

ABSTRACT

A STUDY OF THE PHYSICOCHEMICAL PROPERTIES OF THE TETRAZOLES AND AN INVESTIGATION OF IONIC INTERACTIONS BY SODIUM-23 NUCLEAR MAGNETIC RESONANCE

By

Ronald Erlich

The purpose of this study was twofold. It was desired to study the physicochemical properties of the tetrazoles in an attempt to understand the mechanism of their activity. Since this study was to involve an investigation of metal ion interactions with the tetrazoles, it was also desired to develop and use the techniques of nuclear magnetic resonance on the sodium-23 nucleus as an additional method with which to study ionic interactions in solutions.

Electrical conductance measurements were carried out on six cyclopolymethylenetetrazoles as well as two dihalo derivatives in formic acid at 25°C. Basicity constants as well as limiting equivalent conductances were calculated by the Fuoss-Shedlovsky method from the conductance data. It was shown that while the tetrazoles do not have any detectable proton affinity in aqueous solutions, the unsubstituted cyclopolymethylenetetrazoles act as fairly strong monoprotic bases in formic acid solution. The length of the hydrocarbon chain does not influence the basic strength of the tetrazole ring, but the inductive effect of the halogens essentially divests the ring of its proton affinity.

Complex compounds of magnesium and lithium ions with 1,5-dimethyl-tetrazole in nitromethane solutions were studied by measurement of the donor proton chemical shifts as a function of donor-acceptor mole ratio. Two complexes were found, $(\text{TzMg})^{2+}$ and $(\text{Tz}_4\text{Mg})^{2+}$ depending on concentrations of the reactants. The stoichiometry of the lithium complex appeared to be $(\text{Tz}_4\text{Li})^+$. The fact that the magnitudes of the chemical shifts of the N-methyl and C-methyl protons of the tetrazole were approximately the same may indicate that the coordinating site in the tetrazole ring is the 3-nitrogen, or that in solution the metal may be bound to the 2, 3, or 4-nitrogen, and we are observing an average environment.

Finally, sodium-23 nuclear magnetic resonance studies were carried out with several salts in a variety of solvents. The chemical shift data fall into two classes, little or no concentration dependence for the perchlorate and tetraphenylborate solutions and a marked concentration dependence for the iodide and thiocyanate solutions. The results are consistent with an assumption that contact or solvent shared ion-pairs are formed in the latter case. Through proper selection of the anion, the effects of ion-pairing were minimized, and the solvation of the sodium ion was studied. It was found that the chemical shift for the sodium ion in a given solvent, with respect to a standard of sodium chloride in water, is directly proportional to the electron donating ability of the solvent as measured by Gutmann's donor numbers. Data yield a new value of 33 for the donor number of water which is more in line with other observations. Measurements were also made to study the feasibility of using sodium-23 nuclear magnetic resonance for the investigation of mixed solvent systems. The possible effects of anion solvation on these measurements are also considered.

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By

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To Mom, Dad, and Apple Pie

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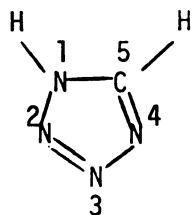
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CHAPTER I
INTRODUCTION AND HISTORICAL

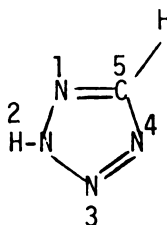
INTRODUCTION

Tetrazoles and substituted tetrazoles have long been the object of intensive investigations because of their unique physiological and physicochemical properties. The first synthetic tetrazole was prepared in 1885 by a Swedish chemist, Bladin (1), when he reacted dicyanophenylhydrazine in sulfuric acid with potassium nitrite and obtained 1-phenyl-5-cyanotetrazole. Well over 400 members of this class of nitrogen heterocycles have since been synthesized and characterized (2).

The tetrazole ring is composed of one carbon and four nitrogen atoms linked by three single and two double bonds. The ring is numbered so that a nitrogen bonded to the carbon is in the 1-position. The remaining three nitrogens are numbered consecutively two through four with the carbon at the 5-position. Tetrazoles may exist in two tautomeric forms I and II (3, 4).



I

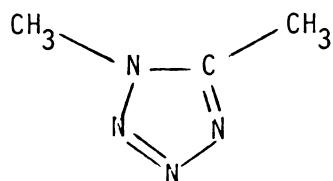


II

It has been shown that 97% of an equilibrium mixture of I and II exists in the form I (5).

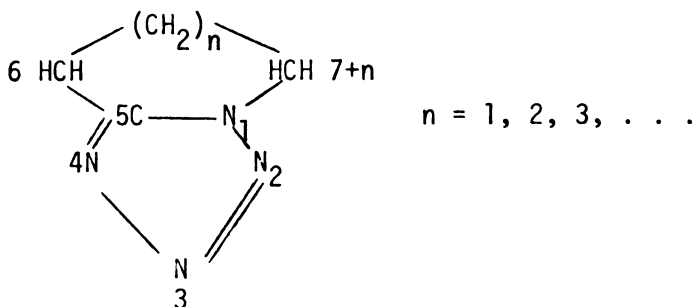
The tetrazole ring is unusual among cyclic systems in that it offers only two points of substitution. Two specific classes of tetrazole

derivatives are involved in the present investigation. In the first of these, both hydrogens on the tetrazole ring are replaced by methyl groups. This compound, 1,5-dimethyltetrazole, is first mentioned in a patent granted to the Knoll Chemical Co. (6).



1,5-dimethyltetrazole

The other special class of the tetrazoles are the cyclopolymethylene-tetrazoles in which a polymethylene chain forms a second ring fused to the tetrazole ring.



cyclopolymethylenetetrazole

It is well known that pentamethylenetetrazole, also known by the trade name Metrazol, is a powerful stimulant and convulsant (7) which has been used extensively in chemotherapy. The physiological activity of the various substituted tetrazoles is recognized and has generated a great deal of research into the nature of physiological interactions. It would be valuable to determine which properties of these compounds give rise to the observed activity. Such an investigation may show how the

tetrazoles affect the central nervous system and may lead to the development of new and more active compounds.

Gross and Featherstone (8) studied the pharmacological activity of a number of tetrazoles and determined that the activity depends on the substitution on the tetrazole ring. Bulky groups on the 5-position cause a decrease in activity while, conversely, large groups on the 1-position usually have the opposite effect. In the case of pentamethylenetetrazole, the introduction of a substituent on the 8-position was found to cause an increase in the analeptic activity only when the substituent is small or closely packed as in the case of methyl and tert-butyl groups.

In 1949, Schueler, et al. (9) attempted to correlate the activity of a number of substituted tetrazoles with their ultraviolet spectra. It was found that compounds of moderate activity and which have alkyl groups substituted on the ring, generally showed little or no absorption down to 220 m μ . The compounds which had aryl substitution and which exerted a depressant effect showed absorption bands in the regions around 290 and 225 m μ , with the 225 m μ band having a much higher absorptivity. A decrease in the depressant activity of the series gave rise to a hypochromic shift of the 225 m μ band, while an increase in the activity gave rise to a hypochromic shift of both bands. Thus there would seem to be some correlation between the absorption spectra of the tetrazoles and their physiological activities.

Dister (10) found that the distribution coefficient (organic)/(aqueous) for pentamethylenetetrazole, substituted pentamethylenetetrazoles, and 1,5-dialkyltetrazoles is larger in basic media and concluded that these tetrazoles are extremely weak bases. Popov and Holm (11) titrated pentamethylenetetrazole in glacial acetic acid with perchloric acid and showed that the solute possesses weak basic properties in this solvent.

Golton (12) studied the distribution of pentamethylenetetrazole between aqueous and carbon tetrachloride layers as a function of pH and determined that the protonization constant for metrazole in water is about 1×10^{-14} .

Popov and Marshall (13) measured the proton affinity of metrazole (pentamethylenetetrazole), substituted pentamethylenetetrazoles, and 1,5-dialkyltetrazoles in formic acid and determined the pK_b of these solutes to have a value of about 2. While the overall correlation was not definite, the most active compound tested was shown to be the most basic and the most inactive compound was the least basic.

Popov and Holm (14) determined the dipole moments of pentamethylene-tetrazole, 8-t-butylpentamethylenetetrazole, and 8-sec-butylpentamethylene-tetrazole in benzene solution to be 6.14 D, 6.20 D, and 6.18 D respectively. Since the 8-t-butyl compound is a very strong convulsant (3 mg/kg minimum convulsant dose (MCD)) while the 8-sec-butyl compound is a very weak convulsant (750 mg/kg MCD). They concluded that there was no correlation between the physiological activity of these compounds and the magnitude of their dipole moments.

In order to study the donor properties of the tetrazoles, Popov, et al. (15) measured spectrophotometrically the formation constants of the pentamethylenetetrazole complexes with iodine monochloride, iodine monobromide, and iodine in carbon tetrachloride solutions. Vaughn, et al. (16) studied the iodine monochloride complexes of 7-methyl-, 9-sec-butyl- and 8-t-butylpentamethylenetetrazole. In all cases the complexes were only slightly stronger than the corresponding complex for the unsubstituted tetrazole. Thus there does not seem to be a correlation between the activity and the donor properties for these studied tetrazoles.

The formation constants for the reaction between the silver ion and the cyclopolymethylenetetrazoles in aqueous solution were determined by

D'Itri (17). His data show that, while the activity of the tetrazoles is varying from 750 mg/kg to 25 mg/kg MCD (7), the formation constant for this reaction remains constant at about 10^3 . Thus correlation between activity and the ability to complex metals, or at least Group I metals, seems to be lacking.

While work on finding possible correlations continued, other work was in progress attempting to elucidate the structure of the metal-tetrazole complexes.

Brubaker (18) prepared and characterized two forms of the solid complex bis(5-aminotetrazolato)copper(II). His data show that complexation rather than simple salt formation is taking place. Complexes with formation constants on the order of 10^{12} were prepared with tetrazole, 5-phenyltetrazole, and 1-ethyltetrazole. It was found that there was very little interaction between 1,5-dimethyltetrazole and copper(II) indicating that a replaceable ring hydrogen is necessary for the complexation to occur.

Several nickel(II) and copper(II) complexes of substituted tetrazoles were prepared by Garber (19). The reflectance spectra indicated that these ions are in an octahedral or tetragonal environment and are, therefore, six coordinate. The authors suggest that two of the six bonds are Ni-C bonds while the other four are Ni-N bonds.

Popov and Holm (11) prepared the silver complexes of several tetrazoles, among them, pentamethylenetetrazole. Upon slow evaporation of the aqueous solution, a solid complex with the formula $\text{Ag}(\text{pentamethylenetetrazole})_2\text{NO}_3$ is obtained. This complex demonstrates that the loss of a ring proton is not necessary for the complexation to occur and that the coordination is probably through one of the nitrogen atoms of the ring.

Popov, et al. (15,20) studied the charge-transfer complexes of substituted tetrazoles with π -electron acceptors and showed that they are extremely weak in solution. They also showed that the formation constants for the ICl-pentamethylenetetrazole complex is three orders of magnitude greater than the formation constant of the benzene-ICl complex. They state that this implies complexation through a ring nitrogen.

The ICl-pentamethylenetetrazole complex was investigated by Baenziger, et al. (21) using x-ray crystallographic techniques. It was found that the tetrazole acts as a unidentate ligand, and the iodine monochloride is bonded to the 4-nitrogen of the ring. Baenziger (22) also studied the zinc(II) complexes with the tetrazoles and showed that again complexation occurs through the 4-nitrogen.

Thus the previous research on the tetrazoles and substituted tetrazoles leaves two major questions unanswered. While measurements of the basicity were made on several of the tetrazoles, no measurements were made on the series of cyclopolymethylenetetrazoles. Since such large changes in activity are realized with such small monotonic changes in the structure of the compounds, it should be easily seen whether the base strengths of the series could be correlated with the activity. Hence, a portion of this research is involved with a determination of the base strengths of the cyclopolymethylenetetrazoles in formic acid solution by conductance measurements.

The second question relates to the structure of the tetrazole complexes. While there is unambiguous evidence indicating that the tetrazole bonds through the 4-nitrogen in two solid adducts, no data are available on the structure of these complexes in solution. Thus another section of this thesis is devoted to attempts to elucidate the structure and stoichiometry of the tetrazole-metal complexes in solution.

In the past, numerous methods have been employed for the study of complexation and solvation in solutions, notably conductance measurements and optical spectroscopy. However, for the type of investigation being reported in this thesis, nuclear magnetic resonance techniques have proven to be quite useful.

Popov and Maxey (23) studied the solvation of the alkali metal cations and ammonium ion in dimethylsulfoxide (DMSO) solutions by observing shifts of the methyl proton resonance of DMSO in pentanol solutions. The solvation number of lithium in DMSO was determined from the plots of the DMSO:LiX mole ratio versus the chemical shift. Similar experiments were carried out with sodium salts. While there was an indication of a break in the curve, it was less well pronounced than with the lithium salts, indicating that solvation did occur, but with a less well-defined stoichiometry. Likewise, Schaschel and Day (24) studied the solvation of the sodium cation in a number of solvents by observing shifts of the solvating agent proton resonances in hydrocarbon solutions. Malinowski, Knapp, and Feuer (25) and Swift and Sayre (26) studied the proton resonance in aqueous sodium chloride solutions as a function of temperature and concentration. Chezick (27) and Fabrikand, et al. (28) determined the coordination number of hydrates by measuring the spin-lattice and spin-spin relaxation times of the water protons in solutions of several alkali salts. Their data indicated a more stable configuration for the water molecules in the hydrates than was found in pure water. Paul and Screenathan (29) investigated the solvate formation for a number of solutes in N,N-dimethylformamide by observing the proton NMR spectra of the solutions. Hammaker and Clegg (30) used the effect of salt concentration on the -OH and -CH₃ proton chemical shifts of methanol to explain the structure making or breaking ability of several solutes. Ion-pair

formation was invoked to explain discrepancies in the data. Similar studies were carried out in water by Schoolery and Adler (31). Nichols and Szwarc (32) investigated the solvation of silver, sodium and lithium ion-pairs in a number of cyclic ethers by proton NMR and concluded that their data indicate coordination between the oxygen atoms of the solvent and the cations of the ion-pairs. Hindman (33) studied the proton magnetic resonance spectra of aqueous solutions of a number of 1:1 electrolytes and related the data to hydration numbers and structuring properties of the cations. Kuntz and Johnston (34) measured the solvent shifts as a function of concentration for nitromethane and halogenated alkanes in a variety of solvents and explained their results by assuming the existence of collision complexes. Luz and Meiboom (35) investigated the solvation of nickel(II) and cobalt(II) ions in methanol-water mixtures by measuring the areas under the bound and free solvent proton resonance peaks using low temperature nuclear magnetic resonance techniques. Similarly, Matwiyoff, et al. (36) used low temperature NMR to study the coordination of nickel(II) and cobalt(II) with N,N-dimethylformamide and acetonitrile. The same type of experiment was performed by Nakamura and Meiboom (37) to study the solvation of magnesium(II) by methanol. Fratiello, et al. (38) investigated the solvation of several cations in solvent mixtures and correlated their results with the basicity of the solvents. Finally, Taylor and Kuntz (39) and Buchson and Smith (40) attempted to study anion solvation by observing the proton magnetic resonance spectra of a variety of ammonium salts in several solvents.

Thus the literature shows that proton nuclear magnetic resonance is a useful tool for the studies of complexation and solvation. In a latter section of this thesis, use is made of this technique for the

determination of the structure and stoichiometry of complexes of 1,5-dimethyltetrazole.

While such studies have been quite useful in the elucidation of the structures of electrolyte solutions, often the information obtained is rather limited since the measurements are carried out on the magnetic resonance of either the solvent protons or the protons of the solvating species dissolved in an inert solvent. In most cases the observed protons are several atoms removed from the interaction site and, consequently, the chemical shifts are only weakly affected by solvation. It is obvious that much better information should be obtained if one directly observes the resonance of the solvated or complexed ions.

The sodium-23 nucleus seems to be particularly well suited for such studies. The relative sensitivity of 0.1 with respect to the proton indicates that the measurements can be carried out in fairly dilute solutions. The small natural line width of the sodium ion resonance in solution allows the use of high resolution nuclear magnetic resonance equipment. Finally the large quadrupole moment of $0.1 \text{ e} \times 10^{-24} \text{ cm}^2$ renders the sodium-23 nucleus a sensitive probe of the neighboring electronic environment.

The first detailed investigation using sodium-23 NMR was done by Jardetzky and Wertz (41) who measured the position, width, and area of the sodium resonances as a function of concentration for a number of compounds in aqueous solution. A 3 N aqueous sodium chloride solution was used as a standard. While they did find that the line broadened somewhat in cases of strong interaction with the sodium ion, they found no chemical shifts which one expects if the electronic environment of the probing nucleus changes. Their results can probably be explained by the fact that they were using a very low radio frequency field (8 MHz) which

would make small shifts difficult to see, as well as a fairly inhomogeneous magnetic field as evidenced by the line-width of 36.7 Hz for the standard solution. Jardetzky and Wertz did however find trends in the line-widths for the more concentrated solutions which they ascribed to ion-pairing.

Subsequent to this work, a number of papers appeared which report the sodium-23 nuclear magnetic resonance measurements of compounds in aqueous solution. Deverell and Richards (42) studied the concentration dependence of the chemical shifts for sodium-23, potassium-39, rubidium-87, and cesium-133 nuclei in aqueous solution. They found a difference in behavior of sodium as compared to the rest of the alkali metal ions, and ascribed this difference in behavior to the high degree of solvation of the sodium ion. They stated that the concentration dependences are caused by interactions between the anions and the cations in solution.

Relaxation times were determined for aqueous solutions of sodium chloride and sodium perchlorate by Eisenstadt and Friedman (43) and in aqueous solutions of the halides of lithium and sodium by Speight and Armstrong (44). The magnetic properties of the alkali metals in aqueous solution were studied by Lutz (45). Finally, work similar to that originally undertaken by Jardetzky and Wertz was done by Griffiths and Socrates (46), but they too found no evidence of any chemical shifts.

Thus it appears that the majority of past work was directed at attempts at finding some interaction between anions and cations in aqueous solutions. However, water is probably the worst possible solvent for such studies due in part to the magnitude of the interaction between water and the cation and in part to the high dielectric constant of water. It would thus appear that more fruitful information might be obtained if one were to work in nonaqueous solvents where the cation-solvent interaction can be varied over a wide range. Literature search

indicated that studies of this type have been rather sparse. The most detailed study found thus far was that of Bloor and Kidd (47) who measured the influence of solvents on the chemical shifts of sodium-23 with respect to aqueous solvents and, with a given solvent, a shift dependence on concentration. However, only sodium iodide was used in their investigations. It was of interest to extend their work to other solutes in order to study the possible influence of the anion on solvation. Thus the third portion of this thesis reports a detailed study of the solvation of the sodium ion in several solvents by sodium-23 nuclear magnetic resonance spectroscopy.

CHAPTER II

CONDUCTANCE WORK

THEORETICAL

The protonation of a weak base in formic acid solution may be written as,



The concentration equilibrium constant for this reaction, K_b , is given by

$$K_b = \frac{[\text{HB}^+][\text{HCOO}^-]}{[B]} \quad (2)$$

If α is the degree of dissociation, we have

$$\begin{aligned} \text{and} \quad (\text{HB}^+) &= (\text{HCOO}^-) = \alpha C_B \\ (B) &= (C_B - \alpha C_B) = C_B(1 - \alpha) \end{aligned}$$

where C_B is the stoichiometric concentration of the base. Substituting these terms into equation 2, we get Ostwald's dilution law

$$K_B = \frac{\alpha^2 C_B}{1 - \alpha} \quad (3)$$

The equation used in the calculation of equivalent conductances is,

$$\Lambda = \frac{1000 L_s}{C} \text{ (ohm}^{-1} \text{ cm}^2 \text{ equivalent}^{-1}) \quad (4)$$

where L_s is the specific conductance defined by,

$$L_s = K/R \text{ (ohm}^{-1} \text{ cm}^{-1}) \quad (5)$$

where K is the cell constant (cm^{-1}) and R is the resistance of the solution in ohms.

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Arrhenius has shown that the degree of dissociation of a weak binary electrolyte can be approximately obtained from the expression

$$\alpha = \frac{\Lambda}{\Lambda_0} \quad (6)$$

Λ_0 being the equivalent conductance of the solution at infinite dilution. If equation (6) is substituted into equation (3) and the resulting expression is rearranged, the following relationship is obtained:

$$K_B = \frac{\Lambda^2 C}{\Lambda_0 (\Lambda_0 - \Lambda)} \quad (7)$$

This equation is only obeyed by weak electrolytes at low concentrations. A more general equation developed by Kohlrausch and Onsager which takes into account higher concentrations, and relaxation and electrophoretic effects is as follows:

$$\Lambda = \Lambda_0 - (A\Lambda_0 + B)\sqrt{C} \quad (8)$$

$$\text{where } A \text{ (relaxation effect)} = \frac{8.205 \times 10^{-5}}{\eta(DT)^{3/2}}$$

$$\text{and } B \text{ (electrophoretic effect)} = \frac{82.43}{\eta(DT)^{1/2}}$$

and where D is the dielectric constant of the solution, η is the viscosity of the solution, and T is the absolute temperature.

