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**Doi, Jon David**

**I. A CARBON-13 NMR STUDY OF METAL ION POLARIZABILITY. II.  
PALLADIUM PORPHYRINS: PHARMACEUTICAL USE AND REACTION  
KINETICS**

*City University of New York*

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I    A  $^{13}\text{C}$  NMR STUDY OF METAL ION POLARIZABILITY

II   PALLADIUM PORPHYRINS:   PHARMACEUTICAL USE AND  
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by

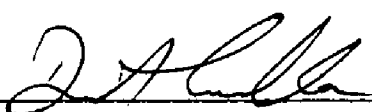
JON D. DOI

A dissertation submitted to the Graduate Faculty  
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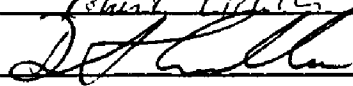
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## Abstract

- I    A  $^{13}\text{C}$  NMR STUDY OF METAL ION POLARIZABILITY  
II   PALLADIUM PORPHYRINS: PHARMACEUTICAL USE AND  
     REACTION KINETICS

by

JON D. DOI

Advisor: Dr. David K. Lavallee

## PART I

Although several methods to determine the effective electron density or polarizing ability of a metal ion are presently available, these techniques have limited applicability. The purpose of this project is to investigate whether  $^{13}\text{C}$  NMR chemical shifts at the para position of the bound ligand, pyridine, correlate well with the effective electron density of the metal center.

The  $^{13}\text{C}$  NMR chemical shifts for the para carbon atom of pyridine bound to these diamagnetic metal ions Zn(II), Cd(II), Ag(I), Co(III) and Rh(III) show an excellent correlation with  $\text{pK}_a$  values calculated from optical basicity data but show a much poorer correlation with experimental  $\text{pK}_a$  values. The strong correlation with

electrostatic force considerations is consistent with the use of  $^{13}\text{C}$  chemical shifts as an empirical measure of the polarizing power of metal ions. From the magnitude of chemical shift values, large changes in polarizability are readily observable but small shift changes, such as those caused by ligand substitution on a particular metal ion, are not likely to be seen.

## PART II

### Chapter 1

Selective lymphatic ablation on rats by use of  $^{109}\text{Pd}$ -hematoporphyrin has been reported. The literature preparation of this complex results in too large a loss in activity for experiments with large animals and human subjects. The palladium complex can be rapidly and quantitatively prepared using N-methylhematoporphyrin in dimethylsulfoxide. Distribution, toxicity and transplantation studies on dogs give promising results but additional experiments need to be run.

### Chapter 2

The mechanism of metalloporphyrin formation involving a metal salt and a N-methylporphyrin follows a two-step pathway: complexation, to form the metalated N-methylporphyrin intermediate and demethylation of the intermediate to form the planar metalloporphyrin. The rates for the reactions involving  $\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$  and N-methyl-tetraphenylporphyrin in acetonitrile, N,N-dimethylformamide and dimethylsulfoxide were determined.

The results in acetonitrile show that the demethylation reaction is rate determining. When combined with results previously reported, the reactivity order:  $\text{Pd(II)} > \text{Cu(II)} > \text{Ni(II)} > \text{Zn(II)} > \text{Mn(II)}$  closely follows the stability of the planar porphyrin products. The results in N,N-dimethylformamide and dimethylsulfoxide are less clear. However, it is apparent that the rate determining step in these solvents is the complexation reaction.

## PREFACE

Before I make my acknowledgements, I would first like to share a few thoughts. My graduate life has been an unforgettable experience. Although I never carried one project to its final conclusion for various reasons, by being exposed to three different types of projects, I believe I learned much more than perhaps I would have from just one involved project. By learning and expanding my knowledge of chemistry, I have found out how little I really know. I also believe that life outside the lab is just as important as research in the lab. I left the security of my home in Minnesota to get married to the woman I love as well as left the state to begin my graduate career in Fort Collins at Colorado State University. The people we met there were wonderful and we made lasting friendships. We also took advantage of the Colorado Rockies to ski. Then, a year later, four graduate students (including myself) made a very difficult decision to follow our mentor to Hunter College in the middle of New York City. For myself, it was the right decision because I was able to do collaborative work that I could have never done in Colorado. But life in New York can be very trying as well as exciting. After a few years here, my wife and I were blessed with God's most wonderful creation, a baby girl named Stephanie Hideko Doi. She made the rough life of a graduate student a little easier to take. And now to thank the people that made it possible to become a

Ph.D. person.

I would first like to thank my research advisor, Dr. David Lavalley for all the help, encouragement, suggestions and friendship that he gave me. He should go far in the chemistry world. We were not just teacher-student but good friends. I would also like to thank Carolyn Lavalley not only for her help in computer programming but also for the many wonderful meals she made us.

I must also note my coworkers for their patience and understanding for putting up with me and my perhaps not so brilliant questions. Ray, H-P-D and especially Marilyn helped me alot.

Since there is more to life than just a test tube, I would like to give thanks to all my friends that made graduate life bearable. G. O., although a bit emotional, he was always there when I needed him. He'll make an excellent physician. Kope was a graduate student who wasn't, but he was great fun and we and our wives had good times together. Alan P. taught me alot, in chemistry and in life. We shall endure. And the rest of you, D. K., "semi", "U.G.", Bertika, "Kool Kev", it would not have been the same without you.

To the people at Brookhaven -Suresh and Tom, and at Columbia Presbyterian -Kazunari, Narohito and Rashid, I learned so much about nuclear chemistry and immunology from you. This Pd-porphyrin drug will work!

And thanks to my only student, Cathy .... you're a good kid and a conscientious worker who is full of kinetics energy.

To my folks, who made me what I am today, by allowing me to ask, "Why?" I owe them alot.

And finally to my wife, Deb. Without her, I wouldn't have made it. She is the most unselfish and caring person I know. I have put her through hell, but it's over now, and it will be better, that's a promise. It is her who makes life . . . . life, and not just existing. We will overcome all adversity together.

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P A R T   I

A  $^{13}\text{C}$  NMR STUDY OF METAL ION POLARIZABILITY

## INTRODUCTION

### METAL ION POLARIZABILITY

A fundamentally important principle which describes the variation in the way transition metal ions react is the effective electron density or polarizability of a metal ion. The effective electron density or polarizability can be defined as the ability of a metal ion to alter the electron density of a bound ligand and depends on both the oxidation state of the metal and on the other ligands bound to the metal in the first coordination sphere. The manner in which a metal atom affects the electron cloud and therefore the properties of a bound ligand can be described as the degree that the metal atom can attract the electrons of the ligand and/or back-donate electron density to it. However, at the same time, the metal ion is influenced by its ligand environment. Some ligands are said to be able to "soften" a metal atom by donating electron density while others are said to be able to "harden" a metal atom by withdrawing electron density(1,2). The types of properties that are attributed to polarization effects are very diverse and include kinetics, equilibrium, spectroscopic and structural features of organometallic and macrocyclic complexes.

Unfortunately, the concept of effective electron density or polarizability is very difficult to measure quantitatively. For example, the reactivity of organometallic compounds are attributed to polarizability changes(3). However, the formal oxidation state of the metal atom in organometallic complexes often inadequately describes the effective electron density at the metal site. The actual role of metal ion polarization is therefore conjectural.

Present methods for evaluating metal atom polarization changes are indirect and limited in scope. They include comparisons of: 1) charge transfer energies(4-7), 2) ligand  $pK_a$  values(8-10), 3) infrared stretching frequencies for stretching modes of  $\pi$  acceptor ligands such as NO, CO, CN and isocyanates(11-14), 4) values of metal- $^1H$  and metal- $^{13}C$  coupling constants for platinum(15-17), mercury(18), and tin (19) compounds and 5)  $^{19}F$  chemical shifts(20-25). These methods each have some validity in deducing trends in polarizability changes for a particular type of complex. The limitations of these methods provide a basis for our premise that it is advantageous to develop a more generally applicable procedure for measuring the polarizing ability of a metal ion utilizing  $^{13}C$  nuclear magnetic resonance spectroscopy.

1) Charge transfer energies have been correlated to  $\pi$  acceptor properties of a series of ligands bound to  $(NH_3)_5Ru^{2+}$ -(4,5) and  $(CN)_5Fe^{3-}$ -(7). The basis for assuming

correspondence between trends in charge transfer energies and trends in polarization is indirect the premise being that the energy of charge transfer (an excited state phenomenon) generally relates to the difference between the extent of charge delocalization in the ground state and excited state. This correlation is limited to the pi-backbonding interaction of the metal atom and ligands without accounting for differences in sigma interactions. A trend of effective electron density increase with metal-to-ligand charge transfer energy decrease appears to be valid when a series of ligands with similar sigma bonding properties (e.g., a series of meta and para substituted pyridines) are bound to the same metal atom. This trend is supported the  $^{13}\text{C}$  NMR work by Lavallee and Baughman(6) on a series of substituted pyridine ligands bound to  $(\text{NH}_3)_5\text{Ru}^{2+}$  and  $(\text{CN})_5\text{Fe}^{3-}$ . Unfortunately, the use of metal-to-ligand charge transfer energies has fairly limited application.

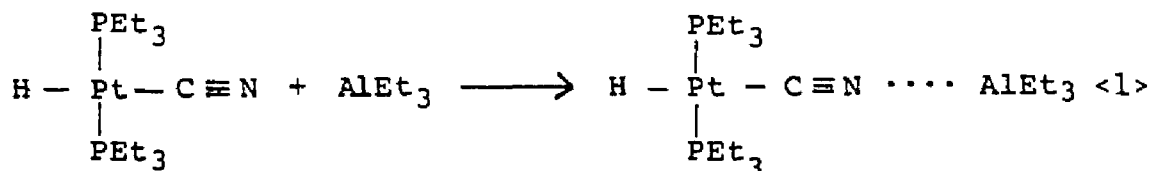
2) Changes in the  $\text{pK}_a$  of bound ligands give some indication of the polarizing ability of the metal atom. Some examples for aquated metal ions are(10):  
 $\text{Cr(III)} (\text{pK} = 4.3) > \text{Co(III)} (2.7)$  and  $\text{Cd(II)} (9.5) > \text{Zn(II)} (6.3)$  (decreased size);  $\text{Co(II)} (9.9) > \text{Co(III)} (2.7)$  and  $\text{Li(I)} (14.4) > \text{Mg(II)} (12.2) > \text{Sc(III)} (4.9)$  (principally an increased charge effect). For non-backbonding metal atoms, such series can be used to define effective charge-radius polarization correlations. Work done by Duffy and Ingram(26) indicates that the polarizing power of metal

atoms does not correlate very well with experimental  $pK_a$  values but rather with predicted  $pK_a$  values based on an optical basicity scale. The number of systems that can be studied by this  $pK_a$  method are limited, however.

3) Infrared shifts for  $\pi$  acceptor ligands such as CO have been related to donor ability of the bound metal atom. The premise of the frequency shift interpretation is that increased metal atom  $\pi$  donation populates the  $\pi$  anti-bonding orbitals of the ligand leading to a weaker CO bond and a lower force constant(27). Caution must be exercised however, because changes in metal-ligand sigma bonding also affect the CO stretching frequency(28). Molecular orbital treatments do indicate that in cases where  $\pi$ -backbonding clearly predominates, proper trends of metal atom  $\pi$ -backbonding ability are produced(29). Fenske has studied a series of complexes of the type:  $M(CO)_{6-x}L_x$  where  $x = 1,2$ ; M is a variety of metals including Mn, Fe and Co; and L is a variety of ligands including the halides. He found a good correlation between the observed carbonyl stretching frequencies and calculated electron densities of the metal atom and its complement of ligands(28). This technique is limited to species which can bind appropriate ligands and to the determination of relative  $\pi$  interactions rather than net effective electron density changes.

4) Metal- $^1H$  and metal- $^{13}C$  coupling constants have been determined for platinum, mercury and tin compounds. Differ-

ences in coupling constant values have been used to deduce substituent effects(17). Studies of Pt-<sup>1</sup>H coupling constants(15), for example, have demonstrated that the coupling constants increase in an order that seems to reflect the Lewis acidity of the substituent trans to the hydride in adducts of trans-cyanohydridobis(triethylphosphine) as shown below:



It can be reasonably argued that the Pt-<sup>1</sup>H coupling constant is probably dominated by a Fermi contact term which is mainly dependent on the s electron density in the Pt-H bond. The Pt-<sup>1</sup>H bond s electron density (and J(Pt-H)) is proposed to increase as the Pt-C bond strength decreases due to stronger bonding of the Lewis acid to the cyanide nitrogen atom(15). The pi interactions of the cyanide ligand with the platinum atom are presumed to be small. The J(Pt-H) value in these adducts may in fact provide a convenient measure of trans electron density changes but this use of coupling constants rests on a considerable number of assumptions.

5) The greater sensitivity of the <sup>19</sup>F nucleus in the NMR experiment compared with the <sup>13</sup>C nucleus accounts for the large literature of <sup>19</sup>F chemical shifts in metal complexes. Parshall's studies of meta and para- fluorophenyl chemical shifts in Ni(II), Pd(II) and Pt(II) complexes are consistent

with pi-backbonding interactions (20,21). Foust and Ford (22) found  $^{19}\text{F}$  chemical shift differences in fluorobenzonitrile complexes of Rh(III) and Ru(II) indicate pi-backbonding for Ru(II) but not for Rh(III). The theoretical interpretation of  $^{19}\text{F}$  shifts, however, is a subject of dispute (23,24). Studies of simple fluorine substituted aromatic molecules indicate that polar field effects (which make it difficult to directly extend  $^{19}\text{F}$  chemical shift changes to effective electron density and to separate sigma from pi effects) are important. Coulson's excellent correlations of  $^{13}\text{C}$  and  $^{19}\text{F}$  chemical shift changes in Pt(II) complexes (25) may then be the exception rather than the rule.

Although the theory of  $^{19}\text{F}$  chemical shifts is at the same level of development as  $^{13}\text{C}$  chemical shift theory, extensive empirical  $^{13}\text{C}$  NMR studies have been undertaken. For this reason and since  $^{13}\text{C}$  NMR spectroscopy is now routine, our efforts have concentrated on the  $^{13}\text{C}$  NMR technique.

#### USE OF $^{13}\text{C}$ NMR TO MEASURE EFFECTIVE ELECTRON DENSITY OR POLARIZABILITY

##### Advantages Of The $^{13}\text{C}$ NMR Technique And The Type Of Probe Molecules Chosen

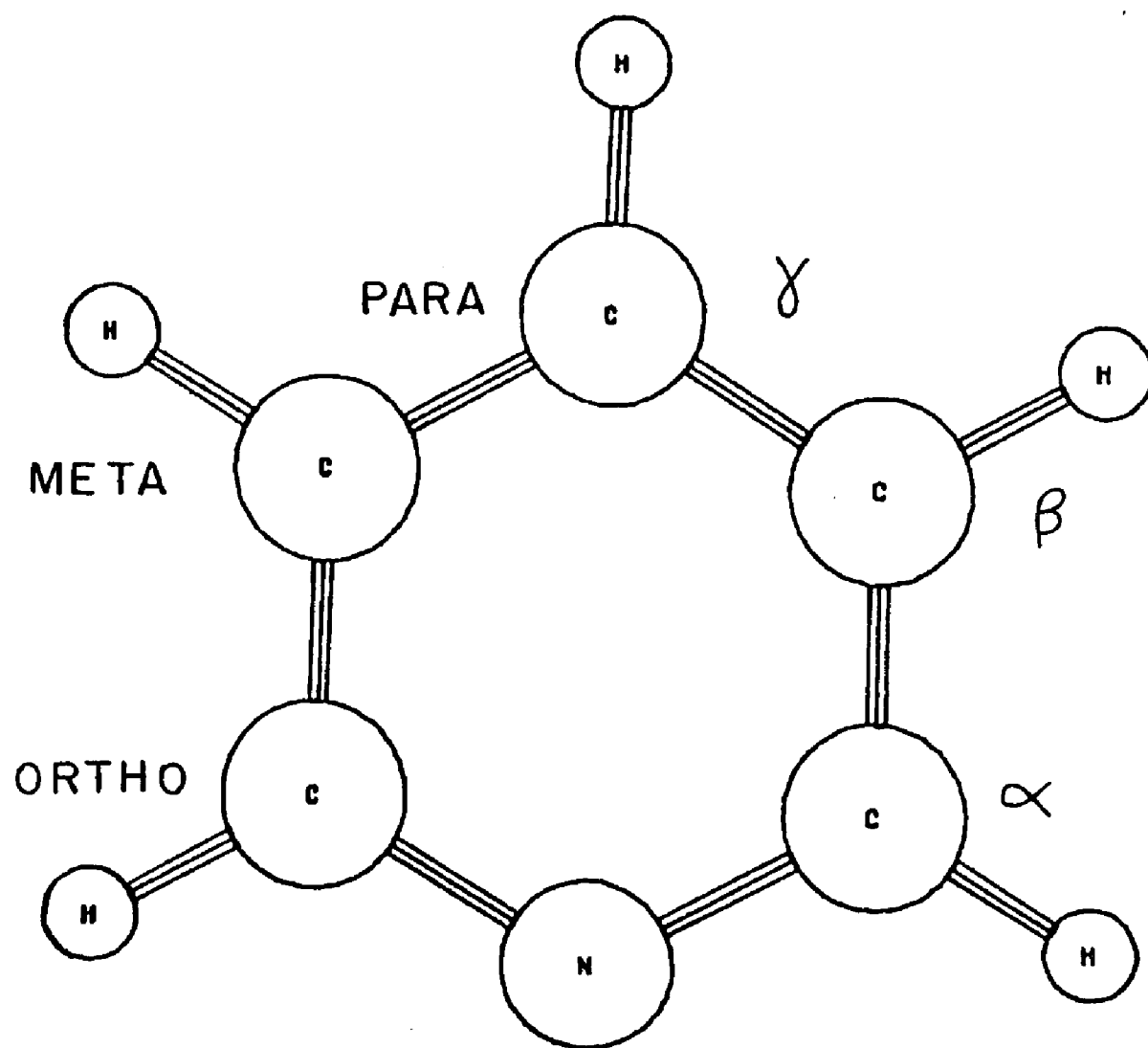
The  $^{13}\text{C}$  NMR chemical shift approach to measure the effective electron density or polarizability of a metal ion has definite advantages over the previously mentioned techniques

because it can be used on many types of metal ion complexes. Probe ligands, benzene and pyridine can be bound to a very large number of metal complexes, including both classical (pyridine) and organometallic (benzene) complexes. A second advantage of the  $^{13}\text{C}$  technique is the excellent resolution and wide chemical shift range available that may allow discrimination between two metal centers that may not be observed by other methods. The chemical shift range of the limited number of classical complexes which have been studied to date is 20 ppm for the para  $^{13}\text{C}$  atom of pyridine and substituted pyridines. (Figure 1 shows the two types of nomenclature used to describe the different carbon positions of pyridine.) The chemical shift resolution can, of course, be improved by using higher field strengths.

#### Empirical Evidence For The Use Of The $^{13}\text{C}$ NMR Technique

Empirical evidence supporting the use of differences in  $^{13}\text{C}$  chemical shifts between a free ligand versus a bound ligand to measure effective electron densities or polarizabilities of metal atoms has been obtained by Coulson(25), Malin(7), Petersen(30), and Lavalley(6). Coulson studied a large number of transX-phenylbis(triethylphosphine) platinum(II) complexes and found an excellent correlation between observed para  $^{13}\text{C}$  chemical shifts and those calculated from a Swain-Lupton substituent effect model(25). Complexes of aromatic nitrogen heterocycle ligands with  $\text{Fe}(\text{CN})_5^{3-}$  show a correlation of  $^{13}\text{C}$  shifts with metal-to-ligand charge trans-

FIGURE 1. AN ORTEP2 REPRESENTATION OF THE PROBE LIGAND, PYRIDINE. THE TWO TYPES OF NOMENCLATURE FOR EACH OF THE CARBON POSITIONS WITH RESPECT TO THE NITROGEN ATOM ARE ILLUSTRATED.



fer energies and expected electron withdrawing power of the ligands (7) as demonstrated by Malin. Petersen has obtained  $^{13}\text{C}$  spectra for complexes of pyridine and substituted pyridine ligands with  $(\text{CN})_5\text{Co}^{2-}$ . He found that the  $(\text{CN})_5\text{Co}^{2-}$  group exerts less withdrawing power than  $(\text{NH}_3)_5\text{Co}^{3+}$ , as one would expect from the greater donor power of  $\text{CN}^-$  relative to  $\text{NH}_3$  (30). Investigations by Lavallee demonstrated that the electron density patterns expected for non pi-backbonding metal atoms  $(\text{NH}_3)_5\text{Co}^{3+}$  and  $(\text{NH}_3)_5\text{Rh}^{3+}$ , are reflected in the  $^{13}\text{C}$  shifts at the para position of bound pyridine ligands. It was also found that electron donating and withdrawing substituents on the ligands affect the  $^{13}\text{C}$  shifts in a manner expected from charge transfer energies (6) (i.e., electron-donating substituents which shield the metal atom have lower metal-to-ligand charge transfer energies while electron-withdrawing substituents deshield the metal atom and have higher charge transfer energies).

#### Theoretical Justification For The $^{13}\text{C}$ NMR Technique

Theoretical support for this  $^{13}\text{C}$  NMR technique is extremely dependent on the choice of the probe ligand and the use of diamagnetic complexes. The choice of the probe is important because in general, the  $^{13}\text{C}$  NMR chemical shift does not reflect electron density at the carbon atom. The studies of Maciel and Pople (31) have shown that systems must fall into a narrow structural class for such a relationship

to hold. Empirical and theoretical justifications for such a correlation have been presented for substituted benzenes(32-34). Hammett type correlations as well as theoretical calculations of electron density changes caused by a variety of substituents correlate well with  $^{13}\text{C}$  chemical shifts in these systems. In these cases, the experimental evidence is consistent with the proposition that the principal effect of the substituent is a change in the paramagnetic shielding term of the  $^{13}\text{C}$  chemical shift as a result of total electron polarization changes. This shielding term,  $\sigma_p^{cc}$ , is one of the contributions to the screening constant for the carbon nucleus,  $\sigma^c$ , as described by Pople(35) in which:

$$\sigma^c = \sigma_d^{cc} + \sigma_p^{cc} + \Sigma\sigma^{CH} + \sigma^{c,ring} \quad <2>$$

where  $\sigma_d^{cc}$  is the contribution from the diamagnetic Langevin-type circulation on carbon,  $\sigma_p^{cc}$  is due to the paramagnetic currents on carbon which results from the mixing of electronic ground and excited states by the magnetic field,  $\Sigma\sigma^{CH}$  is the contribution from currents from hydrogen, and  $\sigma^{c,ring}$  is the contribution from ring currents.

Theoretical investigations have also dealt with the correspondence between electron density and  $^{13}\text{C}$  chemical shifts in aromatic nitrogen heterocycles. Bloor and Eleen(36) compared the  $^{13}\text{C}$  chemical shift data for pyridine with results of CNDO/2 calculations using pi electron densities, total electron densities and more complex expressions involving

bond orders as well as electron densities. A good linear correlation was found using total electron densities while pi electron densities alone or the more complex expressions gave a poorer fit. Tokuhira(37) used several methods for calculating  $^{13}\text{C}$  chemical shifts in pyridine and the diazines and found that sigma and pi effects were both important in obtaining agreement between calculated and experimental  $^{13}\text{C}$  shifts. He determined that the trend for the large shift differences obtained from the various heterocycles could be adequately explained in terms of total electron density changes.

Of special interest are the studies of Friedel and Retcofsky(38) on substituted pyridines, since the pyridine ligand will find wide application as a probe. Their investigations included a diverse set of substituents (e.g., amine, methoxy, aldehyde, cyanide and halides) in ortho, meta and para positions. The correlation between substituent effects and  $^{13}\text{C}$  shifts for pyridine were excellent especially at the para position where anisotropy effects between the substituent and the nitrogen atom are minimal. Since total electron density correlates well with chemical shift changes in mono-substituted benzenes, the correlation between total electron density and  $^{13}\text{C}$  shieldings in substituted pyridines is further supported. Although these studies dealt with the correlation between electron density and substituent effects, the important point to be noted is that a relationship between calculated electron density and  $^{13}\text{C}$

NMR chemical shifts was observed.

#### Consideration Of The Role Of The Nitrogen Heteroatom Of Pyridine

When dealing with pyridine and substituted pyridines, greater caution in interpretation is necessary than for phenyl and substituted phenyl ligands due to the nitrogen heteroatom. The nitrogen distorts the pi electron distribution and generates a local magnetic anisotropy. Its lone-pair dipole forms an electrostatic field and can lead to specific solvation, particularly through hydrogen bonding. It is therefore important to compare the chemical shifts of the complexed ligands relative to those of the free ligand in the same environment. In this study all measurements were done in water.

The electric field effect on complexation to the nitrogen may be large, causing changes in the chemical shift (ortho > meta > para) which are not directly related to the electron density changes. Anisotropy changes at the ortho position of pyridine are also likely to be larger than in bound phenyl ligands. Electric field and anisotropic effects can cause relatively large changes at the ortho position but diminish rapidly with distance (effect  $\propto 1/r^3$ ) (39). For these reasons, the para carbon atom of bound pyridine is the best indicator of metal ion polarizing power. At this position, effects at the complexation site are attenuated to the largest degree and, since

the para carbon is on rotational and reflection symmetry elements, anisotropic charge distribution effects are diminished. The requirement of diamagnetism arises from the fact that a large change in chemical shift even at positions several bonds away from paramagnetic metal ions can arise from pseudocontact interactions.

STUDY TO CORRELATE  $^{13}\text{C}$  CHEMICAL SHIFTS WITH  
 $\text{pK}_a$  VALUES OF METAL-PYRIDINE COMPLEXES

The aim of this project is the use of pyridine to determine whether the effective electron density or polarizability of metal complexes can be determined by the  $^{13}\text{C}$  NMR technique and what the limits of applicability this method may have. One type of property that has been studied by a variety of methods is the  $\text{pK}_a$  variation of bound ligands. An investigation of this sort is attractive because the polarizing power of the metal atoms can be viewed in terms of only sigma effects since non pi-backbonding metals will be used. Therefore, a ligand  $\text{pK}_a$  study was undertaken using the diamagnetic metal ions:  $\text{Co(III)}$ ,  $\text{Rh(III)}$ ,  $\text{Zn(II)}$ ,  $\text{Cd(II)}$  and  $\text{Ag(I)}$  with pyridine as the probe. Experimental  $\text{pK}_a$  values of these metal ions have been determined using such methods as kinetics, glass electrode pH measurements and  $\text{Ag/AgCl}$  electrode pH measurements. A comparison of the results of these accepted methods for determining ligand  $\text{pK}_a$  values (an indicator of the effective electron density of a

metal atom) with the results of the  $^{13}\text{C}$  chemical shift technique using pyridine as the probe molecule will help determine the validity of this procedure.

The  $\text{pK}_a$  of a compound defines its acid strength. The acidity of a metal-aquo complex appears to depend primarily on the polarization of water molecules bound in the first coordination sphere of the metal atom. If this were the only significant effect, then there should be a correspondence between  $^{13}\text{C}$  NMR chemical shift values at the para position of the bound pyridine probe ligand and experimental  $\text{pK}_a$  values of metal-aquo complexes. However, from earlier studies (36,37), it appears that outer-sphere hydration effects significantly influence the acidity of aquated metal complexes. Duffy and Ingram (26) have used calculated optical basicities to determine  $\text{pK}_a$  values that would be expected without participation by water molecules outside the first coordination sphere. Such calculated  $\text{pK}_a$  values deviate considerably from experimentally determined  $\text{pK}_a$  values (26). They were able to obtain related experimental data to support their conclusion by the use of the  $\text{ZnCl}_2 \cdot 4\text{H}_2\text{O}$  molten salt hydrate system in which there is little outer-sphere hydration, but it was not feasible to obtain corresponding data for other metal ions. Angell and coworkers (40) have determined the  $^1\text{H}$  NMR chemical shifts of concentrated solutions of a number of metal ions. They observed an unexpectedly large downfield  $^1\text{H}$  NMR chemical shift for  $\text{Al}(\text{NO}_3)_3 \cdot 10\text{H}_2\text{O}$ , further downfield than nitric

acid. Since they hypothesize that the magnitude of the  $^1\text{H}$  shifts is related to either the polarizing power of metal atoms (for a given anion) or the polarizing effect of anions (for a given metal), then,  $\text{Al}(\text{NO}_3)_3 \cdot 10\text{H}_2\text{O}$ , by virtue of its large downfield shift, has greater polarizing power than nitric acid and therefore is a stronger acid, without the presence of bulk water. The results of Angell, et al., are consistent with Duffy and Ingram's conclusion that bulk water drastically depresses the true acidity of metal-aquo complexes that would be found in the absence of outer-sphere water. Their findings indicate that this phenomenon can decrease the acidity of metal-aquo complexes by greater than ten orders of magnitude (26).

The present work involves the preparation of the aquopyridine complexes of  $\text{Zn}(\text{II})$ ,  $\text{Cd}(\text{II})$ ,  $\text{Ag}(\text{I})$ , and the pentaamminepyridine complexes of  $\text{Co}(\text{III})$  and  $\text{Rh}(\text{III})$ , and the recording of  $^{13}\text{C}$  NMR spectra of these metal-pyridine complexes. The chemical shifts of the aquopyridine complexes of  $\text{Zn}(\text{II})$ ,  $\text{Cd}(\text{II})$  and  $\text{Ag}(\text{I})$  had to be calculated using a non-linear least squares program because an averaged signal of free pyridine, protonated pyridine and complexed pyridine was produced due to rapid exchange of the pyridine on the NMR time scale. The chemical shifts of the pentaamminepyridine complexes of  $\text{Co}(\text{III})$  and  $\text{Rh}(\text{III})$  could be used directly because these complexes are substitution inert. The  $^{13}\text{C}$  NMR chemical shifts of the metal-aquopyridine complexes were compared to experimentally measured  $\text{pK}_\text{a}$  values reported in

the literature (10) and calculated  $pK_a$  values derived from optical basicity values (26). The correlation between  $^{13}\text{C}$  chemical shifts and experimental  $pK_a$  values was not very good due to the effects of outer-sphere water. However, a direct correlation was found between the  $^{13}\text{C}$  chemical shifts at the para position of bound pyridine and calculated  $pK_a$  values. Unfortunately, the chemical shift range observed in this study was relatively small even though the effective electron density differences among the metal atoms were large. These results indicate that systems which involve small changes in metal atom polarization, such as ligand substitution of a certain metal in a particular oxidation state, most likely cannot be studied by this  $^{13}\text{C}$  NMR technique. This method is applicable to systems that undergo a relatively large change in metal atom polarizability. For example, rhodium and iridium organometallic catalysts which have significantly better olefin hydrogenation rates than the commercially used Wilkinson's catalyst have been synthesized by Crabtree and Morris (41-43) and could be investigated by this  $^{13}\text{C}$  NMR technique in order to measure the difference in effective electron density of the metal atom in the catalyst during its catalytic cycle, and the results related to the effectiveness of the catalyst. One may then be able to design catalysts based on effective electron density arguments.

## EXPERIMENTAL PROCEDURES

### REAGENTS

'Gold Label' pyridine, 99+ % pure, was supplied by Aldrich. It was dried over KOH pellets for several days. The previously colorless solution turned yellowish and had some precipitate in it. The solution was filtered, after which anhydrous BaO was added. The solution was then refluxed for three hours and the clean, dry pyridine was distilled from the solution and stored over molecular sieves. Unless otherwise specified, the following chemicals were supplied by Fisher and are A.C.S. certified grade:  $\text{ZnCO}_3$ ,  $\text{CdCO}_3$ ,  $\text{KNO}_3$ ,  $\text{Zn}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$ ,  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{AgNO}_3$  and  $\text{HgO}$ . Anhydrous  $\text{AgClO}_4$ , technical grade  $\text{CoCO}_3$  and  $[\text{Co}(\text{NH}_3)_5\text{H}_2\text{O}]\text{Cl}_3$  were supplied by Alfa.  $[\text{Rh}(\text{NH}_3)_5\text{py}](\text{ClO}_4)_3$  was generously provided by Dr. John Petersen. 'Gold Label' ethylenediaminetetraacetic acid, disodium salt, dihydrate, (EDTA), 99+ % pure, was supplied by Aldrich. Eriochrome Black T indicator and indicator grade Calmagite were supplied by Aldrich. Double vacuum distilled, A.C.S. certified, 70% perchloric acid ( $\text{HClO}_4$ ) was supplied by G. Fredrick Smith. A.C.S. certified concentrated nitric acid ( $\text{HNO}_3$ ) was purchased from Fisher. 99% deuterated acetone- $\text{d}_6$  and dimethylsulfoxide ( $\text{DMSO}$ )- $\text{d}_6$  was supplied by Stohler Isotope. NMR grade tet-

ramethylsilane (TMS), 99.9+ % pure, was purchased from Aldrich. Water used in solutions and preparations was distilled twice; house distilled water was distilled again from alkaline  $\text{KMnO}_4$ . Glass sample tubes (10 mm) and concentric capillaries (2 and 3 mm) for NMR sampling were supplied by Wilmad.

#### INSTRUMENTATION

The NMR spectrometer used was a JEOL PS/PFT-100 equipped with the JEOL EC-100 data system. pH measurements were recorded using a Radiometer/Copenhagen model 26 pH meter equipped with a standard type saturated calomel electrode (S.C.E.) and a glass electrode. Accurate weights were obtained using a Mettler model B6 semi-micro balance (precision:  $\pm 0.01\text{mg}$ ). The Cary 219 spectrophotometer was used for taking visible and ultraviolet absorption spectra. Infrared spectra were recorded on a Perkin Elmer Infracord recording spectrophotometer (range:  $4000\text{--}650\text{cm}^{-1}$ ).

Calculations were carried out using the Hunter College Computing Facility and the IBM 370/168 system of the City University of New York. The terminals used were the ADDS CONSUL 580 and the Perkin Elmer 550.

## PREPARATION OF $[M(H_2O)_5py]^{n+}$ SOLUTIONS

### Preparation Of $Zn(ClO_4)_2$

$ZnCO_3$  was added to an erlenmeyer flask containing some water. Enough concentrated  $HClO_4$  was added to dissolve the  $ZnCO_3$ . Then more  $ZnCO_3$  was added to neutralize the excess  $HClO_4$ . The solution was cloudy with a yellowish tinge to it. It was filtered through a fritted funnel. The  $Zn(ClO_4)_2$  solution was evaporated using a rotary evaporator until a white precipitate formed. The solid  $Zn(ClO_4)_2$  was collected on a Buchner funnel. The filtrate was further evaporated to collect more product. The  $Zn(ClO_4)_2$  was then dissolved in water, and evaporated with the rotary evaporator until it began to precipitate. It was then heated until it redissolved. Then the solution was allowed to cool to room temperature. Beautiful long white needle crystals of  $Zn(ClO_4)_2$  were collected, dried in a vacuum oven and stored in a desiccator.

### Preparation Of $Cd(ClO_4)_2$

The procedure for the synthesis of  $Cd(ClO_4)_2$  was similar to that of  $Zn(ClO_4)_2$ . The  $Cd(ClO_4)_2$  crystallized as very small crystals. It was first air-dried and then further dried in a desiccator (heating in an oven caused the crystals to melt).

Determination Of Zn(II) And Cd(II) Concentrations  
Using An Indirect EDTA Titration

To determine the concentration of the Zn(II) and Cd(II) ions, a displacement titration employing EDTA and using calmagite(44) as the indicator was chosen. Calmagite chelates with Mg(II), but not with Zn(II) or Cd(II). Upon addition of the EDTA, the Zn(II) or Cd(II) binds with it first (the  $pK$  for both ZnEDTA and CdEDTA = 16.6) (45) and only when the Zn(II) or Cd(II) is used up does the EDTA displace the calmagite from the Mg(II) to form the MgEDTA complex ( $pK(\text{MgEDTA}) = 8.7$ ). As the last of the Mg(II) uncomplexes from the calmagite and binds with the EDTA, the calmagite indicator changes from wine-red to blue. If the amount of Mg(II) is known, then the amount of Zn(II) or Cd(II) can be calculated. The calmagite indicator was made by dissolving 0.05 g in 100 ml of water. A pH 10 buffer was made by adding 142 ml of concentrated ammonia to 17.5 g of  $\text{NH}_4\text{Cl}$  and then diluting to 250 ml with water. A 0.0100M solution of EDTA was used. The  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  salt was used as the source of Mg(II) by dissolving 0.100 g in 250 ml of water. The concentration of the Mg(II) was accurately determined by doing a direct titration with EDTA. Using a volumetric pipet, 8.00 ml of the Mg(II) is introduced into a 125 ml erlenmeyer flask. Then, 1 ml of the buffer and 30 ml of water were added. Finally, 6 drops of the indicator were added. In the actual displacement titration, an additional 2.000 ml of either the Zn(II) or Cd(II) solution was added

with a calibrated microtitrator (a device with an accuracy of 0.5  $\mu$ l which delivers up to 2.000 ml).

#### Use Of $\text{AgNO}_3$ Instead Of $\text{AgClO}_4$

When preparation of the Ag(I)-pyridine complex in the same manner as the aquopyridine complexes of Zn(II) and Cd(II) was attempted, a white precipitate formed from the silver perchlorate solution. Since the pH of the solution, was too low ( $\sim 4.00$ ) to precipitate AgOH (The  $K_{sp}$  of AgOH is  $6.76 \times 10^{-9}$  (46) and assuming a  $[\text{Ag}^+] = 0.50\text{M}$ , one would calculate that a pH of less than 6.13 would keep the AgOH in solution.), an experiment was carried out to determine the nature of the precipitate. Pyridine was added to water and the pH was adjusted with concentrated perchloric acid until a pH of 5.3 was reached (pH read with short range pHDrion paper). A white precipitate which formed was filtered using a Buchner funnel. The precipitate was washed with ether, air dried and dissolved in a very acidic perchloric acid solution. A spectrum of it was taken in the ultraviolet region. A spectrum was also taken of pyridine in water at the same pH as the solution containing the white precipitate. From the similarity of the two spectra, it was concluded that the white precipitate contained pyridine. On the basis of the formation constants of the monopyridyl ( $K_1 = 74$ ) and bis(pyridyl) ( $K_2 = 166$ ) complexes of Ag(I) (47), the white precipitate is probably the  $[\text{Ag}(\text{py})_2]\text{ClO}_4$  species. However, as was seen experimentally, the  $[\text{Ag}(\text{py})_2]\text{NO}_3$

complex was soluble in the pH range studied.

#### Gravimetric Determination of Ag(I) Concentrations

Ag(I) concentrations were determined gravimetrically as silver chloride (48).

#### Preparation Of $[Zn(H_2O)_5py](ClO_4)_2$ And $[Cd(H_2O)_5py](ClO_4)_2$ Solutions

Appropriate amounts of Zn(II) or Cd(II) and pyridine stock solutions were measured out using the microtitrator. The pH values of these solutions were then adjusted with  $HClO_4$  using the Radiometer pH meter. The best procedure for preparing these solutions is the following: the desired volume of the pyridine stock solution is put into a 10 ml beaker. Enough  $HClO_4$  is added to bring the pH of the pyridine solution down from 8.5 to approximately 6.5 as monitored by a pH meter. The appropriate amount of either the Zn(II) or Cd(II) stock solution is then added. The pH is then adjusted close to the desired value. The solution is transferred to a 10.00 ml volumetric flask and diluted to volume with water(doubly distilled). The pH is then recorded for the final solution.

#### Preparation Of The $[Zn(H_2O)_5py](NO_3)_2$ , $[Cd(H_2O)_5py](NO_3)_2$ And $[Ag(H_2O)_5py]NO_3$ Solutions

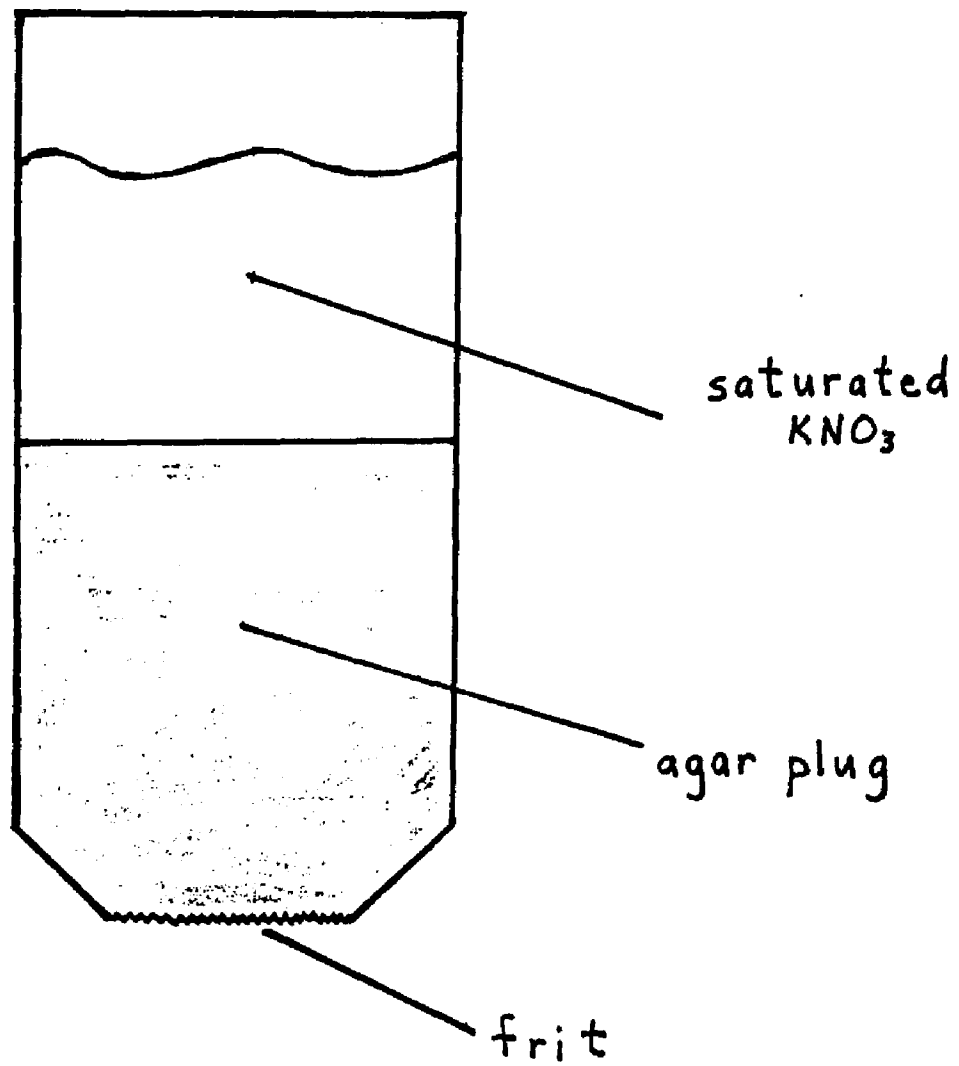
$Zn(NO_3)_2 \cdot 6H_2O$ ,  $Cd(NO_3)_2 \cdot 4H_2O$  and  $AgNO_3$  were dried in a vacuum oven and weighed on the analytical balance in order to obtain the metal ion concentrations. The nitrate salts

of the aquopyridine Zn(II) and Cd(II) complexes were prepared and the pH of the solutions adjusted in the same manner as the perchlorate salts of the same complexes except that nitric acid was used instead of perchloric acid. However, the pH of the  $[\text{Ag}(\text{H}_2\text{O})_5\text{py}]\text{NO}_3$  solution could not be adjusted in the same way as the other solutions because the chloride ions contained within the reference electrode of the pH meter reacted with the silver ions to form silver chloride. The precipitate plugged the porous fiber of the electrode rendering the pH meter inoperative. This problem was solved by making a salt bridge for the S.C.E. so that it would never come in contact with the silver ions in solution. A 4% agar plug(49) saturated with  $\text{KNO}_3$  was made and poured into a 6 cm x 1 cm glass tube with a fritted disk on the bottom. A saturated  $\text{KNO}_3$  solution was poured on top the agar plug (see Figure 2). The S.C.E. was placed into the  $\text{KNO}_3$  solution. The pH of the Ag(I) solutions could then be easily adjusted and recorded.

#### pH MEASUREMENTS

The preparation of the metal-pyridine complexes and adjustment of the pH required care. The pyridine, metal ion solution, and acid had to be added in a particular manner. If the pyridine was added first and the metal ion second, the metal hydroxide would precipitate. If the  $\text{HClO}_4$  being added was too concentrated, a white precipitate would appear

FIGURE 2. A REPRESENTATION OF THE SALT BRIDGE USED FOR THE S.C.E. WHEN DETERMINING THE PH OF THE  $\text{Ag(I)-PYRIDINE}$  SOLUTIONS. A SALT BRIDGE WAS REQUIRED BECAUSE SILVER IONS WOULD REACT WITH THE CHLORIDE IONS IN THE S.C.E. TO FORM  $\text{AgCl}_{(s)}$  WHICH WOULD PLUG THE POROUS FIBER OF THE ELECTRODE. WHEN THE S.C.E. WAS PLACED IN THE  $\text{AGAR/KNO}_3$  SALT BRIDGE, ACCURATE PH MEASUREMENTS COULD BE DETERMINED.



in the vicinity of the acid droplet (presumable the metal-pyridine perchlorate salt caused by the locally high concentration of perchlorate ion). This precipitate would dissolve upon swirling if not too large a quantity of  $\text{HClO}_4$  was added. If the pH of the solution was brought too close to the value desired using the  $\text{HClO}_4$ , then addition of the metal ion would lower the pH below the desired value because the metal ion solution itself is acidic. Another problem was that the pH reading tended to fluctuate if the electrodes were left in the metal-pyridine solutions too long. The pH meter did not read instantaneously as it does for typical aqueous solutions. The needle of the pH meter would come up slowly until it stabilized at some value. In a few moments the needle would begin to go down again. The reading could only be taken in the short span of time that the needle was stable. The pyridine most likely was reacting with the electrodes in some fashion in order to cause this condition. Even with this multitude of problems, the reproducibility of the measurements was better than 0.1 pH units. For some solutions the pH values were checked as they were made, checked again after a batch of solutions had been made and then checked once again after NMR spectra were taken.

In the case of the  $\text{Ag(I)}$ -pyridine solutions, the preparation and use of the salt bridge worked very well. The readings of the  $\text{Ag(I)}$ -pyridine solutions were very stable and the pH meter reacted quite quickly. A few of the  $\text{Zn(II)}$ -pyridine solutions were tested with and without the

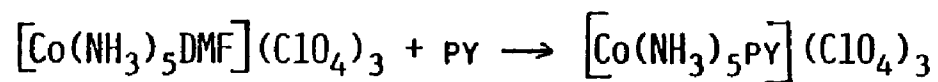
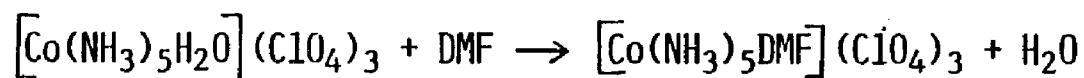
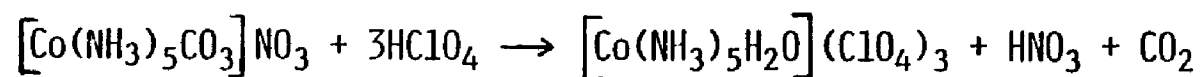
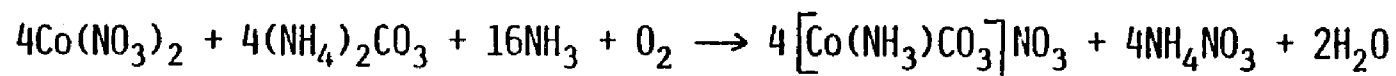
salt bridge to verify that the pH readings of the Ag(I)-pyridine complexes were consistent with those of the Zn(II) and Cd(II) pyridine complexes.

#### SYNTHESIS OF $[\text{Co}(\text{NH}_3)_5\text{py}](\text{ClO}_4)_3$

The synthesis of the precursor,  $[\text{Co}(\text{NH}_3)_5\text{H}_2\text{O}](\text{ClO}_4)_3$ , was attempted by two different methods. One method involved  $[\text{Co}(\text{NH}_3)_5\text{H}_2\text{O}]\text{Cl}_3$  as the starting material.  $\text{AgClO}_4$  was added to a solution of  $[\text{Co}(\text{NH}_3)_5\text{H}_2\text{O}]\text{Cl}_3$  in water until all the chloride ion from the complex was precipitated out as  $\text{AgCl}$ . Since  $[\text{Co}(\text{NH}_3)_5\text{H}_2\text{O}]\text{Cl}_3$  had limited solubility in water, much water was needed to dissolve the material (2 liters of  $\text{H}_2\text{O}$  and about  $40^\circ\text{C}$  was necessary to dissolve 10 g of the cobalt complex). The  $\text{AgCl}$  was filtered from the solution and the solution concentrated to 250 ml. Concentrated  $\text{HClO}_4$  was added to precipitate the aquopentaamminecobalt(III) complex. The brick red precipitate was filtered out of solution and dried. A visible absorption spectrum of this material did not correspond to that reported by Baughman(6). This material was not further characterized.

The aquopentaamminecobalt(III) perchlorate was prepared smoothly by another method involving a four step process(6,50-52) shown in Figure 3.

FIGURE 3. REACTION SEQUENCE TO SYNTHESIZE THE AQUOPENTAAMMINE-COBALT(III) PERCHLORATE COMPLEX. THE FIRST TWO REACTIONS WERE REPORTED IN REFERENCES 50 AND 51. THE LAST TWO REACTIONS WERE REPORTED IN REFERENCES 6 AND 52.



## THE $^{13}\text{C}$ NMR SPECTRA OF THE METAL-PYRIDINE COMPLEXES

Natural-abundance, broad band proton-decoupled  $^{13}\text{C}$  NMR spectra of the metal-pyridine complexes were determined at 25.03 MHz by the pulsed Fourier transform method using the JEOL spectrometer. The control and pulse parameters used to obtain the spectra are listed in Table I. The solutions were sampled in 10 mm tubes containing a 3 mm concentric capillary containing TMS as an external standard and acetone- $\text{d}_6$  for the deuterium lock. The concentrations of metal ions ranged from 0.200M to 0.500M while the pyridine concentration ranged from 0.050M to 1.00M.

## USE OF THE COMPUTER TO CALCULATE THE CHEMICAL SHIFTS OF THE PENTAAQUOPYRIDINE COMPLEXES OF $\text{Zn(II)}$ , $\text{Cd(II)}$ AND $\text{Ag(I)}$

In the metal-pyridine solutions, the species present in the pH range studied are: pyridine (py); protonated pyridine ( $\text{Hpy}^+$ ); and a series of metal-aquo-pyridyl complexes,  $[\text{M}(\text{H}_2\text{O})_5\text{py}]^{n+}$ ,  $[\text{M}(\text{H}_2\text{O})_4(\text{py})_2]^{n+}$  and the other  $[\text{M}(\text{H}_2\text{O})_{6-x}(\text{py})_x]^{n+}$  species. Because the pyridine molecules come on and off the protons and metal complexes rapidly with respect to the  $^{13}\text{C}$  NMR experiment time scale, only one averaged set of pyridine carbon nuclei are observed by the NMR spectrometer. Three peaks are seen in the spectrum: one for the para carbon, one for the two equivalent meta carbons

TABLE I. Control and Pulse Parameters Used in  $^{13}\text{C}$  NMR Experiments<sup>a</sup>

| Parameter             | Value                 |
|-----------------------|-----------------------|
| frequency range       | 5000 Hz               |
| data points           | 8192 or 16,384        |
| irradiating frequency | 25.03 MHz             |
| scans                 | 200 to 24,500         |
| pulse duration        | 12.0 $\mu\text{sec.}$ |
| pulse angle           | 30°                   |
| repetition time       | 3.00 sec.             |

<sup>a</sup>Temperature was kept at  $21 \pm 1^\circ\text{C.}$

and one for the two equivalent ortho carbons. Figure 4 shows a typical spectrum of a metal-pyridine solution.

