ABSTRACT FORM D

Final Report of Grant Funded by the Research and Creative Productions Committee

Title of Research or Creative Production Characterization of New Acetic Solvates and Studies of Acetic Acid Binding Interactions

Department Physical Sciences

Name of Grant Recipient(s) Dan Adsmond

Date of Final Report January 1998 (Mo./Yr.) Date Grant was Funded Dec. 1996 (Mo./Yr.)

Amount for which grant was funded $ 4,396.00 Amount expended $ -4,396

Summary:

The primary goal of the research completed was to improve understanding of the nature of the binding interactions in acetic acid solvates with specific emphasis on binding of acetic acid to sulfa drugs. The four research objectives of the project were met as follows. 1) Six of the nine newly discovered acetic acid solvates were confirmed by NMR. One additional acetic acid solvate, that of methoxysulfadiazine was discovered. Stability studies carried out on all seven solvates indicated that the sulfapyridine and thymolphthalein solvates were the most stable, retaining 100% of their acetic acid for several days. The crystal structure of the 1:1 sulfapyridine:acetic acid solvate was solved. 2) Infrared studies of acetic acid and three of the sulfa drugs provided data indicating that the acetic acid wasn't hydrogen bonding to the amidine group of the sulfa drug as previously predicted. The crystal structure of the sulfapyridine solvate confirmed this by showing that two acetic acid molecules hydrogen bonded to each other in the crystal rather than to the drug molecule. 3) 151 C-H...X hydrogen bonds involving acetic acid were found in a total of 18 published acetic acid solvate structures. The patterns were recorded using graph set notation and further analyzed. 4) Analysis of the data gathered to date suggests a binding of acetic acid to host sulfa drugs through a number of secondary (C-H...X) hydrogen bonds rather than through the predicted primary hydrogen-bond interaction between the carboxylic acid and the amidine groups. Further conclusions await continued data collection and analysis.

Final use of project results, e.g., Where was it published? At what professional meeting was it presented? How was it disseminated to the academic or regional community?

Presented at the Kentucky Academy of Science meeting, Nov. 1997 and will also be presented at the American Crystallographic Association annual meeting, July 1998.

Final Report
Characterization of New Acetic Acid Solvates
and Studies of Acetic Acid Binding Interactions

The primary goal of the research completed was to improve understanding of the nature of the binding interactions in acetic acid solvates with specific emphasis on binding of acetic acid to sulfa drugs.

This primary goal was divided into the following four research objectives.

1. to fully characterize ten newly discovered acetic acid solvates (including solvates of three sulfa drugs) by stability studies, infrared analysis, and, where possible, X-ray structure determination.

2. to carry out infrared binding studies on mixtures of sulfa drugs and acetic acid.

3. to use current crystallographic software to search 26 acetic acid solvate structures (as well as any new crystal structures obtained) for secondary (C-H...O and C-H...N) binding interactions and to gain insight into the role of these secondary interactions in solvate formation.

4. to use the findings from the stability tests, infrared studies, and crystallographic studies mentioned above toward gaining a better understanding of the binding preferences of acetic acid and sulfa drugs.

The following report describes how each of these objectives was met and briefly summarizes some of our main discoveries.

Objective 1

Crystals of the ten compounds previously believed to form acetic acid solvates were grown from acetic acid in order to confirm solvate formation and to determine the stoichiometry (ratio of the components) of the solvate formed. Crystals of 4 additional sulfa drugs were also grown from acetic acid to test their ability to form acetic acid solvates. The results of these experiments are summarized in Table I.

Table I: Results of Acetic Acid Solvate Formation Tests in 14 Compounds.