Shedlovsky (48,49) proposed that for weak electrolytes

$$\Lambda = \alpha \Lambda_0 - S \frac{\Lambda}{\Lambda_0} (\alpha C)^{1/2} \quad (9)$$

where α is the degree of dissociation and S is the Onsager slope given by

$$S = A\Lambda_0 + B$$

A function S_z is then defined where

$$S_z = 1 + Z + 1/2 Z^2 \dots$$

and

$$Z = \frac{S\sqrt{C}}{\Lambda_o^{3/2}}$$

Shedlovsky then proposed the following equation to more accurately predict conductance data:

$$\frac{1}{\Lambda S_z} = \frac{1}{\Lambda_o} + \frac{CS_z f^2 \Lambda}{K_B \Lambda_o^2} \quad (10)$$

where f is the mean activity coefficient calculated by the Debye-Huckel equation. If $\frac{1}{\Lambda S_z}$ is plotted versus $\Lambda S_z f^2$ a straight line is obtained with a slope of $\frac{1}{K_B \Lambda_o^2}$ and an intercept of $\frac{1}{\Lambda_o}$. In practice, an arbitrary value of Λ_o is assumed. Corresponding values of Z and S_z are calculated from the slope and intercept of the data plot. These values are then inserted into equation (10) and a new value of Λ_o is determined. The process is repeated until consecutive values of Λ_o agree to within set limits.

For the determination of cell constants, the method of Lind, et al. (50) was used. These authors devised a method based on the equation

$$\Lambda = 149.93 - 94.65 C^{1/2} + 58.74 C \log C + 198.4 C \quad (11)$$

This equation has been shown to provide conductance data to an accuracy of 0.01% for unassociated electrolytes as is the case for our aqueous KCl solutions.

EXPERIMENTAL

REAGENTS

Formic acid of about 98% purity was obtained from the Baker and Adamson Company. Into each one gallon bottle of the formic acid was added one half pound of anhydrous copper sulfate. This mixture was allowed to stand for at least 48 hours with periodic shaking. The acid was then filtered into a 3-liter round bottom flask and slowly vacuum distilled through a one meter Vigreux column at about 20 mm pressure. The temperature was adjusted so as to maintain smooth boiling of the liquid. The first and last 200 ml of the distillate were discarded.

The middle fraction of the distillate was placed in a 6-liter separatory funnel fitted with a removable center core. The core was inserted and a thermometer was placed in the well. The funnel was then placed in the freezer where the temperature was maintained at 0°C. The liquid had a tendency to supercool, and it was necessary to hold a piece of dry ice against the side of the separatory funnel in order to induce crystallization. Crystal growth was then allowed to proceed until only about 100 ml of liquid was left.

The funnel was removed from the freezer, the thermometer was replaced by a heater constructed of nichrome wire wrapped around a glass core, and enough current was controlled in the wire by means of a Variac to cause a channel to be melted through the solid. By means of suction applied at the bottom of the funnel the small amount of liquid (about 200 ml)

was removed from the crystallized acid. Dry nitrogen was allowed to enter the funnel from the top to equalize the pressure. The heater was replaced with the thermometer, and the entire assembly was placed in dark until the solid was melted. The melting could be speeded by the application of heat from an infrared lamp, but since this heat could aid the decomposition of the acid, it was applied sparingly.

The entire process was repeated three more times. The pure formic acid obtained in this manner had a freezing point of 8.5°C. It was stored frozen under nitrogen atmosphere until ready for use. The specific conductance of this product was $1 \times 10^{-5} \text{ ohm}^{-1} \text{ cm}^{-1}$, slightly better than the best literature value of $6 \times 10^{-5} \text{ ohm}^{-1} \text{ cm}^{-1}$ (51).

The cyclopolymethylenetetrazoles used in this investigation were prepared by the methods of D'Itri (17, 52). In order to remove any possible conducting impurities such as azide ion, etc., the tetrazoles were dissolved in a minimum amount of 50-50 ethanol-water mixture, the solution was acidified with 5 ml of concentrated sulfuric acid, and enough 0.3 M potassium permanganate was added to completely oxidize the azide as evidenced by the permanent purple color of the excess permanganate. The tetrazoles were extracted into chloroform, the solutions were evaporated to dryness, and the products were recrystallized several times from an appropriate solvent mixture (52). Since tri- and tetramethylenetetrazole have reasonable high vapor pressures, they were further purified by sublimation under vacuum. Although pentamethylenetetrazole can be sublimed, the sublimate is gray in color indicating some decomposition. Therefore, only recrystallization from diethylether was used to purify this compound. Other cyclopolymethylenetetrazoles used in this investigation do not have sufficiently high vapor pressures to allow vacuum sublimation even at temperatures near their melting points.

The 6,6'-dichloro- and 6,6'-dibromopentamethylenetetrazole used in this study were obtained from Dr. D'Itri of this laboratory and were used without further purification.

Potassium chloride for the calibration of the conductance cells was obtained by first fusing the pure salt and then grinding this anhydrous substance. Distilled water which had been passed through an Illco-way Research Model 0 ion exchange column was used in preparing the solutions for calibrating the cells.

APPARATUS

In normal conductance measurements, the problems of electrode polarization are usually handled by platinizing the electrodes to increase the surface area. However, this cannot be done with formic acid as a solvent due to the catalysis of the decomposition of the acid by the platinum black. A special conductance apparatus had to be designed for this type of work (53). It employs an oscillator relatively free from harmonic contamination, a frequency selective detector, and a cathode ray tube null indicator which allows one to more accurately balance out large capacitance effects. In this work, the bridge was operated at a frequency of 1000 Hz.

The cells used in this investigation are essentially those described by Daggett, Bair, and Kraus (54). They were constructed of one liter erlenmeyer flasks with an electrode chamber connected at the bottom. A modification was made such that the male 35/45 top reported in the Daggett paper was replaced by a 24/40 female top. This prevents the loss of solution each time the stopper is removed. In this type of cell the resistance of the solution is independent of the volume in the flask as long as the electrode chamber is filled. Thus, accurate measurements can be made on as little as 300 ml of solution.

Unplatinized platinum electrodes were used. The cells were cleaned with a sodium hydroxide solution to remove any traces of oil, rinsed thoroughly with hot distilled water, and then steamed for 1/2 hour on a steaming apparatus. After the steaming, the cells were rinsed with reagent grade acetone and then dried in a stream of purified nitrogen gas.

The cell constants were determined by measuring the conductances of potassium chloride solutions in water. The values obtained for the two cells used in this investigation by applying the method of Lind, et al. (50) were 1.442 ± 0.005 and $0.499 \pm 0.002 \text{ cm}^{-1}$.

During all measurements, the temperature was maintained at $25.00^\circ\text{C} \pm 0.02^\circ\text{C}$ by a Sargeant S-84805 thermostatic bath assembly filled with light mineral oil.

PROCEDURES

Into a one liter volumetric flask was added an appropriate amount of potassium chloride. The flask was then weighed, one liter of deionized water was added to the flask, and the flask was reweighed to determine the exact quantity of water added. The normality of the resulting solution was calculated by the equation

$$C = 10 D_0 W_2 / M_2$$

where D_0 is the density of the solution, taken in this case to be 0.99707, W_2 is the weight percent of the potassium chloride in solution, and M_2 is the molecular weight of the potassium chloride. The calculated concentration values were then inserted into equation 11 and the value of the cell constant determined by using equations 4 and 5 and the measured value of resistance for the cell.

Measurements on the tetrazole solutions were carried out as follows. A given amount of pure formic acid was weighed into each of the conductance cells, the cells were submerged in the oil bath until thermal equilibrium was established, and the specific conductance of the solvent was measured.

Next, known amounts of a stock solution of tetrazole in formic acid were added using a weight buret and the contents of the cell were thoroughly mixed. The temperature was again allowed to equilibrate in the oil bath, and the resistance of the solution was measured. The contents of the flask were again mixed and another equilibrium and reading were made. The process was repeated until resistance values agreed. The addition of the stock solution to the cell was made under laboratory conditions since it was found that brief exposures to the atmosphere caused no observable change in the conductance readings.

The concentration range of solutions used in these measurements varied from $5 \times 10^{-3} \text{ M}$ to $2 \times 10^{-1} \text{ M}$. The upper limit of the concentration was determined by the Fuoss equation

$$C_{\text{max}} = 3.2 \times 10^{-7} D^3$$

where D is the dielectric constant of the solvent. At higher concentrations, the laws of dilute solutions no longer apply. Even for the most dilute solution, the specific conductance of the solvent was less than five percent of the specific conductance of the solution. A solvent correction was made by subtracting the specific conductance of the solvent from that of the respective solutions.

SAMPLE PREPARATION

All samples were prepared by weighing the proper amount of the solute into a five ml volumetric flask and then diluting to the mark with the respective solvent or solvent mixture.

RESULTS AND DISCUSSION

The conductances of a series of tetrazoles and substituted tetrazoles in formic acid were measured to ascertain whether there is a difference in the base strengths of the tetrazoles which may parallel the difference in their biological properties.

It was first shown by two experiments that tetrazoles have a very low proton affinity in water solution. Distilled water was purged with nitrogen until the pH of the solution was 7.00. Enough solid pentamethylenetetrazole was added to make the solution 0.10 M in this solute. No change in the pH of the solution could be detected. Likewise, pentamethylenetetrazole was added to a dilute solution of acetic acid at a pH of 5.00. Again, no change could be detected in the pH of the solution.

Next, conductance data were obtained for solutions of the eight cyclopolymethylenetetrazoles. The data were then analyzed by the methods of Shedlovsky (48,49) by the use of a computer program (55). The program was written in FORTRAN and run on a Control Data 3600 computer.

The results and corresponding deviations for this work are shown in Table I. It should be noted that the relative lack of precision in these data, as compared to conductance data in aqueous solution, can probably be attributed to difficulties encountered in working with formic acid, namely, its instability in the presence of platinum, its extreme hygroscopicity, and its high degree of self-ionization.

Table I. Conductances of Some Cyclopolymethylenetetrazoles in Formic Acid
Solution at 25°C

TRIMETHYLENETETRAZOLE

$10^3 c$	Λ	$10^3 c$	Λ
4.852	31.57	6.26	30.72
10.26	28.29	10.63	28.25
16.91	25.09	15.95	25.41
22.40	23.15	21.43	23.36
26.91	21.89	25.28	22.22
31.58	20.78	30.12	20.99

TETRAMETHYLENETETRAZOLE

$10^3 c$	Λ	$10^3 c$	Λ
6.825	42.32	6.209	42.55
10.59	39.94	10.18	40.04
12.34	38.86	14.46	37.49
18.34	35.76	20.00	34.78
21.39	34.40	23.71	33.24
25.19	32.96	26.58	32.23
29.56	31.50	31.04	30.81
33.69	30.34	42.62	27.91
39.75	28.84	51.09	26.27
49.97	27.16		

PENTAMETHYLENETETRAZOLE

$10^3 c$	Λ	$10^3 c$	Λ
3.628	39.19	4.235	37.83
8.398	36.91	8.295	35.48
14.05	33.39	11.45	33.54
18.98	31.00	15.73	31.32
23.08	29.37	20.50	29.24
27.44	27.91	23.24	28.23
31.14	26.84	27.53	26.87
33.86	26.16	30.32	26.10
38.01	25.21	33.50	25.27
43.23	24.15	36.50	24.58
48.12	23.27	41.64	23.53

Table I (Continued)

HEXAMETHYLENETETRAZOLE

10^3C	Δ	10^3C	Δ
2.790	34.06	3.302	34.65
5.299	33.98	7.165	32.89
8.532	31.92	10.78	30.53
12.37	29.58	14.30	28.57
15.07	28.18	18.16	26.79
17.13	27.26	21.08	25.67
19.65	26.21	24.32	24.56
21.56	25.50	27.12	23.76
24.11	24.66	31.03	22.70
26.33	23.99	33.25	22.17
29.84	23.03	38.87	20.99

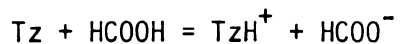
HEPTAMETHYLENETETRAZOLE

10^3C	Δ	10^3C	Δ
2.732	32.58	2.790	34.59
6.064	32.92	6.274	33.92
9.457	30.54	9.737	31.62
12.77	28.72	13.62	29.35
18.37	26.27	16.94	27.73
22.23	24.88	22.29	25.64
26.04	23.70	25.11	24.69
31.57	22.32	29.32	23.49
35.02	21.58	33.44	22.46
46.15	19.62	43.55	20.47

UNDECAMETHYLENETETRAZOLE

10^3C	Δ	10^3C	Δ
5.233	29.71	2.896	29.80
7.794	28.21	4.875	29.69
11.35	26.13	6.872	28.56
13.82	24.88	9.043	27.30
16.54	23.70	11.88	25.72
20.26	22.34	14.07	24.65
22.00	21.77	15.67	23.96
24.48	21.04	17.77	23.11
27.02	20.36	19.96	22.33
29.82	19.69	21.42	21.84
36.42	18.34	28.17	19.97

Table II lists the values of pK_B for the equilibrium



where

$$K_B = \frac{(TzH^+)(HCOO^-)}{(Tz)}$$

along with the standard deviations for these values.

It can be seen that the unsubstituted cyclopolymethylenetetrazoles in formic acid behave as fairly strong electrolytes. The basicity constants shown in Table II do not vary appreciably with the length of the hydrocarbon chain. On the other hand, halogen substitution drastically decreases the basic strength of the cyclopolymethylenetetrazoles to such an extent that it becomes impossible to measure their basicity constants even in formic acid solutions. In fact, it can be seen from Table III that at a given concentration, the molar conductance of the dihalo derivative is nearly three orders of magnitude lower than that of the corresponding unsubstituted cyclopolymethylenetetrazoles.

Comparison of the conductances of the chloro and the bromo derivatives also indicates the greater electron withdrawing effect of the chloride ion.

Table II. Basicity Constants and Limiting Equivalent Conductances at 25°C

TETRAZOLE	Run 1		Run 2	
	pK_B	Λ_o	pK_B	Λ_o
C ₃ MT	1.79 $\pm$ 0.03	41.70 $\pm$ 1.17	1.86 $\pm$ 0.03	43.32 $\pm$ 1.23
C ₄ MT	1.74 $\pm$ 0.03	57.33 $\pm$ 1.10	1.76 $\pm$ 0.03	57.58 $\pm$ 1.53
C ₅ MT	1.88 $\pm$ 0.01	55.05 $\pm$ 1.06	1.84 $\pm$ 0.01	51.87 $\pm$ 0.92
C ₆ MT	1.87 $\pm$ 0.02	46.23 $\pm$ 0.98	1.76 $\pm$ 0.05	44.52 $\pm$ 1.96
C ₁₁ MT	1.91 $\pm$ 0.03	41.86 $\pm$ 1.04	1.83 $\pm$ 0.03	40.07 $\pm$ 0.93

Table III. Conductances of Some Disubstituted Cyclopolymethylenetetrazoles
in Formic Acid Solution

6,6'-DICHLOROPENTAMETHYLENETETRAZOLE

$10^3 C$	Λ
2.776	0.05446
7.888	0.05144
13.18	0.05418
18.07	0.06252
24.08	0.06774
32.00	0.07507
38.47	0.08066
47.14	0.08378

6,6'-DIBROMOPENTAMETHYLENETETRAZOLE

$10^3 C$	Λ
2.657	0.0984
5.308	0.1254
8.573	0.1330
11.65	0.1359
14.34	0.1438
17.83	0.1477
20.03	0.1488

CHAPTER III

A PROTON NMR STUDY OF THE TETRAZOLE-METAL COMPLEXES

EXPERIMENTAL

REAGENTS

1,5-Dimethyltetrazole - The compound was prepared by the methods of Markgraf, et al. (56). Forty-six ml of benzenesulfonylchloride was added dropwise to an ice-cooled solution of 25 grams of acetone oxime in 342 ml of one molar aqueous sodium hydroxide. After this addition was completed, 23.1 g of sodium azide dissolved in a minimum volume of water was added dropwise. The resulting heterogeneous mixture was slowly warmed to room temperature, external heat was applied, and the mixture was allowed to reflux for 24 hours. The solvent was then removed under vacuum, and the residue was extracted several times with hot benzene in order to remove the product.

The compound was recrystallized twice from a benzene-diethylether solution and then sublimed under vacuum to yield about three grams of white, crystalline material (yield 6-7%). The melting point of the tetrazole was 71-73°C (lit (57) 72-73°C).

Nitromethane - Nitromethane was obtained from the Aldrich Chemical Co. Since this material was fairly impure as noted by the large number of peaks in the proton nuclear magnetic resonance spectrum, the following purification procedure was employed. A slurry was prepared with anhydrous methanol and Dowex 50W-X8 acid form cation exchange resin obtained from the Baker Chemical Co. This mixture was added to a 20 cm chromatography column. Nitromethane was then allowed to pass slowly through this

column at a rate of about one drop per second until no further traces of methanol could be found in the eluent as evidenced by the nuclear magnetic resonance spectrum. Additional nitromethane was added to the column until several liters of methanol-free product were collected. The product was then refluxed over barium oxide for 24 hours, and then fractionally distilled through a one meter, helices-packed column. The middle portion of the distillate was collected and stored in amber bottles until used. The nitromethane purified in this manner had a boiling point of 101°C and its NMR and IR spectra agreed with the published data.

Metal Salts - Anhydrous lithium perchlorate was obtained from K and K Laboratories and anhydrous magnesium perchlorate was obtained from Matheson, Coleman and Bell. Both solutes were dried at 110°C under vacuum for several hours prior to use. Sodium tetraphenylborate was obtained from Baker Chemical Co. and was used without further purification.

PREPARATION OF SOLUTIONS

Proper amounts of the respective metal salts and 1,5-dimethyltetrazole were weighed into two ml volumetric flasks and nitromethane was added to the mark. Equal volumes of these solutions were transferred to NMR tubes and a small quantity of TMS was added to each tube.

NMR MEASUREMENTS

The proton nuclear magnetic resonance spectra were obtained on a Varian A56/60D spectrometer. Sweep calibration on the instrument was checked daily by the sideband technique. This technique will be more fully explained in a later chapter in this thesis, but suffice it to say now that the sidebands were generated by using a Hewlett-Packard

4204A oscillator operating at 500 Hz. Tetramethylsilane (TMS) was used as an internal standard. All data were collected at ambient probe temperature which was about 38°C.

RESULTS AND DISCUSSION

The nuclear magnetic resonance spectrum of a solution of 1,5-dimethyltetrazole in nitromethane consists of a singlet at about 2.51 ppm with reference to TMS due to the C-methyl protons, and a singlet at about 3.99 ppm due to the N-methyl protons. Addition of magnesium perchlorate to nitromethane solutions of 1,5-dimethyltetrazole produced downfield shifts in both of the methyl proton resonances. The magnitudes of the shifts are dependent on the mole ratio, tetrazole/magnesium(II).

Several experiments were performed in attempts to determine the stoichiometry of the tetrazole-magnesium(II) complex in solution. In the first of these experiments the concentration of the 1,5-dimethyltetrazole was held constant while the concentration of the metal ion was varied. The results of these measurements are shown in Tables IV and V and in Figs. 1 and 2. If the arms of the curve are extrapolated to an intersection point, there appears to be a break in the curves at a mole ratio of one magnesium(II) ion per tetrazole. This stoichiometry is not surprising in view of the fact that we are forcing this low coordination number through the addition of an excess of magnesium(II).

There is great difficulty in measuring the chemical shift of the lowfield peak since it lies so close to the methyl proton resonance of nitromethane. In the remaining experiments, only the high field resonance was measured.

When nuclear magnetic resonance experiments were performed at constant metal ion concentration, a different type of behavior was found.

Table IV. Experimental Data from the NMR Experiments on 0.1 M
1,5-Dimethyltetrazole Solutions and Varying Magnesium
Perchlorate Concentrations

Conc. Mg ⁺⁺ (M)	HF δ^*	HF Δ^{**}	LF δ	LF Δ
0.0078	151.0 Hz	0.0 Hz	239.3 Hz	0.0 Hz
0.0172	153.2	2.2	240.4	1.1
0.0426	156.2	5.2	242.0	2.7
0.0426	156.7	5.7	243.2	3.9
0.0652	160.0	9.0	245.0	5.7
0.0724	160.2	9.2	245.5	6.2
0.1030	160.0	9.0	245.2	5.9
0.1078	160.3	9.3	245.2	5.9
0.1373	166.0	15.0	249.0	9.7
0.1937	162.1	11.1	246.4	7.1
0.2099	164.5	13.5	248.0	8.7
0.2641	168.5	17.5	250.0	10.7
0.2914	169.9	18.9	251.8	12.5
0.3111	169.0	18.0	251.1	11.8
0.3418	170.1	19.1	252.1	12.8
0.3846	168.0	17.0	250.7	11.4
0.4064	170.8	19.8	252.8	13.5
0.4733	170.5	19.5	252.5	13.2
0.0000	151.0	0.0	239.3	0.0

*Chemical shift (H_z) from TMS

**Chemical shift (H_z) from free ligand resonance

Table V. Experimental Data from the NMR Experiments on 0.2 M
1,5-Dimethyltetrazole Solutions and Varying Magnesium
Perchlorate Concentrations

Conc. Mg ⁺⁺ (M)	HF δ	HF Δ	LF δ	LF Δ
0.021	150.1 Hz	0.1 Hz	239.0 Hz	1.0 Hz
0.035	153.1	3.1	241.0	3.0
0.052	154.5	4.5	242.0	4.0
0.093	158.8	8.8	243.3	5.3
0.114	159.1	9.1	244.6	6.6
0.162	164.1	14.1	248.0	10.0
0.218	167.0	17.0	249.0	11.0
0.251	167.8	17.8	250.0	12.0
0.283	168.5	18.5	250.2	12.2
0.292	167.8	17.8	249.8	11.8
0.325	169.3	19.3	250.5	12.5
0.343	167.8	17.8	250.0	12.0
0.368	169.7	19.7	251.5	13.5
0.394	169.6	19.6	251.3	13.3
0.415	169.7	19.7	250.9	12.9
0.442	169.4	19.4	251.9	13.9
0.456	170.0	20.0	251.5	13.5
0.495	170.7	20.7	251.9	13.9
0.000	150.0	0.0	238.0	0.0

Figure 1. Relationship between the Chemical Shift of the 1,5-Dimethyl-tetrazole Protons and the Concentration of the Mg(II) Ions.
a) N-methyl proton resonance b) C-methyl proton resonance
Ligand concentration constant at 0.1 M.

