperatures over a period of days, and data collection taking at least a week. With the reaction half lives previously mentioned there is very little chance of there being any unreacted acetate left in the cycloamylose matrix.
. On the other hand cycloamyloses can bind non-productively with substrates in much the same way as enzymes\{39\}. Cycloamylose complexes were made with substituted acetanilides as they are much less labile than aryl acetates. The C6A matrix actually protects the acetanilide species from hydrolysis. The rates of hydrolysis are given in Table 1.4. There are considerable structural similarities between acetanilides and phenyl acetates, as is shown in Figure 1.11.


Figure 1.11


Table 1.4. Rates of hydrolysis of $p$-nitro acetanilides and $p^{-n i t r o-}$ phenyl acetates

| Substrate | pH | Temperature | $\mathrm{k} * 10000$ | Reference |
| :--- | :---: | :---: | :---: | :---: |
| p-nitro phenyl acetate | 10.01 | C | Sec . |  |
| p-nitro phenyl acetate+ C6A | 10.01 | 25 | 14.0 | 20 |
| p-nitroacetanilide | 12.47 | 25 | 243 | 20 |
| p-nitroacetanilide +C6A | 12.30 | 70 | 0.90 | 82 |
| p-nitroacetanilide | 11.0 | 25 | 0.0113 | 81,82 |

As both temperature and pH are increased there is an increase in rate. The data presented in Table 1.4 show that the base catalysed hydrolysis of $p$-nitroacetanilide is considerably slower than the base catalysed hydrolysis of $p$-nitrophenyl acetate.
. As the pH is reduced the rate of hydrolysis is significantly reduced (see Table 1.4), and it is to be expected that at $\mathrm{pH} 7-8$ the complexes of $p$-nitroacetanilide with cycloamyloses will be relatively stable, and hence suitable for a crystallographic study. The data only refers to $p$-nitroacetanilide, but it is not unreasonable to suppose that substituted acetanilides in general will be comparitively stable at neutral pHs.

Chapter 2 Crystallographic Structure Determinations Of C6A Complexes.

The last comprehensive review of cycloamylose structures determined crystallographically was written in 1976 by Saenger $\{82\}$. Structural data for a number of different complexes of C 6 A have since been published, and this chapter is intended to summarise, extend and update Saenger's review.

In total nineteen C6A complexes have been solved and published. Cyclohexaamylose complexes tend to crystallise from aqueous solutions into one of a number of almost isomorphous groups. Furthermore, it has been found that within each pseudo isomorphous group the substrate has similar size and properties. Complete structure determinations have shown that in their packing C6A complexes tend to fall into one of six main groups or categories. Recently several C7A structures have been solved $\{22,23\}$, and the first C6A structure from a non-aqueous solvent $\{84\}$ has been reported. The C7A complexes will be discussed in depth elsewhere.

It is to be expected that the packing trends of the C6A and C7A molecules will be related, but it is impossible to transpose structural information from the C6A to the C7A system due to the inherently lower symmetry of the C7A molecule. The C6A complexes have a much higher space group symmetry (usually orthorhombic) than those of C7A , which usually form crystals in a monoclinic or triclinic space group.

A list of the C6A complexes that have been studied crystallographically is given in Table 2.1. The fact that a number of structures have the same space group and almost identical cell dimensions led

Table 2.1. X-ray Crystallograpnic Data For C6A Complexes.

|  | Substrate | Space Gro | oup a | $b$ | $c$ | alpha | beta | gamma | 2 |  | ref. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | A | A | $A^{*}$ |  |  |  |  | $\dot{A}^{3}$ |  |
| 1 | *Water | $\mathrm{P} 2, \mathrm{I}_{1} \mathrm{I}_{1}$ | 14.856 | 33.991 | 9.517 | 90.00 | 90.00 | 90.00 | 4 | 1200 | 86,87 |
| 2 | *Iodine | $\mathrm{P} 21_{1}{ }_{1}{ }_{1}{ }_{1}$ | 14.240 | 36.014 | 9.558 | 90.00 | 90.00 | 90.00 | 4 | 1220 | 88 |
| 3 | *Me thanol | $22_{1} 2_{1} 2_{1}$ | 14.292 | 37.515 | 9.393 | 90.00 | 90.00 | 90.00 | 4 | 1259 | 89,90 |
| 4 | *Krypton (3 Atm.) | $\mathrm{P} 21^{2} 1^{2}{ }_{1}$ | 14.299 | 37.489 | 9.407 | 90.00 | 90.00 | 90.00 | 4 | 1261 | 91,92 |
| 5 | *Krypton (14 Atm.) | $\mathrm{P}_{1} \mathrm{I}_{1} \mathrm{I}_{1}{ }_{1}$ | 14.377 | 37.402 | 9.446 | 90.00 | 90.00 | 90.00 | 4 | 1266 | 91,92 |
| 6 | *l-Propanol |  | 14.292 | 37.515 | 9.393 | 90.00 | 90.00 | 90.00 | 4 | 1259 | 93 |
| 7 | Aceric acid | P2 $1_{1}{ }_{1} 2_{1}$ | 14.34 | 37.62 | 9.43 | 90.00 | 90.00 | 90.00 | 4 | 1272 | 94 |
| 8 | Propionic acid | P2, $2_{1} 2_{1}$ | 14.37 | 39.81 | 9.46 | 90.00 | 90.00 | 90.00 | 4 | 1288 | 94 |
| 9 | Butyric acid |  | 14.38 | 37.99 | 9.44 | 90.00 | 90.00 | 90.00 | 4 | 1289 | 94 |
| 10 | ${ }^{+} \mathrm{p}$-Nitrophenol | $\mathrm{P} \mathrm{F}_{1} \mathrm{I}_{1}{ }^{\text {a }}$ | 24.740 | 13.455 | 15.296 | 90.00 | 90.00 | 90.00 | 4 | 1273 | 49 |
| 11 | *p-Iodophenol | $P 21_{1} 1_{1}{ }_{1}$ | 24.573 | 13.477 | 15.373 | 90.00 | 90.00 | 90.00 | 4 | 1273 | 95 |
| 12 | *p-Hydroxybenzoic acid | $\mathrm{P} 2 \mathrm{~s}^{2} 1_{1}{ }_{1}$ | 24.896 | 13.356 | 15.342 | 90.00 | 90.00 | 90.00 |  | 1275 | 49 |
| 13 | *p-Iodoanaline | $\mathrm{P} 21_{1} \mathrm{~L}_{1}{ }^{\text {! }}$ | 24.569 | 13.681 | 15.475 | 90.00 | 90.00 | 90.00 | 4 | 1300 | 96,97.,98 |
| 14 | *Lithium Polyiodide | P1 | 13.88 | 13.88 | 15.69 | 95.10 | 87.81 | 119.90 | 2 | 1308. | 99,100 |
| 15 | Diethyl ether | PI | 13.95 | 13.87 | 15.67 | 91.3 | 85.51 | 120.29 | 2 | 1303 | 94 |
| 16 | 1 Octanol(Laue) | $6 / \mathrm{mm}$ | 13.86 |  | 15.63 | 90.00 | 90.00 | 120.00 | 2 | 1300 | 94 |
| 17 | Valeric acid(Laue) | 6/mm | 13.85 |  | 15.62 | 90.009 | 90.001 | 120.00 | 2 | 1298 | 94 |
| 18 | 3-Methyl-1-butanol | P2 1 | 23.64 | 13.95 | 16.64 | 90.00 | 95.00 | 90.00 | 4 | 1368 | 94 |
| 19 | Barium Iodide/iodine | P622 | 13.71 |  | 17.04 | 90.00 | 90.00 | 120.00 | 2 | 1387 | 94 |
| 20 | Potassium iodide/iodine | $\mathrm{P6}_{2} 22$ | 15.89 |  | 39.94 | 90.09 | 90.00 | 120.00 |  | 1457 | 94 |
| 21 | Sodium Perchlorate | C2 | 19.87 | 33.71 | 27.79 | 90.010 | 106.86 | 90.0 |  | 1483 | 94 |
| 22 | *Sodium 1-Propanesul phonate | $\mathrm{P} 2,2,2$ | 21.608 | 16.700 | 8.302 | 90.009 | 90.00 | 90.00 | 2 | 1498 | 101 |
| 23 | *Sodium Methyl Orange | P2, $\mathbf{2}_{1}{ }^{2}$ | 22.099 | 16.359 | 8.296 | 90.00 | 90.00 | 90.00 | 2 | 1500 | 102 |
| 24 | *Potassium Acetate | $P 21_{1} 1^{2}$ | 21.89 | 16.54 | 8.30 | 90.0090 | 90.00 | 90.00 | 2 | 1500 | 2 |
| 25 | *Potassium Methyl Orange | P2 $1_{2} 1^{2}$ | 22.120 | 16.419 | 8.292 | 90.009 | 90.00 | 90.00 | 2 | 1502 | 89 |
| 26 | Sodium Hexanoate | $\mathrm{P} 21{ }_{1}{ }_{1}{ }^{2}$ | 21.94 | 16.53 | 16.56 | 90.009 | 90.00 | 90.00 | 4 | 1503 | 94 |
| 27 | *Sodium Benzenesulphonate | P2, 2,12 | 21.832 | 16.529 | 8.356 | 90.009 | 90.00 | 90.00 | 2 | 1507 | 103 |
| 28 | *m-Nitrophenol | $\mathrm{P} 2,1{ }_{1}{ }^{2}$ | 22.231 | 16.865 | 8.152 | 90.009 | 90.00 | 90.00 | 2 | 1523 | 50 |
| 29 | *Cadmium Polyiodide | $\mathrm{P}_{4} \mathrm{I}_{2} \mathrm{I}^{2}$ | 19.93 | 19.93 | 30.87 | 90.00 | 90.00 | 090.00 | - 8 | 1533 | 99,100 |
| 30 | *C6A from a mixed solvent | 821 | 9.505 | 14.505 | 19.738 | 90.010 | 102.88 | 90.00 | 2 | 1295 | 84 |
|  | (Dimethyl sulphoxide/Water) |  |  |  |  |  |  |  |  |  |  |

[^1]McMullan, Saenger, Fayos, and Mootz to divide the complexes into three classes which are approximately isomorphous. Since then other structures have been solved, and the scheme proposed by McMullan has had to be modified in order to take account of the new data. Complete structure determinations are marked by an asterisk, the others are the result of prelimininary crystallographic investigations only.

The packing of the C6A molecule has been found to fall into one of two distinct types. In the first type the central cavity is isolated by the packing of other C6A molecules. These are called cage structures, and have a central cavity which is restricted in size. In the second mode of packing the C6A molecules stack on top of each other with their central cavities forming a long cylindrical channel. These are known as the channel structures.

Recently several structure determinations have been carried out with sufficient accuracy to determine the hydrogen atom positions. They have shed some light on the hydrogen bonding systems existing in crystalline cycloamylose complexes. The neutron diffraction study of the C6A water complex has been carried out, but the results have yet to be published in detail\{85\}. High resolution X-ray studies of various C6A complexes have been reported, and several have managed to find hydrogen atom positions by the use of difference Fourier maps.

Before starting a detailed description of the molecular packing, a reiteration of the importance of both inter- and intramolecular hydrogen bonding is in these systems required. There are four main types of hydrogen bonds that are believed to exist in crystalline cycloamylose complexes.

```
The first and most important type of hydrogen bonding is the intra_molecular ring of hydrogen bonds that occur between the secondary hydroxyl groups(see Chapter 1). These bonds contribute significantly to the rigidity of the cycloamylose molecule, and their disruption reduces the stability of the C6A molecule derived from these bonds\{17, 85,87\(\}\).
```


#### Abstract

The second type of hydrogen bond is believed to exist between primary hydroxyl groups in the C6A molecule and any hydrogen bonding moiety in the guest molecule. This is usually accompanied by a disorder in the primary hydroxyl group and a weakening of the secondary hydroxyl group's hydrogen bonds.


[^2]Group 1 :- The Cage Structures.

Group la. Herringbone Cage Structures. (Nos. 1-9 in Table 2.1)

The complexes in this group are in the $P 2,2,2$, space group with cell dimensions in the order of $a=14.5(0.3) \AA, b=37.0(3.0) \AA$, $c=9.4(0.2) \AA$. The variations in cell edge length within this group are enclosed in parentheses. The a and $c$ axial lengths are not significantly altered by the substrate's size. The tightness of packing along the $b$ axis can vary, and changes to accommodate the guest. When the guest molecule is small the $b$ axis tends to be short, and when the guest is large the $b$ axis tends to be long. These complexes have a low volume per inclusion complex $\left(1200-1290 \AA^{3}\right)$. Typical substrates in this class are water, iodine, krypton, and low molecular weight alcohols. Only small molecules can be included due to the restricted size of the molecular cavity in the lattice, its dimensions being five Angstroms in diameter and eight Angstroms high.

A schematic diagram of the packing of the C6A molecule in the unit cell is given in Figure 2.1. The shaded portıon of the diagram represents the central cavity of the $C 6 A$ molecule.

Saenger prepared crystals of C6A complexed with a series of carboxylic acids differing only in the length of the alkyl group. He found that when the guest was too large to fit into the cavity formed by the herringbone arrangement of C6A molecules the packing of the host changed in order to accomodate the larger guest $\{94\}$. Diagrams of the water and n-propanol complexes are given in Figures 2.2.


Figure $2.2 t$ The CGR motecule in the $n$-prepanci complex.


It has been found that if there is the possibility of hydrogen bonding between the substrate and the primary hydroxyl groups then those hydroxyl groups are disordered, the disorder being between the gauche gauche (gg), (Figure 2.3a), and the gauche trans(gt), (Figure 2.3b), configurations. Primary hydroxyls in the gt configuration open out the secondary hydroxyl end of the molecule, weakening or rupturing several of the hydrogen bonds that are formed between adjacent glucose residues at that end of the molecule (see Figure 1.2).

gg 23a

gt 2.3b

Figure 2.3.
$g g+g t$ CONFIGURATION OF THE PRIMARY HYDRCXYI
in GLUCOSE

In the C6A water complex there are two primary hydroxyl (016 and 056 ) groups in the gt configuration. The oxygens are rotated in towards the central cavity of the cycloamylose molecule, in order to partially satisfy the hydrogen bonding requirements of the included water molecules. The remaining primary hydroxyl groups are in the gg configuration.

Table 2.2 shows the distance between the bridging oxygens across the diameter of the C6A molecule. With the exception of the water
complex most of the complexes are remarkably hexagonal, this being indicated by the small values of delta, which is the difference between the smallest and the largest diagonal. It is apparent that as the substrate becomes larger the distortion of the hexagon decreases.

Table 2.2. Diagonal distances across the bridging oxygens for Group la complexes

Substrate

| 014-044* | $024-054$ | $034-064$ | Delta Ref. |  |
| :---: | :---: | :---: | :---: | :---: |
| $\AA$ | $\AA$ | $\AA$ | $\AA$ |  |


| Water | 8.509 | 8.084 | 9.185 | 1.101 | 87 |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Iodine | 8.406 | 8.488 | 8.795 | 0.389 | 88 |
| Methanol | 8.439 | 8.246 | 8.662 | 0.416 | 90 |
| n-Propanol | 8.435 | 8.353 | 8.572 | 0.219 | 93 |
| Krypton(20atm.) | 8.440 | 8.306 | 8.649 | 0.343 | 85 |

* The first letter is the atomic symbol of the atom, the first number is the number of the glucose residue in the cycloamylose molecule, and the last number is the number of the atom in the residue (see Figure 1.2).

Table 2.3 shows the $02-03^{\prime}$ (The signifies that the atom is on an adjacent glucose residue) distance for those group la complexes which have been published with coordinate lists. This is the distance between the oxygens of the secondary hydroxyl groups on adjacent glucose residues. There is the potential for hydrogen bonding between these hydroxyl groups if their separation is less than about 3.1 Angstroms $\{85\}$. The water complex has a hydrogen bonding potential between four of the hydroxyl groups, and the rest of the complexes in this group have this potential between five of the hydroxyl groups.

Table 2.3. Distance between 02 and 03 on contiguous glucose residues group la complexes.

| Substrate | $012-023$ | $022-033$ | $032-043$ | $042-053$ | $052-063$ | $062-013$ | Ref. |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\AA$ | $\AA$ | $\AA$ | $\AA$ | $\AA$ | $\AA$ |  |
|  |  |  |  |  |  |  |  |
| Water | 2.941 | 3.016 | 2.823 | 3.345 | 4.666 | 3.025 | 86 |
| Iodine | 2.83 | 3.22 | 2.74 | 3.00 | 3.83 | 3.00 | 82 |
| Methanol | 2.955 | 2.912 | 2.956 | 2.949 | 3.145 | 3.066 | 84 |
| Krypton | 2.920 | 2.882 | 2.922 | 2.943 | 3.143 | 3.016 | 86 |
| n-Propanol | 2.931 | 2.899 | 2.902 | 2.971 | 3.127 | 2.996 | 87 |

Due to the herringbone nature of the packing there is no regular array of intermolecular hydrogen bonding. The crystal lattice is held together by hydrogen bonds between the primary and secondary hydroxyl groups on adjacent molecules, the bonds usually pass through a water of crystallisation.

Group lb. Modified Cage Structure (Nos. 10-13 in Table 2.1)

The closing off of the cavity in the last group of structures was achieved by the herringbone packing arrangement. The modified cage structures have the C6A molecules arranged as in Figure 2.4 to close off the cavity. The modified cage structure has two types of cavity in the crystal lattice, one being the C6A cavity, which is indicated by the widely spaced hatching. The other, which is between the cycloamylose molecules, is occupied by three ordered water molecules and is indicated by the closely spaced hatching. The part of the cycloamylose molecule nearest to the viewer is outlined with a heavier line.


Figure 2.4.
PACKING OF GROUP ib COMPLEXES

These complexes are in the $P 2,2,2$, space group, with cell dimensions $a=24.5(0.3) \AA, b=13.4(0.1) \AA$, and $c=15.4(0.1) \AA$. The variation in cell edge length is included in parentheses after the appropriate dimension. The volume per inclusion complex is marginally higher than the previous class (1270-1300 $\AA^{3}$ ). The included molecules in this class tend to be p-disubstituted benzene rings, which are too long to fit into the cavity formed by the herringbone packing.

In this group of structures it is again found that one of the primary hydroxyl groups is disordered between the $g g$ and the gt configurations, stretching the hydrogen bond between the secondary hydroxyl groups of that residue, thus destabilising the C6A molecule.

In group $1 b$ the shape of the C6A macrocycle is significantly altered by the inclusion of these substrates. Unlike the small substrates of group la, these substrates are wide and cause an eliptical distortion of the hexagon formed by the bridging oxygens(04s). Table 2.4 shows the 04-04 distances across the ring for a variety of different included species, and Figure 2.5 schematically shows this distortion in the C6A p -Iodoaniline complex.


The elliptical distortion of the hexagon
clearly iollows the insertion of the Denzene ring which is indicated by the hatchinc.

Figure 2.5.

Table 2.4. Diagonal distances across the bridging oxygens Group lb complexes.

| Substrate | $014-044$ | $024-054$ | $034-064$ | Delta |
| :--- | :---: | :---: | :---: | :---: |
|  | $\AA$ | $\AA$ | $\AA$ | $\AA$ |
| p-Iodophenol | 8.057 | 8.835 | 8.473 | 0.778 |
| p-Nitrophenol | 7.984 | 8.902 | 8.457 | 0.918 |
| p-Hydroxybenzoic Acid | 8.055 | 8.923 | 8.418 | 0.868 |
| p-Iodoaniline | 8.163 | 8.842 | 8.458 | 0.679 |

Delta is the difference between the highest and lowest diagonal distances. n-Propanol has a delta value which is typical for the Group la. complexes other than water and complexes where a phenyl ring is not included into the cavity.