One can show that the observed chemical shift ( $\delta_{obs}$ ) is related to the sum of the products of the chemical shift times the mole fraction of each species, that is:

$$\delta_{obs} = \chi_{py}\delta_{py} + \chi_{Hpy}\delta_{Hpy} + \chi_{Mpy}\delta_{Mpy} + \chi_{Mpy_2}\delta_{Mpy_2} + \dots + \chi_{Mpy_6}\delta_{Mpy_6} \quad <3>$$

where  $\chi$  = mole fraction and  $\delta$  = chemical shift. From the published values for the stepwise formation constants of pyridine complexes of Zn(II) (53), Cd(II) (54) and Ag(I) (47) in aqueous solution, one can infer that at acidic pH values only the monopyridyl and bis(pyridyl) complexes need to be considered in the concentration range studied.

In the case of Zn(II), using the relationships between the formation constants of the metal-pyridine complexes and protonated pyridine and that of the concentration of such species, one can derive a cubic equation with the only variable being the concentration of protonated pyridine and all the other quantities being either constants or measurable observables.

The equation is:

FIGURE 4. THE  $^{13}\text{C}$  NMR SPECTRUM OF A TYPICAL METAL-PYRIDINE AQUEOUS SOLUTION, 300 SCANS. THE ASSIGNMENTS OF THE CARBON POSITIONS ARE SHOWN ABOVE EACH PEAK.



$$\begin{aligned}
 [\text{Hpy}]^3 + \left( \frac{K_1 K_a^2 [\text{H}^+] + K_1 K_a [\text{H}^+]^2 + 2K_1 K_2 K_a^2 [\text{Zn}^{2+}]_0 [\text{H}^+] - K_1 K_2 K_a^2 [\text{py}]_0 [\text{H}^+]}{K_1 K_2 K_a^3 + K_1 K_2 K_a^2 [\text{H}^+]} \right) [\text{Hpy}]^2 \\
 + \left( \frac{K_a [\text{H}^+]^2 + [\text{H}^+]^3 + K_1 K_a [\text{Zn}^{2+}]_0 [\text{H}^+]^2 - K_1 K_a [\text{py}]_0 [\text{H}^+]^2}{K_1 K_2 K_a^3 + K_1 K_2 K_a^2 [\text{H}^+]} \right) [\text{Hpy}] \quad <4> \\
 - \left( \frac{[\text{py}]_0 [\text{H}^+]^3}{K_1 K_2 K_a^3 + K_1 K_2 K_a^2 [\text{H}^+]} \right) = 0
 \end{aligned}$$

where  $K_a$  is the acid dissociation constant of protonated pyridine,  $K_1$  and  $K_2$  are the formation constants of the mono-pyridyl and bis(pyridyl) complexes of Zn(II), respectively, and  $[\text{py}]_0$  and  $[\text{Zn}^{2+}]_0$  are the initial concentrations of pyridine and Zn(II), respectively. The solution to this cubic equation (i.e., the true value of  $[\text{Hpy}^+]$ ) was found(55), and programmed into the computer. This program, called Cubic, is shown in Appendix A.

Equation <3> can be rewritten by expanding the mole fraction term of each species and expressing it in terms of each species' concentration, that is:

$$\delta_{obs} = \frac{[\text{py}]}{[\text{py}]_0} \delta_{\text{py}} + \frac{[\text{Hpy}^+]}{[\text{py}]_0} \delta_{\text{Hpy}} + \quad <5>$$

$$\frac{[\text{Zn-py}]}{[\text{py}]_0} \delta_{\text{Zn-py}} + \frac{[\text{Zn-py}_2]}{[\text{py}]_0} \delta_{\text{Zn-py}_2}$$

Substituting the appropriate observables and constants for

[py], [Zn-py] and [Zn-py<sub>2</sub>] into equation <5> gives:

$$\delta_{obs} = \left( \frac{K_a [Hpy^+]}{[H^+][py]_0} \right) \delta_{py} + \left( \frac{[Hpy^+]}{[py]_0} \right) \delta_{Hpy} + \left( \frac{K_1 K_a [Zn^{2+}] [Hpy^+]}{[H^+][py]_0} + \frac{2 K_1 K_2 K_a^2 [Zn^{2+}] [Hpy^+]^2}{[H^+]^2 [py]_0} \right) \delta_{M-py_x} \quad <6>$$

where it is assumed (as supported by our experimental results) that the chemical shifts of the  $Zn(py)_2^{2+}$  complex does not differ significantly from those of the  $Zn(py)^{2+}$  complex and therefore the two terms were combined into one,  $Zn(py)_x^{2+}$  where  $x = 1$  or  $2$ . The protonated pyridine concentration rather than the concentration of free pyridine was used in the calculations because at acidic pH, the concentration of protonated pyridine is greater than the free pyridine concentration and, it is better to use the larger value in calculating related concentrations for statistical treatment.

Equation <6> has also been incorporated into a subroutine (called YP9) that sets up the cubic equation (<4>), calls the program Cubic to solve the equation and uses equation <6> to fit into the main program that in this case, will calculate the chemical shift of the aquopyridyl complexes of Zn(II), Cd(II) and Ag(I) (a listing of this subroutine is given in Appendix B). YP9 has as its dependent variable, the observed chemical shift for a given solution. There are six parameters in this subroutine, three of which are fixed and three which can be varied and optimized by the main pro-

gram. There are three independent variables which are also fixed. The parameters and independent variables and their values are listed in Table II. The main program with which YP9 and other subroutines are used is the Los Alamos Non-Linear Least Squares Programs(56). With the other available subroutines developed by other workers, such things as: power series functions, exponential functions, four term inverse polynomials, ionic strength dependence, and kinetic rate and activation parameters can be calculated.

The chemical shifts observed for the pentaamminepyridine complexes of Co(III) and Rh(III) were used directly.

TABLE II. Parameters and Independent Variables of Subroutine YP9

| Parameter | Description      | Fixed or Variable | Value               | Independent Variable | Description | Range of Values        |
|-----------|------------------|-------------------|---------------------|----------------------|-------------|------------------------|
| P(1)      | $K_a^a$          | fixed             | 5.22 <sup>b</sup>   | Z(1)                 | $[H^+]$     | 3.50-5.50 <sup>f</sup> |
| P(2)      | $K_1^c$          | variable          |                     |                      |             |                        |
| P(3)      | $K_2^d$          | variable          |                     | Z(2)                 | $[py]_o$    | .500                   |
| P(4)      | $\delta_{py}$    | fixed             | 139.01 <sup>e</sup> |                      |             |                        |
| P(5)      | $\delta_{Hpy^+}$ | fixed             | 148.55 <sup>e</sup> | Z(3)                 | $[M]_o$     | .200-.500              |
| P(6)      | $\delta_{Mpyx}$  | variable          |                     |                      |             |                        |

<sup>a</sup>Acid dissociation constant of protonated pyridine. <sup>b</sup>pK<sub>a</sub> value. <sup>c</sup>Formation constant of the metal pentaquopyridyl complex. <sup>d</sup>Formation constant of the metal tetraaquobis(pyridyl) complex. <sup>e</sup>Values are stated for the para position of pyridine and are given as ppm downfield from TMS. <sup>f</sup>Values are given in terms of molarity.

## RESULTS

### $^{13}\text{C}$ NMR SPECTRA OF THE METAL-AQUOPYRIDINE COMPLEXES

#### Assignment Of NMR Spectra

The assignments of the ortho, meta and para positions of pyridine and the metal-pyridine complexes were made on the basis of the work done by Lavalley, Baughman and Phillips(6). As an aid in making unambiguous assignments for the NMR signals of various pyridine complexes, they made some 2-deuteriopyridine. The  $^{13}\text{C}$  chemical shifts of 2-deuteriopyridine and pentaammine-2-deuteriopyridine complexes of Ru(II) and Co(III) were the same as those containing nondeuterated pyridine within experimental error. The  $^1\text{H}$  NMR spectra of pyridine and 2-deuteriopyridine showed that with one of the ortho protons deuterated, the integrated area of the alpha signal was reduced by half. This is shown numerically in Table III.

A similar type of phenomenon is seen in the  $^{13}\text{C}$  spectra. In this case the line intensities rather than the integrated areas of the signal are employed. When the  $^{13}\text{C}$  spectrum is determined with broad-band proton decoupling, line intensities are recorded which are several times larger than would be expected resulting from just the collapse of a multiplet

TABLE III. Relative Intensities of Pyridine and 2-d-Pyridine  
from  $^1\text{H}$  and  $^{13}\text{C}$  NMR Spectra

| Compound     | $^1\text{H}^a$ |         |          | $^{13}\text{C}^b$ |         |          |
|--------------|----------------|---------|----------|-------------------|---------|----------|
|              | $\alpha$       | $\beta$ | $\gamma$ | $\alpha$          | $\beta$ | $\gamma$ |
| pyridine     | 1.98           | 2.01    | 1.00     | 0.72              | 1.00    | 0.59     |
| 2-d-pyridine | 1.03           | 1.97    | 1.00     | 0.58              | 1.00    | 0.53     |

$^a$  $^1\text{H}$  data from Fleischer, E. B.; Lavalley, D. K. *J. Am. Chem. Soc.* 1972, 94, 2583.  $^b$  $^{13}\text{C}$  data from Reference 6.

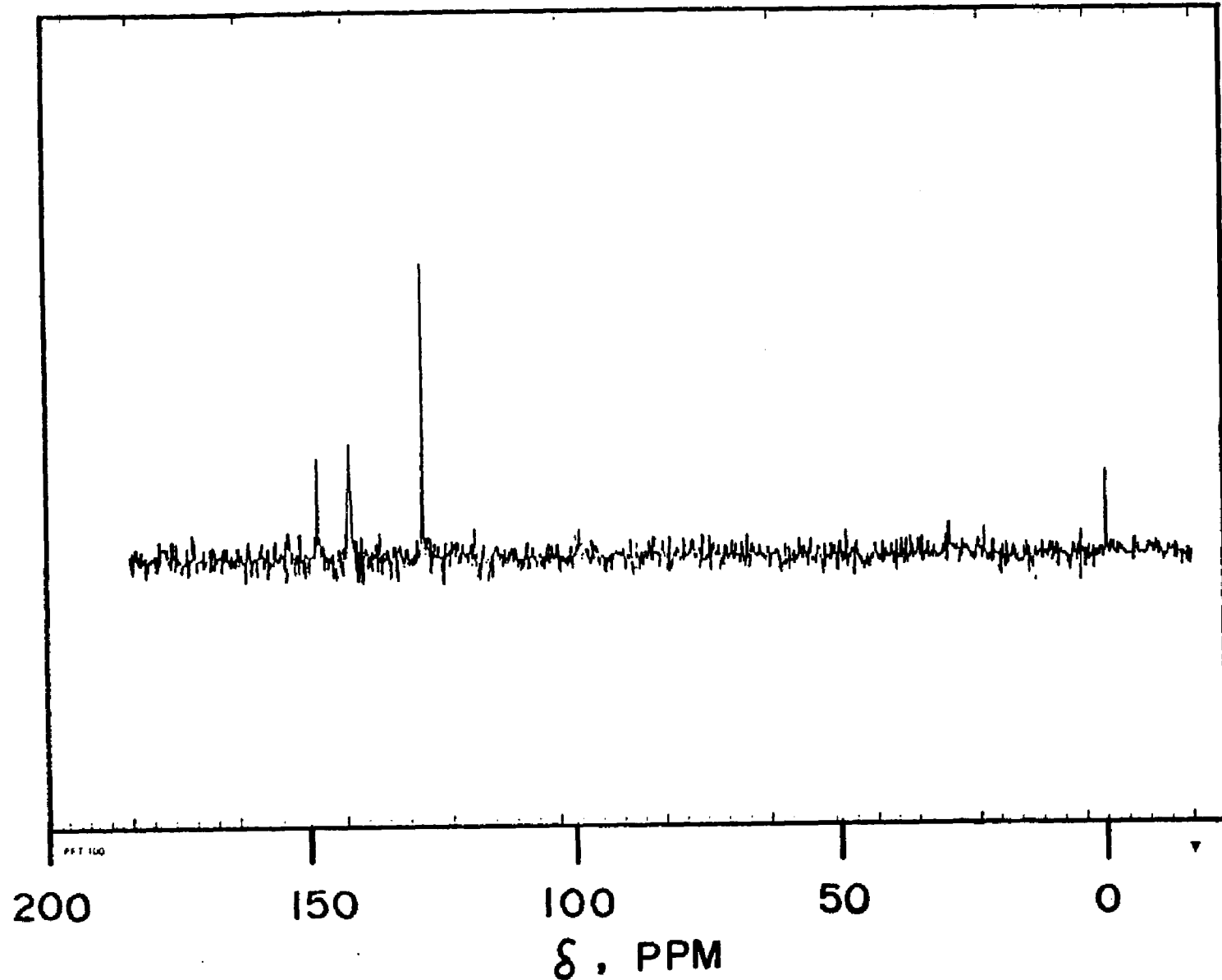
into a singlet. This is due to a signal enhancement which is termed the Nuclear Overhauser Effect (NOE) (57-59).

If one of the alpha protons is now replaced with a deuterium, the NOE should be diminished at this position. As can be seen in Table III, the relative intensity of the alpha signal does decrease in the mono-deuterated pyridine.

The integrated intensities from  $^1\text{H}$  NMR and the line intensities from  $^{13}\text{C}$  NMR spectra of pyridine and 2-deuteriopyridine unambiguously verified that the alpha signal was the most deshielded. The alpha signal should be the most deshielded of the three signals due to its being in closest proximity to the electronegative nitrogen. The gamma proton signal is a triplet due to coupling with the two meta protons while the beta proton signal is a complex pair of doublets due to coupling with both an ortho and para proton. In the  $^{13}\text{C}$  spectrum, the gamma signal (one carbon) is a line of about half the intensity of the alpha and beta signals, each representing two carbons (see Table III). The same type of spectra are observed for complexed pyridine.

In the  $^{13}\text{C}$  spectra of all protonated pyridine, the gamma signal is assigned to be more downfield than the alpha signal. Figure 5 shows this effect. The ortho intensity is greater than the para intensity even though the alpha signal is broadened somewhat because of residual spin-spin coupling to  $^{14}\text{N}$ , that is larger in protonated pyridine than in the free base (60). Pugmire and Grant (61) made the same assign-

FIGURE 5. THE  $^{13}\text{C}$  NMR SPECTRUM OF A PYRIDINE SOLUTION IN  $\text{H}_2\text{O}$  AT A PH OF 1.0, 300 SCANS. NOTE THAT IN THE CASE OF PROTONATED PYRIDINE, THE MOST DOWNFIELD SHIFTED PEAK IS ASSIGNED TO THE GAMMA CARBON SIGNAL. THE ALPHA CARBON IS THE MIDDLE PEAK AND THE BETA CARBON SIGNAL IS THE LEAST DESHIELDED AS IS TRUE IN ALL CASES.



SAMPLE NO. 10  
 DATE 10-15  
 SOLVENT CDCl<sub>3</sub>  
 CONC. 100  
 REFERENCE CDCl<sub>3</sub>  
 NAME 10  
 NUCLEUS <sup>13</sup>C  
 FREQ. 125.76 MHz  
 PULSE 100 100  
 WIDTH 10 10  
 INTERVAL 1.00 SEC  
 RESOLUTION 1.00 SEC  
 DATA POINTS 65536  
 WINDOW EXPONENT 1.00  
 WIND 1.00  
 SPECTRUM WIDTH 100 kHz  
 FILTER 1.00 kHz  
 AMPL. 1.00  
 NO. OF SCANS 128  
 LOCK ON? YES  
 FREQ. 125.76 MHz  
 SIGNAL 1.00  
 LEVEL 1.00  
 SPIN DECOUPLER 1.00  
 FREQ. 125.76 MHz  
 POWER 1.00 W  
 MODULATION 1.00  
 DCN DATA 1.00  
 DATE 10-15-79  
 OPERATOR 10  
 REMARKS

JEOL

ments for protonated pyridines. One possible explanation for this apparently anomalous upfield shift is in terms of a redistribution of electron density in the sigma network. Protonation of the nitrogen of pyridine leads to a large paramagnetic contribution to the nitrogen anisotropy. As electron density is removed from the ring by the inductive effect of the proton, electron density is shifted toward the nitrogen while electron density is severely depleted at the ortho carbon. To counteract this perturbation, electron density is shifted anisotropically towards the ortho carbon mainly from the meta carbon. The N-C bond order is effectively decreased and the electron density distribution is greatly increased at the ortho position. As a result, the ortho shielding of protonated pyridine is far upfield relative to that of pyridine. However, the change in ortho shielding is still a matter of controversy.

#### $^{13}\text{C}$ NMR Spectral Results

Changing the pyridine concentration while leaving the metal ion concentration and the pH constant showed no trend in the chemical shift of the pyridine complexes. Table IV shows the chemical shift of the Zn(II)-pyridine complex as a function of pyridine concentration.

The metal ion concentration was varied from 0.500M to 0.350M to 0.200M at constant pH and pyridine concentration and had no effect (within experimental error) on the  $^{13}\text{C}$  chemical shifts of the pyridine complexes. Likewise,

TABLE IV. The Effect of Varying the Pyridine Concentration on  $^{13}\text{C}$  Chemical Shifts of Pyridine at Constant Zn-pyridine Concentration and pH<sup>a</sup>

| Pyridine Concentration | $^{13}\text{C}$ Chemical Shifts <sup>b</sup> |         |          |
|------------------------|----------------------------------------------|---------|----------|
|                        | $\alpha$                                     | $\beta$ | $\gamma$ |
| 1.000                  | 146.28                                       | 127.68  | 144.67   |
| 0.700                  | 146.25                                       | 127.68  | 144.67   |
| 0.500                  | 146.47                                       | 127.63  | 144.58   |
| 0.100                  | 146.57                                       | 127.85  | 144.67   |

<sup>a</sup>Concentration of the Zn-pyridine complex = 0.500M, pH = 4.64.

<sup>b</sup>ppm downfield from TMS. Reproducibility of shifts =  $\pm 0.05$  ppm.

changes in ionic strength of the solutions by addition of  $\text{LiClO}_4$  produced no effect on the chemical shifts.

For the reasons described above, the following conditions were chosen for the metal-pyridine solutions used in these experiments:

Pyridine Concentration: 0.500M  
Metal Ion Concentration: 0.200M, 0.500M  
Control of Ionic Strength: None  
pH Range: 5.50 - 3.50

A relatively small pH range was chosen due to constraints on the system used. The upper limit is due to the problem of metal-hydroxide formation at higher pH. The lower limit was chosen because of the increasingly large ratio of protonated pyridine to pyridine as one went to lower pH values. At very low pH, there is virtually all protonated pyridine and no free base. Since the metal ions chosen for our study do not compete well with the proton in the binding to pyridine, very little of the metal-pyridine complex can form at low pH. This is indeed the case as borne out by our experimental results. Table V shows the  $^{13}\text{C}$  chemical shifts of aqueous pyridine and solutions of pyridine and the aqueous metal ions at a pH of 2.77. There are no significant differences between free pyridine solutions and solutions containing both pyridine and the metal ions.

Because the nitrate salt of the  $\text{Ag(I)}$ -pyridine complex had to be used, it was necessary to prepare the nitrate salts of the  $\text{Zn(II)}$ -pyridine and  $\text{Cd(II)}$ -pyridine complexes.

TABLE V. The  $^{13}\text{C}$  Chemical Shifts of Pyridine and Metal-pyridine Solutions at pH 2.77

| Species in Solution <sup>a</sup> | $^{13}\text{C}$ Chemical Shift <sup>b</sup> |         |          |
|----------------------------------|---------------------------------------------|---------|----------|
|                                  | $\alpha$                                    | $\beta$ | $\gamma$ |
| pyridine                         | 142.70                                      | 128.95  | 148.57   |
| Zn(II), pyridine                 | 142.89                                      | 128.97  | 148.59   |
| Cd(II), pyridine                 | 143.11                                      | 128.95  | 148.45   |
| Ag(I), pyridine                  | 143.08                                      | 128.82  | 148.31   |

<sup>a</sup>Concentrations of metal ions and pyridine = 0.500M. <sup>b</sup>ppm downfield from TMS. Reproducibility of shifts =  $\pm 0.05$  ppm.

Table VI contains some representative  $^{13}\text{C}$  chemical shifts of aquopyridine complexes of  $\text{Zn(II)}$  and  $\text{Cd(II)}$  in which all parameters except the counterion have been kept constant (i.e., metal ion and pyridine concentrations and pH). When comparing the para position chemical shifts, one notices a small (-0.2 ppm) but consistent downfield shift when going from perchlorate to nitrate.

As a check of our general method of analysis, as well as of the analytical curve-fitting process,  $^{13}\text{C}$  NMR spectra of a series of aqueous pyridine solutions at various pH values were obtained. A list of the chemical shifts of these solutions at the different pH values is shown in Table VII. There are several trends to be noted in this table. As the pH decreases, the meta and para chemical shifts increase smoothly whereas the ortho chemical shifts decrease. Secondly, the ortho and para chemical shift range is much larger than the meta range. These results will be discussed later.

A plot of the  $^{13}\text{C}$  chemical shifts of the para position of pyridine versus pH is illustrated in Figure 6. This amounts to a titration curve of pyridine. Using the acid dissociation expression for protonated pyridine and the following equation:

$$\sigma_{\text{obs}} = x_{\text{py}} \delta_{\text{py}} + x_{\text{Hpy}} \delta_{\text{Hpy}} \quad <7>$$

where  $\delta_{\text{py}} = 139.01 \text{ ppm}$  and  $\delta_{\text{Hpy}} = 148.55 \text{ ppm}$  (See Table

TABLE VI. Effect of Changing the Counterion in the Metal-pyridine Solutions<sup>a</sup> from Perchlorate to Nitrate on the <sup>13</sup>C Chemical Shifts

| Metal ion | pH Value | Counterion  | <sup>13</sup> C Chemical Shift <sup>b</sup> |        |        |
|-----------|----------|-------------|---------------------------------------------|--------|--------|
|           |          |             | α                                           | β      | γ      |
| Zn(II)    | 4.45     | perchlorate | 145.96                                      | 127.89 | 145.18 |
| Zn(II)    | 4.45     | nitrate     | 146.08                                      | 127.78 | 144.97 |
| Cd(II)    | 5.50     | perchlorate | 150.08                                      | 126.97 | 141.77 |
| Cd(II)    | 5.50     | nitrate     | 149.91                                      | 126.90 | 141.50 |
| Cd(II)    | 4.66     | perchlorate | 148.35                                      | 127.48 | 143.28 |
| Cd(II)    | 4.66     | nitrate     | 148.28                                      | 127.31 | 143.08 |

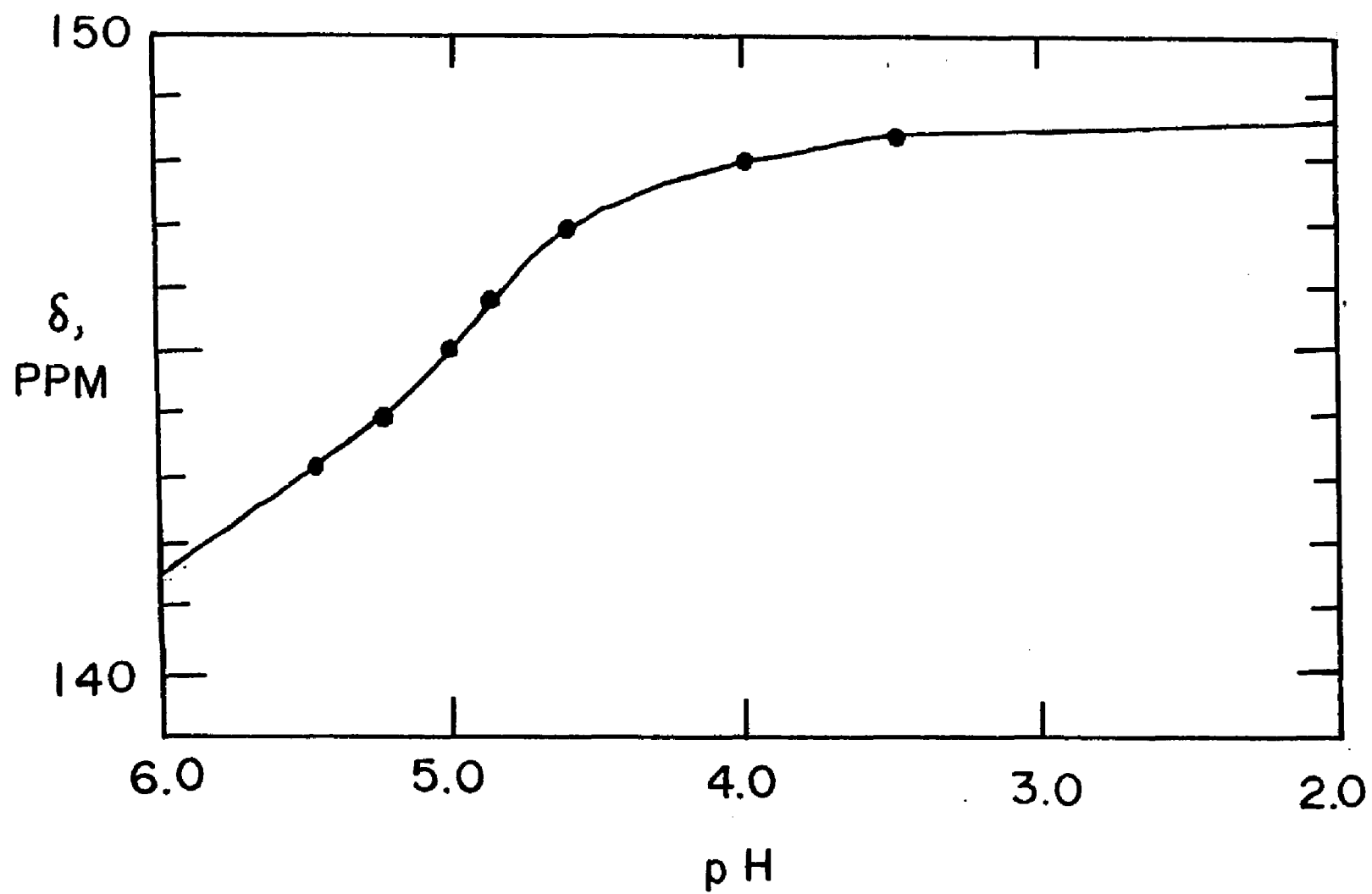
<sup>a</sup>Concentrations of metal ions and pyridine = 0.500M. <sup>b</sup>ppm downfield from TMS. Reproducibility of shifts = ±0.05 ppm.

TABLE VII.  $^{13}\text{C}$  Chemical Shifts of Aqueous Pyridine Solutions<sup>a</sup> at Various pH Values

| pH Value | $^{13}\text{C}$ Chemical Shift <sup>b</sup> |         |          |
|----------|---------------------------------------------|---------|----------|
|          | $\alpha$                                    | $\beta$ | $\gamma$ |
| 8.95     | 149.82                                      | 125.82  | 139.01   |
| 6.00     | 148.91                                      | 126.22  | 140.23   |
| 5.50     | 146.99                                      | 127.09  | 142.99   |
| 5.15     | 145.77                                      | 127.51  | 144.33   |
| 5.00     | 145.28                                      | 127.72  | 144.99   |
| 4.85     | 144.75                                      | 127.92  | 145.67   |
| 4.65     | 143.97                                      | 128.19  | 146.64   |
| 4.00     | 142.99                                      | 128.67  | 148.03   |
| 3.50     | 142.84                                      | 128.87  | 148.42   |
| 0.85     | 142.62                                      | 128.84  | 148.55   |

<sup>a</sup>Pyridine concentration of all solutions = 0.500M. <sup>b</sup>ppm downfield from TMS. Reproducibility of shifts =  $\pm 0.05$  ppm.

FIGURE 6. THE  $^{13}\text{C}$  NMR CHEMICAL SHIFTS FOR THE PARA CARBON ATOM OF AQUEOUS PYRIDINE AS A FUNCTION OF PH. THE FILLED CIRCLES ARE THE EXPERIMENTAL DATA POINTS AND THE SOLID LINE IS THE CALCULATED FIT TO THE ANALYTICAL FUNCTION USED TO DETERMINE THE  $\text{pK}_a$  OF PYRIDINE.



II), one can calculate a  $K_a$  value that best fits the function given in equation <7>. A value of 5.23 for the  $pK_a$  of protonated pyridine was obtained from this analysis. This value is in excellent agreement with literature values (62-64), which average 5.27 in the ionic strength range of our experiment, 0.10M to 1.00M  $NaClO_4$ . This result is encouraging in terms of the use of our experimental procedure and method of analysis for the metal-pyridine solutions.

$^{13}C$  NMR spectra were taken of the aquopyridine solutions of Zn(II), Cd(II) and Ag(I). As with the unbound pyridine solutions, these spectra are simple. There is a single sharp resonance for each of the three types of pyridine carbon atoms (i.e., ortho, meta and para) since the exchange rates for bound and unbound pyridine molecules are fast on the NMR time scale. Spectra comparing the different chemical shifts of the metal-pyridine complexes in solution at one pH value is shown in Figure 7. The  $^{13}C$  chemical shifts of the aquopyridine complexes of Zn(II), Cd(II) and Ag(I) at particular pH values are given in Tables VIII-X, respectively. The most obvious observation drawn from these three tables is that at a given pH, the para position chemical shift decreases in the order:  $Zn(II) > Cd(II) > Ag(I)$ . This result is very significant as will be discussed later.

Several solutions of Zn(II)-pyridine complexes of the same composition and pH were made up and the NMR spectra

FIGURE 7. AN OVERLAY OF THE  $^{13}\text{C}$  NMR CHEMICAL SHIFTS OF PYRIDINE IN EQUILIBRIUM WITH  $\text{Zn(II)}$  (A),  $\text{Cd(II)}$  (B) AND  $\text{Ag(I)}$  (C) IN  $\text{H}_2\text{O}$  AT  $\text{pH} = 4.66$ . TMS WAS USED AS THE REFERENCE. THE ALPHA CARBON SIGNAL SHIFTS TO LOWER FIELD, THE GAMMA CARBON SIGNAL SHIFTS TO HIGHER FIELD, AND THE BETA SIGNAL REMAINS ABOUT THE SAME, WHEN GOING FROM  $\text{Zn(II)}$  TO  $\text{Cd(II)}$  TO  $\text{Ag(I)}$ .

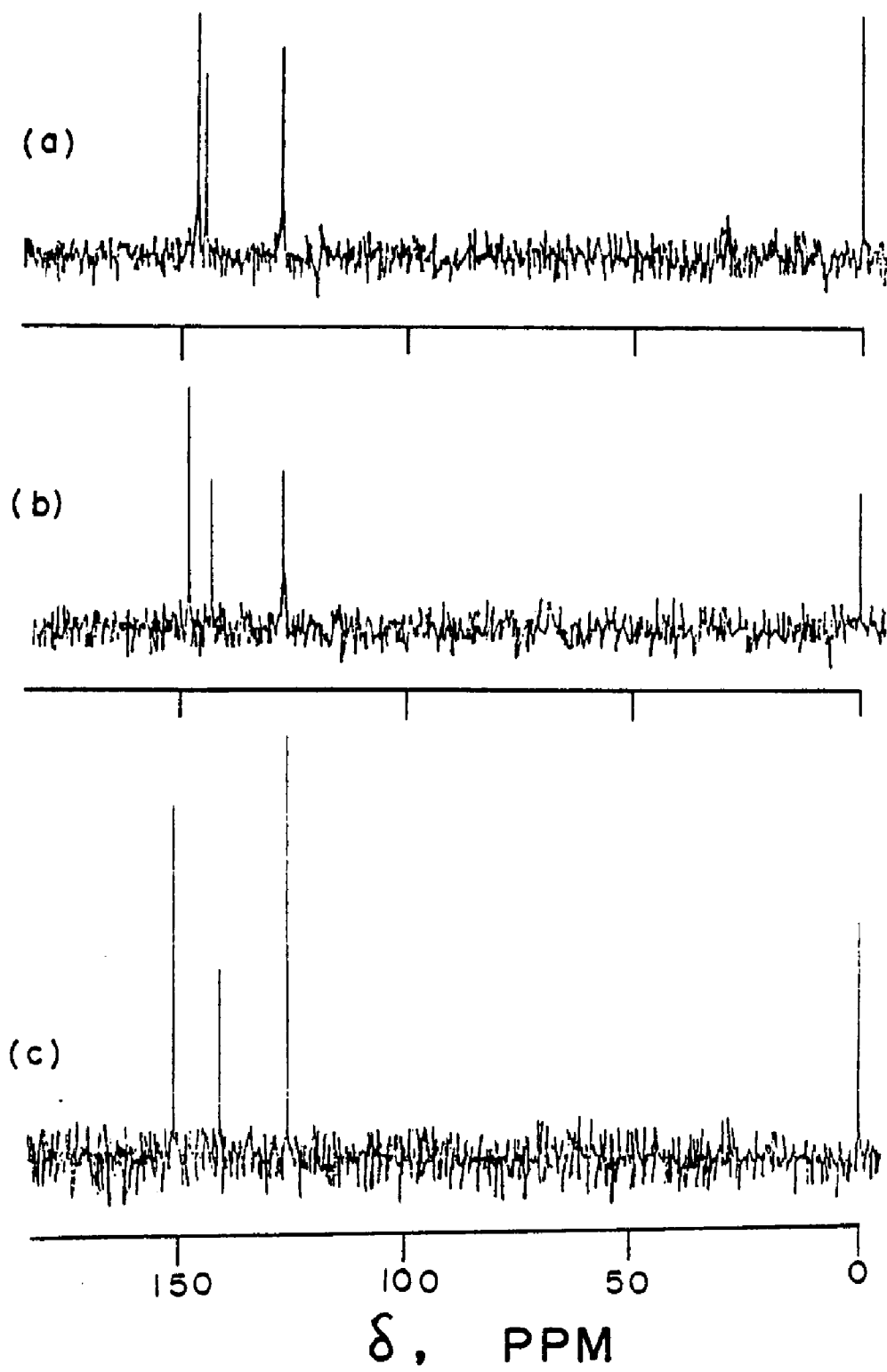


TABLE VIII.  $^{13}\text{C}$  Chemical Shifts of Zn-pyridine Solutions<sup>a</sup> at Various pH Values

| pH Value | $^{13}\text{C}$ Chemical Shift <sup>b</sup> |         |          |
|----------|---------------------------------------------|---------|----------|
|          | $\alpha$                                    | $\beta$ | $\gamma$ |
| 5.51     | 149.06                                      | 127.12  | 142.21   |
| 5.34     | 148.84                                      | 127.19  | 142.48   |
| 5.00     | 147.96                                      | 127.26  | 143.13   |
| 4.62     | 146.47                                      | 127.63  | 144.58   |
| 4.50     | 146.38                                      | 127.94  | 145.04   |
| 4.45     | 145.96                                      | 127.89  | 145.18   |
| 4.33     | 145.77                                      | 128.14  | 145.77   |
| 4.07     | 145.04                                      | 128.43  | 146.57   |
| 3.95     | 144.55                                      | 128.50  | 147.03   |
| 3.60     | 143.74                                      | 128.72  | 147.84   |

<sup>a</sup>Concentrations of aqueous Zn(II) and pyridine solutions = 0.500M.

<sup>b</sup>ppm downfield from TMS. Reproducibility of shifts =  $\pm 0.05$  ppm.

TABLE IX.  $^{13}\text{C}$  Chemical Shifts of Cd-pyridine Solutions<sup>a</sup> at Various pH Values

| pH Value | $^{13}\text{C}$ Chemical Shift <sup>b</sup> |         |          |
|----------|---------------------------------------------|---------|----------|
|          | $\alpha$                                    | $\beta$ | $\gamma$ |
| 5.51     | 150.16                                      | 127.09  | 141.72   |
| 5.49     | 150.08                                      | 126.97  | 141.77   |
| 5.00     | 149.35                                      | 127.36  | 142.45   |
| 4.66     | 148.35                                      | 127.38  | 143.28   |
| 4.00     | 145.72                                      | 128.17  | 145.72   |
| 3.69     | 144.50                                      | 128.41  | 146.86   |

<sup>a</sup>Concentrations of aqueous Cd(II) and pyridine solutions = 0.500M.

<sup>b</sup>ppm downfield from TMS. Reproducibility of shifts =  $\pm 0.05$  ppm.

TABLE X.  $^{13}\text{C}$  Chemical Shifts of Ag-pyridine Solutions<sup>a</sup> at Various pH Values

| pH Value | $^{13}\text{C}$ Chemical Shift <sup>b</sup> |         |          |
|----------|---------------------------------------------|---------|----------|
|          | $\alpha$                                    | $\beta$ | $\gamma$ |
| 5.50     | 152.60                                      | 126.65  | 140.53   |
| 4.65     | 151.43                                      | 126.94  | 141.52   |
| 4.30     | 150.30                                      | 127.24  | 142.50   |
| 4.00     | 148.89                                      | 127.51  | 143.58   |
| 3.75     | 147.55                                      | 127.80  | 144.62   |
| 3.50     | 146.06                                      | 128.09  | 145.79   |

<sup>a</sup>Concentrations of aqueous Ag(I) and pyridine solutions = 0.500M.

<sup>b</sup>ppm downfield from TMS. Reproducibility of shifts =  $\pm 0.05$  ppm.

taken. The results are shown in Table XI, but only the para position is considered here. The average standard deviation of the measurements of these solutions is  $\pm 0.045$  ppm. The reproducibility is very good when considering that within this small standard deviation is not only the error due to the NMR spectrometer but also the possible errors from the preparation of the solutions and obtaining pH values.

The NMR spectra of the pentaamminepyridine complexes of Co(III) and Rh(III) are shown in Figures 8 and 9, respectively. In the pentaamminepyridinecobalt(III) perchlorate spectrum, doublets were observed for both the gamma and alpha carbon atoms of the bound pyridine probe. The  $^{13}\text{C}$  NMR spectrum of this material was taken three times and each time doublets were observed for both positions. All other spectra of pyridine-containing solutions gave singlets at the three carbon positions of pyridine. It is possible, although unlikely, that the NMR spectrometer was accidentally set up for off-resonance-proton-decoupled experiments rather than the broad-band proton decoupled experiments normally run. If the proton decoupling frequency were substantially higher than that required to decouple the proton resonances, then residual coupling or broadening of the carbon signals could result, and the TMS signal might be lowered to the noise level, as was observed in the Co(III) spectrum. Another possibility to explain the doublets observed for the gamma and alpha carbon atoms is the temperature independent paramagnetism that is found in many low spin  $d^6$  metal ions,

TABLE XI. Reproducibility of  $^{13}\text{C}$  Chemical Shifts of Zn-pyridine Solutions<sup>a</sup> of Constant Composition and pH

| pH Value | $^{13}\text{C}$ Chemical Shift <sup>b</sup> | Reproducibility         |
|----------|---------------------------------------------|-------------------------|
|          | $\gamma$ position                           | Standard Deviation(ppm) |
| 5.51     | 142.11                                      |                         |
| 5.51     | 142.21                                      | $\pm 0.053$             |
| 5.51     | 142.13                                      |                         |
| 4.07     | 146.55                                      |                         |
| 4.07     | 146.60                                      | $\pm 0.035$             |

<sup>a</sup>All solutions had a Zn(II) and pyridine concentration = 0.500M.

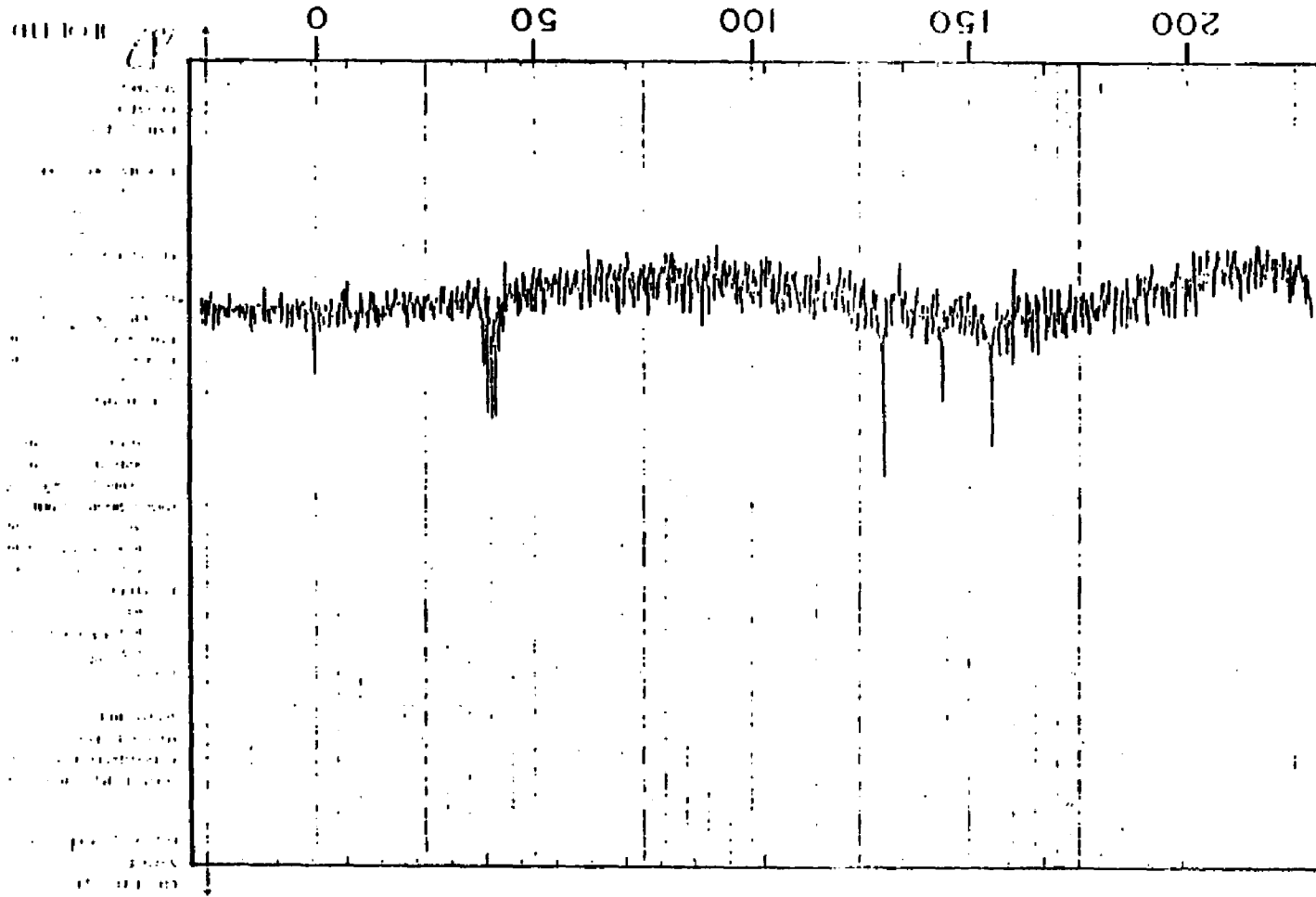
<sup>b</sup>ppm downfield from TMS.

FIGURE 8. THE  $^{13}\text{C}$  NMR SPECTRUM OF 0.2M PENTAAMMINEPYRIDINECOBALT(III) PERCHLORATE IN  $\text{H}_2\text{O}$ , 20,500 SCANS. ACETONE D-6 WAS USED AS THE REFERENCE.



FIGURE 9. THE  $^{13}\text{C}$  NMR SPECTRUM OF 0.04M PENTAAMMINEPYRIDINERHODIUM(III) PERCHLORATE IN  $\text{H}_2\text{O}$ , 35,000 SCANS. TMS WAS USED AS THE REFERENCE.

$\delta$ , PPM



including Co(III). This paramagnetism (explained in detail in the DISCUSSION section) may induce chemical shifts of the protons attached to the Co(III) such that the frequency of the proton signals are out of the range of the proton-decoupler and coupled spectra could result. The doublets are assumed to be due to some form of coupling, therefore the chemical shifts calculated for the gamma and alpha carbon atoms are the unweighted average of the two resonances. The chemical shift data from the  $\text{Co}(\text{NH}_3)_5\text{py}^{3+}$  and  $\text{Rh}(\text{NH}_3)_5\text{py}^{3+}$  complexes (shown in Table XII) can be taken directly from the NMR spectra because these complexes are substitution inert and therefore do not exchange on the NMR time scale. The solutions consist entirely of the metal-bound pyridine complex with no free pyridine.

#### Computer Analysis Of The $^{13}\text{C}$ Chemical Shifts Of The Metal-Pyridine Complexes

Using the Los Alamos Nonlinear Least Squares Program(56) and the subroutines added for this particular problem (see Appendices A and B) on the IBM 370/168 computer system, a least squares fit was performed on the  $^{13}\text{C}$  NMR chemical shift data of the metal-pyridine complexes given in Tables VIII-X. The calculated fits for the para position of the aquopyridine complexes of Zn, Cd and Ag are shown in Figure 10 while the fits of the ortho and meta positions are given in Figure 11. The solid lines represent the least squares fit for the data. The calculated values for the  $^{13}\text{C}$  NMR shifts of the metal-pyridine complexes were determined by

TABLE XII.  $^{13}\text{C}$  Chemical Shift Data of the Pentaamminepyridine Complexes of  $\text{Co(III)}$  and  $\text{Rh(III)}$

| Complex                           | $^{13}\text{C}$ Chemical Shift <sup>a</sup> |         |                     |
|-----------------------------------|---------------------------------------------|---------|---------------------|
|                                   | $\alpha$                                    | $\beta$ | $\gamma$            |
| $\text{Co(NH}_3)_5\text{py}^{3+}$ | 154.27 <sup>b</sup>                         | 129.51  | 143.23 <sup>b</sup> |
| $\text{Rh(NH}_3)_5\text{py}^{3+}$ | 153.27                                      | 129.11  | 142.43              |

<sup>a</sup>ppm downfield from TMS. <sup>b</sup>Chemical shift is the unweighted average of the doublet signal.

FIGURE 10. The  $^{13}\text{C}$  NMR chemical shifts for the para carbon atom of pyridine in equilibrium with  $\text{Zn(II)}$ ,  $\text{Cd(II)}$  and  $\text{Ag(I)}$  as a function of pH. The filled circles are the experimental data points and the solid lines represent the best fit of the chemical shift data to optimized values for stability constants for the metal-pyridine complexes (Table XIII) and the optimized value for the  $^{13}\text{C}$  chemical shift of the metal-pyridine complexes (Table XIV). Larger equilibrium constants lead to a shift in the inflection point of the curve to lower pH as pyridine competes more effectively with the proton.

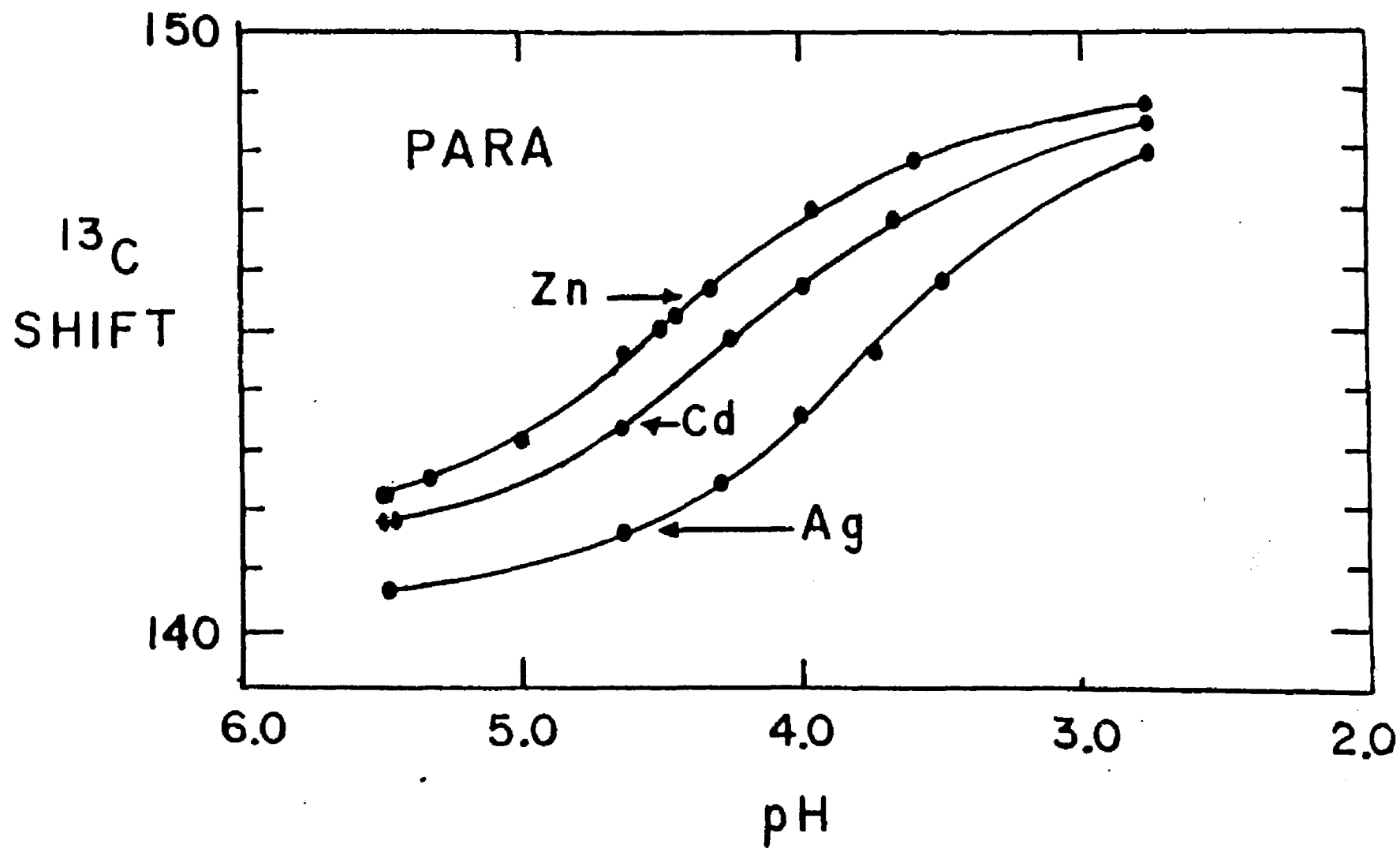
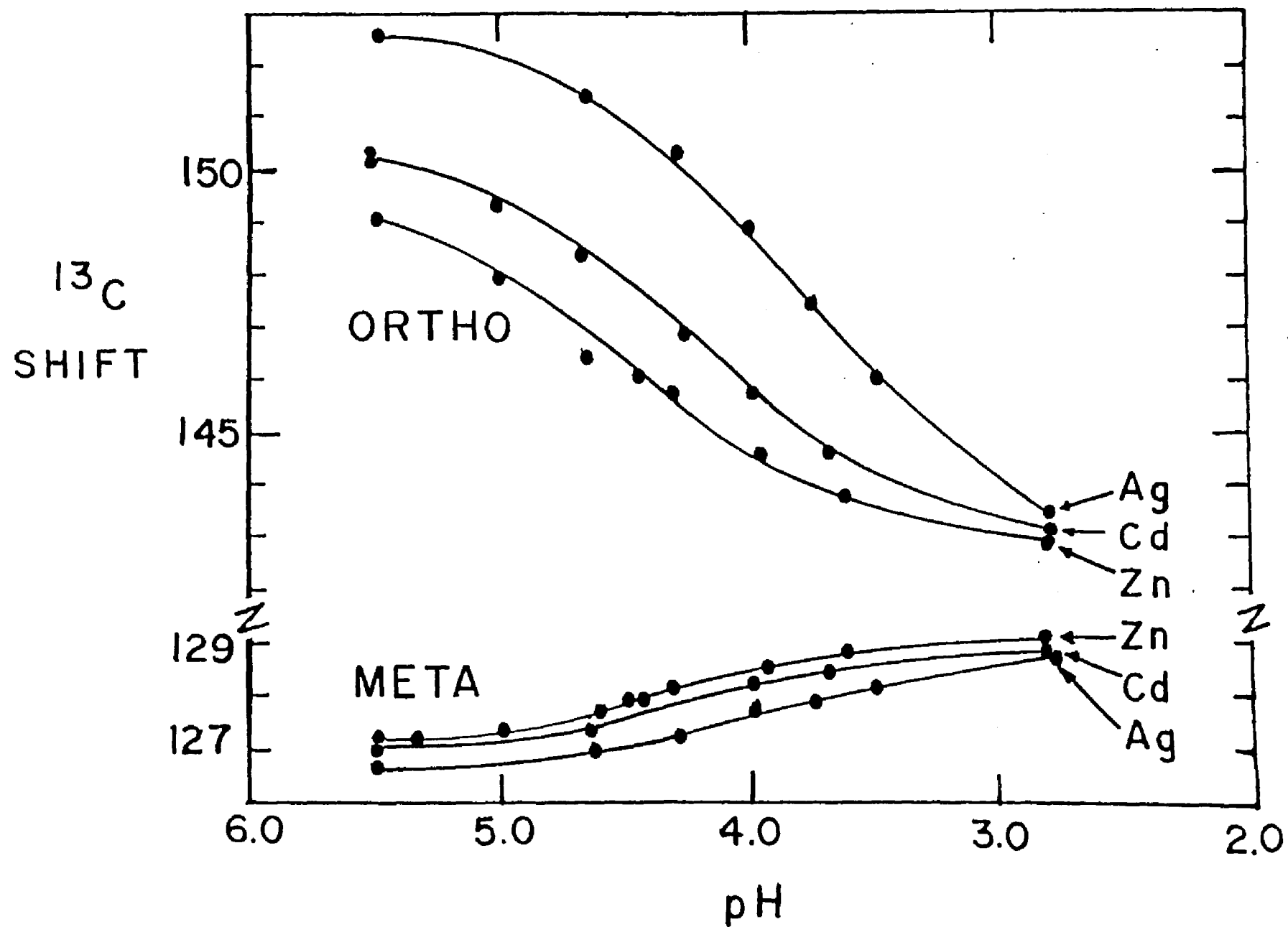


FIGURE 11. The  $^{13}\text{C}$  NMR chemical shifts for the ortho and meta carbon atoms of pyridine in equilibrium with  $\text{Zn(II)}$ ,  $\text{Cd(II)}$  and  $\text{Ag(I)}$  as a function of pH. The filled circles are the experimental data points and the solid lines represent the best fit to the  $^{13}\text{C}$  chemical shift data using the equilibrium constants derived from the para chemical shift data and optimized values for the  $^{13}\text{C}$  NMR chemical shift at the ortho and meta positions for each metal-pyridine complex (Table XIV).



first fixing the formation constants ( $P(2)$  and  $P(3)$  from Table II) taken from literature values(46,51,52) and optimizing the shifts, and then fixing the chemical shifts and allowing the formation constants to vary until neither set of values differed significantly on successive cycles. The calculated formation constants as well as the literature values taken as initial estimates are shown in Table XIII. The calculated formation constants are very similar to values obtained by the more traditional methods used to calculate formation constants; polarography(53), glass electrode(54) and pH measurement(47), and are certainly within the range found in the literature(10). It should be recognized that these constants vary appreciably with the experimental conditions under which the measurements were made.