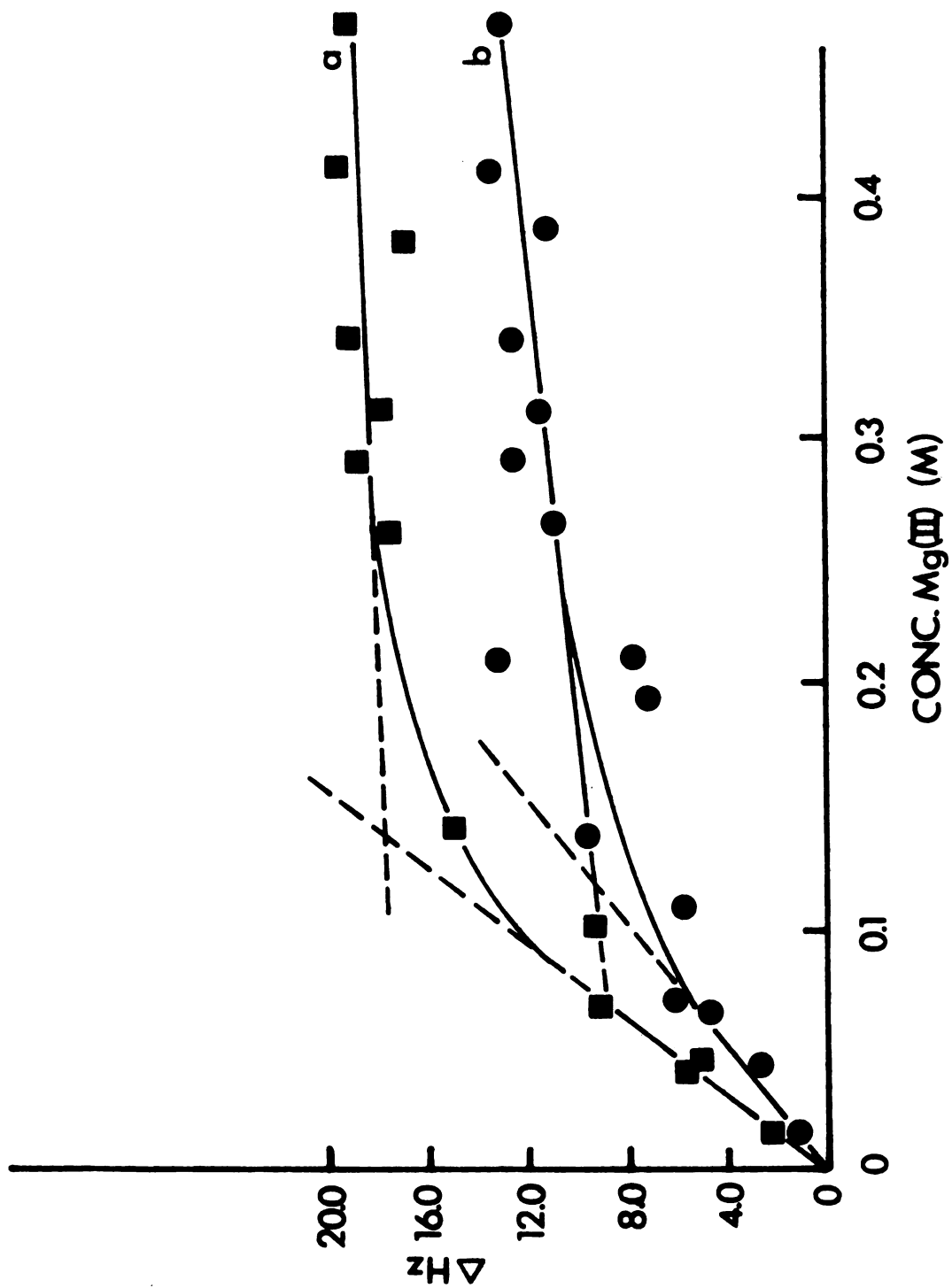


Figure 2. Relationship between the Chemical Shift of the 1,5-Dimethyl-tetrazole Protons and the Concentration of the Mg(II) Ions.
a) N-methyl proton resonance b) C-methyl proton resonance
Ligand concentration constant at 0.2 M.

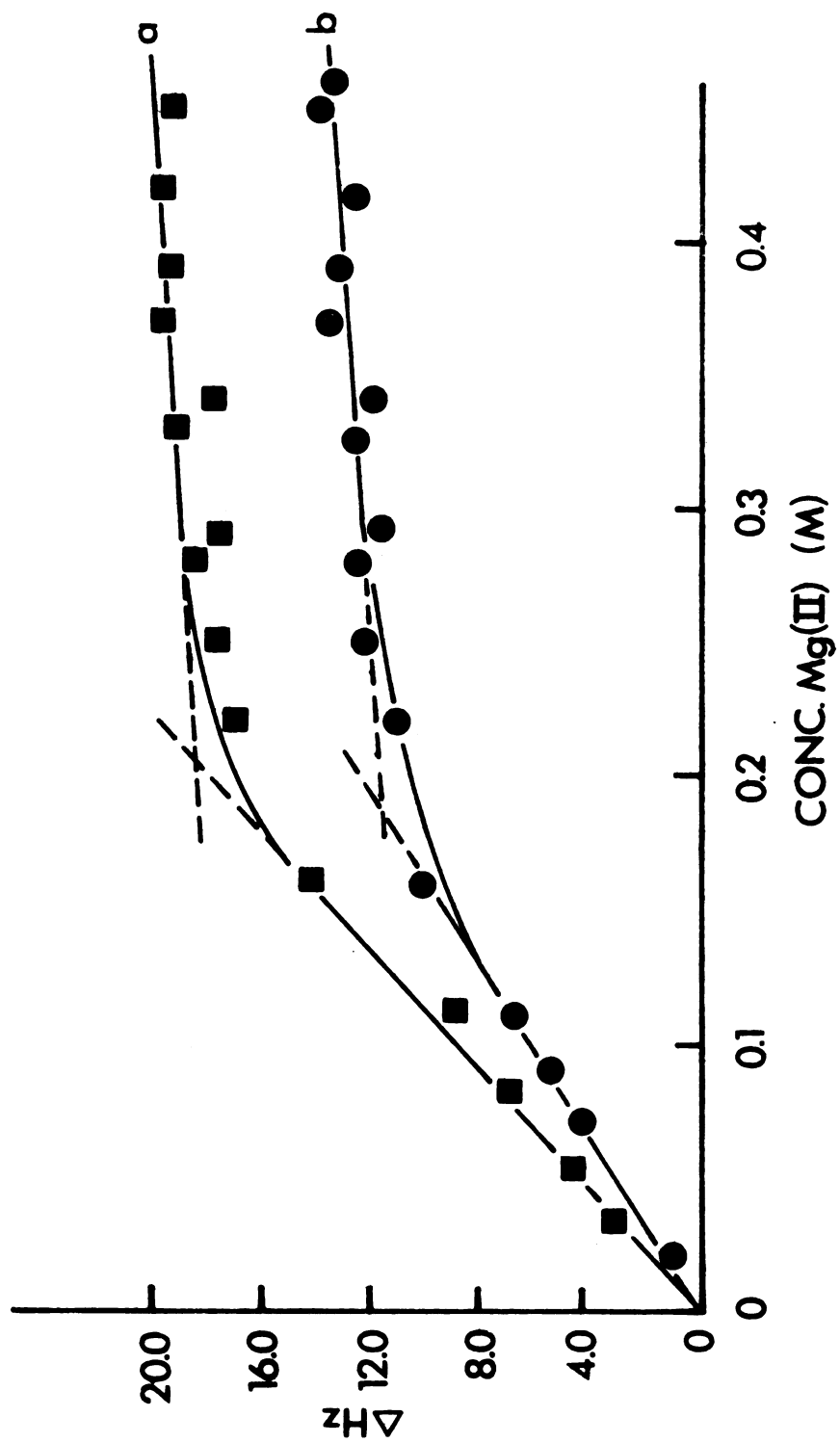


Table VI lists the data for these experiments. The data are also shown in Figs. 3 and 4. It is seen that the large excess of 1,5-dimethyl-tetrazole has led to an increase in the coordination number of the metal from one to four. While breaks in the curves are seen at a mole ratio of about 4:1, it may be that these breaks are fortuitous. Since in progressing to a coordination number of four, the magnesium(II) probably forms the intermediate 1:1, 1:2, and 1:3 complexes with the 1,5-dimethyltetrazole, one may predict that a smooth curve would be found.

It was necessary to show that the observed shifts were actually due to metal-tetrazole interactions and not to some changing properties of the solution because of added solute. In another series of experiments being carried out in this laboratory (57), nuclear magnetic resonance spectra were obtained for the 1,5-dimethyltetrazole-silver(I) system while varying the ionic strength with tetrabutylammonium perchlorate. A series of solutions was prepared in which the concentrations of the tetrazole and the silver ion were held constant at 0.0249 and 0.0254 M respectively, and the concentration of tetrabutylammonium perchlorate was varied from 0.0056 to 0.497 M. The frequency of the N-methyl and C-methyl proton resonances remained essentially constant with the difference between the two extremes of tetrabutylammonium perchlorate concentration being about 1 Hz. Likewise, it was found that the 4.32 ppm solvent proton resonance was independent of either the tetrazole or the silver salt concentration. Therefore, it seems logical to assume that the shifts observed for the tetrazole protons are due to the complexation reaction.

In all previous complexation studies involving 1,5-disubstituted tetrazoles, with one exception, they behaved as unidentate ligands (58).

Table VI. Experimental Data from the NMR Experiments on 0.2 M Magnesium
Perchlorate Solutions and Varying 1,5-Dimethyltetrazole
Concentrations

Conc. Tetrazole (M)	δ	Δ
0.10*	169.5 Hz	19.3 Hz
0.20	166.3	16.1
0.30	163.7	13.5
0.40	161.0	10.8
0.50	159.1	8.9
0.60	157.0	6.8
0.70	156.3	6.1
0.80	155.3	5.1
0.90	155.0	4.8
1.00	154.5	4.3
1.20	153.5	3.3
1.40	153.0	2.8
1.60	152.8	2.6
1.80	152.6	2.4
2.00	152.6	2.4
2.50	152.4	2.2
0.50***	150.2	0.0
0.10**	166.0	15.8
0.20	164.8	14.6
0.30	163.0	13.0
0.50	157.7	7.5
0.70	154.0	3.8
1.00	154.1	3.9
1.50	153.0	2.8
2.00	152.5	2.2

*Run 1

**Run 2

***No Mg^{++} added

Figure 3. Relationship between the Chemical Shift of the 1,5-Dimethyl-tetrazole Protons and the Tetrazole Concentration. Magnesium concentration constant at 0.20 M. Run 1.

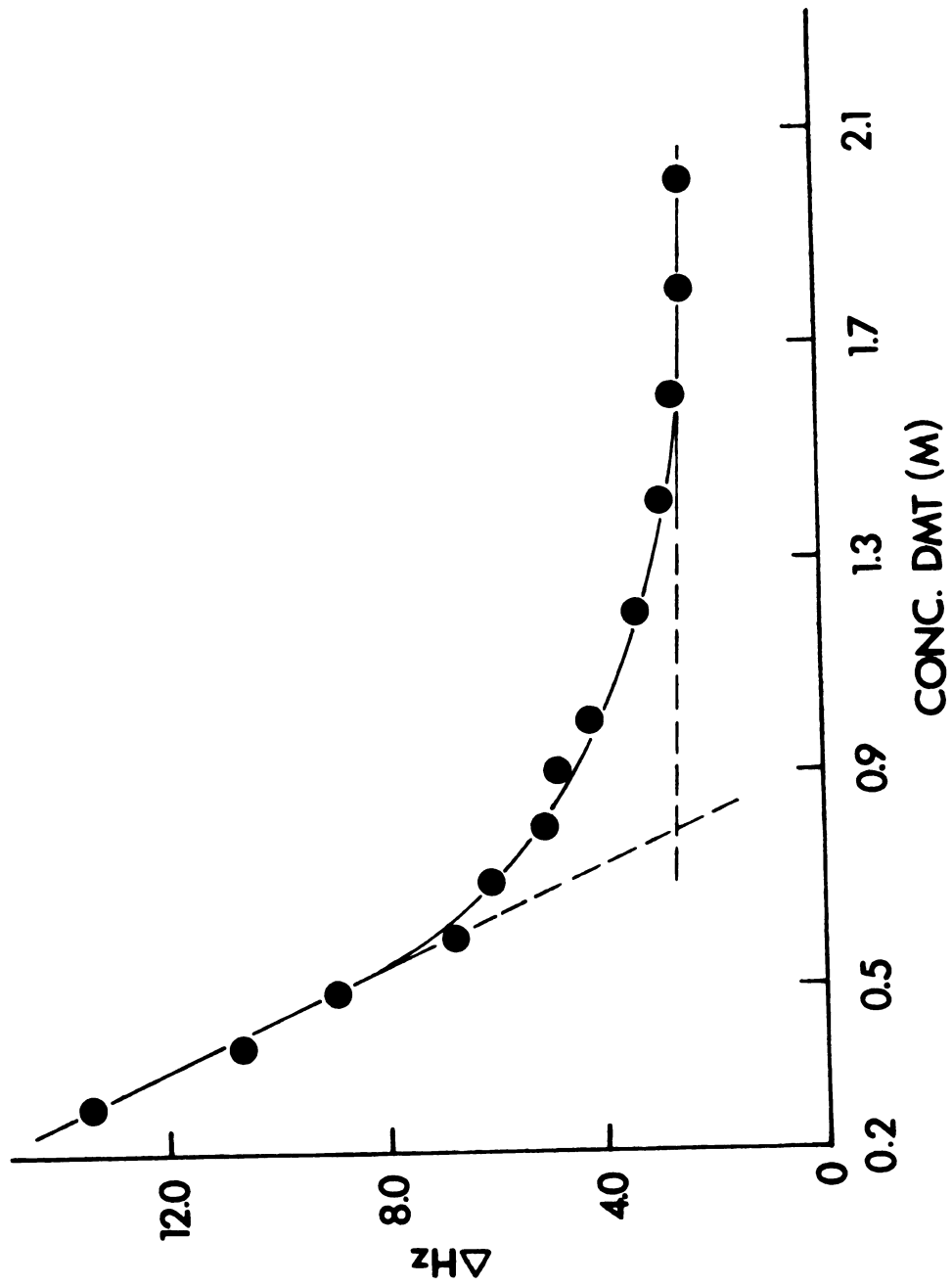
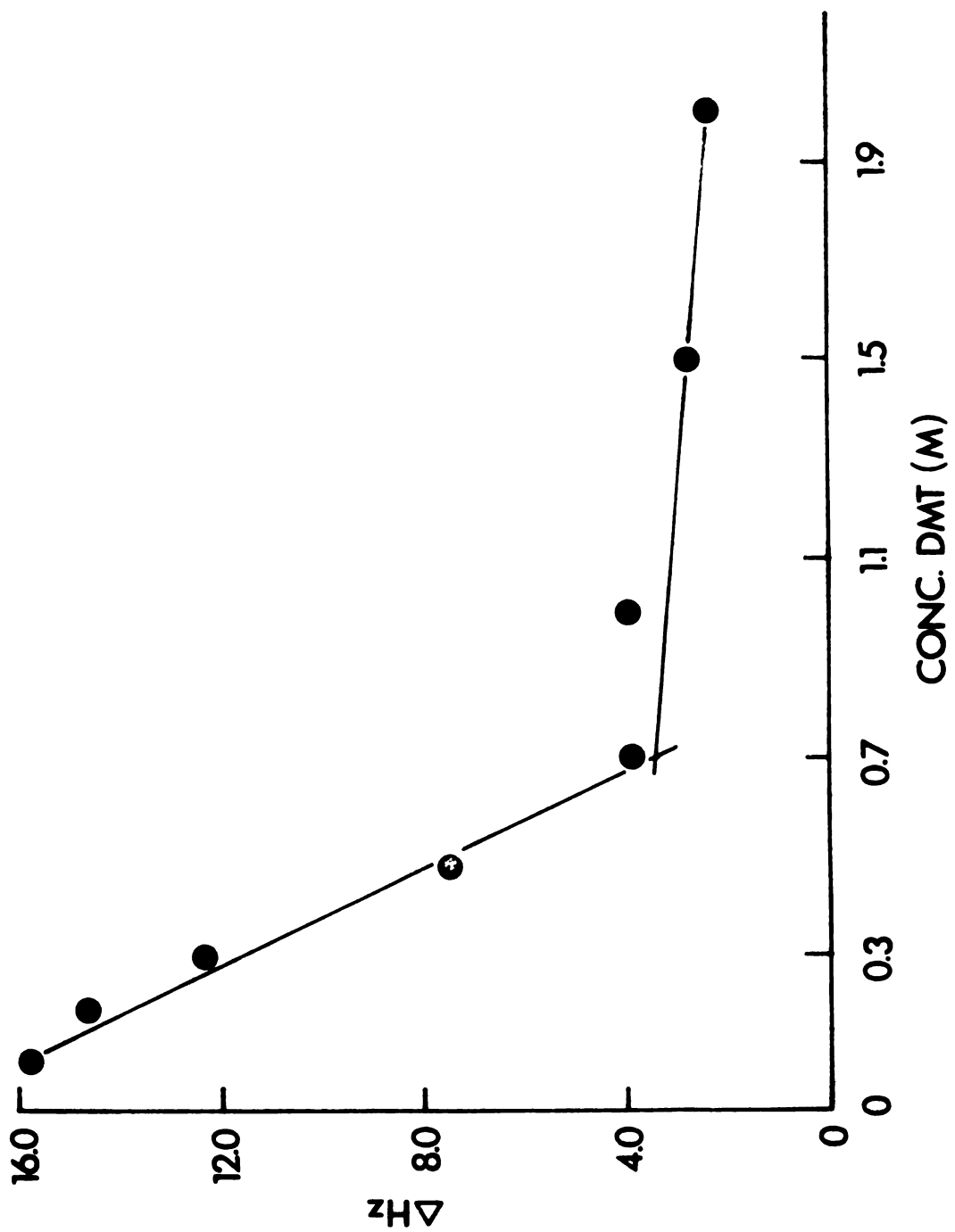


Figure 4. Relationship between the Chemical Shift of the 1,5-Dimethyl-tetrazole Protons and the Tetrazole Concentration. Magnesium concentration constant at 0.20 M. Run 2.



Only the insoluble polymeric pentamethylenetetrazole complexes with the transition metal halides gave indirect evidence of the tetrazole ring acting as a bridging ligand. It is interesting, therefore, to establish the coordination site in the tetrazole ring. As was stated before, the crystal structure of solid pentamethylenetetrazole-ICl showed that the donor site is the 4-nitrogen. It is entirely possible, however, that different donor sites may be found in solutions and/or with other acceptors.

The magnesium(II)-tetrazole complexation data show that the limiting chemical shift of the C-methyl and N-methyl proton resonances are fairly close in value (about 20 Hz and about 16 Hz respectively) which seems to indicate that either the complexation occurs through the 3-nitrogen of the tetrazole ring or that the 2, 3, and 4-nitrogens may act as the donor site and we are observing an average environment. If the complexation occurred solely through the 2-nitrogen, the coupling would occur through three atoms for the C-methyl protons and five atoms for the N-methyl protons. The opposite would occur if the coordination site were solely the 4-nitrogen. In either case it would be expected that the chemical shifts for the N- and C-methyl resonances would be quite different. Although on the basis of this evidence one cannot unambiguously conclude that the coordination site is the 3-nitrogen or a combination of the 2, 3, and 4-nitrogens in solution, these seem to be the most likely explanations of the present results.

Preliminary experiments were also carried out with two other metal salts. An attempt was made to determine whether there is complex formation between the lithium ion and 1,5-dimethyltetrazole by the above techniques. Lithium perchlorate was used and the concentration of

lithium ions was held constant at 0.2 M. The data obtained from the experiment are seen in Table VII and Fig. 5.

Again one may visualize a break in the curve at a stoichiometry of one lithium ion to four tetrazoles. However, as before, this break may be fortuitous. Nevertheless, the data show that some interaction is taking place.

