METAL COMPLEXES OF PENICILLIN AND CEPHALOSPORIN ANTIBIOTICS

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by

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ABSTRACT

The interaction between metal-ions and the penicillin and cephalosporin antibiotics have been studied in an attempt to determine both the site and mechanism of this interaction.

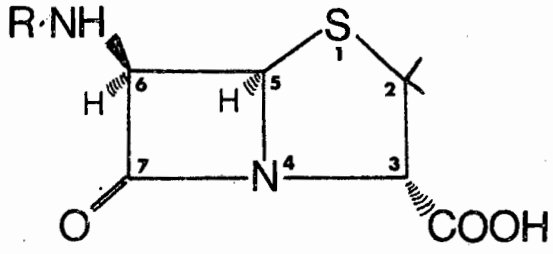
The solution conformation of the Cu(II) and Mn(II) complexes were determined using an n.m.r, line broadening, technique. The ligands benzylpenicillin, 6-aminopenicillanic acid, cephalothin, cephalexin, ampicillin and thiaproline were used to study the effect of structural changes in the ligand on the structure of the complex.

The stability constants of several metal-ions/penicillin complexes were determined potentiometrically. These were correlated with stability constants determined for several related complexes.

Finally the kinetics of the Ni(II) substitution reaction with benzylpenicillin, penicilloic acid, ampicillin and thiaproline was studied.

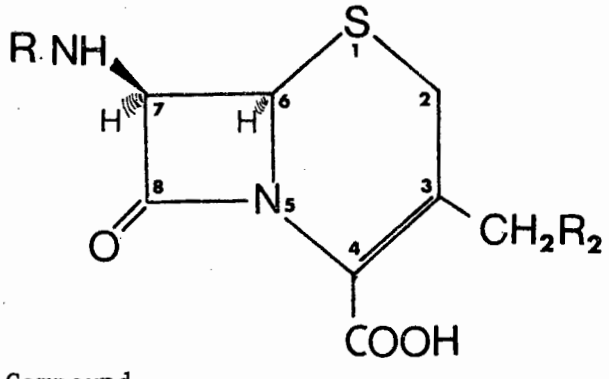
STRUCTURAL FORMULAE OF LIGANDS INVESTIGATED

PENICILLINS

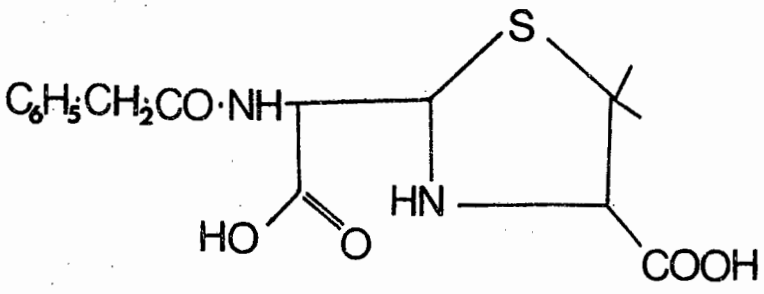


Compound	R
6-amino-penicillanic acid	H
Benzylpenicillin	$C_6H_5 \cdot CH_2 \cdot CO-$
Phenoxymethylpenicillin	$C_6H_5 \cdot O \cdot CH_2 \cdot CO-$
Ampicillin	$C_6H_5 \cdot CH(NH_2) \cdot CO-$

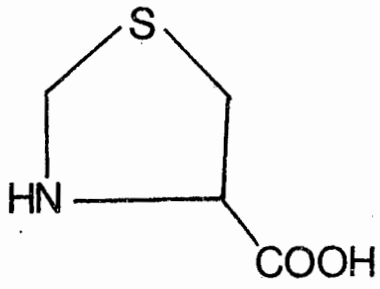
CEPHALOSPORINS



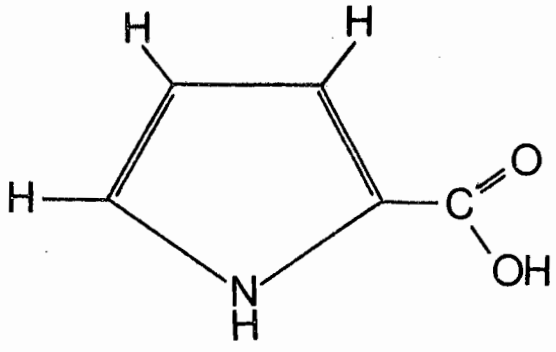
Compound	R_1	R_2
Cephalothin	CH_2CO-	$CH_3 \cdot CO \cdot O-$
Cephalexin	$C_6H_5CH(NH_2)CO-$	H
7-amino-cephalosporanic acid	H	$CH_3 \cdot CO \cdot O-$



Penicilloic Acid



Thiaproline



Pyrrole-2-carboxylic acid

$C_6H_5 \cdot CO \cdot NH \cdot CH_2 \cdot COOH$

Hippuric Acid

GLOSSARY OF SYMBOLS

$A/\hbar$	hyperfine coupling constant.
α	distance of closest approach of two ions.
a	effective radius of complex.
B_1	power of observing r.f. field.
$\beta_{p,q,r}$	overall formation constant. p = ligand, q = metal, r = proton.
D	dielectric constant of medium.
E_A	activation energy of rotation.
ϵ_{1b}	characteristic T_1 enhancement parameter.
ϵ_{2b}	characteristic T_2 enhancement parameter.
f	ratio of nuclei in bound and unbound state.
g	nuclear g factor.
I	nuclear spin quantum number.
Ka	acid dissociation constant.
Ki	stepwise formation constant.
k	Boltzmann's constant.
K_{os}	equilibrium constant for formation of outer-sphere complex.
k_f	formation rate constant.
k_d	dissociative rate constant.
Mo	equilibrium magnetisation.
N	Avagadroe's number
pL	negative logarithm of ligand concentration.
q	number of bound ligand nuclei per metal-ion.
r	metal-proton internuclear distance.
S	total electron spin.
T	absolute temperature.
T_1	longitudinal relaxation time.

T_2	transverse relaxation time.
T_{iM}	i = 1,2 relaxation rate of bound site.
T_{ip}	i = 1,2 paramagnetic contribution to the relaxation rate.
τ_c	dipolar correlation time.
τ_e	scalar correlation time.
τ_M	lifetime of bound state.
τ_r	rotational correlation time.
τ_s	electron spin relaxation time.
η	viscosity of solvent.
μ	ionic strength.
μ	nuclear magnetic moment.
$\Delta\nu$	line width (in H_z) at half height.
ω_I	nuclear Lamor frequency.
ω_s	electron Lamor frequency.
$\Delta\omega_M$	chemical shift of nucleus in bound state.
Z_M	charge on metal-ion.
Z_L	charge on ligand.
$\bar{z}$	average number of ligands bound per metal-ion.

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CHAPTER 1

INTRODUCTION

1.1 The Penicillin and Cephalosporin Antibiotics.

In 1928 an English bacteriologist, Alexander Fleming, noted that a mould, a penicillium species, had growth inhibiting properties against a number of pathogenic bacteria[1]. To the antibacterial culture fluid he gave the name 'penicillin'. Since then an unprecidented amount of work has been done on the isolation, characterisation, and manufacture of penicillins. The discovery of penicillin in turn stimulated an intensive search for other antibiotics resulting in the discovery of chloramphenicol, the tetracyclines and a whole host of other clinically less important antibiotics. One of the last antibiotics which arose from this screening search was cephalosporin[2], which is closely related to the penicillins, having a four-membered β-lactam ring, but instead of the five-membered thiazolidine ring, is fused to a six-membered thiazin ring.

Following on from the discovery of these antibiotics the mechanism whereby they inhibit cell growth has been extensively studied. It has been found that the tetracyclines etc. inhibit DNA synthesis while the penicillins and cephalosporins inhibit bacterial cell wall synthesis.

Bacterial cell walls consist of glycan strands in which two sugars, acetylglucosamine and acetylmuramic acid strictly alternate[3]. From the acetylmuramic acid residues tetrapeptide strands branch off and are cross linked to similar branches of neighbouring glycan strands providing the cell wall with a woven type structure, which is

responsible for its strength. The cross linkages are provided by interpeptide bridges which are to some extent a function of the genus of the bacterium. The case for S. aureus is depicted in Fig. 1.1. During cell wall synthesis the cross-linking of the glycan strands is performed by a transpeptidase enzyme and occurs during the last stages of cell wall synthesis. It is this step that is inhibited by penicillin.

The mechanism of bacterial cell wall synthesis and in particular the last stage of this synthesis the transpeptidisation has been closely studied. The reaction sequence is given in Fig. 1.2. Via a transpeptidase enzyme the terminal anino group of an interpeptide bridge attacks the terminal alanine-alanine residues of an adjacent tetrapeptide string forming a bridge between the two glycan strands and eliminating the terminal D-alanine residue.

FIG. 1.1

STRUCTURE OF PEPTOGLYCAN STRANDS OF S. AUREUS.

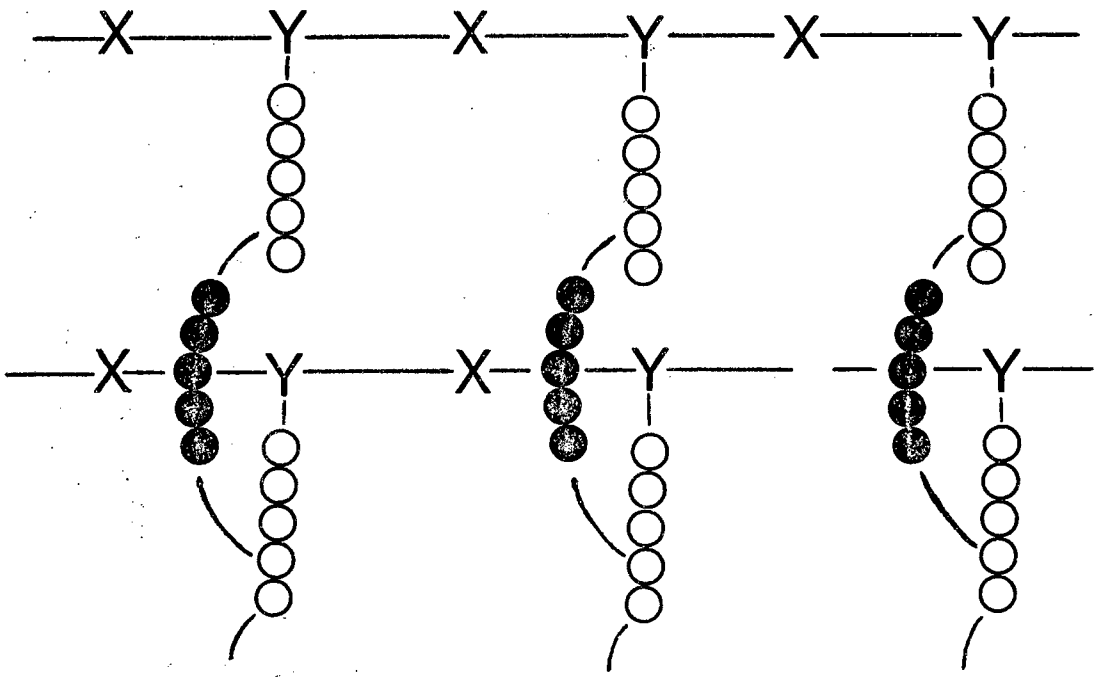
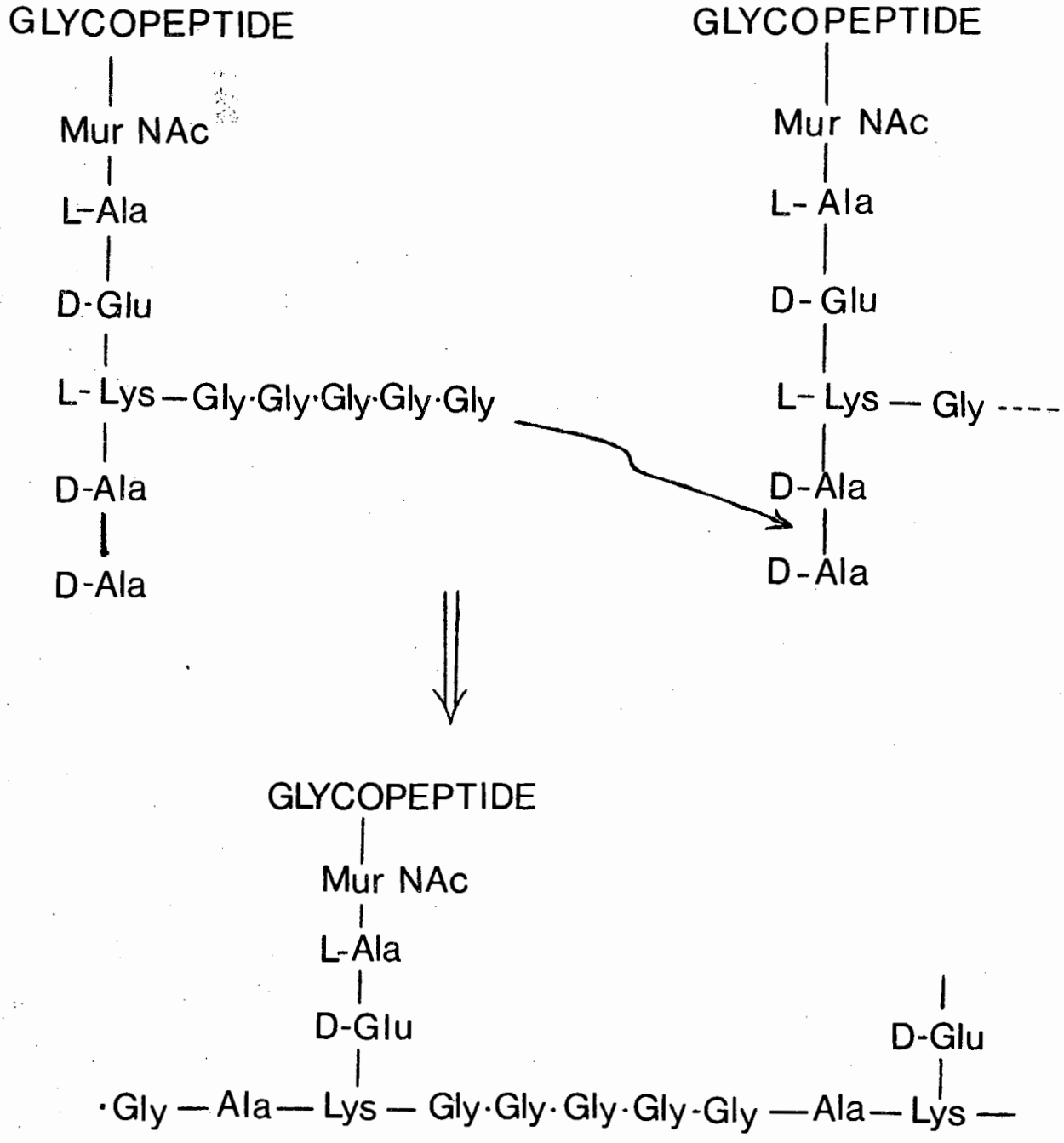


FIG. 1.2

REACTION SEQUENCE FOR TRANSPEPTIDATION OF PEPTIDOGLYCAN STRANDS.