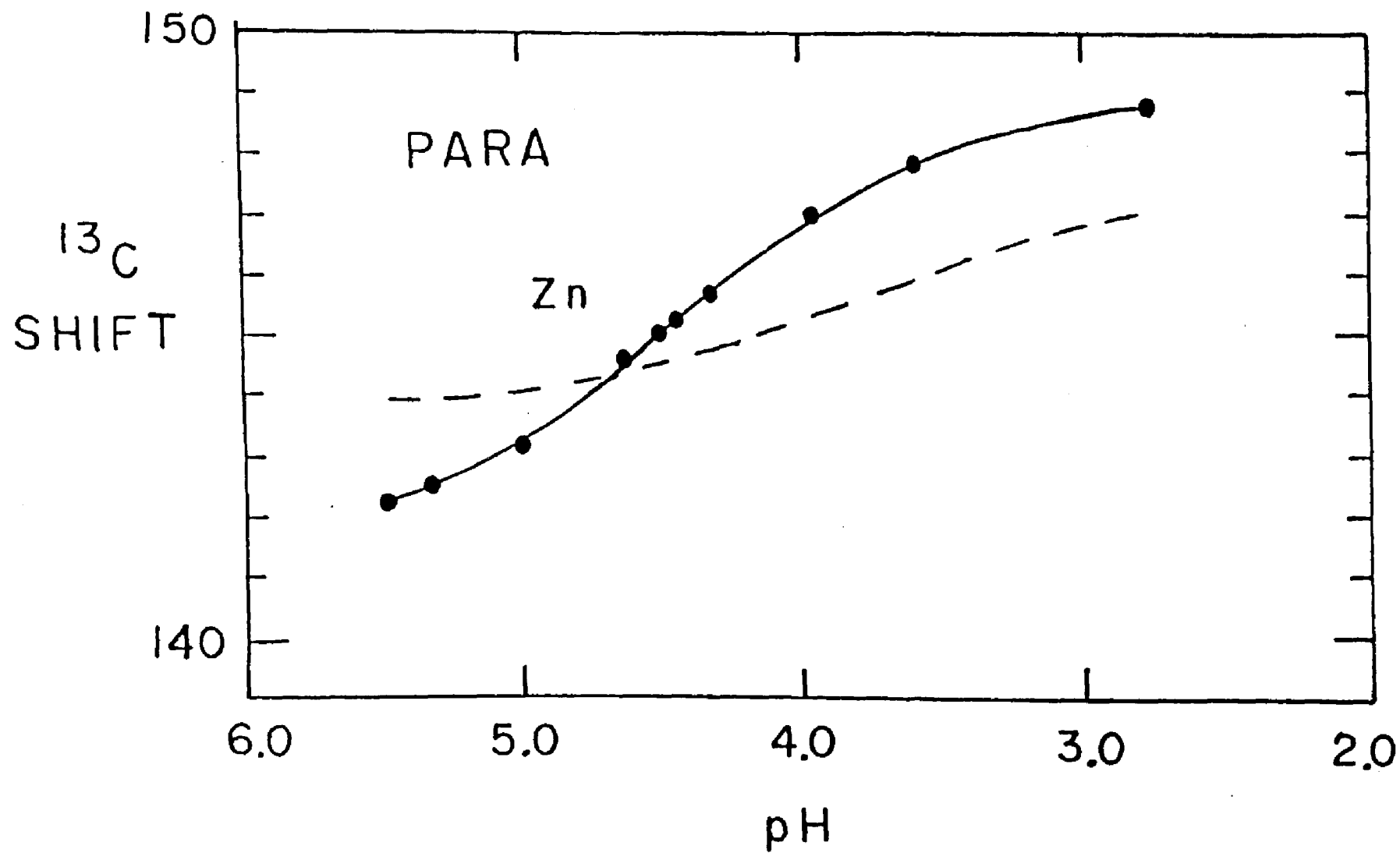
Another indication that the formation constants calculated by the least squares analysis are accurate is that the analytical fit is highly sensitive to the values used for the equilibrium constants. The first stepwise formation constants chosen as initial estimates for the Zn-pyridine complex were  $K_1 = 120$  and  $K_2 = 49.0$  reported by Atkinson and Bauman(63) (using a polarographic determination). The dotted line in Figure 12 shows the fit for the para  $^{13}\text{C}$  chemical shift of pyridine bound to aquated Zn(II) calculated with these formation constants. The fit is extremely poor. However, when the formation constants reported by Sharma and Gaur(53) ( $K_1 = 7.94$  and  $K_2 = 4.27$ ) were used, the calculated fit is excellent as can be seen in Figure 12, the solid

TABLE XIII. Literature and Calculated<sup>a</sup> Values of Formation Constants of Aquopyridine Complexes of Zn(II), Cd(II) and Ag(I)

| Metal ion | FORMATION CONSTANTS |                   |                  |                |
|-----------|---------------------|-------------------|------------------|----------------|
|           | Literature Value    |                   | Calculated Value |                |
|           | K <sub>1</sub>      | K <sub>2</sub>    | K <sub>1</sub>   | K <sub>2</sub> |
| Zn(II)    | 7.94 <sup>b</sup>   | 4.27 <sup>b</sup> | 8.50             | 5.40           |
| Cd(II)    | 32.4 <sup>c</sup>   | 8.91 <sup>c</sup> | 21.4             | 9.3            |
| Ag(I)     | 91.2 <sup>d</sup>   | 170 <sup>d</sup>  | 91.6             | 162            |

<sup>a</sup>Calculated from computer program in Reference 56 and subroutines in Appendices A and B. <sup>b</sup>Reference 53. <sup>c</sup>Reference 54. <sup>d</sup>Reference 47.

FIGURE 12. THE  $^{13}\text{C}$  NMR CHEMICAL SHIFT OF THE PARA CARBON ATOM OF PYRIDINE IN EQUILIBRIUM WITH  $\text{Zn(II)}$  AS A FUNCTION OF pH. THE FILLED CIRCLES ARE THE EXPERIMENTAL DATA POINTS. THE DOTTED LINE IS THE BEST FIT OF THE EXPERIMENTAL CHEMICAL SHIFT DATA TO AN OPTIMIZED VALUE OF THE CHEMICAL SHIFT FOR THE BOUND PYRIDINE USING ONE SET OF LITERATURE VALUES FOR STABILITY CONSTANTS:  $K_1 = 120.2$  AND  $K_2 = 49.0$  (62). THE SOLID LINE REPRESENTS THE BEST FIT WHEN THE EQUILIBRIUM CONSTANTS ARE OPTIMIZED TO GIVE VALUES OF  $K_1 = 8.50$  AND  $K_2 = 5.40$  (53).



line.

These two results are strong evidence that the calculated stepwise formation constants are correct for the conditions used in our experiments.

The final variances obtained are sufficiently small when the chemical shifts of pyridine in the monopyridyl and bis(pyridyl) metal ion complexes are set equal to each other that no attempt was made to allow them to vary independently. It seems reasonable that substitution of a pyridine ligand for a water molecule in the coordination sphere of a non- $\pi$ -backbonding metal ion would not cause a large chemical shift effect. (However, when  $\pi$ -backbonding is involved, there is an effect of one pyridine ligand on another (7).)

Given the positive preliminary results described above, the non-linear least squares program was used to calculate the values for the  $^{13}\text{C}$  chemical shift of the aquopyridine complexes of  $\text{Zn(II)}$ ,  $\text{Cd(II)}$  and  $\text{Ag(I)}$ . These results are given in Table XIV. The standard deviations of the metal-pyridine complexes lie in the same range as the reproducibility of the experiment. This again supports the assumption that these values are statistically valid.

TABLE XIV. Calculated  $^{13}\text{C}$  Chemical Shifts of the Metal-pyridine Complexes<sup>a</sup>

| Metal Complex                              | Calculated Chemical Shift <sup>b</sup> |                   |                   |
|--------------------------------------------|----------------------------------------|-------------------|-------------------|
|                                            | $\alpha$                               | $\beta$           | $\gamma$          |
| $\text{Zn}(\text{H}_2\text{O})_5\text{py}$ | $149.81 \pm 0.04$                      | $127.16 \pm 0.05$ | $142.10 \pm 0.04$ |
| $\text{Cd}(\text{H}_2\text{O})_5\text{py}$ | $150.82 \pm 0.03$                      | $127.15 \pm 0.03$ | $141.59 \pm 0.06$ |
| $\text{Ag}(\text{H}_2\text{O})_5\text{py}$ | $152.85 \pm 0.04$                      | $126.70 \pm 0.02$ | $140.54 \pm 0.05$ |

<sup>a</sup>Indicated ranges refer to standard deviations from the mean calculated from equilibrium expressions described in the text. <sup>b</sup>ppm downfield from TMS.

## DISCUSSION

### BRIEF BACKGROUND OF THE PROBLEM

The pyridine complexes of filled shell  $d^{10}$  metal ions: Zn(II), Cd(II) and Ag(I) and of diamagnetic  $d^6$  metal ions Co(III) and Rh(III) were prepared at different pH values. The  $^{13}\text{C}$  NMR spectra of these solutions were taken. The chemical shifts of the pyridine bound complexes of Zn(II) Cd(II) and Ag(I) had to be calculated due to rapid exchange of the pyridine. A subroutine used to calculate the concentration of protonated pyridine (See CUBIC listed in Appendix A) was developed. Subroutine CUBIC was incorporated into another subroutine (see YP9 in Appendix B) which calculated the  $^{13}\text{C}$  chemical shifts of the metal-pyridine complexes. Because the pyridine complexes of Co(III) and Rh(III) are substitution inert, their shifts could be used directly.

Two other filled shell ions Hg(II) and Cu(I), were considered as candidates for this study. The pH required to prevent precipitation of mercuric hydroxide (calculated from the  $K_{sp}$  of  $\text{Hg}(\text{OH})_2$  (65)) was much too low ( $\sim 1.1$ ) for the mercuric ion to be of use in this study. As expected, when dissolution of HgO was attempted in  $\text{HClO}_4$  solutions above pH 2, copious amounts of white precipitate formed. Cuprous compounds that are stable in water are only sparingly solu-

ble ones such as  $\text{CuCl}$  or  $\text{CuCN}(3)$ . Ionic  $\text{Cu(I)}$  derivatives are unstable since  $\text{Cu(I)}$ -aquo species rapidly disproportionate to copper metal and cupric ion.

#### SIGNIFICANCE OF USING NITRATE VERSUS PERCHLORATE AS THE COUNTERION

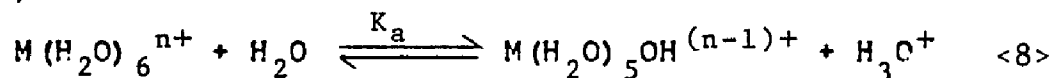
As seen in Table VI, there was a small downfield shift averaging 0.200 ppm when the counterion of the aquopyridine complexes of  $\text{Zn(II)}$  and  $\text{Cd(II)}$  was changed from perchlorate to nitrate. The assumption was at first made that using either of these two anions would have no effect on the  $^{13}\text{C}$  chemical shift values found for the  $\text{Zn(II)}$  and  $\text{Cd(II)}$  complexes because both are usually noninteracting. However, by our experimental results, this assumption may be erroneous.

However, the difference in formation constants between the association of  $\text{Cd(II)}$  and  $\text{Ag(I)}$  with nitrate ion can explain why  $\text{Ag(I)}$  forms primarily an aquo species whether nitrate or perchlorate is used as the counterion, whereas  $\text{Cd(II)}$  remains a cadmium nitrate species when nitrate is the counterion. The formation constant(10) for cadmium nitrate (ca. 1.8) indicates that the major species in solution is a cadmium nitrate complex. But in the case of  $\text{Ag(I)}$ , the stability constant(10) for silver nitrate (ca. 0.54) indicates that  $\text{Ag(I)}$  in aqueous solution remains primarily an aquo complex. For this reason,  $\text{Cd(II)}$ , in the presence of nitrate ion, might have a different chemical shift than in

the presence of perchlorate ion, but Ag(I) should have the same chemical shift whether nitrate or perchlorate is present.

#### FITTING THE $^{13}\text{C}$ DATA TO EXPERIMENTALLY DETERMINED $\text{pK}_a$ VALUES

Table XV shows typical  $\text{pK}_a$  values taken from the literature(10) for the reaction:



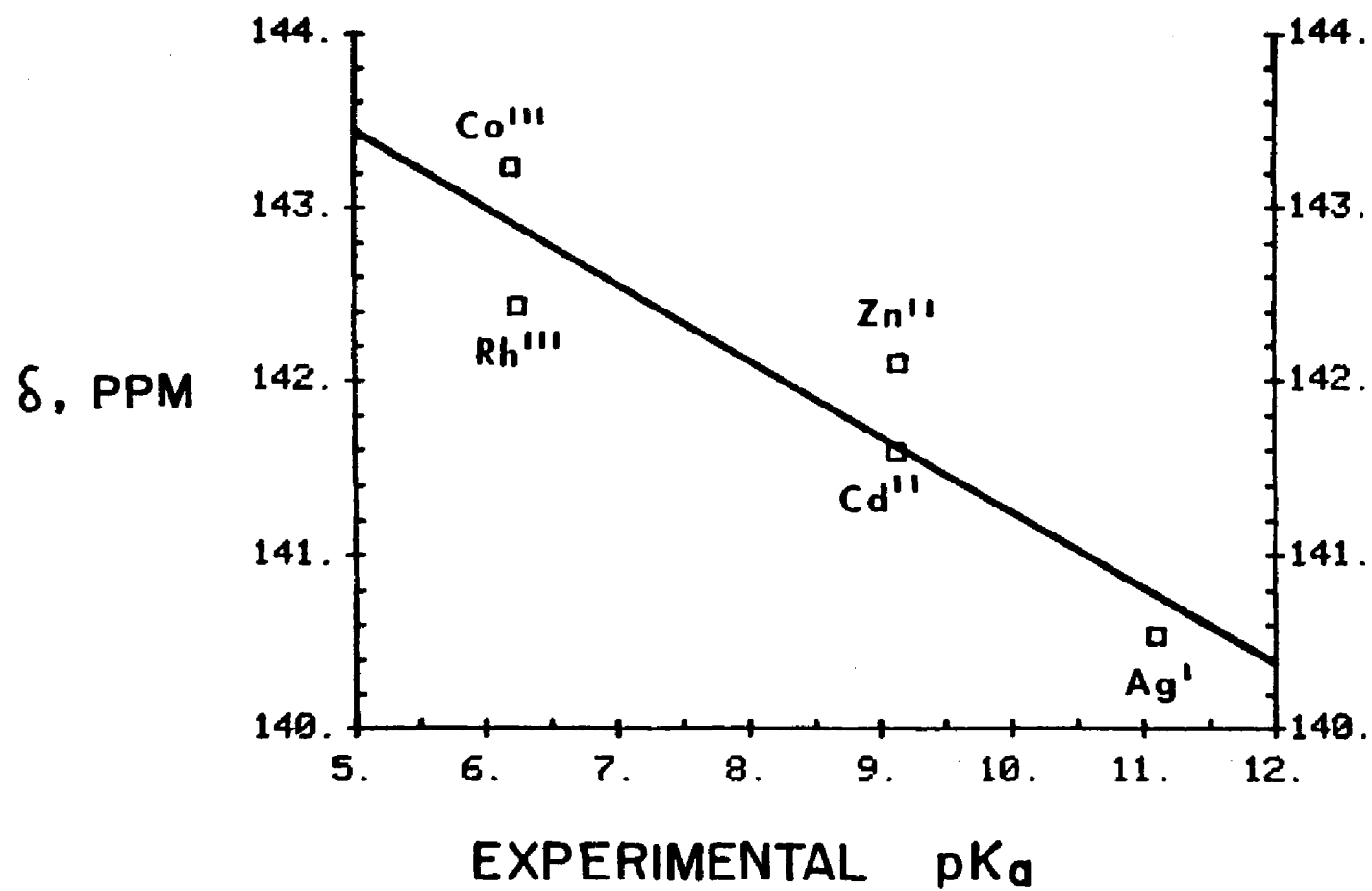
where M stands for the metals used in this study. If the assumption is made that the acidity of an aquo complex depends primarily on the polarization of the water bound in the first coordination sphere by the metal atom, then one would expect a reasonably linear correlation between the  $^{13}\text{C}$  chemical shifts and the  $\text{pK}_a$  values of these metal-aquo complexes. But as can be seen in Figure 13, the correlation is not very satisfactory (standard deviation of the linear regression = 0.4454, correlation coefficient = 0.9232). There are two possibilities for the rather poor correlation; (1) the  $^{13}\text{C}$  NMR technique is not a quantitative indicator of polarizability or (2) the assumption that the  $\text{pK}_a$  of an aquated metal ion is dependent principally on the polarization of bound water while other effects are minor is incorrect. Because of the theoretical and empirical justification supporting the use of the  $^{13}\text{C}$  NMR technique, possibility (2) was investigated.

TABLE XV. Typical Experimental  $pK_a$  Values of Aquated Complexes of Co(III), Rh(III), Zn(II), Cd(II) and Ag(I)

| Metal ion | $pK_a$ Value <sup>a</sup> |
|-----------|---------------------------|
| Co(III)   | 6.20 <sup>b</sup>         |
| Rh(III)   | 6.24 <sup>b</sup>         |
| Zn(II)    | 9.13                      |
| Cd(II)    | 9.12                      |
| Ag(I)     | 11.1                      |

<sup>a</sup>Values taken from Reference 10. <sup>b</sup>Value is for the aquopentaammine species.

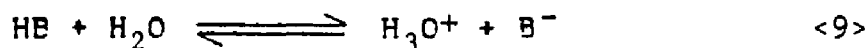
FIGURE 13. THE CORRELATION OF THE  $^{13}\text{C}$  NMR CHEMICAL SHIFTS FOR THE PARA CARBON ATOM OF PYRIDINE COMPLEXES OF  $\text{Co(III)}$ ,  $\text{Rh(III)}$ ,  $\text{Zn(II)}$ ,  $\text{Cd(II)}$  AND  $\text{Ag(I)}$  WITH EXPERIMENTAL  $\text{pK}_a$  VALUES. THE SQUARES ARE THE INDIVIDUAL DATA POINTS AND THE SOLID LINE REPRESENTS THE CALCULATED LEAST SQUARES FIT TO THE DATA POINTS (STANDARD DEVIATION OF THE LINEAR REGRESSION = 0.4445, CORRELATION COEFFICIENT = 0.9232. THE SIZE OF THE SQUARES INDICATE THE REPRODUCIBILITIES OF THE CHEMICAL SHIFT DETERMINATIONS.



## CORRELATION OF $^{13}\text{C}$ CHEMICAL SHIFTS WITH PREDICTED $\text{pK}_a$ VALUES

### Application Of Duffy And Ingram's Optical Basicity Principle

Duffy and Ingram(26) approached the longstanding problem of expressing quantitative ideas like acidity, polarization and electron density numerically and relating them to some parameter which quantifies the polarizing power of the metal ion. They proposed to tackle the problem by regarding metal-aquo complexes simply as oxidic acids and assuming that acidity depends on the state of the oxide ion after dissociation of one or more of its protons(66). The attraction the oxide has for regaining its proton(s) depends on its electron-donating ability. This can be expressed in numerical terms as its optical basicity. Optical basicity may be regarded as a way of quantifying Lewis basicity and can be obtained by either calculation(67) or by experiment(68). Experimentally, optical basicity is calculated from optical spectra of melts and glasses doped with  $d^1s^2$  ions, in particular of shifts in the  $^1S_0 \rightarrow ^3P_1$  transitions of  $\text{Tl}^+$ ,  $\text{Pb}^{2+}$  and  $\text{Bi}^{3+}$ . (An anion with a high optical basicity shows a strong affinity for protons and therefore its parent acid is weak, and vice versa.) The dissociation of an oxyacid HB (which can be neutral or anionic) involves competition between the resulting anion and water for the protons, that is:



It should therefore be expected that the difference between

the optical basicity of an anion,  $\lambda_B$ , and that of water,  $\lambda_{H_2O}$ , can be related to the strength of the acid. Duffy and Ingram found a good correlation between the  $pK_a$  of the acid and optical basicity of the conjugate base. The data for oxyacids lie close to the line made up of the two points for the self-dissociation of  $H_2O$  (corresponding to the protonation and dissociation of water into hydronium ion and hydroxide ion, respectively). The equation of this line is:

$$pK_a + 1.74 = 58.3(\lambda_B - 0.40) \quad <10>$$

In this equation, 0.40 is the  $\lambda$  value for water(67) and 1.74 takes into account the 55M concentration of water. The optical basicity of the conjugate base,  $\lambda_B$ , is a measure of Lewis basicity and can be obtained either from spectroscopic measurements(69) or calculated from the stoichiometry and electronegativities of the constituent elements. Simply put, an acid is considered strong if its  $\lambda_B$  (of its conjugate base) is less than 0.40 and is a weak acid if the value of  $\lambda_B$  is greater than 0.40. The optical basicity of an oxidic group depends on the extent to which the electron-donor power of the oxide species is modified by the cations to which it is attached. It is related quantitatively to: 1) the tendency of the cations A,B,... to reduce the donor properties of the oxide species, which is expressed empirically by "basicity moderating" parameters  $\lambda_A, \lambda_B, \dots$ ; 2) the oxidation numbers  $z_A, z_B, \dots$  of the cations; and 3) the ratio of the cations with respect to the total number of oxides. For an oxyanion of an element Y (with oxidation

state  $y$ ) having the general formula:  $H_nYO_z^{m-}$ , the theoretical basicities are given by the equation:

$$\lambda_B = 1 - (n/2z(1 - 1/\gamma_H) + y/2z(1 - 1/\gamma_Y)) \quad <11>$$

where  $\gamma_H$  and  $\gamma_Y$  are the basicity-moderating parameters of hydrogen and element  $Y$ , respectively (68). For several s- and p- block elements, it has been shown that  $\gamma$  values are closely related to Pauling electronegativity,  $x$  (67), by the following equation:

$$\gamma = 1.36(x - 0.26) \quad <12>$$

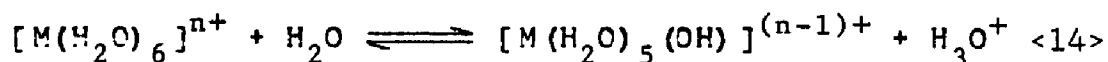
It is at this stage of Duffy and Ingram's proposal that we have modified equation <12> so that it would better reflect the metals used in our study. Allred-Rochow electronegativities were chosen to be used in this equation since they indicate differences in properties of transition and post-transition metals more realistically than do Pauling electronegativities. (Allred-Rochow electronegativities,  $x$ , are empirically derived from effective coulombic force on valence electrons (70), that is:  $\gamma \propto Z^*e^2/r^2$  in which  $Z^*$  is the effective nuclear charge that the electron experiences and  $r$  is its mean distance from the nucleus.) As Duffy and Ingram stated, equation <12> holds for s- and p- block elements, while in our study, transition and post-transition metals are used. The basicity-moderating parameters have therefore been recalculated using Allred-Rochow electronegativity values in such a way as to ensure that

correlations found by Duffy and Ingram using their scale correspond to results for the modified scale. Using the same constraints as Duffy and Ingram(66), equation <13> was derived.

$$\gamma = 1.29(x - 0.26) \quad <13>$$

Equation <13> gives  $\lambda_B$  values that are consistent with expected parameters of strong acids ( $\lambda_B(\text{NO}_3^-) = 0.38$ ) and weak acids ( $\lambda_B(\text{H}_2\text{PO}_4^-) = 0.48$ ). The values of Allred-Rochow electronegativities, basicity-moderating parameters and optical basicities are given in Table XVI for a variety of metal-aquo complexes.

Thus, the dissociation of an oxidic acid is rationalized solely in terms of stoichiometry and basicity-moderating parameters. Duffy and Ingram explored the possibility of extending this optical basicity concept to positively charged acids such as metal-aquo ions. The acid dissociation of a metal hexaquo complex is represented by:



The  $\lambda_B$  values of the conjugate base of these complexes ( $[\text{M}(\text{H}_2\text{O})_5(\text{OH})]^{(n-1)+}$ ) could be calculated from equations <11> and <13> and the predicted value for  $K_a$  of the equilibrium shown in equation <14> could be calculated using equation <10>. The results of such calculations (see Table XVII) indicate the aquo complexes of metal ions such as Al(III) or Zn(II) should be very acidic, comparable with, or even greater than, mineral acids like  $\text{HNO}_3$  while other aquated metals like Ca(II) and Ag(I) are weak acids. It is

TABLE XVI. Allred-Rochow Electronegativity, Basicity-Moderating and Optical Basicity Values of the Aquated Metal Ions: Co(III), Rh(III), Zn(II), Cd(II) and Ag(I)

| Metal ion | Allred-Rochow<br>Electronegativity <sup>a</sup> | $\gamma_Y^b$ | $\lambda_B^c$ |
|-----------|-------------------------------------------------|--------------|---------------|
| Co(III)   | 1.70                                            | 1.86         | 0.335         |
| Rh(III)   | 1.45                                            | 1.54         | 0.363         |
| Zn(II)    | 1.66                                            | 1.81         | 0.376         |
| Cd(II)    | 1.46                                            | 1.55         | 0.391         |
| Ag(I)     | 1.42                                            | 1.50         | 0.422         |

<sup>a</sup>Values taken from Reference 70. <sup>b</sup>Basicity-moderating parameters are obtained from equation <13>. <sup>c</sup>Optical basicity values are calculated for the conjugate base  $[M(H_2O)_5(OH)]^{(n-1)+}$  from equation <11>.

found experimentally though that metal-aquo ions in aqueous solution behave as fairly weak acids (see Table XVII). The reason for this very large difference between predicted and experimental  $pK_a$  values (which amounts to 6-12 pK units) may arise from significant outer-sphere hydration which the metal-aquo complexes undergo in aqueous solution.

#### Evidence Supporting The Significance Of Calculated $pK_a$ Values

The conditions under which metal-aquo ions exist with little outer-sphere hydration are probably met in molten salt hydrates (26), especially when the water:metal ratio is 6:1 or less. Experiments in Duffy and Ingram's lab have indicated that molten salt hydrates readily dissolve weak organic bases and that the protonating power is so great that the acidity is measurable only on the Hammett acidity scale. The Hammett acidity function,  $H_o$ , of a medium is defined (71) as:

$$H_o = -\log(a_{H^+}f_B/f_{BH^+}) \quad <15>$$

where  $a_{H^+}$  is the activity of hydrogen ions and  $f_B$  and  $f_{BH^+}$  are respectively the activity coefficients of an added base B and its conjugate acid  $BH^+$ . The bases are often substituted aromatic amines and the method of determining  $H_o$  depends on the spectrophotometric determination of the concentrations  $c_B$  and  $c_{BH^+}$ , since equation <15> can be rearranged as:

$$H_o = pK_a^B + \log(c_B/c_{BH^+}) \quad <16>$$

where  $pK_a$  is the dissociation constant of the conjugate acid

TABLE XVII. Experimental and Predicted  $pK_a$  Values of Metal-aquo Complexes

| Metal ion            | Experimental $pK_a^a$ | Predicted $pK_a^b$ |
|----------------------|-----------------------|--------------------|
| Co(III)              | 6.20 <sup>c</sup>     | -5.55              |
| Al(III) <sup>c</sup> | 4.97                  | -4.76              |
| Rh(III)              | 6.24 <sup>c</sup>     | -3.91              |
| Zn(II)               | 9.13                  | -3.16              |
| Cd(II)               | 9.12                  | -2.26              |
| Ag(I)                | 11.10                 | -0.437             |
| Ca(II) <sup>c</sup>  | 12.85                 | 1.11               |
| HNO <sub>3</sub>     | -1.71                 | -2.32              |

<sup>a</sup>Data taken from Reference 10. <sup>b</sup>Values obtained from equation <10>. <sup>c</sup>Data taken from Reference 26.

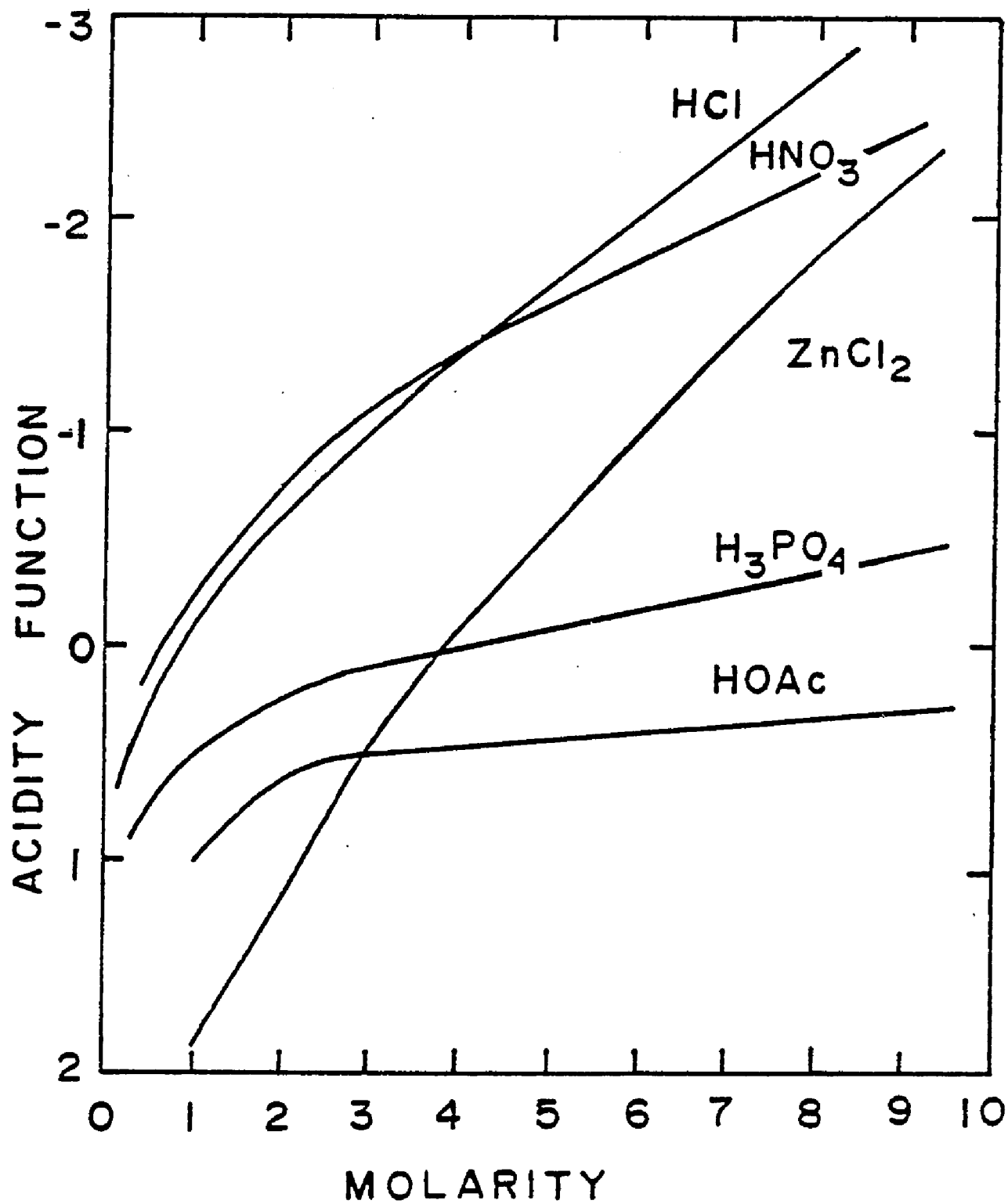
<sup>c</sup>Value is for the aquopentaammine species.

of the indicator base. Because of the constraints on a molten salt hydrate system for the measurement of  $H_0$ , Duffy and Ingram could use only one such system, that of  $ZnCl_2 \cdot 4H_2O$ .

Typical strong acids (e.g.,  $HCl$ ,  $HNO_3$ ,  $H_2SO_4$ ) increase enormously in protonating power as their aqueous solutions become more concentrated. This is in sharp contrast to weak acids (e.g., acetic acid) where there is little increase. Figure 14 shows the variation in  $H_0$  with concentration for a number of acids in aqueous solution, including the new data for the zinc chloride system. It can be seen that in dilute solutions, the protonating power of aquated zinc is very low. However, as the solution becomes more concentrated, the protonating power increases dramatically. Also when comparing the slopes of various strong acids with that of the zinc-aquo complex, the trend of aquated zinc is that of a very strong acid.

In general, powerful Hammett acidity goes together with high Brønsted-Lowry acidity in dilute aqueous solutions. Therefore the zinc-aquo ion should have a low  $pK_a$  (high acidity) and the experimentally determined values averaging 9.1 indicates a mechanism which severely depresses the acidity in dilute solutions. There is no reason to suppose that the  $ZnCl_2 \cdot H_2O$  system is unique among salt-water systems. Actually, strong  $Zn^{2+}-Cl^-$  interactions competing with the  $Zn^{2+}-H_2O$  interaction may even lower the protonating power of these liquids as compared with the nitrates or trifluoro-

FIGURE 14. THE CHANGE IN THE HAMMETT ACIDITY FUNCTION OF VARIOUS ACIDS AS A FUNCTION OF ACID CONCENTRATION, INCLUDING THE DATA FOR THE ZINC CHLORIDE SYSTEM. WEAK ACIDS SHOW LITTLE INCREASE IN PROTONATING POWER AS THEIR AQUEOUS SOLUTIONS BECOME MORE CONCENTRATED, BUT STRONG ACIDS SHOW A DRAMATIC INCREASE. THE TREND OF THE ZINC CHLORIDE SYSTEM IS THAT OF A STRONG ACID.



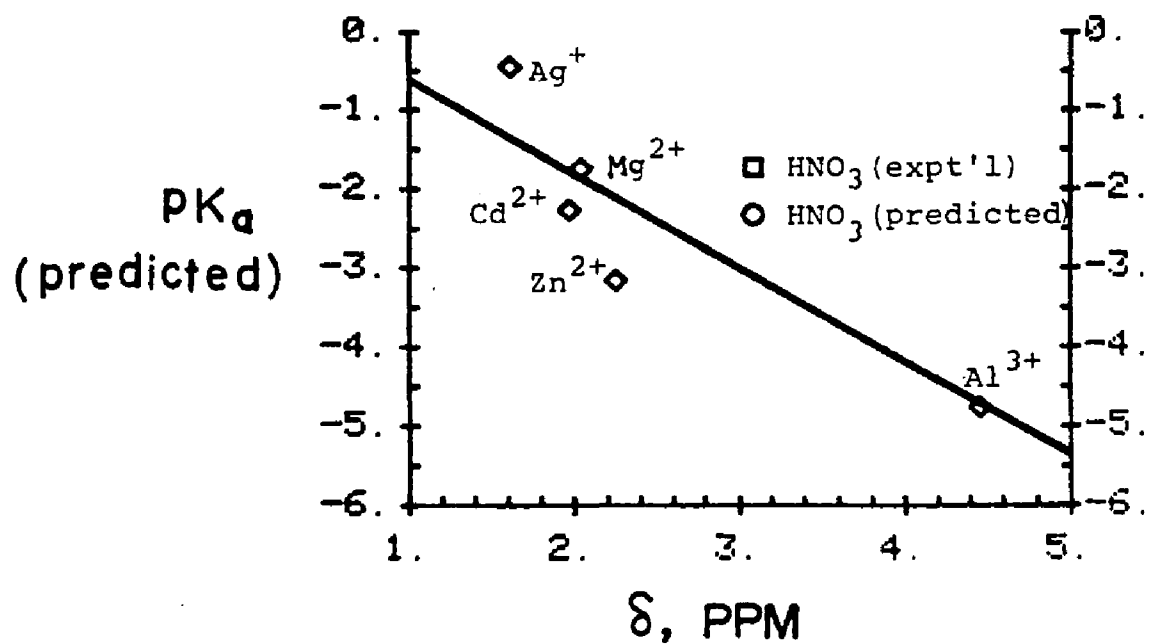
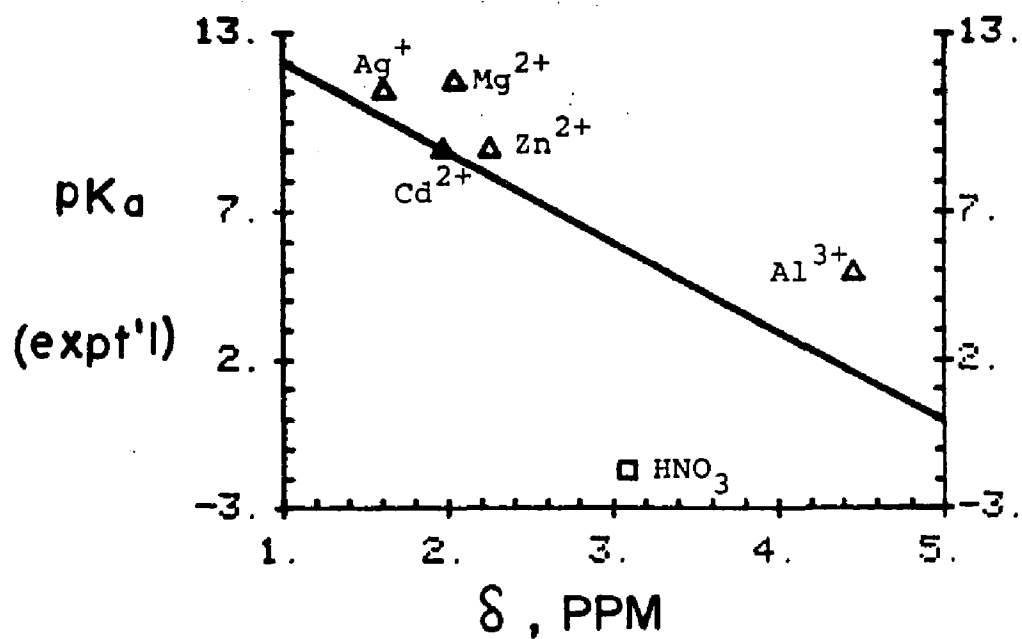
methanesulfonates of equivalent  $[H_2O]/[Zn^{2+}]$  ratios. There is indeed indirect evidence for very high acidity in mixed  $AlCl_3/Al(NO_3)_3$  systems because of their exceptional reactivity (rapid dissolution of Au, Pt, etc.) (40).

Additional Evidence To Support Duffy And Ingram's Predicted  $pK_a$  Hypothesis - Sare, Moynihan And Angell's Work

A  $^1H$  NMR study by Angell and co-workers (40) lent further support for Duffy and Ingram's hypotheses; that their predicted  $pK_a$  values based on optical basicity arguments and their conclusion that many aquated metals are in fact very strong acids, are fundamentally sound. These workers measured the  $^1H$  downfield chemical shifts of the bound water (relative to the tetramethylammonium ion) as a function of temperature for concentrated aqueous solutions of a large number of metal salts and correlated the shifts successfully with the basic properties of the various anions (oxyanions and monoxyanions). A quite unexpected finding by Angell, et al., was the far downfield shift of the  $Al(NO_3)_3 \cdot 10H_2O$  species (4.45 ppm); much further than  $HNO_3$  (3.08 ppm). Since displacement of a hydrogen away from the bound water oxygen (which occurs when a protic acid dissociates) implies a deshielding of the proton hence a downfield shift in for solutions of a given anion should move downfield with increasing cation  $pK_a$ . This indicates, as Duffy and Ingram postulated, that the actual acidity of aquated Al(III) is greater than that of  $HNO_3$ .

Although in his study Angell looked at the effect of changing anions on the downfield shift of a given metal salt, it is possible to use some of his data to investigate the effect of changing cations while keeping the anion constant. The  $^1\text{H}$  downfield chemical shifts for a set of metal nitrates of the form,  $\text{M}(\text{NO}_3)_n \cdot 10\text{H}_2\text{O}$ , are shown in Figure 15. In Figure 15A, the plot of the downfield chemical shift (relative to the tetramethylammonium ion) versus the experimental  $\text{pK}_a$  value of the metal ion is seen. It indicates that the degree of proton deshielding in the water molecules as a result of polarization by the metal atoms (e.g.,  $\text{Al}^{3+} > \text{Zn}^{2+} > \text{Ag}^+$ ) is consistent with expectation. However, the data point for  $\text{HNO}_3$  lies far off the line (almost 7 pK units). If one assumes that the  $^1\text{H}$  chemical shift does correlate with the polarizing ability of a metal atom and that the  $\text{pK}_a$  of  $\text{HNO}_3$  is in the proper range of a strong acid, then one has to accept the  $\text{HNO}_3$  data point as correct. This would indicate that the experimental  $\text{pK}_a$  values of the aquated metals in Figure 15A are in error and do not fully represent the true acidity of these metal-aquo complexes. On the basis of their  $^1\text{H}$  NMR data, Angell suggested that concentrated solutions of certain metal salts form an interesting class of strong protonic acids and for these solutions, the downfield shift is a good indicator of the strong acid character of these complexes. Duffy and Ingram had earlier hypothesized that  $\text{pK}_a$  values predicted from optical basicity are also expressions of the inherent acidity;

FIGURE 15. A COMPARISON OF CORRELATIONS OF DOWNFIELD  $^1\text{H}$  NMR CHEMICAL SHIFTS OF VARIOUS AQUATED METAL SPECIES AS WELL AS NITRIC ACID WITH EXPERIMENTAL (A) AND PREDICTED (B)  $\text{pK}_a$  VALUES. IN 15A, THE POINT FOR NITRIC ACID (SQUARE) LIES FAR OFF THE BEST FIT LINE OF THE OTHER METAL IONS (STANDARD DEVIATION OF THE LINEAR REGRESSION = 4.33, CORRELATION COEFFICIENT = 0.632). IN 15B, BOTH THE EXPERIMENTAL (CIRCLE) AND PREDICTED (SQUARE) NITRIC ACID DATA POINTS LIE MUCH CLOSER TO THE CALCULATED LINE (STANDARD DEVIATION OF THE LINEAR REGRESSION = 0.833, CORRELATION COEFFICIENT = 0.857).

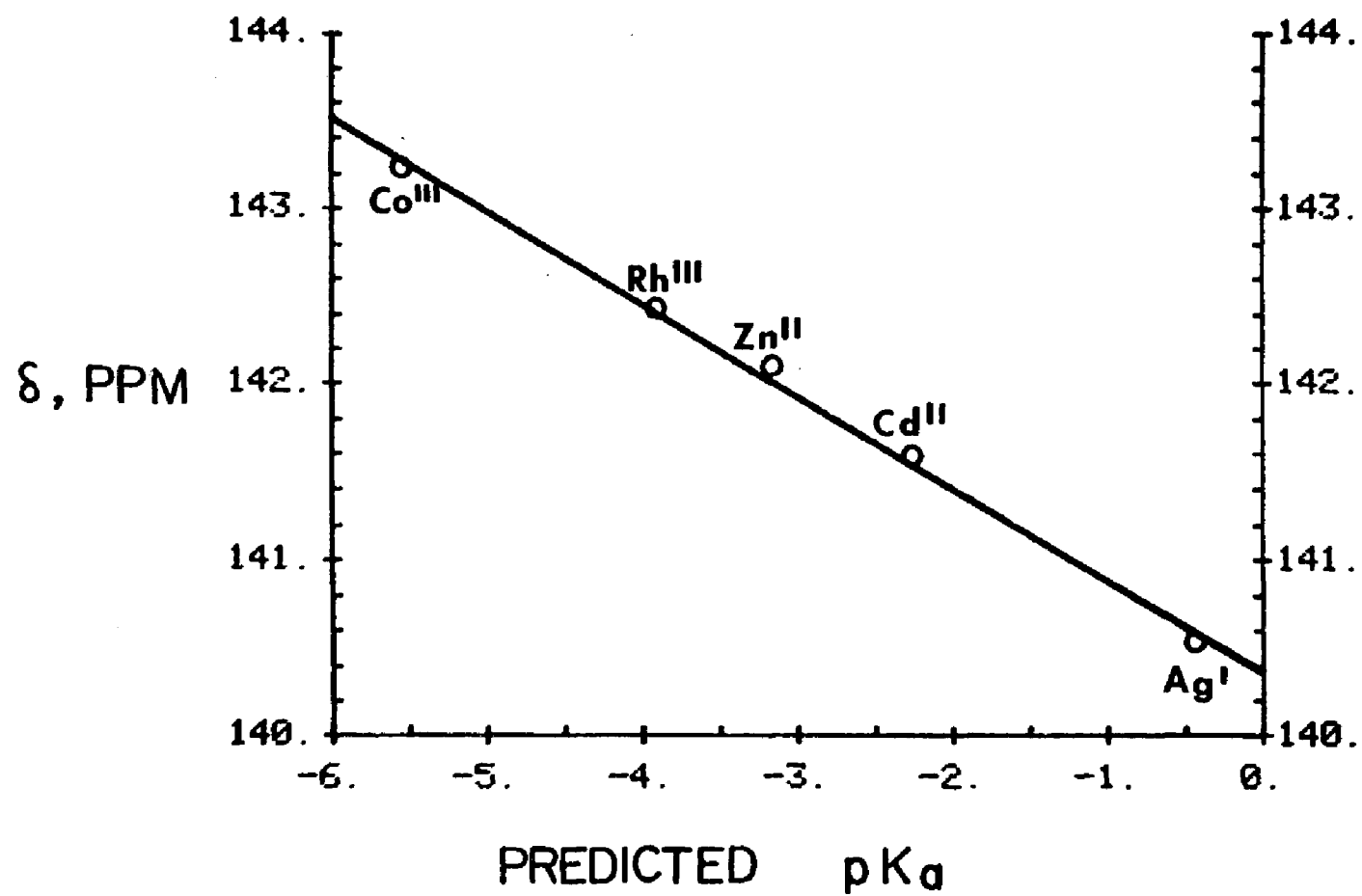


therefore it seemed reasonable to try to relate the predicted  $pK_a$  values with Angell's chemical shifts. This relationship is illustrated in Figure 15B. In this case, the data point for  $HNO_3$  lies much closer to the line connecting the aquated metals (less than 0.5  $pK_a$  units off the line). It should also be noted that the experimental and predicted  $pK_a$  values of  $HNO_3$  lie close together as they should if Duffy and Ingram's optical basicity approach is correct. The predicted  $pK_a$  values show that the metal aquo ions and nitric acid constitute a set of powerful oxidic acids, just as Angell's  $^1H$  NMR data also in effect illustrated.