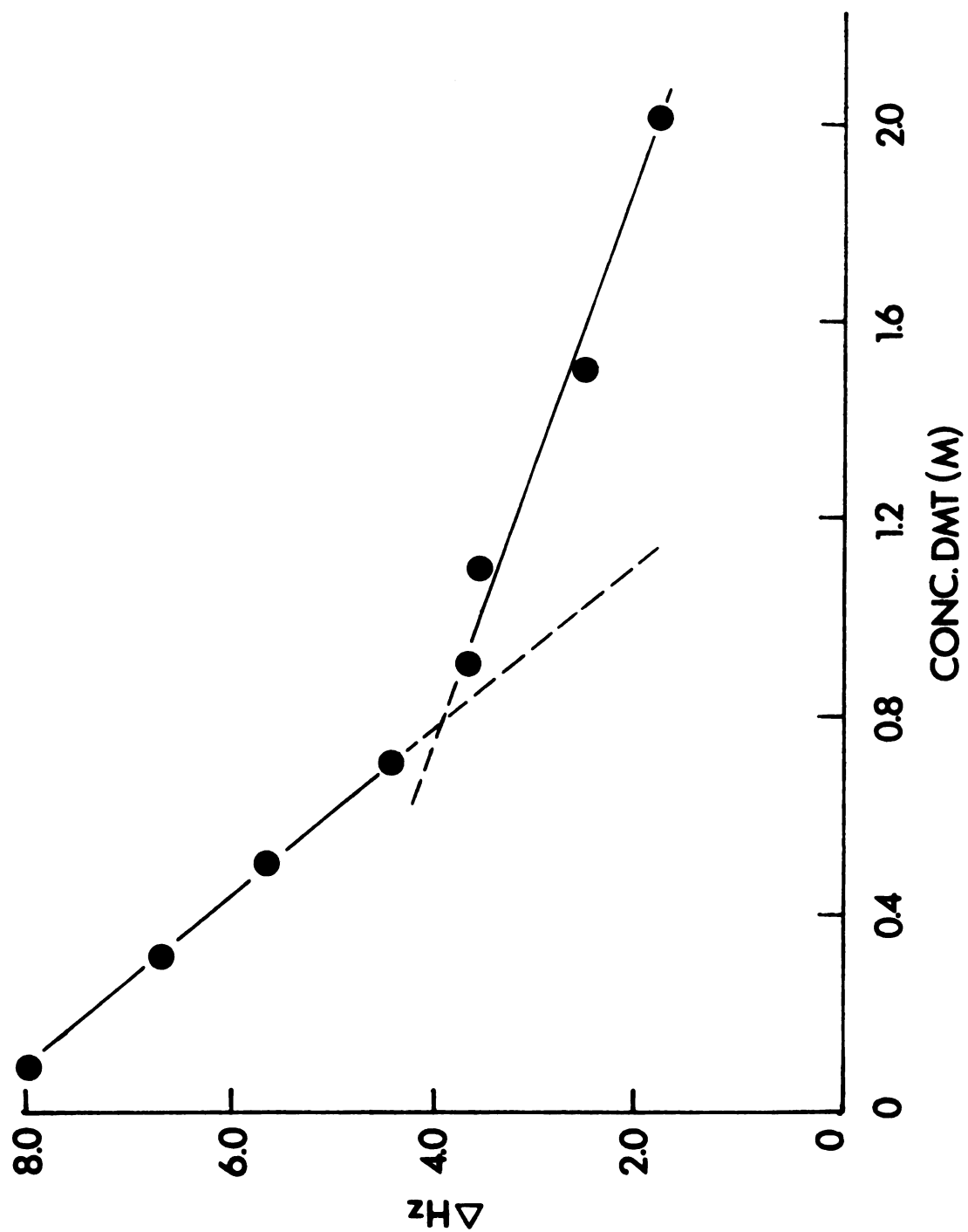
Since tetrazoles are known to be neurologically active and since sodium ions play a very significant role in the neurological makeup of higher organisms, an attempt was made to ascertain whether there is some interaction between the sodium ion and the tetrazoles. As before, nuclear magnetic resonance spectra were obtained on solutions which had constant amounts of either 1,5-dimethyltetrazole or the sodium ion while the other component was varied. However, in these experiments, no shift was found in cases where sodium ion concentration was held constant, and only a linear relationship between chemical shift and concentration was found in cases where the tetrazole concentration was constant. While these results do not show the absence of complex formation, they do point to a very weak complex if indeed one is being formed.

While these data give some insight into the complexation properties of the tetrazoles and in particular, 1,5-dimethyltetrazole, no definite conclusions can be reached. This is due in part to the lack of sensitivity in the nuclear magnetic resonance method employed. The chemical shifts being observed are relatively small since the protons being investigated are several atoms removed from the site of possible interaction. Thus it was felt that a more sensitive technique might supply the necessary information. One such possible technique is the use of nuclear magnetic resonance measurements on nuclei other than the proton.

Table VII. Experimental Data from NMR Experiments on 0.2 M Lithium
Perchlorate Solutions with Varying Concentrations of
1,5-Dimethyltetrazole

Conc. 1,5-Dimethyltetrazole <u>M</u>	δ	Δ
0.10	158.3 Hz	8.3 Hz
0.30	156.9	6.9
0.50	155.9	5.9
0.70	154.7	4.7
0.90	153.9	3.9
1.10	153.8	3.8
1.50	152.8	2.8
2.00	152.0	2.0

Figure 5. Relationship between the Chemical Shift of the 1,5-Dimethyl-tetrazole Protons and the Concentration of the Tetrazole. Lithium concentration constant at 0.20 M.



CHAPTER IV

A SODIUM-23 NUCLEAR MAGNETIC RESONANCE STUDY OF COMPLEXATION AND ION SOLVATION

THEORETICAL

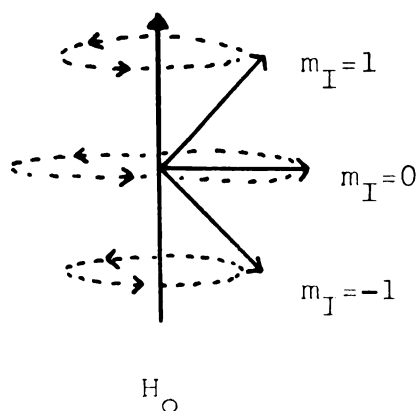
It was seen in the preceeding section of this thesis that useful information on the complexation properties of the tetrazoles can be obtained through the use of proton nuclear magnetic resonance spectroscopy. It was also seen that, in our case, this technique was limited in its usefulness because, even when interactions are known to occur, one only measures chemical shifts on the order of 20 Hz. This lack of sensitivity in the method is due to the fact that the probing nucleus, in this case the proton, is several atoms removed from the site of interaction. In order to have a more sensitive probe of the environment, it is necessary to make use of a nucleus closer to the interaction, ideally, one which takes part in the complexation reaction. There were several criteria used in the selection of the desired probe. However, since this choice of nucleus, and indeed, the majority of this thesis, depends on the principles of nuclear magnetic resonance spectroscopy, it seems to be worthwhile to review those principles at this time.

Let us assume a nucleus composed of spinning charged particles. If the spins of all the particles are paired, the system will possess no net spin, and the nuclear spin quantum number, I , which is the summation of the individual signed spins, will be zero. Since the spins are paired, there is a symmetrical charge distribution on the nucleus. A measure of the charge distribution is given by the nuclear quadrupole moment, eQ , where e is the unit of electrostatic charge, and Q is a measure of the deviation of the charge distribution from spherical symmetry.

The next step up in complexity is the case where a nucleus has a spin quantum number of $1/2$. Since there is only a single positive charge spread over the nucleus, the charge distribution must still be symmetrical and thus the quadrupole moment must still be zero.

In cases where the nuclear spin is ≥ 1 , the distribution of charge will be nonsymmetrical. A positive value of Q indicates that the positive charge is aligned parallel to the direction of the applied field while a negative value of Q indicates that the positive charge is aligned perpendicular to the applied field.

When a nucleus with a non-zero value of the nuclear spin is subjected to an external magnetic field, H_0 , satisfying the equation $\nu_0 = 0.7625 \mu H_0 / I$ where ν_0 is the frequency required for resonance, the axis of the nuclear magnetic moment, μ , will precess about the axis of the applied field in $2I + 1$ possible orientations as shown below for $I = 1$.



The energies of each of the orientations are dependent on the magnitudes of the magnetic moment and the applied field. The energy difference between the states is small, so that at room temperature all levels are almost equally populated. For protons there are two possible states and, at a temperature of 27°C , the ratio of the number of nuclei in the lower state to the number in the upper state is about 1.0000066 at an applied field of 9400 gauss.

Thus for each possible spin state there is an associated energy level which is determined by the value of the applied field. In the nuclear magnetic resonance experiment we observe the energy difference between levels by applying a radio frequency field along with a magnetic field. At a certain frequency of the rf field determined, among other factors, by the magnetic field strength, the nuclei in the lower energy states can remove some of the energy contained in the rf field and thereby can be raised to higher energy levels. It is this change in energy in an applied field that one observes in nuclear magnetic resonance experiments.

It must be remembered, however, that the system is at equilibrium. Since there is an equal probability that a nucleus will absorb energy from the field raising it to a higher spin state and that a nucleus will emit energy and return to a lower state, no net change in energy in the rf field would be noted were it not for the surplus of nuclei in the lower state. Since in a strong magnetic field there is a slight excess of nuclei in the lower state, there will be a net absorption of energy, and thus, an absorption band. As energy is removed from the field, after a finite amount of time, enough nuclei could be excited to the upper state so that the populations of the two states would be equal. The resonance signal which was originally observed would gradually decrease in intensity as the populations changed and, finally, disappear completely. This phenomenon is referred to as saturation.

Under normal operating conditions, saturation does not occur because there are two mechanisms by which nuclei can lose energy without radiation, thereby restoring the surplus in the lower state. These are spin-spin and spin-lattice relaxation. In the spin-spin relaxation, the

nucleus in the upper energy state through collision may give up its energy to another nucleus which is in the lower state. There is no net change in the number of nuclei in each state, but the time a nucleus spends in the excited state is altered. This is an important concept which has an effect on line-width and which will be referred to later.

The second process for non-radiative loss of energy is the spin-lattice relaxation. In this case lattice implies the solvent, electrons, and all other atoms in the system. In the spin-lattice relaxation, the excess energy is transferred to the lattice and the nucleus returns to the lower energy state. This process does cause a net decrease in the population of the higher energy state. Hence, by this mechanism, there is always a surplus of nuclei in the lower spin states and, therefore, a net absorption from the rf field at the corresponding value of H_0 .

The other factors which are important in nuclear magnetic resonance are the chemical shifts and the line-widths. If all the nuclei in a system under investigation were identical, then all resonances would come at exactly the same value of rf field and applied magnetic field. However, proton nuclear magnetic resonance spectra usually show a multiplicity of resonance lines. The fact that not all nuclei of the same type resonate under the same conditions of rf and magnetic fields is primarily due to shielding. Since most nuclei are surrounded by electrons, the external field "seen" at the nucleus is altered by the field set up by the spinning electrons. Depending on the orientation of the nuclei in the system, the electrons' magnetic field can either add or subtract from the externally applied magnetic field. Thus a change in the electron density around the nucleus will change the absolute magnitude of the field it experiences, and thus cause the nucleus to resonate at its own unique value of applied radio frequency field and magnetic field.

Ideally, when the conditions for resonance are met, there should be an extremely narrow absorption signal. However, this is rarely found. The nuclear magnetic resonance phenomenon takes place in a time interval on the order of 10^{-7} seconds. If, during this interval of time, the nucleus under investigation remains in the excited state, there is an easily discernable energy difference between the states, and the experiment will yield a sharp line. If, however, the lifetime of the excited state is smaller than the time of the experiment, the line produced will be broader, the width of the line being inversely proportional to the time spent in the excited state. Thus very long spin-spin relaxation times will give rise to sharp peaks and, conversely, short spin-spin relaxation times will hield broad peaks. As will be shown later, the relaxation time is an indication of the symmetry around the nucleus under investigation and, therefore, the line-width may give some indication of the structure of the species observed in solution.

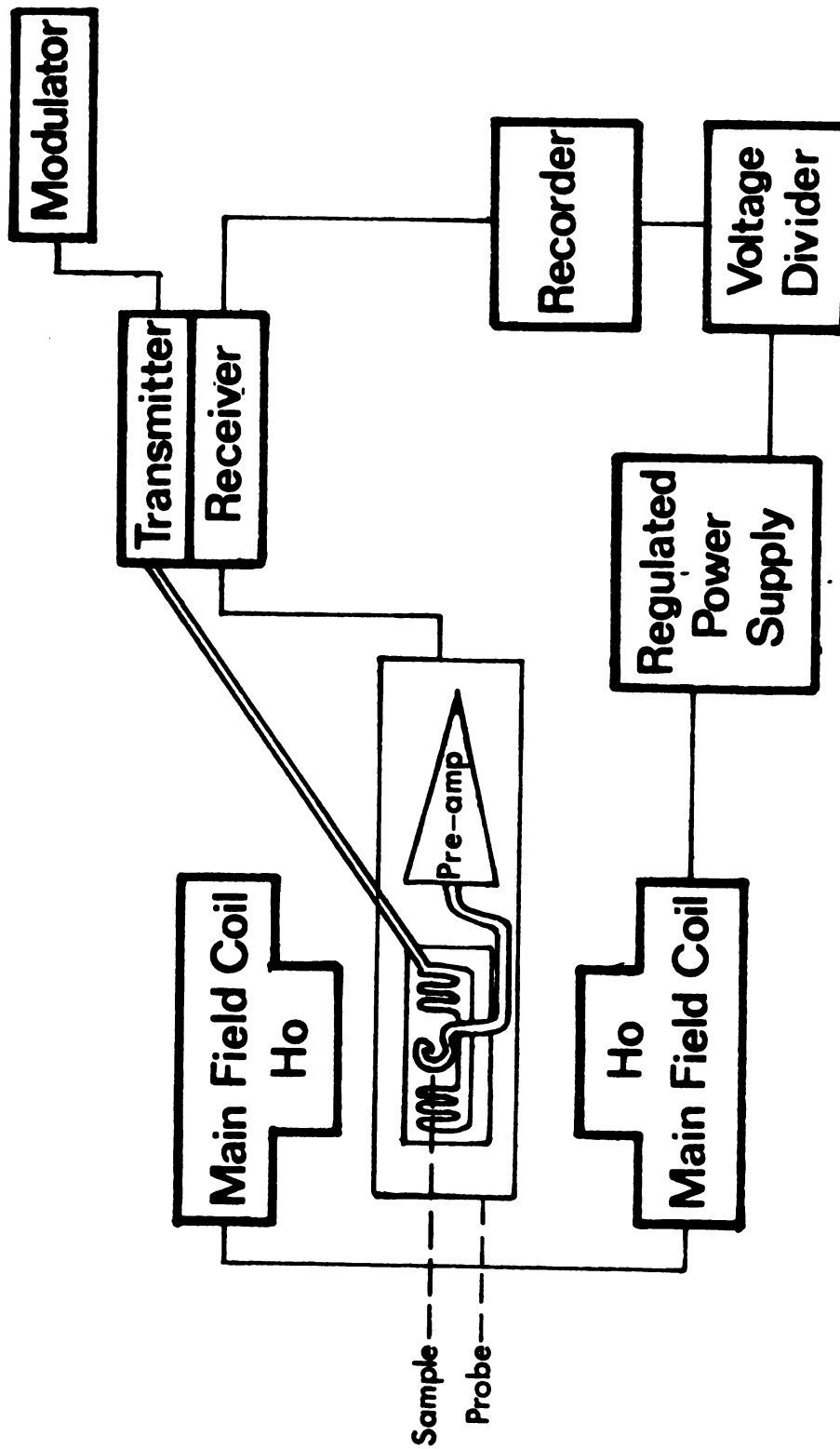
EXPERIMENTAL

INSTRUMENTATION

The basic instrument used in this investigation is the Varian DP-60 nuclear magnetic resonance spectrometer. A V-4310 radio frequency unit was modified for these studies by conversion to phase sensitive detection and subsequent retuning of the unit to 15.879 MHz , the frequency required to observe the sodium resonance at the field of maximum homogeneity, 14,092 gauss.

Throughout this work, the instrumentation was operated in one of two different configurations. The first of these is shown in Fig. 6. The main field, H_0 , is obtained from the main field coils which are activated by a regulated power supply. A modulated radio frequency of 15.88 MHz is supplied to the sample from the transmitter operating in conjunction with the modulator. The returning signal, after being strengthened by the preamplifier, goes to the receiver and then to the recorder. The unique factor in this set-up is the method of field sweep. The recorder operates a voltage divider whose output is fed as an error signal to the magnet power supply causing the main field to increase or decrease in proportion to the sign and magnitude of this voltage. As the recorder scans the spectrum, the linearly increasing voltage produced by the divider causes a smooth increase in the field strength and, thus, the spectrum is generated. Since the value of H_0 at any given position on the chart paper is dependent on the x-axis position of the pen, we have what amounts to a precalibrated chart with

Figure 6. The Recorder Sweep Configuration of the Wide-Line Nuclear Magnetic Resonance Instrumentation.



the magnitude of H_0 scanned being initially set by adjusting the voltage divider circuit and calibrated by the sideband technique.

The second configuration for the instrument replaces the voltage divider with the superstabilizer and slow sweep unit as shown in Fig. 7. In this mode of operation, the error voltage is produced by a combination of the slow sweep unit and the stabilizer. The disadvantage of this system is that the recorder is no longer controlling the value of the main field and, thus, the chart paper is no longer calibrated. However, there is a large advantage in that, with the stabilizer in the control loop, the field is much more stable, and the lower noise level that accompanies this stability allows observation of samples at much lower concentrations.

In order to calibrate the chart paper in the former method and to determine chemical shifts in the latter, the sideband method was employed. Let us assume that we are looking at the absorption signal from a saturated aqueous sodium chloride solution which is used as a reference in this work. As the field is swept, the absorption signal in Fig. 8a is obtained.

When an audio frequency is superimposed on the radio frequency field, images or sidebands of the main frequency are found at plus or minus the audio frequency and at N times the audio frequency where N is an integer. The intensities of the sidebands decrease as N increases. Thus modulation of the radio frequency field of Fig. 8a with a 400 Hz audio frequency yields the spectrum in Fig. 8b. The distance between each of the sidebands of the reference sodium chloride solution is 400 Hz.

The samples used in this investigation are placed in a standard 15 mm test tube with a coaxially mounted 8 mm NMR tube containing the saturated sodium chloride reference solution. When one of these samples

Figure 7. The Slow Sweep Unit-Stabilizer Configuration of the Wide-Line Nuclear Magnetic Resonance Instrumentation.

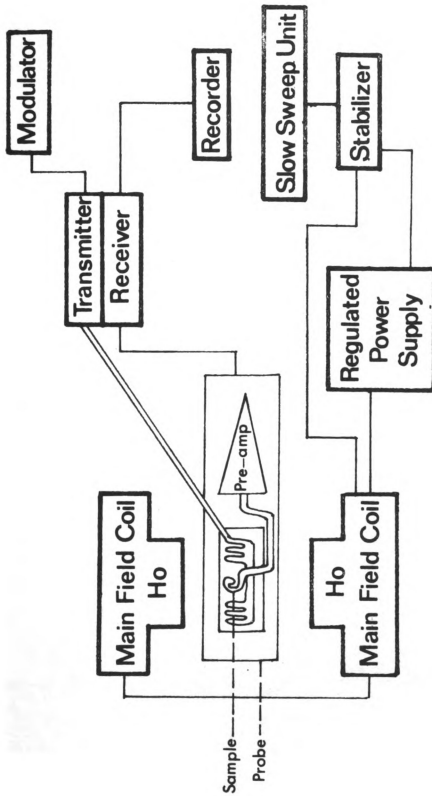
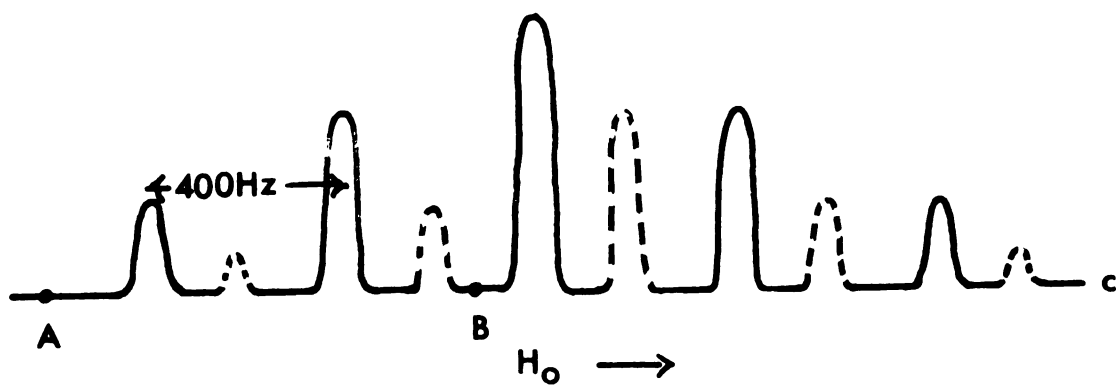
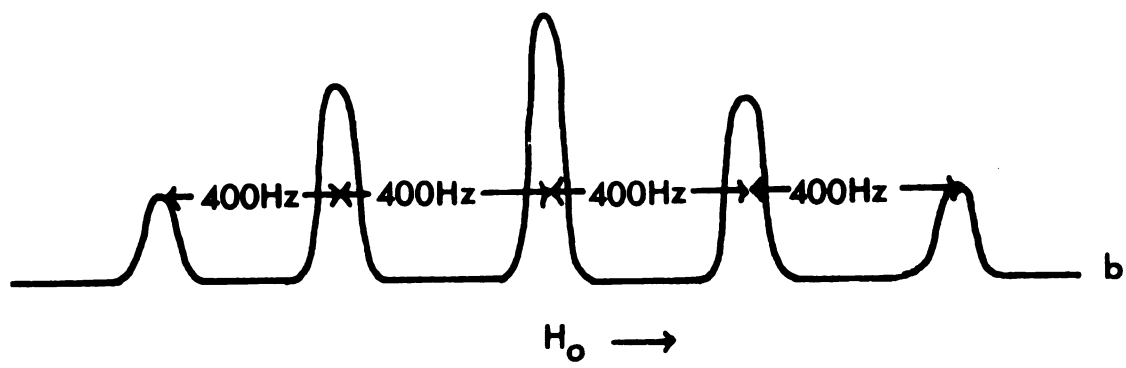
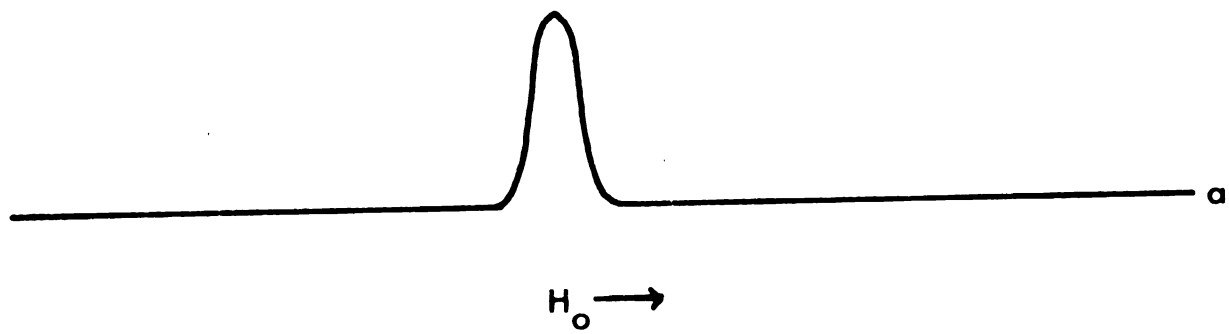


Figure 8. Sample Recorded Spectra. a) only the reference solution present and unmodulated rf field. b) only reference solution present and 400 Hz modulated rf field. c) sample and reference present and 400 Hz modulated rf field.



is run, the spectrum obtained in Fig. 8c is obtained with the dotted line representing the solute absorption signal and the solid line the reference signal. In actual operation, only that part of the spectrum between points A and B are recorded on the chart. Since the distance between the sidebands is known to be 400 Hz by counting the output frequency of the modulator with a Hewlett-Packard 5245 counter and the sweep is known to be linear, the chemical shift of the sample from the reference is determined by linear interpolation.