Host Compound	Solvate (yes or no)	Stoichiometry host comp'd:acetic acid
bromocresol purple	yes	1:2
caffeine	no	
deoxycholic acid	yes	1:1
fluorescein	?	?
2-hydroxy-3,5-diiodobenzoic acid	yes	1:1
mannitol	no	
thymolphthalein	yes	1:1
sulfathiazole	yes	1:2
sulfapyridine	yes	1:1
sulfadimethoxine	yes	1:2
sulfamethoxazole	no	
sulfisomidine	no	
sulfisoxazole	no	
methoxysulfadiazine	yes	1:1

Note that caffeine and mannitol, previously believed to form acetic acid solvates are reported as not forming solvates in Table I. We found that all but a trace amount of acetic acid was removed when the caffeine crystals were washed with ether, indicating that the acetic acid had been actually coating the surface of the crystals rather than being incorporated into the structure. The apparent formation of a mannitol solvate was irreproducible after several attempts and so is also reported as a non-solvate. Testing four new sulfa drugs, the last four listed in Table I, revealed one additional solvate, a 1:1 methoxysulfadiazine/acetic acid solvate.

Fluorescein formed such fine particles in acetic acid solution that it was nearly impossible to remove by filtration. Since the acetic acid had to be left to evaporate for several days before the crystals could be analyzed by NMR, the results from the fluorescein experiments are unreliable.

The stoichiometry of the deoxycholic acid solvate, known from an earlier publication, could not be accurately determined by NMR due to interference of host

peaks in the acetic acid region. Consequently its stability could not be monitored by NMR in the following studies.

Stability studies were carried out on the 7 compounds which formed acetic acid solvates having a definite reproducible stoichiometry. The results of those stability studies are summarized in Table II.

Table II Stability Data of 7 Acetic Acid Solvates With Known Stoichiometry

Substance	Percent Solvent Remaining After x Hours Open at Room Temp					
	2 hours	4 hours	6 hours	8 hours	24 hours	(x) hours
bromocresol purple	100	100	100		60	0 (71)
2-hydroxy-3,5-diiodobenzoic acid	100	100	100	100	50	
thymolphthalein	100	100	100	100	100	100 (96)
sulfathiazole	100	75		50		
sulfapyridine	100	100	100	100	100	100 (292)
sulfadimethoxine	100	100	100	60	0	
methoxysulfadiazine	100	100	100		50	

All seven of the solvates with known stoichiometries retained their acetic acid for two hours. Six of the seven solvates retained their acetic acid for over six hours and two of the seven, the sulfapyridine and thymolphthalein solvates, retained the original amount of acetic acid for several days.

An important part of the characterization of the sulfa drug:acetic acid solvates was to investigate the specific way in which the acetic acid binds to the drug molecule. The specific binding interactions we were interested in were hydrogen bonds which are defined as the attraction between an acidic hydrogen (the hydrogen-bond donor) and an electronegative atom such as oxygen or nitrogen (the hydrogen-bond acceptor). In this document hydrogen bonds will be divided into two categories: 1) primary hydrogen bonds (where the acidic hydrogen is covalently bonded to an oxygen or nitrogen atom)

and 2) secondary hydrogen bonds (where the acidic hydrogen is covalently bonded to a carbon atom). The primary hydrogen bonds are much stronger than the secondary ones.

Infrared (IR) investigations were carried out on the acetic acid solvates of sulfapyridine, sulfadimethoxine, and methoxysulfadiazine in order to gain information about any primary hydrogen-bonding interactions between acetic acid and the sulfa drug. When a carboxylic acid, such as acetic acid, hydrogen bonds to an amidine group, such as those observed on the four sulfa drug molecules, the acid OH stretching peak in the infrared spectrum will shift from the normal broad band observed over a frequency range of 2600-3200 cm^{-1} and split into two smaller peaks observed at 1900 - 2000 cm^{-1} and 2400 - 2500 cm^{-1}. The IR of sulfapyridine, sulfadimethoxine, and methoxysulfadiazine solvates showed no such shifting and splitting of the OH stretching peak suggesting that if the acetic acid molecules were hydrogen bonding to the sulfa drug molecules, they weren't hydrogen bonding to the amidine groups. Only an X-ray crystal structure of such a solvate could provide a definitive answer to questions on specific binding.

Crystals of all sulfa drug solvates were grown in an attempt to obtain crystals of high enough quality for single crystal X-ray structure determination. To date, only crystals of the 1:1 sulfapyridine/acetic acid solvate have been obtained having sufficient quality for x-ray crystallography. The crystal structure of the solvate was solved by Prof. Mark Whitener at Montclair State University. Figures 1-4 highlight the most important features of the crystal structure. The dotted lines in Figures 1-3 represent primary binding interactions and in Figure 4 they represent secondary binding interactions.