#### Our Results

In light of the earlier work of Duffy and Ingram(26) and Angell and co-workers(40), we plotted the para position  $^{13}C$  chemical shifts of the metal-pyridine complexes versus predicted  $pK_a$  values based on optical basicity. As can be seen in Figure 16, the correlation between the two is excellent (standard deviation of the linear regression = 0.06677, correlation coefficient = 0.9983). The relationship between  $^{13}C$  NMR chemical shifts at the para position of bound pyridine and calculated  $pK_a$  values lends strong support for the hypothesis that this  $^{13}C$  NMR technique gives quantitative information about the effective electron density or polarizing power of a metal ion, because the  $pK_a$  values calculated

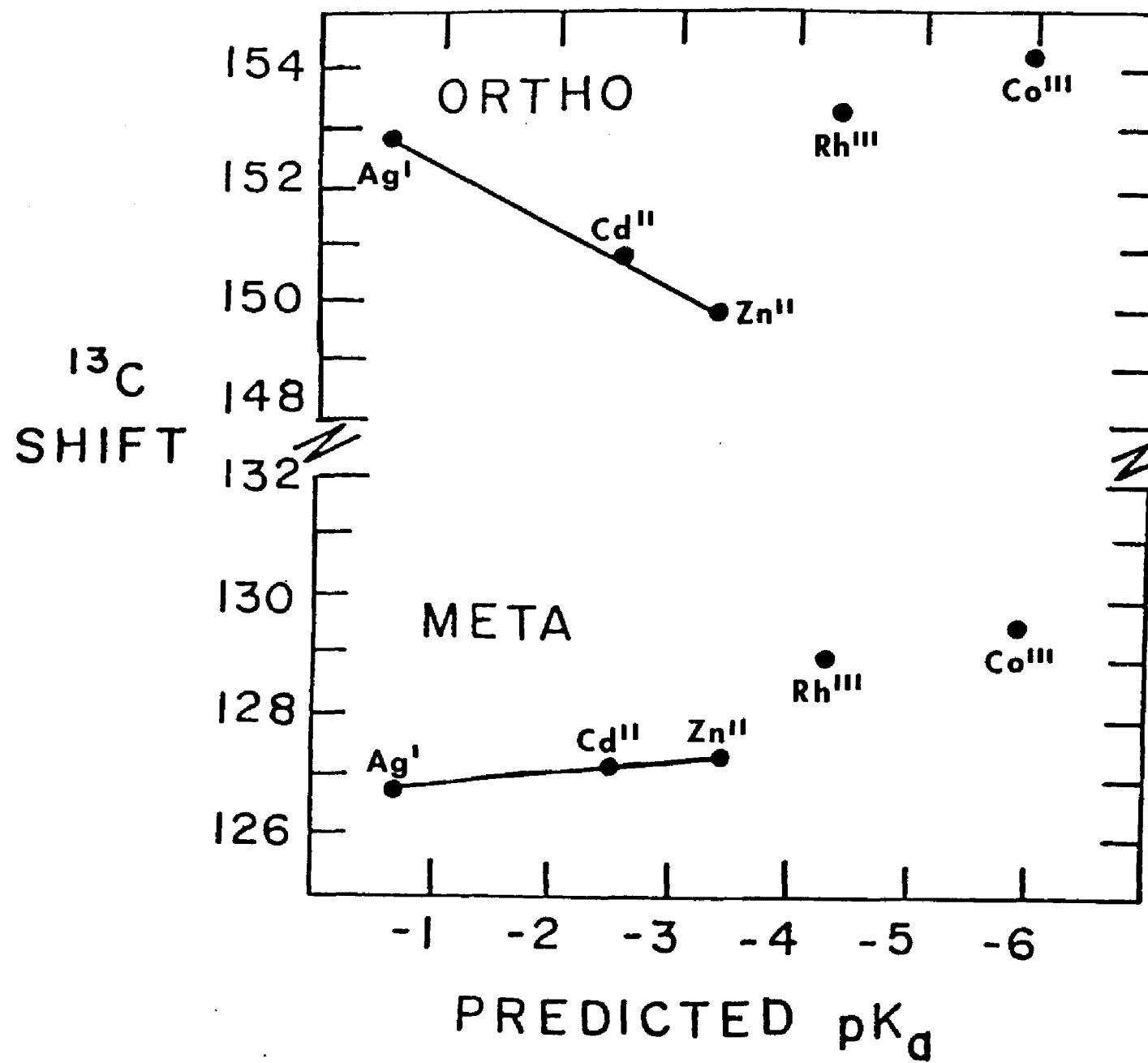
FIGURE 16. THE CORRELATION OF THE  $^{13}\text{C}$  NMR CHEMICAL SHIFTS FOR THE PARA CARBON ATOM OF PYRIDINE COMPLEXES OF  $\text{Co(III)}$ ,  $\text{Rh(III)}$ ,  $\text{Zn(II)}$ ,  $\text{Cd(II)}$  AND  $\text{Ag(I)}$  WITH PREDICTED  $\text{pK}_a$  VALUES. THE OPEN CIRCLES ARE THE INDIVIDUAL DATA POINTS AND THE SOLID LINE REPRESENTS THE CALCULATED LEAST-SQUARES FIT TO THE DATA POINTS (STANDARD DEVIATION OF THE LINEAR REGRESSION = 0.06677, CORRELATION COEFFICIENTS = 0.9983). THE SIZE OF THE CIRCLES INDICATE THE REPRODUCIBILITIES OF CHEMICAL SHIFT DETERMINATIONS.



from optical basicity principles depend primarily on the polarization of water bound in the first coordination sphere by the metal atom. Optical basicity values are derived from the charge and electronegativity of metal ions which are accepted indicators of their polarizing power.

The alpha carbon atom chemical shift seems to correlate well with Duffy and Ingram's predicted  $pK_a$  values for the  $d^{10}$  metal ions (see Figure 17, top portion) but it does so in an inverse manner. The upfield shift (i.e., more shielding) as one goes from Ag(I) to Cd(II) to Zn(II) of the ortho position of bound pyridine can be explained in the same manner as the anomalous upfield shift at the alpha carbon atom of protonated pyridine seen by other groups(6,59,72). In essence, as electron density is removed inductively from the pyridine ring by the metal atom (or proton in the case of protonated pyridine), excess electron density is shifted toward the nitrogen and away from the alpha carbon atom. To counteract this perturbation, electron density is shifted anisotropically toward the alpha carbon mainly from the beta carbon. Because this is an anisotropic effect, it is felt primarily at the alpha carbon, less at the beta carbon and insignificantly at the gamma carbon atom. Because there is an inverse correlation between chemical shift at the ortho position and predicted  $pK_a$  values, this anisotropic effect appears to be related directly to the polarizing power of the metal atoms attached to pyridine. All other effects at the ortho position (i.e., alterations of the anisotropies of

FIGURE 17. THE CORRELATIONS OF  $^{13}\text{C}$  NMR CHEMICAL SHIFTS FOR ORTHO AND META CARBON ATOMS OF PYRIDINE COMPLEXES OF  $\text{Co(III)}$ ,  $\text{Rh(III)}$ ,  $\text{Zn(II)}$ ,  $\text{Cd(II)}$  AND  $\text{Ag(I)}$  WITH PREDICTED  $\text{pK}_a$  VALUES. THE ORTHO  $^{13}\text{C}$  CHEMICAL SHIFTS (TOP PORTION) OF THE  $\text{d}^{10}$  IONS,  $\text{Zn(II)}$ ,  $\text{Cd(II)}$  AND  $\text{Ag(I)}$  CORRELATE STRONGLY WHILE THOSE OF THE  $\text{d}^6$  IONS,  $\text{Co(III)}$  AND  $\text{Rh(III)}$  DO NOT. THE META  $^{13}\text{C}$  CHEMICAL SHIFTS (BOTTOM PORTION) ARE SO SIMILAR FOR THE  $\text{d}^{10}$  IONS THAT THERE IS NO MEANINGFUL CORRELATION WITH PREDICTED  $\text{pK}_a$  VALUES.



the carbon atoms, polarization of the C-H bonds, ring current changes, etc.) either appear to be minor or cancel each other out because of the correlation observed. The shifts of the  $d^{10}$  metal-pyridine complexes at the meta position are virtually identical (see Figure 17, bottom portion). This could mean that the inductive effect of the metal atom on the beta carbon of bound pyridine is more or less balanced by the anisotropic effect caused by the metal atom.

The  $^{13}\text{C}$  chemical shifts for the alpha carbon atom bound to diamagnetic metal ions of the  $d^6$  configuration, such as Co(III) and Rh(III) (Figure 17, top portion), do not show the close correlation of the chemical shift change on complexation with expected polarizing ability of the metal ion as was found for the para  $^{13}\text{C}$  chemical shifts. The origin of this local effect at the ortho position may be temperature independent paramagnetism (T.I.P.), a phenomenon which is pronounced (nearly 0.2 Bohr magnetons(73)) for many low-spin non  $d^{10}$  ions. As a result of participation of open-shell configurations in which unpaired electrons occupy orbitals of different symmetry, through-space contributions to the paramagnetic term of the  $^{13}\text{C}$  chemical shift expression can be significant(74). These effects are expected for octahedral low-spin ions where the lowest energy configuration is  $t_{2g}^6$  but states involving contributions from  $e_g$  orbital occupation are of relatively low energy (i.e., the observed ground state for  $d^6$  ions exhibiting temperature independent paramagnetism involves contributions from such

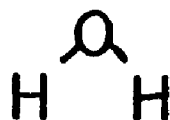
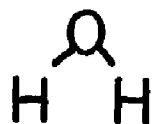
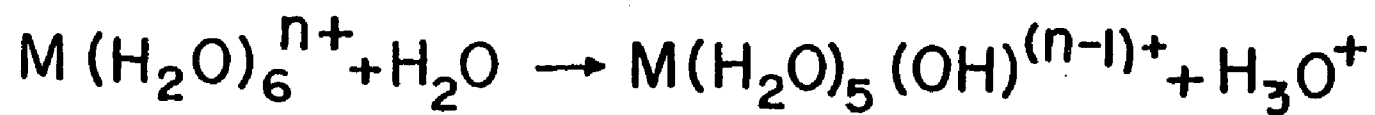
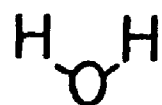
configurations) so that the field-induced mixing in of such low lying excited states (where some of the  $e_g$  orbitals are occupied and unpaired electrons result) with the electronic ground state can occur and T.I.P. is seen.

There were two major assumptions made in this  $pK_a$  study: 1) the replacement of one or two water molecules by one or two pyridine molecules (our probe) did not appreciably change the acidity of the metal-aquo complexes (or if it did change the acidity of these complexes, it did so to the same degree for each metal atom used) and 2) in the case of the Co(III) and Rh(III) complexes, the use of five amines instead of five waters did not significantly change these complexes' acidity. There is some basis for these assumptions. Water, amine and pyridine lie close together in the spectrochemical series. Since the d orbital splittings of these ligands for a given metal ion are approximately the same, then one could say that each of these ligands have about the same effect on the metal ion. There is a second and more compelling reason why these assumptions are mostly likely valid, and that is the correlation between  $^{13}\text{C}$  chemical shifts and predicted  $pK_a$  values, which is based on these assumptions, is excellent. The effect of ligand substitution (when the ligands are similar) on the chemical shift is negligible when compared to the effect of changing metals in different oxidation states.

An important question is why solvation has such an enor-

mously attenuating effect on the true acidity of aquated-metal complexes. A possible explanation was suggested by Duffy and Ingram (26). Keeping in mind that in terms of the pK-optical basicity argument, strong acids are those giving rise to conjugate bases with low  $\lambda_B$  values, it is necessary to consider how solvation affects the optical basicity of conjugate bases. Those bases bearing a negative charge (i.e., derived from oxyacids) will be solvated by the positive ends of the water dipoles (see Figure 18, upper reaction) and the overall basicity will be reduced, thereby stabilizing these conjugate bases. However, in the case of metal-aquo complexes, the conjugate bases formed will bear a positive charge ( $[M(H_2O)_5(OH)]^{(n-1)+}$ ) and therefore will be solvated by the negative ends of the water dipoles (see Figure 18, lower reaction). This can be viewed in terms of hydrogen bonding between the oxygen atoms of the bulk water molecules and the hydrogen atoms on the surface of the inner-sphere water molecules. The basicity-moderating effect of these hydrogen atoms, is no longer focused towards the bound water molecules but rather it is dissipated throughout the solvent. This results in the enhancement of the basicity of the hydroxide group in the first coordination sphere and therefore the original aquo complex behaves as a weaker acid. In the case of many aquated-metal complexes, this effect severely depresses the acidity that would be seen if the outer-sphere bulk water was not present. The  $^{13}C$  NMR chemical shift method can measure the

FIGURE 18. A COMPARISON OF THE EFFECT OF OUTER SPHERE SOLVATION ON THE CONJUGATE BASE OF THE OXYACID,  $\text{HNO}_3$  AND ON THE CONJUGATE BASE OF METAL-AQUO COMPLEXES. A BASE BEARING A NEGATIVE CHARGE, SUCH AS  $\text{NO}_3^-$  (UPPER REACTION), WILL BE SOLVATED BY THE POSITIVE ENDS OF THE  $\text{H}_2\text{O}$  DIPOLES. BASES BEARING A POSITIVE CHARGE, SUCH AS  $[\text{M}(\text{H}_2\text{O})_5\text{OH}]^{(n-1)+}$  (LOWER REACTION), WILL BE SOLVATED BY THE NEGATIVE ENDS OF THE  $\text{H}_2\text{O}$  DIPOLES.



polarizing power of metal atoms through coordinated ligands without having to account for outer-sphere effects.

#### LIMITATIONS OF THE $^{13}\text{C}$ CHEMICAL SHIFT METHOD TO ANALYZE ELECTRON DENSITY CHANGES

Although we found a direct correlation between  $^{13}\text{C}$  NMR chemical shifts at the para position of bound pyridine and predicted  $\text{pK}_a$  values based on optical basicity values which substantiates earlier empirical (6,7,25,30) and theoretical (8,31-37) work, the relatively small chemical shift range found in this study (the chemical shift difference from Co(III) to Ag(I) is 2.89 ppm) limits the general usefulness of this  $^{13}\text{C}$  technique. Large differences in metal ion polarizing ability, such as differences due to metal atoms of different charge, are easily observable using this technique. However, small changes in effective electron density, such as the substitution of one ligand for another on a metal of a certain charge, may not be seen in the chemical shifts of the two complexes. The difference in chemical shifts would most likely fall within the reproducibility of the experiment.

The empirical correlation of  $^{13}\text{C}$  NMR chemical shifts of bound pyridine may be of some interest, however, to investigate the difference in polarizing ability of metal atoms of different oxidation states which are involved in oxidative-addition/reductive-elimination mechanisms of homogeneous

catalysis. Although oxidation states of catalysts change during the oxidative-addition or reductive-elimination steps, no firm evidence has been presented that would indicate changes in polarizing power, i.e., the effective electron density of the metal atom. The oxidation state is a bookkeeping device (to keep charges balanced) rather than a direct indication of the environment of the metal atom of a catalyst that changes during these processes. One could substitute pyridine or benzene for an equivalent ligand of a metal-containing catalyst and observe the chemical shift of the species before and after the oxidative-addition or reductive-elimination steps have occurred. By finding the chemical shift of standards (i.e., the same metal atom with the probe molecule attached and in the two proposed oxidation states of the catalyst) and comparing these shifts to the shifts of the catalyst, one would have a way to determine whether the effective electron density of the metal in the catalyst had actually changed during the oxidative-addition or reductive-elimination steps.

Systems of interest in this regard are pyridine-containing complexes of Rh(I)/Rh(III) and Ir(I)/Ir(III) for which olefin hydrogenation catalysis has been observed by Crabtree and Morris(43). Such complexes as  $[M(\text{cod})L(\text{py})]\text{PF}_6$ , where  $M = \text{Rh(I)}$  or  $\text{Ir(I)}$ ,  $\text{cod} = \text{cyclooctadiene}$  and  $L = \text{PR}_3$ , are interesting because some are remarkably active hydrogenation catalysts for variously substituted olefins; on the scale of two to three orders of magnitude greater in rate than the

commonly used Wilkinson's catalyst  $[\text{RhCl}(\text{PPh}_3)_3]$ . Also, the probe ligand is already on the catalyst so that model compounds (which may change the reactivity and perhaps even the mechanism) will not have to be made. Finally, some of the hydrido intermediates have been isolated (with the metals in the +3 state) (42). Using our correlation of  $^{13}\text{C}$  chemical shifts and polarizability of a metal atom, one may be able to relate the relative effectiveness of the rhodium and iridium catalysts of Crabtree and Morris with that of Wilkinson's catalyst to either the ability of the metal ions to polarize the ligands differently in the two oxidation states or the difference in effective electron density around the metal atoms when going through the catalytic cycle. One may then begin to predict the effectiveness of possible catalysts by effective electron density arguments.

## CONCLUSION

Our study of five diamagnetic pyridine-bound metal complexes indicates a high correlation of the  $^{13}\text{C}$  NMR chemical shifts with predicted  $\text{pK}_a$  values based on optical basicity arguments. This correspondence supports the concept that the polarization of ligands in the first coordination sphere is proportional to the electrostatic potential of the metal ion. If the  $\text{pK}_a$  of an aquated metal complex were due simply

to the polarization of the bound water molecule by the metal atom, then the experimentally determined  $pK_a$  value could be directly related to optical basicities or  $^{13}\text{C}$  chemical shifts of the probe molecule. However, the considerable effect of bulk water molecules outside the first coordination sphere refutes the validity of explanations of  $pK_a$  values based solely on inner-sphere effects. This  $^{13}\text{C}$  NMR technique has many advantages over the systems of molten salt hydrates and concentrated aqueous solutions used by Duffy and Angell, respectively, in that a greater variety of complexes can be synthesized, the conditions required are much less stringent, and outer-sphere effects can be disregarded. Although many systems involving ligand substitution on a particular metal of a certain charge most likely cannot be studied by this technique due to the relatively small range of chemical shift differences among metal-pyridine complexes, certain selected systems that undergo a reasonably large polarizability change at the metal center can easily be investigated.

Work that could be investigated in the future includes:

- 1) examination of the hydrogenation catalyst synthesized by Crabtree and Morris, to see if a large polarizability difference exists at the metal center as the catalyst goes through its cycle;
- 2) trial using the same metal pyridine complexes used in this study in nonaqueous, nonprotic solvents, to see if a larger chemical shift range is observed (i.e., the chemical shift range may be depressed in water

due to its leveling effect on pyridine); and 3) Angell's work using concentrated aqueous solutions could be repeated using this  $^{13}\text{C}$  NMR technique, to see if our results reproduce his results.

APPENDIX A

LISTING OF THE SUBROUTINE "CUBIC"

```
SUBROUTINE CUBIC(A,ZZ,NSETS)
C   THIS SUBROUTINE WILL SOLVE A CUBIC EQUATION AND GIVE
    THE THREE ROOTS (REAL OR COMPLEX)
    DOUBLE PRECISION A(4),ZZ(3)
    DOUBLE PRECISION Q,R,D,B,C,E,ANGLE,THETA,
    COSVAL,SINT,S,TINT,T
C   SET THE MEMBERS OF ARRAY 'ZZ' EQUAL TO ZERO
    DO 1 J=1,3
      ZZ(J)=0.0
1  CONTINUE
C   OPTIONAL LISTING OF THE COEFFICIENTS IN THE ARRAY 'A'
    IF (NSETS.EQ.0) GO TO 2
    WRITE(6,3) A
3  FORMAT(1H0,15X,'A(1) = ',1PD23.15,/,30X,'A(2) = ',
    1PD23.15,/,45X,'A(3) = ',1PD23.15,/,60X,'A(4) = ',
    1PD23.15) Q=(3.*A(3)-A(2)*A(2))/9.
    R=(9.*A(2)*A(3)-27.*A(4)-2.*A(2)*A(2)*A(2))/54
    D=Q*Q*Q + R*R
C   OPTIONAL PRINTOUT OF VARIABLE, D
    IF (NSETS.EQ.0) GO TO 14
    WRITE(6,5) D
5  FORMAT(1H0,30X,'D = ',1PD23.15)
C   TEST OF WHETHER ROOTS ARE REAL OR COMPLEX
```

```

14 IF (D) 6,11,11
C   THIS IS A TRIG. SOLUTION TO FIND THE THREE REAL ROOTS
6 B=(3.*A(3)-A(2)*A(2))/3.
   C=(2.*A(2)*A(2)*A(2)-9.*A(2)*A(3)+27.*A(4))/27.
   E=2.*DSQRT(-(B)/3.)
   ANGLE=(3.*C)/(B*E)
   THETA=DARCOS(ANGLE)/3.
   COSVAL=DCCS(THETA)
C   OPTIONAL PRINTOUT OF VARIABLES, THETA AND COSVAL
   IF(NSETS.EQ.0) GO TO 7
   WRITE(6,8)THETA,COSVAL
8  FORMAT(1H0,30X,'THETA = ',1PD23.15,/,30X,
   'COSVAL = ',1PD23.15)
7  ZZ(1)=E*CCSVAL - A(2)/3.
   ZZ(2)=E*DCOS(THETA + 2.094395) - A(2)/3.
C   NOTE: 2.094395 = (2.*PI)/3. = 120 DEGREES
   ZZ(3)=E*DCOS(THETA + 4.188790) - A(2)/3.
C   NOTE: 4.188790 = (4.*PI)/3. = 240 DEGREES
C   OPTIONAL PRINTOUT OF THE THREE ROOTS IN ZZ ARRAY
   IF(NSETS.EQ.0) GO TO 4
   WRITE(6,10) ZZ
10  FORMAT(1H0,30X,'ZZ(1) = ',1PD23.15,/,30X,'ZZ(2) = ',
   1PD23.15,/,30X,'ZZ(3) = ',1PD23.15)
   GO TO 4
11 SINT=R + DSQRT(D)
   IF(SINT) 16,16,17
16 S=-(DABS(SINT)**.3333333)

```

```
      GO TO 9
17 S=SINT** .3333333
      9 TINT=R - DSQRT(D)
      IF (TINT) 19,19,20
19 T=-(DABS(TINT)** .3333333)
      GO TO 21
20 T=TINT** .3333333
C      OPTIONAL PRINTOUT OF VARIABLES, S AND T
21 IF (NSETS.EQ.0) GO TO 12
      WRITE(6,13) S,T
13 FORMAT(1H0,30X,'S = ',1PD23.15,/,30X,'T = ',1PD23.15)
12 ZZ(1)=S + T - A(2)/3.
      ZZ(2)=0.0
      ZZ(3)=0.0
C      OPTIONAL PRINTOUT OF THE THREE ROOTS IN ZZ ARRAY
      IF (NSETS.EQ.0) GO TO 4
      WRITE(6,15) ZZ(1)
15 FORMAT(1H0,30X,'ZZ(1) = ',1PD23.15,/,30X,
      'ZZ(2) AND ZZ(3) = 0.0')
4 RETURN
      END
```

APPENDIX B

LISTING OF THE SUBROUTINE "YP9"

```
SUBROUTINE YP9 (I)
C   FITTING CHEMICAL SHIFT DATA TO EFFECTIVE ELECTRON
    DENSITY OF METAL IONS
    DIMENSION Y( 200) , X( 5,  200) , W( 200) , IX(40) ,
    PG(40) , DUM(100) , P(40) , SP(40) ,
    YC( 200) , DY( 200) , BM(40, 41)
    DIMENSION ALAB(20) , PART(40) , Z(5)
    DOUBLE PRECISION A(4) , ZZ(3)
    DOUBLE PRECISION DEN1 , DEN2 , DENTOT , TERM1 ,
    HYDCUB , NUM1 , NUM2
    DOUBLE PRECISION NUM3 , HPY , EM , RAT1 , RAT2 , RAT3A , RAT3B
    COMMON N , IK , IW , M , IB , ITEST , IDUM , NDUM , IPR , IFG , IM , YT , TEST
    COMMON WVAR , SSQ , IDF , DET , ISW , IPLT , ISC
    COMMON Y , X , W , IX , PG , DUM , P , SP , YC , DY , BM , ALAB , Z , INTT , PART
    NSETS=0
    IF (I) 13, 14, 14
13 WRITE(6,2)
    2 FORMAT(41H0  Y = P(1)*Z(1) + P(2)*Z(2) + P(3)*Z(3)  )
    GO TO 4
14 CONTINUE
    DEN1 = P(2)*P(3)*P(1)*P(1)*P(1)
    DEN2 = P(2)*P(3)*P(1)*P(1)*Z(1)
    DENTOT = DEN1 + DEN2
```

```

TERM1 = P (2)*P (1)*P (1)*Z (1)*Z (1)
HYDCUB = Z (1)*Z (1)*Z (1)
NUM1 = TERM1/Z (1) - TERM1*P (3)*Z (2)/Z (1) +
TERM1/P (1) + 2.*TERM1*P (3)*Z (3)/Z (1)
NUM2 = HYDCUB - TERM1*Z (2)/P (1) + P (1)*Z (1)*Z (1) +
TERM1*Z (3)/P (1)
NUM3 = HYDCUB*Z (2)
A (1) = 1.0E+00
A (2) = NUM1/DENTOT
A (3) = NUM2/DENTOT
A (4) = - (NUM3/DENTOT)
C   CALL THE SUBROUTINE CUBI C
    CALL CUBIC (A,ZZ,NSETS)
    DO 1 J=1,3
      IF (ZZ(J)) 1,1,3
    3 HPY = ZZ (J)
    1 CONTINUE
C   OPTIONAL PRINTOUT OF VARIABLE HPY
    IF (NSETS.EQ.0) GO TO 7
    WRITE (6,8) HPY
    8 FORMAT(1H0,30X,'HPY = ',1PD23.15)
    7 EM = Z (3)/(1 + P (2)*P (1)*HPY/Z (1) +
      TERM1*P (3)*HPY*HPY/(HYDCUB*Z (1)))
C   OPTIONAL PRINTOUT OF VARIABLE EM
    IF (NSETS.EQ.0) GO TO 9
    WRITE (6,10) EM
    10 FORMAT(1H0,30X,'EM = ',1PD23.15)

```

```
9 RAT1 = P (1) *HPY/ (Z (1) *Z (2))
  RAT2 = HPY/Z (2)
  RAT3A = RAT1*P (2) *EM
  RAT3B = DEN2*2.*EM*RAT2*HPY/HYDCUB
C   THE EQN IS: DEL (EXP) = (PY/PY (0)) *DEL (PY) +
    (HPY/PY (0))
C   *DEL (HPY) + (MPY1/PY (0) + MPY2/PY (0)) *DEL (MPY)
    YT = RAT1*P (4) + RAT2*P (5) + (RAT3A + RAT3B) *P (6)
C   OPTIONAL PRINTOUT OF VARIABLE YT
    IF (NSETS.EQ.0) GO TO 11
    WRITE (6,12) YT
12 FORMAT(1H0,30X,'YT = ',1PD23.15)
11 PART (2) = (RAT3A/P (2) + RAT3B/P (2)) *P (6)
    PART (3) = (RAT3B/P (3)) *P (6)
    PART (6) = RAT3A + RAT3B
4   RETURN
    END
```

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P A R T    I I

C H A P T E R  1

USE OF  $^{109}\text{Pd}$ -HEMATOPORPHYRIN FOR  
SELECTIVE LYMPHATIC ABLATION

## INTRODUCTION

The major problems in transplantation remain rejection and infection. Attempts to prevent rejection by generalized immune suppression may leave the host susceptible to attack by bacterial, viral, or protozoan organisms. This therapeutic dilemma has been the stimulus to devise more selective approaches to immune suppression.

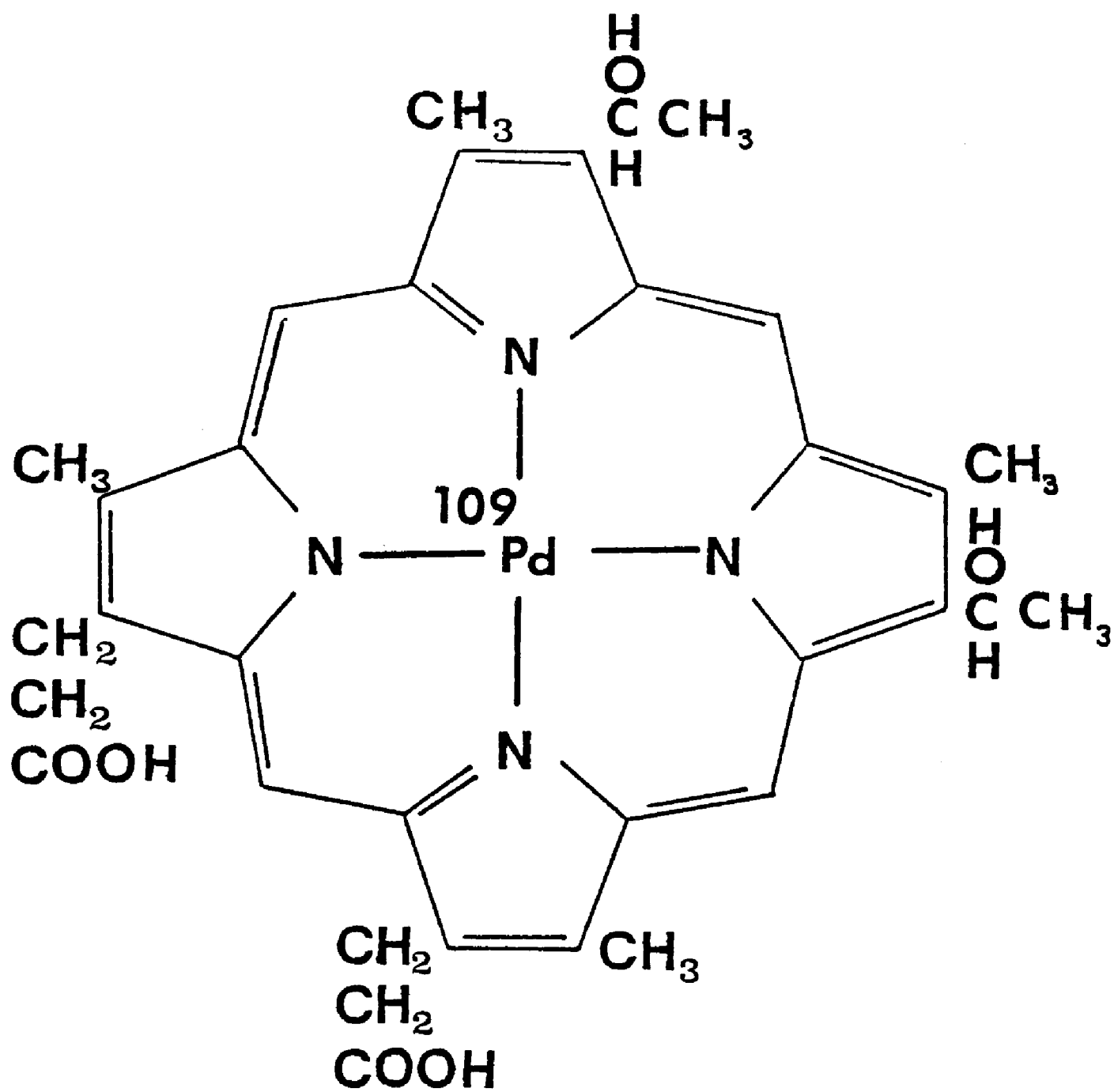
Various porphyrins and metalloporphyrins show marked affinity for lymphatic tissue(1-3). Since lymphatic tissue plays an important role in mediating the immune process responsible for homograft rejection(4), the possibility arose of using radioactive metalloporphyrins for selective lymphatic ablation to control homograft rejection.

Certain criteria should be met for the successful use of internally administered radioactive compounds to control the rejection of transplanted tissue. While procedures directed at total destruction of lymphoid tissue have been shown to decrease the probability of homograft rejection, the resulting generalized immunologic crippling has severely limited the usefulness of these methods(5,6). It would be desirable to selectively destroy lymphoid tissue immunologically committed to react to the antigens of the homograft and to

spare immunologically uncommitted lymphoid tissue which would tolerate the homograft and reconstitute the immune apparatus in the presence of the graft. Evidence indicates that lymphocytes derived from the thymus (thymocytes) may be immunologically uncommitted. They do not respond to antigenic stimulation(7-11), and it takes a thymocyte about 6 weeks after its release from the thymus to gain full immunologic competence(12). Since lymph nodes and the spleen contain a significant number of committed cells(13-16), it was thought that a radioactive compound showing large concentrations in these tissues and relatively lower concentrations in the thymus would be of potential value in the control of rejection of transplanted tissue. In addition to providing large doses of radiation to the lymph nodes and spleen, the radionuclide must show relatively lower concentrations in such critical and radiosensitive organs as intestinal mucosa and bone marrow and must release its energy locally as do alpha-, beta- or low-energy gamma-emitting radionuclides.

Fawwaz, Hemphill and Winchell(17) demonstrated that hematoporphyrin labelled with palladium-109 localizes in lymphoid tissues of rats. Pd-109 is a predominantly beta-emitting radionuclide of energy 1.028 MeV with radiobiologic activity over a scatter area of 1 mm and a half life of 13.43 hours. Hematoporphyrin IX is derived from the naturally occurring metalloporphyrin, iron protoporphyrin (protophemine), by the acid catalyzed removal of the iron and hydration of the 2- and 4- vinyl groups. Figure 1 shows the

FIGURE 1. THE STRUCTURE OF  $^{109}\text{Pd}$ -HEMATOPORPHYRIN.



structure of the  $^{109}\text{Pd}$ -hematoporphyrin radiopharmaceutical drug. The  $^{109}\text{Pd}$ -hematoporphyrin is able to effect selective lymphatic and central bone marrow ablation by its ionizing irradiation. The biodistribution studies shows that the radionuclide has minimal concentration in the gastrointestinal mucosa, distal bone marrow, peripheral blood and in the thymus. Histological examinations of all organs demonstrate that effective cellular (lymphocyte) depletion can be produced rapidly (within 6 days) in all lymphoid organs and in central bone marrow with doses of radioactivity greater than 24  $\mu\text{Ci/kg}$ . Depletion of peripheral circulating leukocytes and lymphocytes was incomplete at all doses tested, and little depletion was seen in the thymus. Recovery began at 15 days and was complete at 60 days after the administration of the radionuclide. The use of palladium-109 alone has no significant effect on heart allograft survival. However, with the addition of two injections of antilymphocyte serum to further deplete peripheral circulating lymphocytes, complete and apparently specific immunologic unresponsiveness to heart allografts occurs in a weakly histocompatible strain combination and significant allograft prolongation occurs in a strongly histocompatible strain combination(18). It was later determined by Hardy, et al.(19), that a sublethal dose of  $^{109}\text{Pd}$ -hematoporphyrin(30  $\text{mCi/kg}$ ), two doses of rabbit antirat lymphocyte globulin (10 mg each) and an allogeneic bone marrow transplant ( $10^8$  cells) resulted in an indefinite prolongation of a major histocompatible strain

combination.

The next logical step in the testing of this selective lymphoid irradiation technique is to do further studies in larger animals in order to confirm the safety of the method and to allow the estimation of the distribution of the radiation dose in higher animals. However, the traditional method for preparation of the palladium-109 complex (i.e., combination of a palladium salt and the free base porphyrin in glacial acetic acid and sodium acetate (20)) results in considerable loss of activity. If this complex is to be applied to lymphatic ablation in human subjects or even large animals like dogs, such a loss of activity would require prohibitively large amounts of initial palladium-109 activity. In addition, a multi-step process involving transfers of material, precipitations, redissolutions and filtering would be impractical on a routine basis.

Studies of N-alkylporphyrins done by Lavalley, et al., (47) have indicated that the rates of the metalation and subsequent dealkylation reactions of these nonplanar porphyrins can be several orders of magnitude faster than the rates of metalation of the corresponding planar porphyrins. With this in mind, we have developed a synthesis for the preparation of N-methylhematoporphyrin. Experiments of the reaction between this N-methylporphyrin and dichloropalladium(II) dihydrate in dimethylsulfoxide have shown that the formation of the desired Pd-hematoporphyrin complex occurs

within seconds at 80°C.

Technical details had to be worked out at Brookhaven National Laboratory for the appropriate manner of handling the large amounts of radioactivity (i.e., greater than one Curie) being emitted by the 109 isotope of palladium after it had been bombarded by neutrons in the Brookhaven High Flux Beam Reactor. A shielded "cave" was designed with the proper apparatus so that all manipulations with the radioactive palladium could be done remotely. The synthetic method of preparation of  $^{109}\text{Pd}$ -hematoporphyrin was varied so that the highest recovery of radioactivity in the final  $^{109}\text{Pd}$ -hematoporphyrin solution (to be injected) could be accomplished.

The animal studies at Columbia Presbyterian Medical Center were carried out using beagles. Three types of animal studies were conducted: distribution, toxicity and transplantation. Distribution and toxicity studies were carried out and compared to studies done using the traditional synthesis of Pd-hematoporphyrin(20). The results of these comparison studies were favorable so transplantation experiments were attempted. These experiments have been promising but are not conclusive. Additional transplantation experiments must be conducted before it is determined whether this selective lymphoid irradiation technique will be tested clinically on humans.

## EXPERIMENTAL PROCEDURES

### SYNTHESIS OF PALLADIUM-HEMATOPORPHYRIN

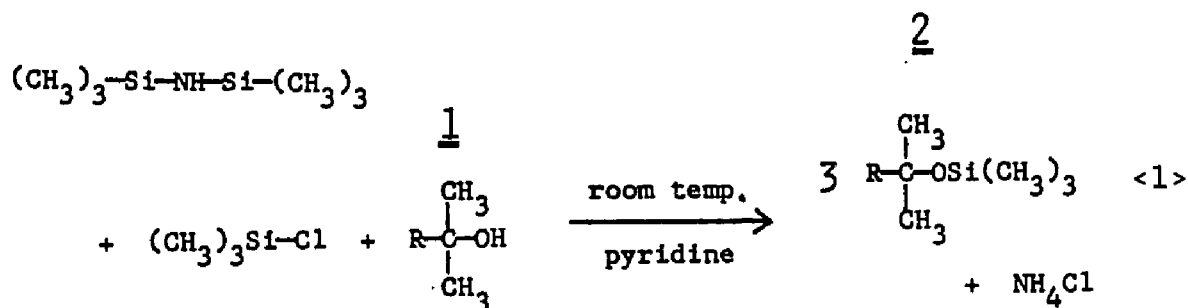
Hematoporphyrin IX free base and hematoporphyrin IX dimethyl ester were purchased from Man-Win Chemicals. The N-methylhematoporphyrin precursor was synthesized by the procedure described below.

#### Synthesis Of The N-methylhematoporphyrin Precursor

Blocking the alcohol groups on  
the periphery of the porphyrin

Trimethylsilyl ethers, readily prepared from alcohols by treatment with a variety of silylating agents, have recently found considerable use in the protection of alcohols. They are thermally stable and also reasonably stable to many organometallic reagents. They are easily cleaved by hydrolysis in acid or base, or by treatment with fluoride ion(63). An attempt to block the hematoporphyrin alcohol side groups involved the use of the silylating reagents, hexamethyldisilazane and trimethylchlorosilane.

The reaction sequence is given below:



where  $\underline{1}$  is hematoporphyrin IX-dimethyl ester and  $\underline{2}$  is the silyl porphyrin ester.

The procedure is as follows.

One gram (1.60 mmoles) of hematoporphyrin IX-dimethyl ester was put into a 100 ml round-bottom flask. Thirty milliliters of dry pyridine were added, dissolving the porphyrin and leaving a very dark red solution. A rubber septum was attached to the top of the round-bottom flask. A needle attached to dry N<sub>2</sub> was inserted through the septum into the solution and the solution was deaerated. With gas-tight syringes, 5.75 ml (27.6 mmoles) of hexamethyldisilazane and 2.88 ml (22.4 mmoles) of trimethylchlorosilane were added simultaneously through the rubber septum. The flask was shaken for 30 seconds and let stand in the dark for five minutes. The progress of the reaction was checked with TLC using silica plates and dichloromethane/acetonitrile as the solvent system. Most of the solvent and the excess silylating reagents were taken off by means of a rotary evaporator. To remove the rest of the unwanted materials, the flask was put on a vacuum line and pumped under high vacuum while the flask was warmed slightly. The silyl-blocked porphyrin was dissolved in dichloromethane but the NH<sub>4</sub>Cl remained a solid and was filtered off by using a medium fritted glass funnel. The major portion of the dichloromethane was evaporated off with dry N<sub>2</sub> and the rest was removed on a vacuum line under high vacuum. The silylated material was used directly for the methylation procedure.

Further characterization procedures (i.e., mass spectroscopy, elemental analysis) were deemed unnecessary because of

the successful completion of the next step.

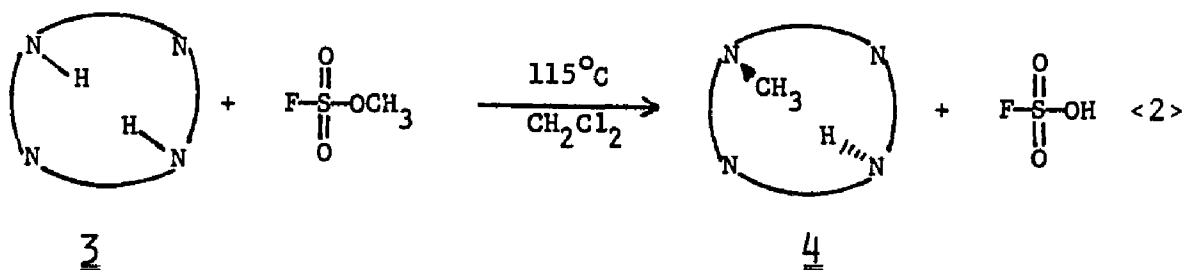
The initial attempt at blocking the two alcohol groups of hematoporphyrin IX-dimethyl ester involved the use of the blocking group, MEM chloride ( $\beta$ -methoxyethoxymethyl chloride). This is a widely used alcohol-protecting reagent. MEM ethers are stable to a variety of reaction conditions and are selectively cleaved under aprotic conditions in the presence of a wide range of alcohol-protected moieties(64). The procedure used is described below.

Ten to fifteen milliliters of dry THF was introduced into a dry 50 ml round bottom flask with a side arm. Dry  $N_2$  was bubbled into the solvent through the side arm. One hundred mg of hematoporphyrin IX-dimethyl ester (.1596 mmoles) was added, forming a dark red solution. Then, 16.85 mg of NaH (a 10% excess) was put into the reaction flask. The flask was kept at  $0^\circ C$  for two hours and allowed to warm to room temperature and left for an additional two hours. Using a microtitrator, 46.8 microliters (.4099 mmoles or a 2.57 fold excess) of the MEM chloride protecting agent was added. The solution was stirred for 20 minutes in an ice bath.

The progress of the reaction was monitored by thin layer chromatography using silica gel plates. Many different solvent systems were tried (e.g., THF, THF/ $CH_2Cl_2$ ,  $CHCl_3$ /heptane,  $CHCl_3$ ) but a difference in R value was never found between the starting material and reaction mixture. Different reaction conditions were tried but again, no change was evident and infrared spectra were identical to those of the starting material. It appears that this reaction to block the alcohol group never proceeded.

# N-methylation of the silyl-blocked hematoporphyrin IX-dimethyl ester

After the alcohol groups of hematoporphyrin IX-dimethyl ester have been blocked by the silyl groups, the next step to form the N-methylhematoporphyrin precursor is to N-methylate the porphyrin with "Magic Methyl" (methylfluorosulfonate). The reaction is shown below.



where 3 is the blocked hematoporphyrin IX dimethyl ester and 4 is the N-methyl porphyrin. The procedure is as follows:

The silyl-blocked hematoporphyrin IX-dimethyl ester was dissolved in a minimum of dichloromethane and transferred to a pressure vessel which was designed for N-methylation of porphyrins(62). The volume of dichloromethane was brought up to 40 ml. With a microtitrator, 148.6 microliters of "Magic Methyl" (1.84 mmoles or a 15% excess) was added dropwise to the stirring porphyrin solution. The pressure vessel was sealed and put into an oil bath heated to  $115^\circ\text{C}$ . The vessel was left stirring in the oil bath for 1.5 hours. The solution was allowed to cool to room temperature after which 40 ml of 6N  $\text{NH}_4\text{OH}$  was prepared and carefully added to the porphyrin solution to neutralize the excess "Magic Methyl". The solution was then washed with water using a 125 ml separatory funnel until the washings were neutral to pH paper. The solvent was removed with a rotary evaporator and the material was further dried using high vacuum on the vacuum line while the flask was slightly warmed.

A 60-200 mesh silica gel column was made up (19 cm in length and 3.5 cm in width) by first putting a

plug of glass wool on the bottom of the glass column and then adding a layer of sand. The silica gel was put in a beaker of dichloromethane and poured into the column that was half filled with dichloromethane. A layer of sand was then placed on top of the silica gel. About 300 ml of dichloromethane was washed through the column. Approximately half of the porphyrin material (500 mg) was dissolved in a minimum of dichloromethane and placed on top of the silica-gel column. The material stayed at the top of the column with this solvent. The solvent was changed to a 1:10 acetonitrile:dichloromethane solvent system. Immediately a blood red band about 3.5 cm in width began to come down the column. A spectrum was taken of this first fraction and was shown to be the hematoporphyrin IX-dimethyl ester starting material (see Figure 2). The solvent system was changed to tetrahydrofuran (THF). A very intense brownish band began to come down the column. Unfortunately, this band streaked the entire column so that all the column had a brown color. However, the majority of the material came off in this fraction with 150 ml of THF. The eluent had only a light yellowish tinge after that. A spectrum of this fraction (see Figure 3) when compared to other N-methyl porphyrins indicated that this fraction was indeed the N-methylhematoporphyrin IX-dimethyl ester. A third fraction was taken off with methanol. A spectrum was taken of this fraction with and without adding lutidine (2,6-dimethylpyridine). Without lutidine, the spectrum resembles a combination of unprotonated and protonated N-methylhematoporphyrin IX-dimethyl ester. Upon addition of lutidine, the spectrum reverted to that of deprotonated N-methylhematoporphyrin IX-dimethyl ester.

The yield of product after these first two steps was ca. 70%.

FIGURE 2. THE VISIBLE SPECTRUM OF SILYL-BLOCKED  
HEMATOPORPHYRIN IX-DIMETHYL ESTER IN  
 $\text{CH}_2\text{Cl}_2$ . THE MATERIAL CAME OFF A SILICA  
GEL CHROMATOGRAPHIC COLUMN AS THE FIRST  
FRACTION WITH 1:10  $\text{CH}_3\text{CN}:\text{CH}_2\text{Cl}_2$ .

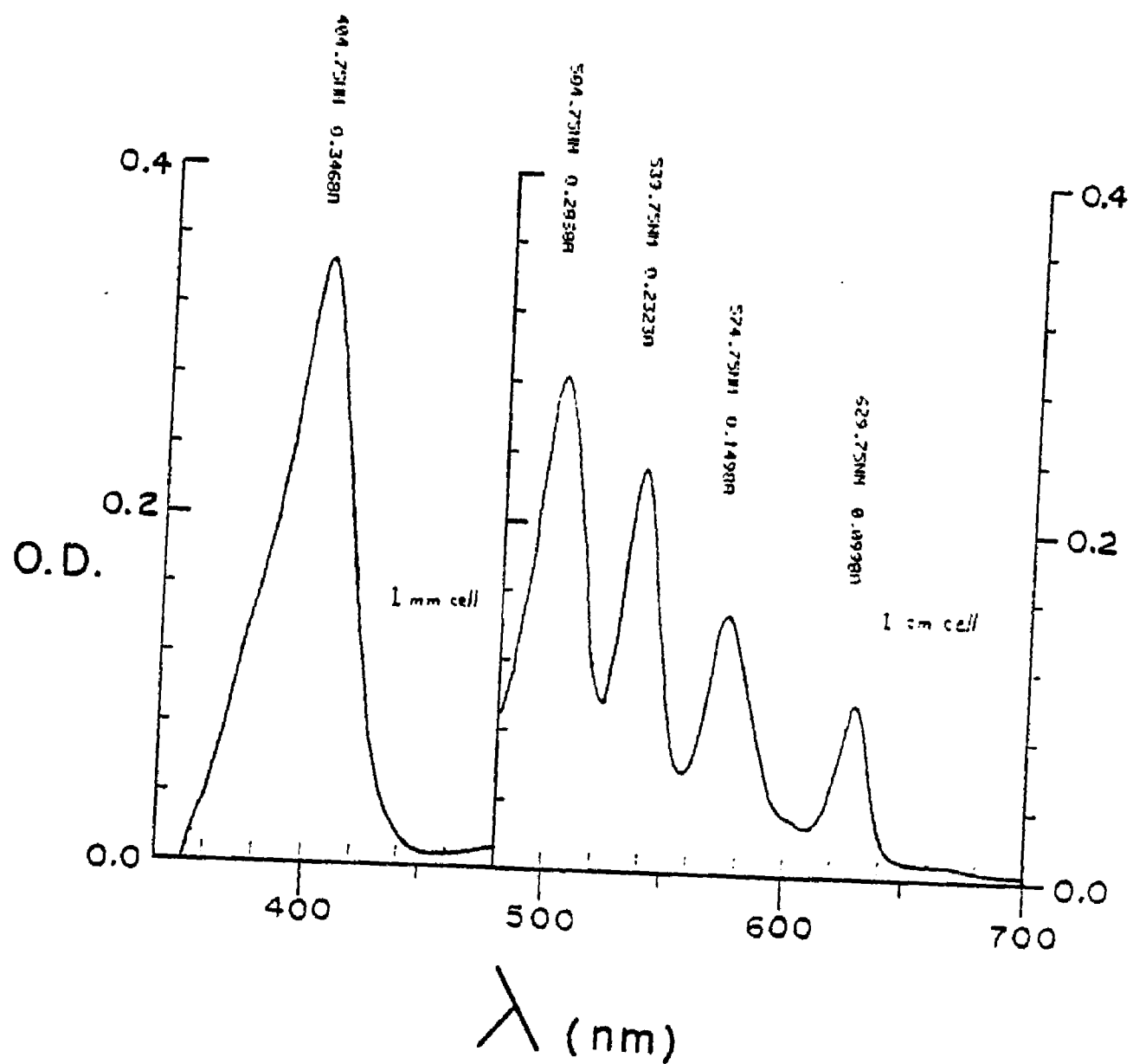
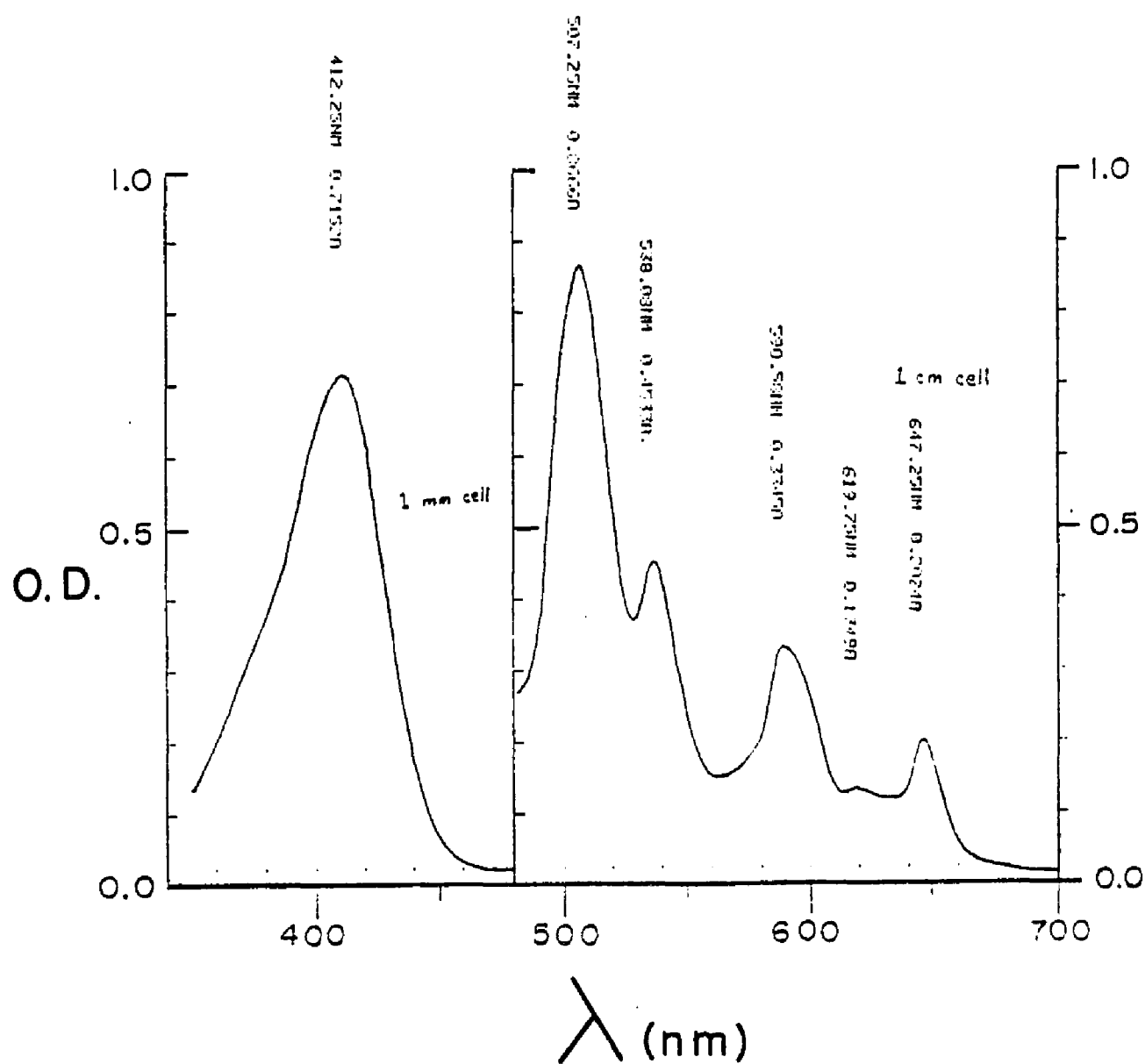


FIGURE 3. THE VISIBLE SPECTRUM OF SILYL-BLOCKED OF  
N-METHYLHEMATOPORPHYRIN IX-DIMETHYL ESTER  
IN  $\text{CH}_2\text{Cl}_2$ . THIS MATERIAL CAME OFF AS THE  
SECOND FRACTION OF A SILICA GEL COLUMN  
WITH THF.