Iodine-127 nuclear magnetic resonance measurements were made on a Varian WL-115 spectrometer.

REAGENTS

Sodium tetraphenylborate used in this investigation was obtained from the J. T. Baker Chemical Co. It was dried under vacuum at 60°C for several hours before use. Some sodium tetraphenylborate was also obtained in the puriss. grade from the Aldrich Chemical Co. This material had an off-white color and left a residue upon dissolution. For these reasons, no further work was done on this material.

Sodium iodide was obtained from G. Fredrich Smith Co., reagent grade sodium iodide was from Matheson, Coleman, and Bell, and sodium thiocyanate was Mallinckrodt Analytical Reagent grade. These three salts were dried for 24 hours at 110°C prior to use.

Benzonitrile was obtained as Fisher aniline-free grade. Dimethylsulfoxide and dimethylformamide were Fisher certified grade, while tetrahydrofuran, ethyl acetate, acetone, and acetonitrile were Baker Analyzed Reagent grade. These solvents were dried over molecular sieves (Linde type 4A) and then used without further purification.

Pyridine used in this investigation was Fisher Certified grade and was dried over KOH pellets for several days.

Hexamethylphosphoramide and nitromethane were obtained from Aldrich Chemical Co. The former was used without further purification, while the nitromethane was purified as described earlier.

RESULTS AND DISCUSSION

AN INVESTIGATION OF ION ASSOCIATION

The chemical shifts of the sodium ion with respect to the sodium resonance of a saturated aqueous sodium chloride solution were determined at several concentrations in a variety of solvents. Sodium perchlorate, tetraphenylborate, iodide, and thiocyanate were used. The data are presented in Table VIII. It is immediately apparent that anions have an important influence on the chemical shift of the sodium ion. If one examines the data for the iodides, it can be seen that the results are essentially in agreement with those of Bloor and Kidd (47). The chemical shifts of the iodides are concentration dependent with the degree of dependency roughly an inverse function of the dielectric constant of the medium.

Chemical shifts of the sodium thiocyanate solutions are also found to be concentration dependent although to a lesser extent than the iodides. Again, these data point toward an inverse relationship between the dielectric constant of the solvent and the concentration dependence of the resulting chemical shift.

A very different type of behavior is found for the solutions of sodium perchlorate and sodium tetraphenylborate. Except in the case of nitromethane solutions where there is a very slight shift with concentration, the sodium-23 resonances of the tetraphenylborate solutions showed no concentration dependences in the 0.1-0.5 M range within the limits of detectability of our instrument (up to ± 0.3 ppm).

Table VIII. Chemical Shifts of ^{23}Na in Different Media

Solvent	NaBPh ₄ c				NaSCN c			
	0.5M	0.25M	0.125M	0.25M	0.125M	0.0625M	0.0313M	
HMPA	-2.9	-3.1	-3.1	0.72	0.90	1.07		
Pyridine	-0.72	-0.72	-0.90	-2.52	-2.52	-2.52	-2.52	
DMSO	0.72	0.72	0.90	0.18	0.72	0.72	1.59	
DMF	5.23	5.23	5.76	4.68	4.86	5.21	5.76	
THF	8.12	8.12	8.31	1.07	3.06	3.60	3.78	
Acetone	8.56	8.56	9.13	5.93	6.49	6.86	7.17	
Acetonitrile	8.00	8.2	7.8	5.58	6.11	6.49	6.68	
Nitrobenzene			7.62*		Insoluble			
Nitromethane	16.1	15.6	15.4		Insoluble			
Methanol	4.53	4.53	4.53	4.50	4.31	4.50	4.50	
Ethyl Acetate	9.4	10.2	9.4		5.03	4.86	4.86	

Table VIII(Continued)

Solvent	NaClO ₄ c					NaI c				
	0.5M	0.25M	0.125M	0.0625M	0.0313M	0.5M	0.25M	0.125M	0.01M	0.005M
HPMA	-1.98	-1.98	-1.98	-1.80	-2.15	-2.6	-2.4	-2.5		
Pyridine	-0.18	-0.18	-0.18	-0.18	-0.18	-3.8	-2.4	-2.4		
DMSO	1.44	1.44	1.44	1.62	1.62		-0.18	0.90		
DMF	5.9	5.8	5.8	5.8	5.9		5.75	5.92		
THF	9.19	9.19	9.01	9.01	8.82	-6.25	-6.36	-5.98		
Acetone	10.3	10.1	9.7	9.9	10.1	3.44	4.50	5.15	6.86	7.75
Acetonitrile	8.3	8.4	8.4	8.1	8.4	4.77	6.07	6.80	7.76	
Nitrobenzene		Insoluble					Insoluble			
Nitromethane					14.0*			Insoluble		
Methanol	5.22	5.22	4.86	4.86	4.86	4.22	4.46	4.68		
Ethyl Acetate	10.8	10.6	10.6	10.6	10.6			-2.54*		

*Saturated Solution

Corresponding solutions of the iodides were much more concentration dependent with downfield shifts of greater than or equal to 2 ppm for the same concentration range. Likewise, for the same dilutions, the sodium perchlorate solutions exhibited no concentration dependences while corresponding sodium thiocyanate solutions showed shifts of up to 3 ppm.

Moreover, while the order (but not the magnitude) of the chemical shifts of various solvents is the same for sodium perchlorate and tetraphenylborate solutions, it is quite different for the sodium iodide and thiocyanate solutions.

It was shown by Saika and Slichter (59) that nuclear magnetic shielding of a nucleus may be divided into three factors. The first of these is the diamagnetic term which is expressed by the Lamb formula

$$\sigma_{\alpha} = \frac{e}{3mc^2} \sum_i \langle \psi_0 | \frac{1}{r_i} | \psi_0 \rangle$$

where e , m , and c have their usual meaning and where the integral is the quantum mechanical average value of $1/r_i$, r_i being the distance of the i th electron from the nucleus. This factor is due to the local diamagnetic currents in the ion.

The second factor is the paramagnetic term and represents the contribution to the shielding by the orbital motions of the valence electrons. In the absence of an external field, the orbital fields have a net average value of zero, but may, in fact, have instantaneous values on the order of thousands of gauss. A small perturbation of these orbital fields by an applied external field produces, therefore, an appreciable magnetic shielding.

The third and final contributing factor to the shielding is the field produced by electrons on neighboring atoms. These electrons are either tightly bound in closed shells or are in the valence shell. The former are hard to polarize and thus do not contribute to the shielding. The latter, however, may contribute to the shielding, but the effect will be rather small compared to the paramagnetic term because the strength of interaction drops off as the inverse cube of the distance. Thus it may be said that, except in the case of protons where the absence of p, d, f, etc. electrons and the spherical symmetry of the s electrons produce only a minor paramagnetic effect, the diamagnetic effect is rather unimportant and the paramagnetic contribution to the shielding accounts for almost 99 percent (59) of the observed shift.

Bloor and Kidd (47) showed that the diamagnetic term may be expressed by

$$\sigma_d = \frac{e^2}{3mc^2} \times \frac{Z_{\text{eff}}}{\alpha_0 n^2} = 17.8 \times 10^{-6} Z_{\text{eff}}/n^2$$

while the paramagnetic term is given by

$$\begin{aligned} \sigma_p &= -[e^2 h^2 L(L+1)]/3m^2 c^2 \Delta e \times \langle 1/r^3 \rangle_{n,\ell} \\ &= -7.67 \times 10^{-16} [(Z_0 Z)^{3/2} L(L+1)]/n^3 \ell(\ell+1)(\ell+1/2) \Delta e \end{aligned}$$

where Δe is taken as the first ionization potential of the ion in question, Z_{eff} is calculated using Slater's rules (60), n is the level to which the electron is added, ℓ is the azimuthal quantum number and L is the net angular momentum of the orbital which the electron enters, and e , h , m , and c have their usual meanings.

If the above equations are solved for the case of the sodium ion, it is seen that the complete donation of one electron would give rise to a paramagnetic shift of -270 ppm and a diamagnetic shift of +9.7 ppm.

Obviously the complete donation of an electron would not occur in the case of an anion-cation or cation-solvent interaction of the type observed in this study. However, these equations do show that if a moiety surrounding a sodium ion is replaced by one of greater electron donating ability, a downfield shift in the sodium resonance may be expected because of the dominance of the paramagnetic term.

Using this interpretation of the observed chemical shifts, one may form a case for the observation of ion-pairing in solution. Due to the large size and charge distribution in anions such as perchlorate and tetraphenylborate, one would not expect these ions to form contact ion-pairs except in solvents of very low dielectric constant because of the low solvating ability of these solvents. As shown by conductance measurements, solvent separated ion-pairs can form, but in this investigation only the immediate environment of the sodium ion is being observed. There would be, therefore, very little competition between the anion and the solvent for a position in the solvation shell of the cation in high dielectric constant media. As a result, one would not expect to see a concentration dependence in the observed chemical shifts.

On the other hand, anions which are easily polarizable or which are highly charged should much more easily form contact or solvent shared ion-pairs in solution. With dilution, the ion-pairs would progressively dissociate and, consequently the chemical shifts would be concentration dependent.

Moreover, within a given solvent, the magnitude of the observed concentration dependence of the chemical shift of the sodium ion resonance for the various solutes investigated should give a good indication of the relative stability of the ion-pair. In acetone and acetonitrile, the iodide and thiocyanate solutions yield about the same

concentration dependence for the sodium resonance, while in tetrahydrofuran, the concentration dependence of the sodium iodide resonance is much greater than that of the thiocyanate resonance. Thus in acetonitrile and acetone solutions, the stabilities of the ion-pairs are about equal in magnitude while in tetrahydrofuran, the iodide seems to form a much more stable ion-pair than does the thiocyanate.

In one experiment, a 0.1 M solution of tetrabutylammonium iodide in acetonitrile was prepared and the iodine-127 resonance signal was observed. It was assumed that, in acetonitrile, because of the large size and charge distribution of the cation, there would be little tendency for ion-pairing to occur. The observed line-width should be indicative of the line-width for either unsolvated iodide ion or a symmetrically solvated iodide ion. A line-width of about two gauss at a field of 20020 gauss and a frequency of 17.0 MH_z was observed.

Another solution was composed of 0.1 M tetrabutylammonium iodide and 0.5 M sodium tetraphenylborate in acetonitrile. Because of the fairly large concentration dependence of the chemical shift in acetonitrile solutions of sodium iodide, it was assumed that the iodide ion is either in the form of contact or solvent shared ion-pairs with the sodium ion. No iodine-127 resonance was detected for this sample.

A possible explanation of these results may be that the presence of the sodium ion in the vicinity of the iodide ion represents a nonsymmetrical perturbation of the iodide's quadrupole field, which, in turn, decreases the relaxation time and, thus, the line-width increases. In this case, the line-width is increased to such an extent that the resonance signal becomes lost in the baseline. We have, therefore, two cases. In a symmetrical field as would be found with solvated or

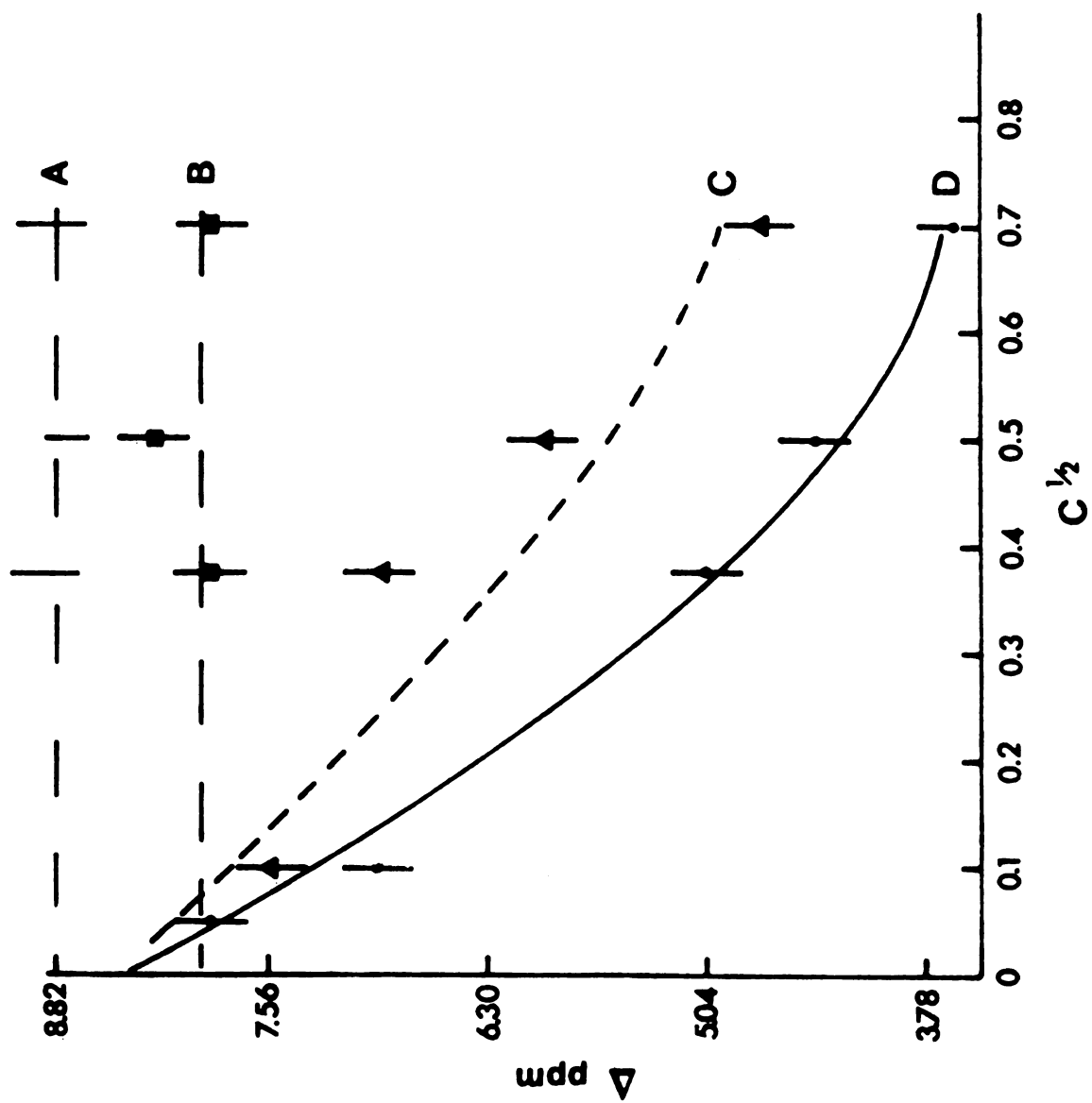
free iodide, we can observe a resonance signal, while in the ion-paired case, the line is so broadened as to disappear.

With these facts in mind, a 0.1 M tetrahydrofuran solution of sodium iodide was prepared and the iodine-127 spectrum was observed. No resonance signal was found, indicating that we are probably observing either contact or solvent shared ion-pairs in solution. The other possible cause of line broadening is an increase in the viscosity of the solution. However, since only solutions of low concentration were used in this investigation, solution viscosity remained fairly constant. It did not seem likely that the perturbation of the iodide quadrupole which may occur upon the formation of a solvent separated ion-pair could be of a great enough magnitude to cause the degree of broadening which we noted.

If ion-pairing is indeed occurring in sodium iodide solutions we are studying, we would expect to find that, within a given solvent, an extrapolation of the chemical shift versus concentration plots should approach the same value at infinite dilution, a value determined by the shift of the solvated sodium ion uninfluenced by a cation-anion interaction. Fig. 9 shows that this was, indeed, the case. The limits of sensitivity of our instrument prevented measurement of solution concentrations less than 0.1 M in many cases. However, the shifts did seem to approach a limiting value of about 8.7 ppm for acetonitrile and acetone solutions, where lower concentrations were able to be studied because of the sharpness of the resonance absorption. The same trend was found for other solvents.

The following experiments were carried out to study the formation of the sodium iodide ion-pair in tetrahydrofuran.

Figure 9. Variation in the Sodium-23 Chemical Shifts with Concentration.
A - sodium tetrphenylborate in acetone; B - sodium tetrphenylborate in acetonitrile; C - sodium iodide in acetonitrile; D - sodium iodide in acetone.



The sodium-23 resonance was observed for a tetrahydrofuran solution of sodium tetraphenylborate. The resonance signal observed corresponded to that expected for the solvated sodium ion in the tetrahydrofuran. Then, a series of measurements were made on solutions containing increasing amounts of tetrabutylammonium iodide in addition to the tetraphenylborate. As shown in Fig. 10, there was a smooth trend in the data as the resonance signal shifted from the initial position to the downfield location of the sodium ion-pair.

In another experiment, increasing quantities of iodine were added to tetrahydrofuran solutions of sodium iodide. The initial resonance was located in the position earlier found for sodium iodide in tetrahydrofuran. Addition of iodine caused the formation of the triiodide ion which should have much less tendency to form contact ion-pairs with the cation than does the iodide ion. Electrical conductance studies on the tetraalkylammonium halides and polyhalides in acetonitrile have shown that while the former show appreciable amounts of ion-pairing, the trihalides behave as completely dissociated electrolytes (61). Indeed, the formation of this anion caused the sodium resonance to shift upfield toward the value one would expect for the solvated cation (Fig. 11). In general, since the formation constant of the triiodide ion in nonaqueous solvents is on the order of 10^7 , it was expected that the formation of triiodide upon addition of iodine to iodide ion would be quantitative (62). The limiting value of the chemical shift for 1:1 mole ratio of $I_2:I^-$ was close, but not identical with the value for the sodium perchlorate and tetraphenylborate solutions. The difference was rather small (~ 1 ppm) and may be due to inherent inaccuracies in our experimental data as shown by some scatter of the experimental points from the best straight line.

Figure 10. Variation in the Sodium-23 Chemical Shift as a Function of the Concentration of Added Tetrabutylammonium Iodide. Sodium concentration equals 0.1 M.