Tripper and Strominger[4] have proposed that penicillin resembles the D-alanine-alanine end of the tetrapeptide string, and as such reacts with the transpeptidase enzyme thereby inhibiting it. However, when comparing stereo drawings of the two the resemblance is not that marked. Lee[5] has therefore made the suggestion that instead of resembling the alanine-alanine residues the penicillin molecule actually resembles an activated conformation of these two residues. The mechanism of action of the transpeptidase enzyme is then suggested to be that it binds the alanine-alanine residues and in so doing distorts the peptide bond between them making it more susceptible to nucleophilic attack. After cleavage of the peptide bond, by a suitably placed thiol group of the enzyme, the terminal alanine residue is released. Finally the thio-ester thus formed reacts with the terminal amino group of the interpeptide string forming a bridge between the glycan strands, regenerating the enzyme.

Penicillin inhibits transpeptidisation by mimicking the alanine-alanine residues and irreversibly binding the enzyme, thus inactivating it. Since penicillin is already "distorted" the reaction between it and the enzyme should be far faster than the enzyme and the alanine-alanine residues. This faster reaction rate is necessary since it has been estimated that at the reaction site penicillin is out numbered by the alanine-alanine residues by a factor of 10 000. Once the penicillin is bound, because of the very unstable β-lactam ring, it is quickly acylated by the enzyme.

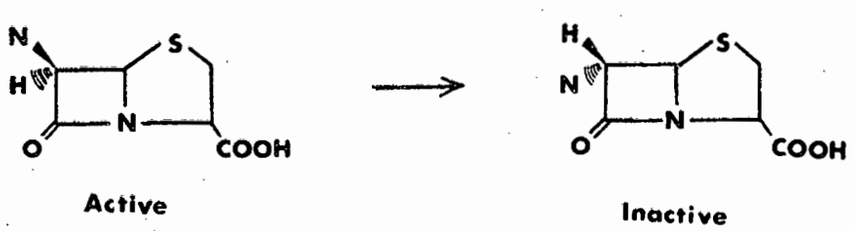
The penicilloyl enzyme, unlike the alanine- enzyme intermediate is stable against nucleophilic attack thus inactivating the enzyme. Presumably this stability against nucleophilic attack is due to steric

inhibition of the entering group by the thiazolidine end of the penicillin which is not lost as in the alanine-alanine cleavage. Ideally this penicilloyl enzyme should be completely stable to nucleophilic attack but it has been observed that penicillin treated cells can resume cross-linking after about 40 min.[6] Certain nucleophiles such as hydroxylamine, H_2O_2 and some thiols increase the rate at which the enzyme is regenerated[7].

From a knowledge then of the mechanism, whereby penicillins inhibit bacterial growth, improved penicillin antibiotics can be designed. The factors necessary for this antibiotic activity are:

1.2 Properties necessary for antibiotic Activity.

(i) Firstly the antibiotic must be able to bind to the enzyme. This property is obvious since unless the antibiotic is bound to the enzyme it will not be able to inhibit it. The structural constraints placed on the antibiotic by this necessity are not very rigorous, however. There exists a wide variation of structures amongst both the active and inactive penicillins and cephalosporins, and in fact little is known as to what factors affect enzyme binding. However, epimerisation at C_6 or C_7 of penicillin or cephalosporin which does not change the chemical properties of the antibiotic does result in loss of activity.



Active Inactive

gram-negative bacteria while ampicillin, which is charged and therefore more hydrophilic, is:

(iv) Stability towards β-lactamases. Many gram-negative bacteria produce β-lactamase enzymes which hydrolyse the β-lactam ring of the antibiotic, thus inactivating it. Therefore the effectiveness of an antibiotic depends on its resistance to these enzymes. It has been found that generally bulky side chained penicillins are more resistant. For example methicillin and oxacillin show a high resistance to these enzymes. These antibiotics, however, show a low affinity for the transpeptidase enzyme and so they are often used in conjunction with a more active antibiotic - they inhibit the β-lactamase while the more active antibiotic inhibits the transpeptidase.

1.3 Metals and Penicillin.

The importance of metal-ions in biological systems has been recognised for some time, and in many cases the structure activity relationship has been elucidated. However, in the less metal-ion specific reactions, where the metal-ion is in equilibrium with the substrate, the functions of the metal are not as well known.

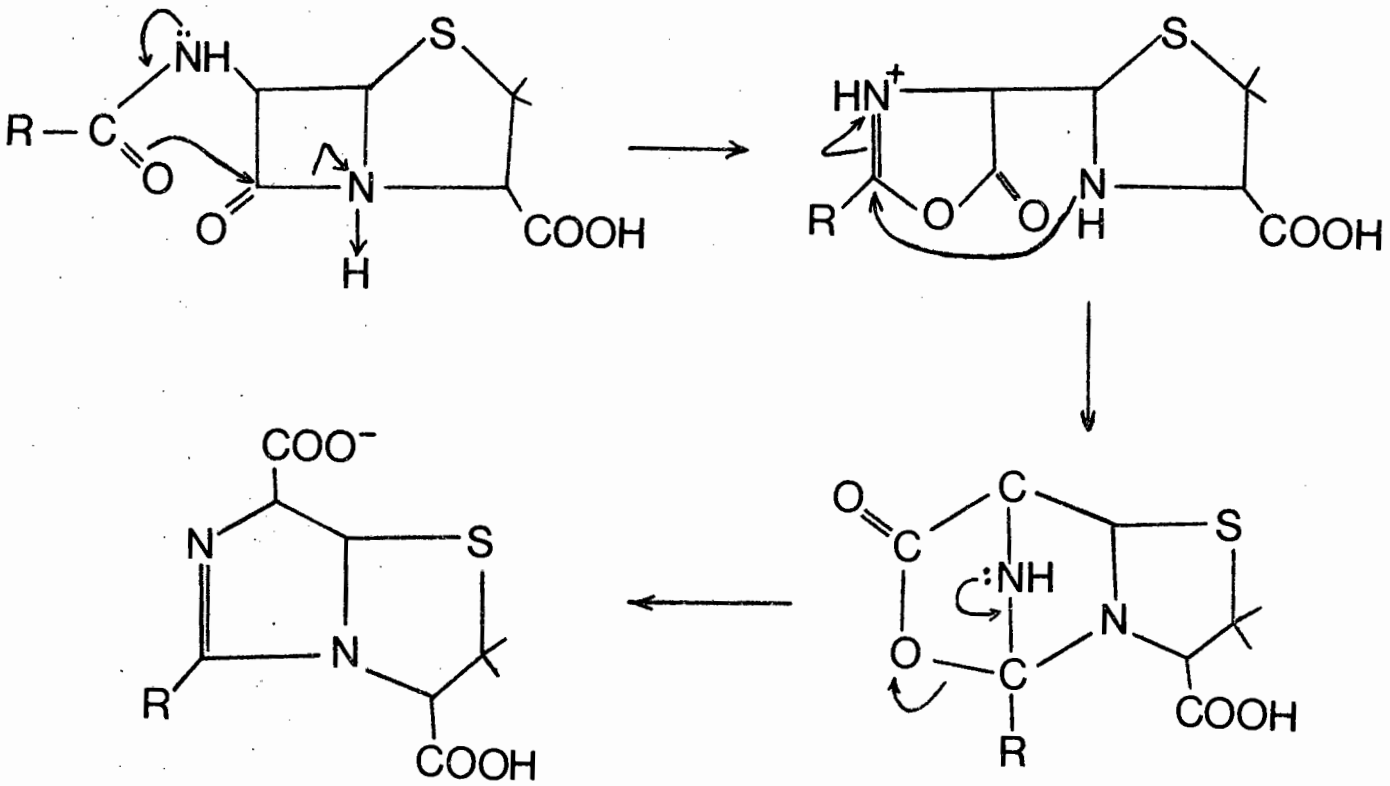
The various roles that metals play in biological reactions range from weak ionic effects to highly specific associations. Two groups of proteins which associate with metal-ions can be differentiated. Firstly the metal-ion may be an indispensable part of the protein to which

it is covalently bound and cannot be removed except by extreme chemical attack. This is the case of the 'metalloproteins' of which the cobaltic complex of vitamin B_{12}, the magnesium complex of chlorophyll and the copper complex of the haemo-cyanins are all examples. The metal-ion in these cases is highly specific, loss of activity accompanying substitution by another metal-ion. A notable exception to this is manganese which, being a better molecular probe is often used as a substitute for magnesium. Metal-ions bound in this way account for a large percentage of the body's total metal content.

Secondly, the metal-ion may be loosely bound to enzymes and as such 'activate' the enzyme. The metal-ion becomes loosely bound to both enzyme and substrate causing configurational or energetic changes which increase the rate of reaction. This type of reaction is much less metal-ion specific, loss of the metal-ion resulting in the enzyme becoming only somewhat inactivated. Metal-ions coordinated in this manner account for approximately 5% of the total metal-ion content of the body. The final fraction, less than 1%, is coordinated to amino acids and other low molecular weight ligands.

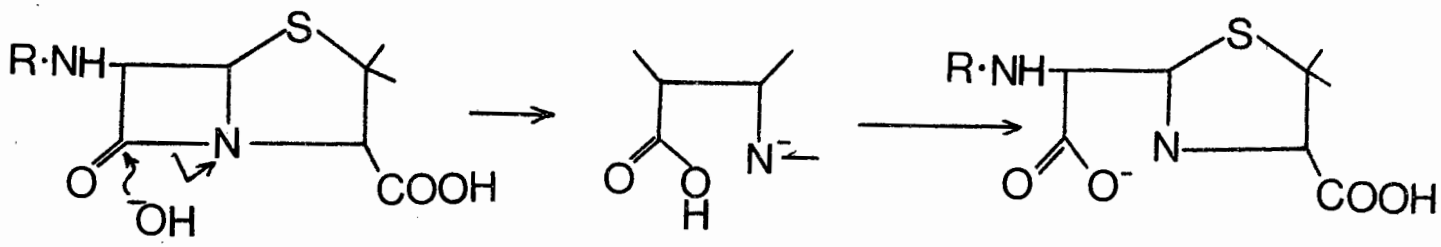
One of the characteristics of the β-lactam antibiotics is that they are not very stable. Both in acid and in alkaline solution they are rapidly inactivated. In acid solution the penicillin is degraded to penillic acid[9] (Scheme 1), while in alkaline solution penicilloic acid[9] is formed (Scheme 2). Both these degradation products are biologically inactive.

SCHEME 1.



Penillic acid

SCHEME 2.



Penicilloic acid

During the earlier studies of penicillin, Carrington[10] noted that many heavy metal-ions such as Mg(II), Pb(II), Zn(II) and Cd(II), all inactivated penicillins in neutral solution. The inactivation by Cu(II), Cd(II) and Zn(II) was found to be fairly rapid. In a subsequent report[11] the inactivating effect of Zn(II), Cd(II), Fe(II) and Fe(III) was studied. Only ferric ions were found to precipitate penicillin from aqueons solution (ferricillin). While these results left little doubt as to the essential incompatability of heavy metal-ions and penicillin, Zn(II) was found to form a salt in ethereal solution which had biological activity. This salt was freely soluble in water and assayed 1,250 U/mg. However, no subsequent reports were made concerning the stability or antibacterial spectrum of this salt.

In 1950, based on the results obtained by the penicillin research groups, Gunther[12] made a more detailed study of the effects of Zn(II), Ni(II) and Cu(II) on penicillin. He was able to isolate and identify the hydrolysis product as penicilloic acid. Following on from this Cressman et al[13] made an extensive study of the Cu(II) 'catalysed' penicillin degradation kinetics. These workers proposed the reaction sequence:

$$\text{Cu(II)} + \text{penicillin} \overset{K_1}{\rightleftharpoons} \text{Cu(II)} - \text{penicillin} \xrightarrow{k_1} \text{Cu(II)} - \text{penicilloic acid} + H^+$$

$$\text{Cu(II)} - \text{penicillin} + {}^-OH \xrightarrow{k_2} \text{Cu(II)} - \text{penicilloic acid.}$$

Where it was assumed that the rapidly formed Cu(II) - penicillin complex is hydrolysed to Cu(II) - penicilloic acid in the rate determining step. The reaction was followed by a pH-stat. technique and values for k_2 and K_1

obtained. By studying the temperature dependence of K_1 a value for ΔS was obtained, which suggested a chelate type structure. By studying the rate of hydrolysis of a variety of penicillins these workers were further able to propose a structure for the Cu(II) - penicillin intermediate. This is given in Fig. 1.3(a).