Hydrolysis of the silyl-blocked  
N-methylhematoporphyrin IX-dimethyl ester

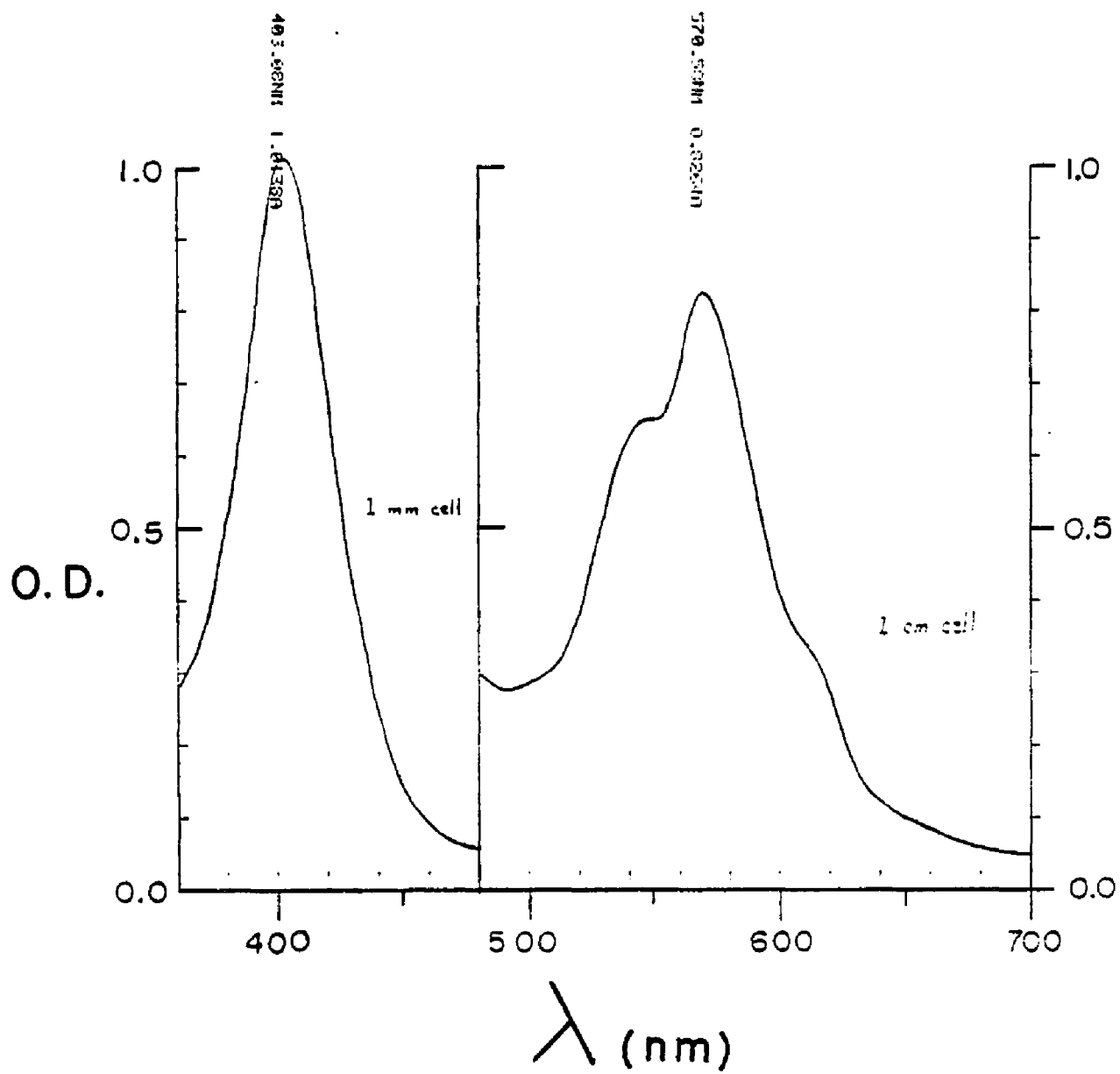
Of the several methods available for hydrolyzing porphyrin esters(63), the KOH - H<sub>2</sub>O/THF procedure was chosen(64). The conditions used in this method would also hydrolyze the silyl protecting groups on the alcohol, leaving the free N-methylhematoporphyrin precursor. The procedure for isolating the free porphyrin as described in reference 64 did not work, and many different attempts were made to isolate the free porphyrin (e.g., precipitation, extraction, column chromatography) but none were completely successful. The use of a molecular exclusion gel column chromatography technique which was eventually chosen did not give a completely pure product nor did it have a particularly good yield but it was the best of the methods attempted. The procedure used is described as follows:

A 200 mg sample of the silyl-blocked N-methylhematoporphyrin IX-dimethyl ester was dissolved in 50 ml of THF. 200 ml of 3N NaOH was prepared and added to the porphyrin solution. Initially, the bottom aqueous base layer was clear and colorless while the upper THF layer held all the porphyrin. The solution was stirred vigorously for at least six hours in the dark. At the end of this period, both the aqueous and organic layers were dark red. (NOTE: According to the procedure, the THF layer was supposed to be colorless after the hydrolysis was complete, but the THF layer was always highly colored. This may have occurred because the quantity of porphyrin hydrolyzed per volume of THF and NaOH used was much greater than that called for in the procedure). The mixture was poured into a 500 ml separatory funnel and the bottom aqueous layer was transferred to a 500 ml round bottom flask which was heated with a heating mantle with an attached Variac set at 50V. A glass tube connected to the house vacuum was inserted into the flask just above the liquid level and the vacuum

was turned on. After most of the water was taken off, the hydrolyzed porphyrin as well as some of the NaOH precipitated out of solution. The porphyrin and solid NaOH were filtered from the solution using a Buchner funnel. The material was first air-dried and then dried further on the vacuum line. When ice-cold methanol was added to the material, the porphyrin dissolved while the majority of the NaOH remained a solid. The porphyrin solution was filtered from the solid NaOH through a fine fritted glass funnel. The methanol was evaporated using heat and house vacuum. The residue was then dried on the vacuum line with warming, and a purple-brown precipitate was obtained. If the precipitate was quite brown (indicating a considerable amount of NaOH) the procedure involving the methanol was repeated. If however, the precipitate was mostly purple, the next step was begun. A 17 cm by 2 cm molecular exclusion column was set up by first pouring dry Bio-Gel P-2 polyacrylamide gel (100-200 mesh, from Bio-Rad) into a beaker of methanol, allowing it to stand and then pouring it into the glass column which was half filled with methanol. Between 100-200 mg of the porphyrin/NaOH material was dissolved in a minimum of methanol and added to the column. Using methanol as the eluent, the porphyrin went down the column quickly and its band remained quite narrow (around 4 cm). The entire colored solution was eluted from the column with less than 8 ml of methanol. A spectrum of this solution (see Figure 4) showed the presence of protonated N-methylhematoporphyrin. A few drops of a solution of NaOH in methanol was added to the porphyrin solution, changing the color from dark red (i.e., protonated) to red-brown (i.e., deprotonated). The solvent was removed using heat and house vacuum, followed by use of the vacuum line and concurrent heating. The porphyrin material was purple and had a crystalline appearance when it was scraped from the flask.

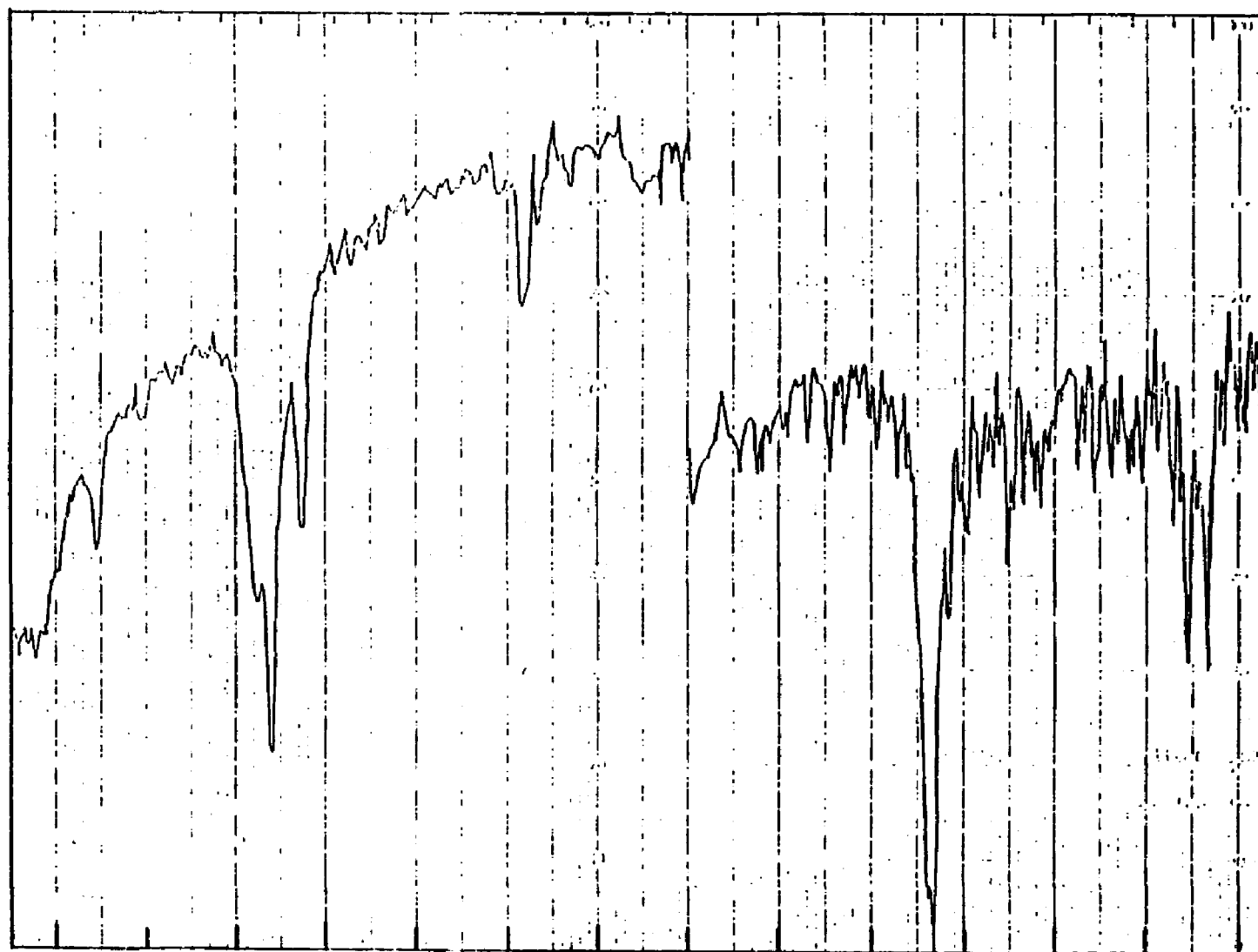
The hydrolysis step had a yield of about 70%, giving an overall yield of 50%. The hydrolysis procedure had such a low yield because some of the porphyrin remained in the THF layer. (NOTE: The P-2 gel column can be overloaded and cannot then be reused because the amide groups of the polyacrylamide gel hydrolyze at pH values above 10. The NaOH

FIGURE 4. THE VISIBLE SPECTRUM OF PROTONATED  
HN-METHYLHEMATOPORPHYRIN, FREE BASE IN  
METHANOL. THE MATERIAL WAS SEPARATED  
FROM EXCESS NaOH BY A P-2 POLYACRYLAMIDE  
GEL COLUMN, USING METHANOL AS THE ELUENT.



which is mixed with the porphyrin may cause this amide hydrolysis to occur.) The high solubility of the N-methyl-hematoporphyrin sodium salt in water and methanol, but insolubility in chloroform or dichloromethane, indicated that hydrolysis did indeed take place; this was confirmed by infrared spectroscopy. An IR spectrum of the hematoporphyrin IX-dimethyl ester (see Figure 5) gave a characteristic ester peak at  $1735\text{ cm}^{-1}$  (either the starting material, the silyl-blocked derivative or the silyl-blocked N-methyl derivative) while the IR spectrum of the N-methylhematoporphyrin (see Figure 6) showed a peak at  $1560\text{ cm}^{-1}$ . The shift to lower energy when going from the propionic acid ester to the sodium salt of the acid can be explained by the fact that the two oxygens of the acid-salt are equivalent (due to resonance) and thus each of the carbon-oxygen bonds can be considered to have a bond order of 1.5 whereas the carbonyl carbon-oxygen bond of the ester is a double bond. The infrared stretching frequencies of the sodium salt of the formate and acetate ions are given to be  $1560$  and  $1578\text{ cm}^{-1}$ , respectively(65). In general, the carboxylate anion of an acid salt has a strong absorption in the range  $1610$ - $1550\text{ cm}^{-1}$  due to its asymmetric stretching mode and a strong absorption near  $1400\text{ cm}^{-1}$  due to its symmetric stretching mode. The correspondence of the infrared spectral data in combination with the solubility data, lends strong evidence that the material prepared is the desired known N-methylhematoporphyrin precursor.

FIGURE 5. AN INFRARED SPECTRUM OF HEMATOPORPHYRIN IX-DIMETHYL ESTER USING A KBr PELLET. THE RANGE IS 10% OF FULL SCALE. THE BREAK AT  $2000\text{ cm}^{-1}$  IS DUE TO DIFFERENT OFFSET VALUES FROM  $4000\text{--}2000\text{ cm}^{-1}$  AND  $2000\text{--}350\text{ cm}^{-1}$ . THE N-METHYLHEMATOPORPHYRIN IX-DIMETHYL ESTER AND THE SILYL-BLOCKED N-METHYLHEMATOPORPHYRIN DIMETHYL ESTER GIVE VIRTUALLY IDENTICAL INFRARED SPECTRA. THE MAJOR PEAK OF INTEREST IS THE CHARACTERISTIC ESTER PEAK AT  $1730\text{ cm}^{-1}$ .



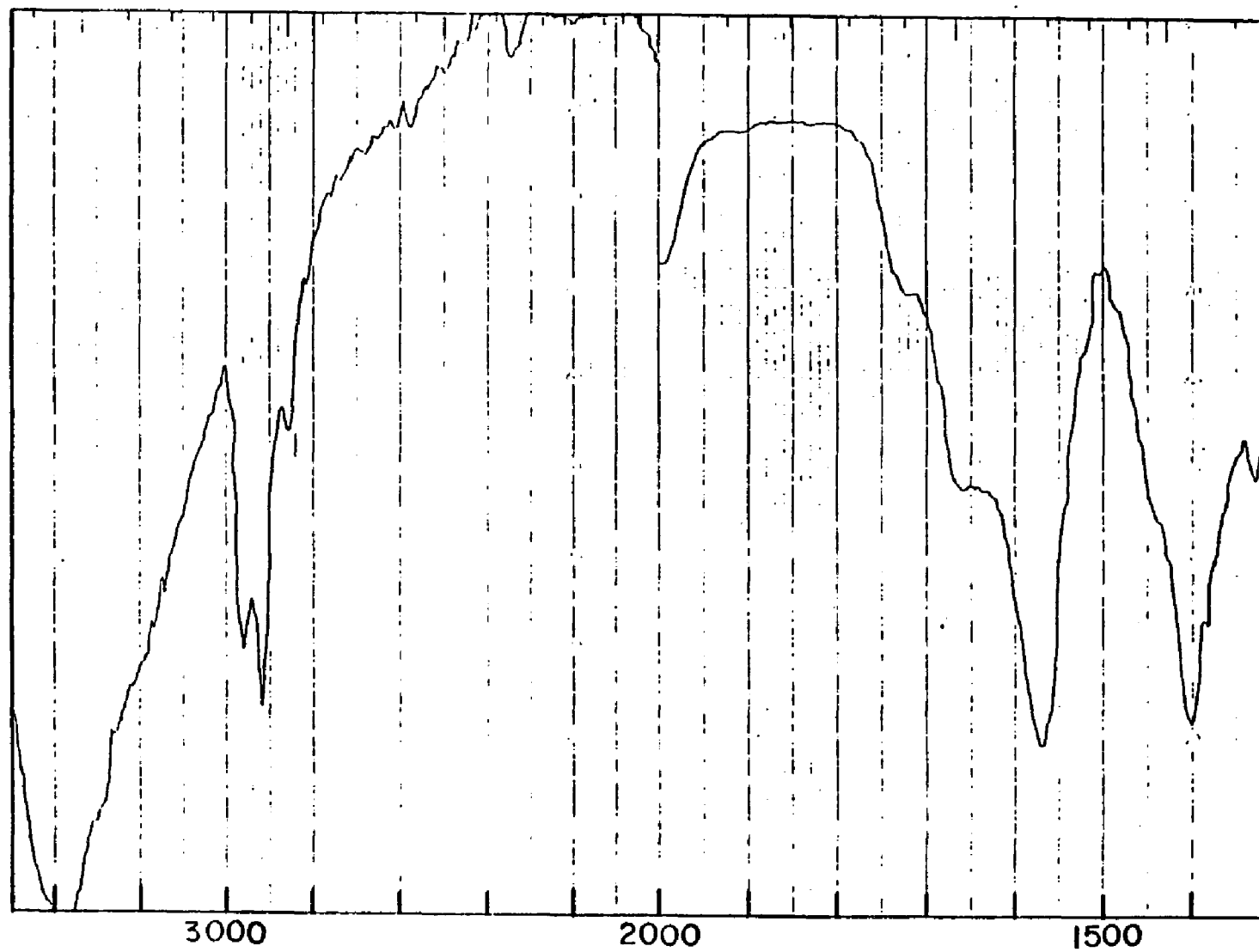
3000

2000

1500

$\lambda, \text{cm}^{-1}$

FIGURE 6. AN INFRARED SPECTRUM OF N-METHYLHEMATOPORPHYRIN, FREE BASE, SODIUM SALT USING A KBr PELLET. THE RANGE IS 20% OF FULL SCALE. THE BREAK AT  $2000\text{ cm}^{-1}$  IS DUE TO DIFFERENT OFFSET VALUES FROM  $4000\text{-}2000\text{ cm}^{-1}$  AND  $2000\text{-}350\text{ cm}^{-1}$ . THE MAJOR PEAKS OF INTEREST ARE THE CARBOXYLATE ANION PEAKS AT  $1570\text{ cm}^{-1}$  AND  $1400\text{ cm}^{-1}$ .



$\lambda$ ,  $\text{cm}^{-1}$

### Synthesis Of The $^{103}\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$ Precursor

The  $^{103}\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$  precursor was synthesized by the following procedure.

Enriched  $^{103}\text{Pd}$  metal (99.8% from Oak Ridge National Laboratory) was dissolved in a 15:1 mixture of concentrated hydrochloric and nitric acid (e.g., for a 20 mg sample of  $^{103}\text{Pd}$ , 3 ml of the acid solution was used). The solution was evaporated to dryness on a steam bath and the residue dissolved and transferred to a test tube with 0.5N hydrochloric acid. It was then evaporated, dried in a stream of air, and evacuated overnight.

### Preparation Of $^{103}\text{Pd}$ -Hematoporphyrin

Although the preparations of the  $^{103}\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$  and the N-methylhematoporphyrin precursors were quite tedious and time consuming, the synthesis of the actual desired product,  $^{103}\text{Pd}$ -hematoporphyrin, from these precursors was quite simple and rapid, as required for successful application of this material as a radiopharmaceutical drug. The procedure is given as follows.

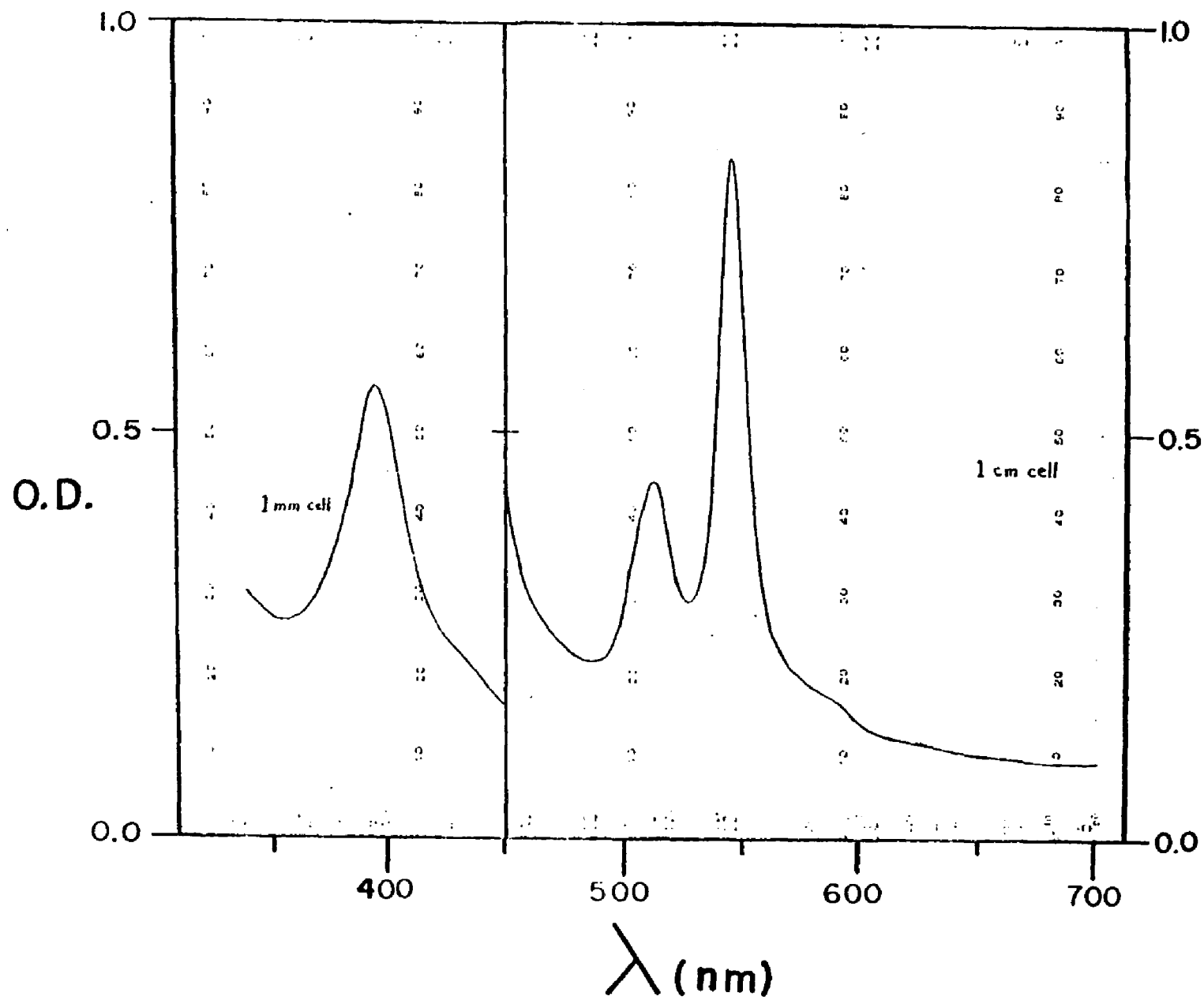
The desired quantity of  $^{103}\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$  is vacuum sealed in a quartz ampoule and irradiated. Using 1.91 mg of  $^{103}\text{Pd}$  and a neutron flux of  $5.5 \times 10^{14}$  neutrons/cm<sup>2</sup> sec in the Brookhaven High Flux Beam Reactor for 16 hours gave a yield of 1.53 Curies (Ci) at E.C.B. (end of bombardment). The sample was allowed to decay for two hours before handling to eliminate species with very high but short-lived activity (principally  $^{36}\text{Cl}$  with a half life of 37.24 minutes and three gamma emissions of energies ranging from 1.642-3.810 MeV). The ampoule of  $^{103}\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$  was transported in a shielded lead pig and transferred to a shielded "cave" prepared for this purpose. Using remote arms, the ampoule was cut open and put into an aluminum block with a cylinder cut out to fit the ampoule. The block was machined to fit over the top of the hot plate. The aluminum block had an

attached thermocouple and an attached Variac for controlling the temperature accurately. 2.00 ml of DMSO were added to the ampoule through a polyethylene tube which led inside the "cave". The aluminum block was heated to 70°C and the solution was stirred for a half an hour to allow the  $^{109}\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$  to go into solution.

A 13.23 mg sample of N-methylhematoporphyrin (20% excess) was dissolved in 1.00 ml of 0.010N sodium acetate by heating to 70°C for 10 minutes with continuous stirring.

The porphyrin/sodium acetate solution was then added dropwise using a syringe through polyethylene tubing into the ampoule containing the  $^{109}\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$ /DMSO solution. The polyethylene tubing was rinsed twice with 1.00 ml portions of the sodium acetate solution. The color of the solution was a very deep red. The solution was allowed to stir at 70°C for 30 minutes and then the solvents were removed by vacuum and heat. An inverted funnel attached to a vacuum line was lowered over the ampoule to provide the vacuum and the temperature was raised to 150°C. It took about 1.5 hours to remove the solvents. The aluminum block was then cooled to 80°C and 5.00 ml of 0.050N NaOH was added through the polyethylene tube. The solution was allowed to stir at 80°C for 15 minutes. The color of the solution remained a very deep red. The solution was then transferred into a syringe using the remote arms. The syringe was mounted on a 0.45 micron millipore filter. The solution was forced through the filter into a glass vial by pressurized nitrogen. The filter was rinsed twice with 2.00 ml of the NaOH solution. The vial was capped with a rubber septum and transferred into a lead pig. A rough reading of the activity was taken on a Capintec Radioisotope Calibrator set for the 8.8 KeV radiation given off by the Pd-109 isotope. The activity of the solution was found to be 920 millicuries (mCi) while that left on the filter was 63 mCi (or 6.5% of the total activity). A more accurate reading of the activity was determined using a gamma spectrometer equipped with a Germanium-Lithium detector. A visible spectrum taken of the aliquot after the  $^{109}\text{Pd}$  had cooled matched that of a spectrum taken of "cold" Pd-hematoporphyrin from a reaction done with "cold"  $\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$  and N-methylhematoporphyrin (see Figure 7).<sup>2</sup> The  $^{109}\text{Pd}$ -hematoporphyrin solution was ready for injection less than three hours after the ampoule was cut open.

FIGURE 7. THE VISIBLE SPECTRUM OF PD-HEMATOPORPHYRIN IN DIMETHYL-SULFOXIDE. THE 700-450NM PORTION OF THE SPECTRUM WAS TAKEN IN A 1 CM CUVETTE. THE 450-340NM PORTION OF THE SPECTRUM (THE SORET REGION) WAS TAKEN OF THE SAME SOLUTION IN A 1MM CUVETTE.



(NOTE: A change in procedure which could be used in the future involves the elimination of the solvent removal step. In this case, the entire procedure would be completed in less than one hour.)

#### DISTRIBUTION, TOXICITY AND TRANSPLANTATION STUDIES IN DOGS USING THE $^{109}\text{Pd}$ -HEMATOPORPHYRIN DRUG

Three different types of studies involving the injection of radioactive  $^{109}\text{Pd}$ -hematoporphyrin solutions into dogs were undertaken: 1) distribution, to see where and in what quantity the material localized in the dogs; 2) toxicity levels, checked by white blood cell and lymphocyte counts with respect to time and 3) tissue transplantation, in which both kidneys of the recipient beagle were removed and one kidney from a different breed of dog (usually a mongrel) was transplanted into the host. As with the toxicity studies, the white blood cell and absolute lymphocyte counts were taken as well as the creatinine and blood uric nitrogen counts, which are indicators of whether the kidney is functioning or has been rejected.)

#### Activity Distribution Study On Beagles

To compare the  $^{109}\text{Pd}$ -hematoporphyrin prepared by this new method with the  $^{109}\text{Pd}$ -hematoporphyrin prepared by the acetic acid method, localization in dogs was chosen as the cri-

terion. Distribution studies were therefore investigated. Although the procedure for preparing the solution to be injected were different for each dog (specifics are given in the RESULTS Section), generally, a large excess (from 150 to 300 mg) of hematoporphyrin was dissolved in 0.050N NaOH. Two to three millicuries of the "hot"  $^{109}\text{Pd}$ -hematoporphyrin solution was combined with the free base hematoporphyrin. This solution was injected into the front paw of each beagle. Blood samples were taken periodically up to 24 hours when the dogs were sacrificed. A necropsy was performed and slices from various organs were analyzed for  $^{109}\text{Pd}$  by beta radiation counting. The blood clearance time was found by analyzing the activity of the blood samples taken at various times. The relative distributions were found in a number of tissues - liver, kidney, spleen, muscle, mesenteric lymph nodes, popliteal lymph nodes, lung, ovary, duodenum, adrenal, pancreas and bone marrow. In all, seven distribution studies were done on female beagles.

#### Toxicity Study Of The $^{109}\text{Pd}$ -hematoporphyrin On Beagles

Different doses of radioactivity were given to dogs in order to determine the  $\text{LD}_{50}$  (lethal dose at which 50% of the animals die) of the  $^{109}\text{Pd}$ -hematoporphyrin material and to compare the toxicity of this new preparation of  $^{109}\text{Pd}$ -hematoporphyrin with the material from the traditional acetic acid preparation. Each beagle was injected with a total quantity of hematoporphyrin (from both the "hot"

$^{109}\text{Pd}$ -hematoporphyrin and the "cold" free base hematoporphyrin) of 15 mg per kg of dog. The dogs weighed between 9 and 19 kg, so the amount of free base hematoporphyrin given varied from 110 to 220 mg. The dosage of radioactivity given to the dogs varied from 6.5 mCi/kg of dog to 13.4 mCi/kg. The white blood cell and absolute lymphocyte counts were taken before the injection of the  $^{109}\text{Pd}$ -hematoporphyrin material and every 3 to 15 days after injection up to 50 days, the length of the experiment. Nine beagles were used in these toxicity studies.

#### Transplantation Study On Beagles

After the preliminary distribution and toxicity studies were carried out, actual transplantation studies were begun. Three methods of protocol were used: 1) injection of  $^{109}\text{Pd}$ -hematoporphyrin four days before the transplantation operation; 2) injection of  $^{109}\text{Pd}$ -hematoporphyrin four days before the transplant plus 2 shots of antilymphocyte globulin (20 mg/kg dog) before the transplant; and 3) injection of the above two materials plus a bone marrow transplant immediately following the transplantation operation.

For any of the protocol procedures described above, each dog was given 9 mCi/kg dog of  $^{109}\text{Pd}$ -hematoporphyrin along with enough "cold" free base hematoporphyrin to bring the total quantity of hematoporphyrin to 15 mg/kg dog. Four days after the  $^{109}\text{Pd}$ -hematoporphyrin was administered, the transplantation operation was carried out. In all cases,

the donor kidney was from a mongrel. Three to four surgeons and at least two technicians were present for the operation. The left kidney from the recipient was removed. The left kidney of the donor was removed, flushed with saline and transplanted to the recipient where the left kidney had been previously. After the kidney had been transplanted, the recipient's right kidney was removed. The operation under sterile conditions took between 2.5 to 4 hours. After the operation, both the white blood cell and absolute lymphocyte counts were taken frequently. Also the creatinine and blood uric nitrogen levels were checked to monitor the health of the transplanted kidney.

#### INSTRUMENTATION

Spectrophotometric determination of various porphyrin solutions were carried out on a Cary 14 spectrophotometer at room temperature.

Infrared spectra were taken on a Beckman 4240 spectrophotometer. Most samples were prepared as a KBr pellet. A typical sample was prepared using a few milligrams of material and 100 mg of dry KBr. The samples were pressed under 10 tons per square inch of pressure for several minutes. On a few occasions, a solution sample was made in dichloromethane.

$^1\text{H}$  NMR spectra were taken on a Varian EM-360 60MHz continuous wave spectrometer.

Weighings up to 10 grams were performed on a Mettler H-15 semimicro balance. Bulk weighings were carried out on a Sartorius single pan balance.

#### MATERIALS

The following list contains the materials used for this work and their sources.

|                                                      |                      |
|------------------------------------------------------|----------------------|
| acetonitrile                                         | Fisher Certified     |
| ammonia                                              | Fisher Reagent       |
| bio-gel P-2 (100-200 mesh)                           | Bio-Rad              |
| dichloromethane                                      | Fisher Certified     |
| dimethylsulfoxide                                    | Fisher Certified     |
| hematoporphyrin IX-dimethyl ester                    | Man-Win Chemicals    |
| hematoporphyrin IX, free base form                   | Man-Win Chemicals    |
| hexamethyldisilazane                                 | Petrarch Systems     |
| 2,6-lutidine                                         | Eastman              |
| methanol                                             | Fisher Certified     |
| $\beta$ -methoxyethoxymethyl chloride                | Aldrich              |
| methylfluorosulfonate ("Magic Methyl")               | Aldrich              |
| 4A molecular sieves                                  | Davison Chemicals    |
| $\text{PdCl}_2$ (anhydrous)                          | Alfa                 |
| $\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$            | Brookhaven Nat'l Lab |
| $\text{Pb}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$ | Alfa                 |
| potassium bromide (ultra pure)                       | Alfa                 |
| pyridine (99%+)                                      | Aldrich (Gold Label) |
| silica gel (grade 62, 60-200 mesh)                   | Davison Chemicals    |
| silica gel TLC sheets (#6060)                        | Eastman              |

|                             |                  |
|-----------------------------|------------------|
| sodium acetate              | Fisher Certified |
| sodium hydroxide            | Fisher Certified |
| tetrabutylammonium fluoride | Aldrich          |
| tetrahydrofuran             | Fisher Certified |
| tetramethylsilane           | Aldrich          |
| trimethylchlorosilane       | Petrarch Systems |

## RESULTS

### ACTIVITY RECOVERED IN $^{109}\text{Pd}$ -HEMATOPORPHYRIN SYNTHESIS

After synthesis of the  $^{109}\text{Pd}$ -hematoporphyrin remotely, the activity was calculated using results obtained with the instrumentation described in the experimental section. The amount of activity recovered determined the type and number of animal experiments that would be carried out at Columbia Presbyterian Medical Center. The quantity of Pd-108 that was used, the amount and duration of neutron flux, the number of millicuries of Pd-109 recovered in the 0.050N NaOH solution, the number of millicuries left on the millipore filter, the percentage of the theoretical limit of activity recovered and the date of each experiment are given in Table I. The theoretical yield of Pd-109 activity produced is calculated from the following equation.

$$A_D = \phi \cdot \sigma_p \cdot N_p (1 - \exp(-\lambda_D \cdot t_i)) (\exp(-\lambda_D \cdot t_c)) \quad <3>$$

where  $A_D$ =activity of daughter (Pd-109) in millicuries,  $\phi$ =neutron flux= $1.8-5.5 \times 10^{14}$  n s $^{-1}$  cm $^{-2}$ ,  $\sigma_p$ =cross section of parent (Pd-108)= $8.3 \times 10^{-24}$  barnes ( $10^{24}$  barnes=1 cm $^2$ ),  $N_p$ =number of parent atoms,  $\lambda_D$ =decay constant of daughter= $.693/t_{1/2}$  ( $t_{1/2}$ =13.46 hrs.),  $t_i$ =time of irradiation,  $t_c$ =time of cooling, and  $3.7 \times 10^7$  is a factor which changes  $A_D$  from disintegrations/sec to millicuries. The early experiments gave a relatively low yield of activity so some parameters

TABLE I. Parameters Used in Producing Pd-109, Activity Retained in Injecting Solution and on Filter, and Date of Each Experiment

| Date     | Mass of Pd-108 used (mg) | Length of Bombardment (hours) | Neutron Flux <sup>a</sup> (neutrons/sec) | ACTIVITY OF Pd-109 <sup>b</sup>      |                                |                                                              |
|----------|--------------------------|-------------------------------|------------------------------------------|--------------------------------------|--------------------------------|--------------------------------------------------------------|
|          |                          |                               |                                          | Retained in injecting solution (mCi) | Left on millipore filter (mCi) | Percentage Yield retained in injecting solution <sup>c</sup> |
| 10/16/80 | 2.11                     | 5.5                           | 0.5                                      | 58                                   | 16.6                           | <i>d</i>                                                     |
| 12/10/80 | 5.81                     | 5.5                           | 1.5                                      | 335                                  | 507                            | <i>d</i>                                                     |
| 1/27/81  | 6.33                     | 1.0                           | 16                                       | 287                                  | 159                            | 64.6                                                         |
| 2/11/81  | 5.67                     | 1.8                           | 16                                       | 549                                  | 10.0                           | 76.6                                                         |
| 2/25/81  | 6.25                     | 1.8                           | 24                                       | 203                                  | 572                            | 20.4                                                         |
| 3/03/81  | 5.44                     | 1.8                           | 24                                       | 174                                  | 153                            | 20.0                                                         |
| 3/20/81  | 5.11                     | 1.8                           | 24                                       | 311                                  | 207                            | 38.1                                                         |
| 3/27/81  | 5.63                     | 2.5                           | 24                                       | 756                                  | 284                            | 60.5                                                         |
| 4/17/81  | 4.76                     | 2.5                           | 24                                       | 792                                  | 118                            | 75.0                                                         |
| 5/01/81  | 4.64                     | 2.5                           | 24                                       | 387                                  | 376.5                          | 37.6                                                         |
| 5/15/81  | 4.36                     | 2.5                           | 24                                       | 766                                  | 53                             | 79.2                                                         |
| 6/12/81  | 3.58                     | 5.5                           | 16                                       | 2484                                 | 83.2                           | 103                                                          |
| 7/10/81  | 1.91                     | 5.5                           | 16                                       | 1555                                 | 85.3                           | 211                                                          |
| 7/24/81  | 1.74                     | 5.5                           | 16                                       | 1022                                 | 147                            | 152                                                          |
| 8/14/81  | 3.38                     | 2.5                           | 24                                       | 717                                  | 24.5                           | 95.6                                                         |
| 9/18/81  | 1.80                     | 5.5                           | 16                                       | 1254                                 | <i>e</i>                       | 180                                                          |
| 10/07/81 | 2.03                     | 5.5                           | 16                                       | 1408                                 | 83.1                           | 180                                                          |

<sup>a</sup>The source was the Brookhaven High Flux Beam Reactor. <sup>b</sup>Calculated from the Germanium-Lithium detector on a gamma spectrometer. <sup>c</sup>The theoretical activities were calculated from equation <13>. Note that the absolute percentages cannot be correct but it is the percentages that are relative to each other that are important. <sup>d</sup>Could not calculate.

<sup>e</sup>Did not take reading.

were changed in order to increase the yield. Also, attempts were made to minimize the amount of activity left on the millipore filter. As can be seen from Table I, the parameters have been nearly optimized.

## RESULTS OF STUDIES USING DOGS

### Distribution And Toxicity Studies

To determine whether the  $^{109}\text{Pd}$ -hematoporphyrin synthesized by this new procedure was the same material as that produced by the acetic acid method, distribution and toxicity studies were done using dogs.

This distribution study involved seven beagles. Different variables in the protocol were changed in order to see the effect on the distribution of the activity in the dog. Descriptions of the seven beagles and the protocols used are listed below.

Beagle 1: 150 mg of the "cold" hematoporphyrin were dissolved in 20.0 ml of 0.050N NaOH and centrifuged. No undissolved material remained. This solution and then a saline rinse were injected into the dog. Five minutes later, the "hot"  $^{109}\text{Pd}$ -hematoporphyrin/DMSO solution and saline rinse were injected. (NOTE: At this time the experimental procedure for the  $^{109}\text{Pd}$ -hematoporphyrin synthesis did not involve the evaporation of the DMSO and subsequent addition of 0.050N NaOH.)

Beagle 6: Approximately 0.5 ml of the  $^{109}\text{Pd}$ -hematoporphyrin/DMSO solution was added to 4.5 ml of the 0.050N NaOH. The mixture was centrifuged to check for precipitation. None occurred. 150 mg hematoporphyrin were dissolved in 15.0 ml of the NaOH. The two solutions were then combined and injected together.

Beagle 7: 150 mg of hematoporphyrin were dissolved in 20.0 ml of the dilute NaOH and injected into the beagle. Four hours later 0.5 ml of the  $^{109}\text{Pd}$ -hematoporphyrin/DMSO was injected.

Beagle 9: 150 mg of hematoporphyrin were dissolved in 20.0 ml of the dilute NaOH. The "hot"  $^{109}\text{Pd}$ -hematoporphyrin/0.050N NaOH solution was added directly to the hematoporphyrin solution and then centrifuged. No precipitation occurred. The entire mixture was then injected into the dog.

Beagle 16: 300 mg of hematoporphyrin were dissolved in 30.0 ml of the NaOH solution. The  $^{109}\text{Pd}$ -hematoporphyrin/NaOH solution was combined with the hematoporphyrin solution, mixed well and injected together.

Beagle 17: 150 mg of hematoporphyrin were dissolved in 20.0 ml of the dilute base and injected into the dog. One hour later the  $^{109}\text{Pd}$ -hematoporphyrin/NaOH solution was injected.

Beagle 18: 245 mg of hematoporphyrin were dissolved in 25.0 ml of a 20% DMSO in 0.050N NaOH solution. The  $^{109}\text{Pd}$ -hematoporphyrin/20%DMSO in .050N NaOH solution was combined with the hematoporphyrin solution, mixed well and injected.

Of the seven beagles used in the distribution study, two -Beagles 9 and 16- gave results that were similar to distribution studies using the acetic acid preparation. These two dogs had protocols which were the most similar of the seven (the only difference being the amount of hematoporphyrin injected) indicating that this is the procedure which should be used. The results of the distribution study on these two beagles are given in Table II. Dogs 1, 17 and 18 had inordinately large amounts of activity in the liver while the kidney of Dog 6 and the lung of Dog 7 contained a much larger quantity of activity than expected.

The toxicity study consisted of nine beagles. Both the

TABLE II. Distribution of Administered  $^{109}\text{Pd}$ -Hematoporphyrin in Dogs

| Tissue                | Dog 9 <sup>a</sup>    |                         |                      |                         |
|-----------------------|-----------------------|-------------------------|----------------------|-------------------------|
|                       | Counts/g <sup>c</sup> | Activity/g <sup>d</sup> | Count/g <sup>c</sup> | Activity/g <sup>d</sup> |
| Blood (0.5 hours)     | 5.55                  | 427                     | 4.77                 | 367                     |
| Blood (2 hours)       | 4.00                  | 308                     | 3.80                 | 292                     |
| Blood (17.5 hours)    | 1.73                  | 133                     | 1.52                 | 117                     |
| Blood (22 hours)      | 1.65                  | 127                     | 1.24                 | 95.7                    |
| Mesenteric lymph node | 5.80                  | 487                     | 8.01                 | 616                     |
| Popliteal lymph node  | 2.04                  | 157                     | 1.83                 | 141                     |
| Liver                 | 8.24                  | 634                     | 11.4                 | 874                     |
| Kidney                | 5.87                  | 452                     | 4.61                 | 534                     |
| Spleen                | 4.57                  | 351                     | 5.74                 | 442                     |
| Muscle                | .0939                 | 7.22                    | .142                 | 10.9                    |
| Lung                  | .979                  | 75.3                    | 1.15                 | 88.3                    |
| Ovary                 | 1.48                  | 114                     | 1.25                 | 96.3                    |
| Duodenum              | .846                  | 65.1                    | 1.04                 | 79.9                    |
| Adrenal               | 2.84                  | 219                     | 3.21                 | 247                     |
| Pancreas              | .303                  | 23.3                    | .394                 | 30.3                    |
| Bone Marrow           | .748                  | 57.5                    | .222                 | 17.1                    |

<sup>a</sup>150 mg of hematoporphyrin injected with  $^{109}\text{Pd}$ -hematoporphyrin.

<sup>b</sup>300 mg of hematoporphyrin injected with  $^{109}\text{Pd}$ -hematoporphyrin.

<sup>c</sup>(Corrected counting rate/gram of tissue)  $\times 10^{-4}$ . <sup>d</sup>(Fraction of administered activity/gram of tissue)  $\times 10^6$ .

white blood cell count and the absolute lymphocyte count were taken prior to the injection of the  $^{109}\text{Pd}$ -hematoporphyrin/hematoporphyrin/.050N NaOH solution and periodically after the injection, up to 50 days after injection. An activity of  $^{109}\text{Pd}$ -hematoporphyrin of 13 mCi/kg dog or greater was lethal to the dog whereas activities ranging from 6-11 mCi/kg dog were not lethal. It was also found that the white blood cell and absolute lymphocyte counts of the dogs who survived decreased to a minimum from 10 to 14 days after injection before rising again. Some representative numbers for the white blood cell and absolute lymphocyte counts for the various activity dosage levels can be found in Table III.

#### Kidney Transplantation Study

Eight dogs have been used thus far in this kidney transplantation study. Beagles 12 and 14 were given only the  $^{109}\text{Pd}$ -hematoporphyrin drug. The data on these dogs are given in Table IV. Beagle 1 was sacrificed 15 days after the transplant operation when it was certain that the kidney had been rejected. The second beagle died at five days after the operation because of a blood clot at the entrance of the kidney. Beagles 19, 21 and 23 were given both the two shots of antilymphocyte globulin and the  $^{109}\text{Pd}$ -hematoporphyrin. Table V contains the data for these dogs. Beagle 19 lived for 35 days before she was sacrificed although she was never healthy after the transplant opera-

TABLE III. The Effect of the Dosage Level of Pd-109 Activity and Antilymphocyte Globulin on the White Blood Cell and Absolute Lymphocyte Counts of Beagles at Different Time Periods after Injection

| Dog #3 (6mCi/kg) |                  |                               | Dogs #2,4,5 <sup>c</sup> (9mCi/kg) |      |                  | Dogs #8,11<br>(9mCi/kg + 2 shots ALG <sup>d</sup> ) |        |                  |
|------------------|------------------|-------------------------------|------------------------------------|------|------------------|-----------------------------------------------------|--------|------------------|
| Day              | WBC <sup>a</sup> | Abs. Lym.<br>(%) <sup>b</sup> | Day                                | WBC  | Abs. Lym.<br>(%) | Day                                                 | WBC    | Abs. Lym.<br>(%) |
| 0 <sup>e</sup>   | 7200             | 38                            | 0 <sup>e</sup>                     | 8287 | 39               | 0 <sup>e</sup>                                      | 13,775 | 29               |
| +3               | 5900             | 17                            | +2                                 | 6017 | 17               | +2                                                  | 2275   | 10               |
| +6               | 2560             | 50                            | +5                                 | 3150 | 29               | +3                                                  | 1015   | 4                |
| +10              | 2320             | 22                            | +13                                | 1990 | 63               | +10                                                 | 655    | 38               |
| +14              | 1640             | 50                            | +20 <sup>f</sup>                   | 3120 | 42               | +14 <sup>h</sup>                                    | 4920   | 45               |
| +17              | 1560             | 42                            | +23 <sup>f</sup>                   | 2880 | 35               | +17 <sup>h</sup>                                    | 4075   | 33               |
| +28              | 4900             | 30                            | +28 <sup>g</sup>                   | 3700 | 50               | +20 <sup>i</sup>                                    | 4120   | 21               |
| +35              | 9150             | 21                            | +35 <sup>g</sup>                   | 5350 | 31               | +30                                                 | 6375   | 20               |
|                  |                  |                               |                                    |      |                  | +38 <sup>h</sup>                                    | 5800   | 26               |

TABLE III. (continued)

| Dog #10<br>(10.4mCi/kg + 2 shots ALG) |        |                  | Dog #13 (11mCi/kg + ALG) |          |                  | Dog #15 (13mCi/kg) |        |                  |
|---------------------------------------|--------|------------------|--------------------------|----------|------------------|--------------------|--------|------------------|
| Day                                   | WBC    | Abs. Lym.<br>(%) | Day                      | WBC      | Abs. Lym.<br>(%) | Day                | WBC    | Abs. Lym.<br>(%) |
| 0 <sup>e</sup>                        | 12,550 | 26               | 0 <sup>e</sup>           | 8600     | <i>j</i>         | 0 <sup>e</sup>     | 16,740 | 20               |
| +2                                    | 2050   | 6                | +2                       | 8400     | 7                | +2                 | 14,400 | 8                |
| +6                                    | 1960   | 20               | +7                       | 8800     | 11               | +3                 | 10,275 | 1                |
| +9                                    | 1440   | 30               | +13                      | 5300     | 12               | +7                 | 5400   | 22               |
| +20                                   | 880    | <i>j</i>         | +25                      | Delivery |                  | +9                 | 1750   | 50               |
| +30                                   | 6000   | 18               | +27                      | 1650     | 25               | +11                | Died   |                  |
|                                       |        |                  | +36                      | 47,000   | 5                |                    |        |                  |
|                                       |        |                  | +47                      | 32,050   | 9                |                    |        |                  |

<sup>a</sup>White blood cell count. <sup>b</sup>Percentage of WBC count. <sup>c</sup>Data is the average of the dogs listed.

<sup>d</sup>Antilymphocyte globulin was given to the dogs at days -4 and -2 (15mg/kg). <sup>e</sup>Day that

<sup>109</sup>Pd-hematoporphyrin was injected. <sup>f</sup>Averages of dogs #4 and #5. <sup>g</sup>Data from Dogs #2 only.

<sup>h</sup>Data from Dog #11 only. <sup>i</sup>Data from Dog #8 only. <sup>j</sup>Reading was not taken.

TABLE IV. The White Blood Cell Count and % Absolute Lymphocyte<sup>a</sup> of Beagles #12 and #14 after being given <sup>109</sup>Pd-hematoporphyrin (9mCi/kg) in Preparation of a Kidney Transplant

| Dog # 12 |                  |               |                   | Dog # 14 |        |               |                   |
|----------|------------------|---------------|-------------------|----------|--------|---------------|-------------------|
| Day      | WBC <sup>b</sup> | Abs. Lym. (%) | Comment           | Day      | WBC    | Abs. Lym. (%) | Comment           |
| 0        | 24,600           | 14            | c                 | 0        | 13,225 | 20            | c                 |
| +2       | 12,000           | 11            |                   | +2       | 14,400 | 4             |                   |
| +3       | 26,980           | 3             |                   | +3       | 18,200 | 4             |                   |
| +4       | 15,850           | 8             | kidney transplant | +4       |        |               | kidney transplant |
| +6       | 21,050           | 2             |                   | +6       | 5050   | 3             |                   |
| +9       |                  |               | organ rejected    | +8       | 500    | 1             | d                 |
| +10      | 3840             | 2             |                   |          |        |               |                   |
| +13      | 8850             | 10            |                   |          |        |               |                   |
| +16      | 5628             | 11            |                   |          |        |               |                   |

<sup>a</sup>Percentage of WBC count. <sup>b</sup>White blood cell count. <sup>c</sup>Reading taken prior to injection of <sup>109</sup>Pd-hematoporphyrin. <sup>d</sup>Died of lung problems. The graft remained intact.