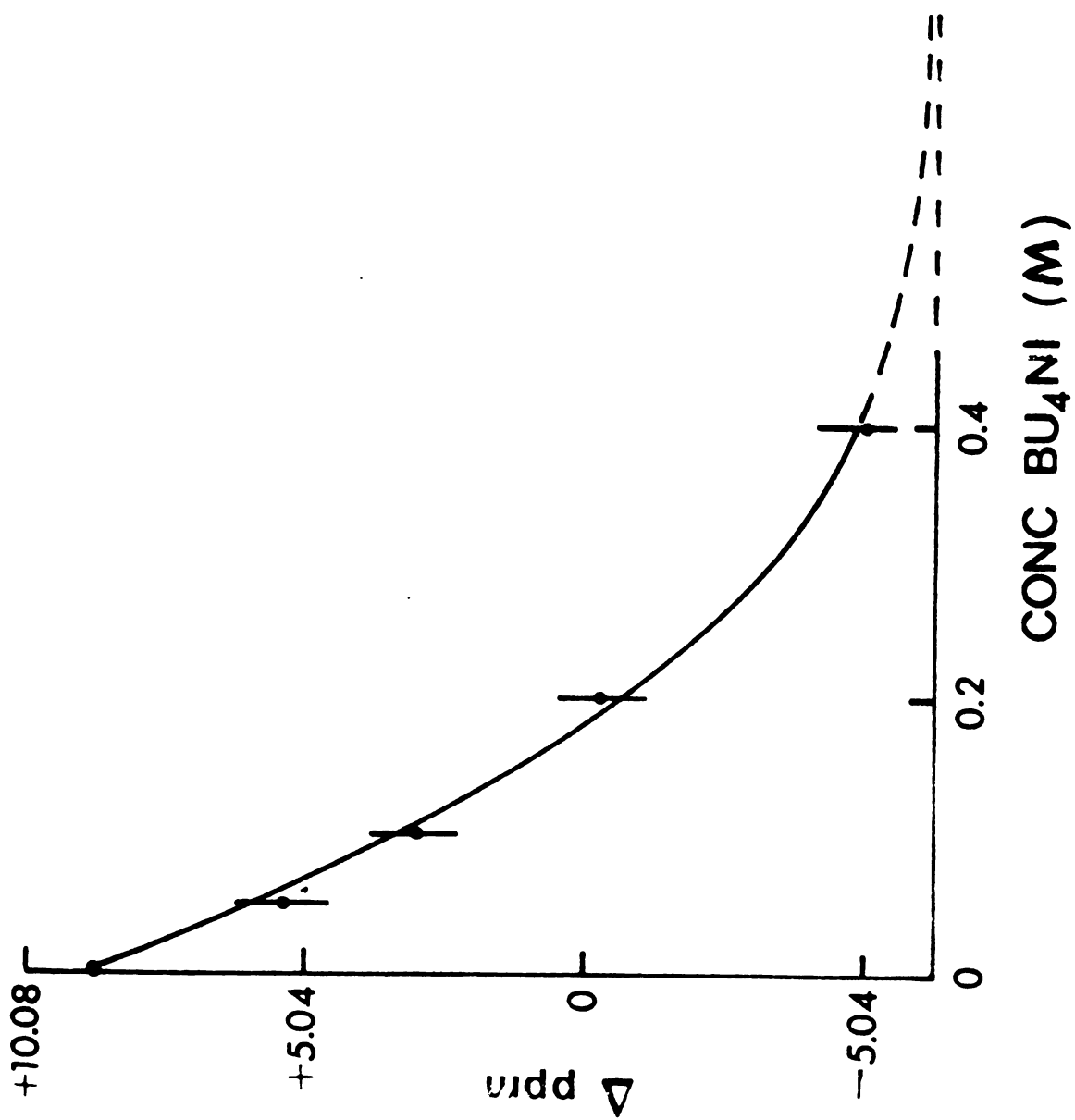
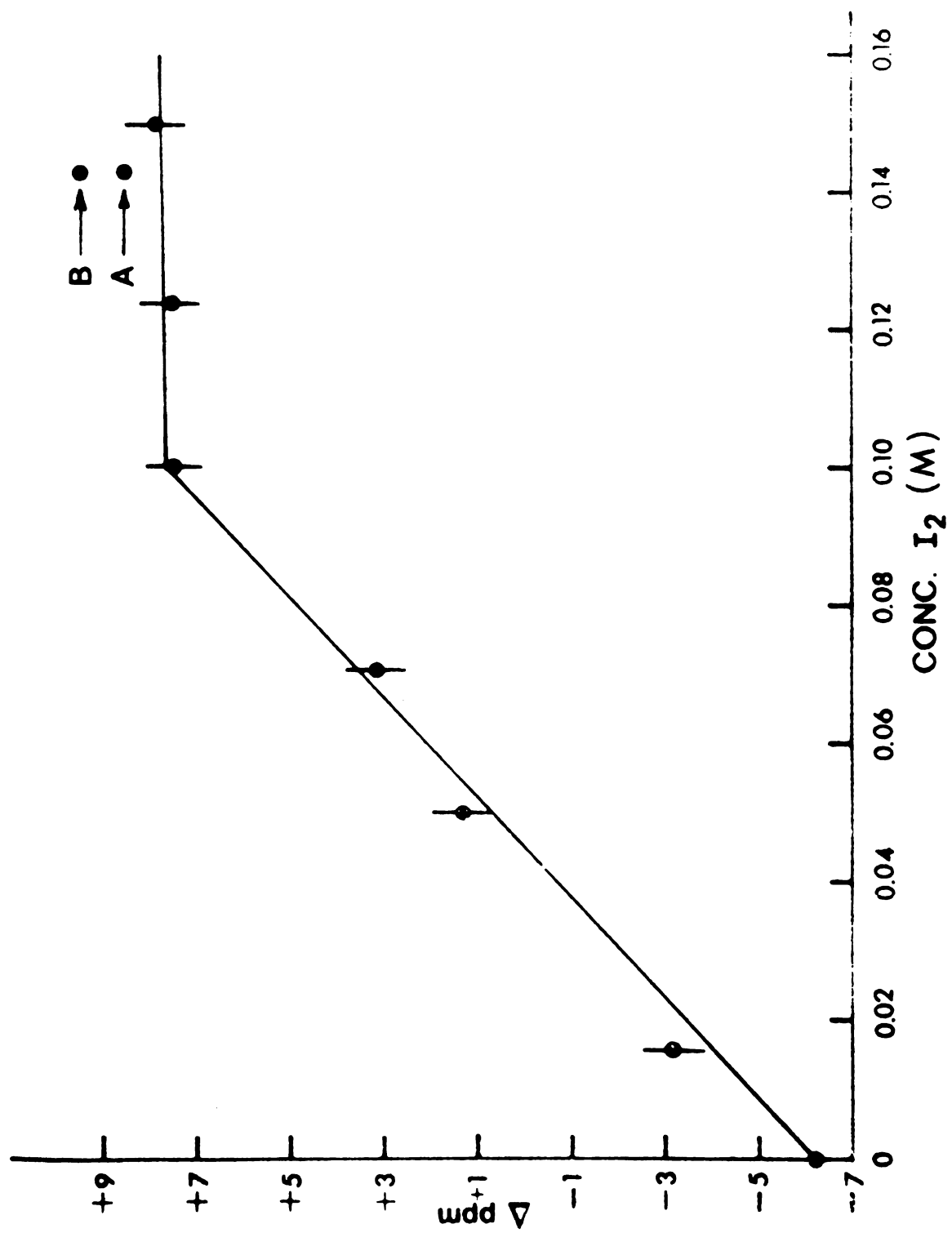


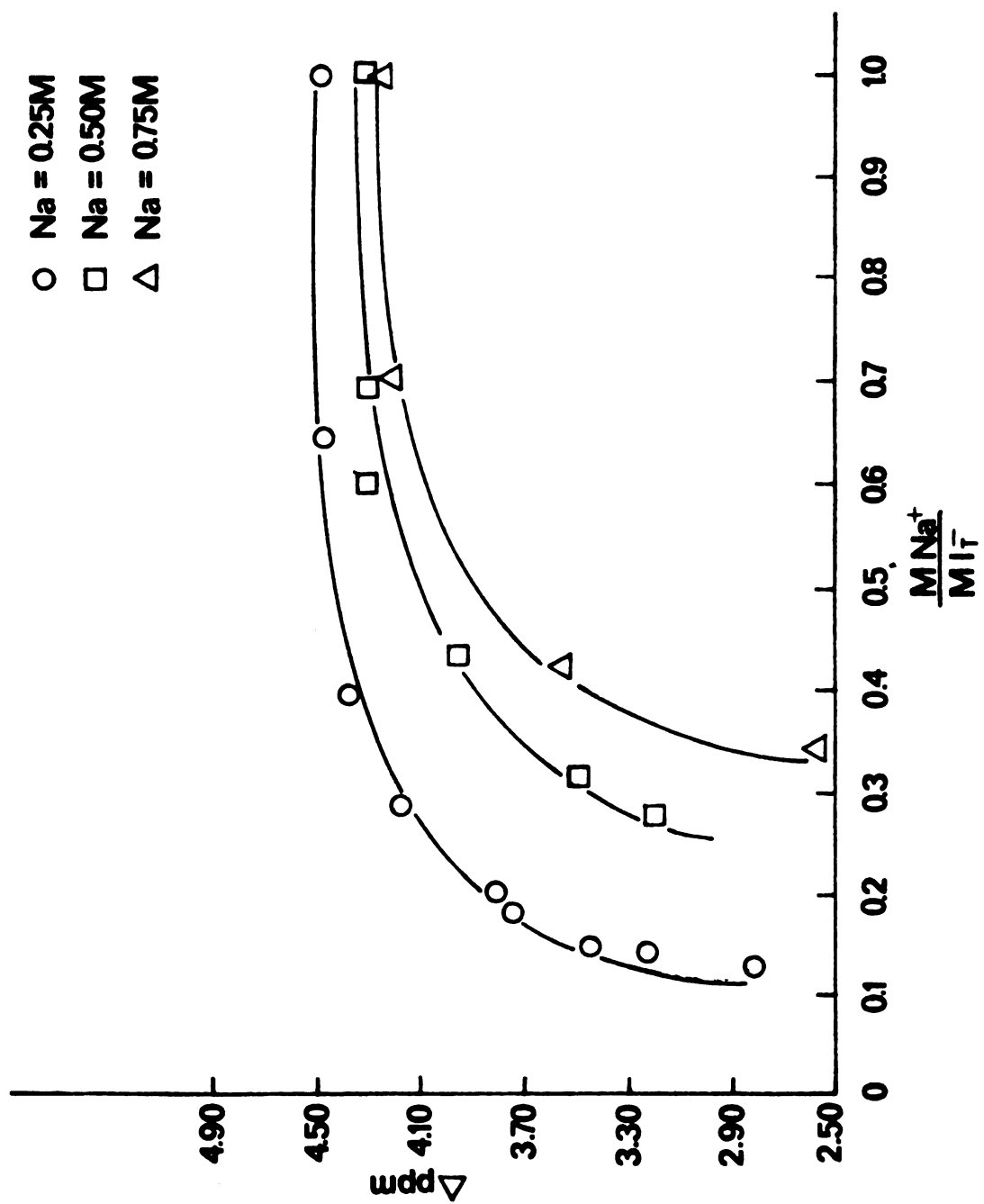
Figure 11. Variation in the Sodium-23 Chemical Shift in a 0.1 M Solution of Sodium Iodide in Tetrahydrofuran as a Function of the Concentration of Added I_2 . A - 0.1 M sodium perchlorate in tetrahydrofuran; B - 0.1 M sodium tetraphenylborate in tetrahydrofuran.



It should be emphasized again that it appears that the magnitude and direction of the chemical shifts of sodium-23 do not allow differentiation between free solvated ions and solvent separated ion-pairs.

In a recent paper by Taylor and Kuntz (63), the methyl proton resonance of triphenylmethylphosphonium iodide ($\text{CH}_3\text{Ph}_3\text{P}^+\text{I}^-$) in 1-bromonaphthalene solutions was studied as a function of added methanol concentration. The addition of three percent methanol to a 0.04 M solution of the solute caused a 38 Hz upfield shift in the methyl proton resonance. The authors attempted to explain their data by invoking the concept of anion solvation. They state that methanol hydrogen-bonds to the iodide anions and, therefore, permits an enhanced solvent-cation interaction by reducing the effects of ion association. Since methanol is known to solvate anions by hydrogen bonding, this explanation seemed reasonable. However, from the previous work it seemed that other explanations might also fit these data. Thus, we wished to further study this system by the techniques of Na-23 NMR. In Fig. 12, the shift of the sodium-23 resonance has been plotted versus the ratio of the the sodium concentration to the total iodide concentration in a solution of sodium iodide in methanol. The iodide ion concentration was varied by the addition of tetrabutylammonium iodide. The first fact which becomes apparent is the concentration dependence of the chemical shift of the sodium-23 resonance (Table VIII). At a mole ratio of 1.0, obviously we simply have a solution of sodium iodide in methanol. It can be seen that, for each initial concentration of sodium iodide, there is a very slight change in chemical shift with changing concentration of the added iodide ion until a certain point is reached where the shift rapidly decreases toward the low value expected for the sodium iodide

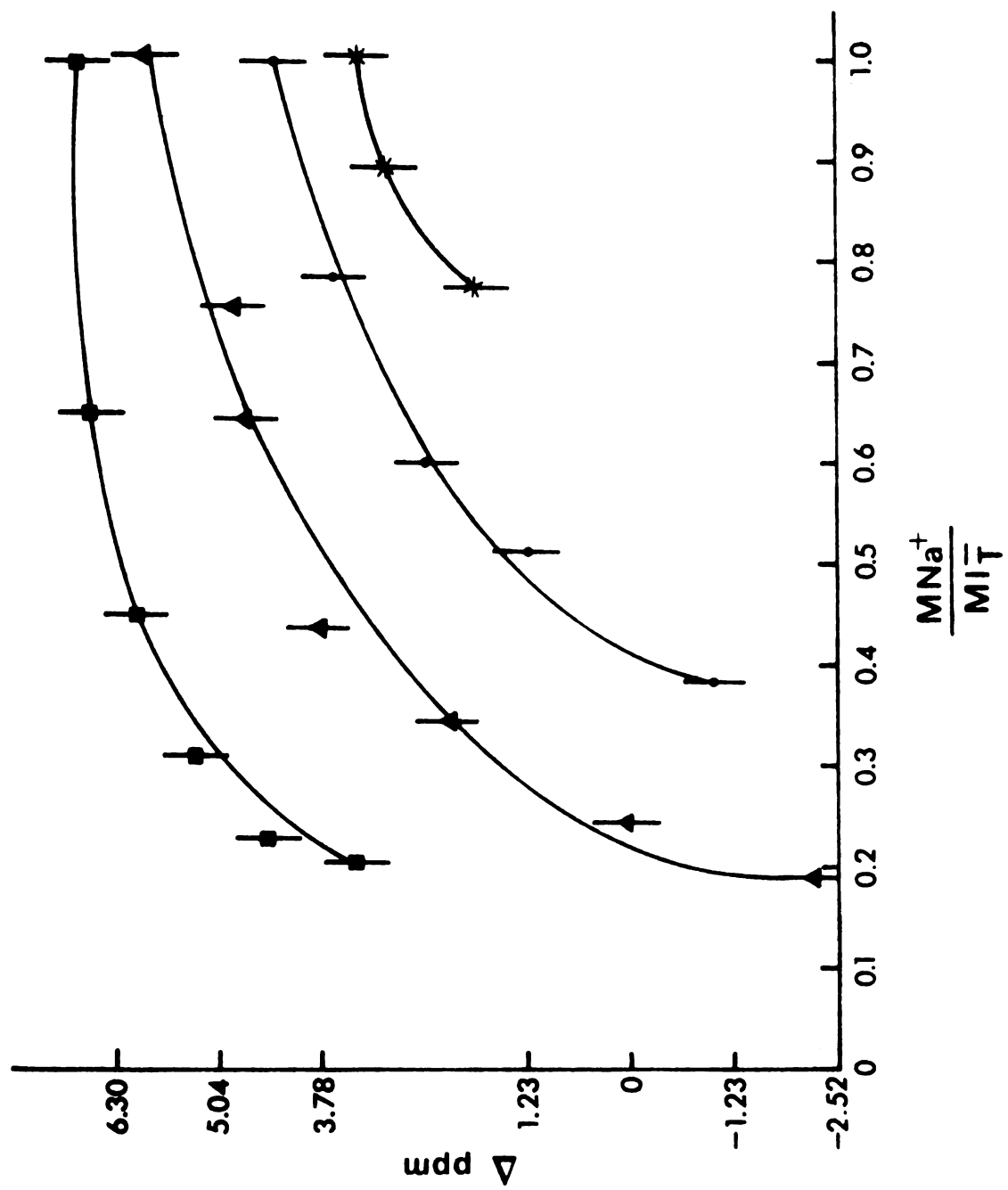
Figure 12. Variation in the Sodium-23 Chemical Shift in Methanol Solutions as a Function of the Ratio of the Concentration of Sodium in Solution to the Total Concentration of Iodide Ion.



ion-pair. These data may be explained as was done by Kuntz and Taylor (63) by assuming that with low concentrations of the iodide ion (high Na^+/I^- ratio) the latter is effectively solvated by the methanol and is not free to interact with the sodium ion. Thus the sodium shift remains relatively constant. At a $\underline{\text{M MeOH}}/(\underline{\text{MNa}^+} + \underline{\text{MI}^-})$ ratio of about 13, it appears that there is not enough methanol to solvate both the iodide and sodium ions. The iodide ion is therefore free to form a contact or solvent shared ion-pair with the sodium ion resulting in a large downfield shift. This experiment does fit with the experiment of Kuntz and Taylor in that they used an inert solvent in their experiment so that they had a limited amount of methanol in solution. However in our experiment, methanol was used as the solvent and the resulting curve is the same, methanol remaining in at least a 2:1 excess over the total sodium and iodide concentration.

When the same experiment is run in acetonitrile, the data displayed in Fig. 13 are obtained. These data appear to be very similar to those obtained in a methanol solvent system. The results appear to be in conflict with the statement by Kuntz and Taylor that "it is very unlikely that these solvents (acetonitrile) coordinate significantly with either the cation or the anion". We postulate that these curves may result from the fact that there are two or more species present in solution. In the high mole ratio portion of the curves, there is a large excess of solvent compared to the concentration of the anion. Under these conditions, in the competition between the solvent and the anion for the cation, probability favors the formation of the solvated cation with the possibility of formation of solvent separated ion-pairs. Since in the solvent separated ion pair, effects of the anion on the electron density around the sodium ion must be felt through a sphere of solvent

Figure 13. Variation in the Sodium-23 Chemical Shift in Acetonitrile Solutions as a Function of the Ratio of the Concentration of Sodium in Solution to the Total Concentration of the Iodide ion. $\square$ equals 0.1 M; Δ equals 0.25 M; $\bullet$ equals 0.5 M; $\star$ equals 0.75 M.



one or more molecules thick, one would not expect that the formation of a solvent separated ion-pair will have a large effect on the chemical shift of the sodium-23 resonance. As the ratio decreases (the relative amount of solvent is decreasing), the probability is shifted in the direction of formation of a contact ion-pair which results in a downfield shift of the resonance. At any given value for the ratio, the set of points gives the concentration dependence of the chemical shift which was described earlier. The shape of the curves at different sodium ion concentrations is due to the presence of more than one type of species in solution. At low concentrations of sodium iodide, there is a large excess of solvent and, therefore, contact ion-pairing will not take place until a large amount of the anion is added. However, at higher initial sodium ion concentrations, the concentration of the solvent needed for the formation of contact ion-pairs is reached much sooner.

It was previously shown in the theoretical section that the chemical shift is brought about by changes in the electron density around the ion. If the effects of cation-anion interactions are removed from the system under investigation, changes in the electron density around the sodium nucleus are due to interactions with the solvent. We have already seen that there is little tendency for the perchlorate or the tetraphenylborate anions to associate with the sodium ions as evidenced by the lack of a concentration dependence of the chemical shift with these salts. Thus, the variation of the Na^+ chemical shift with the solvent can be explained by the varying abilities of the solvents to alter the electron density around the sodium-23 nucleus, which should be related to the donor ability of the solvents. An empirical approach to this donating or complexing ability of the solvent is given by Gutmann's donor numbers (64a). Gutmann postulates that the enthalpy of complex formation between a solute and a solvent is proportional to the donating

ability of the solvent. He formulates a scale of donor numbers which are equal to the enthalpy in kcal/mole of the reaction between the solvent and antimony pentachloride in 1,2-dichloroethane solutions. As Gutmann shows, these numbers can be of use in predicting the solvating ability of liquids, their relative donating abilities, and many other aspects in the properties of nonaqueous solutions. It is reasonable to expect, therefore, that with the effect of the anion removed, there might be a relationship between the donor numbers and the relative chemical shift for the sodium ion. A plot of chemical shift versus the donor number for the case of perchlorate solutions is shown in Fig. 14, and for tetraphenylborate solutions in Fig. 15.

The correlation between the chemical shift and donor number in both these cases is evident. The only discrepancy is water. This donor number was determined by indirect methods and may be in error (64b). According to our data, water should have a donor number of 33 instead of the value of 18 as reported by Gutmann. The linearity of the plots seem to place donor numbers in a more generally useful position than the previously discussed enthalpies of complex formation. Also, the shifts supply useful information about the solvating abilities of several solvents, at least vis a vis the sodium ion.

A STUDY OF MIXED SOLVENT SYSTEMS

We now wish to see if the techniques used in this work can be extended to the investigation of mixed solvent systems. In their study of the chloride ion in mixed solvent systems, Langford and Stengle (65) imply that the sodium-23 resonance cannot be used for this type of investigation because the chemical shifts would depend not only on the solvent but also on the anion. We have already shown that in the case

Figure 14. Plot of Sodium-23 Chemical Shifts for Perchlorate Solutions Versus the Donor Number of the Solvent. 1 - nitromethane; 2 - acetonitrile; 3 - acetone, 4 - ethyl acetate; 5 - tetrahydrofuran; 6 - dimethylformamide; 7 - dimethyl sulfoxide; 8 - pyridine; 9 - hexamethylphosphoramide; 10 - water.