Table 2.5. Distances between 02 and 03 on contiguous glucose residues Group 1b complexes.

| Substrate | $012-023$ | $022-033$ | $032-043$ | $042-053$ | $052-063$ | $062-013$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\AA$ | $\AA$ | $\AA$ | $\AA$ | $\AA$ | $\AA$ |
| p-Nitrophenol | 2.650 | 2.847 | 2.736 | 2.836 | 3.068 | 2.860 |
| p-Iodophenol | 2.687 | 2.827 | 3.105 | 2.838 | 2.961 | 2.883 |
| p-Hydroxybenzoic Acid | 2.674 | 2.897 | 3.114 | 2.845 | 2.878 | 2.824 |
| p-Iodoaniline | 2.967 | 2.875 | 2.697 | 2.757 | 3.045 | 2.923 |

Where the included molecule is a substituted benzene ring, the distances between the secondary hydroxyl group's oxygen atoms are much closer to a distance which is normally considered reasonable for hydrogen bonding(Table 2.5), although the 0-0 distances of 3.1 Angstroms could correspond only to very weak hydrogen bonding $\{85\}$.

The molecular packing in this group of structures greatly facilitates the inter-molecular hydrogen bonding by bringing the primary and secondary hydroxyl groups into close proximity. There is a regular and dense array of hydrogen bonds between the ends of the adjacent molecules. Few of these hydrogen bonds pass through an imtermediate water molecule.

Group 2 Channel Stuctures

There is a wide variation in space groups and cell dimensions of group two complexes, but it has been found that the packing arrangements break down into one of four types. In all of these modifications the C6A molecules stack vertically on top of one another a manner analagous to the way that a stack of coins is formed. The C6A molecules can pack head to tail or head to head, tail to tail to form the molecular stack. The secondary hydroxyl end of the C6A molecule is referred to as the head of the molecule and the primary hydroxyl end of the molecule, the tail of the molecule. These stacks of C6A molecules can either form a hexagonal close packed array, or that can adopt a second more complex packing arrangement.

Group 2a. (Nos 14-17 in Table 2.1.)

In this group the guests are typically large organic molecules, e.g. valeric acid and l-octanol, which are too large to fit into a cage type of structure. According to Saenger\{88\} C6A molecules will stack head to tail and the molecular stacks are hexagonally close packed as in Figure 2.6. No complete structure determination has been carried out on any member of this class. The cell dimensions and space groups of these complexes vary considerably and there is no readily apparent pattern.

Group 2b. (No. 20 in Table 2.1)

Only one complex of this sort has been reported for the C6A system $\{99,100\}$. The complex is in space group Pl, with cell dimensions a $=13.88 \AA, \dot{b}=13.88 \AA, c=15.69 \AA, \alpha=94.10^{\circ}, \beta=87.8^{\circ}, \gamma=119.90^{\circ}$. The volume per inclusion complex is $1308 \AA^{3}$. There is head to head, tail to tail stacking, with the C6A stacks hexagonally close packed. The packing is illustrated in Figure 2.7. The guest molecule in this group is a polyiodide ion, which runs up the central cavity of the C6A stack.

The primary hydroxyl groups are all in the $g g$ configuration. There is a complex arrangement of hydrogen bonds between the secondary hydroxyl groups on adjacent molecules. These groups are directly hydrogen bonded to each other, with the exception of several of the secondary hydroxyl groups which are bonded to the lithium cation $\{100\}$. The hydrogen bonding between the primary hydroxyl groups on adjacent molecules is more complex and is usually indirect, passing through a water molecule.


SECTION THROUGH XX


Figure 2.6.


Figure 2.7.

The diagonal distances between the bridging oxygens are shown in Table 2.6. There is very little distortion of the C6A molecules from hexagonality. Table 2.7 shows the distances between the secondary hydroxyl groups on adjacent glucose residues. The lengthening of one of the bonds to $3.30 \AA$ probably results from the hydration of the lithium ion. There are two molecules per assymmetric unit, and the tables tabulate the distances for each molecule.

Table 2.6. Diagonal distances across the bridging oxygens Group 2b complexes.

| $041-044$ | $042-045$ | $043-046$ | Reference |
| :---: | :---: | :---: | :---: |
| $\AA$ | $\AA$ | $\AA$ |  |
| $\AA$ | $\AA$ | $\AA$ |  |


| Molecule 1 | 8.31 | 8.53 | 8.53 | 100 |
| :--- | :--- | :--- | :--- | :--- |
| Molecule 2 | 8.54 | 8.42 | 8.65 | 100 |

Table 2.7. Distance between 02 and 03 on contiguous glucose residues Group 2 b complexes

|  | $012-023$ | $22-023$ | $032-043$ | $042-053$ | $052-063$ | $062-013$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\AA$ | $\AA$ | $\AA$ | $\AA$ | $\AA$ | $\AA$ |
| Molecule 1 | 2.78 | 2.91 | 2.82 | 2.89 | 2.96 | 2.90 |
| Molecule 2 | 3.05 | 2.90 | 3.01 | 2.86 | 3.30 | 3.11 |

Group 2c. (Nos. 21-27 in Table 2.1.)

The complexes in this group are all in the $P 2,2,2$ space group with the exception of the sodium hexanoate complex, which is in the $\mathrm{P} 2,2,2$, space group with a cell dimension twice the $c$ axial length of other members of this group. The cell dimensions in this group are $\mathrm{a}=21.8(0.5) \AA, \mathrm{b}=16.40(0.40) \AA, \mathrm{c}=8.30(0.20) \AA$. The variation in cell dimension within this group is shown in parentheses after the appropriate dimension. The volume per inclusion complex of this group lies in the range $1498-1523 \dot{A}^{3}$, which is much larger than that found in the cage structures.

Either the cation, if the included species is ionic, or the second guest molecule is included interstitially between the stacks. The guest is included in the cavity and can vary in size from the acetate ion in the potassium acetate complex to the large methyl orange anion.

Some of these guests are small enough to fit into the Group la cage structure, but do not, probably due to the absence of a favourable site for interstitial guest or cation coordination\{94\}. The interstitial species is too large to fit into the gap between the rings as in group 2 b , so there is a modification of the packing to allow the formation of a suitable small cavity outside the C6A molecule. The lithium cation in group $2 b$ is believed to be small enough to fit into the region between cycloamylose molecules, and the cations in this group are too large to fit into this space and must lie between the cycloamylose stacks.

The packing in this group is more complex. The C6A molecules are stacked head to tail, but these stacks are not in a hexagonal
close packed arrangement. The columns of C6A molecules lie parallel to the $c$ axis with centres on $0,0, Z$, and $0.5,0.5, Z$. The molecules in the stack that lies on the origin have their heads pointing along the $c$ axis but those in the stack that is centred on $x=0.5 \quad y=0.5$ have their heads pointing towards $-c$. The packing is illustrated in Figure 2.8.

Only the methyl orange and the $\underline{m}$-nitrophenol complexes in group $2 c$ have a disorder in the primary hydroxyl group similar to that found in types $1 a$ and $1 b$, but there is still the potential for a good ring of hydrogen bonding around the secondary hydroxyl groups. The shape of the substrate can cause a distortion of the cycloamylose molecule, but there is no distortion of the hydrogen bonding. Table 2.8 shows the $02-03^{\prime}$ distances which indicate a good hydrogen bonding arrangement.

Table 2.8. Distance between 02 and 03 on contiguous glucose residues Group 2c complexes.

| Substrate | $012-023$ | $022-033$ | $032-043$ | Ref. |
| :--- | :---: | :---: | :---: | :---: |
|  | $\AA$ | $\AA$ | $\AA$ | $\AA$ |
| Sodium Propanesulphonate | 2.83 | 2.94 | 2.88 | 101 |
| Sodium Methyl Orange | 2.84 | 2.87 | 2.78 | 101 |
| Potassium Acetate | 2.839 | 2.853 | 2.863 | 2 |
| Potassium Methyl Orange | 2.84 | 2.89 | 2.81 | 101 |
| Sodium Benzene Sulphonate | 2.83 | 2.94 | 2.88 | 101 |
| m-Nitrophenol | 2.788 | 2.821 | 2.858 | 50 |

The diagonal distance across the bridging oxygens is shown in Table 2.9. As can be seen there is only distortion of the C6A molecule when a phenyl ring is included in the cavity.


Figure 2.8.

Table 2.9. Diagonal distances across the bridging oxygens
Group 2c complexes.

| Substrate | $014-044$ | $042-045$ | $043-046$ | Delta | Ref |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | $\AA$ | $\AA$ | $\AA$ | $\AA$ | 0 |
|  |  |  |  |  |  |
|  | A |  |  |  |  |
| Sodium Propanesulphonate | 8.45 | 8.40 | 8.59 | 0.19 | 96 |
| Sodium Methyl Orange | 8.56 | 8.08 | 8.82 | 0.74 | 96 |
| Potassium Acetate | 8.469 | 8.308 | 8.667 | 0.359 | 2 |
| Potassium Methyl Orange | 8.50 | 8.31 | 8.67 | 0.36 | 96 |
| Sodium Benzene Sulphonate | 8.52 | 8.19 | 8.81 | 0.62 | 96 |
| m-Nitrophenol | 8.444 | 8.346 | 8.745 | 0.399 | 99 |

The hydrogen bonding between the C6A molecules in this group is good, with bonds being formed between the primary and secondary hydroxyl groups in the stack. The hydrogen bonds are direct and do not pass through a water molecule.

In the methyl orange complex the methyl orange anion is longer than the $c$ axis with which it is parallel. This leads to the substrate being disordered, which is readily apparent from the photographs, where there is diffuse streaking superimposed on sharp reflections parallel to the $c$ axis. This suggests an ordered C6A framework, and a disordered substrate, with the disorder being parallel to the $c$ axis. In this case the disorder has been resolved by Harata $\{102\}$. This has been observed for other channel type structures\{94\}.

Group 2d. (No. 28 in Table 2.1)

Again only one complex of this sort has been reported for the C6A system $\{94,95\}$. This complex is in the $\mathrm{P}_{2} 2_{1}^{2} 2$ space group with $\mathrm{a}=\mathrm{b}=$ $19.93 \AA, c=30.87 \AA$. The volume per inclusion complex is $1533 \AA^{3}$. Again there is head to head stacking, but with a more complex packing of the individual stacks. The packing is illustrated in Figure 2.9. The guest molecule in this case is a cadmium polyiodide ion. The diagonal distance between the bridging oxygens is tabulated in Table 2.10 , and the $02-03^{\prime}$ distances between secondary hydroxyls on adjacent residues are shown in Table 2.11.

Table 2.10. Diagonal distance across the bridging oxygens Group 2d complexes.

| $014-044$ | $024-054$ | $034-064$ | Delta |
| :---: | :---: | :---: | :---: |
| $\AA$ | $\AA$ | $\AA$ | $\AA$ |
| $\AA$ | $\AA$ | $\AA$ | $\AA$ |
| 8.45 | 8.42 | 8.65 | 0.23 |
| 8.64 | 8.50 | 8.68 | 0.18 |


| Molecule 1 | 8.45 | 8.42 | 8.65 | 0.23 |
| :--- | :--- | :--- | :--- | :--- |
| Molecule 2 | 8.64 | 8.50 | 8.68 | 0.18 |

Table 2.11. Distance between 02 and 03 on contiguous glucose residues Group 2d complexes.

| $012-023$ | $022-033$ | $032-042$ |
| :---: | :---: | :---: |
| $\AA$ | $\AA$ | $\AA$ |


| Molecule 1 | 2.88 | 2.94 | 2.97 |
| :--- | :--- | :--- | :--- |
| Molecule 2 | 2.98 | 3.00 | 3.10 |



Figure 2.9.

In this group there are strong intermolecular hydrogen bonds between the secondary hydroxyls on adjacent molecules. These bonds are direct. There are four direct inter molecular hydrogen bonds between primary hydroxyl groups, the rest of the hydrogen bonding between the primary hydroxyls is through water molecules.

The Glucose Residue in Cycloamylose Structures.

The average bond angles and distances within the glucose moiety for the C6A methanol complex are displayed in Figure 2.10a. These are close to the Scott and Arnott "Mean Glucose" Figure 2.10b \{104\}. In unsubstituted $\alpha-D-$ glucose the angle $C 3-C 4-04$ is 108.7 , which is close to the tetrahedral angle, and the reduction of this angle to 105.6 in C6A could be due to the steric strain effects caused by the annular structure of the molecule\{85\}. The standard deviations of the bond angles in Figure 2.10a is 0.3 and the standard deviation in bond length is $0.005 \AA$. The standard deviations of the Scott Arnott residue are considerably lower than those quoted above.

There is a small deviation of the atoms $C 2, C 3, C 5,05$ from their least squares plane, typically less than $0.02 \AA$, which shows that the glucose molecules are in the Cl chair configuration\{85\}. Atoms Cl and C 4 are on average $0.675 \AA$ and $0.629 \AA$ on opposite sides of this plane $\{85\}$.


AVERAGE BOND DISTANCES AND ANGLES FOR C6A METHANOL COMPLEX


Figure 2.10 b
SCOTT AND ARNOTT GLUCOSE RESIDUE

The Planarity Of The Glycosidic Linking Oxygens.

The displacement of the bridging oxygens(04s) from their least square best plane is given in Table 2.12.

Table 2.12. Deviation in Angstroms of the 04 atoms from their least squares plane.

| Substrate | 014 | 024 | 034 | 044 | 054 | 064 |
| :--- | ---: | :--- | :--- | :--- | :--- | :--- |
| Water | 0.023 | 0.092 | -0.136 | 0.057 | 0.057 | -0.094 |
| Iodine | 0.001 | 0.166 | -0.193 | 0.047 | 0.123 | -0.144 |
| Methanol | -0.174 | 0.154 | 0.005 | -0.144 | 0.128 | 0.032 |
| Krypton | -0.159 | 0.135 | 0.013 | -0.138 | 0.117 | 0.033 |
| n-Propanol | -0.144 | 0.131 | -0.003 | -0.113 | 0.128 | 0.026 |
| p-Nitrophenol | 0.121 | 0.040 | -0.163 | 0.133 | 0.022 | -0.152 |
| p-Hydroxy Benzoic acid | 0.145 | 0.110 | -0.154 | 0.145 | 0.004 | -0.152 |
| p-Iodoaniline | 0.038 | -0.046 | 0.010 | 0.034 | -0.041 | 0.006 |
| Sodium 1-Propanesulphonate | 0.003 | -0.017 | 0.014 | 0.003 | -0.017 | 0.014 |
| Sodium Methyl Orange | -0.002 | -0.002 | 0.004 | 0.004 | 0.006 | 0.006 |
| Potassium Acetate | 0.116 | -0.116 | 0.000 | 0.116 | -0.116 | 0.000 |
| Potassium Methyl Orange | 0.012 | 0.012 | -0.008 | -0.008 | -0.004 | -0.004 |
| Sodium Benzenesulphonate | 0.024 | -0.012 | -0.013 | 0.024 | -0.012 | -0.013 |

This table shows that there is only slight puckering of the cycloamylose macrocycle. The distance between these oxygens is in the region of 4.5 Angstroms, and the deviation from the plane is not more than 0.16 Angstroms. The substrate has no systematic effect on the puckering of the cycloamylose macrocycle.

Chapter Three The Formation And Crystal Data Of Some C7A Complexes.

Preparation Of Substituted Acetanilides.

Substituted acetanilides were prepared by refluxing the corresponding aniline with acetic anhydride and glacial acetic acid in the established literature manner\{105\}. These acetanilides were recrystallised from toluene three times, and were treated with activated charcoal.

The melting points of the acetanilides that were prepared are listed in Table 3.1 , along with the literature values $\{106\}$. ${ }^{1} \mathrm{H}$ n.m.r. spectra of the $\underline{p}$ and $\underline{m}$-nitro- and the $\underline{p}$-bromo acetanilides were consistent with their structure.

Table 3.1. Literature and observed melting points for the prepared substituted acetanilides.

| Acetanilide | Literature | Observed |
| :--- | :---: | :---: |
| M.P. $\{106\}$ | M.P. |  |
| p-bromo- | $C^{\circ}$ | $C^{\circ}$ |
| m-chloro- | 168 | 168.5 |
| p-chloro- | $72-3$ | 72 |
| ㅍ-nitro- | 178.4 | $178-9$ |
| P-nitro- | 154 | 153 |

The Formation of Crystals Of Cycloamylose Complexes.

There are several ways of forming crystals for crystallographic studies. Cycloamylose complexes are usually formed in aqueous solutions which limits crystal formation techniques to solute diffusion, vapour diffusion, slow evaporation, and the cooling of a saturated solution.

Solute Diffusion.

Cycloamyloses have the ability to complex with almost any small hydrophobic species. The solubility of the cycloamylose complexes are, with few exceptions, much lower than the solubility of the free cycloamylose. The diffusion of the guest molecule across the liquid interface between an ethereal solution of the guest molecule and an aqueous solution of the cycloamylose can produce good crystals, but often forms the cycloamylose ether complex. The crystals formed in this way are usually small\{82\}.

Vapour Diffusion

This technique is only possible where the guest molecules have an appreciable vapour pressure. None of the substituted acetanilides that were investigated had a high enough vapour pressure to successfully use this method. Attempts to form an ethanol complex of C7A in this manner were unsuccessful, the resultant complex having the form of a fine powder rather than single crystals. This method has been successfully used to form the C7A n-propanol complex\{23\}.

A difficulty often encountered in slow evaporation is the tendency for crystals to deposit as a microcrystalline crust on the wall of the container just at the surface of the solution. As the solvent evaporates the solution recedes leaving the crust in a position where it is not effective in inducing good crystal growth\{107\}. This is the problem that was encountered when attempts were made to form cycloamylose complexes by this method.

Slow Cooling of A Saturated Solution.

This is the most commonly used technique to form crystals of cycloamylose complexes. A saturated solution of the cycloamylose guest mixture is prepared at $100 \mathrm{C}^{\circ}$ and this solution is cooled slowly in a large insulated water bath over a period of three days. The substituted acetanilides that were prepared above were complexed with cycloamyloses by the slow cooling technique. Several of these complexes, the $p$-bromo-, $\underline{p}$-chloro-, $\underline{p}$-nitro- and the $\underline{m}$-nitro acetanilide C7A complexes were investigated crystallographically.

Crystal Handling Techniques.

Crystals of cycloamylose complexes decompose if the water of crystallisation is allowed to escape. In order to study the crystal in the X-ray beam special handling techniques had to be used to avoid the breakdown of the crystal lattice.

The standard protein crystallographic technique of sealing the crystal in a fine ( 0.5 mm diameter) Lindemannglass tube, which has a
low absorbtion coefficient for X-rays was used. The system adopted is illustrated in Figure 3.1. Araldite was found to be the most satisfactory sealant at the plasticine end of the tube, as the solvent in plasticine tended to interact with the piceln wax that is normally used to seal the tubes, which resulted in the tube sagging. This was more pronounced in hot weather.


FIGURE 3.1 THE SEALING OF A CRYSTAL IN A LINDEMANN TUBE

Cycloheptaamylose $\underline{p}$-Substituted Acetanilide Complexes.

Cycloheptaamylose is believed to form a $1: 1$ complex with a wide range of organic molecules\{85\}. Saturated equimolar solutions of C7A with various meta- and para- substituted bromo-, chloro- and nitroacetanilides at $100 C^{\circ}$ were prepared and allowed to cool. At this ratio of guest to host it was found that the guest molecule crystallised from the solution and no complex crystals were formed. The proportion of host to guest was increased to $2: 1$ and crystals of complexed C7A were formed. Attempts to form complexes of these guests with C6A failed even at a 10:1 ratio of host to guest. Table 3.2 shows the results of the attempts to form complexes with both C6A and C7A.