TABLE V. The White Blood Cell Count, % Absolute Lymphocyte<sup>a</sup>, Creatinine and Blood Uric Nitrogen Levels of Beagles #19 and #23 after being given <sup>109</sup>Pd-hematoporphyrin (9mCi/kg) and 2 shots of Antilymphocyte Globulin (20mg/kg) in Preparation for a Kidney Transplant

| Day | D o g # 1 9      |             |            |                  | Comment                               |
|-----|------------------|-------------|------------|------------------|---------------------------------------|
|     | WBC <sup>b</sup> | Abs.Lym.(%) | Creatinine | BUN <sup>c</sup> |                                       |
| -4  |                  | 28          |            |                  | <i>d</i>                              |
| -3  | 8300             |             |            |                  | 1 <sup>st</sup> shot ALG <sup>e</sup> |
| -2  | 8200             | 10          |            |                  | 2 <sup>nd</sup> shot ALG              |
| -1  | 10,650           | 8           |            |                  |                                       |
| 0   | 9350             | 5           |            |                  | transplant                            |
| +1  |                  |             | 1.7        | 35               | <i>f,g,h</i>                          |
| +2  | 10,275           | 7           | 1.5        | 38               | <i>f,g</i>                            |
| +6  |                  |             | 1.3        | 20               | <i>g,i</i>                            |
| +8  | 7800             | 6           | 1.5        | 17               | <i>i</i>                              |
| +10 | 2100             | 13          | 6.1        | 105              | <i>h,j</i>                            |

TABLE V. (continued)

| Day | D o g # 1 9 |               |            |     | Comment                  |
|-----|-------------|---------------|------------|-----|--------------------------|
|     | WBC         | Abs. Lym. (%) | Creatinine | BUN |                          |
| -4  | 16,350      | 30            |            |     | <sup>k</sup>             |
| -2  | 11,350      | 8             |            |     | 1 <sup>st</sup> shot ALG |
| -1  | 12,150      | 6             |            |     | 2 <sup>nd</sup> shot ALG |
| 0   | 7750        | 8             |            |     | transplant               |
| +2  | 17,880      | 5             |            |     |                          |
| +4  | 17,600      | 6             |            |     | <sup>f</sup>             |
| +6  |             |               | 4.4        | 119 | <sup>f</sup>             |
| +8  | 6600        | 5             | 3.9        | 119 | <sup>f, g</sup>          |
| +10 | 7900        | 4             | 3.5        |     | <sup>f, g, h, l</sup>    |
| +13 | 3200        | 7             | 3.2        | 107 | <sup>f, g, h</sup>       |
| +16 | 3000        | 8             | 2.4        | 56  | <sup>f, g, h</sup>       |
| +20 | 12,300      | 6             | 2.5        | 38  | <sup>f, g, h</sup>       |
| +23 | 9800        | 7             | 3.0        | 37  | <sup>f, g</sup>          |
| +27 | 9700        |               | 5.5        | 87  | <sup>f, g, h, l</sup>    |
| +29 | 7400        | 14            | 7.3        | 98  | <sup>f, h</sup>          |
| +31 |             |               | 9.1        | 118 | <sup>f, h</sup>          |
| +32 |             |               |            |     | biopsy                   |
| +34 |             |               | 12.1       | 144 | <sup>f</sup>             |
| +35 | 61,800      | 7             | 13.4       | 180 | <sup>m</sup>             |

<sup>a</sup>Percentage of WBC count. <sup>b</sup>White blood cell count. <sup>c</sup>Blood uric nitrogen level. <sup>d</sup>Injected with <sup>109</sup>Pd-hematoporphyrin (10.4mCi/kg because of a miscalculation). <sup>e</sup>Antilymphocyte globulin. <sup>f</sup>Given nourishment intravenously. <sup>g</sup>Given an antibiotic. <sup>h</sup>Vomited. <sup>i</sup>Creatinine and BUN levels are normal. <sup>j</sup>Dog was sacrificed. Biopsy revealed that graft had been rejected. Only 75% of the mesenteric lymph nodes had been destroyed. <sup>k</sup>Injected with 9mCi/kg <sup>109</sup>Pd-hematoporphyrin. <sup>l</sup>Given a blood transfusion. <sup>m</sup>Dog was sacrificed. Autopsy revealed dog had massive infection. Biopsy revealed that graft may not have been rejected.

tion. This dog was given nourishment intravenously for most of the time period after the operation. She was also given antibiotics and a blood transfusion. However, the major cause of death was not from the rejection of the kidney but rather from massive infection. Beagle 23 was sacrificed at ten days after the operation. This dog was in excellent condition from the time immediately after the operation until the day she was sacrificed. The creatinine and blood uric nitrogen levels increased dramatically on the tenth day. Examination showed massive internal bleeding and the dogs could not be saved. A biopsy indicated that the kidney graft had been rejected and that only three-fourths of the mesenteric lymph nodes were destroyed by the drugs given. One-fourth of them remained intact. The  $^{109}\text{Pd}$ -hematoporphyrin did not seem to be performing adequately. Beagle 21 died the first day after the operation due to anesthesia problems. This problem did not occur because of the administration of the  $^{109}\text{Pd}$ -hematoporphyrin or the antilymphocyte globulin but rather from the surgery itself. Beagles 20, 22 and 24 were given just the two antilymphocyte globulin injections with no Pd-109. The statistics of Beagle 24 are given in Table VI. This drug itself should have prolonged the lifetime of the dogs by a factor of about two but at the three doses given, 15 (the amount used with the  $^{109}\text{Pd}$ -hematoporphyrin in beagles 19, 21 and 23), 20 and 30 mg antilymphocyte globulin/kg dog, there was no prolongation. The kidney graft was rejected in nine

TABLE VI. The White Blood Cell Count, % Absolute Lymphocyte, Creatinine and Blood Uric Nitrogen Counts for Beagle 24 after being given 2 shots of Antilymphocyte Globulin (20mg/kg) in Preparation for a Kidney Transplant

| Day | WBC <sup>a</sup> | Abs.Lym. (%) <sup>b</sup> | Creatinine | BUN <sup>c</sup> | Comments                 |
|-----|------------------|---------------------------|------------|------------------|--------------------------|
| -3  | 1650             | 57                        |            |                  |                          |
| -2  | 9950             | 9                         |            |                  | 1 <sup>st</sup> shot ALG |
| -1  |                  |                           |            |                  | 2 <sup>nd</sup> shot ALG |
| 0   | 7400             | 5                         |            |                  | transplant               |
| +1  | 18,000           | 6                         | 3.2        | 70               |                          |
| +4  | 8000             | 7-11                      | 1.6        | 38               |                          |
| +6  |                  |                           | 1.2        | 19               | e                        |
| +8  | 16,050           | 1                         | 1.1        | 33               |                          |
| +11 | 11,350           | 12                        | 5.2        | 94               | f                        |
| +13 |                  |                           |            |                  | sacrificed               |

<sup>a</sup>White blood cell count. <sup>b</sup>Percentage of WBC count. <sup>c</sup>Blood uric nitrogen. <sup>d</sup>Antilymphocyte globulin. <sup>e</sup>The creatinine and BUN counts were normal at this point. <sup>f</sup>Rejection of the kidney probably took place.

days, the same amount of time the control dogs rejected the grafts. It is obvious that the antilymphocyte globulin was not performing well either. More experiments will be necessary to further test the effectiveness of the antilymphocyte globulin drug. It has already been shown by the previous experiments done on rats, that the  $^{109}\text{Pd}$ -hematoporphyrin complex itself will not indefinitely prolong the lifetime of the graft. It was necessary to use the antilymphocyte globulin as a supporting drug and also, in some cases, a bone marrow infusion.

## DISCUSSION

### BRIEF BACKGROUND

The use of  $^{109}\text{Pd}$ -hematoporphyrin for selective lymphatic ablation has been reported and found to be effective at preventing homograft rejection in rats and dogs. However, the literature method for the preparation of the radioactive palladium complex results in considerable loss of activity, requiring too much initial activity to be of practical use in experiments involving large animals and human subjects. It involves a multi-step process which includes transfers of materials, precipitations, redissolutions and filtering, but does not guarantee the purity of the final  $^{109}\text{Pd}$ -hematoporphyrin product. It would, therefore, be impractical for use on a routine basis. By using N-methyl-hematoporphyrin as a precursor, we have developed a method of preparation of the  $^{109}\text{Pd}$ -hematoporphyrin complex which is complete in less than one minute, has a quantitative yield of a very pure product, and has a minimum of steps in the process.

### SYNTHESIS OF THE N-METHYLHEMATOPORPHYRIN PRECURSOR

The synthesis of the porphyrin precursor, N-methylhemato-

porphyrin, has been worked out in detail. The yields of the silyl-blocked hematoporphyrin IX-dimethyl ester and the silyl-blocked N-methylhematoporphyrin IX-dimethyl ester complexes are very good. However, after the base hydrolysis of the silyl-blocked N-methylhematoporphyrin IX-dimethyl ester complex, the yield of the desired N-methylhematoporphyrin product is not very high (less than 70%). Also, this product is never completely pure because there is always some sodium hydroxide present with it. Both the relatively low yield and partially impure product are most likely due to the extremely high basicity of the N-methylhematoporphyrin complex. (The  $pK_3$  of a very similar porphyrin, N-methyldeuteroporphyrin, was found to be very close to 11(70).) The commonly used methods of hydrolysis could not be used because of the high basicity of this N-methylporphyrin. If any amount of acid was added, the secondary alcohol groups on the periphery of the porphyrin would dehydrate to vinyl groups (i.e., change from hematoporphyrin to protoporphyrin). The high basicity also caused this N-methylporphyrin to become protonated when being eluted through the molecular exclusion column with methanol, probably from trace water contained in the methanol. In order to isolate the product as the free base form (sodium salt) and not the protonated species, a strong base was required to compete favorably with the N-methylporphyrin for the proton. A small excess of sodium hydroxide was necessary, so the porphyrin product was slightly contaminated with the excess base. Fortu-

nately, the sodium hydroxide byproduct did not in any way hinder the use of the N-methylhematoporphyrin complex as the porphyrin precursor. If this material would ever be needed in large quantities, a better method to hydrolyze the silyl-blocked N-methylhematoporphyrin IX-dimethyl ester would have to be found.

#### PREPARATION OF THE $^{109}\text{Pd}$ -HEMATOPORPHYRIN COMPLEX

The synthesis of the  $^{109}\text{Pd}$ -hematoporphyrin complex by this new route of using N-methylhematoporphyrin as a precursor was found to be vastly superior to the traditional method of preparation. Complexation of N-methylporphyrins with a variety of metals and subsequent demethylation have been found to be quantitative and, with the proper choice of nucleophile, can be many orders of magnitude faster than the traditional method of metalation of planar porphyrins. With the procedure used to synthesize the  $^{109}\text{Pd}$ -hematoporphyrin complex, it is absolutely certain that only the palladium which is bound to the porphyrin is allowed into the injecting solution. Any free palladium would be precipitated by the sodium hydroxide and filtered from the injecting solution by the millipore filter. This insures that the solution is free from unbound palladium contaminants. This is important because upon injection into an animal, ionic palladium species would go directly to the kidneys. However, it is questionable whether the evaporation of the DMSO/NaAc

and the redissolution of the subsequent solid material in NaOH is actually necessary. This procedure is presently followed because work by Fawwaz has indicated that materials which are dissolved in DMSO tend to accumulate in the kidneys(71). Therefore, the DMSO is removed as a precaution. Further tests should be carried out to determine whether a 10% solution of DMSO would in fact change the distribution of the  $^{109}\text{Pd}$ -hematoporphyrin complex in an animal. This procedure produces the reaction product rapidly and with a minimum loss of activity (less than 5%). The only other product of the reaction is the formation of methylated DMSO which is presumably decomposed by water to produce methanol (which is removed when the DMSO/NaAc is evaporated). The N-methylhematoporphyrin precursor is very stable in the absence of metal ions. No decomposition of the solid is seen after several months.

#### DISTRIBUTION STUDIES

To compare the  $^{109}\text{Pd}$ -hematoporphyrin prepared by this new method with that of the traditional acetic acid method, localization in dogs was chosen to be the criterion. Spectroscopic comparisons of the two materials indicated their similarity but the possible effects of any residual byproducts of the two syntheses and the unlikely possibility that the dissolution properties of the two materials might differ, have prompted us to investigate distribution patterns.

Comparison of the activity distribution patterns of the beagles shown in Table II with those previously observed in beagles that were given the  $^{109}\text{Pd}$ -hematoporphyrin synthesized by the acetic acid route(72) gave comparable results. The necessary characteristics of the distribution: good localization in the mesenteric lymph nodes; reasonably but not overly large localization in the spleen; reasonably rapid blood clearance; and relatively low localization in most other organs, have been reproduced by this new method of synthesis. The one major difference between the values given in Table II and those in reference 72 is that the absolute values for the fraction of administered activity per gram of tissue in our study are lower. This is simply due to the difference in weights between the beagles in the two studies and the resulting difference in tissue weights (i.e., the beagles in our study weighed significantly more than those used in Fawwaz's study(72)) and therefore, the effective concentrations of activity per gram of tissue reported in our study is less.

#### TOXICITY STUDIES

Toxicity studies were also performed to compare results using this new method of synthesis with the traditional one. In this case there apparently is a significant difference. The LD of the  $^{109}\text{Pd}$ -hematoporphyrin prepared by the traditional acetic acid method was close to 40 mCi/kg rat while

the LD<sub>50</sub> for our preparation was approximately 12 mCi/kg dog. The LD<sub>50</sub> of the <sup>109</sup>Pd-hematoporphyrin prepared by the acetic acid preparation in dogs has not been determined due to the difficulty of retaining the large amount of activity needed for a toxicity study. The LD<sub>50</sub> of the material synthesized by our preparation in rats has not been done yet but is presently being worked on. Although the LD<sub>50</sub>s of a substance in a rat and dog would not be expected to be identical, available data suggest that they should be reasonably close to each other. Therefore, an LD<sub>50</sub> of 40 mCi/kg rat is probably significantly different from an LD<sub>50</sub> of 12 mCi/kg dog, although the actual difference may not be that large. This apparently much lower LD<sub>50</sub> dose using our preparation of the <sup>109</sup>Pd-hematoporphyrin complex may be due to the differences in purity between the two materials. The <sup>109</sup>Pd-hematoporphyrin prepared by our method clears the blood slightly faster than the material from the traditional synthesis. This implies that this <sup>109</sup>Pd-hematoporphyrin gets into the organs at a faster rate than the other material which could also account for the increased toxicity of this material. Because of the faster blood clearance time, however, the dose at which this <sup>109</sup>Pd-hematoporphyrin complex is effective for selective lymphatic ablation may be lower than the effective dosage of the traditionally synthesized <sup>109</sup>Pd-hematoporphyrin. If this were true, the quantity of activity that the organs would have to be subjected to could be decreased. A comparison between the

effective dose of the  $^{109}\text{Pd}$ -hematoporphyrin complex synthesized by the two methods is hampered because of the difficulty in retaining, in the acetic acid preparation, the large amount of activity necessary to implement lymphatic ablation in dogs. .

#### TRANSPLANTATION STUDIES

The results of the kidney transplantation study have been disappointing so far because of the lack of useful information from the eight beagles used. The first two beagles were only given the  $^{109}\text{Pd}$ -hematoporphyrin complex before the transplantation operation. From the studies on rats done earlier, it was not expected that the  $^{109}\text{Pd}$ -hematoporphyrin complex itself would prolong the lifetime of the transplanted kidney and in fact, it did not. The kidney of the first dog was rejected at about nine days as indicated by the creatinine and blood uric nitrogen levels. (The normal levels of creatinine and blood uric nitrogen are  $<1.5$  and  $<20$ , respectively while counts of  $>4.0$  and  $>60$  indicate that rejection of the transplanted organ has taken place.) The homograft of a control dog given no drugs before the transplantation operation would be rejected at about nine to ten days.

Of the three dogs given both the  $^{109}\text{Pd}$ -hematoporphyrin and the antilymphocyte globulin drugs, only one beagle (#19) gave some information about the drugs and the homograft.

This dog lived for 35 days although she was never very healthy after the transplantation operation. The necropsy revealed that the major cause of death was infection rather than rejection of the kidney. If the dog was healthy, she may have remained alive for quite some time. The other two beagles given the  $^{109}\text{Pd}$ -hematoporphyrin and antilymphocyte globulin (#21 and #23) both died within ten days from causes unrelated to the transplanted kidney. The necropsy on Beagle 23 revealed that only 75% of the mesenteric lymph nodes were destroyed. For this procedure to succeed, all the mesenteric lymph nodes must be destroyed by the  $^{109}\text{Pd}$ -hematoporphyrin drug. In the case of this dog at least, the  $^{109}\text{Pd}$  activity was not completely effective.

From the readings of the absolute lymphocyte and white blood cell counts before and after the transplantation operation, it was evident that the antilymphocyte globulin drug was not performing adequately. The purpose of antilymphocyte globulin is to destroy the immunocompetent lymphocytes that are present in the circulatory system. This drug in conjunction with the  $^{109}\text{Pd}$ -hematoporphyrin should decrease the absolute lymphocyte percentage - the percentage with respect to the white blood cell count - close to zero. But this was not occurring. For that reason, three more beagles were injected with just the antilymphocyte globulin drug before the kidney was transplanted to see the effect of this drug alone. From previous work on rats, it was expected that the antilymphocyte globulin drug itself would double

the lifetime of the graft, but at the three dosage levels given (Beagle 20 - 15 mg antilymphocyte globulin/kg dog, Beagle 24 - 20 mg/kg and Beagle 22 - 30 mg/kg), no prolongation was evident. More experiments need to be run to determine the effective dose of antilymphocyte globulin in a dog.

The healthiness of the test beagles that have been used in these experiments before the administering of any drug is open to question. A significant percentage of the beagles were either sick or susceptible to infection so that the real purpose of the experiments was masked by symptoms of the unhealthy animals. It is mandatory that the animals be in excellent health before these types of experiments can be undertaken. Certainly more transplantation experiments need to be run to demonstrate the effectiveness of the  $^{109}\text{Pd}$ -hematoporphyrin and antilymphocyte globulin drugs. Also, another experiment to try is the administering of the  $^{109}\text{Pd}$ -hematoporphyrin and antilymphocyte globulin drugs in conjunction with a bone marrow infusion. It is this combination of drugs that had the most success on rat heart transplants from donor to recipient which had major histocompatibility differences. One would hope to get similar results from the studies on dogs.

## CONCLUSION

The transplantation study thus far is inconclusive. However, from experiments on rats and the few useful results that have been gotten from the dog experiments, the outlook of this method to eradicate organ transplant rejection by selective lymphatic ablation is promising. The advantages of this procedure to prevent transplanted organ rejection are enormous. Perhaps the greatest benefit is that recipients of transplanted organs would not have to remain on immunosuppressive drugs for the rest of their lives as is necessary for present day procedures. They would therefore be free of the debilitating effects of the immunosuppressive drugs used today. Another great advantage is that organ donors and recipients would not have to be so closely cross-matched as is mandatory at the present time. The donor and recipient could have large histocompatibility differences but the organ might not be rejected by this selective lymphatic ablation method of immunosuppression. Also, only a portion of the immune system would be suppressed by this method. The recipient's body would still be able to muster a defense against disease and bacterial infection. These advantages make it imperative to fully explore the possibilities of this selective lymphatic ablation technique.

P A R T    II

CHAPTER 2

KINETIC STUDY OF Pd-TETRAPHENYLPORPHYRIN  
IN ACETONITRILE, DIMETHYLFORMAMIDE  
AND ACETONITRILE

## INTRODUCTION

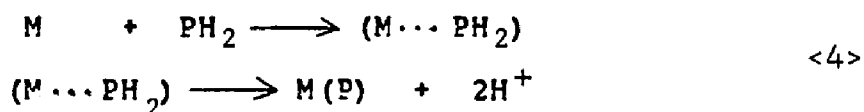
Derivatives of porphyrins occur widely in nature and have an important role in photosynthesis, biological oxidation-reduction reactions and oxygen transport. A wide variety of metal ions can be bound by the 4-coordinate macrocyclic porphyrin ligand. Examples of some important metalloporphyrins and related compounds are chlorophylls - which contain Mg(II); protohaem - the prosthetic group of hemoglobin and myoglobin in which Fe(II) is complexed with protoporphyrin IX; and vitamin B<sub>12</sub> - which is biologically active as the 5'-deoxyadenosyl derivative and has Co(III) incorporated in the center of the porphyrin ring. The mechanism by which the metal ion incorporates into the natural porphyrins in biological systems is obviously of scientific interest and importance. However, a number of problems are encountered when attempting to study these natural systems.

Solubility is always a difficult problem because metal salts dissolve preferentially in polar solvents while porphyrins more readily dissolve in nonpolar environments. Detergents have been added to increase the solubility of the two species but can markedly affect the rate of metalation depending on the type of detergent added (anionic or

cationic). Another problem with natural systems is the very slow incorporation of the metal ion into the porphyrin without the enzymes that are normally present in vivo.

Synthetic porphyrins are being used extensively to eliminate some of these problems. Porphyrins such as meso-tetrapyridylporphine, tetrasulfonated tetraphenylporphine and tetrakis(4-methylpyridyl)porphine are water soluble and can be used as model systems. Through the study and characterization of these systems it may be possible to extend the results to biologically important porphyrins in their natural environment.

The mechanism of metal ion incorporation into porphyrins has been extensively studied for many years. Because of the multitude of conditions used, comparisons are difficult to make. However, several conclusions have been drawn. Almost without exception, porphyrin metalation studies have indicated that an associative mechanism involving the free base porphyrin ( $\text{PH}_2$ ) takes place. This mechanism is shown below.



Though the mechanisms have been generalized as either dissociative ( $\text{S}_{\text{N}}1$ ) or displacement ( $\text{S}_{\text{N}}2$ ) reactions(21,22), there are nearly as many detailed mechanisms as there are kinetic studies.

Although the absolute magnitude of rates of metal ion insertion into a number of porphyrins in a variety of sol-

vents does not necessarily remain constant, the order of metal ion reactivity generally holds. Hydrated metal ions inserted into tetrakis-(4-N-methylpyridyl) porphine in water at pH 4 and 50°C(23) follow the order: Cu(II) > Zn(II) > Co(II) > Mn(II) > Ni(II). Metal halides complex with  $\alpha, \beta, \gamma, \delta$ -tetraphenylporphine in dimethylformamide at 70°C(24) in the order Cu(II) > Cd(II) > Zn(II) > Pd(II) > Co(II) > Ni(II) > Mg(II). Tetrapyrroldiporphine incorporating a metal ion in aqueous nitric acid solutions(25) shows the rates to follow the order: Cu(II) > Zn(II) > Mn(II) > Co(II) > Fe(II) > Ni(II) > Cd(II). These examples show the consistency of the metal order with the exception of the order of Co(II) and Mn(II). It is interesting to note that the relative rates of incorporation of metal ions follow the order of the relative rates of water exchange in the coordination sphere of the metal ions with: Cu(II) > Zn(II) > Mn(II) > Co(II) > Ni(II) (26).

The nature of the reacting metal ion species has also been found to be extremely important in the kinetics of metalloporphyrin formation. Several studies of metal ion incorporation into a variety of porphyrins using the acetate salts of the metal ion in the solvent acetic acid found a half order dependence on the metal ion(27,28). The half order dependence could be explained by a predissociation of the metal acetate dimer (i.e.,  $M_2(Ac)_4 \longrightarrow 2M(Ac)_2$ ), followed by the monomer reacting with the porphyrin.

In addition to the studies that describe the dependences of the metal ion complexation reaction to porphyrins on various components in the system, a number of other studies have attempted to isolate intermediates of this porphyrin metalation reaction. In 1960, Fleischer and Wang proposed the controversial sitting-atop (SAT) type complex as an intermediate to metalloporphyrin formation(29). Since then, there has been evidence supporting a SAT intermediate (27,30-32), evidence for the non-existence of SAT complexes (33-39), and a proposition that the intermediate is actually an ion pair instead of a SAT(40). The use of luminescence spectra of N-methyl complexes provided conclusive evidence for the formation of ion pairs rather than SAT complexes under conditions which had been postulated to produce SAT complexes(38). However, this study does not preclude the possibility that there is a SAT complex or some other species formed as a reactive intermediate during the porphyrin metalation reaction. These few examples illustrate some of the factors that influence the rate of metal complexation with porphyrins. For a more complete review of porphyrin metalation kinetics, see references 41 and 42.

Even with the number of studies undertaken using planar porphyrins, several questions remained unanswered. Are the two pyrrolic nitrogen protons attached, partially dissociated or completely dissociated when the metal ion inserts into the porphyrin? Does the rate determining step involve an equilibrium between the planar and puckered forms of the

porphyrin ligand? Does the metal ion associate as an ion pair or form a SAT intermediate before insertion occurs?

N-methylporphyrins, which have a methyl group substituted for one of the pyrrolic nitrogen protons, have been used to help answer some of these questions. The size of the N-methyl group deforms the porphyrin molecule so that it no longer has a planar structure(43,44). This eliminates the equilibrium step between the planar and puckered forms of the porphyrin (because it is already non-planar) which would elucidate whether this step is rate determining. Whether the loss of solvent ligands on the metal and subsequent binding to the porphyrin or the loss of the central protons on the porphyrin and insertion of the metal ion is rate determining should also be explained by results obtained using these nonplanar N-methylporphyrins.

If deformation of the porphyrin is rate determining and this step is eliminated by the use of N-methylporphyrins, then the rate of metal complexation should follow the solvent exchange rates of the metal ions. However, if the loss of the pyrrolic nitrogen protons is the controlling step, the metal insertion rate would be independent of the metal chosen.

Only a few studies involving the kinetics of metal complexation to N-methylporphyrins have been done. Shah, Shears and Hambright compared the rates of Zn(II) and Cd(II) complexation with etioporphyrin III and its N-methyl deriva-

tive in dimethylformamide(45). They found the rate to be first order in both the metals and the porphyrins. The rate of reaction was  $10^5$  faster for the incorporation of the metal ions into the N-methyletioporphyrin III than into the planar etioporphyrin III. It has also been shown by Coughy, Fujimoto and Johnson that the rates of complexation with the statically distorted N-methylporphyrin were much faster than those observed for planar porphyrins(46). These results are consistent with the mechanism involving the deformation of the porphyrin as the rate determining step and not the loss of the proton(s).

A much more comprehensive study was carried out by Bain-Ackerman and Lavalley on the kinetics of metalloporphyrin formation(47). The porphyrin chosen was N-methyltetraphenylporphyrin with the metal ion series Cu(II), Zn(II), Co(II), Mn(II) and Ni(II). The anhydrous metal perchlorate salts were used so that all the metal ions had the same ligand before complexation; the solvent. Dimethylformamide was used as the solvent because of the appreciable solubility of many porphyrins(48) and inorganic salts(49) in it. The results of this study indicated that the metal incorporation reaction was first order in metal ion and in porphyrin. The order of the rate constants were: Cu(II) > Zn(II) > Co(II) > Mn(II) > Ni(II). This order agrees with that found by Lowe and Phillips for metal ions inserting into the tetra(N-methyl-4-pyridinium)porphyrin in aqueous solution at pH 4 and 50°C(23) and also agrees with the

$\alpha, \beta, \gamma, \delta$ -tetraphenylporphyrin complexation reaction done in dimethylformamide by Longo, et al., using metal halides(24). The order of the rates of metal complexation also coincides with the relative rates of water exchange as well as dimethylformamide exchange. This was shown by the observation that the observed rate constant,  $k_{obs}$ , closely followed the solvent exchange rate,  $k_d$ , for the series of metal ions chosen, with the exception of Mn(II). These relatively constant ratios,  $k_{obs}/k_d$ , support the loss of the first solvent molecule from the solvated metal ion as the rate determining step. It was also shown in this study through a deuterium isotope experiment that the breaking of the pyrrolic N-H bond in N-methyltetraphenylporphyrin was not involved in the rate determining step in its reaction with Co(II). Onady and Lavallee, using free base tetraphenylporphyrin, determined that there was no kinetic isotope effect for the reaction of Zn(II) with the protonated and deuterated forms of the porphyrin in dimethylformamide(50).

Another type of study involving N-methylporphyrins is the metal ion assisted demethylation reaction(51-55). Kinetic studies and product analysis on the demethylation reactions of the N-methyltetraphenylporphyrin complexes of Cu(II), Ni(II), Zn(II) and Mn(II) (52-54) and the N-methyletioporphyrin complex of Zn(II) (51) demonstrate that the reaction proceeds by simple abstraction of  $-CH_3^+$ . This reaction involves a metal ion assisting in the dissociation of an electrophile bound to one of the pyrrolic nitrogen atoms of

the porphyrin. The reactivity order:  $\text{Cu(II)} > \text{Ni(II)} > \text{Zn(II)} > \text{Mn(II)}$  is in reasonable agreement with the stability order of the planar metalloporphyrin products(56). The order found for the demethylation reaction is certainly quite different from that found for metalation reactions(23,57). The dependence on nucleophile concentration and the compensation of  $\Delta H^\ddagger$  and  $T\Delta S^\ddagger$  (an isokinetic effect) point to the same mechanism. Also, the tendency to promote demethylation does not correspond to relative rates of planar or N-methyl porphyrin metalation. This suggests that the rate determining steps of these two reactions (i.e., metal complexation and demethylation) are affected differently by the various metal ions studied.

These demethylation reaction studies have biological implications. Alkylating agents having the potential for tumor localization, which would alkylate at a controllable rate to allow time both for placement in neoplastic tissue and clearance from the blood, would have great chemotherapeutic value. N-alkylporphyrins act as alkylating agents with reaction rates which are predictably sensitive to the nature of the metal ion and choice of alkyl group, and they also show nucleophilic site selectivity(58,59). Lavalley has found that one metalloporphyrin, N-methyltetra(p-sulfophenyl)porphinatocopper(II), shows reproducible activity in the P385 lymphocytic leukemia test system as determined by the Screening Section of the National Cancer Institute and therefore has potential as an antitumor agent.

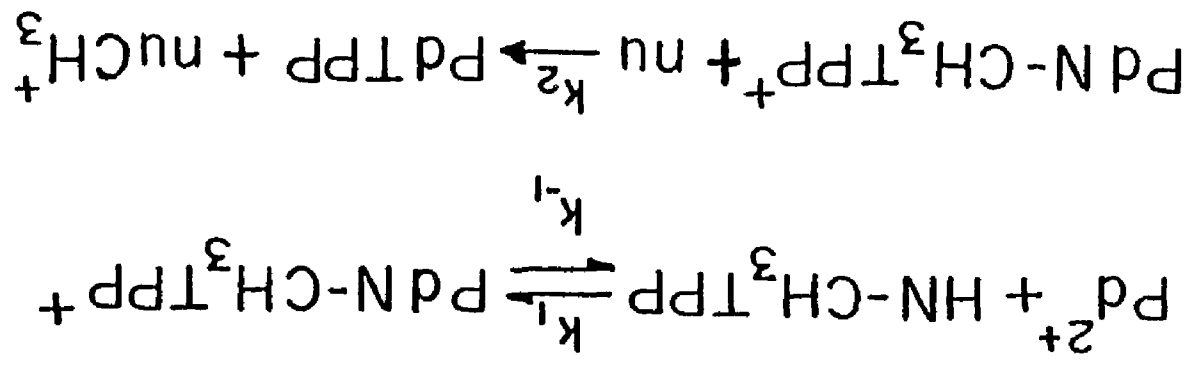
In addition to the application of N-alkylporphyrin complexes as therapeutic agents, elucidation of the kinetics of their dealkylation reactions would be useful in the synthesis of radiolabelled metalloporphyrins for imaging and therapeutic use.

In the presence of a nucleophile, metal ions can react with N-alkylporphyrins by a two step process to produce non N-alkylporphyrin products. This two step mechanism is shown in Figure 8, where Nuc represents the nucleophile. Either the first reaction, the metal complexation reaction, or the second reaction, the demethylation reaction, can be rate determining.

N-methylporphyrins form complexes up to  $10^5$  times more rapidly than non-alkylated porphyrins(46,48). With the proper choice of nucleophile (for the alkyl group) and solvent, the dealkylation reaction can occur rapidly and the overall metalloporphyrin formation can be much faster by this two-step sequence than by the direct reaction of the metal ion with a non-alkylated porphyrin(49-51). A direct application of this principle is the formation of  $^{109}\text{Pd}$ -hematoporphyrin for lymphatic ablation studies in large animals by this two-step process as described in CHAPTER 1.

It is our intention to expand the above mentioned studies on metalloporphyrin formation by studying the second row transition metal ion, Pd(II), in its reaction with an N-me-

FIGURE 8. THE REACTION SCHEME FOR METALLOPORPHYRIN FORMATION FROM Pd(II) AND N-METHYLTETRAPHENYLPORPHYRIN. THE TOP REACTION IS THE COMPLEXATION REACTION WHICH FORMS THE Pd N-METHYLTETRAPHENYLPORPHYRIN COMPLEX. THE BOTTOM REACTION INVOLVES THE DEMETHYLATION OF THE COMPLEX BY A NUCLEOPHILE TO FORM THE PLANAR Pd-TETRAPHENYLPORPHYRIN PRODUCT.



thylporphyrin. The porphyrin chosen for this work is N-methyltetraphenylporphyrin. This compound was chosen because of its ease of synthesis, its reasonably low  $pK_a$  value (in comparison with other N-methylporphyrins) and because it has no ionizable side chain substituents which might interact with the metal. The metal salt used was  $Pd(NO_3)_2 \cdot xH_2O$  because of the relatively inert nitrate ion. So that direct comparisons can be made with previous work, the metal complexation study was done in dimethylformamide and the demethylation reaction was studied in acetonitrile. The nucleophile used to abstract the  $-CH_3^+$  group was di-n-butylamine.

As in the previous metal complexation work, the rate of Pd(II) insertion into an N-methylporphyrin should follow the solvent exchange rate of the Pd(II) ion. On the other hand, the demethylation rate of the Pd(II) N-methylporphyrin complex to form the planar Pd(II) porphyrin product should parallel the stability of the planar metalloporphyrin product. The kinetic data presented in this study will be compared with the work of Longo, et al., on metal incorporation into planar  $\alpha, \beta, \gamma, \delta$ -tetraphenylporphyrin(24); the work of Bain-Ackerman and Lavalley on metal complexation with N-methyltetraphenylporphyrin(47); and the work by Lavalley on the demethylation reaction of metalated N-methyltetraphenylporphyrin complexes(52-54).

A kinetic study on the Pd(II) incorporation into N-methyltetraphenylporphyrin in dimethylsulfoxide is also

reported because the reaction to prepare the  $^{109}\text{Pd}$ -hematoporphyrin complex discussed in CHAPTER 1 was carried out in this solvent and it would be interesting to learn whether the metal complexation step or the demethylation step is rate determining. Kuila and Lavallee have shown that changes on the periphery of the porphyrin have little effect on the demethylation rates of reaction (59). They studied the demethylation reactions of  $\text{Cu N-CH}_3\text{TPP}$ ,  $\text{Cu N-CH}_3\text{TPPS}$  (tetrasulfonate) and  $\text{Cu N-methyldeuterioporphyrin}$  and found little difference in rates with these differently structured porphyrins. The kinetic data in dimethylsulfoxide will then be compared with results in the other two solvents in order to determine the solvent effect on the  $\text{Pd(II)}$  insertion reaction into  $\text{N-methyltetraphenylporphyrin}$ .

The kinetic results in acetonitrile indicate that the demethylation of the  $\text{Pd N-methyltetraphenylporphyrin}$  intermediate is rate determining. The complexation reaction is too fast to be observed using a spectrophotometer. When the  $\text{Pd(II) N-methylporphyrin}$  demethylation rate is compared with the demethylation rates of metalated  $\text{N-methylporphyrin}$  complexes previously studied, the reactivity order is in agreement with the stability of the planar metalloporphyrin products. The conclusions drawn from the kinetic data in dimethylformamide and dimethylsulfoxide are less clear. Although the demethylation reaction is apparently fast and the complexation reaction is rate determining in these solvents, the details of the mechanistic pathway have not been

elucidated. Further work needs to be done to identify the reactive palladium species and to determine the effects of the solvents and non-coordinating base on the reaction involving Pd(II) and N-methyltetraphenylporphyrin.

## EXPERIMENTAL PROCEDURES

### PURIFICATION AND PREPARATION OF SOLUTIONS

The N-methyl- $\alpha,\beta,\gamma,\delta$ -tetraphenylporphyrin was supplied by Sam Borkowsky. It was prepared from methylfluorosulfonate and tetraphenylporphine in refluxing dichloromethane, purified by column chromatography using alumina as the solid support, recrystallized (66), and characterized as previously reported (52).

Acetonitrile was purified by adding 4A molecular sieves and calcium hydride (lumps) to a flask containing reagent grade acetonitrile, stirring overnight, filtering off the molecular sieves and calcium hydride and adding  $P_2O_5$  powder, again stirring overnight, and finally refluxing one hour and distilling. The purified and dry acetonitrile was stored over molecular sieves. (NOTE: Only 0.5 to 1.0% W/V  $P_2O_5$  should be added. An excess catalyzes the formation of a bright orange acetonitrile polymer.)

Dimethylformamide (DMF) was stirred with barium oxide (BaO) for 2 hours under  $N_2$ , distilled from the BaO and collected into a receiving flask containing 4A molecular sieves. There were no abundant impurities (i.e., dimethylamine or formic acid) as evidenced by the  $^1H$  NMR spectrum

taken.

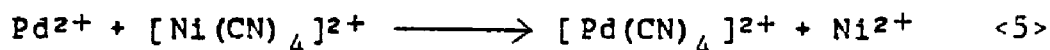
Dimethylsulfoxide (DMSO) was stirred with BaO for 30 minutes, distilled under vacuum and stored under molecular sieves.

Di-n-butylamine was stored with potassium hydroxide (pellets) and calcium hydride (lumps) for two days. The solution was filtered, transferred to another flask and fractionally distilled. The di-n-butylamine was collected in a receiving flask containing molecular sieves.

2,6-Lutidine and 2,6-di-t-butylpyridine were refluxed with BaO and distilled from it. The 2,2,6,6-tetramethylpiperidine was used as received from Aldrich.

Anhydrous  $\text{PdCl}_2$  and  $\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$  were found to be insoluble in  $\text{CH}_3\text{CN}$  so  $\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$  was used. Stock  $\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$  solutions were made up by dissolving the desired amount of the palladium salt in the appropriate solvent, filtering through a sintered glass funnel and then diluting to volume in a volumetric flask.

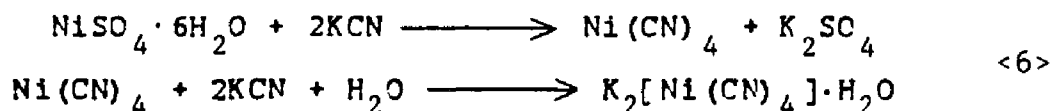
The concentrations of the palladium stock solutions were determined by an indirect EDTA titration method described by Vogel (67). The essential reaction is given below.



All the  $\text{Pd}^{2+}$  ions are bound by the  $[\text{Ni}(\text{CN})_4]^{2-}$  to free the same stoichiometric amount of  $\text{Ni}^{2+}$ . The free  $\text{Ni}^{2+}$  is com-

pletely bound as the NiEDTA complex by the addition of excess  $\text{EDTA}^{2-}$ . The excess  $\text{EDTA}^{2-}$  is then titrated with a standardized  $\text{MnSO}_4$  solution. One can then calculate the amount of  $\text{Pd}^{2+}$  that was initially present.

Potassium tetracyanonickelate(II) was prepared by the method of W.C. Fernelius(68). The reaction sequence is as follows:



The  $\text{MnSO}_4$  solution was standardized by an EDTA titration using Eriochrome Black T (EBT) as an indicator(69). Palladium was present in amounts not exceeding 25-30 mg. Any acid from the palladium salt was neutralized by adding 0.1M NaOH (as determined by a blue color with bromocresol green indicator). A 5.0 ml of 0.1M HCl and 30-60 mg of  $\text{K}_2[\text{Ni}(\text{CN})_4] \cdot \text{H}_2\text{O}$  were added and the solution diluted to 40 ml. Then 5 ml of buffer solution of pH=10 (ammonia/ammonium chloride solution) were added and the solution mixed well. Excess 0.01000M EDTA solution, 0.1 g of ascorbic acid and a few drops of EBT were then added. (NOTE: Ascorbic acid was used to keep the palladium in the divalent state.) The excess EDTA was titrated with the standardized  $\text{MnSO}_4$  solution until the color of the solution changed from blue to pink.

#### TYPICAL OPERATING PROCEDURE USED FOR KINETICS

Solutions in 1 cm cuvettes were placed in the cell compartment of a Cary 14 or a Varian 635 spectrophotometer, with the temperature maintained by a circulating temperature bath. When using the Beckman DU-8, the temperature of the samples was regulated by a thermoelectric (Peltier) heater and cooler. The solutions were allowed to equilibrate for at least a half an hour. The proper quantity of di-n-butylamine or non-coordinating base (2,6-lutidine, 2,6-di-t-butylpyridine or 2,2,6,6-tetramethylpiperidine) was pipetted into the porphyrin solution in one cuvette. The  $\text{Pd}(\text{NO}_3)_2$  solution was placed in another cuvette. A spectrum of the N-methyltetraphenylporphyrin solution was taken of a sample made from the proper amount of porphyrin stock solution and solvent to make the same concentration as the porphyrin in the kinetic determination. The solvent was used as a blank. A spectrum was also taken of the  $\text{Pd}(\text{NO}_3)_2$  solution to be used so that the optical density of the excess metal solution could be subtracted from the spectrum of the final product. The  $\text{Pd}(\text{NO}_3)_2$  solution was pipetted into the cuvette containing the the porphyrin solution, the cell was shaken and the run started. The spectrophotometer was set at the appropriate wavelength and a timer was started after the injection of the metal solution. The reaction was taken through six half-lives in most cases. A spectrum of the product was taken at the end of the run and compared to a spectrum of the expected product (58).

# TREATMENT OF KINETIC DATA

In all cases, the kinetics were conducted under pseudo first order conditions with the  $\text{Pd}(\text{NO}_3)_2$  and amine or non-coordinating base being held in large excess (greater than 20 times) compared to the porphyrin.

The first order rate law can be represented by equation <7>

$$-d[A]/dt = k[A] \quad <7>$$

and the integrated form shown in equation <8>.

$$[A] / [A]_0 = \exp(-kt) \quad <8>$$

The fraction of A left at any time t is represented in the left side of equation <8>. This is also equal to  $(OD_\infty - OD_t) / (OD_\infty - OD_0)$ , where  $OD_\infty$  and  $OD_0$  are the final and initial optical density readings and  $OD_t$  is the optical density at any time, t. With this substitution for  $[A] / [A]_0$  in equation <8>, one gets equation <9>.

$$(OD_\infty - OD_t) / (OD_\infty - OD_0) = \exp(-kt) \quad <9>$$

The experimental observable is OD. Solving equation <9> for  $OD_t$  yields equation <10>.

$$OD_t = (OD_\infty - (OD_\infty - OD_0)\exp(-kt)) \quad <10>$$

By putting appropriate variable and parameter names in for the exponential function given in equation <10>, one gets the proper form that is usable to the Prophet computer system.

$$Y(t) = (B1 - (B1 - B2) * \exp(-B3 * X)) \quad <11>$$

where  $B1=OD_{\infty}$ ,  $B2=OD_0$  and  $B3=k_{obs}$ . The non-linear least squares program within Prophet calculates  $OD_t$  from the parameters and variables given and compares its value for  $OD_t$  to the observed value. It minimizes the sum of the squares of the differences,  $\sum (OD_t(\text{expt'l}) - OD_t(\text{calc}))^2$ .  $OD_{\infty}$  and  $OD_0$  were arbitrarily set to 1 and the observed rate constant,  $k_{obs}$ , was given a value of 0.001 so that Prophet was able to fit the function shown in equation <11>. All three parameters are allowed to vary simultaneously. Generally, five iterations were necessary to perform the fits.

Activation parameters were determined with the use of the Prophet computing system. The Eyring relationship (shown in equation <12> is utilized in the calculation of  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  where  $\kappa$  is the Boltzmann constant =  $1.381 \times 10^{-16}$  erg/ $^\circ$ K,  $h$  is Planck's constant =  $6.626 \times 10^{-27}$  erg sec and  $R$  is the gas constant = 1.987 cal/mole $^\circ$ K.

$$k = (\kappa T/h) \exp(-\Delta H^\ddagger/RT) \exp(\Delta S^\ddagger/R) \quad <12>$$

Putting the Eyring relationship in a form that can be used on Prophet gives the following equation:

$$\ln(k/T) = (-\Delta H^\ddagger/R(1/T)) + (\ln(\kappa/h) + \Delta S^\ddagger/R) \quad <13>$$

By plotting  $\ln(k/T)$  versus  $(1/T)$ , one gets a straight line where the slope =  $-\Delta H^\ddagger/R$  and the y-intercept =  $\ln(\kappa/h) + \Delta S^\ddagger/R$ .

## INSTRUMENTATION

The kinetic determinations were done on four UV-Visible spectrophotometers: the Cary 14, Cary 219, Varian 635 and Beckman DU-8. Only one kinetic run could be done at one time on the Cary 14 while four could be done (plus a blank) on the Varian 635 and five runs (plus a blank) could be monitored on the Beckman DU-8. The Cary 219 was used primarily for overlay spectra.

The pipettes used for kinetics were the Gilson PIPETMAN continuously adjustable digital microliter pipettes. The two pipettes used were the P-200D (20-200  $\mu$ l) and the P-1000D (100-1000  $\mu$ l). The accuracy of these pipettes is better than 1% while the precision is greater than 0.2%. The pipettes were calibrated for each solvent by dispensing measured volumes and weighing the solvent on an analytical balance. The volume of liquid actually delivered could then be calculated.

The other instruments used have been described previously (see EXPERIMENTAL PROCEDURES, CHAPTER 1).

## MATERIALS

In addition to the reagents listed previously, the following materials and their sources are listed below.

|                                           |                     |
|-------------------------------------------|---------------------|
| ammonium chloride                         | Alfa                |
| ascorbic acid                             | Aldrich             |
| barium oxide                              | Fisher Technical    |
| bromocresol green                         | M. Coleman and Bell |
| calcium hydride                           | Fisher Certified    |
| di-n-butylamine                           | Fisher Reagent      |
| di-t-butylamine                           | Eastman             |
| N,N-dimethylformamide                     | Fisher Certified    |
| EDTA, disodium salt                       | G.F. Smith          |
| Eriochrome Black T                        | Aldrich             |
| hydrochloric acid                         | Fisher Reagent      |
| hydroxylamine hydrochloride               | Alfa                |
| $\text{MnSO}_4 \cdot 6\text{H}_2\text{O}$ | Fisher Certified    |
| $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ | Fisher Certified    |
| phosphorus pentoxide                      | Fisher Certified    |
| potassium cyanide                         | Alfa                |
| potassium hydroxide                       | Fisher Certified    |
| 2,2,6,6-tetramethylpiperidine             | Aldrich             |
| triethanolamine                           | Aldrich             |

## RESULTS

### SOLVENTS AND CONDITIONS USED

The kinetics of the insertion of Pd(II) into N-methyltetraphenylporphyrin in  $\text{CH}_3\text{CN}$ , DMF and DMSO were investigated using the nitrate salt of Pd(II). The metal dependence, amine dependence and temperature dependence were acquired and evaluated in each solvent. All runs were done under pseudo first order conditions with the metal concentration being in at least a 25-fold excess. The porphyrin concentration ranged from  $1-15 \times 10^{-4}\text{M}$ . The metal concentration range was  $1-25 \times 10^{-3}\text{M}$ . The amine concentration was varied from .010M to 1.0M. The non-coordinating bases used in the kinetic studies were 2,6-lutidine and the more sterically hindered bases, 2,6-di-t-butylpyridine and 2,2,6,6-tetramethylpiperidine. The concentration of the non-coordinating base, when used, was kept between 0.0100M and 0.300M. The results will be presented for each solvent individually.

### KINETIC RESULTS IN ACETONITRILE

The kinetics in acetonitrile were run by monitoring the appearance of the Pd-tetraphenylporphyrin product. At a

concentration of around  $10^{-5}\text{M}$  porphyrin, the kinetics were run at 537 nm. When the concentration of the porphyrin was closer to  $10^{-6}\text{M}$ , the kinetic data were obtained at 412 nm.

The results for the metal and amine dependence at the temperatures, 25, 37 and 50 degrees Centigrade are given in Tables VII, VIII and IX, respectively. The amine concentration was varied over a 40-fold range; from 1M to 0.0250M. The metal concentration was kept at either  $1.00 \times 10^{-2}\text{M}$  or  $1.00 \times 10^{-3}\text{M}$ . At a concentration of Pd(II) of  $1.00 \times 10^{-2}\text{M}$ , the second order rate constant was found to be  $(2.45 \pm 0.34) \times 10^{-3} \text{ M}^{-1}\text{s}^{-1}$  at  $25.0^{\circ}\text{C}$ . When the Pd(II) concentration was  $1.00 \times 10^{-3}\text{M}$ , the rate constant was  $(3.59 \pm 0.48) \times 10^{-3} \text{ M}^{-1}\text{s}^{-1}$ . These results were determined using four stock solutions of different concentrations.

Figure 9 shows a kinetic run done by overlaying spectra from 700-450 nm during the course of the reaction at about 5 minute intervals. This overlaid kinetic run indicates the instantaneous formation of an intermediate (presumably the Pd N-methyltetraphenylporphyrin complex) because no indication of the porphyrin starting material is seen. Isosbestic points at 544 and 521 nm indicate that the porphyrin intermediate complex goes directly to the planar metalloporphyrin product with no further intermediates formed at any significant concentration.