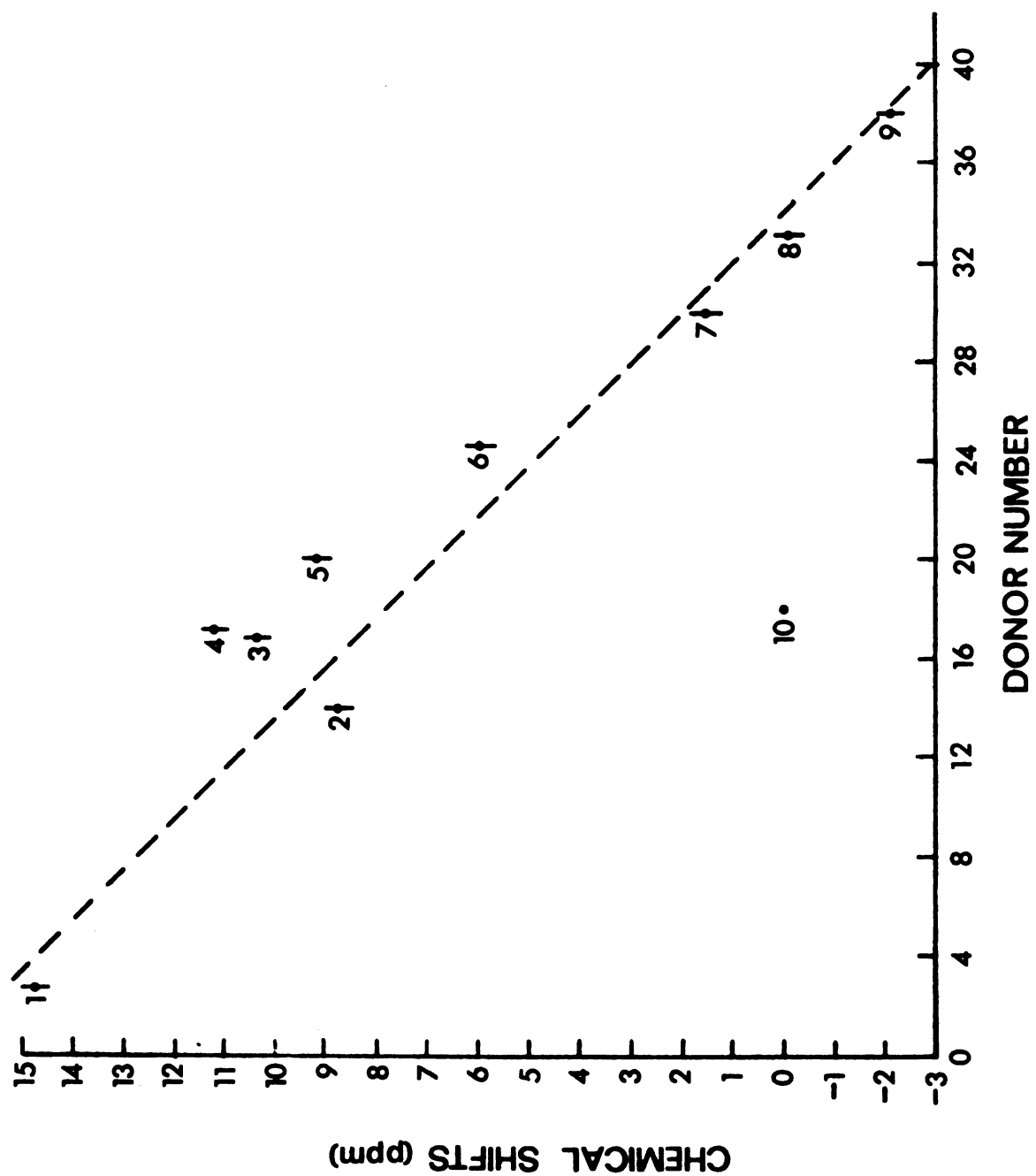
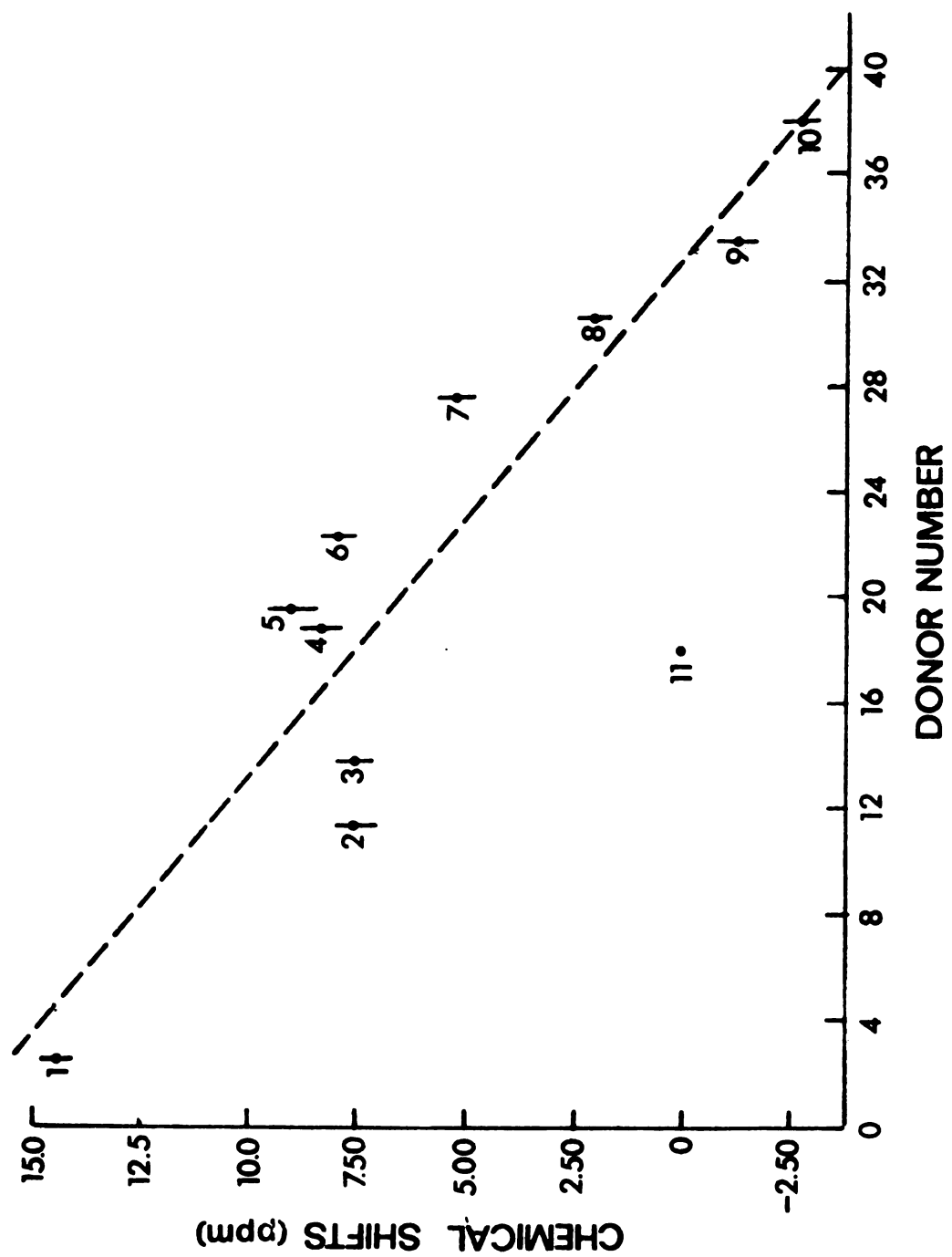


Figure 15. Plot of Sodium-23 Chemical Shifts for Tetraphenylborate Solutions Versus the Donor Number of the Solvent. 1 - nitromethane; 2 - benzonitrile; 3 - acetonitrile; 4 - acetone; 5 - ethyl acetate; 6 - tetrahydrofuran; 7 - dimethylformamide; 8 - dimethylsulfoxide; 9 - pyridine; 10 - hexamethylphosphoramide; 11 - water.



of tetraphenylborate solutions, there is little tendency for anion-cation interactions to occur. Thus we have used this solute in our present investigation.

The iso-solvation point may be defined as follows. In a mixed solvent study, the chemical shift varies smoothly from the value it would have in pure solvent A to that value it would have in pure solvent B. At some point in the study, the chemical shift has progressed 50% of the way from one value to the other. This point is defined as the iso-solvation point where there is equal competition of each of the solvent components for the cation. It is seen from Fig. 16 that for the acetonitrile-dimethylsulfoxide system the iso-solvation point occurs when the solvent is composed of about 20 mole percent dimethylsulfoxide. It follows, therefore, that sodium ion is preferentially solvated by dimethylsulfoxide probably due to the fairly high negative charge residing on the oxygen atom of this molecule. In Fig. 17, the results are shown for the same type of experiment carried out on the nitromethane-dimethylsulfoxide system. Again, due to the localization of the negative charge on the oxygen, we would expect to find the solvation to favor the dimethylsulfoxide. As can be seen, the iso-solvation point is reached at about five mole percent dimethylsulfoxide. It appears therefore that the solvating abilities of these solvents are in the order dimethylsulfoxide > acetonitrile > nitromethane.

The sodium-23 chemical shift was also measured for sodium tetraphenylborate in pyridine-acetonitrile mixtures. As is seen in Fig. 18, the iso-solvation point for this experiment occurs at about 40 mole percent pyridine. While this shows pyridine to be the preferred solvating agent, there appears to be fairly equal competition of the solvents for a place in the sodium ion solvation shell.

Figure 16. Variation of Chemical Shift of Sodium-23 Resonance as a Function of the Solvent Composition for Dimethylsulfoxide-Acetonitrile Mixtures.

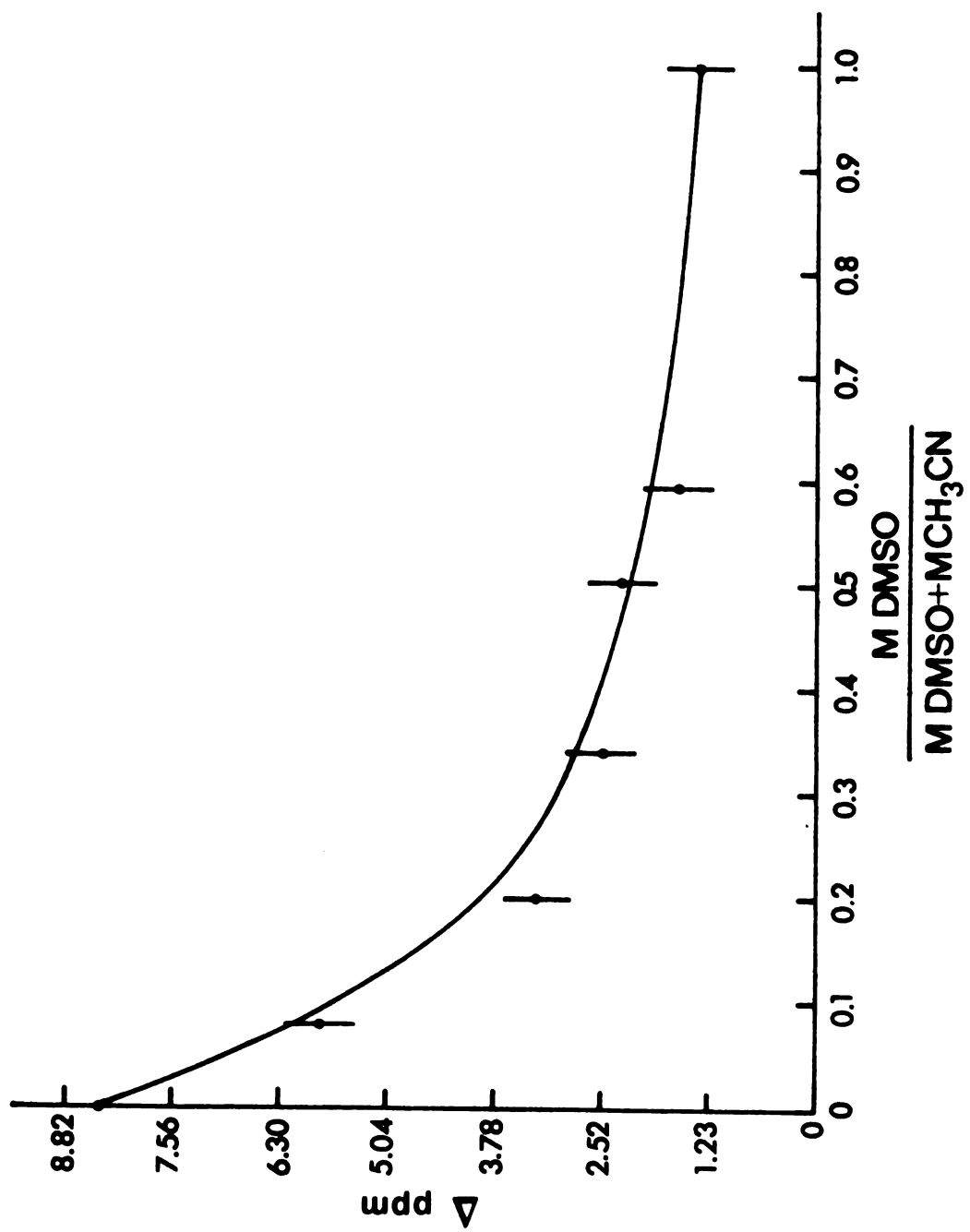


Figure 17. Variation of the Chemical Shift of the Sodium-23 Resonance as a Function of the Solvent Composition for Nitromethane-Dimethylsulfoxide Mixtures.

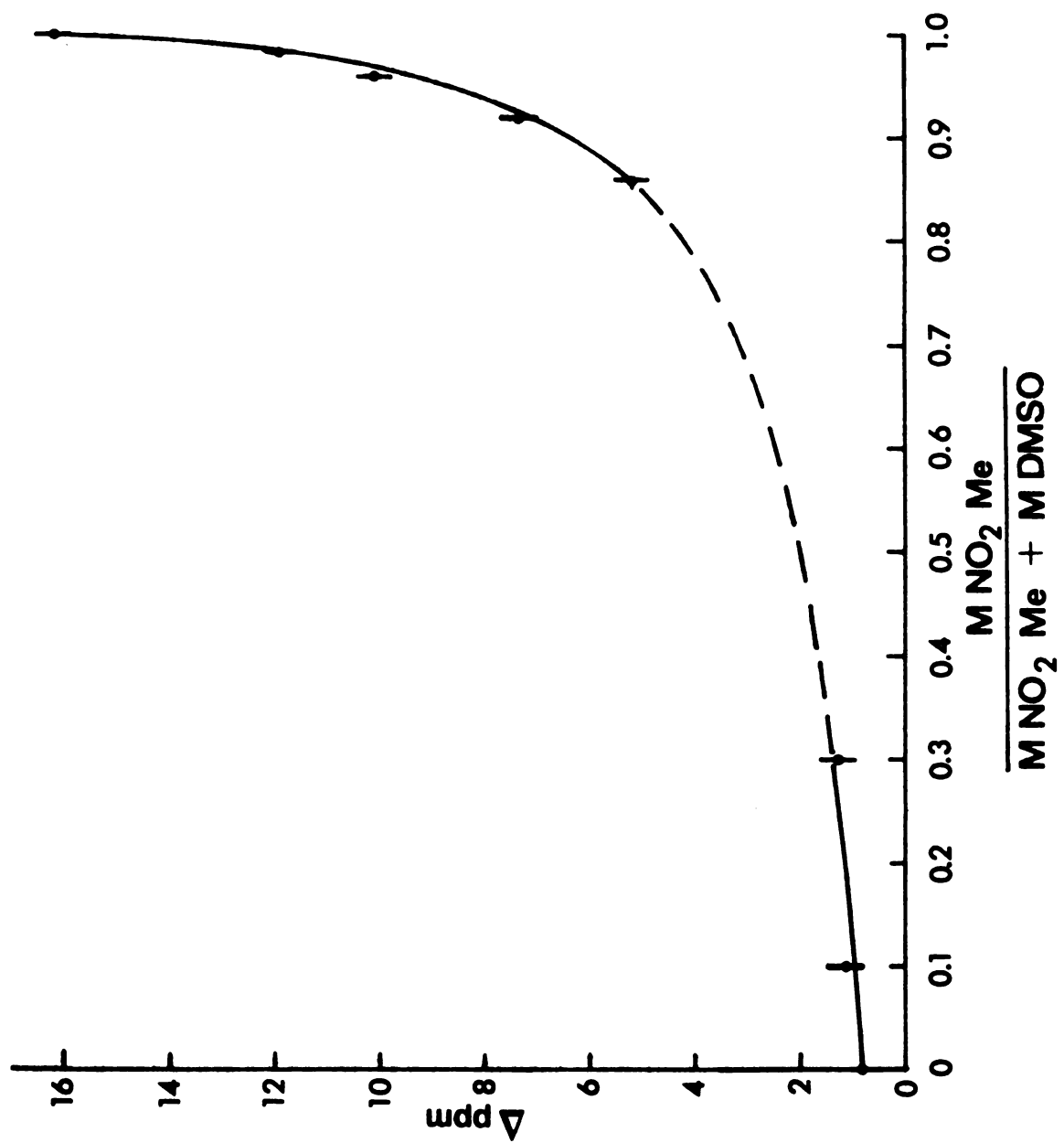
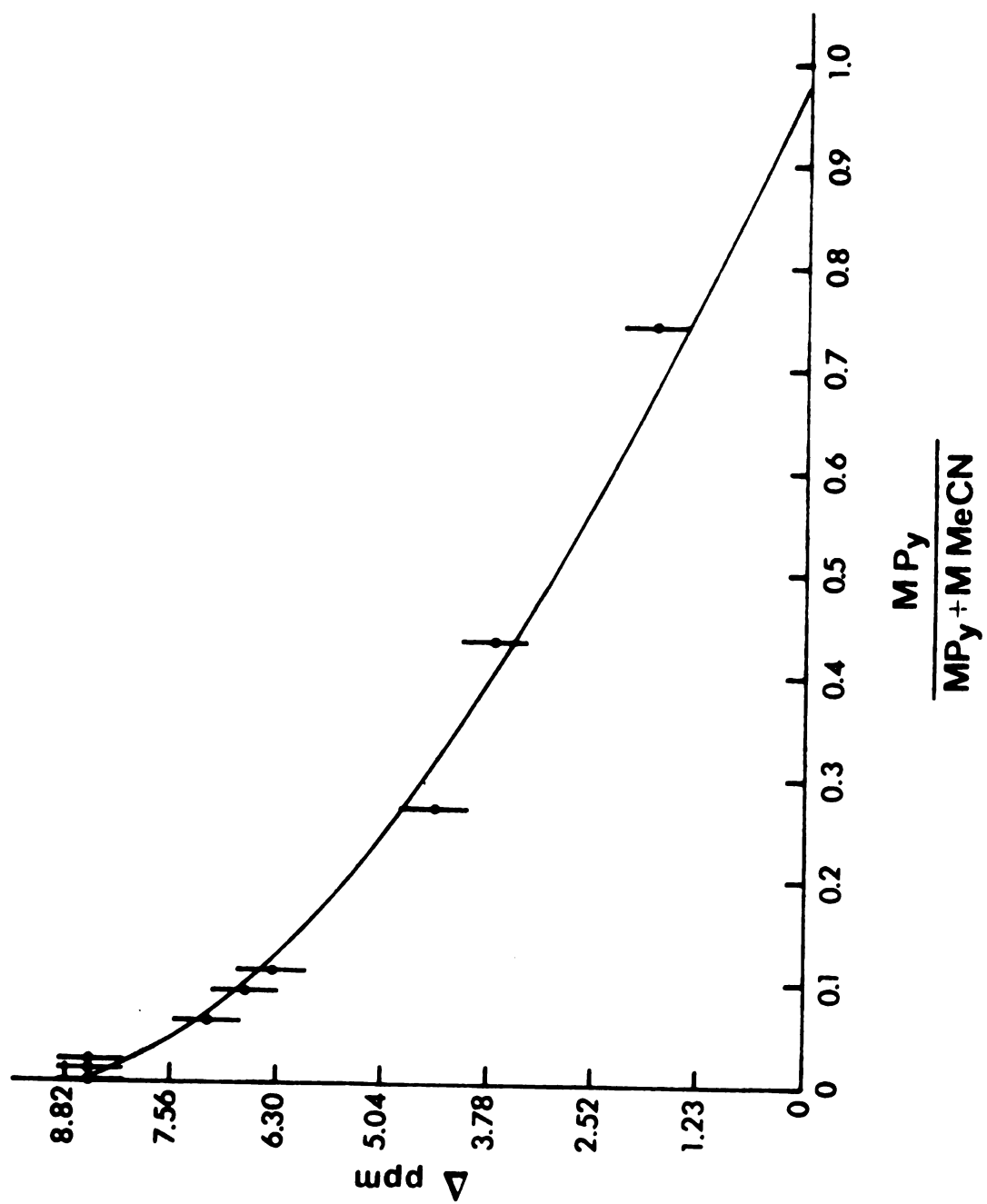


Figure 18. Variation of the Sodium-23 Chemical Shift as a Function of the Solvent Composition for Pyridine-Acetonitrile Mixtures.

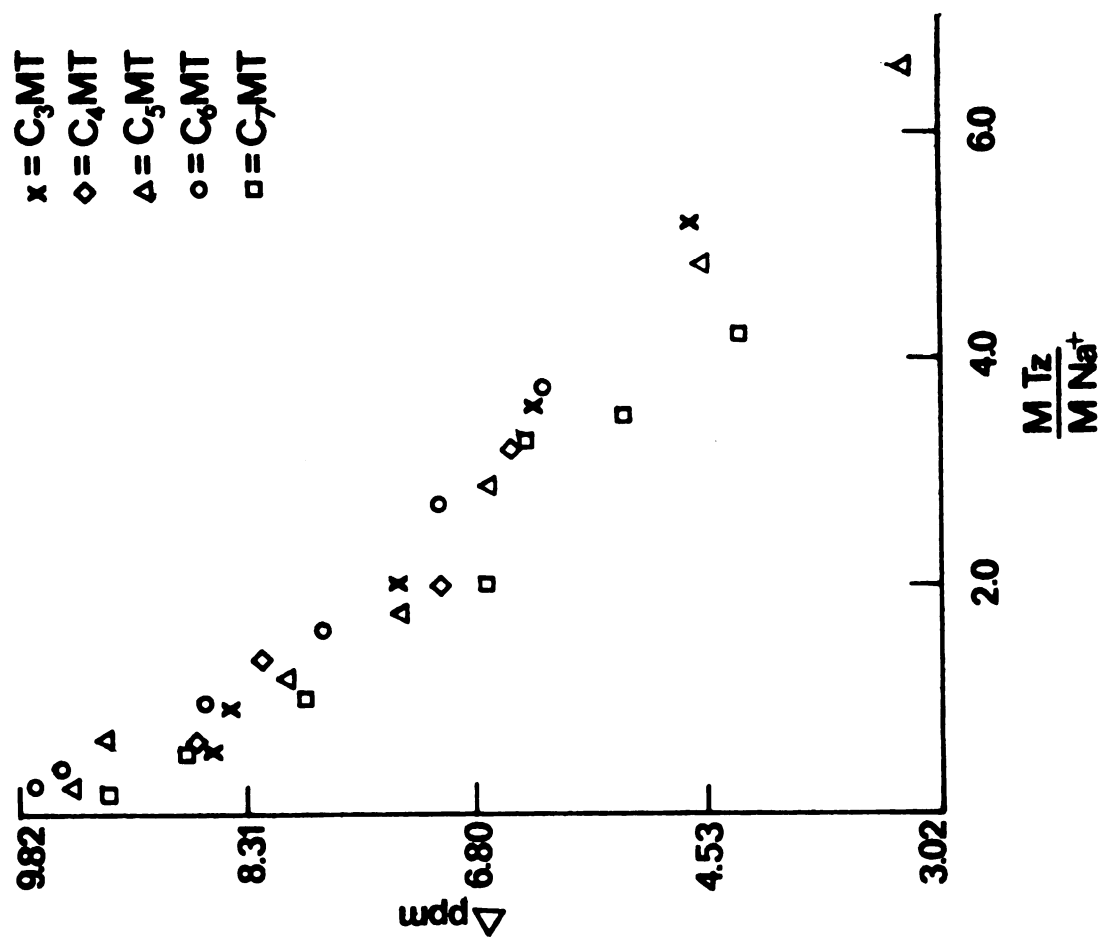


A STUDY OF SODIUM-TETRAZOLE INTERACTIONS

With the techniques of measuring small chemical shifts now established, an attempt was made to study the complexation of sodium by the tetrazoles. As was previously noted, sodium is a very important ion in human physiology. The potential across a biological membrane is determined by the concentration of several types of ions both inside and outside the cell, the two most important of these being potassium and sodium. The human neuron may be looked at as a sodium pump which allows potassium to concentrate within the cell while actively transporting sodium to the outside. It is the potential difference across the membrane caused by the sodium concentration gradient across the membrane which gives rise to the electrical activity of the neuron. It is possible that, through some mechanism, the cyclopolymethylenetetrazoles are interfering with the sodium pump allowing the sodium to re-enter the cell causing it to discharge. This chemically induced firing of the neurons manifests itself in the convulsive symptoms characteristic of the cyclopolymethylenetetrazoles.