Typically 0.1 g . of cycloamylose was used with the corresponding number of moles of the guest molecule. A saturated solution of the complex was then prepared at boiling point. This usually required $2-4 \mathrm{ml}$ for C6A and 6-8 ml for C7A.

Table 3.2. The results of crystallisations with different ratios of guest to host.

Cycloamylose
Ratio of host:guest
p-bromoacetanilide
p-chloroacetanilide
p-nitroacetanilide
m-nitroacetanilide

C7A
$1: 1 \quad 2: 1$
G $\quad$ C
G C
G C
G C

C6A
$1: 1 \quad 5: 1 \quad 10: 1$
G G
G/H
G/H
G G G/H
G G G/H

G - Guest molecule crystals formed
C Complex crystals formed
G/H Both guest and host crystals formed

The rationale behind increasing the guest to host ratio is as follows. In the solution of guest molecule with cycloheptaamylose there is an equilibrium $\{20\}$,

C7A + Guest $\rightleftharpoons$ C7A.Guest - (1)

The equilibrium under normal circumstances lies to the right. There is both free C7A and guest in the solution $\left(K_{\text {diss }}\right.$ is of the order of 0.001 M . typically\{20\}). The solubility of the complex is usually much less than that of the cycloamylose molecule. Acetanilides are insoluble and in this system at $1: 1$ ratio of guest to host the concentration of uncomplexed guest in the solution exceeds the saturation concentration of the acetanilide before the saturation concentration of the complex is reached. This disturbs the equilibrium in equation (1), and the guest molecule is removed from the complex in order to restore the equilibrium. This reduces the concentration of the complex, and thus reduces the probability of the complex forming crystals. At a $1: 1$ ratio of guest to host this process continues until the concentration of the guest is less than its saturation concentration at room temperature. Cooling the solution to $5 C^{\circ}$ did not alter this. The only way of combating this is to force the equilibrium further to the right by increasing the concentration of the cycloamylose.

The problem is more acute for the C6A complexes as the solubility of C6A is ten times that of C7A. The solubility of the complex is usually less than that of the free cycloamylose, and greater than that of the uncomplexed guest. This usually means that the saturation concentration of a C6A complex is several times that of the corresponding C7A complex, and hence the probability of the complex solution
reaching the guest's saturation concentration before the complex's saturation concentration is greater.

The crystals of the $p$-bromo- and the $p$-chloro- acetanilides complexes were clear and colourless. The crystals of the $p$-nitroacetanilide complex(C7APNA) were clear and pale yellow in colour. The size of these crystals ranged from $0.6 \times 0.2 \times 0.2 \mathrm{~mm}$ for C7APNA to $0.4 \times 0.1 \times 0.1 \mathrm{~mm}$ for the C7A p-bromoacetanilide complex(C7APBA) complex. The morphology of the $p$-substituted acetanilide complex is illustrated in Figure 3.2. Crystals of the C7A water complex(C7ANAT) complex were opaque and varied in size up to $3 \times 3 \times 3 \mathrm{~mm}$ The crystal morphology for the C7ANAT complex is shown in Figure 3.3.


FIGURE 3.2 THE MORPHOLOGY OF THE P-SUBSTITUTED ACETANILIDE COMPLEXES.


FIGURE 3.3 THE MORPHOLOGY OF C7ANAT CRYSTALS

Chemical Analysis Of The C7APNA Complex.

The calculated and observed analysis figures for the C7APNA complex are shown in Table 3.3. The crystals were dried in vacuo for 24 hours at $80 \mathrm{C}^{\circ}$.

Table 3.3. Chemical analysis of the C7APNA complex.

|  | Calculated <br> C7APNA. | Calculated <br> C7APNA. $5 \mathrm{H}_{2} \mathrm{O}$ | Observed |
| :--- | :---: | :--- | :---: |
|  | $\mathrm{C}_{50} \mathrm{H}_{78} \mathrm{O}_{\mathbf{3 8}} \mathrm{N}_{2}$ | $\mathrm{C}_{50} \mathrm{H}_{88} \mathrm{O}_{43} \mathrm{~N}_{2}$ |  |
| C\% | 45.66 | 42.73 | 42.65 |
| $\mathrm{H} \%$ | 5.93 | 6.26 | 6.11 |
| N\% | 2.13 | 1.99 | 1.95 |
| N:C | $1: 21.43$ |  | $1: 21.87$ |

The ratios of nitrogen to carbon for the calculated and observed analyses are, to within experimental error the same. This ratio is a more sensitive indication of the degree of complexation than a direct comparison of the observed and calculated percentages of carbon, nitrogen and oxygen, as this ratio is independent any water of crystallisation that may be present. Residual water of crystallisation would have the effect of lowering the observed nitrogen and carbon percentages. Calculations show that there are probably five water molecules per cycloamylose complex still present in the 'dried' sample.

Crystal Data For The C7APNA And C7APBA Complexes.

Precession and de Jong Bouman photographs were taken of these crystals in order to determine their space group and cell dimensions. The C7APNA crystals, which were the largest, diffracted strongly out to the Weissenberg limit with Cu radiation. The C7APBA crystals diffracted to $a \sin Q / l a m b d a$ of 0.45 . The C7APCA crystals ceased to diffract after approximately 24 hours in the X-ray beam, and a full set of cell dimensions was not collected. The zones that were photographed had identical reciprocal dimensions to the C7APBA and C7APNA complexes. The cell dimensions of the C7APNA and C7APBA are tabulated in Table 3.4.

Table 3.4. Crystal data for the C7APNA and C7APBA complexes. (Cell edge lengths in Angstoms.)

| Complex | C7APBA | C7APNA |
| :--- | :--- | :--- |
| a | 15.17 | $15.20(0.04)$ |
| b | 15.58 | $15.67(0.05)$ |
| c | 15.73 | $15.70(0.04)$ |
| Alpha | 88.17 | $87.69(0.48)$ |
| Beta | 97.88 | $98.15(0.15)$ |
| Gamma | 103.46 | $103.18(0.38)$ |
| Space Group | Pl | P 1 |
| Z | 2 | 2 |
| Radiation | Cu | $3604 \AA^{3}$ |
| Cell Volume | $3579 \AA^{3}$ | 1.33 |

The cell lengths for the C7APBA crystal were measured from precession photographs, and the interaxial angles were determined from both precession and de Jong-Bouman photographs. These dimensions have an error of $0.3-0.4 \%$, which is the result of a $0.1 \%$ error in the meas-
urement process, the remainder being systematic errors, such as thickness of film, incorrect crystal to film distance, and film shrinkage $\{108\}$. The cell lengths for the C7APNA complex were taken from data that was determined during the scanning of the intensity films(see later in this chapter). The figures in parentheses after the crystal data are the standard deviations of the measurements made during the scanning of the films.

In the light of the preliminary investigations, a data set was collected for the p-nitroacetanilide complex. The intensity data were recorded on an equi-inclination Weissenberg camera. Layers hk0-hk; $\overline{12}$ and h01-h31 were collected and scanned by the S.R.C. microdensitometer service then at Chilton, which has subsequently moved to Daresbury.

The Scanning of The Weissenberg Intensity Films.

The films were scanned by an Optronics Photoscan P1000 rotating drum scanner. The optical density measurements were made on a $100 \mu \mathrm{~m}$ raster. The top film of each pack is pre-scanned to locate regions of optical density significantly higher than the background level, and to separate genuine reflections from background or other spurious regions of high density. The film is then rescanned carefully, and the optical densities are integrated to obtain the reflection intensities. Subsequent films in the pack have a different prescan using information calculated during the top film's prescan.

The reflections are then assigned indices. The main disadvantage of this method of scanning films is that weak and less than reflections are not located. However, this method copes well with photographs taken from a poorly aligned crystal.

The interlayer scaling and the Lorentz polarisation corrections were determined by the Shelx program suite \{109\} , ~ w h i c h ~ c a n ~ also do linear absorbtion corrections. Linear absorbtion corrections were not carried out on any of the C7A complexes that were scanned at Chilon, as none of these complexes contained atoms heavier than oxygen.

This program produced and refined cell dimensions which were measured from the film for each layer. A mean of this data was taken, and these are the cell dimensions for the C7APNA complex that are tabulated in Table 3.4.

5500 unique reflections were measured. The highest
observed $\sin \theta / \lambda$ for a reflection $w$ as 0.63 . The number of observed reflections is 0.6 of the possible maximum, but most of the unobserved reflections were at $\operatorname{ligh} \sin \theta$

The Complex of C7A With Water.

A saturated solution of cycloheptaamylose $\left(\begin{array}{lll}\mathrm{C}_{42} & \mathrm{H}_{70} & \mathrm{O}_{35}\end{array}\right)$ at 100 $C^{\circ}$ was cooled slowly and large opaque white crystals were formed. Intensity data were recorded photographically using a Heisenberg camera with nickel filtered Cu radiation. Layers hk0-hk12 and h01-h91 were photographed, and the films were scanned by the S.R.C. microdensitometer service. The cell dimensions that were determined by the scanning process were averaged, and the standard deviations calculated. These are tabulated, along with the cell dimensions that other workers have found $\{110\}$, in Table 3.5. All of the workers found that the crystals were in the $\mathrm{P} 2{ }_{1}$ space group.

Table 3.5. Measured and reported cell dimensions for the C7ANAT complex. (Cell edge lengths in Angstroms.)
Observed D. French D. Rhorer H. Zacharias F. Takesagua Iowa Pittsburgh New Orleans Pittsburgh.

| a | $20.88(0.06)$ | 20.93 | 20.776 | 21.140 | 20.890 |
| :--- | ---: | ---: | ---: | ---: | ---: |
| b | $10.17(0.07)$ | 10.24 | 10.169 | 10.236 | 10.125 |
| c | $15.12(0.04)$ | 15.27 | 15.106 | 15.109 | 15.096 |
| Beta $110.3(0.4)$ | 112.0 | 110.7 | 111.88 | 111.16 |  |
| Volume 3011 | 3034 | 2985 | 3033 | 2977 |  |
| Z | 2 | 2 | 2 | 2 | 2 |

[^3]The Complex of Cycloheptaamylose With Iodine.

The slow cooling of a saturated solution of cycloheptaamylose and iodine in a $1: 2$ molar ratio from $100 C^{\circ}$ produced complex crystals (C7AI). The crystals were dark brown in colour, similar to the C6A iodine complex\{82\}. The data were collected on a Nonius Enraf CAD-4 four circle diffractometer, using zirconium filtered molybdenum radiation. Preliminary precession and de Jong-Bouman photographs indicated a centered cell(C2). Table 3.6 shows the cell dimensions that resulted from the least squares refinement of 13 reflections.

Table 3.6. Crystal data for the C7A iodine complex. (Cell edge lengths in Angstroms.)
a
$19.532(0.007)$
b
24.499(0.007)
c. 15.744(0.006)

Alpha . 90.00
Beta 109.40 (0.03)
Gamma 90.00
Cell Volume $\quad 7106 \AA^{3}$
Z 4
Space Group $\mathrm{P}_{2} 1$

Table 3.7. Reflection statistics for the C7A iodine complex.

Number of Reflections.
Sigma>F 852
3Sigma>F>Sigma 899
F>3 Sigma 984

The Complex of Cycloheptaamylose With m-Nitroacetanilide.

Crystals of the cycloheptaamylose m-nitroacetanilide complex (C7AMNA $\mathrm{C}_{50} \mathrm{H}_{78} \mathrm{O}_{38} \mathrm{~N}_{2}$ ) were prepared by the slow cooling of saturated solution of guest and host in a $1: 2$ ratio from $100 \mathrm{C}^{\circ}$. These crystals were not easily formed, and several attempts varying the ratios of guest to host from $1: 1.5$ to $1: 2.5$ were required to grow crystals of a suitable size for X-ray analysis.

Data were collected on a Nonius Enraf CAD-4 four circle diffractometer using zirconium filtered Molybdenum radiation. Data was collected in the theta range $1-18^{\circ}$. Preliminary de Jong-Bouman and precession photographs indicated that the crystal was in the monoclinic P2, system. The cell dimensions are tabulated in Table 3.8. The crystal did not diffract well and there was an indication of twinning, the result of this was that each reflection was composed of three reflections that could not be resolved. An omega scan somewhat reduced this problem, as the two smaller peaks were not scanned when this scan mode was used.

Table 3.8. Crystal data for the C7AMNA complex. (Cell edge lengths in Angstroms.)
a 19.519 (0.015)
b $\quad 24.215(0.009)$
c $\quad 15.810(0.008)$
Beta 108.82 (0.06)
Volume $\quad 7075 \AA^{3}$
Z 4

Space Group P2 1

The Solution Of The Cyclohexaamylose Structures

The first cyclohexaamylose structure to be determined was that of the potassium acetate complex\{2\}. The structure was solved by a combination of Patterson methods and a suitably generated starting model. The uv0 section of the Patterson map was found to have a sixfold arrangement of peaks similar to that expected from a benzene molecule, which is consistent with the observation that the C6A molecule is to a first approximation hexagonally symmetric. A molecule of C6A was generated from a set of glucose coordinates arranged hexagonally with the linkage that had been determined chemically, and with the 02-03' distance between adjacent glucoses set at $2.85 \AA$. This model was then placed in the orientation indicated by the Patterson map, and the structure then refined relatively smoothly.

The first group la structure was solved using the heavy atom method. The iodine complex of $C 6 A$ was formed, and the structure was solved by difference maps phased on the iodine atom positions. This took a number of years, the first data set being reported in $1957\{111\}$, and the final structure not being reported until $1972\{88\}$. After this all of the group lastructures were treated as being isomorphous with the iodine complex, and simply refined using the least squares method, usually omitting the primary hydroxyl groups\{87\}. The first structure in each of the other groups was solved using the heavy atom method, and subsequent complexes in that group were solved on the basis that the C6A molecules were isomorphous.

The Solution Of The Cycloheptaamylose $p$-Nitroacetanilide complex.

The solution of a structure of the size of C7APNA is complex. An ab initio solution by direct methods is not feasible at the present moment due to the large number of non hydrogen atoms in the unit cell (approx. 180), and the absence of a heavy atom. Isomorphous replacement initially looked promising, as it is feasible to replace the nitro group on the acetanilide by a bromine, and this complex is in fact isomorphous (Table 3.1). There is the possibility that the substrate may be sevenfold disordered, each of the possible positions having a weight of 0.14 . Such a disorder has been reported for a C7A complex\{22\}. The fact that the photographs of the p-nitroacetanilide extend out to a much higher $\sin \theta / l a m b d a$ than those of the $\underline{p}$-bromoacetanilide complex suggests that there is some disorder. The bromine atoms would certainly have a high temperature factor. The iodine atoms in the C6A iodine complex\{88\} and the iodine atom in the C6A p-iodoaniline complex\{96-8\} both have higher temperature factors than any other atom other than the oxygens in the primary hydroxyl groups. This and the potential disorder of the substrate would dramatically reduce the efficacy of the isomorphous replacement technique.

The principal method of structure solution that remains is the trial and error translation and rotation of a model of the structure in the unit cell. On the basis of a C6A molecule having a diameter of $13.5 \AA$ the diameter of the C7A molecule was calculated to be 15 A . The cell edges of the triclinic cell fall between 15.2 and $15.7 \AA$ (see Table 3.1) which gives no direct indication of the packing of the C7A molecule. The fact that there are two unrelated molecules of the complex in the unit cell further complicates the solution of the structure. Two pieces of experimental data greatly reduced the complexity of the problem.

The first observation was that the precession photographs of the hk0 zone showed a cell having pseudo C2 symmetry, with the interaxial angle close to, but not equal to $90^{\circ}$, and the breakdown of the mm symmetry only occuring at higher values of $\sin Q$. The relationship between the two cells is shown in Figure 4.1 , $a$ and $b$ refer to the triclinic cell, and $a^{\prime}$ and $b^{\prime}$ refer to the pseudo monoclinic cell. The cell dimensions that have been calculated for the pseudo centred cell are shown in Table 4.1, and these are compared with the cell dimensions that have been reported for the complex with $\underline{m}$-bromobenzoic acid\{112\}, which is in the space group C 2 .


Figure 4.1 Pseudo Centred Cell Of C7APNA
Table 4.1. Cell dimensions of the pseudo centred cell of C7APNA. (Cell lengths in Angstroms.)

| Triclinic | Pseudo Centred | C2 cell $\{112\}$ |
| :--- | :---: | ---: |
| C7APNA | C7APNA | m-Bromobenzoic acid |


| a | 15.20 | 19.1 | 19.23 |
| :--- | :--- | :--- | :--- |
| b | 15.67 | 24.1 | 24.58 |
| c | 15.70 | 15.7 | 15.80 |
| Alpha | 87.69 | 91.8 | 90.00 |
| Beta | 98.15 | 96.6 | $109.5 \rightarrow$ |
| Gamma | 103.18 | 94.6 | 90.00 |

The most informative and direct indication of the packing of the C7A molecules came from the Patterson map generated from the Patterson series

$$
P(x, y, z)=1 / V \sum \sum \sum|F h k 1|^{2} \operatorname{Cos}(2 \pi(h x+k y+1 z))
$$

h k 1

The Ovw section of the map is shown in Figure 4.2 . The peaks have a considerable weight, the peak heights being 99 at the origin, 16 at the $2.5 \AA$ vector and 11 at the $8 \AA$ vector. As there are no heavy atoms in the structure these peaks must be the result of a vector which occurs often in the structure, a high multiplicity vector.


There is such a vector in the glucose molecule with a high multiplicity. This vector is illustrated in Figure 4.3. A model of the C7A molecule was examined and it was found that the $2.5 \AA$ vectors were almost parallel to the C7A macrocycle's sevenfold axis. As this vector is parallel to the sevenfold axis an approximate orientation for the host molecule with respect to the unit cell axes $c$ an been determined. This peak was reported for the C6A potassium acetate structure, $2.35 \AA$ \{2\} from the origin.


THE PEAR HEIGHT AT THE ORIGIN OE THE YATTERSON FUNCTION IS SET AT QS, CONTOUR INTERVAL LEVELS APE SET AT 5,20 , AND 15 WNTTS

Figure 4.2

The approximate height of the C7A molecule has been calculated as approximately $7.0 \AA\{46\}$, so it is quite reasonable to expect that the $8 \AA$ peak is the result of the C7A-C7A vector. This shows that the C7A molecules are stacked in a channel type structure, similar to the group two structures. The spread of the $8 \AA$ vector peak also suggests a two fold relationship between the C7As as the direct stacking of one C7A on top of the other would result in a much less diffuse peak, and would affect the intensities of the $1=2 n+1$ reflections.

The Generation Of A Model Of Cycloheptamylose.

Cycloamyloses are comparitively rigid molecules by virtue of hydrogen bonds between the secondary hydroxyl groups (see Chapter 2). The hydrogen bonds in the C7A molecule have been shown to be stronger than those in the C6A molecule by n.m.r.\{25\}. The average length of the hydrogen bond in C6A was estimated as $2.85 \AA$ by Sundararajan and Rao \{17\}. They suggested in the same paper that a reasonable distance for the hydrogen bonds in C7A would be 2.70 A . The knowledge of the hydrogen bond length greatly increases the accuracy of the model by restricting he configurations that the cycloamylose molecule can adopt, and thus simplifies the generation of such a model.

The first data required to generate a model of a cycloamylose molecule is a set of the glucose molecule's molecular coordinates. In chapter 2 it was shown that there is a distortion of each of the glucose rings from the ideal geometry as suggested by Arnott\{104\}, where these residues go together to form a C6A molecule. An average of the coordinates of each of the glucose rings in the C6A n-propanol, and the C6A iodine structures was taken, as these were, at the time, the best structure determinations. This model would take into account the gg gt
configurational change that can occur in the primary hydroxyl groups, as the model would have its primary hydroxyl groups in a position intermediate between the $g g$ and the gt configuration, thus allowing them to move into the correct conformation in later refinement. A diagram of the average glucose residue is shown in Figure 4.4 .