The original prediction before the infrared studies was that the best donor, the acetic acid hydrogen, would hydrogen bond to the best acceptor present, the amidine nitrogen of the sulfapyridine. Figure 1 shows that in the 1:1 sulfapyridine/acetic acid solvate, acetic acid binds to a neighboring acetic acid molecule instead of the sulfapyridine host molecule even though the amidine nitrogen of sulfapyridine is a better acceptor than acetic acid. This acetic acid dimer, with two acetic acid molecules binding to each other in a bidentate fashion, was observed in 6 other published acetic acid solvate structures but previously appeared only when there was not a better hydrogen-bond acceptor than itself present on the host molecule. This curious result confirmed our interpretation of the infrared studies which seemed to indicate that acetic acid was binding to itself rather than to the amidine group of sulfapyridine as previously predicted.

Figure 2 is a stereo view showing all the molecules in the unit cell, the repeating building block of the crystal. The acetic acid molecules may be seen hydrogen bonding to each other in the center of the unit cell while the amidine groups of neighboring sulfapyridine molecules are seen hydrogen bonding to each other at the sides of the unit cell in a bidentate fashion similar to that observed between acetic acid molecules.

Figure 3 shows all the primary hydrogen bonding interactions of sulfapyridine in the 1:1 sulfapyridine/acetic acid solvate crystal. The bidentate interaction between