It was decided to begin this investigation by looking for direct interactions between the sodium ion and the tetrazoles. Presumably, if an interaction was occurring, there would be a change in the electron density surrounding the sodium ion, and a chemical shift would be noted. A series of solutions were prepared containing a fixed quantity of sodium tetrphenylborate (chosen for the low degree of anion interference) and varying amounts of the cyclopolymethylenetetrazoles in nitromethane. Nitromethane was chosen as the solvent because, while not found in large quantities in biological systems, it has a very low donor number, and thus would not mask a weak interaction between sodium and the tetrazoles. The results of this experiment are shown in Fig. 19. It was at once

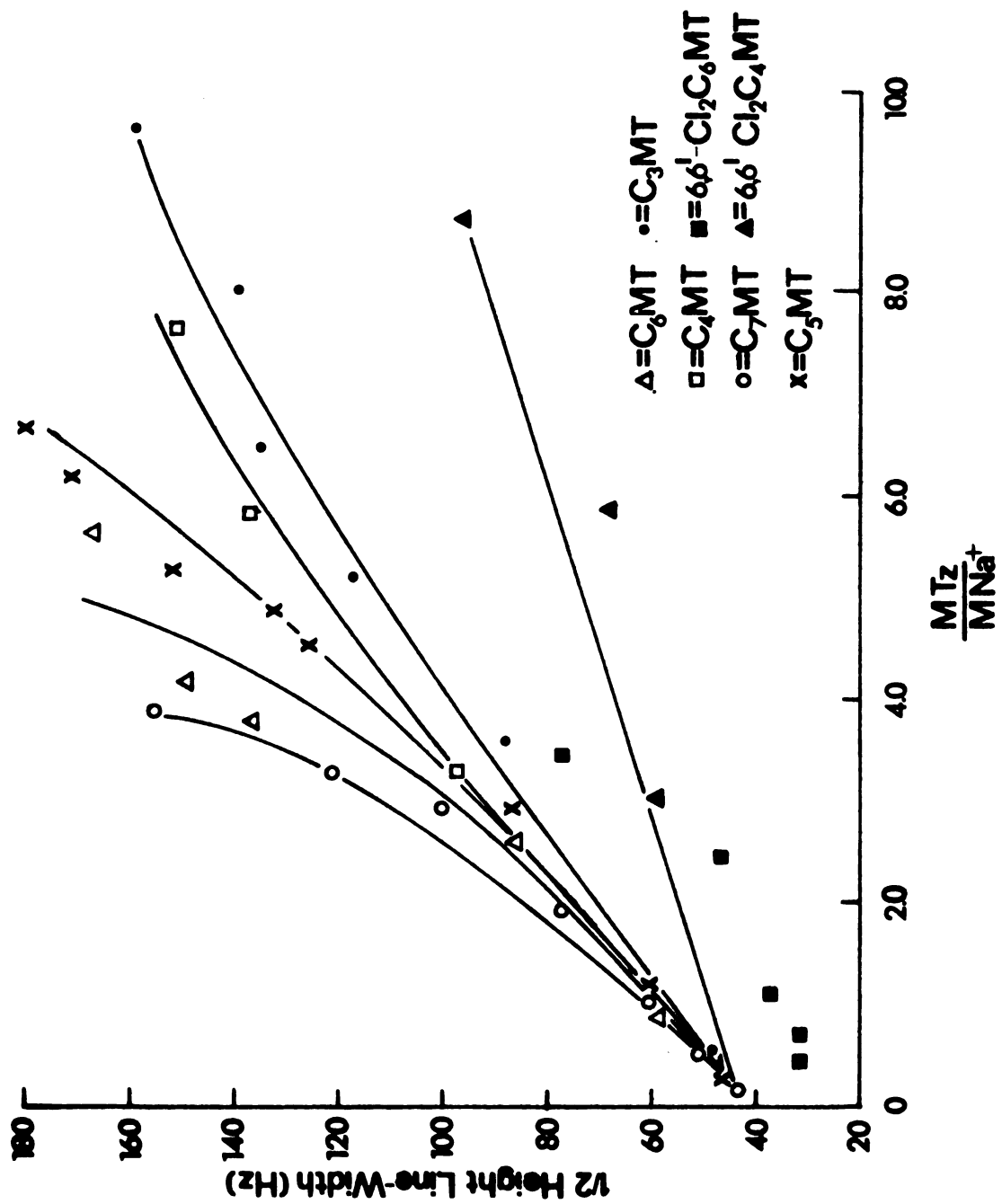
Figure 19. Variation in the Chemical Shift of the Sodium-23 Resonance as a Function of the Ratio $\underline{M} \text{ Tz} / \underline{M} \text{ Na}$. Sodium concentration equals 0.5 $\underline{M}$.



apparent that there is an interaction between sodium ion and the tetrazoles, as evidenced by the large change in the sodium-23 chemical shift with increasing concentration of the tetrazole. However it was also seen that all the cyclopolymethylenetetrazoles studied have about the same effect on the sodium chemical shift. Since the biological activity of these cyclopolymethylenetetrazoles ranges over about two orders of magnitude (7), we were not able to see any differentiation of the tetrazoles on the basis of chemical shift. However, it can be seen that the scatter of the experimental data from the best line through the points seems to be increasing at higher mole ratios of tetrazole to sodium since, at the higher ratios, the lines were becoming extremely broad, and the exact measurement of position on a very broad line is difficult at best. We, therefore, decided to study this line broadening phenomenon. Solutions were prepared containing a fixed amount of sodium tetraphenylborate in nitromethane with varying amounts of tetrazole, and measurements were made of the half-height line-width for the sodium resonance. The results of this investigation are shown in Fig. 20. While there is a large amount of scatter of the data, it can be seen that each of the tetrazoles displayed a different rate of broadening of the sodium-23 resonance line. Moreover, the rate of broadening was directly proportional to the convulsant activity of these drugs.

We wished to see if this correlation could be extended to tetrazoles other than the unsubstituted cyclopolymethylenetetrazoles. Measurements were made on 6,6'-dichlorohexamethylenetetrazole solutions, and the data indicated that this compound should have an activity between that of hepta- and hexamethylenetetrazole. Similarly, the data for 6,6'-dichlorotetramethylenetetrazole indicated that it should be a weaker convulsant than trimethylenetetrazole. Both findings are in

Figure 20. Variation in the Half-Height Line-Width of the Sodium Resonance as a Function of the Ratio $\underline{M}_{Tz}/\underline{M}_{Na}$. Sodium concentration equals 0.5 $\underline{M}$.



accord with the results of Stone on the activities of these compounds (7). Finally, measurements were made on 1,5-dimethyltetrazole solutions. The resonance line showed very little broadening in agreement with pharmacological data indicating no activity for this compound.

While these data are interesting, several facts make them less than useful for understanding the mechanism of activity of the tetrazoles. First of all, fairly high concentrations of the tetrazoles are needed in order for the differentiation to become apparent. This concentration level may be much higher than the quantity of the drug found at the in vivo site of interaction. Moreover, the solvent used in this investigation is chosen to accentuate any interaction. The same experiment run in water would probably give much less, if any, differentiation within reasonable concentration limits. Finally, the line-width is a function of the asymmetry around the sodium-23 nucleus. More rapid broadening of the resonance line may be expected as one proceeds through the series of tetrazoles just on the basis of increasing bulk of the ligand leading toward increasing perturbation of the sodium quadrupole.

More work on the problem of the mechanism of tetrazole drug action is preceeding at the present time and this will be mentioned in the section on future work.

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APPENDIX I

A DISTRIBUTION STUDY OF THE CYCLOPOLYMETHYLENETETRAZOLES

INTRODUCTION

It has been found that the physiological effect of the cyclopoly-methyltetrazoles increases as a function of the number of carbon atoms in the methylene chain (7). This dependency on chain length may be attributed to chemical effects such as reactivity or reactivity rate at the site of interaction, or it may be due to purely physical effects such as the speed with which the tetrazoles reach the reaction site.

The tetrazoles must have an effect either within the cell or at an axon, dendrite, or synapse. Biological studies on the tetrazoles have revealed that an isolated neuron, when placed in a tetrazole solution, exhibits rapid and repeated electrical discharges (66). In the absence of other neurons, these data would seem to indicate that the tetrazoles have an effect within the cell or cell membrane rather than a disruptive effect on intercellular information transfer. If this conclusion is valid, the tetrazole must first pass into or through the cell membrane.

One of the major theories for the passage of materials through a membrane postulates an actual dissolution of the material in the membrane. Thus a study of the rate of the tetrazole's progress toward the reactive site must, of necessity, involve a study of the solubility of the cyclopolymethylenetetrazoles within a lipid membrane.

Two problems are encountered in the study of this solubility. Since water and lipid are immiscible, equilibrium between the aqueous and lipid phases would be obtained only with great difficulty due mainly to

the limited methods of forming intimate mixtures of the solvents. Also, good quantitative methods for the determination of the cyclopolymethylene-tetrazoles do not exist.

The ideal solvent for this work would have the solvent properties of lipids and yet have a high enough vapor pressure so that rapid removal of the solvent and subsequent gravimetric determination of the solute can be easily achieved. Carbon tetrachloride fits these requirements and has the added benefits of availability and ease of purification.

Ordinarily one would obtain the distribution coefficient for the tetrazoles between water and carbon tetrachloride and attempt to relate this coefficient with the pharmacological properties of the solute. However, preliminary work in carbon tetrachloride solutions by vapor pressure osmometry indicates some association of the tetrazoles probably through dipole-dipole interaction. Moreover, survey work on aqueous solutions of the tetrazoles by vapor pressure osmometry indicates that polymers, or at least dimers, are also forming in the aqueous layer.

Thus, the distribution ratio will be determined at this time since it is a function only of the total concentration of the tetrazoles in each layer and is independent of the formation of higher order species.

EXPERIMENTAL

REAGENTS

Carbon tetrachloride was purified by refluxing overnight a mixture of carbon tetrachloride and an aqueous basic solution of potassium permanganate. The aqueous layer was removed, and the product was washed several times with distilled water. It was then allowed to reflux for 24 hours over barium oxide and then distilled. The middle fraction was retained.

The cyclopolymethylenetetrazoles were prepared by the methods of D'Itri (55) and purified as mentioned earlier.

An isotonic salt solution was prepared by dissolving 8.500 grams of reagent grade sodium chloride in a liter of solution.

PROCEDURE

The tetrazoles were weighed into 2 tared, stoppered, conical 15 ml centrifuge tubes. To one tube was added five milliliters of carbon tetrachloride and to the other was added five milliliters of water. Both tubes were placed in a water bath at 25°C and shaken every five minutes. When dissolution was complete, or after 15 minutes in the case of slightly soluble species, five milliliters of water were added to the carbon tetrachloride solution, and five milliliters of carbon tetrachloride were added to the water tube. The tubes were again placed in the water bath for 20 minutes and were shaken every five minutes to achieve equilibrium. After this period of time the tubes were centrifuged to obtain complete separation of the layers.

Three samples of one milliliter volumes were removed from the carbon tetrachloride layer of each tube and placed in preweighed, covered 50 milliliter beakers. Each beaker was reweighed immediately after adding the solution. The six beakers for each run were then placed in a vacuum oven and a partial vacuum was applied. The beakers were slowly heated to about 50°C to avoid bumping of the solution. The beakers were dried to constant weight when the solvent was apparently completely removed. The molality of the carbon tetrachloride layer was calculated based on the weight of the residue and the weight of the solvent removed. Likewise, the molality of the aqueous layer was determined. The distribution ratio for each run was then established. By noting the agreement of the ratio for the carbon tetrachloride first mixture and the water first mixture, establishment of distribution equilibrium was confirmed.

In some cases, substitution of salt solutions for the aqueous phase was made.

For completeness, the solubilities of the cyclopolymethylenetetrazoles in both water and carbon tetrachloride were determined by the same evaporation method.

The data for all these experiments are shown in the following tables. However, as stated before, the occurrence of higher ordered species in solution made further calculations difficult. For this reason, the data are displayed with no further discussion.

Table IX. Solubility of the Cyclopolymethylenetetrazoles in Carbon
Tetrachloride at 25°C

Tetrazole	Molal Solubility
Trimethylenetetrazole	0.0095 M
Tetramethylenetetrazole	0.041
Pentamethylenetetrazole	7.9
Hexamethylenetetrazole	3.7
Heptamethylenetetrazole	15.0*

*This value is rather doubtful as the solute tends to liquify in the presence of solvents and the highly viscous residue after solvent evaporation may be a miscible mixture of the tetrazole and carbon tetrachloride.

Table X. Solubility of the Cyclopolymethylenetetrazoles in Water at 25°C

Tetrazole	Molal Solubility
Trimethylenetetrazole	2.2 M
Tetramethylenetetrazol	2.5
Pentamethylenetetrazole	approaches infinite
Hexamethylenetetrazole	0.40
Heptamethylenetetrazole	0.20
Octamethylenetetrazole	insol
Nonamethylenetetrazole	insol
Undecamethylenetetrazole	insol

Table XI. Distribution Data on the Cyclopolymethylenetetrazoles

Tetrazole	Grams Sample	M_{CCl_4}	M_{H_2O}	$D(\frac{\text{nonaqueous}}{\text{aqueous}})$
C_3 MT	0.3063	0.0064	0.5442	0.0118
C_3 MT	0.3074	0.0062	0.5465	0.0114
C_3 MT	0.7162	0.0122	1.2770	0.0096
C_3 MT	0.7164	0.0152	1.2725	0.0120
C_3 MT	1.2640	0.0120	2.2688	0.0053
C_3 MT	1.2677	0.0170	2.2676	0.0075
C_4 MT	0.3016	0.0071	0.4745	0.0149
C_4 MT	0.3046	0.0073	0.4790	0.0151
C_4 MT	0.7306	0.0102	1.1604	0.0088
C_4 MT	0.7386	0.0125	1.1696	0.0107
C_4 MT	1.2406	0.0297	1.9495	0.0149
C_4 MT	1.2565	0.0271	1.9805	0.0137
C_5 MT	0.3063	0.0697	0.3329	0.2093
C_5 MT	0.3162	0.0610	0.3611	0.1688
C_5 MT	0.7583	0.2213	0.7468	0.2964
C_5 MT	0.7607	0.2267	0.7418	0.3056
C_5 MT	0.5122	0.1245	0.5441	0.2288
C_5 MT	0.5160	0.1212	0.5548	0.2184
C_5 MT	1.0188	0.3993	0.8419	0.4743
C_5 MT	1.0184	0.3991	0.8416	0.4743
C_5 MT	1.1744	0.4682	0.9579	0.4880
C_5 MT	1.1813	0.4797	0.9497	0.5051
C_5 MT	1.3095	0.5731	0.9872	0.5806
C_5 MT	1.3495	0.6343	0.9482	0.6689
C_5 MT	1.5015	0.7805	0.9365	0.8334
C_5 MT	1.5074	0.7918	0.9271	0.8540
C_5 MT	1.7051	0.9611	0.9451	1.0169
C_5 MT	1.7150	0.9713	0.9431	1.0299
C_5 MT	1.9842	1.2221	0.9355	1.3063

Table XI (Continued)

Tetrazole	Grams Sample	M_{CCl_4}	M_{H_2O}	$D(\frac{\text{nonaqueous}}{\text{aqueous}})$
C ₆ MT	0.3086	0.1541	0.1614	0.9548
C ₆ MT	0.3093	0.1527	0.1646	0.9274
C ₆ MT	0.7160	0.7410	0.1948	2.4182
C ₆ MT	0.7217	0.7465	0.1935	2.4631
C ₆ MT	1.2023	0.8736	0.1959	4.4594
C ₆ MT	1.2038	0.8750	0.1962	4.4594
C ₇ MT	0.2683	0.1762	0.0436	4.0446
C ₇ MT	0.2899	0.2014	0.0296	6.7950
C ₇ MT	0.2612	0.1892	0.0145	13.0912
C ₇ MT	0.2622	0.1855	0.0215	8.6516
C ₇ MT	0.2942	0.2062	0.0273	7.5666
C ₇ MT	0.3906	0.2102	0.0286	7.3591
C ₇ MT	0.5242	0.3820	0.0252	15.1495
C ₇ MT	0.5290	0.3843	0.0247	14.0486
C ₇ MT	1.1416	0.8407	0.0412	20.4100
C ₇ MT	1.1186	0.8373	0.0188	44.4565
C ₇ MT	0.7357	0.5278	0.0478	10.8405
C ₇ MT	0.7597	0.5479	0.0457	11.9957
C ₇ MT	1.2982	0.9501	0.0562	16.9030
C ₇ MT	1.3083	0.9535	0.0629	15.1496

Table XII. Distribution Data with Aqueous Sodium Chloride Solutions

Tetrazole	Grams Sample	C _{NaCl}	M _{CCl₄}	M _{H₂O}	D
C ₅ MT	0.7354	0.14 M	0.2157	0.7225	0.2986
C ₅ MT	1.9492	0.14	1.2324	0.8685	1.4189
C ₅ MT	1.9298	0.14	1.1810	0.9219	1.2810
C ₅ MT	0.7226	1.0	0.3241	0.5223	0.6089
C ₅ MT	0.7350	1.0	0.3391	0.5265	0.6440
C ₅ MT	0.7277	2.5	0.4713	0.3065	1.5380
C ₅ MT	0.7264	2.5	0.4658	0.3133	1.4870
C ₅ MT	0.7255	4.0	0.5554	0.1701	3.2653
C ₅ MT	0.7259	4.0	0.5577	0.1670	3.3388
C ₅ MT	0.7275	5.0	0.5682	0.1527	3.7221
C ₅ MT	0.7274	5.0	0.5701	0.1495	3.8141
C ₅ MT	0.7201	0.14	0.2362	0.6680	0.3535
C ₅ MT	0.7187	0.14	0.2331	0.6709	0.3474

APPENDIX II

SUGGESTIONS FOR FUTURE WORK

SUGGESTIONS FOR FUTURE WORK

The recent aquisition of a pulsed nuclear magnetic spectrometer system with a super conducting magnet allows the study of solutions in lower concentration ranges and measurement of resonance lines much too broad for the present instrumentation. Also, the number of nuclei available for study is significantly increased. The sophistication of the new instrumentation suggests that the following experiments can be carried out.

1. The number of anions used in these studies can be increased because the low solubility of other solutes in the solvents investigated is offset by the increased sensitivity of the instrument.
2. The present investigation should be extended to lower concentrations especially in the case of tetrahydrofuran solutions of sodium iodide.
3. A systematic investigation on the relaxation times for sodium ion in the various solvents should be undertaken. There are indications that the width of the sodium resonance may be proportional to the solvation number in the case of the perchlorates and tetraphenylborates.
4. The work on the sodium iodide solutions should be repeated by iodine-127 resonance techniques. This may help determine the type of ion-pair forming in solution.
5. Boron, chlorine, carbon, and high field proton techniques should be used in an attempt to explain the small, but constant difference in chemical shift between the sodium perchlorate and tetraphenylborate solutions in which ion pairing was assumed to be negligible.

6. Work on the interaction between alkali metals and crown compounds, heretofore impossible because of the magnitude of the resonance line-width, should be undertaken to determine the nature of these interactions.

While not normally in the realm of analytical chemistry, several biologically oriented experiments should be undertaken which may clarify the mechanism of the biological activity of metrazole and the other convulsant tetrazoles.

1. Using a section of frog skin as a membrane, it may be possible to determine the rate of sodium transport across the membrane by increasing the concentration of sodium ion on one side of the membrane and following sodium concentration versus time on the other side by a sodium ion sensitive electrode. It can then be determined whether the addition of a cyclopolymethylenetetrazole significantly alters this rate of transport.

2. An alternative is allowing a colony of organisms to grow in a substrate of known sodium concentration for a known period of time. The size of the colony can be followed by turbidimetric methods, and the sodium uptake of the colony in the substrate can be determined by the removal of the colony by centrifugation and then determination of the depletion of sodium concentration by standard methods. The rate of sodium uptake per organism per unit of time can be then compared both with and without tetrazoles in the substrate.

Finally, a more active line of communication between students and faculty in the Departments of Chemistry, Biochemistry, and Biophysics should be established to make the best use of the talents available in three separate groups all attempting to explain the same phenomena.

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