This glucose residue was then built up into a C7A molecule by assuming sevenfold symmetry. The distances between the 02 s and the 03 s on adjacent glucoses was set at $2.75 \AA$, which is longer than was predicted theoretically $(2.70 \AA\{17\})$, and considerably shorter than the average $02-03^{\prime}$ distances for C6A(chapter 2). The C7A molecule that was generated in this way is illustrated in Figure 4.5 , and the coordinates are tabulated in Table 4.2.

Figure 4.5 Model of the $67 A$ molecule.


|  |  |  |  |  | 042 | -1.10 | $-6.35$ | -2.03 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | C11 | -1.75 | 5.74 | 0.2 .8 | 043 | -3.64 | -4.98 | -2.018 |
|  | C12 | -0.97 | 6.27 | -0.87 | 044 | -3.84 | -3.06 | -0.00 |
|  | C13 | 0.35 | 5.45 | -1.11 | 045 | -1.6.6 | -5.63 | 1.45 |
|  | C14 | 1.10 | 5.40 | 0.21 | 046 | -4.08 | -5. 14 | 2.93 |
|  | C15 | 0.24 | 4.97 | 1.37 | C51 | 4.07 | -4.41 | 0.28 |
|  | C16 | 0.92 | 5.11 | 2.73 | C52 | 3.60 | -5.23 | -0.87 |
|  | 012 | -1.77 | 6.20 | -2.03 | C53 | 2.05 | -5.06 | -1.11 |
|  | 013 | 1.12 | 6.06 | -2.08 | C54 | 1.35 | -5.34 | 0.21 |
|  | 014 | 2.13 | 4.42 | -0.00 | C55 | 1.94 | -4.58 | 1.37 |
|  | 015 | -0.95 | 5.79 | 1.45 | C56 | 1.39 | -5.00 | 2.73 |
|  | 016 | 1.45 | 6.40 | 2.93 | 052 | 4.28 | -4.82 | -2.03 |
|  | C21 | -5.50 | 2.21 | 0.28 | 053 | 1.62 | -5.95 | -2.08 |
|  | C 22 | -5.51 | 3.15 | -0.87 | 054 | -0.00 | -4.91 | -0.00 |
|  | C23 | -4.04 | 3.67 | -1.11 | 055 | 3.37 | -4.80 | 1.45 |
|  | C24 | -3.54 | 4.23 | 0.21 | 056 | 1.47 | -6.39 | 2.93 |
|  | C25 | -3.74 | 3.29 | 1.37 | C61 | 5.98 | 0.43 | 0.28 |
|  | C26 | -3.42 | 3.91 | 2.73 | C62 | 6.33 | -0.45 | -0.0.7 |
|  | 022 | -5.95 | 2.48 | -2.03 | C63 | 5.23 | -1.5.5 | -1.11 |
|  | 023 | -4.04 | 4.66 | -2.08 | C64 | 5.02 | -2.27 | C. 21 |
|  | 024 | -2. 13 | 4.42 | -0.00 | C65 | 4.79 | -1.34 | 1.37 |
| $\infty$ | 025 | -5.12 | 2.87 | 1.45 | L66 | 4.78 | -2.03 | 2.73 |
| 1 | 026 | -4.10 | 5.12 | 2.93 | 062 | 6.44 | 0.35 | -2.03 |
|  | C31 | -5.20 | -2.98 | 0.28 | 063 | 5.6.6 | -2.44 | -2.08 |
|  | C32 | -5.90 | -2.34 | -0.87 | 064 | 3.84 | -3.06 | -0.00 |
|  | C33 | -5.39 | -0.87 | -1.11 | 065 | 5.85 | -0.36 | 1.45 |
|  | C34 | -5.51 | -0.13 | 0.21 | 066 | 5.92 | -2.84 | 2.93 |
|  | C35 | -4.90 | -6.87 | 1.37 | C71 | 3.39 | 4.94 | 0.30 |
|  | C36 | -5.19 | -0. 2.4 | 2.73 | C72 | 4.30 | 4.67 | -0.87 |
|  | 032 | -5.65 | -3.11 | -2.03 | C73 | 4.48 | 3.12 | -1.11 |
|  | 033 | -6. 16 | -0. 26 | -2.08 | C74 | 4.91 | 2. 51 | 0.21 |
|  | 034 | -4.79 | 1.09 | -0.00 | C.75 | 4.04 | 2.91 | 1.37 |
|  | 035 | -5.43 | -2.21 | 1.15 | C76 | 4.57 | 2.47 | 2.73 |
|  | 036 | -6.56 | -0.01 | 2.93 | 072 | 3.74 | 5.25 | -2.03 |
|  | C41 | -0.91 | -5.93 | 0.2 .8 | 073 | 5.44 | 2.91 | $-2.08$ |
|  | C42 | -1.85 | -6.07 | -0.87 | 074 | 4.79 | 1.09 | -0.00 |
|  | C43 | -2.68 | -4.76 | -1.11 | 075 | 3.93 | 4.35 | 1.45 |
|  | Cく4 | -3.33 | -4.39 | C. 21 | 076 | 5.91 | 2. 1.6 | 2.93 |
|  | $\mathrm{C}, 5$ | -2.37 | -4.37 | 1.37 |  |  |  |  |
|  | Lid | -3.05 | -4.21 | 2.73 |  |  |  |  |

The Solution Of The Structure By Trial And Error Methods.

This model of the C7A molecule was combined with the data derived from the Patterson map to form the starting point of a systemmatic search of the unknown parameters that would specify the C7A molecules' positions in the unit cell, and their orientations with respect to one another.

The orientation and position of a rigid body with respect to a given origin and axial system can be specified by six parameters. These are the three translational parameters $T x, T y, T z$, where the subscript refers to the direction of the translation, and $R x, R y, R z$, the three rotational parameters, the rotations being about $x, y$ and $z$ (as illustrated in Figure 4.6).

The origin of the unit cell in Pl is not specified by the symmetry elements of the space group, and an adjustment of all of the atomic coordinates by $x / a, y / b, z / c$, simply alters the phase values for all of the reflections by an amount which depends on $x y z$ and hkl. As the origin is indeterminate in Pl the translational parameters required to locate the centre of gravities of two objects is reduced to three, $T x, T y, T z$, where $T x$ is the separation of the centres of gravity in the $x$ direction, Ty their separation in the $y$ direction, and $T z$, their separation in the $z$ direction. Each rigid body has three rotational parameters that define its orientation with respect to the axial system. Therefore to uniquely define two rigid bodies in the Pl space group nine parameters must be specified,(Tx,Ty,Tz,Rx1,Rx2,Ryl,Ry2,Rzl,Rz2). The model that has been generated of the C7A molecule is treated as a rigid body.

Each molecule was positioned with its centre of gravity on the origin before the rotations were carried out. The rotations were carried out on the molecule as illustrated in Figure 4.6. The Rz rotation was the first rotation that was applied, followed by Ry and Rx.


Figure 4.6 The axes of the C7A model that were used for the rotations and translations.

The Patterson map suggests that, for the orthogonal coordinate system illustrated in Figure 4.6 the rotations $R x 1=R x 2$, and Ryl $=$ Ry2 due to the sharpness of the $2.5 \AA$ peak, and the absence of any other peak in the $2-3 \AA$ region of the vector map. There is one ambiguity that can not be resolved directly from the Patterson, namely the method of stacking. This means that Rxl can be equal to Rxl or $\mathrm{Rxl}+180$. Therefore the number of totally unknown parameters is reduced to two ( $\mathrm{Rz} 1, \mathrm{Rz} 2$ ), which are the rotations about the sevenfold axis. As the model is sevenfold symmetrical the values of these rotations need only be explored in the range $0-2 \pi / 7$. There is also the parameter Rxl, which has one of two possible values. There are two enantiomeric possibilities, corresponding to a C7A molecule made up of $D$ - and $L$ glucoses. These two are non-equivalent, and both of these possibilities were searched.

Using a low resolution data set ( 50 selected high and low E reflections with a $\sin \Theta / l a m d a<0.2$ ) an $R-f a c t o r ~ s e a r c h ~ o f ~ t h e ~ t w o ~ r o t a-~$ tions in the range $0-51^{\circ}$ was carried out. The values of Rzl and Rz2 were altered in five degree increments. The results are shown for the head to head, stacking in Figure 4.7. Similar calculations were carried out for head to tail stacking, but the $R$-factors were much higher. At $\mathrm{Rzl}=21^{\circ}$ and $\mathrm{Rz} 2=14^{\circ}$ there is a large minimum in the map, which corresponds to the most likely solution of the structure. This region was then searched on a finer grid, allowing the other seven parameters to vary until a minimum was reached.

At this stage higher resolution data was introduced (500 reflections with $\sin \Theta / l a m d a<0.25)$, and the refinement process about the minimum was then repeated. The final $R$-factor was 0.43 for 500 reflections.

This model of the structure was not sufficiently accurate to allow a meaningful least squares refinement of the atomic parameters. The step refinement program in the X-ray program package\{113\} was used at this stage to further refine the atomic coordinates. After several cycles of refinement this program reduced the model to an almost unrecognisable collection of atoms. Thus step refinement was alternated with Modelfit\{114\}, which is a program that takes rough model coordinates and restores them to a stereochemically more reasonavle representation of the C7A molecule. The resolution was increased again at this stage to 1500 selected high and low E reflections with a $\sin Q /$ lamda <0.3. The resulting $R$-factor for 1500 reflections was 0.30 .

The alternation of refinement with the fitting of the refined coordinates to a reasonable geometry was continued using least squares refinement instead of step refinement. During these cycles of refinement
the resolution was increased to include 3000 reflections with $\sin \Theta / l a m d a$ of less than 0.3 , and the $R$-factor was reduced to 0.28 . At this stage difference maps were used to locate the first of the p-nitroacetanilide molecules, along with 18 water molecules. Further cycles of modelfit and least squares refinement reduced the $R$-factor to 0.23 , when a careful study of the difference maps revealed the second p-nitroacetanilide molecule. Up to this stage an overall temperature factor had been used.


Chapter 5 Attempts to solve the C7ANAT structure.

The solution of the C7APNA complex had been greatly facilitated by the information gathered from the Patterson map. As the C7A molecule in C7ANAT is likely to be similar in shape to the C7A molecule in C7APNA the Patterson map was calculated. The Patterson function for C7ANAT has four symmetry related peaks, at $2.5 \AA$. In Chapter Four these peaks were shown to define the orientation of the C7A molecule in the unit cell. These peaks are shown in Figure 5.1 (the contours are in 5 unit intervals). The origin peak is 120 units high. The alignment of the C7A molecule along this vector confirms the reports that the C7ANAT structure has a herringbone packing arrangement $\{110\}$, around the 2 , axis in a manner similar to the Group la structures of the C6A system, but with a different space group and cell dimensions.


Figure 5.1.

There are several large peaks in the $u 0 \mathrm{w}$ section of the Patterson map, Figure 5.2, but these could not be ascribed to structural or packing features that one would expect to find in this section. The Harker section (u0.5w illustrated in Figure 5.2) could not be analysed in terms of a projection of the structure.

In order to define a rigid body in space with respect to an axial system, six parameters must be specified (Tx,Ty,Tz,Rx,Ry,Rz). The

translations $\mathrm{Tx}, \mathrm{Ty}$ and Tz are the translations along the cell edge of the centre of gravity of the model, and $R z$ is the rotation of the C7A model about its sevenfold axis. Ry and $R x$ are the two other rotations orthogonal to this. In the space group $P 2_{1}$, a translation up the $y$ axis(Ty) has no effect on the magnitude of the structure factor; i.e. there is no need to vary Ty. The Patterson map reduces to four the number of possible values of Rx and Ry. These are illustrated schematically in Figure 5.3. The space group symmetry reduces these four possibilities to two as 1 and 2 are equivalent as a result of the screw axis as are 3 and 4. The head of the arrow is at the secondary hydroxyl end of the molecule, and the primary hydroxyl end of the molecule lies closer to the origin. These two non equivalent possible orientations of the C7A molecule will subsequently be referred to as the 'up' and the 'down' orientations.


FIGURE 5.3 FOUR POSSIBILITIES FOR THE ALIGNMENT OF THE C7A MOLECULE

These two orientations also cover all of the enantiomorphic possibilities, as a molecule of C7A made up of L-glucoses lying along vector three is exactly equivalent to a molecule of C7A made up of D-glucoses lying along vector one. This means that the six parameters aforementioned are reduced to three $(\mathrm{Tx}, \mathrm{Tz}, \mathrm{Rz})$, making a translation rotation search feasible.

The Translation Rotation Search.


#### Abstract

The previous section analysed the parameters that must be explored in a translation/rotation search. Before carrying this out, however, a re-examination of the validity of the starting model is required. The model of C7A, which was generated with the sevenfold sy_mmetry should bear a reasonable resemblance to the C7A molecule in a complex. In Chapter 2 it was shown that the 'empty' C6A (a C6A molecule complexed with water) molecule is much more distorted than the C6A molecule with a non aqueous guest. This distortion of the host molecule is likely to be repeated in the C7A system. N.m.r. evidence\{40\} has shown that the conformation of the C7A molecule changes to accommodate the guest. There is no way that the distortion can be estimated or incorporated into a model. For this reason no alterations to the model that was used in the C7APNA system could be made prior to using the model for the translation/rotation search of C7ANAT, as no significant improvement could be made to this model.


A low resolution data set was used, because the objective of the translation/rotation search was to locate the position of the C7A molecule, and not to resolve molecular details. Fifty reflections with a sin$⿴ /$ /lamda < 0.15 were used for the search. Translation parameters were searched on a $0.5 \AA$ raster within the area $x=0-0.5 a$ and $z=0-0.5 c$, and the rotation about the ring axis Rz was searched in $5^{\circ}$ intervals in the range $0-50^{\circ}$, as the model is sevenfold symmetric (Chapter Four). In order to check both the 'up' and the 'down' orientations of the model a total of 4600 structure factor calculations had to be performed.

The volume of calculation necessitated that a specific structure factor calculation program be written both to generate the displaced and rotated model and calculate the structure factors. Instead of calculating conventional R-factors the following correlation factor was measured in order to reduce the complexity of the calculation.

Correlation Factor $=\frac{\sum\left|F_{0}^{2}-F_{c}^{2}\right|}{\sum F_{0}^{2}}$

The results of this search were mapped out for each value of Rz , thus each map contained a plot of the correlation factors for $a=0$ - 0.5 and $c=0-0.5$. An example of one of these plots is given in Figure 5.4. there were over 100 minima located in the search. The correlation factor has been multiplied by 100 , with the contour intervals being at 5 unit intervals. Each of these minima were then searched on a finer grid ( $0.1 \AA$ in translation and $1^{\circ}$ in Rz ). This reduced the number of minima to 15 with a correlation factor of 0.55 or less. A conventional $R$-factor was calculated for each of these, and the $R$ factors varied between 0.36 and 0.32 .

Attempts to further refine these 15 possibilities using the rigid body refinement routine in the $X$-Ray System $\{113\}$, using data sets of slowly increasing resolution failed, due to the inadequacy of the initial model. The distortion of the C7A macrocycle from heptagonality, probably due to the opening out of the glucose residues caused by the primary hydroxyl groups altered conformation is the most likely difference between the model and the real structure.


Other Attempts To Solve The C7ANAT Structure.

The difficulties encountered in the translation/ rotation search lead to a consideration of other methods that might give information about the translational parameters of the model in the ac plane of the unit cell. Any direct information about Tx and Tz would greatly simplify the problem.

The translation of a model of C7A orientated with the correct values of $R x$ and Ry along the a axis will have no effect on the magnitude of the calculated structure factors of the $0 \mathrm{kl}, 0 \mathrm{kO}$, and the 001 reflections. The only factor that influences the magnitude of the h00 reflections is the displacement of the correctly orientated model along the a axis. The displacement of the molecule along $c$ has no effect on the h00 reflections. Thus a structure factor search using only the h00 reflections and translating the model along the a axis should indicate likely values of $T x$. A similar calculation on $T z$ and the 001 reflections should indicate plausible values for Tz . This search was carried out for both the 'up' and the 'down' models, with two different values of $\mathrm{Rz}\left(0^{\circ}\right.$ an $25^{\circ}$, SEE NDTE ON PAGE 95. and $25^{\circ}$ ) . The results of this search are shown in Figures 5.5. These translational minima were then searched thoroughly using a low resolution data set. Again none of these trial structures refined.

The Difference Patterson Function

The Patterson map contains peaks that correspond to the vector displacements of every atom in the unit cell from every other atom in the unit cell. Where there is a rotational symmetry element in the unit cell, the vectors can be separated into two types, the intramolecular vectors, vectors between atoms within the molecule, and intermolecular vectors, vectors between atoms in symmetry related molecules.

FIGURE 5.5


FIGURE 5.5
Translation of the model of C:7A along the a axis. $R$-Fartor for the hoo reflections vs displacement
along a. - I. 1somer




The C7A molecule is a rigid species, whose geometry is well
known. The orientation of the molecule has been determined from the Patterson map. The rotation about the $z$ axis $(\mathrm{Rz})$ and the displacement of the molecule with respect to the screw axis are unknown. If a model of the C7A molecule is placed in the unit cell with a correct orientation, but an incorrect displacement, then the intramolecular vectors of the model and the real structure should be identical. The subtraction of these vectors should leave a Patterson map with intermolecular vectors only.

$$
\begin{equation*}
P_{(x y z)}=1 / v \sum \sum \sum\left[F o^{2}-F c^{2}\right] \cos (2 \pi(h x+k y+1 z)) \tag{1}
\end{equation*}
$$

$x$ y $z$
The difference Patterson function, equation 1 , is extensively used in protein crystallography to locate the heavy atom positions \{115\}. The importance of this technique in this field has long been realised. Rotation and translation searches of unit cells using Patterson type functions have also been attempted, but so far have only succeeded in solving simple structures $\{116-9\}$.

Figure 5.6 shows the situation that arises when a trial structure is put into the unit cell in the wrong position in the unit cell with respect to the screw axis. The diagram is a projection onto a plane normal to the 21 axis, $X$ is the position of the centre of gravity of the molecule. $T$ and $T^{\prime}$ refer to the true position of the molecule, $T^{\prime}$ being related to $T$ by the screw axis. $F$ and $F^{\prime}$ are the trial input positions of the molecule. Assuming that the model of the C7A molecule is relatively accurate, then the intramolecular vectors that arise from the model will be close to those of the true structure.

The intermolecular vectors are different and are known for the model structure, and unknown for the true structure. The difference vector map should contain information about the relative displacements of the two molecules.


FIGURE 5.6 THE DISPLACEMENT OF THE CENTRE OF GRAVITY OF A MOLECULE WITH RESPECT TO THE ORIGIN

The difference map should contain a peak at two delta, where delta is the separation of the true and false molecules. This is because vectors between the atoms in symmetry related molecules in the correct structure will differ by two delta from the corresponding vector in the false molecule. As the vertical displacement is 0.5 b in both cases this peak should appear on the $v=0$ section, at two delta from the origin.

The C6A DMSO complex $\{84\}$ is in the same space $\operatorname{group}\left(P 2_{1}\right)$ as the C7ANAT complex, and for this reason served as a convenient test of the validity of this technique. Observed structure factors were generated from the coordinate list, and the structure factors were calculated for reported coordinates displaced by a fixed amount, as shown in Figure 5.7. The difference Patterson map was calculated using $\left|F_{0}^{2}-F c^{2}\right|$ as the coefficients in the Patterson synthesis. The results are shown in Figure 5.8.