In Figures 10 and 11, plots of the observed rate constant versus the di-n-butylamine concentration are shown at Pd(II)

TABLE VII. Kinetic Results for the Reaction of Pd(II) with N-CH<sub>3</sub> Tetraphenylporphyrin in Acetonitrile at 25°C.

| [amine] <sup>a</sup> | [Pd(II)]<br>x 10 <sup>3</sup> | [N-CH <sub>3</sub> TPP]<br>x 10 <sup>5</sup> | k <sub>obs</sub> x 10 <sup>3</sup><br>s <sup>-1</sup> | k <sub>obs</sub> /[amine]<br>x 10 <sup>3</sup> , M <sup>-1</sup> s <sup>-1</sup> | t <sub>1/2</sub><br>(min.) |
|----------------------|-------------------------------|----------------------------------------------|-------------------------------------------------------|----------------------------------------------------------------------------------|----------------------------|
| 1.000                | 10.00                         | 2.67                                         | 2.04                                                  | 2.04                                                                             | 5.66                       |
| 0.800                | 10.00                         | 2.89                                         | 1.70                                                  | 2.12                                                                             | 6.79                       |
| 0.600 <sub>b</sub>   | 10.00                         | 3.10                                         | 1.39                                                  | 2.31                                                                             | 8.33                       |
| 0.600 <sub>b</sub>   | 10.00                         | 3.23                                         | 1.38                                                  | 2.30                                                                             | 8.36                       |
| 0.373 <sub>b</sub>   | 10.00                         | 3.59                                         | 0.880                                                 | 2.36                                                                             | 13.1                       |
| 0.200                | 10.00                         | 0.296                                        | 0.517                                                 | 2.59                                                                             | 22.3                       |
| 0.100                | 10.00                         | 0.304                                        | 0.306                                                 | 3.06                                                                             | 37.7                       |
| 0.100                | 10.00                         | 0.304                                        | 0.302                                                 | 3.02                                                                             | 38.2                       |
| 0.100                | 10.00                         | 0.527                                        | 0.300                                                 | 3.00                                                                             | 38.7                       |
| 0.100                | 10.00                         | 0.304                                        | 0.275                                                 | 2.75                                                                             | 42.0                       |
| 0.100                | 10.00                         | 0.304                                        | 0.291                                                 | 2.91                                                                             | 39.7                       |
| 0.100                | 10.00                         | 0.166                                        | 0.306                                                 | 3.06                                                                             | 37.7                       |
| 0.0500               | 10.00                         | 0.296                                        | 0.136                                                 | 2.72                                                                             | 84.9                       |
| 0.0500               | 10.00                         | 0.296                                        | 0.138                                                 | 2.76                                                                             | 83.7                       |
| 0.0500               | 10.00                         | 0.166                                        | 0.143                                                 | 2.86                                                                             | 80.9                       |
| 0.400                | 1.00                          | 0.166                                        | 1.21                                                  | 3.02                                                                             | 9.56                       |
| 0.400                | 1.00                          | 0.130                                        | 1.23                                                  | 3.06                                                                             | 9.42                       |
| 0.400                | 1.00                          | 0.332                                        | 1.20                                                  | 3.00                                                                             | 9.61                       |
| 0.200                | 1.00                          | 0.296                                        | 0.633                                                 | 3.16                                                                             | 18.2                       |
| 0.100                | 1.00                          | 0.296                                        | 0.367                                                 | 3.67                                                                             | 31.5                       |
| 0.0500               | 1.00                          | 0.296                                        | 0.201                                                 | 4.02                                                                             | 57.5                       |
| 0.0500               | 1.00                          | 0.296                                        | 0.209                                                 | 4.18                                                                             | 55.3                       |
| 0.0250               | 1.00                          | 0.296                                        | 0.101                                                 | 4.04                                                                             | 114                        |
| 0.0250               | 1.00                          | 0.296                                        | 0.0983                                                | 3.93                                                                             | 118                        |

<sup>a</sup>Amine used was di-n-butylamine.

<sup>b</sup>2,6-lutidine was added first (such that its concentration was 0.200M) and later, the given amount of amine was added.

TABLE VIII. Kinetic Results for the Reaction of Pd(II) with N-CH<sub>3</sub> Tetraphenylporphyrin in Acetonitrile at 37°C.

| [amine] <sup>a</sup> | [Pd(II)]<br>x 10 <sup>3</sup> | [N-CH <sub>3</sub> TPP]<br>x 10 <sup>5</sup> | k <sub>obs</sub> x 10 <sup>3</sup><br>(s <sup>-1</sup> ) | k <sub>obs</sub> /[amine]<br>x 10 <sup>3</sup> , M <sup>-1</sup> s <sup>-1</sup> | t <sub>1/2</sub><br>(min.) |
|----------------------|-------------------------------|----------------------------------------------|----------------------------------------------------------|----------------------------------------------------------------------------------|----------------------------|
| 0.200                | 10.00                         | 0.296                                        | 1.23                                                     | 6.15                                                                             | 9.39                       |
| 0.200                | 10.00                         | 0.296                                        | 1.19                                                     | 5.95                                                                             | 9.70                       |
| 0.100                | 10.00                         | 0.304                                        | 0.685                                                    | 6.85                                                                             | 16.9                       |
| 0.100                | 10.00                         | 0.304                                        | 0.771                                                    | 7.71                                                                             | 15.0                       |
| 0.0500               | 10.00                         | 0.296                                        | 0.328                                                    | 6.56                                                                             | 35.2                       |
| 0.0500               | 10.00                         | 0.296                                        | 0.320                                                    | 6.40                                                                             | 36.1                       |
| 0.0250               | 1.00                          | 0.296                                        | 0.290                                                    | 11.6                                                                             | 39.8                       |
| 0.0250               | 1.00                          | 0.296                                        | 0.290                                                    | 11.6                                                                             | 39.8                       |

<sup>a</sup>Amine used was di-n-butylamine.

TABLE IX. Kinetic Results for the Reaction of Pd(II) with N-CH<sub>3</sub> Tetraphenylporphyrin in Acetonitrile at 50°C.

| [ amine] <sup>a</sup> | [ Pd(II)]<br>x 10 <sup>3</sup> | [ N-CH <sub>3</sub> TPP]<br>x 10 <sup>5</sup> | k <sub>obs</sub> x 10 <sup>3</sup><br>s <sup>-1</sup> | k <sub>obs</sub> /[ amine]<br>x 10 <sup>3</sup> , M <sup>-1</sup> s <sup>-1</sup> | t <sub>1/2</sub><br>(min.) |
|-----------------------|--------------------------------|-----------------------------------------------|-------------------------------------------------------|-----------------------------------------------------------------------------------|----------------------------|
| 0.200                 | 10.00                          | 0.296                                         | 3.49                                                  | 17.4                                                                              | 3.31                       |
| 0.200                 | 5.00                           | 0.296                                         | 3.70                                                  | 18.5                                                                              | 3.12                       |
| 0.200                 | 1.00                           | 0.296                                         | 4.19                                                  | 21.0                                                                              | 2.76                       |
| 0.200                 | 1.00                           | 0.296                                         | 4.22                                                  | 21.1                                                                              | 2.74                       |
| 0.100                 | 1.00                           | 0.296                                         | 2.59                                                  | 25.9                                                                              | 4.46                       |
| 0.100                 | 1.00                           | 0.296                                         | 2.45                                                  | 24.5                                                                              | 4.71                       |
| 0.0500                | 1.00                           | 0.296                                         | 1.34                                                  | 26.8                                                                              | 8.62                       |
| 0.0500                | 1.00                           | 0.296                                         | 1.33                                                  | 26.6                                                                              | 8.68                       |
| 0.0250                | 1.00                           | 0.296                                         | 0.762                                                 | 30.5                                                                              | 15.2                       |
| 0.0250                | 1.00                           | 0.296                                         | 0.754                                                 | 30.2                                                                              | 15.3                       |

<sup>a</sup>Amine used was di-n-butylamine.

FIGURE 9. OVERLAID SPECTRA OF A KINETIC RUN FOR THE DEMETHYLATION OF THE PRESUMED INTERMEDIATE, Pd N-METHYLTETRAPHENYLPORPHYRIN IN ACETONITRILE. WAVELENGTHS WERE SCANNED FROM 700-380NM DURING THE ENTIRE COURSE OF THE REACTION. EACH SCAN TOOK ABOUT FOUR MINUTES. THE ARROWS INDICATE INTENSITY CHANGES AS THE REACTION PROCEEDS.

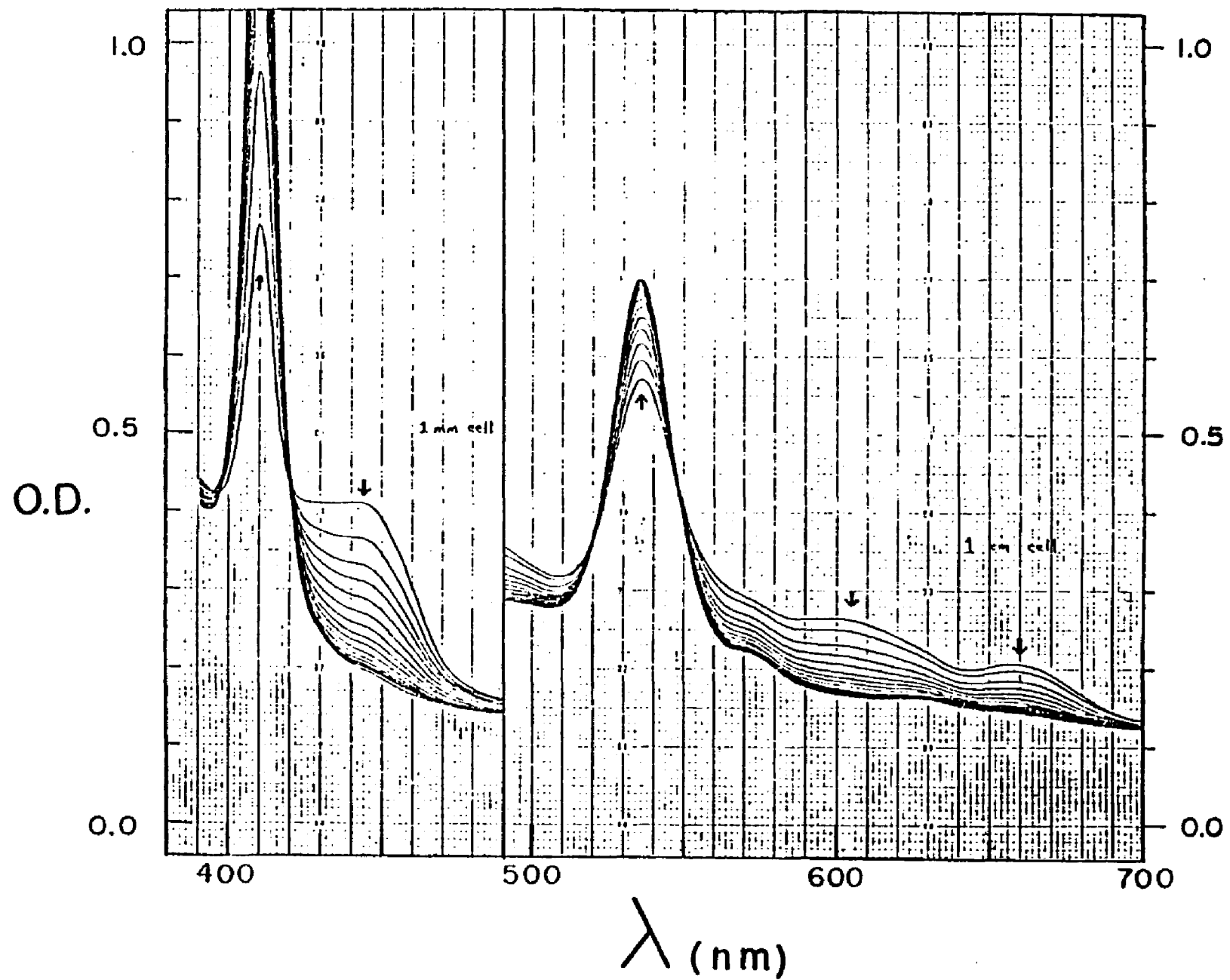


FIGURE 10. THE PLOT OF THE OBSERVED FIRST-ORDER RATE CONSTANT FOR THE DEMETHYLATION OF Pd N-METHYLTETRAPHENYLPORPHYRIN BY DI-N-BUTYLAMINE IN ACETONITRILE AT 25°C AS A FUNCTION OF THE AMINE CONCENTRATION. THE CONCENTRATION OF Pd(II) IS KEPT CONSTANT AT 0.0100M. A LINEAR CORRELATION IS OBSERVED (STANDARD DEVIATION OF THE LINEAR REGRESSION =  $6.250 \times 10^{-5}$ , CORRELATION COEFFICIENT = 0.9975).

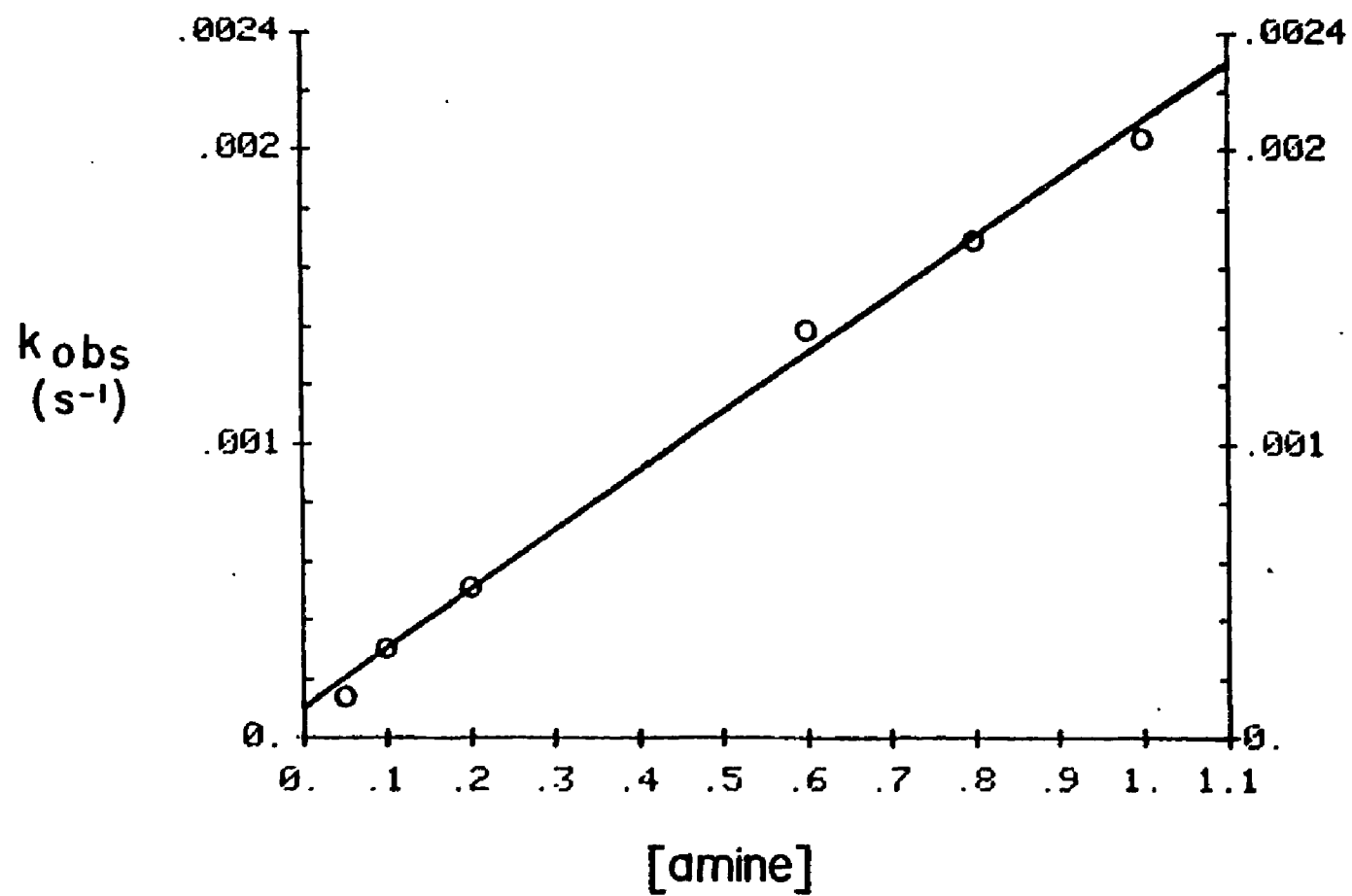
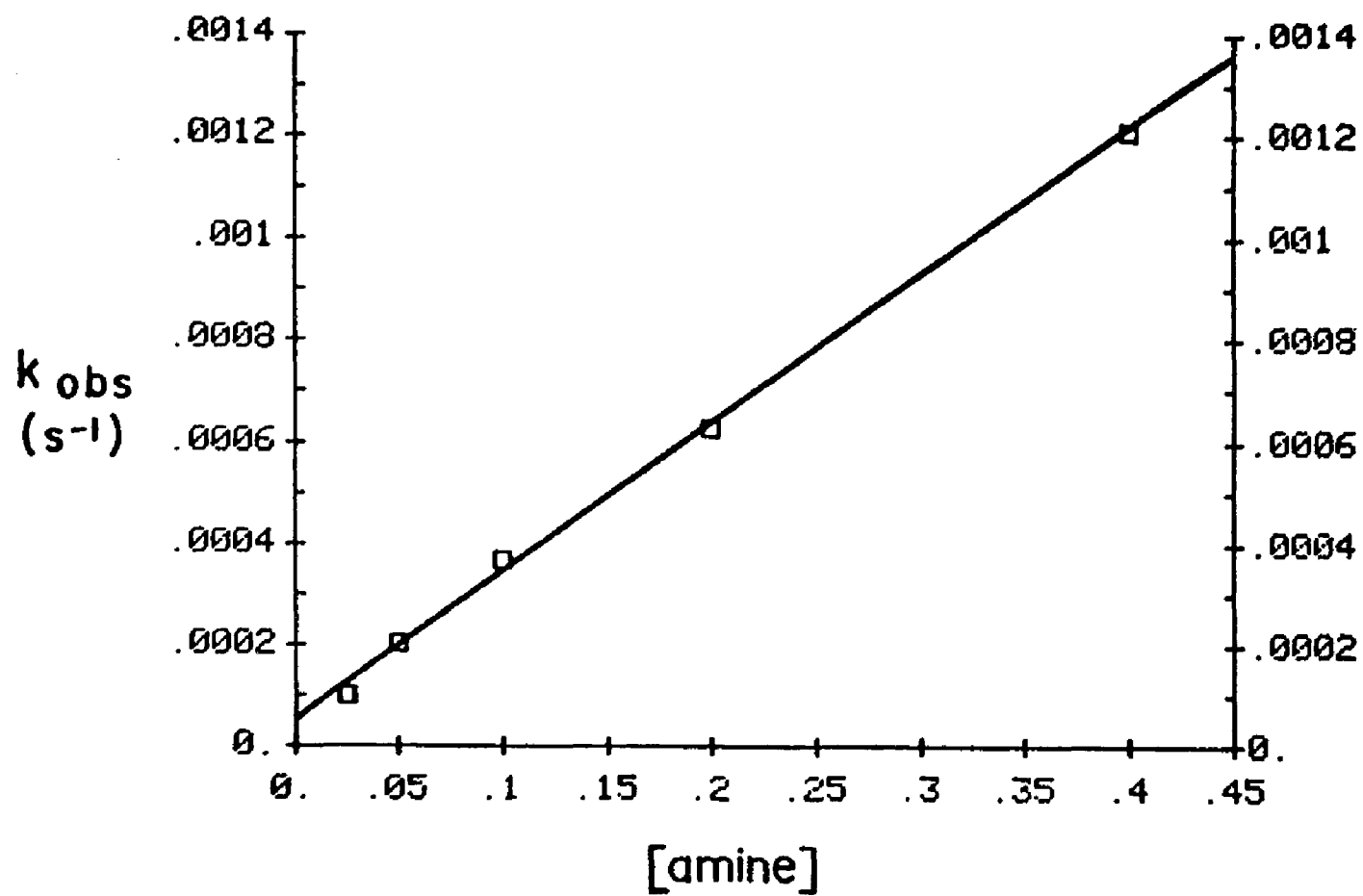


FIGURE 11. THE PLOT OF THE OBSERVED FIRST-ORDER RATE CONSTANT FOR THE DEMETHYLATION OF Pd N-METHYLTETRAPHENYLPORPHYRIN BY DI-N-BUTYLAMINE IN ACETONITRILE AT 25°C AS A FUNCTION OF THE AMINE CONCENTRATION. THE CONCENTRATION OF Pd(II) IS KEPT CONSTANT AT 0.00100M. A LINEAR CORRELATION IS OBSERVED (STANDARD DEVIATION OF THE LINEAR REGRESSION =  $2.135 \times 10^{-5}$ , CORRELATION COEFFICIENT = 0.9991),



concentrations of  $1.00 \times 10^{-2}$  and  $1.00 \times 10^{-3}$ , respectively. Both plots show a linear dependence with the slopes being  $2.00 \times 10^{-3}$  and  $2.91 \times 10^{-3}$ , and the intercepts are  $9.57 \times 10^{-5}$  and  $5.18 \times 10^{-5}$  for Figures 10 and 11, respectively.

The data in Tables VII-IX were used to plot  $\ln(k/T)$  versus  $1/T$ , where  $k$  is the second order rate constant (i.e.,  $k = k_{\text{obs}}/[\text{amine}]$ ), and thus determine the temperature dependence of this system. In Figure 12, the data plotted correspond to 0.0100M Pd(II) and 0.200M di-n-butylamine. The solid line is a least squares fit of the data. The enthalpy of activation,  $\Delta H^\ddagger$ , calculated from the slope of the line according to the Eyring formulation described earlier, was  $14.0 \pm 0.4$  kcal/mole. The entropy of activation,  $\Delta S^\ddagger$ , was calculated from the intercept as  $-23.3 \pm 1.2$  eu. At a Pd(II) concentration of  $1.00 \times 10^{-3}$ M and an amine concentration of 0.0250M, the temperature dependence was plotted in Figure 13. In this case, the  $\Delta H^\ddagger$  was  $14.8 \pm 0.2$  kcal/mole while the  $\Delta S^\ddagger$  was calculated to be  $-19.7 \pm 0.7$  eu.

The rate was not entirely independent of the metal concentration. At all temperatures, when the Pd(II) concentration was decreased from  $1.00 \times 10^{-2}$ M to  $1.00 \times 10^{-3}$ M, the second order rate constant increased by about 18%. That is the reason the temperature dependence was calculated separately for each of the two Pd(II) concentrations used.

#### KINETIC RESULTS IN N,N-DIMETHYLFORMAMIDE

As in acetonitrile, the kinetics in dimethylformamide

FIGURE 12. LINEAR TEMPERATURE DEPENDENCE FOR THE DEMETHYLATION OF Pd N-METHYLTETRAPHENYLPORPHYRIN IN ACETONITRILE WHEN THE Pd(II) CONCENTRATION IS KEPT CONSTANT AT 0.0100M WHICH IS REPRESENTED BY THE EYRING FORMULATION AS  $\ln(k/T)$  VERSUS  $1/T$ . THE SLOPE IS A FUNCTION OF THE ENTHALPY OF ACTIVATION WHICH IS EQUAL TO  $14.0 \pm 0.4$  KCAL/MOL. THE INTERCEPT IS A FUNCTION OF THE ENTROPY OF ACTIVATION WHICH IS EQUAL TO  $-23.3 \pm 1.2$  EU. (NOTE: K IS IN UNITS OF  $M^{-1}s^{-1}$ .)

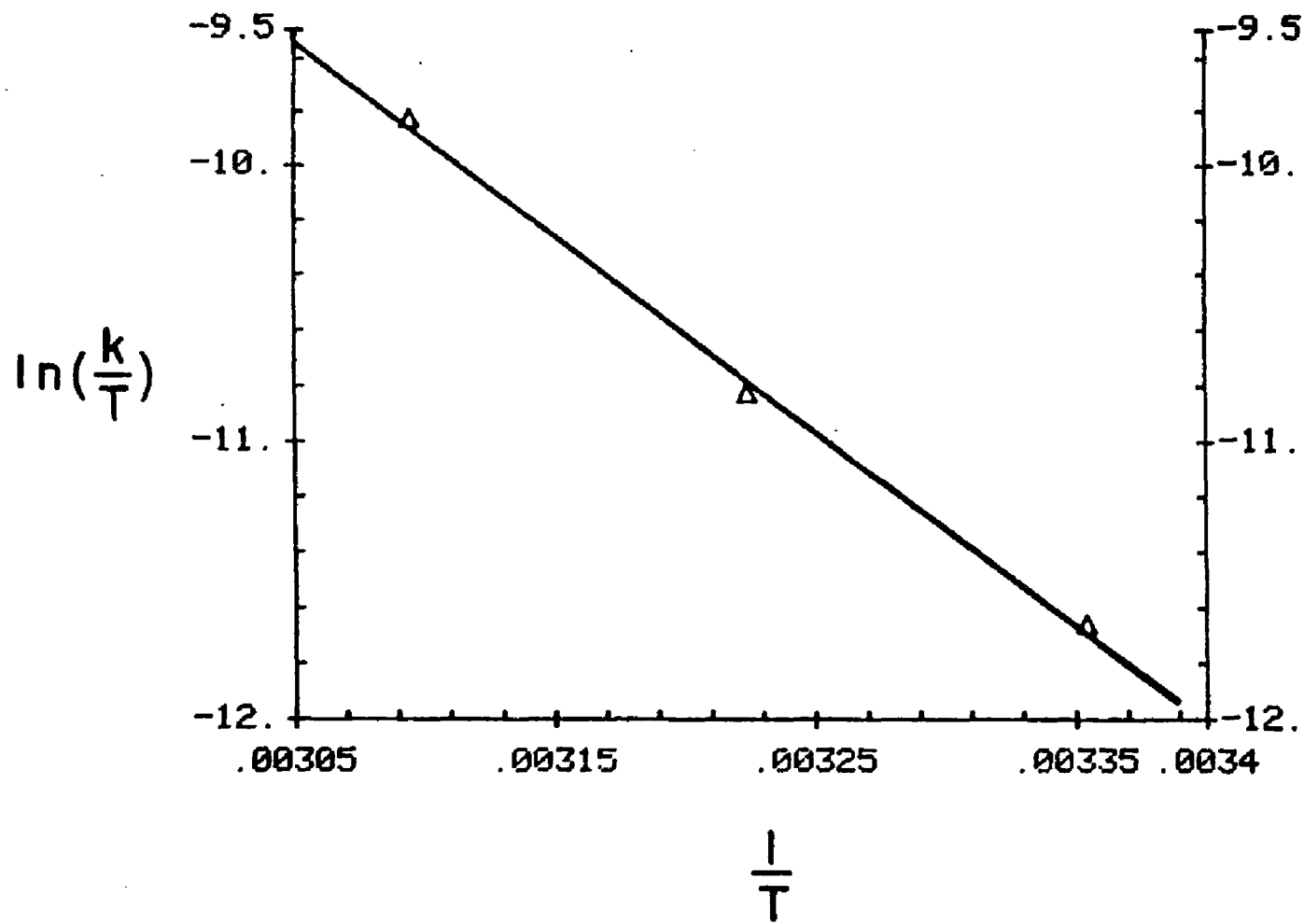
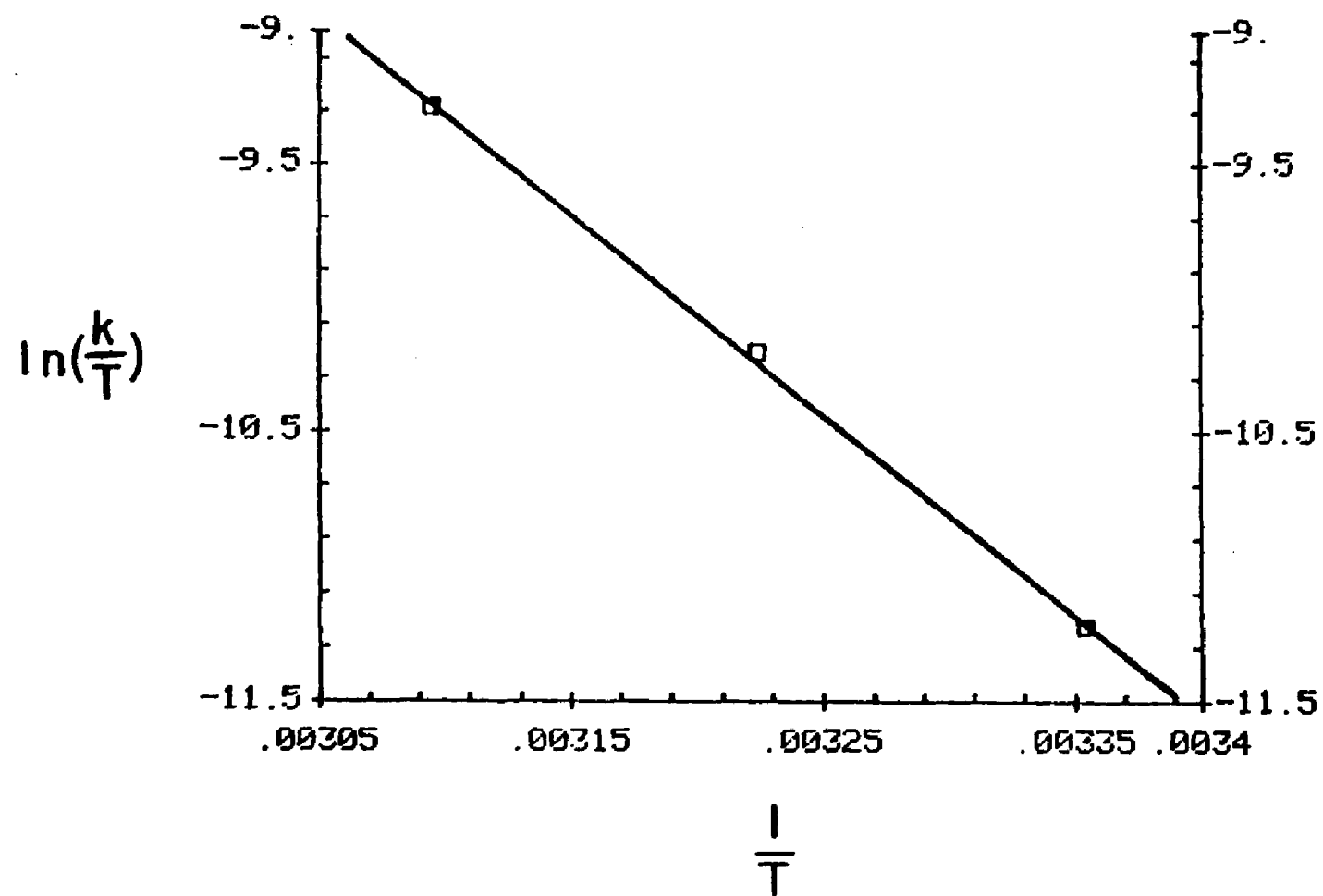


FIGURE 13. LINEAR TEMPERATURE DEPENDENCE FOR THE DEMETHYLATION OF Pd N-METHYLTETRAPHENYLPORPHYRIN IN ACETONITRILE WHEN THE Pd(II) CONCENTRATION IS KEPT CONSTANT AT 0.00100M WHICH IS REPRESENTED BY THE EYRING FORMULATION AS  $\ln(k/T)$  VERSUS  $1/T$ . THE SLOPE IS A FUNCTION OF  $\Delta H^\ddagger$  WHICH IS EQUAL TO  $14.8 \pm 0.2$  KCAL/MOL. THE INTERCEPT IS A FUNCTION OF  $\Delta S^\ddagger$  WHICH IS EQUAL TO  $-19.7 \pm 0.7$  EU. (NOTE:  $k$  IS IN UNITS OF  $M^{-1}s^{-1}$ .)



were run by monitoring the appearance of the product. The spectrophotometer was set at 523.1 nm when the porphyrin concentration was around  $10^{-5}M$  and was set at the Pd-tetraphenylporphyrin Soret peak at 415.6 nm when the N-methyltetraphenylporphyrin concentration was about  $10^{-6}M$ .

Experimental data for the metal, amine and temperature dependences appear in Table X. The Pd(II) concentration was varied over a 24-fold range from 0.024M to 0.001M. The amine concentration ranged from zero to 0.600M.

An overlay kinetic run was produced (see Figure 14) by scanning from 700 nm to 450 nm while the reaction was taking place. The reaction appeared to go smoothly from the porphyrin starting material to the planar metalloporphyrin product. An isosbestic point at 533 nm indicated that no intermediates were formed at any appreciable concentration.

There were several problems with this system which prevented the experimental data from yielding straightforward results. Kinetic data from reactions containing only Pd(II) and N-methyltetraphenylporphyrin were irreproducible. The metalated N-methylporphyrin complexes were being demethylated apparently from the DMF or from hydrolysis products of the DMF. Also, in solutions without amine present, palladium hydroxide and/or oxide would begin to form at higher temperatures, create a colloidal suspension (which would increase the optical density) and then precipitate out of

TABLE X. Kinetic Results for the Reaction of Pd(II) with N-CH<sub>3</sub> Tetraphenylporphyrin in N,N-Dimethylformamide

| [Pd(II)]<br>x 10 <sup>3</sup>                                                                       | [N-CH <sub>3</sub> TPP]<br>x 10 <sup>5</sup> | [amine] | k <sub>obs</sub> x 10 <sup>3</sup><br>(s <sup>-1</sup> ) | t <sub>1/2</sub><br>(min.) | temp.<br>(°C) |
|-----------------------------------------------------------------------------------------------------|----------------------------------------------|---------|----------------------------------------------------------|----------------------------|---------------|
| Changing concentration of Pd(NO <sub>3</sub> ) <sub>2</sub> · xH <sub>2</sub> O and di-n-butylamine |                                              |         |                                                          |                            |               |
| 23.86                                                                                               | 4.079                                        | 0.100   | 1.808 <sup>c</sup>                                       | 6.39                       | 25.0          |
| 11.93                                                                                               | 4.079                                        | 0.100   | 1.409 <sup>c</sup>                                       | 8.20                       | 25.0          |
| 2.214                                                                                               | 4.079                                        | 0.100   | 0.6202                                                   | 18.62                      | 25.0          |
| 1.214                                                                                               | 4.089                                        | 0.0968  | 0.4127 <sup>c</sup>                                      | 27.99                      | 25.0          |
| 23.86                                                                                               | 4.079                                        | 0.200   | b                                                        |                            | 25.0          |
| 11.93                                                                                               | 4.079                                        | 0.200   | b                                                        |                            | 25.0          |
| 2.214                                                                                               | 4.079                                        | 0.200   | 0.5318                                                   | 21.71                      | 25.0          |
| 2.214                                                                                               | 3.933                                        | 0.300   | 0.5282                                                   | 21.87                      | 25.0          |
| 2.214                                                                                               | 3.933                                        | 0.300   | 0.5317                                                   | 21.72                      | 25.0          |
| 1.660                                                                                               | 3.933                                        | 0.300   | 0.3869 <sup>c</sup>                                      | 29.85                      | 25.0          |
| 1.660                                                                                               | 3.933                                        | 0.300   | 0.3734 <sup>c</sup>                                      | 30.93                      | 25.0          |
| 1.107                                                                                               | 3.933                                        | 0.300   | 0.3122 <sup>c</sup>                                      | 37.00                      | 25.0          |
| 1.107                                                                                               | 3.933                                        | 0.300   | 0.2959 <sup>c</sup>                                      | 39.03                      | 25.0          |
| 1.660                                                                                               | 3.933                                        | 0.600   | 0.2917 <sup>c</sup>                                      | 39.6                       | 25.0          |
| 3.320                                                                                               | 1.748                                        | 0.300   | 0.4801                                                   | 24.1                       | 25.0          |
| 3.320                                                                                               | 1.748                                        | 0.300   | 0.4680                                                   | 24.7                       | 25.0          |
| 3.320                                                                                               | 1.748                                        | 0.100   | 0.5479                                                   | 21.1                       | 25.0          |
| 3.320                                                                                               | 1.748                                        | 0.100   | 0.5462                                                   | 21.15                      | 25.0          |
| 2.214                                                                                               | 4.079                                        | 0.050   | 0.5780                                                   | 261.1                      | 25.0          |
| 2.214                                                                                               | 4.079                                        | 0.100   | 1.198 <sup>e</sup>                                       | 9.641                      | 25.0          |
| 2.214                                                                                               | 4.079                                        | 0.050   | 1.375 <sup>e</sup>                                       | 8.400                      | 25.0          |
| 2.214                                                                                               | 1.020                                        | 0.200   | 0.6522 <sup>e</sup>                                      | 17.71                      | 25.0          |
| 2.214                                                                                               | 1.020                                        | 0.200   | 0.6258 <sup>e</sup>                                      | 18.46                      | 25.0          |
| 2.214                                                                                               | 4.079                                        | 0.0050  | 3.486 <sup>e</sup>                                       | 3.313                      | 25.0          |
| 1.955                                                                                               | 4.079                                        | 0.200   | 1.023 <sup>e</sup>                                       | 11.29                      | 25.2          |
| 3.910                                                                                               | 4.079                                        | 0.100   | 2.248 <sup>e</sup>                                       | 5.138                      | 25.2          |
| 3.910                                                                                               | 1.020                                        | 0.100   | 1.005 <sup>e</sup>                                       | 11.49                      | 25.2          |
| 2.214                                                                                               | 4.079                                        | 0.200   | 1.391 <sup>e</sup>                                       | 8.303                      | 25.2          |

TABLE X. (continued)

| [Pd(II)]<br>x 10 <sup>3</sup> | [N-CH <sub>3</sub> TPP]<br>x 10 <sup>5</sup> | [amine] <sup>a</sup> | k <sub>obs</sub> x 10 <sup>3</sup><br>s <sup>-1</sup> | t <sub>1/2</sub><br>(min.) | temp.<br>(°C) |
|-------------------------------|----------------------------------------------|----------------------|-------------------------------------------------------|----------------------------|---------------|
| 2.214                         | 4.079                                        | 0.200                | 1.576 <sup>e</sup>                                    | 7.329                      | 25.2          |
| 2.214                         | 4.079                                        | 0.200                | 1.317 <sup>e</sup>                                    | 8.770                      | 25.2          |
| 1.549                         | 0.800                                        | 0.600                | d                                                     |                            | 25.2          |
| 2.214                         | 4.079                                        | 0.600                | d, e                                                  |                            | 25.2          |
| 2.214                         | 4.079                                        | 0.400                | d, e                                                  |                            | 25.2          |
| 2.214                         | 4.079                                        | 0.200                | 4.120 <sup>e</sup>                                    | 2.803                      | 37.5          |
| 2.214                         | 4.079                                        | 0.200                | 3.523 <sup>e</sup>                                    | 3.278                      | 37.5          |
| 2.214                         | 4.079                                        | 0.200                | 5.559 <sup>e</sup>                                    | 2.078                      | 37.5          |
| 2.214                         | 4.079                                        | 0.200                | 4.659 <sup>e</sup>                                    | 2.479                      | 50.0          |
| 2.214                         | 4.079                                        | 0.200                | 8.542 <sup>c, e</sup>                                 | 1.352                      | 50.0          |

Changing the concentration of N-methyltetraphenylporphyrin

|       |        |       |                     |       |      |
|-------|--------|-------|---------------------|-------|------|
| 2.214 | 4.079  | 0.100 | 0.6202 <sup>c</sup> | 18.62 | 25.0 |
| 2.214 | 2.914  | 0.100 | 0.3914              | 29.51 | 25.0 |
| 2.214 | 1.748  | 0.100 | 0.4533              | 25.48 | 25.0 |
| 2.214 | 0.2039 | 0.100 | 0.2539              | 45.49 | 25.0 |
| 1.107 | 0.4079 | 0.100 | 0.1981              | 58.30 | 25.0 |
| 1.107 | 0.2039 | 0.100 | 0.2243              | 51.49 | 25.0 |

Changing the temperature

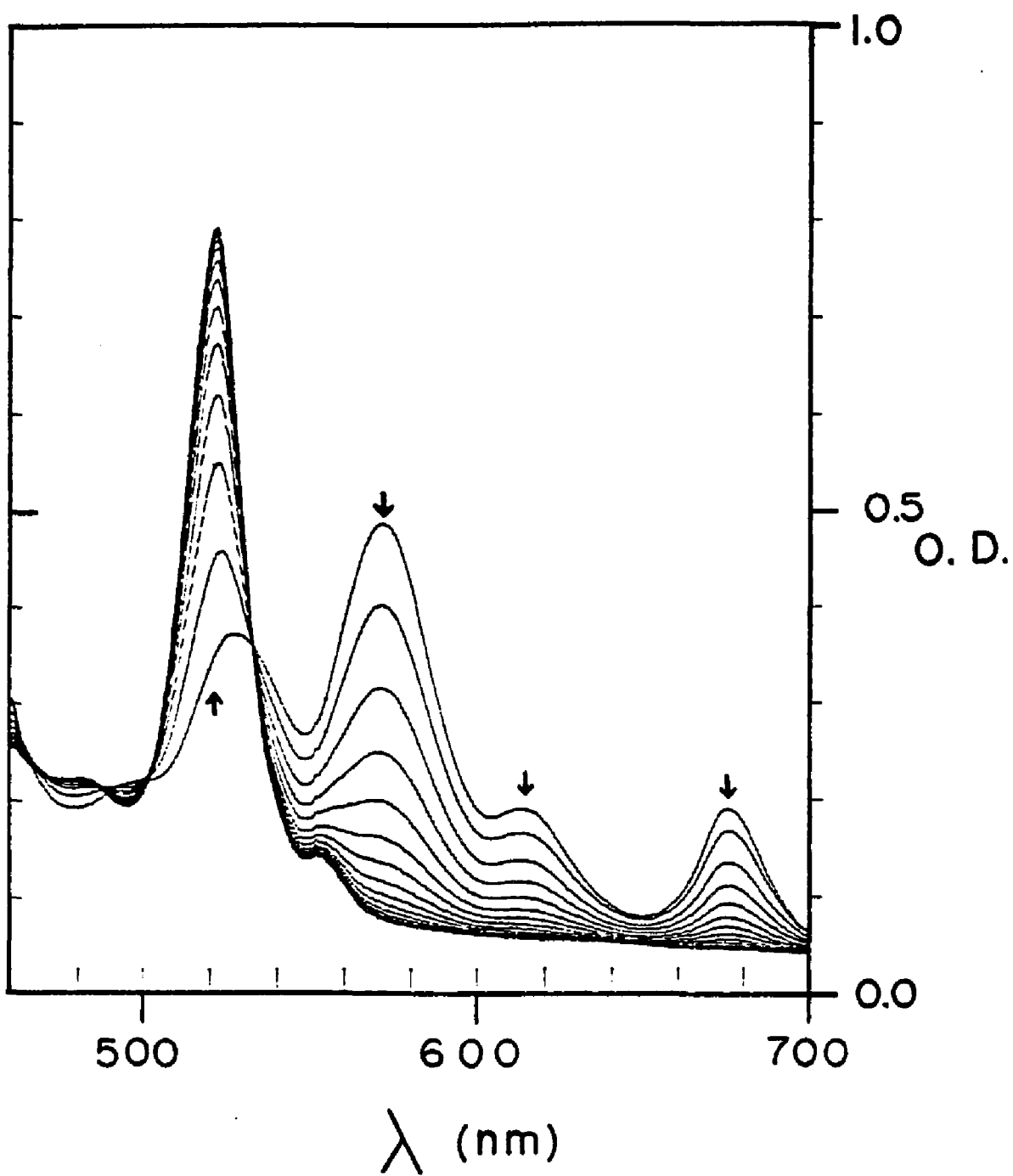
|       |       |       |                     |       |      |
|-------|-------|-------|---------------------|-------|------|
| 2.214 | 4.079 | 0.200 | 0.5318              | 21.72 | 25.0 |
| 2.214 | 4.079 | 0.200 | 0.2080 <sup>c</sup> | 55.52 | 37.5 |
| 2.214 | 4.079 | 0.200 | 0.2405 <sup>c</sup> | 48.02 | 37.5 |
| 2.214 | 4.079 | 0.200 | 0.3504              | 32.96 | 37.5 |
| 2.214 | 4.079 | 0.200 | 0.3992              | 28.93 | 37.5 |
| 2.214 | 4.079 | 0.200 | 2.345               | 4.925 | 50.0 |
| 2.214 | 4.079 | 0.200 | 2.872               | 4.022 | 50.0 |

TABLE X. (continued)

| [Pd(II)]<br>x 10 <sup>3</sup> | [N-CH <sub>3</sub> TPP]<br>x 10 <sup>5</sup> | [amine] <sup>a</sup> | k <sub>obs</sub> x 10 <sup>3</sup><br>s <sup>-1</sup> | t <sub>1/2</sub><br>(min.) | temp.<br>(°C) |
|-------------------------------|----------------------------------------------|----------------------|-------------------------------------------------------|----------------------------|---------------|
| No amine present              |                                              |                      |                                                       |                            |               |
| .7380                         | 4.079                                        | --                   | 0.2024 <sup>e</sup>                                   | 57.06                      | 25.0          |
| 1.107                         | 3.933                                        | --                   | 0.4885 <sup>c,e</sup>                                 | 23.64                      | 25.0          |
| 2.214                         | 4.079                                        | --                   | 5.943 <sup>c,e</sup>                                  | 1.943                      | 25.0          |
| 2.214                         | 4.079                                        | --                   | 14.69 <sup>c,e</sup>                                  | .7862                      | 25.0          |
| 2.214                         | 4.079                                        | --                   | e, f                                                  |                            | 25.0          |
| 2.214                         | 4.079                                        | --                   | e, f                                                  |                            | 25.0          |
| 2.214                         | 4.079                                        | --                   | 0.4544 <sup>c</sup>                                   | 25.42                      | 25.0          |
| 2.214                         | 4.079                                        | --                   | 0.8025                                                | 14.39                      | 25.0          |
| 2.214                         | 3.933                                        | --                   | 0.8715                                                | 13.25                      | 25.3          |
| 0.960                         | 4.371                                        | --                   | 0.2622 <sup>c</sup>                                   | 44.05                      | 58.0          |
| 0.960                         | 4.371                                        | --                   | 0.3611                                                | 31.99                      | 62.7          |

<sup>a</sup>Amine used was di-n-butylamine. <sup>b</sup>Opaque black solution was observed. <sup>c</sup>Data from these kinetic runs did not exactly fit the first order function (correlation coefficients varied from .9873 to .9976). <sup>c</sup>Reaction never went to completion. <sup>e</sup>The kinetics were run using a new stock Pd(NO<sub>3</sub>)<sub>2</sub> · xH<sub>2</sub>O solution. <sup>f</sup>Data from these kinetic runs gave impossible results (i.e., calculated OD<sub>0</sub> to be a negative value).

FIGURE 14. OVERLAID SPECTRA OF A KINETIC RUN INVOLVING Pd(II) AND N-METHYLTETRAPHENYLPORPHYRIN IN DIMETHYLFORMAMIDE. WAVELENGTHS WERE SCANNED FROM 700-460NM DURING THE ENTIRE COURSE OF THE REACTION. EACH SCAN TOOK ABOUT FOUR MINUTES. THE ARROWS INDICATE INTENSITY CHANGES AS THE REACTION PROCEEDS.



solution (which would decrease the optical density). This secondary reaction was taking place while the palladium/porphyrin reaction was being observed. The addition of amine suppressed the palladium hydroxide and/or oxide reaction. However, kinetic runs which had amine concentrations greater than 0.400M never went to completion. Also, the reactions which had amine in them generally did not exactly fit a first order plot. A typical example is shown in Figure 15. The crosses are the experimental data points while the solid line is the calculated fit. Equation <11> was used to calculate the least squares fit on the kinetic data.

The temperature dependence was determined using the best possible kinetic runs (as determined by most closely fitting the first order curve) and is shown in Figure 16. Note that in this case,  $\ln(k_{\text{obs}}/T)$  versus  $1/T$  was plotted to determine the temperature dependence. The enthalpy and entropy of activation were found to be  $13.40 \pm 0.06$  kcal/mole and  $-26.7 \pm 0.3$  eu, respectively.

#### KINETIC RESULTS IN DIMETHYLSULFOXIDE

In the same manner as in the other two solvents discussed, the kinetic experiments were performed by monitoring the appearance of the Pd-tetraphenylporphyrin product. The kinetics were run at 523.1 nm at  $10^{-5}$ M porphyrin concentration and at 416.4 nm at  $10^{-6}$ M concentration of porphyrin.

FIGURE 15. THE CORRELATION OF OPTICAL DENSITY CHANGES AS A FUNCTION OF TIME FOR A TYPICAL KINETIC RUN IN DIMETHYLFORMAMIDE WHICH CONTAINED DI-N-BUTYLAMINE. THE CROSSES ARE THE EXPERIMENTAL DATA POINTS AND THE SOLID CURVE REPRESENTS THE BEST FIT TO EQUATION (7), THE EXPONENTIAL FUNCTION WHICH DESCRIBES A FIRST ORDER REACTION. ALTHOUGH THE FIT IS GOOD (STANDARD DEVIATION OF THE LINEAR REGRESSION = 0.004098, CORRELATION COEFFICIENT = 0.9987), THE EXPERIMENTAL POINTS DEVIATE FROM A FIRST ORDER REACTION IN A SMALL BUT NONRANDOM FASHION.