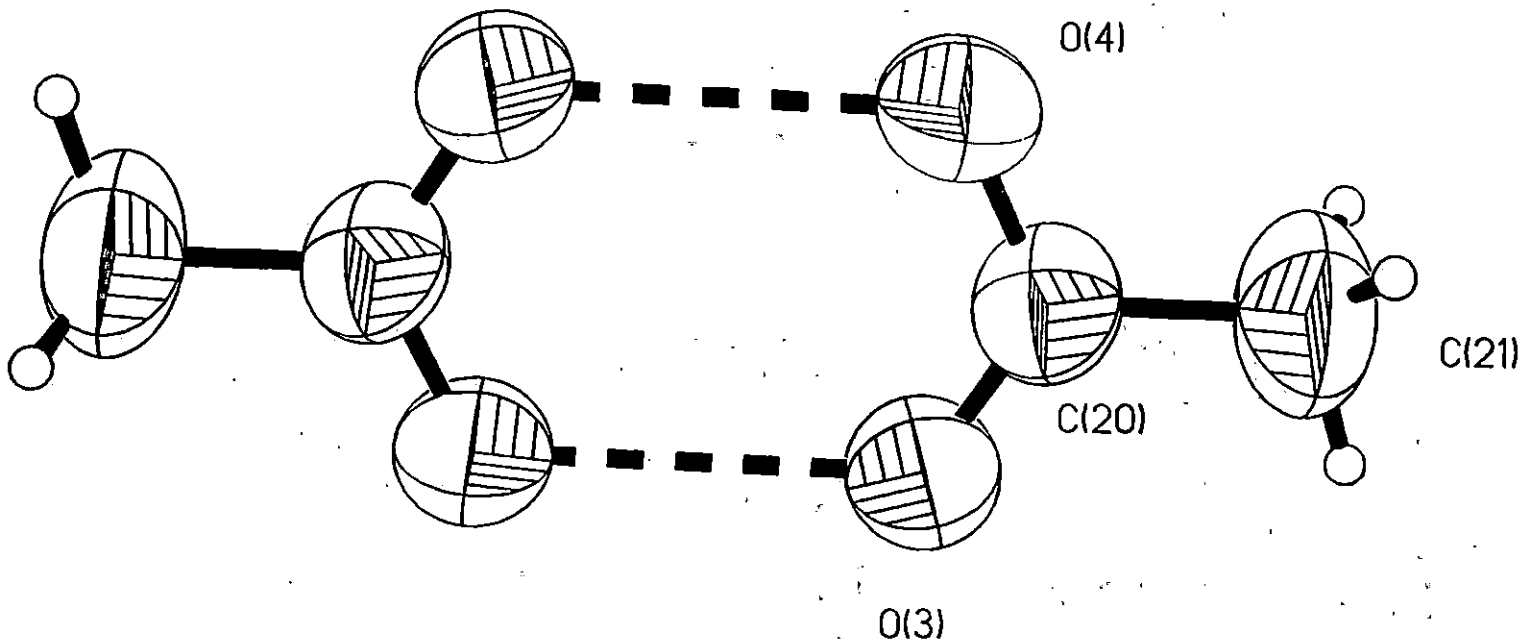


Figure 1: The Acetic Acid Dimer Observed in the 1:1 Sulfapyridine/Acetic Acid Solvate.

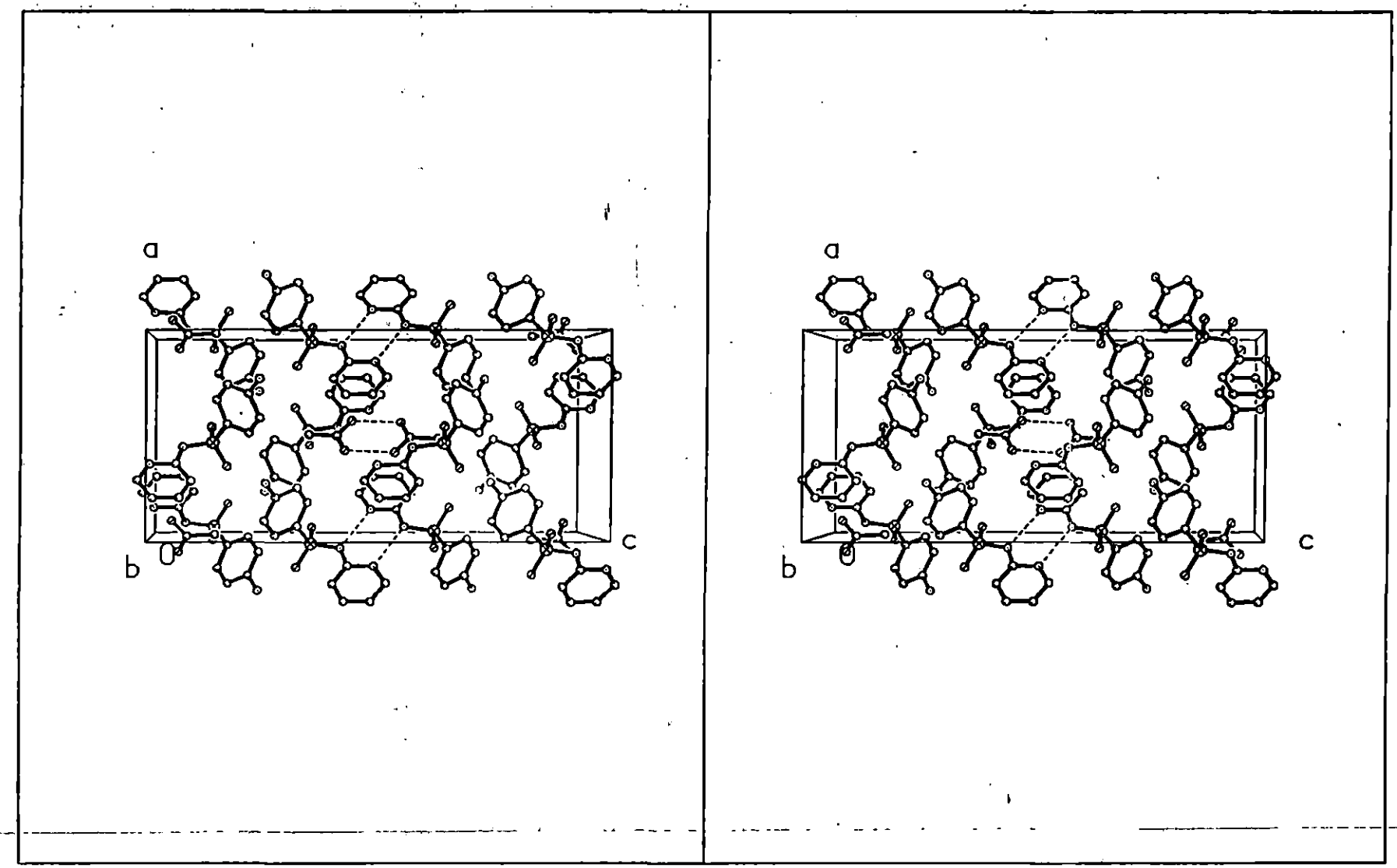


Figure 2: Stereoview of the Unit Cell of the 1:1 Sulfapyridine/Acetic Acid Solvate.