FIGURE 5.7 DISPLACEMENT OF THE C6ADMSO COMPLEX
WITH RESPECT TO THE ORIGIN.


FIGURE 5.8 DIFFERENCE MAP FOR DISPLACED DMSO COMPLEX

For the test case this method shows the displacement of the trial structure from the real one. There is however an ambiguity caused by the centre of symmetry in the Patterson map, but this can be resolved by generating a second map from a second set of coordinates displaced from the first by a known amount. The origin peak is 15 units high, and the depth of the hollow is 7 units. There is no other peak of a comparable size in the map.

This technique was applied to the C7ANAT structure. Structure factors were calculated for two different displacements of each of a number of trial orientations of the C7A molecule with respect to the origin. Both the 'up' and 'down' orientations of the C7A molecule had Rz varied in $5^{\circ}$ intervals in the range $0-50^{\circ}$ to generate the trial orientations. A difference Patterson map was calculated for each of the possible configurations detailed above, and no peaks were consistent with the known displacement of the C7A molecule appeared on the two maps.

The only reason that can explain the failure of the above attempts to solve the C7ANAT structure is that the structure is considerably distorted from the model. The C7A iodine complex was examined in the hope that it would be isomorphous with the C7ANAT structure, as had been found in the C6A system\{88\}, but this was not the case. It was reported in September 1978 \{124\} that the C7ANAT structure has been solved by Saenger using the hydrogen iodide complex which is isomorphous and solving this complex using the heavy atom method. Work in this field was discontinued for this reason.

Chapter 6 Attempts to solve the C7Aiodine complex.

The initial motivation to investigate the C7A-iodine complex was the failure of all of the attempts to solve the C7ANAT structure. The C6A water complex had been solved by the isomorphous replacement of the included water molecules by an iodine molecule\{87\}. The C6A iodine complex was then solved using the conventional heavy atom method\{88\}. It was hoped that the C7A iodine complex would be isomorphous with the C7ANAT complex, and that the heavy atom method could be used to solve first the iodine structure, and then the C7ANAT structure.

The crystal was found not to be isomorphous with C7ANAT and the data was collected, as described in Chapter three. Preliminary inspection of the precession photographs indicated that the space group was C2, isomorphous with the 2,5-diiodobenzoic acid C7A(C7ADBA) complex that has been solved by Hamilton, Steinrauf and VanEtten\{112\}. There were unfortunately no atomic coordinates published with the structural report, and a request for the atomic parameters has yet to be acknowedged.

The crystals were small and diffracted weakly. The collection of data (see Chapter 3) proceeded smoothly. A subsequent and careful inspection of the photographs showed that three hk0 reflections with $h+$ $\mathrm{k}=2 \mathrm{n}+1$ were present. The true space group must be $\mathrm{P}_{1}$, although the symmetry is close to $C 2$. In $C 2$ symmetry the $h+k=2 n+1$ reflections should be absent, here they are very weak. In his thesis $\{100\}$ Noltemeyer confirmed this, saying that there were several weak reflections of the type $h+k=2 n+1$, and the space group was $P 2_{1}$.
symmetry, and the most significant peaks, which are on the $w=0$ section are shown in Figure 6.1. The peaks in the sharpened Patterson map could only be interpreted as four iodine atoms related by a pseudo two fold axis, as illustrated in Figure 6.2. The coordinates are listed in Table 6.1.

Table 6.1 . The iodine atom coordinates before and after refinement.
Initial Refined Coordinates

I1

| $x / a$ | 0.0706 | 0.0616 |
| :--- | :--- | :--- |
| $y / b$ | 0.0000 | 0.0000 |
| $z / c$ | 0.0933 | 0.1043 |

I2

| $\dot{x} / \mathrm{a}$ | -0.0272 | -0.0236 |
| :--- | ---: | ---: |
| $y / b$ | 0.0000 | -0.0281 |
| $z / c$ | 0.2372 | 0.2332 |

The $y$ coordinates of the iodine atoms, that were determined from the Patterson map, were close to zero. This introduced a pseudo mirror plane, normal to $b$, into the Fourier map phased on the iodine positions. The iodine atom positions were refined using the least squares refinement program in the X-Ray system\{113\} using all of the data. The final iodine atom coordinates are tabulated in Table 6.1. The positional parameters of the atoms refined well, but the temperature factors are very high, typically three times those reported by Saenger in the C6A iodine structure. After the refinement there were no peaks in the difference Fourier synthesis with magnitudes greater than 2 which might correspond to other iodine atom sites.

| PEAK. | $x$ | $y$ | 2 | PEAK MEIGHT. |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | -0.055 | 0.0 | 0.545 | 13 |
| 2 | 0.036 | 0.0 | 0.322 | 15 |
| 3 | 0.100 | 0.0 | 0.70 | 16 |
| 4 | -0.082 | 0.0 | 0.120 | 17 |

01


FIGURE 6.1 SHARPENED PATTERSON MAP UOW SECTION


FIGURE 6.2 IODINE ATOM POSITIONS

The cell dimensions of the C7A iodine complex are close to those reported by Hamilton et. al. for C7ADBA\{112\}. Thus it is reasonable to suppose that the structures will be almost identical(see Chapter 2). The ring axis of the C7A molecule in the C7ADBA structure is parallel to the crystallographic $c$ axis. This is consistent with the coordnates of the iodine atoms that resulted from the refinement.

With the two iodine atoms held in a fixed position with respect to the origin, there are two enantiomeric possibilities, as the iodine atoms define the enantiomorph. There are also two ways of placing the C7A molecule in the unit cell with respect to the iodine atom positions, either with the primary hydroxyl groups close to the origin, or with the secondary hydroxyl groups close to the origin. The latter two possibilities are shown in Figure 6.3. A search of the rotation and translational parameters of the C7A molecule in each of the four trial structures was carried out using a modified version of the structure factor program that was written for the C7ANAT structure. The C7A molecule positions were refined using a low resolution data set (sin $Q$ / lambda < 0.15) holding the iodine atoms fixed.

These four refined possibilities were then further refined in the 1976 version of $X$-ray\{113\}. The C7A molecule was treated as a rigid body and refined using the least squares method with a higher resolution data set $(\sin Q / l a m b d a<0.2)$, again holding the iodine atoms' atomic coordinates invariant. The R-factors at this stage varied between 0.47 and 0.42 for the four possible structures. These four trial structures were then refined further with the individual glucose residues being treated as rigid bodies. This failed to yield a significant improvement in the correlation between the observed and calculated structure


FIGURE 6.3 TWO POSSIBLE ORIENTATIONS OF THE IODINE ATOMS AND THE CTA MOLECULE.
factors. Step refinement of the four trial structures, followed by Modelfit\{114\}, a method which had been successful in the C7APNA structure again failed to improve the R-factor. Difference Fourier maps based on the phases calculated for the iodine atoms did not show a ring of electon density surrounding the iodine atoms in a position that the C7A molecule could be expected to be found.

The most disturbing feature of the refinement was the isotropic temperature factors of the iodine atoms, which were very high(U = $0.34 h^{A^{2}}$. Anisotropic refinement of the temperature factors of the iodine atoms did not significantly improve the R-factor, and the iodine atoms did not exhibit significant anisotropy.

The only explanation for the difficulties encountered in the attempts to solve the structure is that the iodine atoms are disordered, and that the disorder is not systematic. The reflections of the type $h+k$ $=$ odd are. so weak that the iodine atoms cannot make a significant contribution to them. This suggests that the twofold symmetry of the iodine atoms is preserved in spite of the disorder.

Chapter 7. The Structure of The C7APNA Complex.

The alternation of Modelfit\{l|4\}, with block diagonal least squares refinement (the X-Ray system) had reduced the R-factor to 0.23 for a structure with a reasonable geometry. Both guest molecules and eighteen water molecules had been located using difference Fourier maps. An overall temperature factor had been used up to this stage, and it seemed appropriate to change from an overall to individual isotropic temperature factors. Least squares refinement of the temperature factors alone reduced the $R$-factor to 0.18 . At this stage more water molecules could be located on the difference Fourier map. Further least squares refinement of both the isotropic temperature factors and the positional parameters of the atoms reduced the $R$-factor to 0.132 . The $p$-nitroacetanilide molecules were treated as rigid bodies in these cycles of refinement.

The isotropic temperature factors in the C7APNA structure are comparable with those found for C6A structures (typically $U=0.05 \dot{A}^{2}$
). The highest temperature factors found in the C7A molecule are those of the primary hydroxyl group, which is the only group with free rotation about a single bond. The temperature factors for the atoms in substrate one are typically about 0.10 . The population parameters of both substrates were set to 0.75 , after analysing the peak heights of 0181 and 0182 and comparing them with the peak height of a primary hydroxyl group with a similar temperature factor.

Blocked matrix least squares refinement was used in the above refinement of the structure, with the positional parameters and the temperature factors for five atoms forming a block. A larger block size would have been desirable, but was not possible as the computers in Edinburgh had restrictions placed on both core space and CPU time.

The coordinate list is shown in Table 7.1. Figures 7.1, 7.2, 7.3 show the general view of the C7APNA complex. Figures $7.4,7.5,7.6$ show the packing of the molecule in the unit cell.

The numbering scheme adopted for this structure is as follows. The letter denotes the atom type. The first number is 1 or 2 , referring to the two different C7A molecules in the unit cell. The second number defines the residue number within that molecules, and the third number defines the atom number within that residue.

The glucose residues in the C7APNA complex are all in the Cl chair conformation. All other X-ray crystallographic, spectroscopic, and o.r.d. data have shown that the glucose residues in cycloamylose are in this conformation.

The bond lengths and angles are shown in Tables 7.2 and 7.3 respectively. Sigma, the estimated standard deviation (e.s.d.) of a bond or angle, calculated from the e.s.d.s of atom positions derived from the least squares correlation matrix is $0.04 \AA$ for a $C-C$ bond, $0.035 \AA$ for a $\mathrm{C}-\mathrm{O}$ bond and $1^{\circ}$ for bond angles. Saenger reported that some of the corresponding bonds in different glucose residues in the C6A iodine complex $\{85\}$ varied by 5 e.s.d.s, which he attributed to the strain induced by the cyclisation of the cycloamylose molecule\{85\}.

It has been noted in other cycloamylose and oligosaccharide structures that the $\mathrm{Cl}-05$ and the $\mathrm{Cl}-04^{\prime}$ bond lengths are significantly shorter than the $C 5-05$ and $C 4-04$ bonds. The average lengths of these bonds in the C7APNA structure do not follow this trend, although the


Table 7.1 Parameter list for C7APNA $R=0.13$
Water molecules are listed overleaf

|  | x/a | y/b | z/c | $u * 100 \AA^{2}$ Pop. Patam. |  |
| ---: | ---: | ---: | ---: | ---: | ---: |
| 0501 | -0.34322 | 0.46366 | -0.63428 | 7.672 | 0.576 |
| 0502 | -0.47840 | 0.45435 | 0.46042 | 8.579 | 0.169 |
| 0503 | 0.17756 | 0.16739 | -0.18466 | 19.600 | 0.211 |
| 0504 | -0.46722 | 0.32488 | 0.29036 | 9.438 | 0.697 |
| 0505 | 0.04214 | -0.15465 | -0.24839 | 11.431 | 0.507 |
| 0506 | -0.37810 | 0.30018 | -0.83740 | 7.537 | 0.424 |
| 0507 | -0.56329 | -0.23796 | -0.18430 | 8.244 | 0.550 |
| 0508 | -0.43999 | 0.25019 | -0.18176 | 10.538 | 0.406 |
| 0509 | -0.43508 | 0.28969 | -0.23015 | 4.913 | 0.381 |
| 0510 | -0.45893 | -0.43695 | -0.28445 | 12.003 | 0.360 |
| 0511 | -0.26869 | 0.45451 | -0.18019 | 7.337 | 0.799 |
| 0512 | 0.06948 | 0.25367 | -0.18593 | 11.968 | 0.497 |
| 0514 | -0.55332 | -0.16163 | -0.72035 | 6.276 | 0.792 |
| 0515 | 0.09593 | -0.44470 | -0.69716 | 6.055 | 1.000 |
| 0516 | -0.48960 | -0.36833 | -0.33862 | 19.223 | 0.275 |
| 0517 | -0.55517 | -0.37463 | 0.06242 | 9.174 | 0.444 |
| 0518 | -0.47541 | -0.39312 | -0.36789 | 18.339 | 0.246 |
| 0519 | -0.45573 | 0.49709 | -0.86305 | 17.820 | 0.224 |
| 0520 | -0.46128 | 0.41247 | -0.51113 | 9.417 | 0.500 |
| 0521 | -0.65415 | -0.34538 | -0.51537 | 15.915 | 0.500 |
| 0522 | -0.61963 | -0.33266 | -0.78179 | 8.603 | 0.500 |
| 0523 | -0.34195 | 0.44144 | -0.91711 | 11.259 | 0.500 |

Coordinates for the PNA molecules frow the electon density maps

| 0181 | 0.10504 | 0.04359 | 0.24404 |
| ---: | ---: | ---: | ---: |
| 0182 | 0.10570 | -0.08974 | 0.23979 |
| 0183 | -0.11696 | -0.15897 | -0.14894 |
| N 181 | 0.09096 | -0.02304 | 0.20851 |
| N 182 | -0.10096 | -0.02308 | -0.11064 |
| C181 | -0.04783 | 0.05897 | 0.00000 |
| C182 | 0.00400 | 0.05667 | 0.09085 |
| C183 | 0.03913 | -0.02538 | 0.13191 |
| C184 | 0.00400 | -0.09718 | 0.07836 |
| C185 | -0.04348 | -0.08974 | 0.00000 |
| C186 | -0.06087 | -0.02051 | -0.03191 |
| C187 | -0.13196 | -0.07949 | -0.17021 |
| C188 | -0.15696 | -0.05385 | -0.23830 |


| 0281 | -0.11013 | 0.00513 | -0.67021 |
| ---: | ---: | ---: | ---: |
| 0282 | -0.10643 | 0.14103 | -0.68085 |
| N 281 | -0.08913 | 0.07692 | -0.63830 |
| C 281 | -0.05000 | 0.07179 | -0.55319 |
| C 282 | -0.03043 | 0.13846 | -0.51064 |
| C 283 | 0.01087 | 0.12821 | -0.43617 |
| C 284 | -0.02826 | -0.01538 | -0.51489 |
| C 285 | 0.01087 | -0.02564 | -0.43617 |
| C 286 | 0.03261 | 0.04615 | -0.39362 |
| N 282 | 0.07609 | 0.03846 | -0.30851 |
| C 283 | 0.09783 | 0.17949 | -0.26596 |
| C 287 | 0.10435 | 0.10256 | -0.25532 |
| C 288 | 0.14130 | 0.05897 | -0.18085 |

[^4] cifference map.

Figure 7.1 C7APNA Projection onto the bc plane


Figure 7.2 C7APNA projection onto the ac plane



Figure 7.3b
Projection of C7APNA molecule two on to the $a b$ plane.




| E0na | ＊Ȯこというe Numíur |  |  |  | fiesiこue |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| C1－C2 | 1 | 1.36 | 1.49 | 1.51 | 1.53 | 1.62 | 1.51 | 1.49 |
|  | 2 | 1.57 | 1.54 | 1.52 | 1.53 | 1.57 | 1.56 | 1.61 |
| C2－C3 | 1 | 1.63 | 1.43 | 1.52 | 1.41 | 1.52 | 1.57 | 1.58 |
|  | 2 | 1.48 | 1.49 | $1 . \angle E$ | ：． 79 | 1.53 | 1.43 | 1.51 |
| c3－c34 | 1 | 1.51 | 1.59 | 1.57 | 1．65 | 1.54 | 1.56 | 1.53 |
|  | 2 | 1.58 | 1.52 | 1.42 | 1.50 | 1.55 | 1.59 | 1.55 |
| C4－C5 | 1 | 1.56 | 1.56 | 1.54 | 1.54 | 1．5 | 1.50 | 1.63 |
|  | 2 | 1.49 | 1.58 | 1.68 | 1.59 | 1.59 | 1.56 | 1.54 |
| C5－C6 | 1 | 1.53 | 1.62 | 1.60 | 1.52 | 1.58 | 1.59 | 1．57 |
|  | 2 | 1：56 | 1.58 | 1.53 | 1.59 | 1.62 | 1.63 | 1． 4.6 |
| C1－C5 | 1 | 1.52 | 1.41 | 1.42 | 1.45 | 1.41 | 1.45 | 1.56 |
|  | 2 | 1.42 | 1.43 | 1.41 | 1.44 | 1.42 | 1.39 | 1.38 |
| C1－04． | 1 | 1.54 | 1.41 | 1.43 | 1.44 | 1.35 | 1.42 | 1.41 |
|  | 2 | 1.47 | 1.44 | 1.50 | 1.44 | 1.38 | 1.44 | 1.45 |
| C2－02 | 1 | 1.40 | 1.42 | 1.48 | 1.45 | 1.49 | 1.48 | 1.42 |
|  | 2 | 1.43 | 1.47 | 1.42 | 1.45 | 1.42 | 1.44 | 1.55 |
| C3－03 | 1 | 1.46 | 1.45 | 1.50 | 1.50 | 1.42 | 1.50 | 1.45 |
|  | 2 | 1.49 | 1.48 | 1.53 | 1.49 | 1.46 | 1.46 | 1.44 |
| C4－04 | 1 | 1.45 | 1.42 | 1.41 | 1.37 | 1.46 | 1．$\angle C$ | 1.46 |
|  | $=$ | 1．39 | 1.44 | 1.40 | 1.37 | 1.35 | 1.35 | 1.45 |
| C5－05 | 1 | 1.38 | 1.53 | 1.44 | 1.42 | 1.36 | 1． 48 | 1.38 |
|  | 2 | 1.51 | 1.47 | 1.38 | 1.46 | 1.42 | 1.37 | 1.36 |
| c6－06 | 1 | 1.42 | 1.40 | 1.39 | 1.34 | 1.43 | 1.41 | 1.44 |
|  | 2 | 1.39 | 1.40 | 1.37 | 1.45 | 1.40 | 1.47 | 1.43 |