Besides the study of the rate of hydrolysis several workers have attempted to isolate solid metal complexes of the penicillins[14,15]. Th(IV), Fe(III), Co(II) and Al(III) complexes of penicillin have been prepared, but because of the hygroscopic nature of the penicillin and its inherent instability these complexes never gave very good elimental analysis. Mixed tetracycline[14,15], sulphanilamide[16] and isoniazid[14] metal penicillin complexes have also been prepared. These have been studied from two approaches, firstly their physical characteristics and structure have been investigated and secondly their antibacterial activity evaluated. On the physical side Th(IV) was found to form very sensitive precipitates from aqueous solution with penicillin but not with methylpenicillin, suggesting that the carboxylic acid group is involved in chelation with this metal-ion. The I.R. spectrum of the Co(II) - penicillin complex, on the other hand, shows a marked decrease in the absorbance of the 1786 cm^{-1} band, which is due to the β-lactam, suggesting the involvement of this group in chelation. Unfortunately the spectrum is not well resolved and nothing definite could be said about the resonance band of the side chain amide group. Finally the Fe(III) hydroximate complex of penicillin has been used in the colorametric determination of penicillin[17].

On the clinical side several metal complexes of penicillin have been found to have antibacterial activity. The Co(II)-penicillin complex has been studied by many workers and found to have an activity as much as five times that of penicillin[18]. The complex results in higher and prolonged penicillin concentrations in the blood. The Al(III) and Fe(III) complexes have been found to have a low toxicity and a prolonged time action[16]. The same was found for the mixed metal sulphonamide penicillin complexes[16]. The Al(III)-sulphonamethoxy pridazine-penicillin complex[19] has been found to give blood levels almost as prolonged as penicillin procaine and without producing the skin sensitizing effect that penicillin procaine does.

The interaction between metal-ions and the cephalosporin antibiotics has not been as well studied as the metal-penicillin interaction. Cephalosporins are more stable than their penicillin counterparts and their hydrolysis is not 'catalysed' by having metal-ions. In fact metal-ions such as Cu(II), Co(II), Ni(II) and Zn(II) are used in the purification of cephalosporin[20]. The cephalosporin is precipitated from solution as the metal complex, which on treatment with dilute acid yields the free antibiotic.

1.4 Objectives of the Research.

From the previous section we have seen that metal-ions have a considerable effect on the *in vitro* stability and *in vivo* storage[21], fate[22], of the penicillin antibiotics. Because of this and because of the scarcity of work that has been done in this field it was felt that a more

detailed study was necessary.

There are several questions, relating to this subject, which need to be answered and this was the objective of the research:

(i) Why do Cu(II) and Zn(II) 'catalyse' the hydrolysis of penicillin while Co(II) and Ni(II) do not?

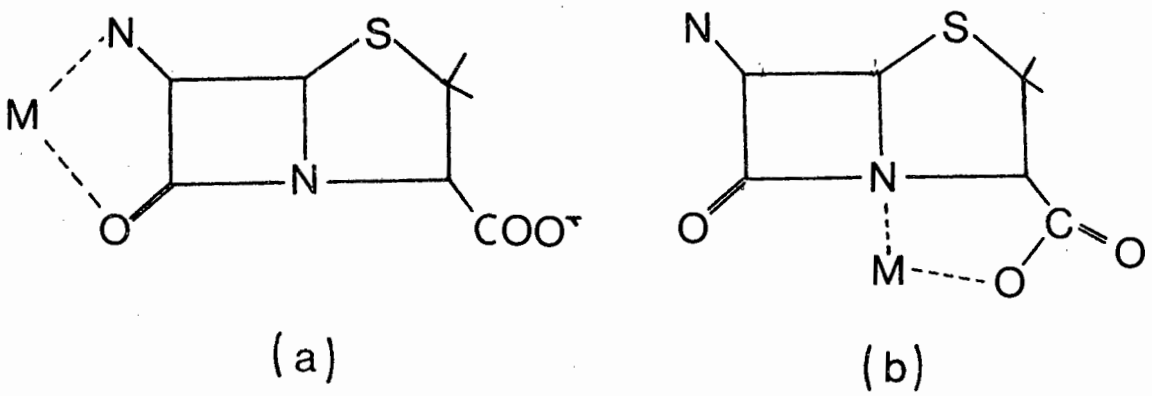
(ii) What is the mechanism whereby this hydrolysis takes place and

(iii) What is the structure of the metal complex formed?

The answer to these three questions lies in the answer to the third, 'What is the structure of the metal complex'? So far, on kinetic grounds, Cressman et al[13] have proposed structure 1.3(a), the added ring strain and also the increased electrophilicity of the β-lactam ring being responsible for the increased rate of hydrolysis. On the other hand, structure 1.3(b)[23] has been proposed as a likely site of chelation. The main objective of this research was then to determine which of these two most likely structures is the correct one.

FIG. 1.3

TWO MOST LIKELY STRUCTURES FOR THE METAL PENICILLIN COMPLEX.



This problem was approached from several different angles. Firstly the solution conformation of the Cu(II) and Mn(II) complexes were determined using n.m.r. As these two metal-ions are paramagnetic, those protons closest to the metal-ion are selectively broadened. This selective broadening was used to determine the conformation of the complex in solution. This was repeated with several penicillin and cephalosporin antibiotics, and also several structurally related compounds in an attempt to determine the effect of structural changes in the ligand on the structure of the complex.

Secondly the stability constants of several metal-ion/penicillin complexes were determined potentiometrically. These were correlated with stability constants determined for several related complexes in an attempt to determine the structure of the penicillin complex in solution.

Finally the Ni(II), penicillin substitution reaction kinetics was studied using a stopped flow reactor. Unfortunately the kinetics had to be limited to Ni(II) reactions as the other metal-ions of interest react at rates beyond the scope of this reactor.

CHAPTER 2

NUCLEAR MAGNETIC RELAXATION STUDIES

2.1 Introduction.

In the presence of certain paramagnetic ions the transverse relaxation times (T_2's) of nuclei provide a sensitive probe to molecular structure. Although the theory of this type of interaction has been known for many years[24] and much has been done with metals such as Nickel (II)[25];it is only with the advent of modern spectrometers that the metals copper, manganese, etc., have found extensive use. Most of the applications have been qualitative, dealing with macro-biological systems, while the applications to small organic molecules has been limited.

Of the small molecular systems, nucleotides and nucleosides have received the most attention. A whole host of these have been studied with a variety of metals both diamagnetic and paramagnetic. Much of the work has been of a qualitative nature; it being noted that certain of the ligand nuclei are shifted or broadened more than others. This type of investigation is important in that it can lead to a knowledge of the metal ligand association constants and also give an idea of the structure of the complex in solution. Of the work done here, that of Cohn and Hughes[26] provides a good example. These workers studied the effect of Cu(II) and Mn(II) on the ^{31}P and ^{1}H n.m.r. spectra of ADP and ATP and were able to conclude that the metal-ions interacted with both the phosphates and adenosine ring. The work of Kotowycz and Hayamiza[27] is interesting in that these workers studied the effect of Mn(II) on the ^{13}C spectra of nucleotides. The results that can be obtained from a study of this kind are more valuable than those that can be obtained from the ^{1}H spectra since

the molecule has more carbon atoms than protons and also these are usually more geometrically fixed in space. It is, however, far more time consuming. On the quantitative side an excellent series of studies on the binding of metal-ions to ATP have been carried out by Sternlicht and Shulman[28]. These provide a classic example of the analysis of relaxation rates to yield molecular information. These workers were able to determine all the parameters governing relaxation and hence calculate the distance of the metal-ion from the protons of the purine ring. A similar, though far less rigorous study of the Mn(II) thiamine diphosphate complex[29] has been done where it was possible to 'map out' the average solution conformation of the complex.

The difficulty in the quantitative distance calculations is in the accurate determination of the correlation times which modulate the metal-ion-nucleus interaction. This difficulty can be overcome (see later) by determining relative internuclear distances, which are independent of these correlation times, rather than the absolute distances. Of course in doing this some information is lost since it now needs the metal interaction with 4,rather than 3, nuclei to be known before the metal-ion can be uniquely placed in space. This is the type of study which we have undertaken with the penicillin and cephalosporin antibiotics to determine both the site and conformation of their metal complexes in solution. Certain sections of the work have already been published.[30,31]

2.2 Theory.

The basic theory of n.m.r. is dealt with in many standard texts.[32]

What follows is a discussion of those aspects of the effect of paramagnetic substances on the nuclear magnetic resonance which are relevant to the present study.

The present interest is in the line shape of an n.m.r. signal and in particular the width at half height. If the power of the observing radio frequency is low the theoretical line shape becomes Lorenztian and independent of T_1.

$$L = \frac{M_o(\gamma B_1)\, T_2}{1 + (\omega - \omega_I)^2\, T_2^2} \qquad \ldots\ldots\ldots (2.1)$$

Where ω_I is the nuclear Lamor frequency, ω is the frequency of the irradiating r.f. field of intensity γB_1, and M_o is the equilibrium magnetisation.

The width at half height is given by:

$$\Delta\nu = \frac{1}{\pi T_2} \qquad \ldots\ldots\ldots (2.2)$$

Hence any factors which affect T_2 will be reflected in their effect on the line width.

2.2.1 Mechanism of Nuclear-Spin Relaxation.

In addition to interaction with the lattice, nuclei can interact amongst themselves. Each magnetic nucleus gives rise to a magnetic field of magnetude μ/r^3, which is about 14 gauss for a proton at a distance of $1A^o$. Hence the nuclei experience not only the applied magnetic field H_o but also

the vector sum of these local magnetic fields, which will be different at different points in space. If H_{loc} is the spread of these local magnetic fields, the spread in the Lamor precession frequencies will be:

$$\Delta\nu = \frac{\mu H_{loc}}{I\hbar} \qquad \ldots\ldots\ldots (2.3)$$

If the precessing nuclei are in phase at a particular instant in time, after a time $(\Delta\nu)^{-1}$ they will be out of phase again. Since T_2 is the time constant for this dephasing, H_{loc} will contribute to it. This is why the line width of solid samples is so great while in solution, where the molecules undergo rapid reorientation and so these local fields are averaged out to be very small, the line widths are far narrower.

Dephasing of nuclei can also occur by a simultaneous flip-flop mechanism. In this one nucleus excites another, the energy coming from the exciting nucleus so that the total energy of the pair is conserved. The relative phase of these two nuclei change in a time $(\Delta\nu)^{-1}$ and so contribute to T_2. Inhomogeneity of the applied field will also cause line broadening since this will produce its own H_{loc}. This was one of the major factors to be overcome in the building of high resolution instruments. It is now further reduced by sample spinning, which helps average out these local fields.

2.2.2 Relaxation in Paramagnetic Systems[33]

Since the magnetic moment of an unpaired electron is $\approx 10^3$ times greater than the nuclear magnetic moment, nuclear relaxation in paramagnetic

systems is far faster than in diamagnetic systems.

The strong local fields produced by the unpaired electron can be coupled to the nuclei by simple dipole-dipole interactions, or sometimes by a hyperfine or scalar coupling transmitted through chemical bonds. This mechanism is strongly attenuated, and is only effective in aromatic systems or where the nucleus in question is no more than a few bonds removed from the paramagnetic centre eg. H_2O[28].

Solomon[34] and Bloembergen[35] have formulated expressions for the relaxation times T_1 and T_2, of nuclei bound to a paramagnetic centre;

$$\frac{1}{T_{1_M}} = \frac{2}{15} \frac{\gamma_I^2 g^2 S(S+1)\beta^2}{r^6} \left(\frac{3\tau_c}{1+\omega_I^2 \tau_c^2} + \frac{7\tau_c}{1+\omega_s^2 \tau_c^2} \right)$$

$$+ \frac{2}{3} S(S+1)(A/\hbar)^2 \left(\frac{\tau_e}{1+\omega_s^2 \tau_e^2} \right) \quad \ldots\ldots\ldots (2.4)$$

and

$$\frac{1}{T_{2_M}} = \frac{1}{15} \frac{\gamma_I^2 g^2 S(S+1)\beta^2}{r^6} \left(4\tau_c + \frac{3\tau_c}{1+\omega_I^2 \tau_c^2} + \frac{13\tau_c}{1+\omega_s^2 \tau_c^2} \right)$$

$$+ \frac{1}{3} S(S+1)(A/\hbar)^2 \left(\frac{\tau_e}{1+\omega_s^2 \tau_e^2} + \tau_e \right) \ldots\ldots\ldots (2.5)$$

The first term in the above expressions arises from dipole-dipole interactions between the electron and nuclear spins while the second term represents the scalar or hyperfine interaction. The correlation times,

τ_c and τ_e, which modulate these interactions are given by:

$$\frac{1}{\tau_c} = {}^1/_{\tau_s} + {}^1/_{\tau_M} + {}^1/_{\tau_r} \qquad \ldots\ldots\ldots (2.6)$$

$${}^1/_{\tau_e} = {}^1/_{\tau_s} + {}^1/_{\tau_M} \qquad \ldots\ldots\ldots (2.7)$$

Where τ_s is the electron-spin relaxation time, τ_r the rotational correlation time, and τ_M the lifetime of the bound state.

The paramagnetic metals can be divided into two classes depending on their value of τ_s, those which broaden a spectrum and those which shift the spectrum. Mn(II), Cu(II) and Gd(III)have relatively long electronic relaxation times ($\tau_s \simeq 10^{-8}$ sec.) and so τ_c will be dominated by τ_r ($\tau_r \simeq 10^{-10}$ sec, $\tau_m \simeq 10^{-8}$ sec.) and give rise to large $\frac{1}{T_{2M}}$ values, which broaden the spectrum. Ni(II) and Co(II), on the otherhand have very short electronic relaxation times ($\tau_s \simeq 10^{-12} - 10^{-13}$ sec.) and so τ_c will be dominated by these. This results in ${}^1/_{T_{2M}}$ being two orders of magnitude less than those of Mn(II) and Cu(II).