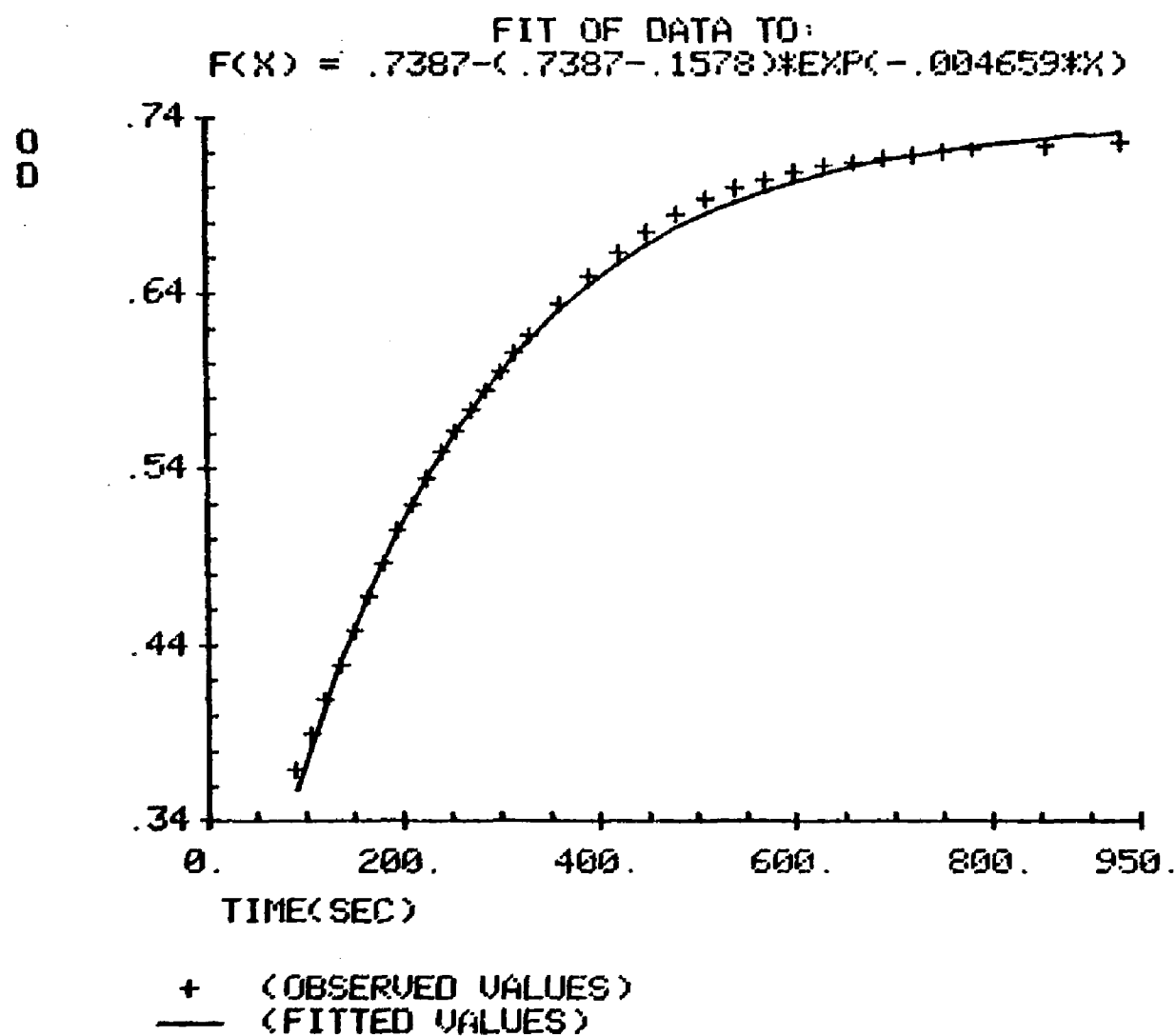
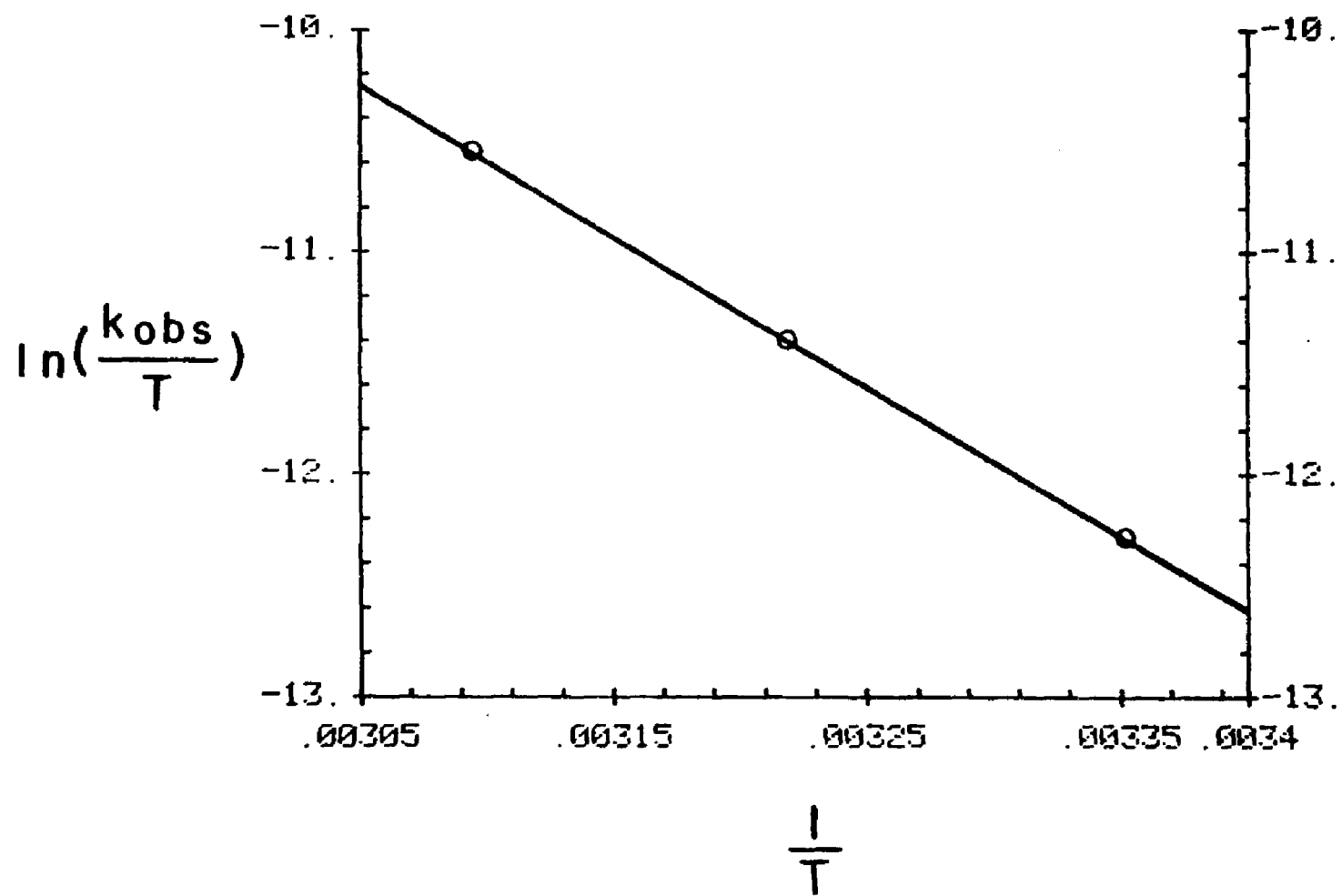


FIGURE 16. LINEAR TEMPERATURE DEPENDENCE FOR THE REACTION INVOLVING Pd(II) AND N-METHYLTETRAPHENYLPORPHYRIN IN DIMETHYL-FORMAMIDE. ONLY THREE KINETIC RUNS WERE USED FOR THIS TEMPERATURE DEPENDENCE. THESE RUNS CLOSELY FOLLOWED THE FIRST-ORDER PLOT AND ALL HAD CONSTANT CONCENTRATIONS OF Pd(II) ( $2.214 \times 10^{-3}M$ ), PORPHYRIN ( $4.079 \times 10^{-5}M$ ) AND AMINE (0.200M). THE EYRING FORMULATION,  $\ln(k_{obs}/T)$  VERSUS  $1/T$ , WAS USED TO CALCULATE THE ACTIVATION PARAMETERS. THE SLOPE IS A FUNCTION OF  $\Delta H^\ddagger$  WHICH IS EQUAL TO  $13.40 \pm 0.06$  KCAL/MOL. THE INTERCEPT IS A FUNCTION OF  $\Delta S^\ddagger$  WHICH IS EQUAL TO  $-26.7 \pm 0.3$  EU. (NOTE:  $k_{obs}$  IS IN UNITS OF  $s^{-1}$ .)



Overlaid spectra of a kinetic run involving Pd(II) and N-methyltetraphenylporphyrin is shown in Figure 17. Spectra were taken by scanning from 700 nm to 460 nm as the reaction was taking place. Upon close inspection of the spectra, two reactions were observed. The first reaction was quite fast while the second one was much slower.

The experimental data for the first reaction at various Pd(II) concentrations and temperatures is shown in Table XI. The non-coordinating base used was 2,2,6,6-tetramethylpiperidine. The Pd(II) concentration was varied from 0.01860M to 0.002325M. No obvious temperature dependence was seen from these data.

Table XII lists the data for the metal and temperature dependences of the second reaction using 2,6-lutidine as the non-coordinating base. The Pd(II) concentration was varied over a range of 0.01860M to 0.003719M. The temperature dependence ( $\ln(k_{\text{obs}}/T)$  versus  $1/T$ ) is plotted in Figure 18 in which the open circles represent the experimental points and the solid line is a least squares fit. The enthalpy of activation was calculated to be  $24.1 \pm 2.1$  kcal/mole while the entropy of activation was  $-0.50 \pm 0.07$  eu.

The kinetic data for the second reaction using 2,2,6,6-tetramethylpiperidine is given in Table XIII. For these sets of reactions, the Pd(II) concentration ranged from 0.01860M to 0.002325M. The plot of  $\ln(k_{\text{obs}}/T)$  versus inverse temperature are shown graphically in Figure 19. The

FIGURE 17. OVERLAID SPECTRA OF A KINETIC RUN INVOLVING Pd(II) N-METHYLTETRAPHENYLPORPHYRIN IN DIMETHYLSULFOXIDE. WAVELENGTHS WERE SCANNED FROM 700-460NM DURING THE ENTIRE COURSE OF THE REACTION. EACH SCAN TOOK ABOUT FOUR MINUTES. THE ARROWS INDICATE INTENSITY CHANGES AS THE REACTION PROCEEDS. NOTE THAT TWO REACTIONS ARE VISIBLE.

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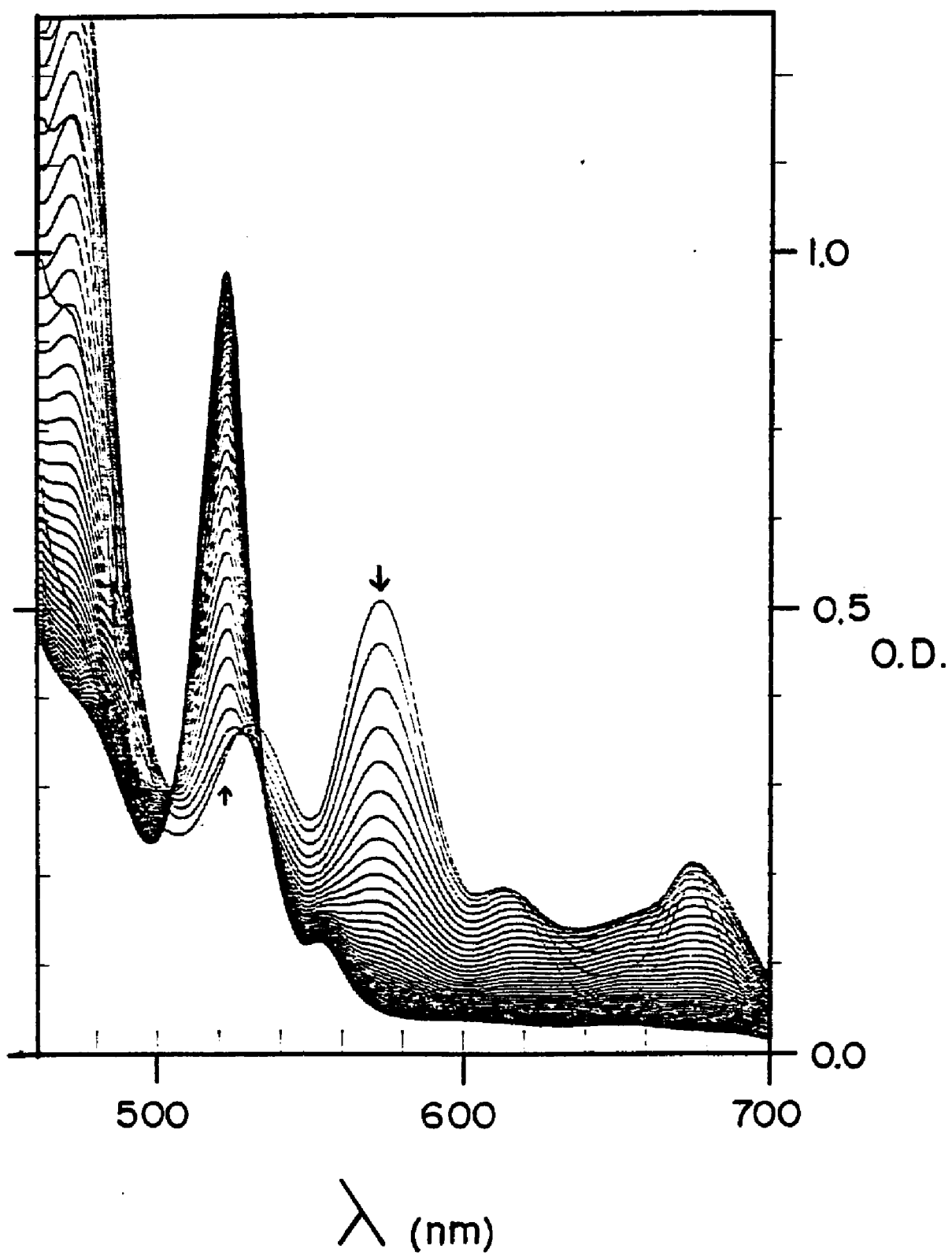


TABLE XI. Kinetic Results of the First, Fast Reaction Involving Pd(II) and N-Methyltetraphenylporphyrin in Dimethylsulfoxide Using 2,2,6,6-tetramethylpiperidine as a Non-Coordinating Base

| [Pd(NO <sub>3</sub> ) <sub>2</sub> ] | [N-CH <sub>3</sub> TPP]<br>x 10 <sup>5</sup> | [2,2,6,6-tetra-<br>methylpiperidine] | k <sub>obs</sub> x 10 <sup>3</sup><br>(s <sup>-1</sup> ) | t <sub>½</sub><br>(sec.) | temp.<br>(°C) |
|--------------------------------------|----------------------------------------------|--------------------------------------|----------------------------------------------------------|--------------------------|---------------|
| .01860                               | 6.955                                        | 0.200                                | 7.108                                                    | 97.50                    | 15.0          |
| .009300                              | 6.955                                        | 0.200                                | 6.072                                                    | 114.1                    | 15.0          |
| .004650                              | 6.955                                        | 0.200                                | 6.701                                                    | 103.4                    | 15.0          |
| .01860                               | 6.955                                        | 0.200                                | 16.16                                                    | 42.88                    | 25.0          |
| .009300                              | 6.955                                        | 0.200                                | 12.22                                                    | 56.71                    | 25.0          |
| .004650                              | 6.955                                        | 0.200                                | 15.69                                                    | 44.17                    | 25.0          |
| .002325                              | 6.955                                        | 0.200                                | 19.17                                                    | 36.15                    | 25.0          |
| .01860                               | 6.955                                        | 0.200                                | 7.271                                                    | 95.31                    | 25.0          |
| .01860                               | 6.955                                        | 0.200                                | 9.642                                                    | 71.87                    | 25.0          |
| .009300                              | 6.955                                        | 0.200                                | 4.999                                                    | 138.6                    | 25.0          |
| .009300                              | 6.955                                        | 0.200                                | 5.747                                                    | 120.6                    | 25.0          |
| .01860                               | 6.955                                        | 0.200                                | 9.877                                                    | 70.16                    | 37.5          |
| .01860                               | 6.955                                        | 0.200                                | 11.95                                                    | 57.99                    | 37.5          |
| .01860                               | 3.477                                        | 0.200                                | 15.13                                                    | 45.80                    | 37.5          |

TABLE XII. Kinetic Results of the Second Reaction Involving Pd(II) and N-Methyltetraphenylporphyrin in Dimethylsulfoxide Using 2,6-Lutidine as a Non-Coordinating Base

| [Pd(NO <sub>3</sub> ) <sub>2</sub> ] | [N-CH <sub>3</sub> TPP] |                | $k_{obs} \times 10^3$<br>(s <sup>-1</sup> ) | $t_{\frac{1}{2}}$<br>(sec.) | temp.<br>(°C) |
|--------------------------------------|-------------------------|----------------|---------------------------------------------|-----------------------------|---------------|
|                                      | $\times 10^5$           | [2,6-lutidine] |                                             |                             |               |
| .01860                               | 5.155                   | .2870          | .03719 <sup>a</sup>                         | 18,630                      | 35.0          |
| .01860                               | 5.155                   | .2870          | .03731 <sup>a</sup>                         | 18,570                      | 35.0          |
| .003719                              | 5.155                   | .2870          | .05040 <sup>a</sup>                         | 13,750                      | 35.0          |
| .003719                              | 5.155                   | .2870          | .05050 <sup>a</sup>                         | 13,720                      | 35.0          |
| .003036                              | 3.156                   | .3514          | .03654 <sup>a</sup>                         | 18,970                      | 35.0          |
| .01860                               | 5.155                   | .2870          | .3737 <sup>a</sup>                          | 1,854                       | 50.0          |
| .01860                               | 5.155                   | .2870          | .3523 <sup>a</sup>                          | 1,967                       | 50.0          |
| .003719                              | 5.155                   | .2870          | .1728 <sup>a</sup>                          | 4,010                       | 50.0          |
| .01860                               | 5.155                   | —              | .2957 <sup>a</sup>                          | 2,344                       | 50.0          |
| .01860                               | 5.155                   | —              | .3080 <sup>a</sup>                          | 2,250                       | 50.0          |
| .01860                               | 2.577                   | .2870          | .2864 <sup>a</sup>                          | 2,420                       | 50.0          |
| .01860                               | 5.153                   | .2152          | 7.012                                       | 98.83                       | 80.0          |
| .01860                               | 5.153                   | .2152          | 6.613                                       | 104.8                       | 80.0          |

<sup>a</sup>These kinetic runs did not exactly fit the first order function (correlation coefficients varied from .9759 to .9967).

FIGURE 18. LINEAR TEMPERATURE DEPENDENCE FOR THE REACTION INVOLVING Pd(II) AND N-METHYLTETRAPHENYLPORPHYRIN IN DIMETHYL-SULFOXIDE USING 2,6-LUTIDINE AS A NON-COORDINATING BASE. THE EYRING FORMULATION,  $\ln(k_{obs}/T)$  VERSUS  $1/T$ , WAS USED TO CALCULATE THE ACTIVATION PARAMETERS. THE SLOPE IS A FUNCTION OF  $\Delta H^\ddagger$  WHICH IS EQUAL TO  $24.1 \pm 2.1$  KCAL/MOL. THE INTERCEPT IS A FUNCTION OF  $\Delta S^\ddagger$  WHICH IS EQUAL TO  $-0.50 \pm 0.07$  EU. (NOTE:  $k_{obs}$  IS IN UNITS OF  $s^{-1}$ .)

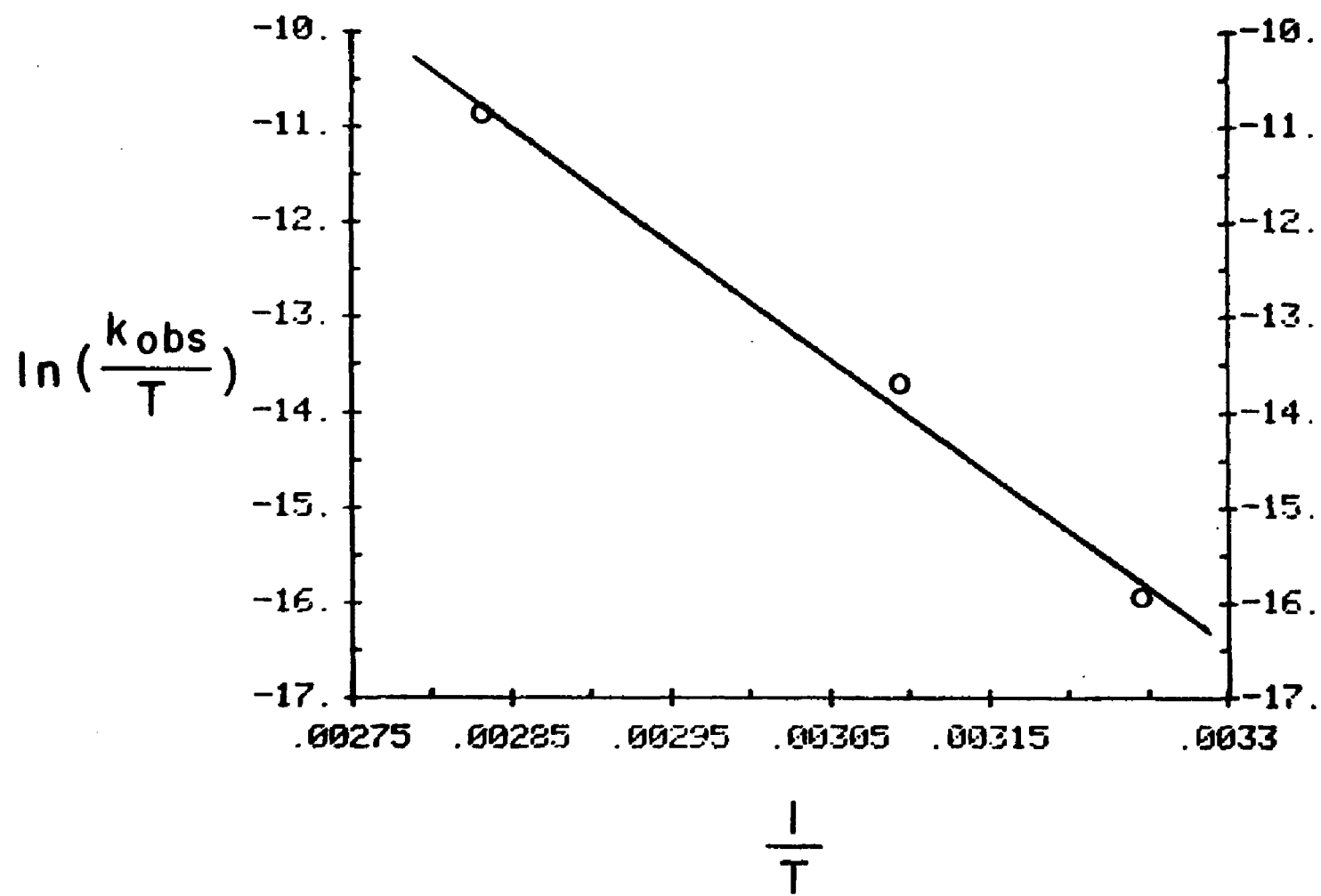
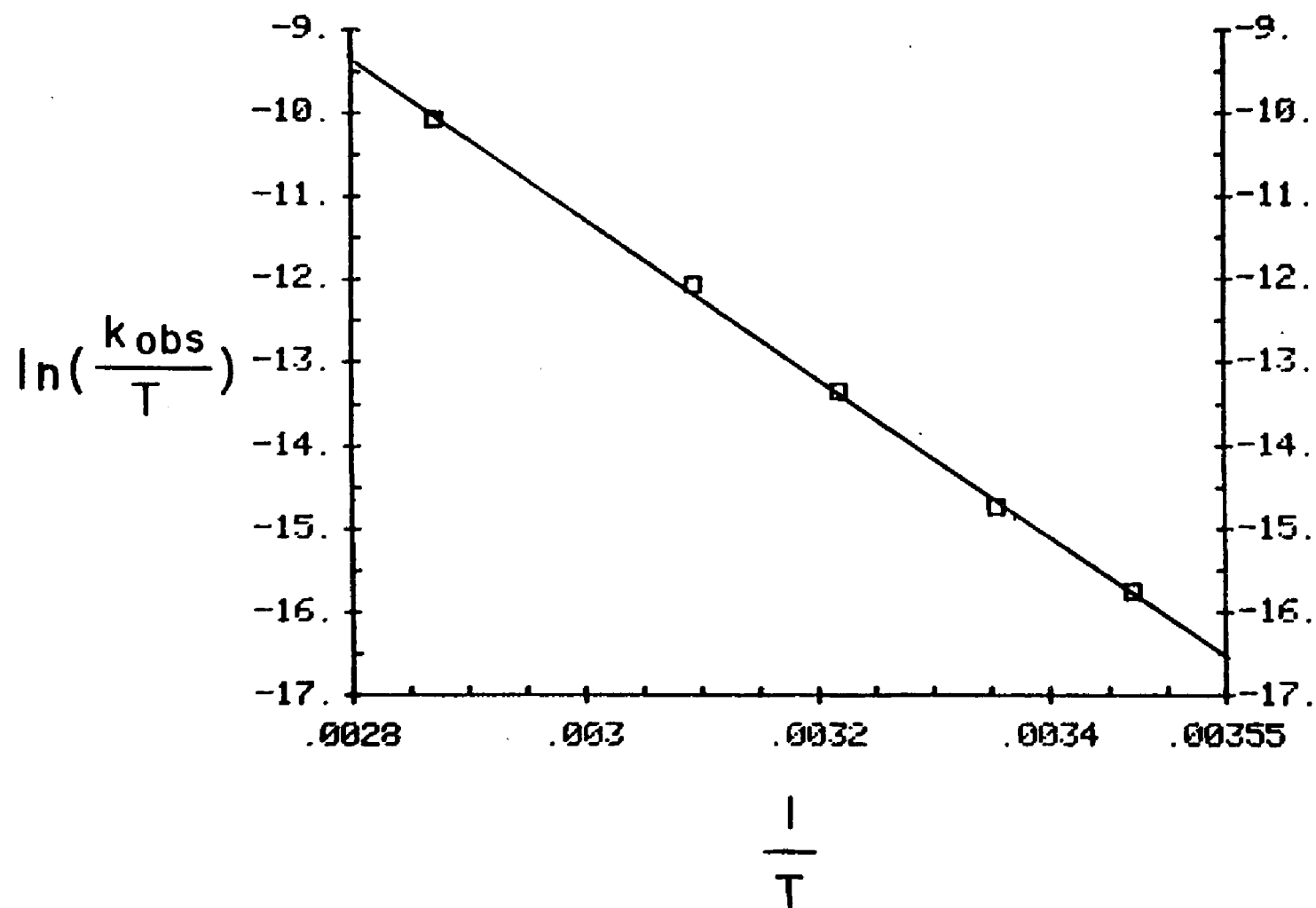


TABLE XIII. Kinetic Results of the Second Reaction Involving Pd(II) and N-Methyltetraphenylporphyrin in Dimethylsulfoxide Using 2,2,6,6-Tetramethylpiperidine as a Non-Coordinating Base

| [Pd(NO <sub>3</sub> ) <sub>2</sub> ] | [N-CH <sub>3</sub> TPP]<br>x 10 <sup>5</sup> | [2,2,6,6-tetra-<br>methylpiperidine] | k <sub>obs</sub> x 10 <sup>3</sup><br>(s <sup>-1</sup> ) | t <sub>1/2</sub><br>(sec.) | temp.<br>(°C) |
|--------------------------------------|----------------------------------------------|--------------------------------------|----------------------------------------------------------|----------------------------|---------------|
| .009300                              | 6.955                                        | 0.200                                | .04831                                                   | 14,350                     | 15.0          |
| .004650                              | 6.955                                        | 0.200                                | .03471                                                   | 19,960                     | 15.0          |
| .01860                               | 6.955                                        | 0.200                                | .1209                                                    | 5,732                      | 25.0          |
| .01860                               | 6.955                                        | 0.200                                | .1166                                                    | 5,943                      | 25.0          |
| .009300                              | 6.955                                        | 0.200                                | .1221                                                    | 5,676                      | 25.0          |
| .00930                               | 6.955                                        | 0.200                                | .1202                                                    | 5,765                      | 25.0          |
| .01860                               | 6.955                                        | 0.200                                | .5872                                                    | 1,180                      | 37.5          |
| .01860                               | 6.955                                        | 0.200                                | .5456                                                    | 1,270                      | 37.5          |
| .01860                               | 6.955                                        | 0.200                                | .5282                                                    | 1,312                      | 37.5          |
| .009300                              | 6.955                                        | 0.200                                | .4471                                                    | 1,550                      | 37.5          |
| .004650                              | 6.955                                        | 0.200                                | .4230                                                    | 1,638                      | 37.5          |
| .002325                              | 6.955                                        | 0.200                                | .4317                                                    | 1,605                      | 37.5          |
| .01860                               | 6.955                                        | 0.100                                | .6901                                                    | 1,004                      | 37.5          |
| .01860                               | 3.477                                        | 0.200                                | .5990                                                    | 1,157                      | 37.5          |
| .004650                              | 3.477                                        | 0.100                                | .4265                                                    | 1,625                      | 37.5          |
| .004650                              | 3.477                                        | 0.050                                | .5018                                                    | 1,381                      | 37.5          |
| .01860                               | 6.955                                        | 0.200                                | 2.227                                                    | 311.2                      | 50.0          |
| .009300                              | 6.955                                        | 0.200                                | 1.619                                                    | 428.0                      | 50.0          |
| .009300                              | 6.955                                        | 0.200                                | 1.751                                                    | 395.8                      | 50.0          |
| .01860                               | 6.954                                        | 0.200                                | 13.48                                                    | 51.41                      | 75.0          |
| .01860                               | 6.954                                        | 0.200                                | 15.03                                                    | 46.11                      | 75.0          |
| .01860                               | 6.954                                        | 0.200                                | 16.06                                                    | 43.18                      | 75.0          |
| .01860                               | 3.477                                        | 0.200                                | 14.16                                                    | 48.94                      | 75.0          |

FIGURE 19. LINEAR TEMPERATURE DEPENDENCE FOR THE REACTION INVOLVING Pd(II) AND N-METHYLTETRAPHENYLPORPHYRIN IN DIMETHYL-SULFOXIDE USING 2,2,6,6-TETRAMETHYLPIPERIDINE AS A NON-COORDINATING BASE. THE FIT IS REPRESENTED BY THE EYRING FORMULATION AS  $\ln(k_{obs}/T)$  VERSUS  $1/T$ . THE SLOPE IS A FUNCTION OF THE ENTHALPY OF ACTIVATION WHICH IS EQUAL TO  $19.1 \pm 0.3$  KCAL/MOL WHILE THE INTERCEPT IS A FUNCTION OF THE ENTROPY OF ACTIVATION WHICH IS EQUAL TO  $-12.3 \pm 0.4$  EU. (NOTE:  $k_{obs}$  IS IN UNITS OF  $s^{-1}$ .)



activation parameters,  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  were calculated to be  $19.09 \pm 0.33$  kcal/mole and  $-12.3 \pm 0.4$  eu, respectively as determined from methods of calculation previously described.

THE DIFFERENCE BETWEEN USING 2,6-LUTIDINE AND  
2,2,6,6-TETRAMETHYLPYPERIDINE AS A NON-COORDINATING  
BASE IN DIMETHYLFORMAMIDE AND DIMETHYLSULFOXIDE

A distinct color change was observed upon the addition of the non-coordinating base, 2,6-lutidine, to Pd(II) solutions in DMF and DMSO. The color of the Pd(II) solution in both these solvents was bright golden. Upon addition of the 2,6-lutidine, the color immediately changed to pale yellow. When 2,2,6,6-tetramethylpyperidine was used as a base, no difference in color of the Pd(II) solution was observed.

## DISCUSSION

### CHOICE OF SOLVENTS FOR THE KINETICS OF THE REACTION BETWEEN $\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$ AND N-METHYLTETRAPHENYLPORPHYRIN

The kinetic experiments involving  $\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$  and N-methyltetraphenylporphyrin were performed in three solvents, acetonitrile, N,N-dimethylformamide and dimethylsulfoxide.

Dimethylformamide has been shown to be a good solvent for kinetic studies because of the solubility of both the porphyrin ligands and the metal salts. Acetonitrile and dimethylsulfoxide also are acceptable solvents although the porphyrin ligands are less soluble in these solvents than in DMF and therefore must be studied at relatively low concentrations. However, this is not a severe problem because the porphyrin is used as the limiting reagent and generally kept at concentrations of  $10^{-5}$  molar or less, in any case. Dimethylformamide has the additional benefit of being a basic solvent and can bind the small amounts of hydrogen ions generated during the complexation reaction. It also has the ability to hydrogen bond with water. Small amounts of water can be tied up in the bulk solvent and thus will be less able to solvate the metal ions and influence their environment. Thus, the use of DMF eliminates the need for the addition of a non-coordinating base.

A special property of porphyrin ligands should be noted. It is well known that substituting a methyl group for one of the central hydrogen atoms causes an increase in the basicity of the porphyrin. Generally, the N-methyl derivative's  $pK$  value differs from the corresponding N-unsubstituted porphyrin by about 10  $pK$  units(63,73). The increased basicity of the N-methyl porphyrins could create problems. During the course of metal ion insertion reactions, protons are dissociated from the porphyrins (one in the case of the N-methyl porphyrins and two from the planar porphyrins). These protons can then protonate as yet unreacted porphyrin to give a mono- or di- protonated species. If the porphyrin is quite basic, it will readily take up these protons. Once protonated, the porphyrin cannot be metalated. Therefore, it would be necessary to add a non-coordinating base to scavenge the released protons. However, the addition of a non-coordinating base may add another problem in that it may cause precipitation of the metal ion species before it can react with the porphyrin. The  $pK_3$  of N-methyldeuteroporphyrin (the reaction of the monoprotonated porphyrin deprotonating to form the neutral porphyrin) is close to 11(70). The  $pK_3$  of planar deuteroporphyrin is much lower; around 5.5(21,46). The  $pK_3$  of N-methylhematoporphyrin would likely be similar to that of N-methyldeuteroporphyrin. N-methyl-tetraphenylporphyrin, on the other hand, is only slightly basic; its  $pK$  is 5.6(52). (The  $pK_3$  of tetraphenylporphyrin is 4.4(74).) Because N-methyltetraphenylporphyrin is much

less basic than either N-methyldeuteroporphyrin or N-methylhematoporphyrin, it is easier to work with. It is less apt to be protonated in nonprotic solvents which may contain trace amounts of water.

The reaction of Pd(II) with N-methyltetraphenylporphyrin was run under pseudo-first order conditions in all cases with the metal ion being in at least a 20-fold excess over the porphyrin concentration.

Experiments to determine the ionic strength dependence on systems similar to the Pd(II)/N-methyltetraphenylporphyrin system presently being studied were performed by Bain-Ackerman(47). She found that when the ionic strength was varied over a 6-fold range(in the case of Mn(II)) to a 22-fold range(in the case of Co(II)), the rate of metal complexation varied only slightly. Longo, et al., also studied the effect of ionic strength on the rate of the reaction between Cu(II) and tetraphenylporphyrin, using sodium nitrate and lithium perchlorate over a .5-fold range and found no dependence on the reaction rate(24). Since the rate has been found to be essentially independent of ionic strength, no attempt was made to control the ionic strength of solutions used in our studies.

#### KINETIC STUDY IN ACETONITRILE

Acetonitrile was chosen as a solvent to add to previous

less basic than either N-methyldeuteroporphyrin or N-methylhematoporphyrin, it is easier to work with. It is less apt to be protonated in nonprotic solvents which may contain trace amounts of water.

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#### KINETIC STUDY IN ACETONITRILE

Acetonitrile was chosen as a solvent to add to previous

studies done by Lavalley (52-54). He studied the metal ion promoted demethylation reactions of N-methyltetraphenylporphyrin by Ni(II), Zn(II), Mn(II) and Cu(II). The reaction of the metal ion, Pd(II), with the same N-methyl porphyrin will indicate whether the same mechanism is followed by this second row transition metal ion as with the previously studied first row transition metal ions. If the same mechanism is in effect with Pd(II), this study will then determine if Pd(II) follows the same trend as the other metal ions based on the predictions of the earlier studies.

The demethylation reaction of the Pd N-methyltetraphenylporphyrin complex was the rate determining step as evidenced by the overlaid spectra taken (see Figure 9). This is the same result that was found by Lavalley in his study. Further proof that the demethylation reaction was rate determining was determined by producing the Pd N-methyltetraphenylporphyrin intermediate in situ, adding the nucleophile, di-n-butylamine, and getting the same observed rate constant as when following the kinetics of the Pd(II) and N-methyltetraphenylporphyrin starting materials at the same amine concentration.

As can be seen by the plots of  $k_{obs}$  versus amine concentration in Figures 10 and 11, the kinetic data show a first order dependence on the di-n-butylamine concentration from a range of 0.0250M to 1.00M. This resembles the results for the reactions of the Zn(II) and Mn(II) porphyrins but not

the Cu(II) and Ni(II) porphyrins. In the cases of Cu(II) and Ni(II), a two-path reaction scheme involving an axial coordination equilibrium between the solvent and amine is postulated to explain the kinetic results. This mechanism entails the reaction of either solvated  $M-N-CH_3TPP$  or  $(HNR_2)M-N-CH_3TPP$  with di-n-butylamine to form M-TPP at two different rates (where  $M = Cu(II), Ni(II)$ ). It was not necessary to invoke this two-step process with the Pd(II) metal ion.

If the demethylation reaction is totally dominant in the proposed two-step mechanism (complexation and demethylation) then the observed rate constant should be independent of the reactants in the complexation step,  $Pd(NO_3)_2 \cdot xH_2O$  and N-methyltetraphenylporphyrin. The reaction was independent of the porphyrin concentration but had a slight inverse dependence on the Pd(II) concentration, that is, the second order rate constant increased on the average of 18% when the Pd(II) concentration was decreased from 0.0100M to 0.00100M. The small inverse dependence on the concentration of Pd(II) may be due to the ionic strength change when the metal ion concentration is decreased or to the binding of the amine by the Pd(II) thereby reducing the effective amine concentration that can act as a nucleophile. In order to compare the kinetic results of the demethylation reaction of the Pd(II) complex with the first row transition metal ions previously studied, the activation parameters were determined from the temperature dependence data. The results are shown in Table

XIV. The reactivity order for the five metal ions is: Pd(II) > Cu(II) > Ni(II) >> Zn(II) >> Mn(II). This reactivity sequence closely follows the stability order of the planar metalloporphyrins which is: Pd(II) > Cu(II) > Ni(II) > Zn(II) > Mn(II). The definition which best describes the stability of a metalloporphyrin is its resistance to demetalation by acid(56). Pd(II) is in the class of metalloporphyrins that is most stable to acid. This signifies that Pd-porphyrins are not completely demetalated in 100% H<sub>2</sub>SO<sub>4</sub> (25°C, 2 hr.). Mn(II), on the other hand, belong in the class of metalloporphyrins that is least stable to acid, which means that Mn-porphyrins are completely demetalated in a H<sub>2</sub>O/CH<sub>2</sub>Cl<sub>2</sub> solvent system.

The inclusion of Pd(II) with the previously studied first row transition metal ions discounts another theory that had been proposed to explain the reactivity order. It was suggested that a simple electrostatic promotion of CH<sub>3</sub><sup>+</sup> dissociation was operative based on the ionic radii of the dipositive metal ions (i.e., reactivity of Cu(II) (0.69Å) > Ni(II) (0.72Å) > Zn(II) (0.74Å) > Mn(II) (0.80Å) (54)). Since Pd(II) has an ionic radius of 0.86Å, it would be predicted to have the lowest reactivity based on this electrostatic model, however, it has the highest reactivity.

It should also be noted that the order found for the demethylation reaction is much different from that found by Lowe and Phillips(23) and Bain-Ackerman and Lavalley(47)

(Cu(II) > Zn(II) > Mn(II) > Ni(II)) and by Longo, et al., (24) (Cu(II) > Zn(II) > Pd(II) > Ni(II)) for complexation reactions. This would suggest that the rate determining steps of the complexation and demethylation reactions are affected differently by the various metal ions studied.

There appears to be an isokinetic effect for the activation parameters of the metal complexes listed in Table XIV. The isokinetic temperature was found to be 82.3°C. This compensation for larger  $\Delta H^\ddagger$  values by more favorable  $\Delta S^\ddagger$  values so that the  $\Delta G^\ddagger$  values remain approximately the same has been found in a number of cases for reactions which likely go by the same mechanism (75-77). This would indicate that for the metal ions studied, the mechanism by which demethylation of metalated N-methylporphyrins takes place is the same.

#### KINETIC STUDY IN N,N-DIMETHYLFORMAMIDE

The kinetics of the reaction between  $\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$  and N-methyltetraphenylporphyrin in DMF were studied for comparison with results that Bain-Ackerman and Lavalley found for reactions of the first row transition metal ions, Cu(II), Zn(II), Co(II), Mn(II) and Ni(II). They found a correlation between the rates of complexation of these metal ions with N-methyltetraphenylporphyrin and the solvent exchange rates of the metal ions. An object of this study was to determine whether the second row transition metal ion, Pd(II), follows

TABLE XIV. Activation Parameters for the Demethylation of Metalated N-Methyltetraphenylporphyrin Complexes<sup>a</sup>

| Metal ion           | $\Delta H^\ddagger$<br>kcal/mole | $\Delta S^\ddagger$ (318k)<br>eu | $\Delta G^\ddagger$<br>kcal/mole |
|---------------------|----------------------------------|----------------------------------|----------------------------------|
| Pd(II)              | 14.4±0.6                         | -21.5±1.6                        | 20.8                             |
| Cu(II) <sup>b</sup> | 16.9±1.0                         | -13.1±2.9                        | 21                               |
| Ni(II) <sup>b</sup> | 18.0±1.0                         | -14.0±3.0                        | 23                               |
| Zn(II) <sup>b</sup> | 41.6±2.0                         | 54.6±6.2                         | 26                               |
| Mn(II) <sup>b</sup> | > 51                             | > 65                             |                                  |

<sup>a</sup>Error limits are the standard deviations from calculated least-squares fit of  $\ln(k/T)$  versus  $1/T$ . Eyring activation parameters are specified.

<sup>b</sup>Data taken from Reference 54.

the same trend.

The results of the reaction involving  $\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$  and N-methyltetraphenylporphyrin in DMF were difficult to interpret. The problems encountered in this system (discussed in the RESULTS Section) will make the conclusions drawn from this data more speculative in nature than the conclusions based on the kinetic data in acetonitrile. However, even with the problems inherent with this system, several conclusions could be drawn.

To exactly match the conditions of Bain-Ackerman and Lavalley(47), it would have been preferable to do the kinetic study using only the metal ion and N-methylporphyrin in the DMF, but this was not possible. The rates were not reproducible possibly because of the hydrolysis of the Pd(II) to produce a palladium hydroxide complex. For the majority of kinetic runs, the data did not fit the exponential curve of a first order reaction exactly (see Figure 15). The palladium oxide/hydroxide species would certainly react differently than the solvated Pd(II) in the complexation reaction. Hydrolysis of the metal ions studied by Bain-Ackerman and Lavalley was not seen because of the much smaller acid dissociation constants for these first row transition metal ions(79). The formation of a palladium hydroxide or oxide precipitate could occur at pH values of the experiments. It was necessary to add di-n-butylamine to suppress precipitate formation but this addition of amine

added another variable to the system.

The demethylation of metalated N-methyltetraphenylporphyrin complexes in DMF occurred at a much slower rate for the first row transition metals(47) than was observed for palladium in this study. The possibilities are two: 1) The DMF itself acts as a nucleophile or 2) Pd(II) causes the hydrolysis of DMF to dimethylamine which then acts as a nucleophile. It is unlikely that the DMF is acting as a nucleophile because there is no reason to expect any difference between Pd(II) and the first row transition metal ions in this respect. It is more likely that Pd(II) causes the hydrolysis of DMF to dimethylamine. Unfortunately, the product analysis after demethylation would not differentiate between the two possibilities because the final product in each case would be trimethylamine (in the presence of trace water).

Although the rates of reaction decreased upon the reduction in concentration of Pd(II), a linear correlation between the observed rate constant and the Pd(II) concentration was not found. Also, the complexation reaction had an inverse, though not linear, dependence on the concentration of di-n-butylamine. These results would suggest that the complexation reaction of Pd(II) to N-methyltetraphenylporphyrin under pseudo-first-order conditions is not simply first order in metal ion concentration nor independent of amine concentration as was found by Bain-Ackerman and Laval-

lee in their study of Cu(II), Zn(II), Ni(II), Co(II) and Mn(II)(47). An additional reaction (or set of reactions) must be taking place which complicates the complexation mechanism. It is known that Pd(II) binds nitrogen ligands quite well(83). One could reasonably expect that solvated Pd(II) could bind with the di-n-butylamine in solution. This could give a series of palladium-amine complexes which have one, two, three or four amines attached. Since it has been shown that the rate determining step in the complexation reaction is ligand dissociation (either first or second) of the metal ion(47), then the nature of the Pd(II) species is extremely important in determining the porphyrin metalation rate. It is likely that the ligand dissociation of the solvated Pd(II) species would be much different than that of the palladium-amine species. This, in turn, would affect the N-methylporphyrin complexation rate.

With the information stated above, one could postulate several possible mechanisms which could explain the data. Two of these possible mechanisms will be briefly described.

The first mechanism involves an equilibrium between solvated Pd(II) and palladium-amine species. Several reactive Pd(II) species could exist in solution. This would explain the less than perfect first order fits, the non first order dependence on Pd(II) and the dependence on di-n-butylamine. An equilibrium between solvated and amine-bound Pd(II) could also explain the suppression of a palladium hydroxide or

oxide precipitate upon addition of amine to the solution. It is likely that a palladium-amine complex would be more stable to hydrolysis than solvated Pd(II). If one did not envisage any binding of the amine to the Pd(II), then one might expect more palladium oxide or hydroxide precipitate formation due to the high basicity of the added amine.

The second possible mechanism involves the formation of an outer-sphere ion pair between the Pd(II) species and the porphyrin. This would explain the non first order dependence on Pd(II) if one assumes a high equilibrium constant for the ion pairing process. The dependence on amine could also be explained by the same palladium-solvent and palladium-amine equilibria as described previously.

These two mechanisms can be postulated based on the kinetic results, but there is no direct evidence to support or disprove these posulated mechanisms. Before more conclusive statements concerning possible mechanisms can be made, it is mandatory that the reactive species (singular or plural) be identified. In this system, defining the reactive species would be very difficult to accomplish.

The only definite conclusion that can be made about this study in DMF is that the reaction pathway is much different than that in acetonitrile. As indicated by the overlaid spectra (see Figure 14), there is no spectral evidence that the Pd N-methyltetraphenylporphyrin intermediate is formed at appreciable concentrations. The spectra are those of the

starting material going smoothly to product. In acetonitrile, the demethylation reaction is rate determining while in DMF, it appears that the complexation reaction is the controlling one. No comparisons can be made with Bain-Ackerman's or Longo's studies because the complete reaction pathway is not known in this study. It cannot be established whether the rate constants or activation parameters of the Pd(II) reaction with N-methyltetraphenylporphyrin can be related to previous studies (24,47) until it has been determined that the reaction mechanism is the same.

#### KINETIC STUDY IN DIMETHYLSULFOXIDE

Dimethylsulfoxide was chosen as the third solvent because the reaction used in the selective lymphatic ablation study described in CHAPTER 1 is done in DMSO. It is therefore particularly important to elucidate the mechanism of the reaction involving  $\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$  and N-methyltetraphenylporphyrin in this solvent. The rates of reaction when using N-methyltetraphenylporphyrin instead of N-methylhematoporphyrin should not be changed appreciably. This follows from earlier studies of Kuila and Lavallee(59).

The results of this study of the reaction involving Pd(II) and N-methyltetraphenylporphyrin in DMSO are not straightforward either. Several unexpected and puzzling results were observed.

- 1) If one looks closely at the overlaid spectra of the reaction between the Pd(II) and porphyrin using 2,2,6,6-tetramethylpiperidine as a noncoordinating base (see Figure 17), one notices two reactions occurring; one relatively fast reaction which is apparently over in 5-6 scans (approximately 15-20 minutes) and has no isosbestic points (the major changes in absorbance are at 650 nm and 470 nm), and a much slower reaction which has two isosbestic points. If the first reaction was not visible, then this overlay series would appear to be that of the complexation reaction. The complexation reaction would be slow and the demethylation reaction, fast, as was the case in DMF. But a reaction before the complexation reaction is, in fact, observed. This first reaction changes the entire spectrum by very little.
- 2) There is no dependence of the first reaction and only a slight dependence of the second reaction on the Pd(II) concentration. If the second reaction was, as initially postulated because of spectral evidence, the complexation reaction, then one would expect a first order dependence on the Pd(II) concentration. If either of the two reactions were the demethylation reaction, then one would not expect any observable dependence under pseudo-first-order conditions because the solvent is the nucleophile.
- 3) From the kinetic data there appears to be little if

any temperature dependence of the first reaction (although very few data points were used in the calculation of  $OD_{\infty}$ ,  $OD_0$  and  $k_{obs}$  because of interference from the second reaction). One possible explanation of this phenomenon is that the first reaction is an equilibrium reaction in which the equilibrium constant is very large, very small or for which  $\Delta H^0 = 0$ . A large  $K_{eq}$  indicates a large negative free energy value ( $\Delta G^0$ ) which implies a large negative enthalpy ( $\Delta H^0$ ). A temperature dependence would not be observed in this case because a relatively small change in temperature (i.e., from 288.1K to 310.6K) would not result in an observable change in the equilibrium position. A small equilibrium constant implies a large positive free energy value and for the same reason as when  $K_{eq}$  is large, a small change in temperature would not greatly affect the equilibrium position. An enthalpy of zero would also result in a non dependence on temperature.

- 4) As the Pd(II) concentration is decreased, the change in optical density (i.e.,  $OD_{\infty} - OD_t$ ) decreases linearly even though the observed rate constant remains the same in the first reaction. This would seem to indicate that the first reaction is independent of the porphyrin. In other words, if the porphyrin were involved in the first reaction (assuming a large equilibrium constant for this reaction), and its concentration were kept constant, the change in optical density would also

remain constant because the porphyrin is the limiting reagent. The first reaction must involve Pd(II) but yet its rate is independent of the Pd(II) concentration.

- 5) The rate is inversely proportional to the concentration of the two bases used, 2,6-lutidine and 2,2,6,6-tetramethylpiperidine, but not in a first order fashion. Also, the rates of reaction when 2,6-lutidine was used were slower than when 2,2,6,6-tetramethylpiperidine was used.

Unfortunately, very few definite conclusions can be drawn from these kinetic results. There is clearly no evidence that the Pd N-methyltetraphenylporphyrin intermediate is formed at appreciable concentrations. In this regard, the spectral results of the reaction involving Pd(II) and N-methyltetraphenylporphyrin in DMF and DMSO are similar to each other but much different than the results in acetonitrile.

One could postulate mechanisms for the reactions in DMSO similar to those in DMF (i.e., formation of a palladium-hydroxide complex as the reactive species and/or the formation of a palladium-porphyrin outer-sphere ion pair) but without knowledge of the identity of the reactive Pd(II) species, such mechanisms cannot be strongly supported.

Reaction with the solvent itself is possible. DMSO can undergo proton abstraction in the presence of a relatively strong base such as 2,2,6,6-tetramethylpiperidine. The dim-

syl anion could then react with the Pd(II) cation to form a palladium-hydroxyl species or act as a nucleophile to demethylate the Pd N-methyltetraphenylporphyrin complex.

Addition of a non-coordinating base to these solutions appears to greatly complicate the interpretation of the kinetic data but is necessary in order to take up the protons from the acidic Pd(II) solution. If the protons were not removed from solution, the N-methyltetraphenylporphyrin would be protonated and the rate of metal ion complexation would become very slow. A well defined, anhydrous palladium salt with poorly-coordinating anions would have been a much more preferable starting material than  $\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$  (e.g., such as the perchlorate or trifluoromethanesulfonate salt) but the preparation of such salts is unknown.

#### SOLVENT EFFECTS ON THE Pd(II) N-METHYLTETRAPHENYLPORPHYRIN REACTION

Only a few general conclusions can be offered about the effect of the solvent on the reaction involving Pd(II) and N-methyltetraphenylporphyrin because in the three solvents studied different reactive palladium species are formed. In acetonitrile, the palladium ion is at least partially solvated. However in dimethylformamide, the reactive species is likely to be one or more palladium-amine complexes while in dimethylsulfoxide, a variety of palladium-hydroxide complexes could be the reactive palladium species. Since the

solvated metal ion is not present in all three solvents and because the rate determining step of the complexation reaction involves the loss of one or two ligands from the metal, direct comparisons cannot be definitely made.

It is likely that differences in solvents play an important role in determining whether the complexation or the demethylation reaction is rate determining. Acetonitrile, a solvent of low solvating power, binds weakly to metal ions which would tend to make the complexation reaction fast (since complexation rates depend on solvent exchange rates). Dimethylformamide has a superior ability to solvate metal ions and binds with them to a greater extent which would result in a slower complexation rate. Dimethylsulfoxide solvates metal ions even better and therefore binds with them stronger than the other two solvents which would tend to decrease the complexation rate even more. This would explain the difference in kinetics observed by Lavallee in acetonitrile, where he found the complexation reaction to be very fast, and of Bain-Ackerman and Lavallee in DMF, where they were able to readily monitor the complexation reaction. The correlation between the rate of complexation and the ability of the solvent to solvate metal ions is also found in this study where the complexation rate of Pd(II) to N-methyltetraphenylporphyrin was very fast in acetonitrile, decreased to a point where it could be readily studied by conventional techniques in dimethylformamide and decreased even further in dimethylsulfoxide. This suggests that the

major effect of the solvent medium is on the nature of the metal ion. The proper choice of a solvent should allow one to study both the complexation and demethylation reaction, from evidence of the solvent dependence.

### CONCLUSION

The kinetic results in acetonitrile show a first order dependence on the di-n-butylamine concentration. This is evidence that the demethylation reaction is rate determining while spectral data show the complexation reaction is very fast. Comparison of the results of this study with those of previous work under similar conditions gives the reactivity order for the five metal ions studied: Pd(II) > Cu(II) > Ni(II) >> Zn(II) >> Mn(II). This order closely follows the stability order of the planar metalloporphyrin products.

The kinetic results in dimethylformamide and dimethylsulfoxide make interpretation in these solvents much more difficult. Spectral evidence in both solvents indicate no formation of the Pd N-methyltetraphenylporphyrin intermediate at any appreciable concentration. This indicates that the complexation reaction is rate determining and that the demethylation reaction is relatively fast. A definite mechanism that is supported by conclusive evidence cannot be postulated at this time because the identity of the reactive palladium species in dimethylformamide and dimethylsulfoxide is

unknown due to the complexity of this system. Several possible palladium species have been discussed but no direct evidence to confirm the identity of the reactive palladium species has thus far been sought. Further work needs to be done to elucidate the complete mechanistic pathway in dimethylformamide and dimethylsulfoxide. Identification of the reactive palladium species may be elucidated by observing the species formed when the palladium salt and non-coordinating base are combined in dimethylformamide and dimethylsulfoxide without the addition of the porphyrin.

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