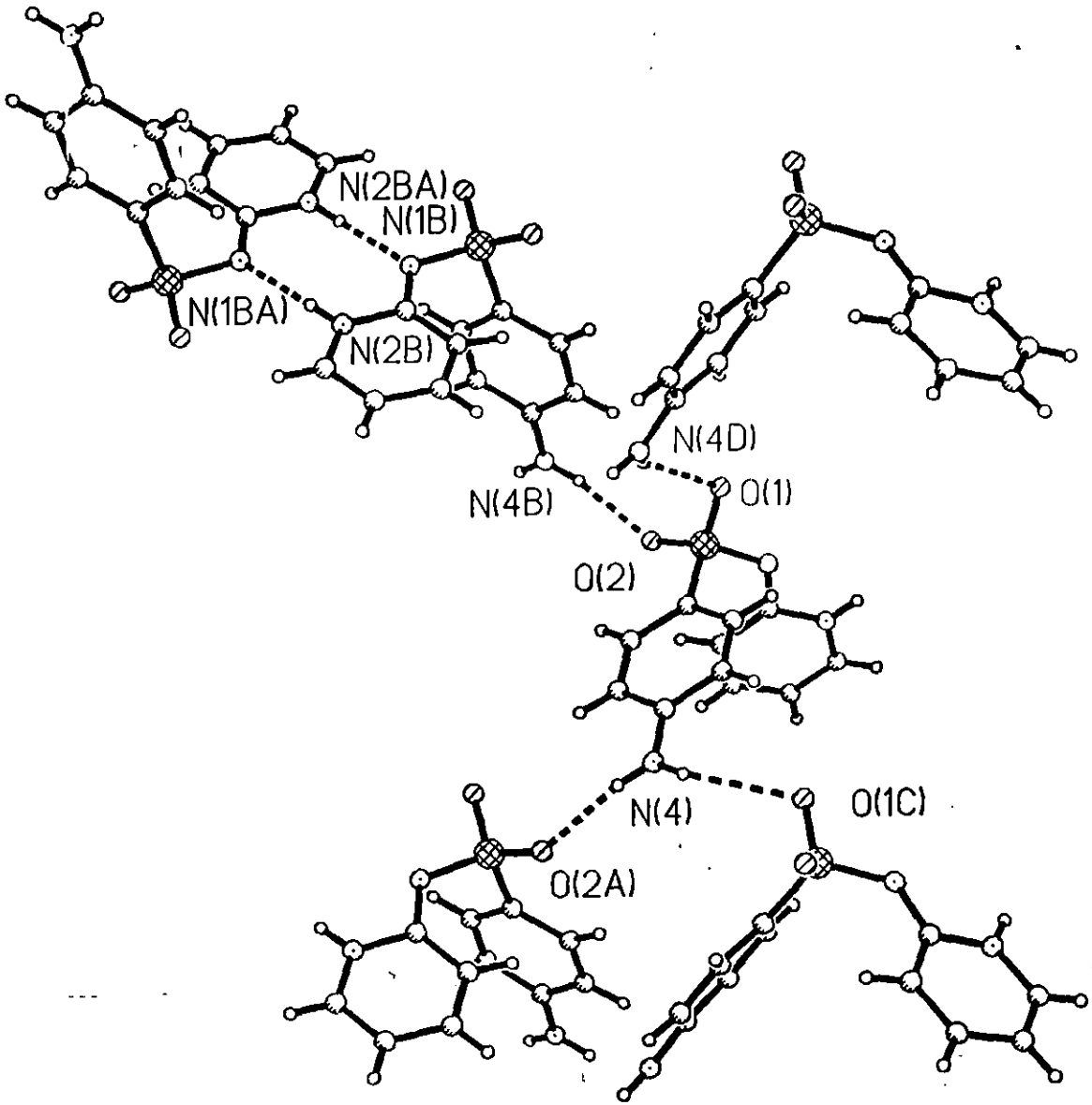


Figure 3: The Primary Binding Interactions of Sulfapyridine in the 1:1 Sulfapyridine/Acetic Acid Solvate.