In the case of Mn(II) and Cu(II), the two metals used in the present study, and under the experimental conditions used (i.e. 100 MH_z spectrometer);

$$\omega_s = 4 \times 10^{11} \text{ rad.sec.}^{-1} \quad \text{and} \quad \omega_I \simeq 6 \times 10^8 \text{ rad.sec.}^{-1}$$

hence $\omega_s^2 \tau_c^2 >> 1$ and $\omega_I^2 \tau_c^2 << 1$

Also it has been shown by Shulman, Sternlicht[28] and other workers[36]

that the scalar contribution to the relaxation rates of nuclei not directly bonded to the paramagnetic ion, are negligible. This is also supported by the absence of any detectable shift in the resonance frequencies of any of the protons studied. Under these conditions then, equations 2.4 and 2.5 reduce to;

$$1/T_{1M} = \frac{2}{5} \frac{\gamma_I^2 g^2 \beta^2 S(S+1)}{r^6} \cdot 3\tau_c \quad \ldots\ldots\ldots (2.8)$$

$$1/T_{2M} = \frac{1}{15} \frac{\gamma_I^2 g^2 \beta^2 S(S+1)}{r^6} \cdot 7\tau_c \quad \ldots\ldots\ldots (2.9)$$

$\gamma_I^2 g^2 \beta^2 S(S+1)$ Is a constant for a given nucleus and paramagnetic ion and is equal to 2.3×10^{-30} and 2.0×10^{-31} for Mn(II) and Cu(II) respectively.

2.2.3 Effect of Chemical Exchange on Relaxation Rates.

The effect of chemical exchange on relaxation rates has been well characterised by Luz and Meilboom[37], and Swift and Connick[38]. The relevant equations are:

$$\frac{1}{T_{1OBS}} = \frac{1}{T_{1A}} + \frac{f}{T_{1M} + \tau_M} \quad \ldots\ldots\ldots (2.10)$$

and

$$\frac{1}{T_{2OBS}} = \frac{1}{T_{2A}} + \frac{f}{\tau_M} \left[\frac{1/T_{2M} (1/T_{2M} + 1/\tau_M) + \Delta\omega_M^2}{(1/T_{2M} + 1/\tau_M)^2 + \Delta\omega_M^2} \right] \quad (2.11)$$

Where the subscript A and M denote the free and bound states respectively and f is the ratio of bound nuclei to free nuclei. If we assume that only one ligand is bound to each metal-ion, and if the ligand concentration is in large excess of the metal then $f = [M]/[L]$.

There are several limiting cases of the above equations:

(i) If $\Delta\omega_M^2 >> 1/T_{2M}^2, 1/\tau_M^2$ then

$$1/T_{2p} = f/\tau_M \qquad \ldots\ldots\ldots (2.12)$$

where $$\frac{1}{T_{2p}} = \frac{1}{T_{2OBS}} - \frac{1}{T_{2A}} \qquad \ldots\ldots\ldots (2.13)$$

Relaxation occurs through a change in the precessional frequency and T_{2p} is controlled by τ_M.

(ii) If $1/\tau_M^2 >> \Delta\omega_M^2 >> (\tau_M T_{2M})^{-1}$ then

$$1/T_{2p} = f\,\tau_M\,\Delta\omega_M^2 \qquad \ldots\ldots\ldots (2.14)$$

Chemical exchange is rapid and so T_{2p} is controlled by the change in the precessional frequency.

(iii) If $1/T_{2M} >> \Delta\omega_M^2$; $1/\tau_M^2$ then

$$1/T_{2p} = f/\tau_M \qquad \ldots\ldots\ldots (2.15)$$

Relaxation by T_{2M} is fast, i.e. exchange is slow, and so T_{2p} is controlled by the rate of chemical exchange.

(iv) If $(T_{2M}\ \tau_M)^{-1} >> 1/T_{2M}^2$; $\Delta\omega_M^2$ then

$$1/T_{2p} = f/T_{2M} \qquad \ldots\ldots\ldots (2.16)$$

Here exchange is fast and so relaxation is controlled by T_{2M}.

When dealing with Mn(II) or Cu(II), since $\Delta\omega_M$ is small (i.e. these metals broaden rather than shift), only cases (iii) and (iv) are important. Under conditions of fast chemical exchange T_{2p} is controlled by T_{2M}, while under conditions of slow exchange T_{2p} is controlled by τ_M. The latter condition allows kinetic information about the solution complex to be obtained while the former condition enables structural information about the complex to be obtained. This was the condition under which the present study was conducted.

2.2.4 Temperature Dependence of Relaxation Rates.

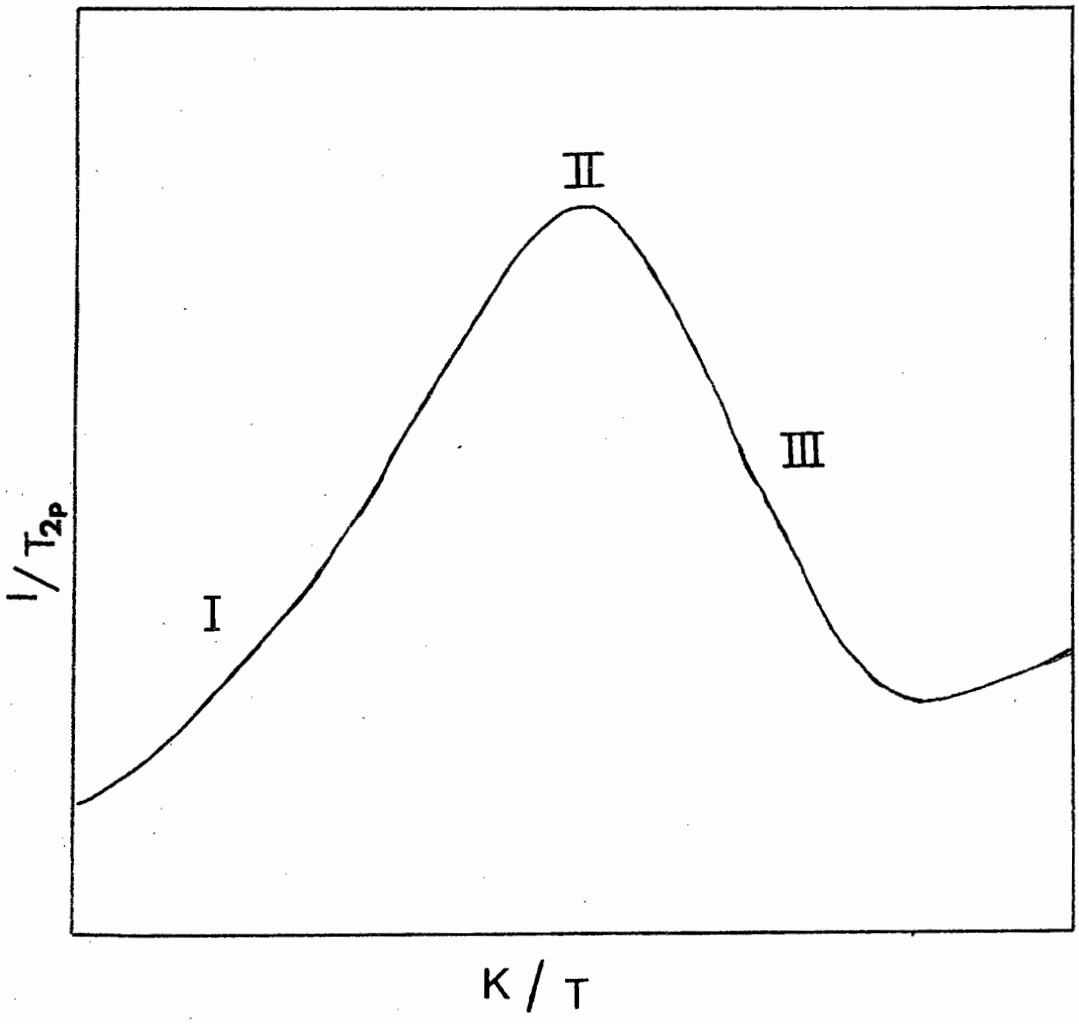
As we have seen above, the observed relaxation rates are represented by equations 2.15 or 2.16 depending on which condition of chemical exchange holds. Thus depending on the rate of exchange the temperature dependence of T_{2p} will be governed by the temperature dependence of τ_M or T_{2M}. This is illustrated in Fig. 2.1.

Region I is the fast exchange region where T_{2p} is controlled by T_{2M}, which is a function of τ_r. τ_r is given by:

$$\tau_r = \tau_r^0 \exp.(E_A/RT) \qquad \ldots\ldots\ldots (2.17)$$

FIG. 2.1

THEORETICAL CURVE SHOWING THE TEMPERATURE DEPENDENCE OF T_{2p}.



Where E_A is the activation energy of rotation.

Substitution of this into equation 2.9 gives:

$$1/T_{2M} = K \exp.(E_A/RT) \qquad \ldots\ldots\ldots (2.18)$$

Where K is a constant for a particular metal-ion, proton pair.

$$\therefore \quad \text{Ln } T_{2p} = -E_A/RT + \text{constant} \qquad \ldots\ldots\ldots (2.19)$$

Thus an increase in temperature results in a narrowing of the n.m.r. resonances in this region.

Region III is the slow exchange region where T_{2p} is controlled by τ_M. The temperature dependence of τ_M is given by the Eyring relation:

$$\tau_M = (kT/h)^{-1} \exp.(\Delta H^{\ddagger}/RT - \Delta S^{\ddagger}/R) \qquad \ldots\ldots\ldots (2.20)$$

When this is substituted into equation 2.15 we obtain:

$$\text{Ln}T_{2p} = \Delta H^{\ddagger}/RT + \text{constant} \qquad \ldots\ldots\ldots (2.21)$$

Thus an increase in temperature results in an increase in the line widths of the spectrum.

Region II, the intermediate region, can only be represented by the complete Swift and Connick relation, with τ_r, τ_M and τ_s all having a significant contribution. The temperature dependence of T_{2p} is therefore extremely complex and difficult to analyse.

The basis of the present work on molecular conformations depends on the condition of fast chemical exchange being met. That this condition holds can now be demonstrated by the temperature dependence of T_{2p}, (see Figs. 2.3, 2.7 and 2.10).

2.2.5 Evaluation of τ_c.

In practice an accurate determination of τ_c is often difficult.

For this reason relative internuclear distances are calculated rather than absolute internuclear distances. However, there are several methods available whereby estimates of τ_c may be obtained.

(i) From Stokes' Law: An estimate of the rotational correlation time may be obtained from Stokes' Law;

$$\tau_r = \frac{4\pi \eta a^3}{3kT} \qquad \ldots\ldots\ldots (2.22)$$

where η = viscosity of solvent

a = effective radius of complex.

This method has been used to obtain estimates of τ_r for the thiaproline and penicillin complexes.

(ii) From measurements of the relaxation rates of the solvent. This method determines the correlation time of the water protons in the first hydration sphere of the complex, which is then assumed to be applicable to the ligand nuclei as well. This assumption is valid for small, fairly rigid ligands, where the relevant correlation time is that of rotation.

If it is assumed that fast exchange conditions apply to both the free aqua and ligand complexes, then the enhancement parameter ε_{1b} is defined by:

$$\varepsilon_{1b} = \frac{q^*}{q} \frac{T_{1M}}{T_{1M}^*} \qquad \ldots\ldots\ldots (2.23)$$

Where * denotes the metal/ligand/H_2O system and q the hydration number.

It is further assumed that:

(a) the $M-OH_2$ distance is the same for the free metal and complex.

(b) the hyperfine contribution to T_{1M}^* and T_{1M} is negligible

then:

$$\varepsilon_{1b} = \frac{q^*}{q} \frac{f(\tau_c^*)}{f(\tau_c)} \qquad \ldots\ldots\ldots (2.24)$$

where $f(\tau_c) = 3\tau_c/(1 + \omega_I^2 \tau_c^2)$

In order to solve for τ_c we need to know both q, q* and τ_c. q and τ_c can be obtained from tables but q* must either be guessed at or determined independently.

In a similar manner we may write for ε_{2b}:

$$\varepsilon_{2b} = \frac{q^*}{q} \frac{T_{2M}}{T_{2M}^*} = \frac{q^*}{q} \frac{f(\tau_e^*)}{f(\tau_e)} \qquad \ldots\ldots\ldots (2.25)$$

where $f(\tau_e) = \tau_e/(1 + \omega_s^2 \tau_e^2)$

Note that in this case the scalar term will dominate T_{2M} and so $\tau_c = \tau_M$ and not τ_r. For this reason determinations of the solvent spin-spin relaxation rates does not aid conformation determinations. Also, because water relaxation rates are fast they cannot be accurately determined by progressive saturation, the only method of determining T_1 in these laboratories. Finally the accuracy of the method depends on the magnitude of ε_{1b}. This in turn depends on the change in the correlation time, i.e. the change in the rate of rotation in going from free metal to

complex. For small ligands this change is fairly small. This method has been used however, to great advantage in the study of macromolecular systems where the change in the correlation time is of the order of 10^4.

2.2.6 Extraneous Factors which Affect Relaxation Times.

There are several other factors, mainly instrumental, which can affect the line shape of an n.m.r. resonance. These should however, be constant during an experiment and so not effect the results i.e. any instrumental factors are subtracted from the line width in the form of $T_{2(0)}$. These factors are:

(i) Magnetic field inhomogeneity. On the Varian XL 100 spectrometer, the one used in this study, the magnetic field homogeneity is better than 0.2 H_z.

(ii) Change in bulk susceptibility. Due to the addition of paramagnetic metals to the sample the bulk susceptibility may change. Simple calculations, however, show this to be negligible at the metal-ion concentrations used here.

(iii) Viscosity of the medium. As the viscosity of the medium increases so the molecular tumbling rate decreases. This means that the local magnetic fields and inhomogeneities will not be averaged out as well and so the spectral lines will become broader.