| Eund Angle | Nこleculミ Number | 1 | 2 | 3 | $\begin{gathered} \text { =esicut } \\ 4 \end{gathered}$ | 5 | 6 | 7 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| C2－C1－05 | 1 | 113.6 | 109.2 | 105.6 | 106.2 | 102.5 | 107.6 | 107.3 |
|  | 2 | $108 . E$ | 157． | 106.9 | 106.9 | 106．2 | 106.6 | 102.5 |
| C2－こ1－C4． | 1 | 109.0 | 112.4 | 107.2 | 106.1 | 108．6 | 109．8 | 107.2 |
|  | 2 | 106.0 | 111.1 | 107.2 | 10\％．$\%$ | 111.0 | 106．C | 105.5 |
| 05－C1－C4． | 1 | 98.7 | 108．？ | 10E．s | 112.2 | 114.8 | 110.6 | 10．0 |
|  | 2 | 110.4 | 10E． 1 | 109． 1 | 111.0 | 111．？ | 111.6 | 111．9 |
| C1－C2－C3 | 1 | 110.7 | 110．\％ | 11C．0 | 112.3 | 108.5 | 107.7 | 105.9 |
|  | 2 | 107.9 | 105.8 | 111.2 | 109.2 | 108.0 | 112.1 | 105.5 |
| CイーCこーここ | 1 | 11.5 | 0？．2 | 127.7 | 107.2 | ：¢E．$\%$ | 15？．？ | 1．27．1 |
|  | 2 | 107．6 | 105.4 | 10E．0 | 108．？ | 102.2 | 108.5 | 104.5 |
| C3－C2－52 | 1 | 111.2 | 112.5 | 108．5 | 110.1 | 108.2 | 108.1 | 110.2 |
|  | 2 | $111 . \mathrm{E}$ | 109.2 | 112.6 | 112．0 | 108.9 | 107.3 | $109.2$ |
| C2－C3－C4 | 1 | 108．$=$ | 110.9 | 105.7 | 109.5 | 101.9 | $105 . ?$ | 107.4 |
|  | 2 | 109.2 | 107.5 | 111.4 | 110.8 | 107.1 | 107.7 | 105.6 |
| C2－C3－03 | 1 | 107.7 | 112． C | 107．0 | 112.8 | 108.7 | 106．E | 105.5 |
|  | 2 | 104.8 | 104.6 | $111 . \mathrm{E}$ | 108． 6 | 106.1 | 113.2 | 106.9 |
| C4－C3－03 | 1 | 107.9 | 107.0 | 103．7 | 102.8 | 105.9 | 105.1 | 104．2 |
|  | 2 | 108.6 | 101.6 | 103.1 | 107．E | 105．2 | 154.4 | 10.0 |
| c3－C4－C5 | 1 | 108.7 | 105.6 | 104．3 | 100.6 | 110.4 | 103.3 | 103.4 |
|  | 2 | 107.4 | 103.3 | 106.5 | 105.6 | 110.9 | 103.1 | 1CS． 1 |
| C3－C4－C4 | 1 | 105.6 | 109.2 | 109．4 | 106.9 | 102.2 | 106.9 | 106.7 |
|  | 2 | 107.7 | 1 CE ． 0 | $112 . E$ | 108．9 | 108.4 | 111.2 | 105.5 |
| C5－C4－C4 | 1 |  | 107.4 | 10E．L |  | $108.5$ | $106.3$ | $108.2$ |
|  | 2 | $107.4$ | 104.5 | 109.1 | 106.7 | 109.2 | 112.5 | $109.7$ |
| C4－C5－C6 | 1 | 111.1 | 105.5 | 109.4 | 107.8 | 108．0 | 111.0 | 105.9 |
|  | 2 | $111 . \mathrm{F}$ | 110.5 | 10E．2 | 107．E | 104．9 | 1．E．A | 110.2 |
| C4－C5－05 | 1 | 110.6 | 10E．？ | 111.2 | 111.5 | 110.2 | 109.4 | 107.4 |
|  | 2 | 105.6 | 106.6 | 110.3 | 108．7 | 110.6 | 112.5 | 112.3 |
| C6－C5－05 | 1 | 107.5 | 103.3 | $103.5$ |  |  | $101.9$ | $10 \varepsilon .2$ |
|  | 2 | 108．4 | 104.3 | 109．1 | 99.7 | 105.4 | 104.3 | $105.9$ |
| C5－06－06 | 1 | 108.3 | 112.9 | 110.2 | 110.7 | 10E． 4 | 111.7 | 110.7 |
|  | 2 | 110.2 | 109.5 | $10 \% .5$ | 111.7 | 107.5 | 108． 1 | 114.2 |
| C4－04－C1＂ | 1 | 112.8 | 116.8 | 118.4 | 112.7 | 116.1 | 114.4 | 113.9 |
|  | 2 | 116.4 | 114.2 | 117.4 | 116.1 | 116.7 | 11E． 1 | 117.1 |
| C1－05－c5 | 1 | 116.6 | $113 . \%$ | 115.7 | 110.7 | 114.4 | 110.4 | 117.3 |
|  | 2 | 114.8 | 114.6. | 119.0 | 111.6 | 114.2 | 112.0 | 116.7 |

C7APNA bond lengths are within 3 e.s.d.s of the usual values of these bonds in the C6A structures $\{85\}$.

The average value of the angle $C 1^{\prime}-04-C 4\left(116^{\circ}\right)$ in the C7APNA structure is less than that found in the C6A system $\left(119^{\circ}\right)\{2,85,88)$, and close to that found in disaccharides $\left(116^{\circ}\right)\{104\}$. The e.s.d. for bond angles in the C7APNA structure is such that no conclusions can be drawn from this data. Sundarajan and Rao predicted that the bond angle across the bridging oxygen would be $110^{\circ}$. The bond angles about the bridging oxygens are significantly greater than this, suggesting that their model may be inaccurate.

In other cycloamylose structures the angles $\mathrm{C} 4-04-\mathrm{Cl}^{\prime}$ and Cl-05-C5 are significantly larger than $109^{\circ}$, whereas the angles C3-C4-04 and C3-C4-04 are less than $109^{\circ}$. In $\alpha-D-$ glucose these angles are closer to 109 (see Chapter 2). The difference of the values of these angles in C6A and $\alpha-D-g l u c o s e$ is probably due to the steric strain effects that result form the cyclisation of the molecule.

In conclusion, the bond lengths and angles in the C7APNA structure are consistent with all other crystallographic studies of C6A molecules within the limits of the accuracy of the structure determination.

The Planarity And Geometry of The Bridging Oxygens.

The bridging oxygens are almost coplanar. The low deviations of these atoms from their least squares best plane, Table 7.4, is also a characteristic of the C6A system (see Chapter 2 Table 2.12). The best planes of the 04 atoms of the two C7A molecules are close to being para1lel, the angle between them being one degree. The best planes of the 04 s make angles of $8^{\circ}$ with the b axis and $1^{\circ}$ with the a axis.

Table 7.4. The deviation of the bridging oxygens from their least squares best plane in Angstroms.

Molecule Residue

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Molecule 1 | -0.07 | 0.02 | 0.05 | -0.04 | 0.04 | 0.07 | 0.01 |
| Molecule 2 | -0.03 | -0.04 | 0.05 | 0.01 | -0.06 | 0.02 | 0.05 |

The diagonal distances across the C7A molecule are distorted in a symmetric manner due to the substrate, which causes an elliptical deformation. The diagonal distance has been calculated by taking the distance between $0 n 4$ to the mid-point of $0(n+3) 4$ and $0(n+4) 4$. The substrate lies between residue 7 and residue 3 for molecule 1 and between residue 2 and residue 6 in molecule 2. The diagonal distances are shown in Table 7.5.

Table 7.5. Diagonal distances across the C7A molecules in Angstoms.
Molecule Residue

| Number | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Molecule 1 | 9.87 | 10.09 | 9.62 | 9.63 | 10.09 | 9.86 | 9.51 |
| Molecule 2 | 9.94 | 9.53 | 9.63 | 10.05 | 9.73 | 9.63 | 10.01 |

The difference between the maximum and minimum diagonal distance is $0.58 \AA$, which is significantly less than that found for group 1 b complexes(Table 2.4 p 45 ). The substrates do not cause as big a distortion of the C7A molecule as do similar substrates in C6A complexes, due to the large diameter of the C7A molecule.

The distances between 04 s on adjacent glucose residues are shown in Table 7.6 , along with the angle between the bridging oxygens. This angle should be 128.6 if the bridging oxygens form a heptagon.

Table 7.6. Distances, in Angstroms, and angles between the adjacent bridging oxygens

Molecule Residue Number

|  |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $04-04^{\prime}$ | 1 | 4.28 | 4.43 | 4.44 | 4.38 | 4.26 | 4.42 | 4.39 |
|  | 2 | 4.41 | 4.39 | 4.38 | 4.33 | 4.45 | 4.37 | 4.31 |
| $0^{\prime}-04-04^{\prime \prime}$ | 1 | 127.9 | 124.9 | 130.4 | 130.7 | 125.2 | 128.1 | 132.5 |
|  | 2 | 132.5 | 127.1 | 127.8 | 126.1 | 129.3 | 131.3 | 126.1 |

In all of the substituted benzene ring C6A complexes the guest molecule lies parallel to the longest diagonal between the bridging oxygens. In C7A this can not occur as the C7A molecule does not have two fold symmetry. The PNA molecules are aligned towards 0164 and 0214 for substrates one and two respectively.

Endocyclic Torsion Angles In The C7APNA Complex.

The torsion angle $A-B-C-D$ is zero if the bonds $A-B$ and $C-D$ are cis coplanar, and positive if, looking along the central bond(B-C) from $B$, the far bond ( $C-D$ ) is rotated clockwise with respect to the near bond ( $A-B$ ).

The endocyclic torsion angles for the glucose residues in C7APNA are shown in Table 7.7. Saenger noted that there could be variations in the value of a particular torsion angle in a C6A structure of up to twelve degrees. The values of the torsion angles for the torsion angles in C7APNA vary up to $18^{\circ}$ between corresponding angles in different residues, which can be partially attributed to the high standard deviations in atomic parameters which can have a significant effect on the value of the torsion angle.

Table 7.7. Encocyciic and Exccyciic cihecral angles fur trie cha mulecule in the CTAFNA compiex.


Exocyclic Torsion Angles.

The exocyclic torsion angles can be divided into two groups, those about the bridging oxygen, and those that define the conformation of the primary hydroxyl group. The conformation of the primary hydroxyl groups are defined by $05-C 5-C 6-06$ and $C 4-C 5-C 6-06$ and are tabulated in Table 7.7. The values of these torsion angles show that the primary hydroxyl groups in the C7APNA structure are gauche with respect to both 05 and C4. This is the gg conformation(Figure 2.3 page 42 ), which is the most stable for the C6A system. In this conformation the primary hydroxyl groups are pointing away from the molecular cavity. This was found to be the case in most of the C6A complexes where the complexant is a substituted phenyl ring(see Chapter 2).

The second group of exocyclic torsion angles are those about the bridging oxygens(04). These torsion angles are shown in Table 7.7. The average values of the torsion angles about 04 for ten C6A structures $\{85\}$ are shown in Table 7.8 , along with the average torsion angle for the C7APNA complex.

Table 7.8. The average values of the exocyclic torsion angles about 04 for ten C6A complexes, and C7APNA.

| Torsion Angle | C6A 485$\}$ | C7APNA |
| :---: | :---: | :---: |
| $\mathrm{C} 2-\mathrm{Cl}-04^{\prime}-\mathrm{C4}^{\prime}$ | 107.6 | 116 |
| 05-C1-04'-C4' | -132.7 | -126 |
| $\mathrm{Cl}-04^{\prime}-\mathrm{C4}{ }^{\prime}-\mathrm{C} 3^{\prime}$ | 130.6 | 131 |
| $\mathrm{C1}-04^{\prime}-\mathrm{C4}{ }^{\prime}-\mathrm{C} 5^{\prime}$ | $-110.3$ | 117 |

C6A has the form of a truncated cone, with the maximum width at the secondary hydroxyl group end of the molecule. Torsion angles of $120^{\circ}$ and $-120^{\circ}$ would generate a cycloamylose molecule that is cyclindrical \{85\}. The average values of the torsion angles for C7A are closer to $120^{\circ}$ than those of C6A, which indicates that C7A is closer to being cylindrical than C6A. The average values of the 04 torsion angles for each of the C6A molecules are remarkably similar with a variation of $6^{\circ}$, although the variation of these angles within each individual complex is quite high, $\pm 25^{\circ}$ for group la complexes and $\pm 10^{\circ}$ for Group 1 b complexes. The variation in 04 torsion angles for the seven residues in the C7APNA complex is similar to that of the C6A when it is complexed with a substituted phenyl ring.

Two other torsion angles involving the bridging oxygen are commonly reported. These are $\phi$ and $\phi^{\prime}$ (defined as $04-\mathrm{C4}-04^{\prime}-\mathrm{C} 4^{\prime}$ and C1-04'-C4'-04' respectively). These torsion angles define the glucose residues with respect to each other, and hence to the ring axis\{96\}. In group la complexes these torsion angles vary considerably from residue to residue within the molecule, indicating that there is a buckling of the C6A molecule, which has been attributed to either molecular packing forces $\{85\}$ or to strain in the C6A molecule $\{85\}$.

When C6A is complexed with a phenyl ring the C6A molecule becomes more regular(see Chapter 2). This is reflected both in the torsion angles and in the completion of the secondary hydroxyl groups ring of hydrogen bonds. The variation in $\phi$ and $\phi^{\prime}$ from residue to residue in the C6A water complex is $50^{\circ}$ whereas in the C6A $\underline{p}$-iodoaniline complex the variation is $14^{\circ}$.

The values of $\phi^{\prime}$ and $\phi^{\prime}$ in the C7APNA complex are shown in Table 7.9. They show that although the bridging oxygens are almost coplanar, the angle between the ring axis and each of the glucose residues changes. The guest molecule is at an angle with respect to the ring axis, and the glucose residues that are close to the $N$-acetyl group are moved slightly towards the ring axis.

Table 7.9. The dihedral angles $\phi$ and $\phi^{\prime}$.

| Torsion | Molecule | Residue. |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Angle |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 |  |
| $\varnothing$ | 1 | 178 | 162 | 131 | 139 | 163 | 149 | 149 |  |
|  | 2 | 178 | 161 | 152 | 147 | 147 | 145 | 116 |  |
|  |  |  |  |  |  |  |  |  |  |
| $\varnothing^{\prime}$ | 1 | -178 | -164 | -139 | -124 | -141 | -156 | -148 |  |
|  | 2 | -166 | -160 | -154 | -150 | -142 | -144 | -124 |  |

When $\phi_{N}$ or $\phi_{r}^{\prime}$ is greater in magnitude than $\phi_{n-1} \phi_{n-1}^{\prime}$ then the secondary hydroxyl groups of residue $n$ are closer to the C7A molecular axis than those of $n-1$.

Intramolecular Hydrogen Bonds.

The numerous crystallographic structure determinations of C6A complexes have shown that there is hydrogen bonding between atoms 02 and 03 on adjacent glucose residues. In some group la complexes atoms 02 and 03 are too far apart for hydrogen bonds to form, but in these structures there are at least three strong hydrogen bonds between the secondary hydroxyl groups. In all other groups there is a complete ring of hydrogen bonds between the secondary hydroxyl groups of the molecule. Saenger has examined several structures at high resolution, both with X-rays and neutrons, and has conclusively shown that hydrogen bonds can form between two hydroxyl groups that are separated by up to $3.15 \AA\{85\}$. He also showed that the hydrogen atoms involved in these secondary hydroxyl groups hydrogen bonds can be attached to either 02 or $03\{85\}$.

It has been predicted theoretically and spectroscopically that the hydrogen bonds between secondary hydroxyl groups in C7A are stronger than those in C6A(see Chapter 1). This is indicated by a shorter 02-03 distance. In the C7APNA complex there is a complete ring of hydrogen bonds between the secondary hydroxyl groups in both molecules, which are shorter, and hence stronger than those found in C6A. The 02-03 distances are shown in Table 7.10.

Table 7.10. 02-03 distances between adjacent glucose residues.
Molecule Number
Bond ( $\dot{A}$ )

$$
012-023022-033 \quad 032-043 \quad 042-053052-063 \quad 062-073 \quad 072-013
$$

| Molecule | 1 | 2.80 | 2.70 | 2.71 | 2.82 | 2.79 | 2.78 | 2.90 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Molecule | 2 | 2.79 | 2.71 | 2.82 | 2.77 | 2.85 | 2.78 | 2.74 |

Intermolecular Hydrogen Bonds.

Intermolecular hydrogen bonds have been observed in the group 2 C6A structures, both between secondary and primary hydroxyl groups on adjacent molecules and between either secondary or primary hydroxyl groups on adjacent molecules. All of the C7A structural data with the exception of the C7A water structure, that have so far been reported have had strong hydrogen bonding between the secondary hydroxyl groups on adjacent molecules, which are related by a two fold or pseudo two fold axis. The difference between C6A and C7A structures in this respect must be due to a more favourable geometric relationship between the secondary hydroxyl groups.

The secondary hydroxyl groups can act as a donor for one hydrogen bond and an acceptor for two hydrogen bonds\{121\}. The formation of the intramolecular hydrogen bonds uses half of the donors at the secondary hydroxyl groups end of the molecule. The rest of the donors form hydrogen bonds with the adjacent C7A molecule. There are strong hydrogen bonds formed between 03 s on adjacent molecules. The 03-03 bond lengths are shown in Table 7.11.

Table 7.11. Bond lengths between 03 s on adjacent C7A molecules.

| Bond | Bond Length (A ) |
| :--- | :---: |
| $13-0213$ | 2.99 |
| $23-0273$ | 2.85 |
| $33-0263$ | 2.80 |
| $43-0253$ | 2.87 |
| $53-0243$ | 2.77 |
| $63-0233$ | 2.83 |
| $73-0223$ | 2.85 |

In all cases the angles between the intermolecular hydrogen bonds are between $110^{\circ}$ and $125^{\circ}$. There are other $02-03$ intermolecular contacts of less than $3.1 \AA$ which have contact distances of between 2.95 and $3.15 \AA$. The angles between 02 and 03 on facing molecules do not preclude hydrogen bonding between these atoms, although the angles of the type $01 \mathrm{~N} 2-02 \mathrm{M} 3-01 \mathrm{~N} 3$ are in the order of $60-70^{\circ}$, which precludes any simultaneous hydrogen bonds between these atoms. There is no possibility of hydrogen bonding between 02 s on neighbouring rings as angular disposition of other atoms bonded to the 02 s is incompatible with a bond being formed. It is reasonable to suppose that there can be hydrogen bonding either between $01 N 3$ and 02 M 3 or between 01 N 2 and 02 M 3 , and that these bonds can not exist simultaneously but that the bonds between 03s will predominate.

Inter Ring Hydrogen Bonds Between Primary Hydroxyl Groups.

Some primary hydroxyl groups have contact distances of less than 3.1 A with primary hydroxyl groups on other C7A molecules in the unit cell. The angle $\mathrm{C} 6-06--06^{\prime}$ is in all cases reasonable for hydrogen bonding, although in some cases the hydrogen bond would be weak. The contact distances for these bonds are shown in Table 7.12.

Table 7.12 Inter ring primary hydroxyl hydrogen bonds.

Bond
$0146-0256$
$0126-0156$
$0226-0266$

Bond Length $(\AA)$ Bond
0176-0226
0136-0176
0246-0276

Bond Length $(\AA)$
2.80
2.99
2.79

Hydrogen Bonding of Included Water Molecules.

The water of crystallisation forms an extensive hydrogen bonded network around the cycloamylose molecules. There are often two sites for included water molecules close to each other, both in a satisfactory position for hydrogen bonding with the host molecule. There are a number of contacts between water molecules of less than $2.4 \AA$. It is probable that these sites cannot be simultaneously occupied, as this distance is too short for hydrogen bonding\{120\}.

There are several clusters of ordered water molecules around 0504 and 0501, and a similar grouping is found around 0514 and 0515 . The former cluster joins 0276 and 0166 together and the latter joins 0246, 0216 and 0146 together. These are illustrated schematically in Figure 7.7, and illustrated in Figures 7.8 and 7.9 . All reasonable inter/hydroxyl distances having distances of less than $3.1 \AA$ are shown Table 7.13. There are distances of less than $3.1 \AA$ which have poor angular relationships with other hydrogen bonds or with bonds to the carbon atom of the hydroxyl group. These have not been included in the table. The location of the waters of crystallisation and their relationship with the C7A molecule are shown in Figure 7.13.

Figure 7.7 Schematic Representation of the
Principal hydroen bonded systems.


= gure 7.8 Intermolecular hydroger bond ng scheme.