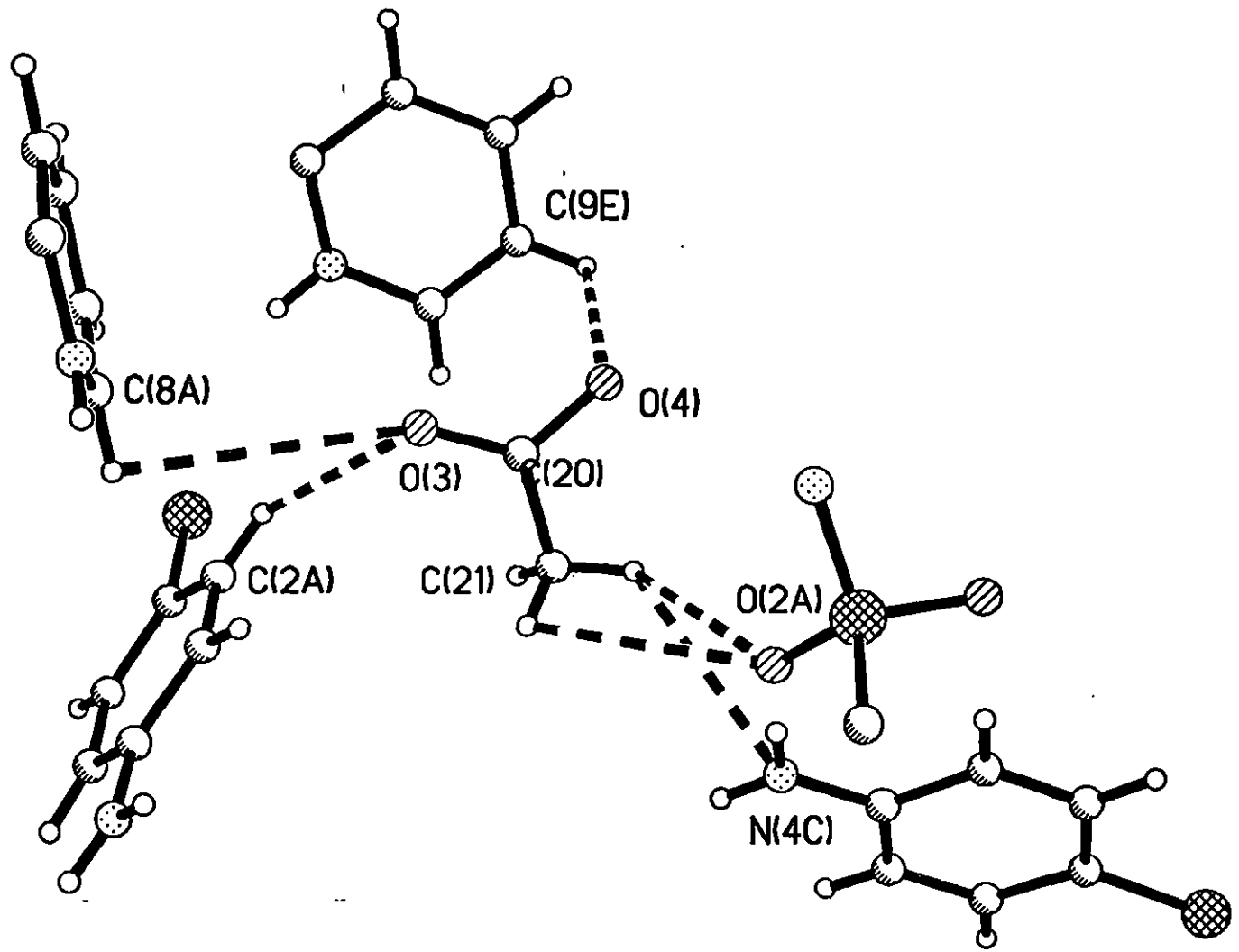


Figure 4: The Secondary (C-H...X) Binding Interactions of Acetic Acid in the 1:1 Sulfapyridine/Acetic Acid Solvate.

amidine groups previously seen in Figure 2 is shown in the upper left corner. In addition, each of the amino hydrogens (attached to N4) in the sulfapyridine bind to sulfonyl oxygens (O1 & O2) of neighboring sulfapyridines forming chains having a repeating unit of 8 atoms.