2.2.7 Calculation of Relative Internuclear Distances.

In a paramagnetic metal complex those nuclei closest to the

metal-ion will be preferentially broadened. For the class I 'broadening' metals like Cu(II) and Mn(II) the degree of broadening is given by equations 2.9 and 2.16. When these are combined we get:

$$\Delta\nu = \frac{1}{\pi T_{2p}} = \frac{f}{15\pi} \cdot \frac{\gamma_I^2 g^2 \beta^2 S(S+1)}{r^6} \cdot 7\tau_c \quad \ldots\ldots\ldots (2.26)$$

Since the width at half height, $\Delta\nu$, can be measured directly from the n.m.r. spectrum it is theoretically possible to calculate the metal-ion-proton internuclear distance. The problem, however, is in the determination of τ_c. To overcome this difficulty relative internuclear distances are determined rather than absolute internuclear distances. This is done by determining the broadening produced by different metal concentrations. A plot of $\Delta\nu$ against f (equation 2.26) should then yield a straight line with a slope proportioned to $1/r^6$. Therefore we have:

$$\left(\frac{r_A}{r_B}\right)^6 = \frac{(\text{Slope})A}{(\text{Slope})B} \quad \ldots\ldots\ldots (2.27)$$

Where A and B denote two ligand protons.

This is the equation used in all the distance determinations in this study.

2.3 Results and Discussion.

Since accurate determination of T_1 is not possible in our laboratories the assumptions made in the theory of the paramagnetic ion-proton interaction were tested using model compounds of increasing complexity. This was necessary since Espersen et al[39] have shown that the assumption that the scalar contribution to T_2 is negligible is not always true for Cu(II). The first model compound studied was pyrrole-2-carboxylic acid which has only one site of coordination and only one possible conformation and hence all the proton-metal distances are known and can be checked against those obtained from n.m.r. The second was thiaproline which has again only one coordination site but has two possible conformations. The penicillins represent the most complex case in which there are several possible sites and conformations.

2.3.1 Pyrrole-2-Carboxylic Acid/Mn(II).

The spectrum of pyrrole-2-carboxylic acid is shown in Fig.2.2(a) along with the assignments based on coupling constant data.[40] Because of the coupling between the different protons this spectrum is too complex for an effective metal broadening experiment and so had to be partially decoupled. This is shown in Fig. 2.2(b) where A and C were decoupled from B and B from C. With the spectrum in this form the broadening experiments were carried out. The spectrum after the addition of $1.6\ 10^{-5}$ M Mn(II) is shown in Fig. 2.2(c). Qualitatively it can be seen that the H_A resonance is far broader that those of H_B and H_C. Inspection of a model of the complex shows that the H_A proton is much closer to the

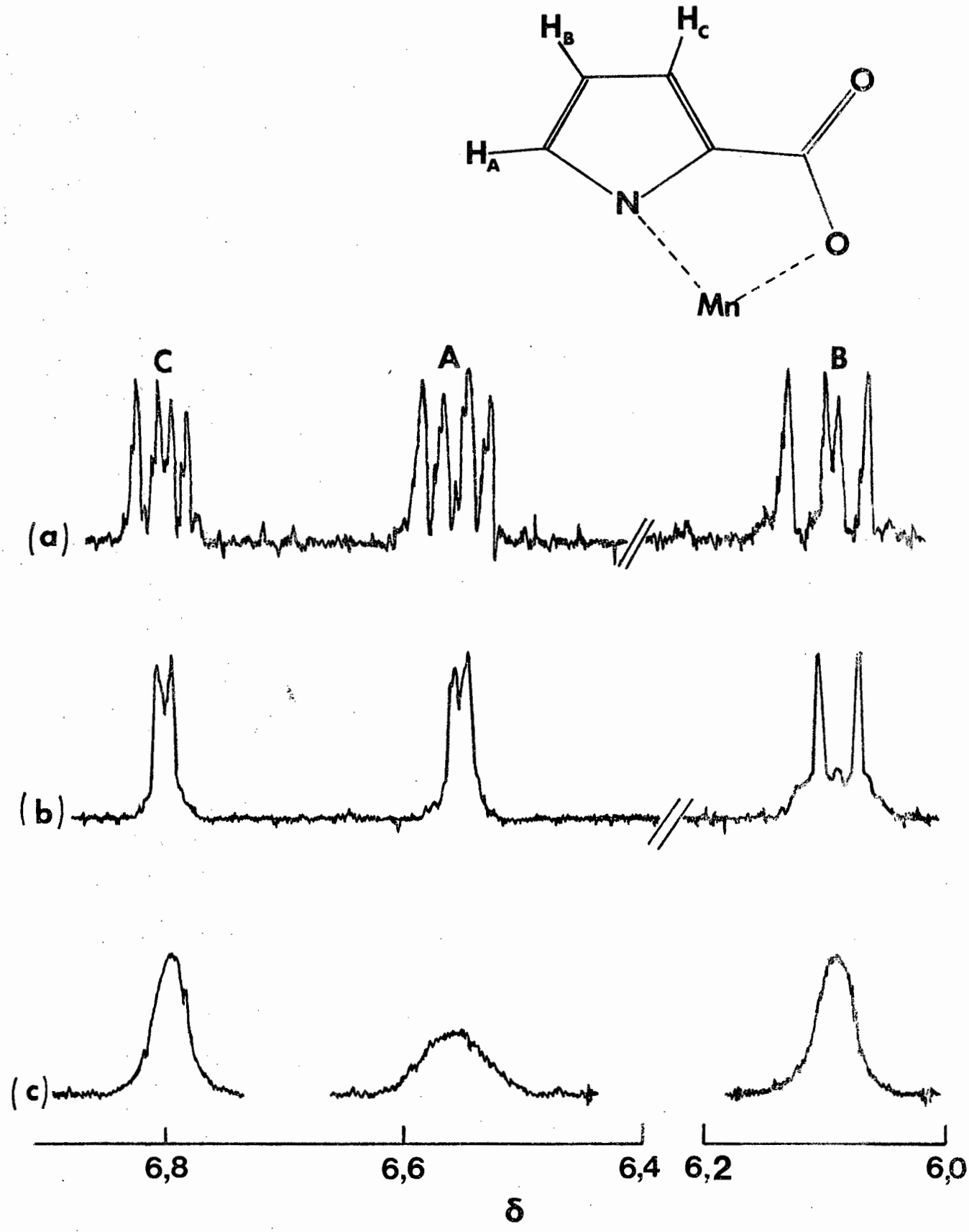


FIG. 2.2

(a) ^{1}H N.m.r spectrum of 0.1 M pyrrole-2-carboxylic acid, pH 5.5, 23°C;

(b) partially spin-decoupled spectrum, A and C from B, B from C;

(c) (b) + 1.6×10^{-5} M Mn(II).

metal-ion than either H_B or H_c. The order of proton-metal internuclear distance is $H_A << H_c < H_B$ which is the same order as the broadening of the n.m.r. peaks. The broadening can in fact be used in reverse to assign the n.m.r. spectrum.

One of the conditions which has to be satisfied in order for quantitative internuclear distance determinations is that of fast chemical exchange. This was tested for by a variable temperature study on a partially broadened spectrum of pyrrole-2-carboxylic acid. The resultant plot of $(f\ T_{2p})^{-1}$ vs. $1/_K$ (Fig. 2.3) has a positive slope which indicates that we are in the region of fast chemical exchange.

The results of the broadening observed during the metal titration are shown in Fig. 2.4 in the form of a plot of T_{2p}^{-1} vs. f. From the relative slopes of these curves the relative metal-proton internuclear distances can be calculated. These are compared to the internuclear distances measured from a Drieding model of the complex in table 2.1. Since there is likely to be fast flipping between the two conformations of the chelate ring, in which the metal-ion is either above or below the pyrrole ring, the metal-ion is assumed to be in the plane of the pyrrole ring. The agreement between the measured and experimentally observed internuclear distances are all within 6%. The low error here is a result of the sixth power of r in equation 2.27, which turns a 60% error in the relative slopes into a 10% error in the relative distances.

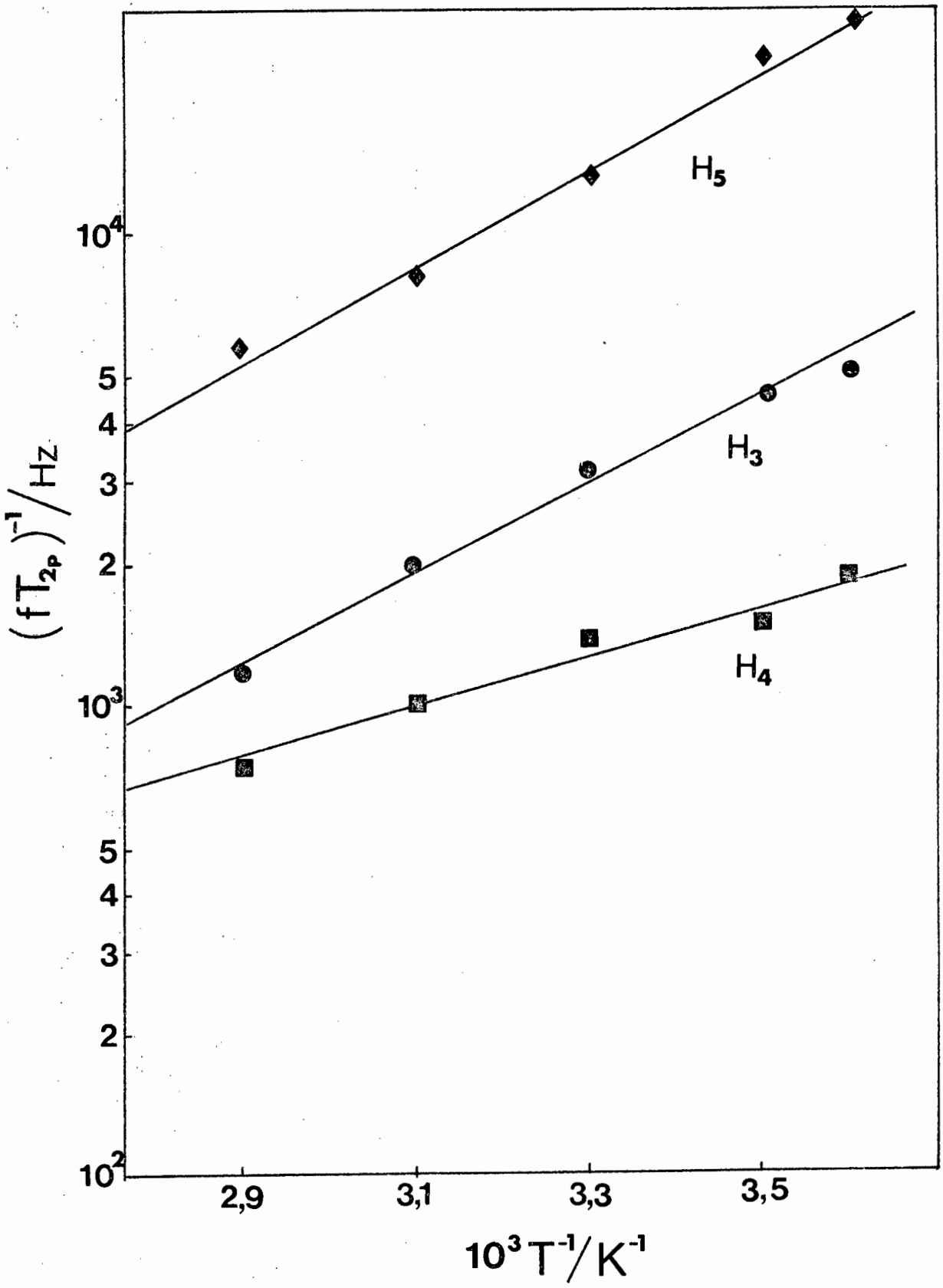


FIG. 2.3

Temperature dependence of f T_{2p} for pyrrole-2-carboxylic acid - Mn(II), pH 5.5.