0

0

0

0517 025



Table 7.13 Hydrogen bonded contacts of the water molecules of crystallisation.

| Bond | Bond Length | Bond | Bond Length |
| :---: | :---: | :---: | :---: |
| 0501-0276 | A |  | A |
| $0501-0504$ | 2.75 | $0501-0520$ | 2.80 |
| $0504-0166$ | 2.71 | $0504-0266$ | 3.10 |
| $0506-0136$ | 2.70 | $0504-0506$ | 2.66 |
| $0508-0133$ | 2.61 | $0506-0523$ | 2.49 |
| $0508-0233$ | 2.70 | $0508-0262$ | 2.58 |
| $0511-0153$ | 2.95 | $0511-0122$ | 2.95 |
| $0514-0522$ | 2.80 | $0514-0256$ | 2.85 |
| $0514-0176$ | 2.76 | $0515-0246$ | 2.83 |
| $0515-0166$ | 2.80 | 2.72 | $0515-0216$ |


#### Abstract

The substrate was located using difference Fourier maps. The electron density defining the substrate is almost planar, and sections through this plane were inspected. These are illustrated in Figure 7.10 The $\underline{p}$-nitroacetanilide(PNA) molecule is superimposed on this electron density. The structure of PNA has not been published, so the coordinates of the PNA molecule were calculated from two other structures (nitroaniline\{122\}, and acetanilide\{123\}). The PNA molecules were assumed to be planar, although small rotations of the $N$-acetyl group have been noted \{123\}.


The orientations of the PNA molecule are illustrated in Figures 7.1, 7.2, and 7.3. The phenyl rings of the guest molecule are at a considerable angle to the plane of the bridging oxygens, $61^{\circ}$ for substrate 1 and $63^{\circ}$ for substrate 2. These angles are similar to those found in the C7A $p$-iodophenol complex\{23\}, $60^{\circ}$ and $54^{\circ}$ respectively. In the C6A structures this angle is in the range $80-90^{\circ}$ ( $80.6^{\circ}$ for p-nitrophenol\{49\}, and $85.6^{\circ}$ for $p$-hydroxybenzoic acid $\{49\}$ ).

It is possible to rationalise the larger tilt of the guest molecule in C7A complexes with forces causing the formation of the complex. Tilting the substrate with respect to the bridging oxygens has the effect of concentrating the hydrophobic portion of the guest molecule in the hydrophobic portion of the host molecule. From this result it would appear that the hydrophobic forces stabilise the complex. The C7A molecule has a larger diameter than the C6A molecule, thus allowing the guest molecule more freedom to optimise the geometry of complexation. An alternative explanation for the increased tilt is the strength of the


Figure. .10 Section of diffezence Eourier map with the PNA. molecules ommitted showing the electron density representing she PNA molecule.
intermolecular hydrogen bonds between the secondary hydroxyl group 5 . C7A forms $1: 1$ complexes in solution. When the C7A molecules crystallise they usually do so as dimers, and the only way to include both the guest molecules in the cavity of the dimer is to tilt them so that they do not collide.

The C7A p-iodophenol complex is much harder to understand in terms of the forces causing complexation. The C7A $\mathrm{p}^{-i o d o p h e n o l ~ c o m p l e x ~}$ \{23\} is unusual in that there are three guest molecules per two C7A molecules. The host molecules in this complex are hydrogen bonded in much the same way as the C7APNA complex. The angles of two of the guests with respect to the $\mathrm{C7A}$ 's molecular axis are much higher than those found in the C6A complex of $p$-iodophenol and comparable with the angles found in C7APNA. The phenyl rings are level with the primary hydroxyl groups of the C7A molecule. The third $\mathbb{P}^{-i o d o p h e n o l ~ m o l e c u l e ~ i s ~ p a r a l l e l ~}$ to the plane of the bridging oxygen molecules midway between the the dimer's secondary hydroxyl groups. It is possible that the presence of the third guest molecule causes the other two $\underline{p}^{\text {-iodophenol molecules to }}$ be forced away from it down the host's molecular axis. The C7A structures so far have not elucidated or simplified the analysis of the driving forces of complex formation.

Chapter 8 Complex Formation.

Cycloamyloses form complexes with such a bewildering variety of substrates that it is difficult to identify the interaction that causes complexation. Any hypothesis seeking to explain complexation must be able to explain the inclusion of compounds as different as Kr and p-iodoaniline.

The following have been suggested as driving or stabilising forces for complex formation\{46\}.
a) The release of energy by the reduction of steric strain in the cycloamylose molecule.
b) London Dispersion/van der Waal's forces.
c) Hydrogen bonding between the guest and host, where possible.
d) Hydrophobic interactions.

The strength with which a variety of different guest molecules bind to the host molecule has been examined in order to determine which of the above forces is the most important in the guest host interaction. It is now generally accepted that the stabilisation of the complex is due to all of the above interactions, providing that the guest can interact in a suitable manner. Recently a theoretical discussion of the complexing forces in the cycloamylose system has been published\{125\}.

Steric Strain Release As a Driving Force In Complex Formation.

There is a wealth of structural information available for complexed C6A, both with water and with other substrates\{85\}. Saenger noticed that the conformation of the C6A molecule is much more regular
when it complexed with a guest molecule, than when it is complexed only with water, and that there are better hydrogen bonds between the secondary hydroxyl groups when there is a large guest molecule in the cavity.

The conformation of the empty C6A molecule (the C6A water complex) is referred to as the 'tense' state, and that of the complexed C6A is referred to as the 'relaxed state'. Diagrams of these tense and relaxed states are shown in Figure 8.1. Saenger believes that, on the inclusion of a guest molecule, the host molecule changes from a tense to a relaxed state. This change in conformation with complex formation is supported by spectroscopic evidence\{28\}. Saenger has proposed that there are three possible routes between the tense and relaxed C6A,
a) The substrate replaces the included water molecules directly.
b) The empty C6A molecule gains activation energy, transforming the host molecule into an almost hexagonal state (a low energy conformation), while the water molecules pick up energy and become disordered. These disordered molecules are then replaced by the substrate.
c) The guest forms an intermediate complex with the empty C6A host and enters the cavity after the complex has gained activation energy.

[^5]Figure 8.1a The C6A molecule in the 'tense' configuration.


-144-



- O(6) H
- $\sim O(2) \mathrm{H}, \mathrm{O}(3) \mathrm{H}$

Figure 8.2 Complex formation steric strain relief
The two water molecules in the C6A water complex are not located of the toroidal axis of the C6A molecule, but are displaced by $0.6 \dot{A}$ in order to achieve van der Waals contact with the wall of the cavity. The contact point of these molecules on the interior of the cavity is opposite to the most rotated glucose residue in the structure $\{121\}$ 。

Saenger calculated that the included molecules in the C6A water complex are 'high energy' water molecules, because they are unable to have as full a complement of hydrogen bonds as the water in the bulk solvent $\{121\}$. The release of these high energy water molecules into the bulk solvent during complex formation is a favourable process as they can then have their full quota of hydrogen bonds. The release of the steric strain and the release of the high energy water molecules constitute the first type of driving force for complex formation.

Evidence For Strain In The C6A Water Complex

Distortion And Strain Across The Linking Oxygen

Figure 8.la shows the physical shape of the tense molecule. Saenger evaluated the Flory function, which is a plot of the potential energy contours for the rotation of the bonds about the glycosidic linking oxygens as a function of the torsion angles $\phi$ and $\phi$, The torsional angles $\phi$ and $\phi^{\prime}$ are defined as the clockwise rotation about the Cl to $04^{\prime}$ bond from $04 \mathrm{Cl} 04^{\prime} \mathrm{C4}$ ' and anticlockwise around $\mathrm{C4}$ ' to $04^{\prime}$ from $04^{\prime \prime} \mathrm{C} 4^{\prime} 04^{\prime} \mathrm{Cl}$ respectively. The definition of the torsion angles are illustrated in Figure 8.3.


Figure 8.3 The definition of the torsion angles

The Flory diagram(Figure 8.4) shows that there is a strained configuration across one of the linking oxygens, which is the point that is displaced from the potential energy minimum. When the molecule is complexed the linkage angles are all close to the minimum energy configuration. There is probably a further stability conferred upon the host molecule by the formation of the remaining two hydrogen bonds between the secondary hydroxyl groups. This analysis examines the strain that exists across the bridging oxygens, but ignores the strain that is absorbed by the rest of the glucose residue.


Figure 8.4 The Flory diagram for the C6A water complex

The Distortion And Strain In The Glucose Residue.

A measure of the distortion of a glucose residue has been developed by French and Murphy\{126,127\} for the modelling of amylose structures. Equation 1 defines the Torsion Angle Index(TAI) that French and Murphy developed.

Torsion Angle Index $=|Q 1|+|Q 2|-|Q 3|-|Q 4|+|Q 5|+|Q 6|-(1)$

Q1 $=$ the torsion angle between $05-$ C1-C2-C3 see Figure 8.5
Q2 $=$ the torsion angle between C1-C2-C3-C4 see Figure 8.5
Q3 $=$ the torsion angle between C2-C3-C4-C5 see Figure 8.5
Q4 $=$ the torsion angle between $\mathrm{C} 3-\mathrm{C} 4-\mathrm{C} 5-05$ see Figure 8.5
Q5 $=$ the torsion angle between $\mathrm{C} 4-\mathrm{C} 5-05-\mathrm{Cl}$ see Figure 8.5
Q6 $=$ the torsion angle between $C 5-05-C 1-C 2$ see Figure 8.5


Figure 8.5 Definition of the torsion angles

The $04-04^{\prime}$ vector is referred to as the virtual bond. As the virtual bond becomes larger the torsion angles Q1-Q6 change systematically, Q1, Q2, Q5, Q6 decreasing and Q3 and $Q 4$ increasing in value\{126\}. French and Murphy calculated the virtual bond length and torsion angle index for glucose residues in a wide range of environments, and plotted one against the other (Figure 8.6). They conclude that a change in the virtual bond length is coupled to the TAI in a predictable manner, and that it is a sensitive indication of the degree of distortion that is present in the glucose moiety.

When a plot of the virtual bond length is made against the torsion angle index for C6A complexes(Figure 8.7) it was found that many of the points do not come close to the line that French and Murphy constructed. In fact there seems to be little order in the cycloamylose plot. Harata 778 , took a much larger sample of C6A complexes and found that the resultant plot follows the general line of the French plot but with a much larger scatter of points from the median line. Points that deviate significantly from this median line show that strain across the virtual bond is not the sole cause of the distortion. This treatment does not give an accurate picture of the strain in cycloamyloses which seems to be more meaningfully analysed by investigating the strain across the bridging oxygen than by examining the strain within the glucose residue. The above results indicate that the part of the cycloamylose molecule under the most stress is the linking oxygens.


| CHA1 | C6A Potassium acetate complex (Residue 1) |
| :---: | :---: |
| CHA2 | C6A Potassium acetate complex (Residue 2) |
| CHA 3 | C5A Potassium acetate complex (Residue 3) |
| PLA | Planteose |
| NEG | Methyl a-D-glucooyranoxide |
| ASR | Amot-Scott average residue |
| 2AL | 3 Maltose Jonohydrate |
| RAF | Raffinose pertanycrate |
| $\pm \mathrm{AC}$ | x-Lactose mononydrate |
| GUR | a-D-Glucose urea |
| ADG |  |
| MeBM | Methy 3 -mattoxide |
| <ES | 1-Kestose |

Figure 8.6 TAI plot after French And Murphy\{127\}


Figure 8.7

TAI plot for C6A scructures Exocyclic Torsion Angles.

In light of the shortcomings of torsion angle analysis for the cycloamylose system a more simple approach was adopted. It was found that a significant result could be derived by analysing the exocyclic torsion angles of the glucose residues in the C6A complexes. A modified torsion angle index (MTAI), as defined in Equation 2, is used.
$\mathrm{MTAI}=\mathrm{Q} 1+\mathrm{Q} 2+\mathrm{Q} 3+\mathrm{Q} 4$
where Q1 is the torsion angle between $C 2-C 1-04^{\prime}-C 4^{\prime}$ see Figure 8.8
Q2 is the torsion angle between $\mathrm{Cl}-04^{\prime}-\mathrm{C4}^{\prime}-\mathrm{C} 3^{\prime}$ see Figure 8.8
Q3 is the torsion angle between $05-\mathrm{Cl}-04^{\prime}-\mathrm{C}^{\prime}$, see Figure 8.8
Q4 is the torsion angle between $\mathrm{Cl}-04^{\prime}-\mathrm{C} 4^{\prime}-05^{\prime}$ see Figure 8.8

Figure 8.8 Definition of the exocyclic torsion angles


The MTAI has been evaluated for four C6A complexes and the results are tabulated in Table 8.1

Table 8.1. Modified Torsion Angle Index For Some Complexes of C6A.

| Bridging oxygen | $1+2$ | $2+3$ | $3+4$ | $4+5$ | $5+6$ | $6+1$ |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: |
| between residues |  |  |  |  |  |  |
| Water | 18.4 | -11.4 | -12.5 | -69.9 | 39.6 | -26.0 |
| Krypton | 3.6 | 9.7 | -2.6 | -50.1 | 45.8 | -27.8 |
| P-Iodoaniline | -3.8 | 2.4 | -22.12 | 10.2 | 1.8 | 2.1 |
| Sodium 1-propanesulphonate | 2.1 | -2.3 | 3.0 | 2.1 | -2.3 | 3.0 |

A positive value of the MTAI indicates that the glucose residue after the + sign in the above table has its secondary hydroxyl groups closed in towards the centre of the cavity. This is clearly illustrated in Figure 8.1. Where there is a large positive and a large negative value of the MTAI on adjacent rings then there is little possbility of hydrogen bonding between these rings. The large negative value of the MTAI for the linking oxygen in the water complex between residues 4 and 5 indicate clearly that there is a substantial distortion from hexagonality. Residue 5 is opened out with respect to residue 4 , as is indicated by the large negative MTAI.

In the water complex of C6A Q2 and Q4 have positive values and Q1 and Q3 have negative values. The MTAI is a measure of the angle between the cycloamylose ring axis and the glucose residues on either side of the bridging oxygen( $04^{\prime}$ ). The cycloamylose molecule would have the form of a cylinder if the exocyclic torsion angles were close to -120 and 120 which would give a MTAI of $0\{85\}$.

The MTAI index gives a more direct indication of the strain that the C6A molecule is under. The sum of the magnitudes of the MTAIs is an indication of the stability of the complex, as stable complexes have a low value of this sum, and unstable compexes have a higher value of this sum.

The Cause Of The Distortion In The C6A Molecule.

The distortion of the water complex of $C 6 A$ is harder to rationalise. The formation of the water complex requires the removal of two water molecules from the bulk of the water. This is a very unfavourable process due to the highly hydrogen bonded nature of water. On purely steric grounds the C6A cavity could contain more than two water molecules, but does not due to the inability of the cycloamylose cavity to fulfil the hydrogen bonding requirements of an additional water\{121, 95\}. There is thus no possibility of offsetting the energy required to separate the water molecule from the bulk by forming hydrogen bonds with the cycloamylose molecule.
. The cycloamylose cavity is thus subject to a 'partial vacuum', and the macrocycle is already distorted due to two of the primary hydroxyl groups being in the gt configuration, in order to hydrogen bond with the included water molecules. If further distorted in order to reduce the volume of the cavity. The hydrogen bonds between the secondary hydroxyl groups, which are already weak, are fractured by this and the rigidity of the cycloamylose molecule is lost, with the result that the molecule becomes more easily distorted, and moves in such a way to maximise the energy derived from hydrogen bonding with the included water molecules, and minimise the volume of the cavity. The fracture of the secondary hydrogen bonds is not an unfavourable process, as it is likely that strong hydrogen bonds will be formed with the bulk solvent.

The Mechanism Of Complex Formation Proposed By Saenger

Saenger proposed that steric strain release is the major driving force for complex formation between the empty and complexed cycloamylose, and suggested that the following mechanism for the change of bonding between the solute and solvent for complex formation.

1) The approach of the substrate to the cycloamylose molecule.
2) The breakdown of the water structure inside the cycloamylose rings and the removal of one of the water molecules from the ring interior.
3) The breakdown of the water structure around that part of the substrate molecule that is going to be included into the cavity.
4) The interaction of the substituents on the substrate molecule with the groups on the ring or interior of the cycloamylose molecule.
5) The formation of hydrogen bonds, if any, between the substrate and cycloamylose molecule. This is extremely fast and can not be the rate determining step.
6) Reconstitution of the water structure around the exposed parts of the substrate:cycloamylose complex after inclusion.

The water structure is modified around the partners of the reaction in steps 2,3 and 6 . Steps 1,2 , and 6 are general steps, and should not give rise to substrate specificity within the same class of compounds. When kinetic specificity with respect to substrates is found, then steps 3 or 4 are much more likely to be rate determining $\{15,85\}$.
(b) Energy derived from London Dispersion/Van Der Waals' Forces.

London/van der Waals' forces are weak attractive forces that usually arise from dipole-dipole interactions. The size of these interactions is dependent on the polarisability of the substrate. For a series of structurally related substrates an approximately linear correlation exists between the molar refraction of the substrate and the cycloamylose/substrate dissociation constant $\{16\}$. In addition to this the dissociation constants for a series of $p$-substituted benzoic acids are correlated by a Hammett type relationship which is thought to be related to the polarisability of the substrate $\{20\}$.

Bergeron maintained $\{128,9\}$ that irrespective of how the guest penetrates the cavity, provided that it releases the strain and displaces the high energy water, it should bind. The more effectively that the guest releases the strain, the more effectively it should bind.

Bergeron et al. $\{128,9\}$ suggested that the following observations constituted a flaw in the steric strain release system of stabilising the complex. They observed that benzoic acid binds ninety times more strongly than phenol, and suggested that the guest enters from the secondary hydroxyl end, with the guest's functional group being in the proximity of the primary hydroxyl groups on the host. This, they maintained, showed that a molecule with a larger dipole was bound more firmly to the host, and hence that there was a dependance on a dipolar interaction. This is contradicted by kinetic evidence\{20\}, which shows that the hydrolysis of phenyl acetate is catalysed by the C6A molecule. The catalysis is due to the secondary hydroxyl groups, which suggests that the guest molecule enters the cavity phenyl ring first from the secondary hydroxyl end of the host, as has been shown in the C7APNA complex.

They also noticed that $\underline{p}$-nitrobenzoic acid bound less strongly than benzoic acid, and that $p$-nitrophenol binds more strongly than phenol. They also noticed that the $p$-nitrophenolate anion binds more strongly than the neutral p-nitrophenol molecule.

From these observations they concluded that a dipole dipole interaction is resonsible for the formation of the complex. A hydrogen bonding interaction between the guest and the secondary hydroxyl groups on the host can explain all of the above data. It is possible that the hydroxyl group in the benzoic acid could be in the optimum configuration for hydrogen bonding with the host's secondary hydroxyl groups, as the guest's hydroxyl group is directed away from its molecular axis. The phenol molecule has its hydroxyl group pointing along its molecular axis and away from the host's hydroxyl groups.

The addition of a nitro group could bring the hydroxyl moiety in the phenol molecule into a better hydrogen bonding configuration, and reduce the effectiveness of the hydrogen bonding to the benzoic acid.


#### Abstract

Recent n.m.r. studies $\{42\}$ have shown that substrates bind more effectively when they are charged; i.e. when the substrate is highly polarised. These forces are contributary to the complex stability, but are not likely to make a major contribution to the stabilisation of the complex, as water is itself a very good solute of dipoles, and the differences between the solute solute and the solute solvent interactions is probably small.


(c) Hydrogen Bonding.

This is more likely to be a bonus which helps to further stabilise the complex. This is because the hydrogen bonds between the guest and host are not sufficiently in excess of the hydrogen bonds between water and the separate guest and host molecules to account for the observed strength of binding. Furthermore stable complexes are formed with substrates such as benzene, for which hydrogen bonding is not possible. Reducing the polarisability of the water should have the effect of increasing the strength of the interaction, but this is not found to be the case $\{43\}$.
(d) Hydrophobic Interactions.

These interactions are thought to exist where the solvation of the complex is energetically more favourable than the solvation of the individual components. These forces are not the result of a strong interaction between the component parts, but occur from the large internal cohesion of the water.