The lack of any primary hydrogen bonding between the acetic acid molecule and the sulfapyridine host prompted a search for possible secondary (C-H...X) hydrogen-bonding interactions that could help account for the inclusion of acetic acid in the crystal. Figure 4 shows the six secondary hydrogen-bonding interactions that were found joining each acetic acid molecule to the neighboring sulfapyridine molecules. Three of the six interactions are formed by methyl hydrogens of the acetic acid molecule (attached to C21) donating to a sulfonyl oxygen and an amino nitrogen of neighboring molecules. Each of the other three secondary hydrogen-bonding interactions involves an aromatic sulfapyridine hydrogen donating to an acetic acid oxygen.

Objective 2

To gain information about the initial binding interaction between acetic acid and the sulfa drug we carried out infrared studies on intimate mixtures of acetic acid and three of the sulfa drugs having a 1:1 and 1:2 ratio of the substance to acetic acid. We were interested in whether the acetic acid would hydrogen bond to the amidine group of the sulfa drug in these mixtures. Each mixture was ground together with acetic acid for up to ten minutes and then analyzed by IR. Still the OH stretching peak was unshifted and unsplit indicating that the acetic acid was binding to itself rather than to the amidine group of the sulfa drug.

To further investigate potential binding of acetic acid to the amidine group, we heated mixtures of each of the sulfa drugs with a 1:1 mixture of acetic acid in a closed vial at 50-60 °C for up to 21.5 hours. The IR still indicated that no such binding was occurring.

Based on the IR studies and the crystal structure of sulfapyridine, we now believe that the acetic acid in both methoxysulfadiazine and sulfadimethoxine solvates is not binding to the amidine group either. The solving of the crystal structures of these two solvates will provide confirmation of the hypothesis.

Objective 3

A prerequisite to identifying secondary (C-H...O and C-H...N) hydrogen bonds is knowledge of the hydrogen atom positions. This information was available for 18 of the 26 published acetic acid solvates previously studied. The VISTA software package included in the Cambridge Structural Data Base was used to find the secondary C-H...O and C-H...N hydrogen bonds in all 18 of these structures.

In all, 151 C-H...X hydrogen bonds were found in the 18 structures. The type of donor and acceptor atom was noted for each hydrogen bond and recorded in a table.

Acetic acid acted as a hydrogen-bond donor in 59 of these hydrogen bonds and as a hydrogen-bond acceptor in 102. The donating hydrogens in these 102 hydrogen bonds where acetic acid was the acceptor may be divided into three categories, the largest being aromatic protons on the host molecule, accounting for 66 hydrogen bonds. Non-aromatic protons on the host donated 26 hydrogens and other acetic acid molecules donated the remaining 10. Table III summarizes the various types of C-H...X binding interactions that were discovered.

Table III: Summary of the 151 C-H...X Hydrogen Bonds Appearing in 18 Acetic Acid Solvates

H-Bond Type	% of total H-Bonds	# of Structures Appearing	# Times per Acetic Acid Molecule
(AA)CH...X(Host)	32	15/18	2
(AA)O...HC(Host)	61	17/18	2
(AA)CH...O(AA)	7	6/18	4