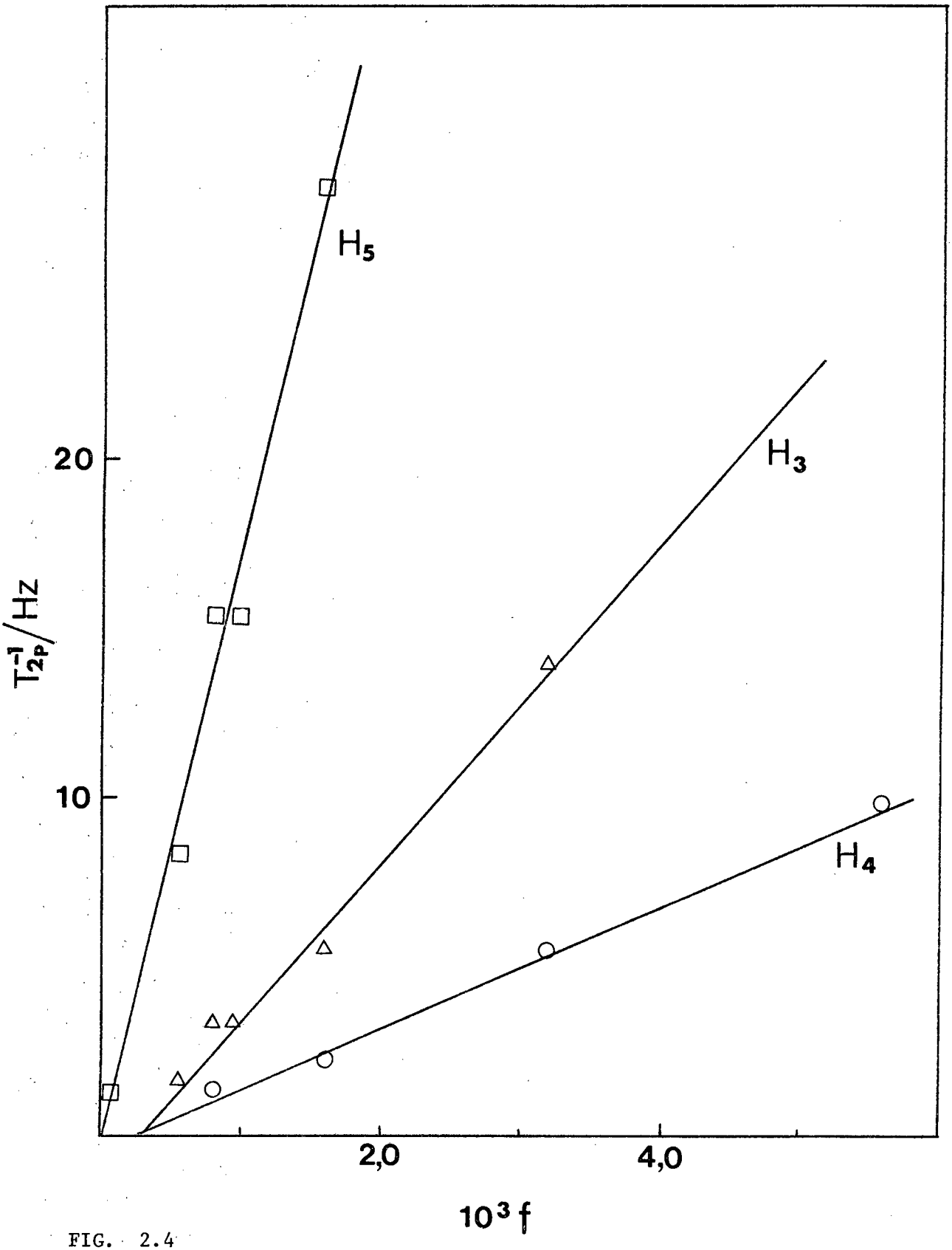


FIG. 2.4

Measured values of T_{2p} for pyrrole-2-carboxylic acid - Mn(II) as a function of f, pH 5.5, 23°C.

TABLE 2.1

Comparison of Measured and Experimentally Determined proton-metal distances for pyrrole-2-carboxylic acid-Mn(II).

Proton	Measured (A^o)	Experimental (A^o)[a]
3-H	4.7	4.6
4-H	5.2	5.3
5-H	3.6	3.6

(a) Relative to 3-H.

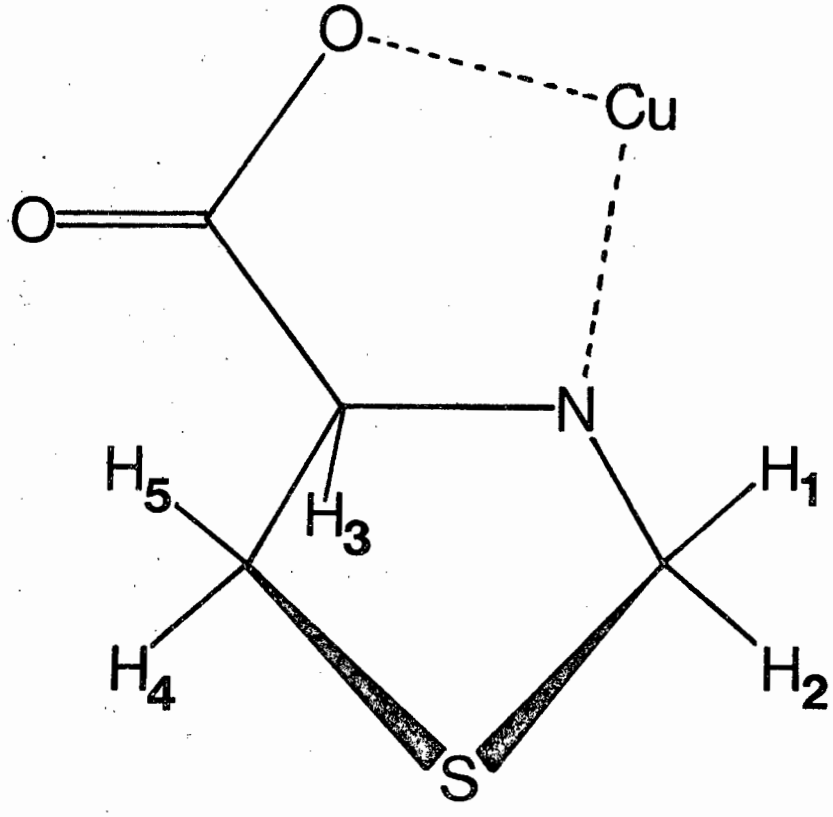
The agreement between experiment and theory shown here lends confidence to the assumptions made in the derivations and the use of paramagnetic ion probes in n.m.r. to determine internuclear distances.

2.3.2 Thiaproline - Cu^{2+} /Mn^{2+}.

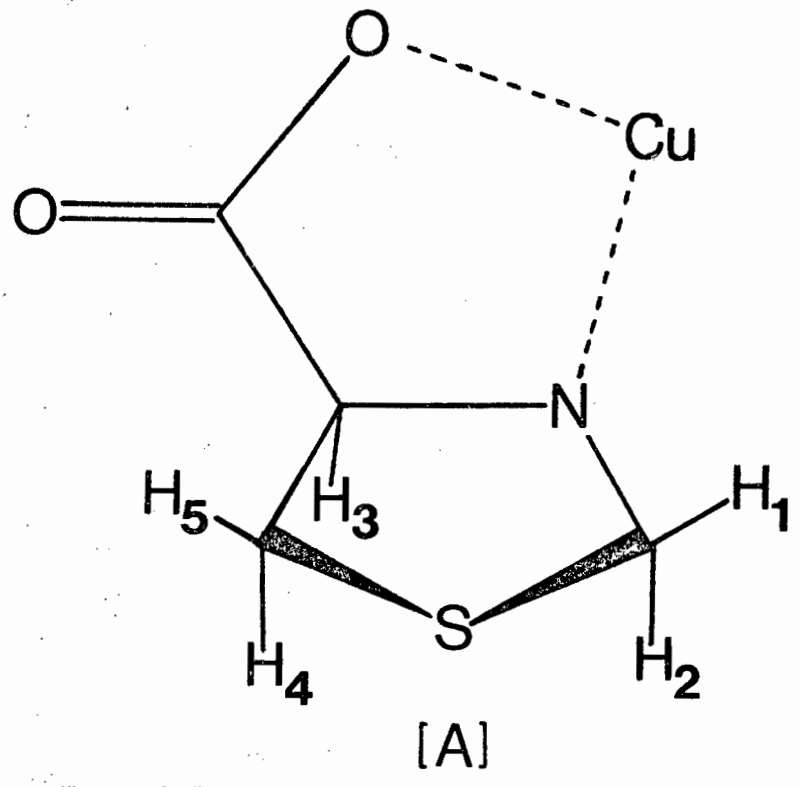
Like pyrrole-2-carboxylic acid thiaproline has only one metal coordination site. The five-membered thiazolidine ring can however adopt two different conformations. These are shown in Fig. 2.5. The five-membered chelate ring can also adopt two different conformations but these need not be considered here as the position of the metal-ion relative to the thiaproline protons is the same in both cases.

The spectrum of thiaproline is shown in Fig. 2.6(a). Protons 1 and 2 give rist to a classic AB quartet while protons 3,4 and 5 give an ABX pattern. The analysis of the ABX system was carried out according to the method of Diehl[41] which allowed the assignment of the spectrum as shown. The assignment of the H_1 and H_2 protons is far more difficult and cannot be done without the aid of an additional n.m.r. technique such as the nuclear Overhauser effect. Inspection of the partially broadened spectrum, Fig. 2.6(b), shows that of the two AB doublets the low field one is broader. Since H_1, in the complex, is always closer to the metal-ion than H_2 the broader doublet must be assigned to H_1. This again illustrates the qualitative use of paramagnetic broadening agents in the assignment of n.m.r. spectra.

The test for fast chemical exchange i.e. the plot of $(f\ T_{2p})^{-1}$ against reciprocal temperature is shown in Fig. 2.7. The positive slope of these lines shows that the complex is undergoing fast chemical exchange. This is to be expected since τ_M is ~ 10^{-8} sec. for Mn(II) and Cu(II), while τ_r is ~ 10^{-11} sec. τ_r may be estimated for the thiaproline complex using



[B]



[A]

FIG. 2.5

Schematic drawing of the Cu(II)-thiaproline complex showing the two possible conformations.

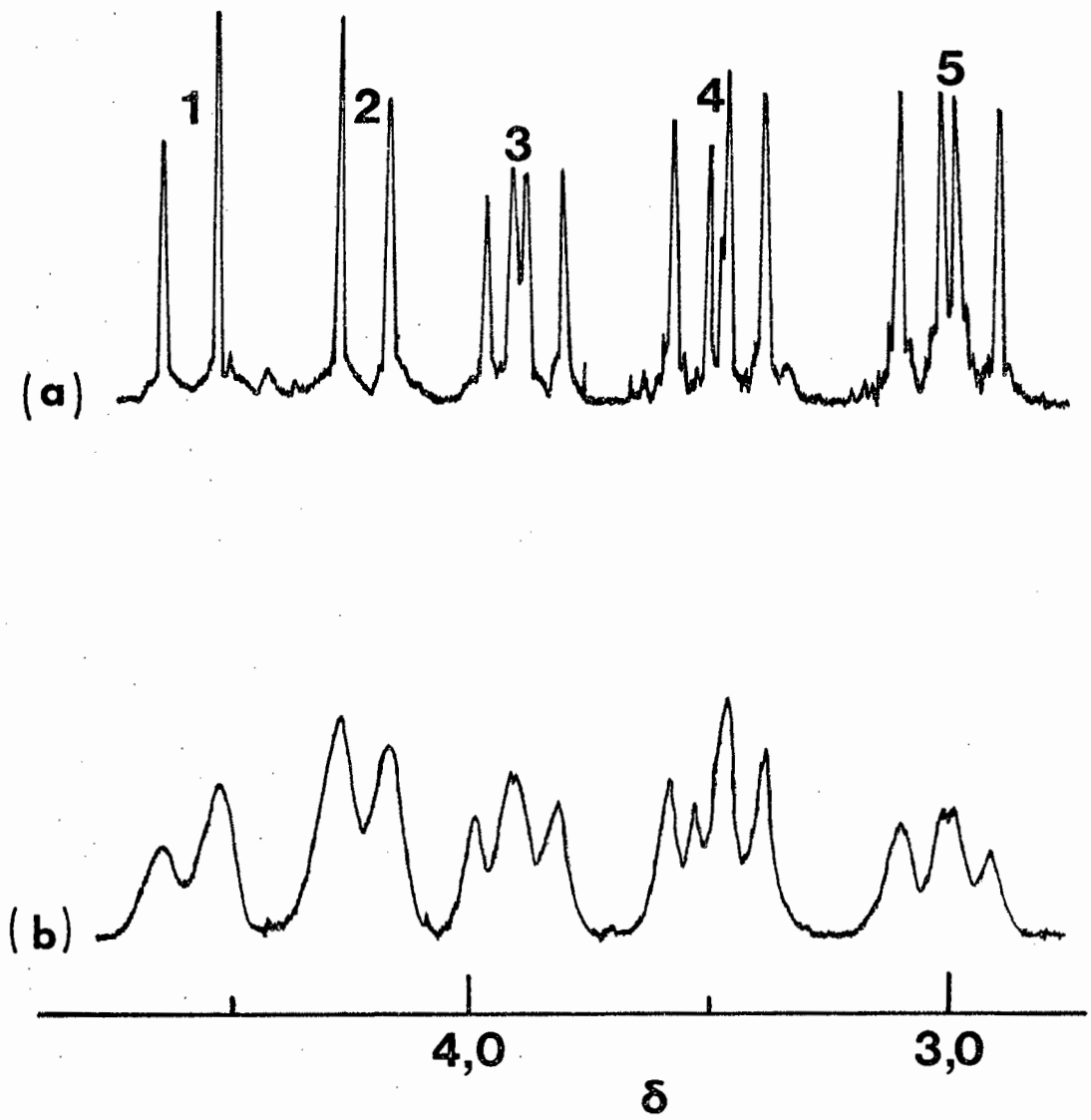


FIG. 2.6

^{1}H N.m.r spectrum of thiaproline in the presence and absence of Mn(II);

(a) 0.1 M thiaproline, pH 8.0, 23°C;

(b) as (a) + 4 x 10^{-5}M Mn(II); J_{12} 9.5, J_{43} 8.2, J_{45} 10.5, J_{53} 7.2 H_z.

Stokes' Law (equation 2.22) and the effective radius of the complex, as measured from a Drieding model. The activation energy for rotation of the complex is given by the slope of the reciprocal temperature plot. Using τ_r, τ_r° can then be calculated (equation 2.17). These parameters are found to be similar to those for $Mn(H_2O)_6^{2+}$. (See Table 2.2).

TABLE 2.2

Parameters obtained from analysis of relaxation rates of the Mn(II) - thiaproline system.