In order to understand what happens it is necessary to look at the way that the guest and host interact with water. The substrate, which is usually non polar, must first of all make a cavity in the water structure, which is an energetically unfavourable process. The water is reoriented in forming the solvation shells, and becomes highly structured in the volume immediately around the substrate. The formation of this, energetically favourable, structured hydrogen bonded entity balances out the unfavourable enthalpy terms associated with the formation of the cavity in the solvent.

The top and bottom of the cycloamylose molecule is clustered with hydroxyl groups, which can take up when solvated a structure analagous to the bulk water structure. The solvation of the cavity is not so easy to rationalise. The interior of the cavity of C6A contains water which is strained as the result of not being able to have its complete quota of hydrogen bonds. Thus the energy required to form the solvent cavity can not entirely be recouped due to the unfavourable solvation of the cavity. This was neatly illustrated by Schenk and Sand $\{29\}$, who showed that the solubility of C6A was increased by the addition of relatively small amounts of less polar solvents such as ethanol, which can be solvated by the cavity more favourably than water.

The displacement of water from the cavity and the subsequent formation of the original water structure should decrease the energy content of the system, and in part account for the stability of the complexes. This hypothesis is supported by the fact that complex formation is enhanced by hydrophobic substituents and hindered by hydrophilic ones $\{33\}$.

Spectrophotometric measurements on the C6A and C7A complexes with azo dyes indicate that a hydrophobic interaction is significant in the formation of a complex\{130\}. Another direct indication of the importance of hydrophobic interactions is found from chemical shift changes that occur in the n.m.r. spectra when $C 7 A$ and sulphathiazole form a complex. These shifts to higher field indicate a predominantly hydrophobic interaction\{131\}.

Kamiyama and Bender\{132\} have shown that hydrophobic binding similar to that observed in enzyme substrate interactions may be possible, especially when the guest is too large to fit into the cavity. They discussed the interaction of 1-adamantane carboxylate with C6A and C7A in terms of the entropy and enthalpy of formation. The guest is too large to form an inclusion complex with C6A, and can just fit into the C7A molecule's cavity. An association complex was formed with C6A as well as the inclusion complex with C7A. From this they concluded that the association complex(a complex where the guest is outside the cavity) was stabilised by a hydrophobic interaction.

Theoretical Examinations Of Complex Formation.

A theoretical examination of complex formation has been carried out by Tabushi et al.\{125\}. Their conclusions are interesting for two reasons. Firstly, they suggest that the C6A molecule is in a more stable conformation when it is in a tense state, and secondly they conclude that the main driving force for complex formation is a dipole dipole interaction.

There are, however, several aspects of the treatment of their model that are disquieting. In particular, they have optimised the atomic coordinates of two of the C6A complexes, and have not treated the C6A water complex in the same way. This would not be serious if direct comparisons between the empty and complexed C6A had not been made. Unfortunately direct comparisons have been made. They have investigated the C6A methyl orange complex, and have calculated the energies of complex formation for a $1: 1$ complex. However, it has been reported that C6A forms a $2: 1$ complex with methyl orange\{37\}.

Their method of analysis is basically sound, and the calculations are meaningful. They have considered both the enthalpy and entropy of the various components that go to make up a complex. There are however several parts of the system which will be extremely difficult to assess accurately, e.g. the entropy associated with the tense and complexed C6A molecules.

Conclusion
It is likely that driving force for complex formation is a
combination of steric strain relief with a concomitant release of the
high energy water and hydrophobic interactions, with van der Waals
forces and hydrogen bonding between the guest and host molecules
stabilising the complex.

It is apparent from this section that there is a tremendous variety and complexity in the interactions that occur between the guest and the host molecules. When this is finally rationalised, the mechanism and properties of cycloamyloses will be much more easily predicted and explained.

Chapter 9 The Effect Of Cycloamylose Size On Its Catalytic Properties.

There are three principal factors that affect the rate of hydrolysis of substituted phenyl acetates in the presence of cycloamyloses. The first of these is the position of the directing group(the substituent) on the phenyl ring. This was discussed in Chapter one. The remaining two factors, which are interdependant, are the bulk of the directing group and the size of the of the cycloamylose molecule. It is obvious that C6A cannot complex efficiently with an aryl acetate which has a large substituent.

The structures of the $p$-iodophenol complexes of C7A\{23\} and C6A 995 have been determined. Unfortunately in the C7A case there is a 3:2 ratio of guest to host, which alters the disposition of the guest in the cavity, and makes a direct comparison of the guest's orientation in the C7A and C6A complexes meaningless. The C6A p-nitrophenol complex has been solved by Harata\{49\}. The directing group and its position on the ring is the same as that for PNA. A comparison of the disposition of the guest molecules, the included portions of which are essentially the same, may provide some insight into the difference in rate observed for the same phenyl acetate in the presence of different cycloamyloses. The two complexes are illustrated in Figures 9.1.

It is readily apparent from these diagrams that the guest molecule in the C7A complex is included further into the cavity than the guest in the C6A complex. It can be appreciated from these diagrams that in the C6A case the p-substituted phenyl acetates will not come into the close proximity of, and are held rigidly away from, the secondary hydroxyl groups in the host molecule. When the substrate is complexed with C7A where there is a larger cavity there is more possibility of the

Figure 9.1a Cross section of the C7APNA complex (molecule 1)


Figure 9.1 b Cross section of the C 6 A p-nitrophenol complex

$-162-$


Figure 9.2
substrate coming close to the secondary hydroxyl groups, and hence experiencing a larger rate enhancement than the C6A case. It is therefore interesting to note that kinetic data\{46\} confirms this hypothesis with the rate of hydrolysis of $p$-nitrophenyl acetate being three times faster in the presence of C7A than in C6A.

The rate enhancement for m-nitrophenyl acetate is greater in the presence of C6A than in the presence C7A. The disposition of $\underline{m}$ nitrophenol in the C6A cavity is shown in Figure 1.6a (page 18). The steric interaction of the guest molecule in this structure keeps the substrate further out of the cavity. There will be a force pulling the substrate into the cavity, which countered by the steric interaction between between the secondary hydroxyl groups and the phenolic grouping. In C7A the rate enhancement is lower than in C6A for the hydrolysis of m-nitrophenyl acetate. This can be explained by the acetate group having the room in the cavity to rotate away from the secondary hydroxyl groups thus reducing the rate enhancement.

The effect of altering the size of directing group on catalysis is shown in Figure 9.2. There are two balanced forces at play in the formation of a complex. Firstly, the cycloamylose molecule wanting to include as much of the substrate as possible in order to reduce the volume of the substrate that is surrounded by structured water, which is energetically unfavourable. Secondly there will be a resistance to this caused by the steric interaction of the acetate group, and the secondary hydroxyl groups. As the size of cycloamylose molecule is incr-eased there is a larger possibility of the substrate altering its disposition within the cavity, reducing the interaction of the substrate with the secondary hydroxyl groups. This is illustrated in the graph in Figure 9.2.

```
    Thet-butyl substituted phenyl group is extremely bulky, and the
size of the C6A cavity is not big enough to accomodate it, and does not
allow the optimum contact of the acetate group with the secondary hydro-
xyl groups. In C7A the tertiary butyl group can fit into the cavity
neatly, and the ester experiences a greater rate enhancement.
In conclusion the comparison of the \(p\)-nitrophenol C6A complex
and the C7APNA complex shows that the predictions of the physical nature
of catalysis on the basis of kinetic and spectroscopic data are
fundamentally correct.
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List Of Abbreviations

A Angstroms
C6A Cyclohexaamylose
C7A Cycloheptaamylose
C8A Cyclooctaamylose
C9A Cyclononaamylose
n.m.r. Nuclear magnetic resonance
e.s.r. Electon spin resonance
o.r.d. Optical rotatory dispersion

DMSO Dimethyl sulphoxide
CA Cycloamylose
C7APNA The cycloheptaamylose p-nitroacetanilide complex

C7APBA The cycloheptaamylose p-bromoacetanilide complex

C7AMNA The cycloheptaamylose m-nitroacetanilde complex
C7APCA The cycloheptaamylose p-chloroacetanilide complex
C7ANAT The cycloaheptaamylose water complex
C7AI The cycloaheptaamylose complex

## Obaerved and calculated atructure factors for C7APNA Values given are h,k,1, $10^{*}$ Fo and $10^{*}$ PC



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10605 $\begin{array}{lll}-6 & 416 & 385 \\ -5 & 105 & 16 \\ 4 & 251 & 104 \\ 3 & 467 & 93 \\ 2 & 652 & 06 \\ 1 & 404 & 10 \\ 0 & 132 \\ 1 & 298 \\ 2 & 144 \\ 3 & 200 \\ 4 & 106 \\ 5 & 174 \\ 6 & 134 \\ 7 & 184 \\ 8 & 66 \\ 9 & 186 & 130\end{array}$



Observed and calculated structure factora for C7APNA
Values given are $h, k, 1,10^{*}$ Po and $10^{*} \mathrm{Fc}$

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Observed and calculated structure factors for C7APNA
Valuea given are $h, k, 1,10^{*}$ Fo and $10^{\star}$ Fc

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Observed and calculated structure factors for C7APNA
Values given are $h, k, 1,10 \star F 0$ and $10 \star \mathrm{Fc}$

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## Structure of the complex cycloheptaamylose-p-nitroacetanilide

THE cycioamyioses ( Cn A ) are $\alpha-1,4$-linked cyclic oligomers of D-glucopyranose, which have attracted considerable attention as enzyme models ${ }^{1}$. These doughnut-shaped molecules have the primary hydroxyl groups from the 6-position of the glucose residues at one side of the torus, and the secondary hydroxyl groups from the 2 - and 3 -positions at the other. On the inside of the cavity there is a ring of CH groups, a ring of glycosidic oxygens, and a further ring of CH groups, resulting in a hydrophobic ether-like interior. The ability of the cycloamyloses to form stable complexes with a variety of organic compounds by inclusion within the hydrophobic cavity has prompted their use as models for the active sites of enzymes. Of particular interest is the observation that they can accelerate the release of phenols from a variety of aryl esters ${ }^{2}$ and of anilines from anilides ${ }^{3}$ by a reaction pathway similar to that observed for the hydrolytic enzyme $\alpha$-chymotrypsin. Furthermore, a marked degree of substrate specificity is observed: thus, the cleavage of meta-substituted aryl acetates is accelerated more than that of their para-analogues ${ }^{2}$. It has been suggested that this specificity is due to the closer positioning of the nucleophilic secondary hydroxyl group of the cycloamylose to the ester carbonyl in the meta complex than in the para complex ${ }^{2}$. To test the vaiidity of this hypothesis we have made an X-ray crystallographic study of a series of meta- and para-substitured acetanilides and report here on the $1: 1$ compiex of C7A with p-nitroacetanilide (PNA).


Stezowski has solved the structure of two other C7A inclusion complexes, those of $n$-propanol and $p$-iodophenol, and the structure of the 2,5 -diiodobenzoic acid complex has also been reported ${ }^{5}$. Cyclohexaamylose inclusion complexes have been studied by Saenger ${ }^{6}$ and Harzata ${ }^{7}$.
Crystals of C7A-PNA were obtained by slow cooling of $2: 1$ mixtures of C7A and PNA in water; for X-ray photography 'wet' crystals were sealed in Lindemann glass tubes. The crystals are triclinic, $\left(\mathrm{C}_{6} \mathrm{H}_{10} \mathrm{O}_{5}\right)_{7} \cdot \mathrm{C}_{8} \mathrm{H}_{8} \mathrm{~N}_{2} \mathrm{O}_{3} \cdot n \mathrm{H}_{2} \mathrm{O}$, where $n$ is about 7, $a=15.13 \AA \quad b=15.54 \AA, c=15.69 \AA, \alpha=88.65^{\circ}$, $\beta=98.16^{\circ}, \gamma=103.14^{\circ}$, space group PI, $Z=2$. The crystals show pseudo-monoclinic diffraction symmetry ( C 2 ), similar to several complexes reported by Hamilton and Steinrauf ${ }^{8}$, and suggesting that the two C7A molecules in the PI cell are related by an approximate twofold axis.
Intensity data were recorded on Weissenberg films ( $\mathrm{CuK}_{\alpha}$ radiation) and measured by the SRC Microdensitometer Service to give 5,500 independent reflections with maximum $\sin \theta / \lambda$ around 0.5 . A model of the C7A molecule was constructed assuming seven-fold symmetry and using the geometry of individual glucose units as found in two C6A structures ${ }^{6}$. The Patterson function suggested that the sevenfold axes of the two C7A molecules in the unit cell are almost parallel (or antiparallel) and close to the $c$ axis; it also gave an indication of the displacement between the molecules. The antiparallel arrangement, with approximate C2 symmetry, was used, and structure factors were calculated for a series of models with


Fig. 1 One molecule of C7A, with its inciuded molecule of PNA, projected along the approximate sevenfold axis. The larger circles represent oxygen atoms and the smaller carbon. The atoms of the PNA molecule are shaded. Dotted lines represent hydrogen bonds.
each of the ring o,ientations (about the sevenfold axis) varied in $5^{\circ}$ steps. Trial and error adjustment of the ring separation and tilt then led to an approximate solution with $R=0.40$ for 500 reflections and $\sin \theta / \lambda<0.24$.

Refinement involved many stages, mostly using the XRAY72 system of programs ${ }^{9}$; we used step refinement, later least-squares procedures, and at various stages Fourier and difference Fourier summations. The higher resolution data were graduaily introduced, and bond lengths and angles were maintained close to standard values by the program 'Modelfit' ${ }^{10}$. One molecule of PNA was found in the difference Fourier calculated with 2,900 reflections ( $E>0.5$ and $\sin \theta / \lambda<0.4)$ at $R=0.25$; the second PNA molecule appeared in a subsequent difference Fourier calculated with the full data set; water molecules were also found.


Fig. 2 c-Axis projection of half the structure. The other C7A molecules lie immediately above those shown here.


Fig. 3 b-Axis projection of one column of C7A molecules. Seven hydrogen bonds link the $O(3)$ 's of neighbouring molecules up the stack.

At present, $R=0.17$ for 5,500 observed reflections (one isotropic vibration parameter for all atoms). The PNA molecules have been assigned a site occupancy of 0.75 on the basis of the peak heights in the electron density maps, but it is possible that the sites are fully occupied by atoms with higher vibration parameters. In all, 194 non-hydrogen atoms have been located, 77 in each C7A, 13 in each PNA, and 14 water molecules. Figures 1-3, prepared with the program Pluto ${ }^{11}$ illustrate the structure. Formally estimated standard deviations of atom positions are about $0.05 \AA$ in the C7A molecules. Further refinement is planned.

All glucose units have the Cl chair conformation and dimensions which do not differ significantly from standard values. Each C7A molecule has the expected torus shape with the PNA molecule in the cavity. The PNA molecule is tilted so that its $\mathrm{N}-\mathrm{N}$ axis makes an angle of $30^{\circ}$ with the sevenfold axis ot the C7A ring (see Fig. 3); in this way the oxygen atoms of the nitro group are approximately in the plane of the seven primary hydroxyl groups ( $\mathrm{O}(6)$ 's) and the acetyl group is level with the secondary hydroxyl groups ( $O(2$ )'s and $O(3)$ 's). Each anilide nitrogen makes a hydrogen bond with a water molecule within
the cavity. The C7A molecules are only slightly distorted from sevenfoid symmetry. The torsion angles $\phi$ and $\phi^{\prime},\left(0(4)_{n}\right.$ -$\mathrm{C}(1)_{n}-\mathrm{O}(4)_{n-1}-\mathrm{C}(4)_{n+1}$ and $\mathrm{C}(1)_{n}-\mathrm{O}(4)_{n+1}-\mathrm{C}(4)_{n+1}-\mathrm{O}(4)_{n+2}$ $n$ is the glucose residue number) have mean values of 173 and $-175^{\circ}$ with variations around each C7A molecule of $=10^{\circ}$. In cyclohexaamyloses the mean values of $\phi$ and $\phi^{\prime}$ are 166 and $-169^{\circ}$ with similar variations.
There are no hydrogen bonds or other contacts $<3.2 \AA$ between the C7A molecule and the PNA in its cavity; one of the associated water molecules is very weakly hydrogen bonded $(3.2 \AA$ ) with a glucose $\mathrm{O}(3) \mathrm{O}(3)$ of each glucose unit is hydrogen bonded to $\mathrm{O}(2)$ of its neighbour; the mean distance is $2.79 \AA$, the range $2.6-3.0 \AA$. Very similar hydrogen bonding occurs in cyclohexaamyloses ${ }^{\circ}$
Pairs of C7A molecules associate face to face with hydrogen bonds between their $O(3)$ 's (mean distance 2.80 , range $2.7-$ $3.0 \AA$ ). The closest approach between PNA molecules is $3.8-$ $4 \AA$ (between acetyl groups; if the PNA molecules were not tilted the acetyl groups would be impossibly close). These face-to-face pairs of C7A molecules are stacked along the $c$ axis (Fig. 3) to produce the characteristic 'channels. ${ }^{8}$ One pair of primary hydroxyl groups $(\mathrm{O}(6)$ 's) of neighbouring molecules within a stack is directly linked by hydrogen bonds, and there are additional links through water molecules. Such head-tohead stacking is found in all other C7A complexes ${ }^{4.5}$ but only in one C6A complex ${ }^{\circ}$. Neighbouring columns are close-packed (Fig. 2) and there are several hydrogen-bonded links between hydroxyl groups of neighbouring columns and others through water molecules. $O(4)$ and $O(5)$ atoms are not invoived in any hydrogen bonds $<3.0 \AA$.

The PNA molecule has been found, as expected, in the cavity of the C7A. There are no specific interactions apart from very weak hydrogen bonding through a water molecule, so we conclude that van der Waals forces are responsibie for holding the PNA molecule in the cavity. The tilt (Fig. 4) was initially a surprise, but it ailows the PNA molecule to occupy most of the available space in the C7A cavity while keeping the poiar nitro and amide groups close to the hydroxyl groups of C7A and the nonpolar benzene ring in contact mainly with CH groups. If a molecule of $m$-nitroacetanilide were similarly held in the C7A cavity its acetyl group would probably approach the secondary hydroxyl groups; however, conclusions about this must await the structure determination of the $m$-nitroacetanilide complex which we are pursuing.

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## List Of Postgaduate Courses Attended.

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X-Ray Group Seminars (1974-8)
Imp Computing Course (E.R.C.C.)
EMAS Computing Course (E.R.C.C.)
Spectroscopy Course (Dr. S. Cradock)
Shell Lectures (1975)
N.m.r. Spectroscopy Course (Dr. Harris)
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[^0]:    The above mechanism is similar to those found for chymotrypsin\{39\} and penicillinase\{65\}. The existance of the covalently bound species $S^{\prime}$.CA has been shown in solution\{5\}\}. Both cycloamylose and enzyme catalysed reactions exhibit the same rate dependance on the concentration of catalyst. As the concentration of the catalyst is

[^1]:    * Refers to a complete structure determination

[^2]:    The remaining two types of hydrogen bonding occuring in these systems are intermolecular hydrogen bonding between cycloamylose molecules, which only occurs frequently in channel type structures, and hydrogen bonding between hydroxyl groups in the cycloamylose and water of crystallisation.

[^3]:    2200 Unique reflections were measured to a $\sin \theta / \lambda$ limit of 0.60 , and this represented 0.7 of the total number of reflections to this limit.

[^4]:    Table 7. la Parameters of the Plif molecule frow the

[^5]:    The actual change in conformation is, according to Saenger, the main driving force for complex formation. The relief of strain is independant of the nature of the substrate, and this qualitative approach can account for the large variety of substrates that form complexes. His scheme is illustrated below in Figure 8.2.