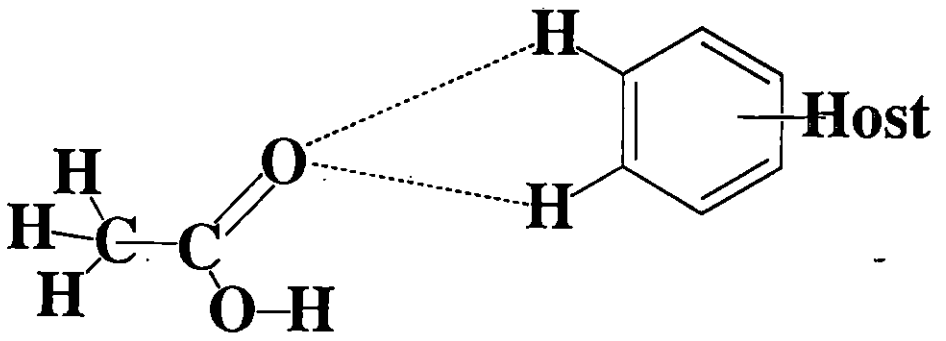
It appears that secondary hydrogen bonding plays a very important role in acetic acid solvate formation with an average of nine secondary C-H...X interactions occurring per acetic acid molecule. On the average seven of these C-H...X interactions were between the acetic acid molecule and the host molecule while two were between neighboring acetic acid molecules.

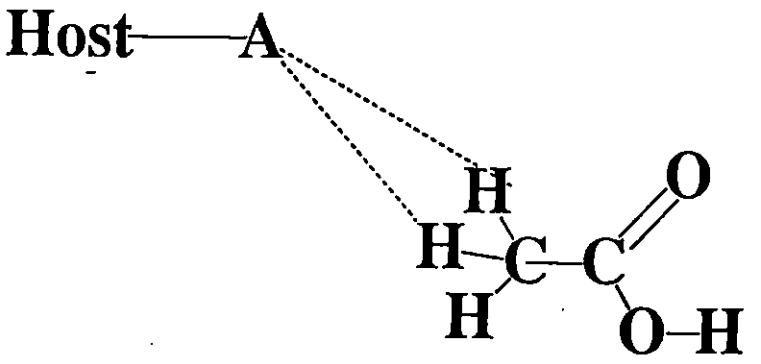
The observed secondary C-H...X hydrogen bonds included a variety of bidentate interactions between an acetic acid molecule and a host molecule. The two most commonly observed patterns are shown in Figure 5. The first, a ring formed by two neighboring aromatic protons binding to a single acetic acid oxygen, occurred 27 times in the 18 structures investigated. The second pattern, a ring formed when two methyl protons on the same acetic acid molecule donated to a single host acceptor, appeared a total of 10 times in the 18 structures.

Objective 4

The literature shows preferential binding between carboxylic acid groups and amidine groups in crystals where both groups are present. In fact all five published crystal structures containing both a carboxylic acid group and a sulfa drug with an amidine group show those two groups binding to each other. One would expect, then, that solvates involving sulfa drugs containing one or more amidine groups and acetic acid (containing a carboxylic acid group) would exhibit the previously observed binding

Figure 5: Commonly Observed Bidentate C-H---X Hydrogen bond patterns





interaction between the two and that the carboxylic acid-amidine binding interaction would be the driving force for acetic acid solvate formation. What we found, however, was that six sulfa drugs with the requisite amidine group did not even form acetic acid solvates and that in the four sulfa drugs that did form solvates there was no evidence for acid-amidine binding. Infrared studies revealed no apparent binding between acetic acid and the amidine group of the sulfa drug either in a solvate crystal or in a mixture where acetic acid and the sulfa drug had been intimately ground together. The crystal structure of the 1:1 sulfapyridine:acetic acid solvate confirmed these results.

Crystal structures of three different polymorphs of sulfapyridine are known. Upon receipt of data from the authors, the hydrogen-bond patterns and crystal packing of the solvate will be compared with those of the non-solvates to determine the effect of the acetic acid incorporation on the crystal structure.

Our research also revealed the prevalence of secondary binding interactions in acetic acid solvates in general and in the sulfapyridine/acetic acid solvate. It appears that the energy lost by the lack of acid-amidine binding may be at least partially compensated for by the many secondary binding interactions.

Results of this work have been presented in the form of a talk by Sherri Gorrell at the 1997 meeting of the Kentucky Academy of Science. Sherri will also present a poster at the annual meeting of the American Crystallographic Association in Washington D.C. in July of 1998. Currently results are being compiled and further analyzed for publication in a refereed journal.