α (effective radius) (A°)	4.0
E_A / kcal mol^{-1}	4.6
τ_r (298 K) (S)	6.5×10^{-11}
τ_r (298 K) (S) $Mn(H_2O)_6^{2+}$ [33]	3.0×10^{-11}
E_A / kcal mol^{-1} $Mn(H_2O)_6^{2+}$ [33]	4.5

The results of the metal-ion titration are shown in Fig. 2.8 from which the relative metal-ion proton internuclear distances of the complex may be obtained. These are given in Table 2.3 together with the internuclear distance measured from a Drieding model of the complex. The two conformations of the thiazolidine ring, have different metal-ion proton distances. However there is rapid flipping between these two conformations and so only the time average of the two is determined by n.m.r. Inspection of the measured distances for the two conformations shows that

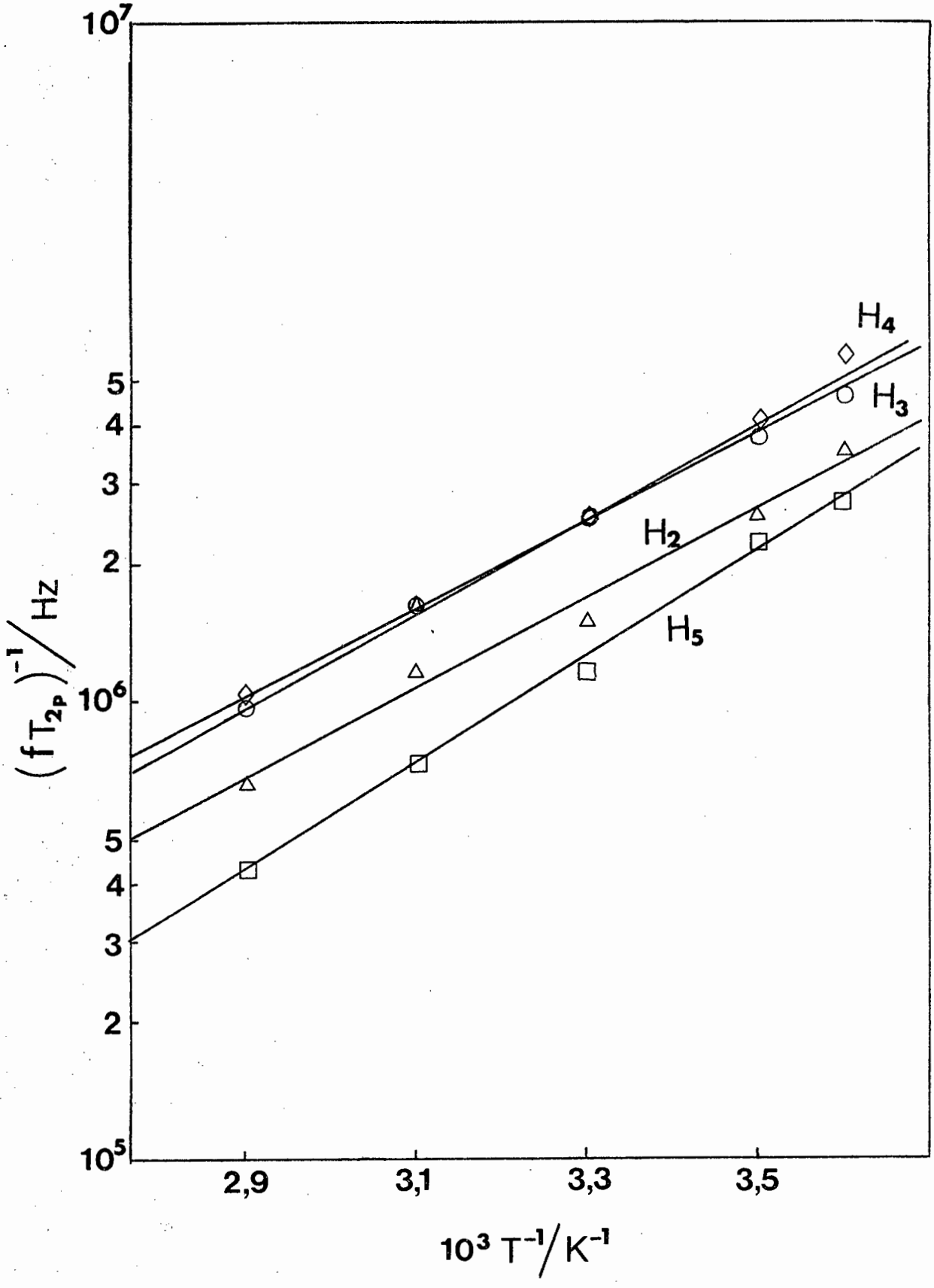


FIG. 2.7

Temperature dependence of f T_{2p} for thiaproline - Mn(II), pH 8.0.

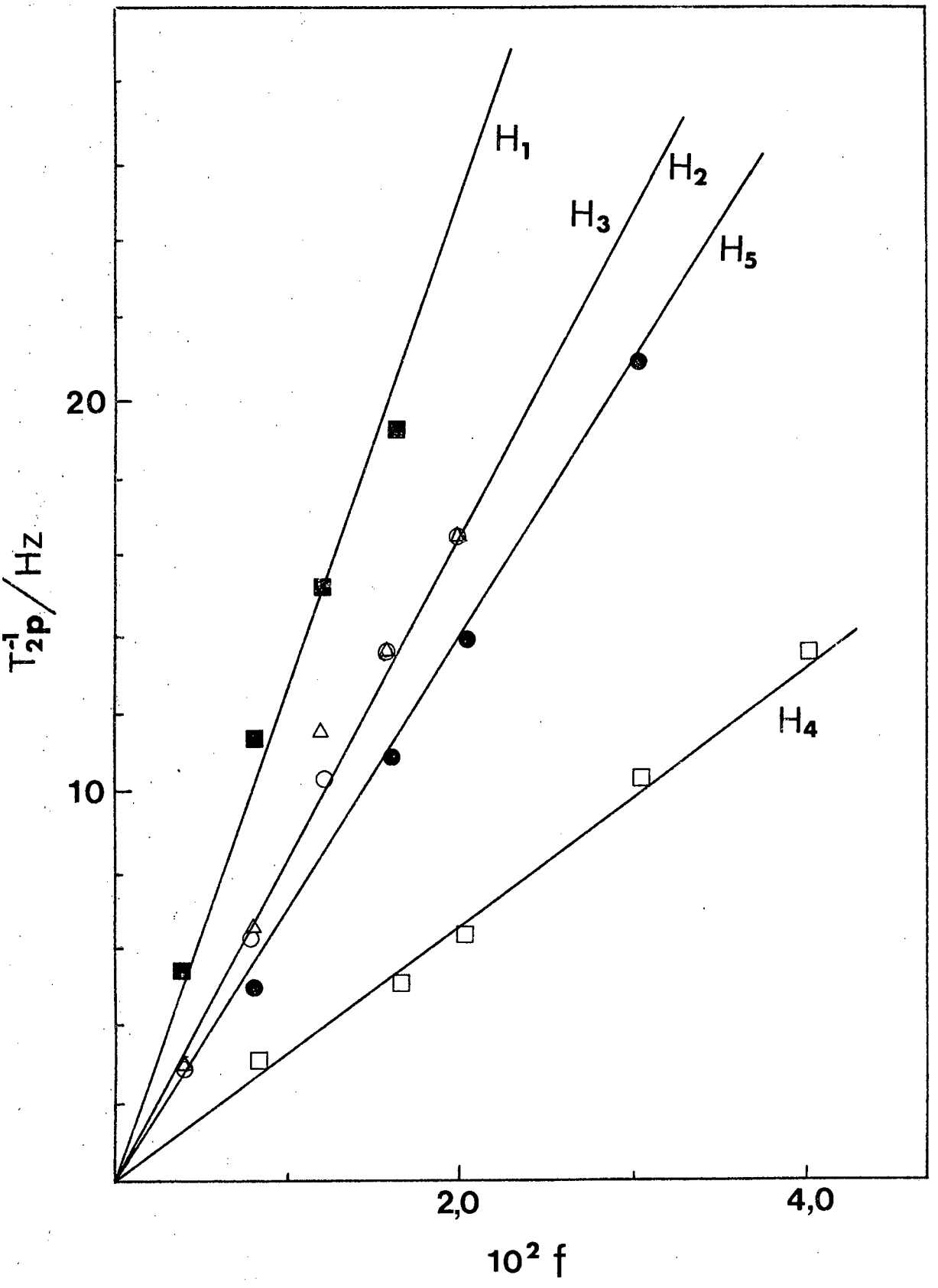


FIG. 2.8

Measured values of T_{2p} for thiaproline - Mn(II) as a function of f, pH 8.0, 23°C.

only H_5 is sensitive to the conformational change. The experimentally determined metal-ion proton distance for this proton indicates that perhaps the [A] conformation is the more energetically favoured of the two, but no numerical value can be given to this.

TABLE 2.3

Comparison of Measured and Experimentally determined proton-metal distances for thiaproline - Mn(II) and Cu(II) systems.

Proton	Measured distances (A^o) for two conformations [b]. [A]	[B]	Experimental distances [a] (A^o) Mn(II) / Cu(II)
H_1	3.0	2.7	3.1
H_2	3.3	3.8	3.6
H_3	3.6	3.5	3.5
H_4	4.8	4.7	4.3
H_5	3.9	4.7	3.7

(a) Relative to 3-H (b) Conformation [A] has H_5 equatorial.

Using the value of τ_r calculated from Stokes' Law and having experimentally determined T_{2p} for a particular f. An estimate of the absolute metal-ion proton distance may be obtained. It must be emphasised, however, that this is only an estimate since the rotational

correlation time τ_r is not accurately known. This is why relative metal-ion-proton distances have been used in the conformational analysis. The value of r for H_3 obtained using this method is 4.8. A^o, which is in satisfactory agreement with the value of 3.5 determined experimentally, considering the error, both in τ_r and f. Errors in these two quantities do not play a part in the calculation of relative internuclear distances.

The above experimental proceedure was repeated using Cu(II) as the paramagnetic broadening agent. This metal-ion was found to give the same results as Mn^{2+}.

2.3.3 Benzylpenicillin and 6 APA/Mn^{2+} /Cu^{2+}.

The n.m.r. spectrum of benzylpenicillin is shown in Fig. 2.9. The resonances have been assigned previously in deuterochloroform solution.[42] The H_6 proton was assigned from its coupling to the amine proton of the side chain while the α and β methyl groups were assigned using the nuclear Overhauser effect. The results of our broadening studies show these assignments to be the same in aqueous solution.

The variable temperature study of the Mn^{2+} - penicillin complex is shown in Fig. 2.10. Once again the complex is undergoing fast chemical exchange and an estimate of the activation energy of rotation can be obtained. This is 6.4 kcals/mole which is higher than that for the Mn^{2+}/ thiaproline complex. This is to be expected since the penicillin complex is more bulky. With Cu(II), but not with Mn(II), rapid hydrolysis of the penicillin occurred at about 340°K which is consistent with the observation of Cressman[13] that Cu(II) specifically promotes hydrolysis of penicillins.

The results of the Mn^{2+} and Cu^{2+} titrations are shown in Fig.s 2.11 and 2.12 respectively. Although several sites of coordination of the metal-ion are possible the two most probable are shown in Fig. 1.3. From kinetic evidence Cressman[13] proposed structure 1.3(a) although structure 1.3(b) could not be precluded. Qualitatively it can be seen (Fig. 2.9) that the side chain methylene protons are not appreciably broadened whereas H_3 broadened rapidly. Also H_5 broadened faster than H_6.

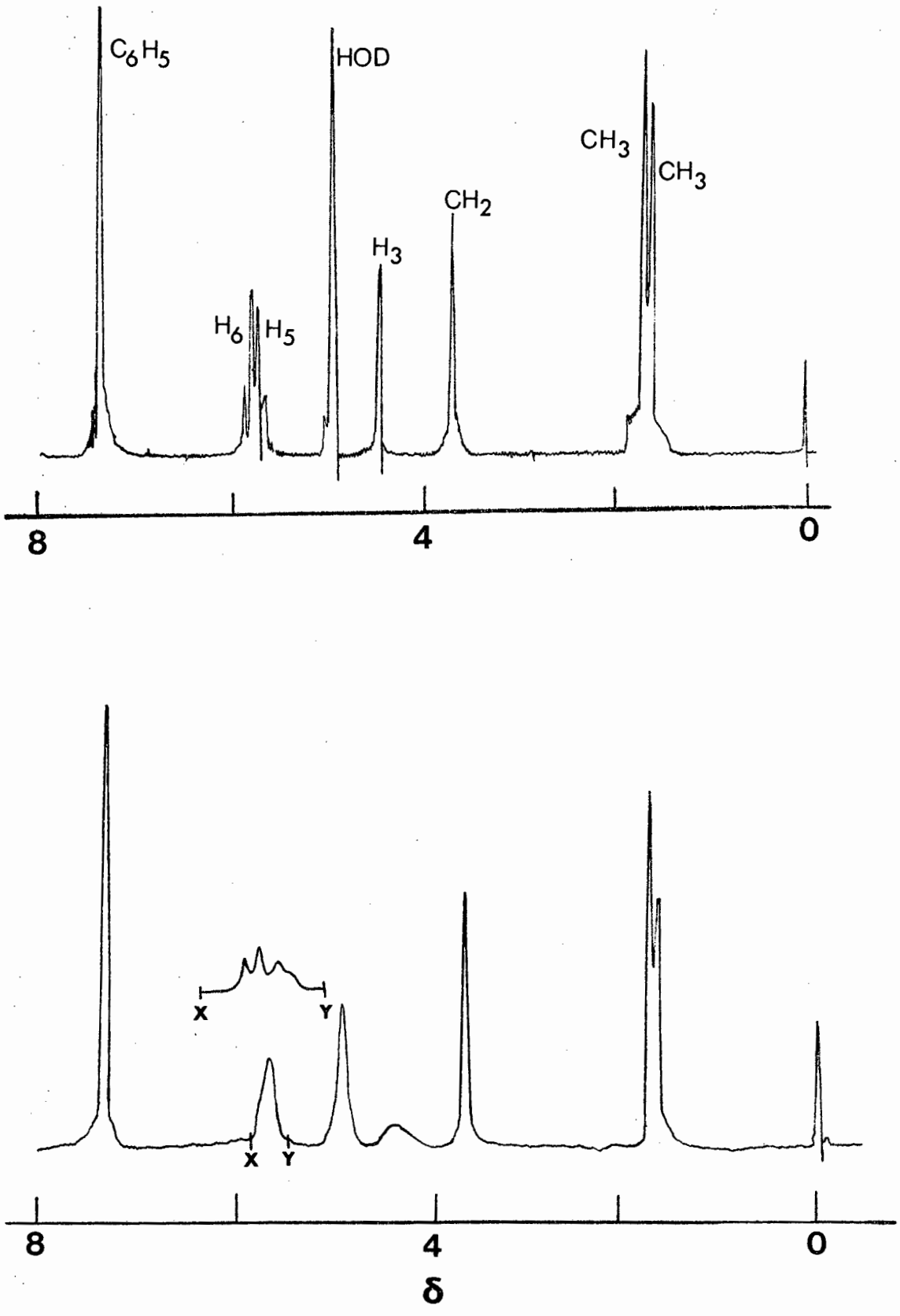


FIG. 2.9

^{1}H N.m.r spectrum of benzylpenicillin in the presence and absence of Mn(II);

(a) 0.1 M penicillin, pH 5.5, 23°C;

(b) as (a) + 3 x 10^{-5} M Mn(II). The insert shows the region XY expanded.

Hence metal binding cannot occur at the side chain but must rather be as in structure 1.3(b). The measured internuclear distances for this structure are given in Table 2.4, along with the experimentally determined internuclear distances. The thiazolidine ring may adopt two different conformations (the [B] conformation is shown in Fig. 2.13), and the internuclear distances for each is given in Table 2.4. The experimental data, which represents the time average of these two conformations, does not show a preference for either. Similarly the time average conformation of the side chain is determined to be as in Fig. 2.13.

TABLE 2.4

Comparison of measured and experimentally determined proton-metal distances for benzylpenicillin and 6APA, Mn(II) and Cu(II) complexes.

Proton	Measured distances (A^o) for two conformations[b] [A]	[B]	Experimental[a] distances (A^o) for Benzylpenicillin-Mn(II) / Cu(II)	Experimental[a] distances (A^o) for 6APA-Mn(II)
3-H	3.2	3.6	3.4	3.4
5-H	2.8	2.8	3.0	3.0
6-H	4.1	4.1	4.1	4.3
α-CH_3	4.5	2.7	3.5	3.9
β-CH_3	5.0	4.5	4.3	4.7
CH_2	-	-	5.8	-

(a) Relative to 3-H. (b) Conformation [A] has α-CH_3 equatorial.

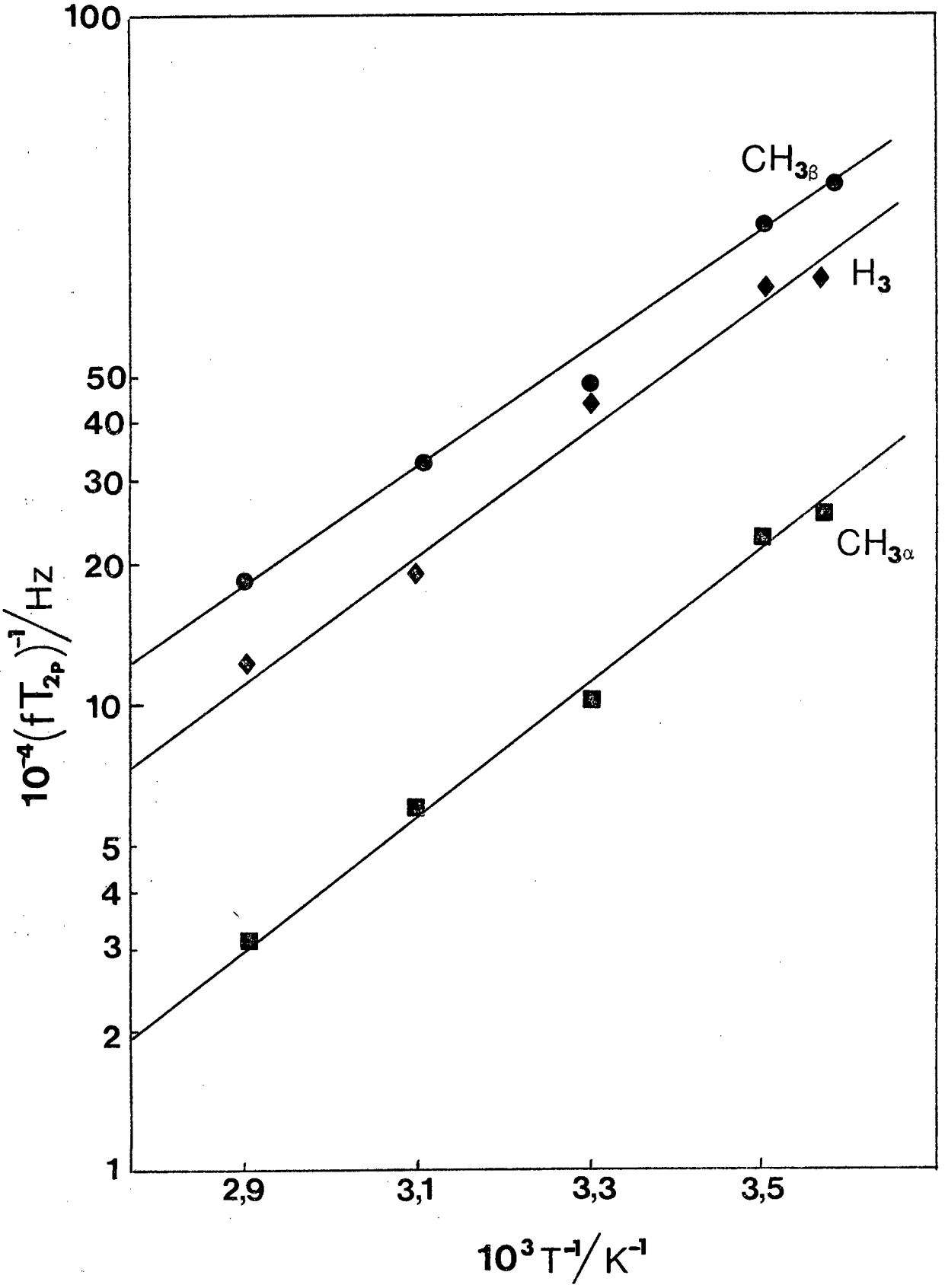


FIG. 2.10

Temperature dependence of f T_{2p} for benzylpenicillin - Mn(II), pH 5.5.

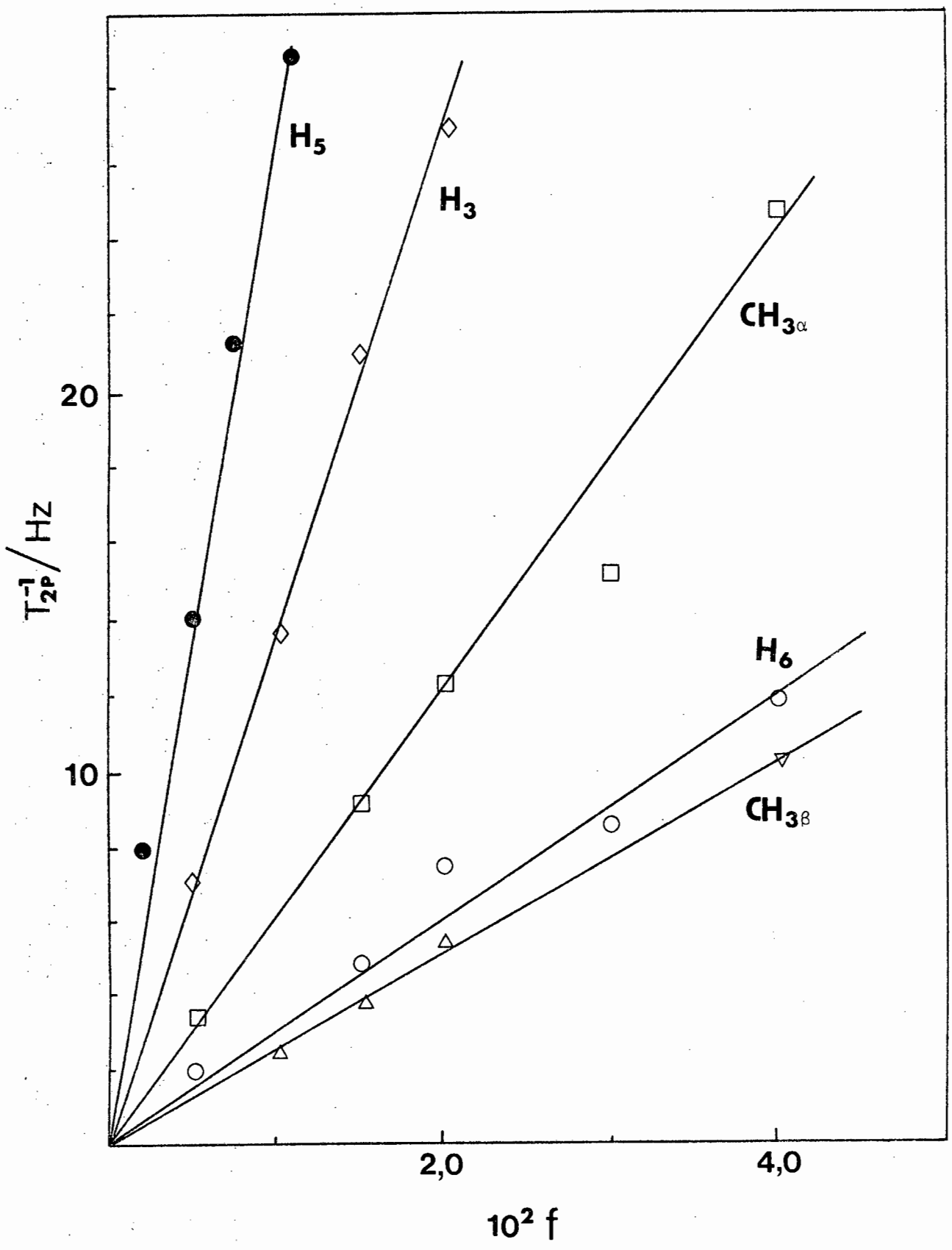


FIG. 2.11

Measured values of T_{2p} for benzylpenicillin - Mn(II) as a function of f, pH 5.5, 23°C.

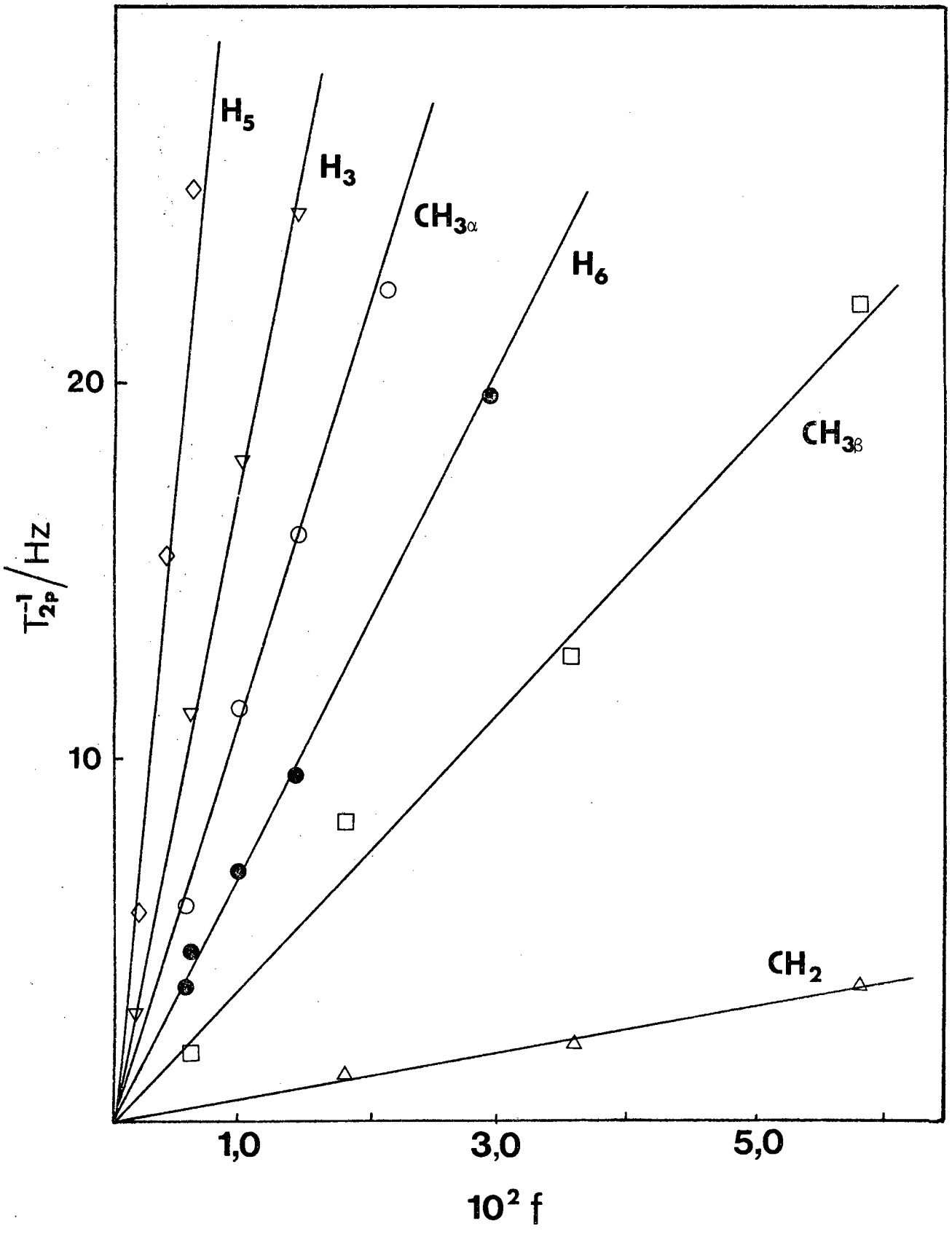


FIG. 2.12

Measured values of T_{2p} for benzylpenicillin - Cu(II) as a function of f, pH 5.5, 23°C.

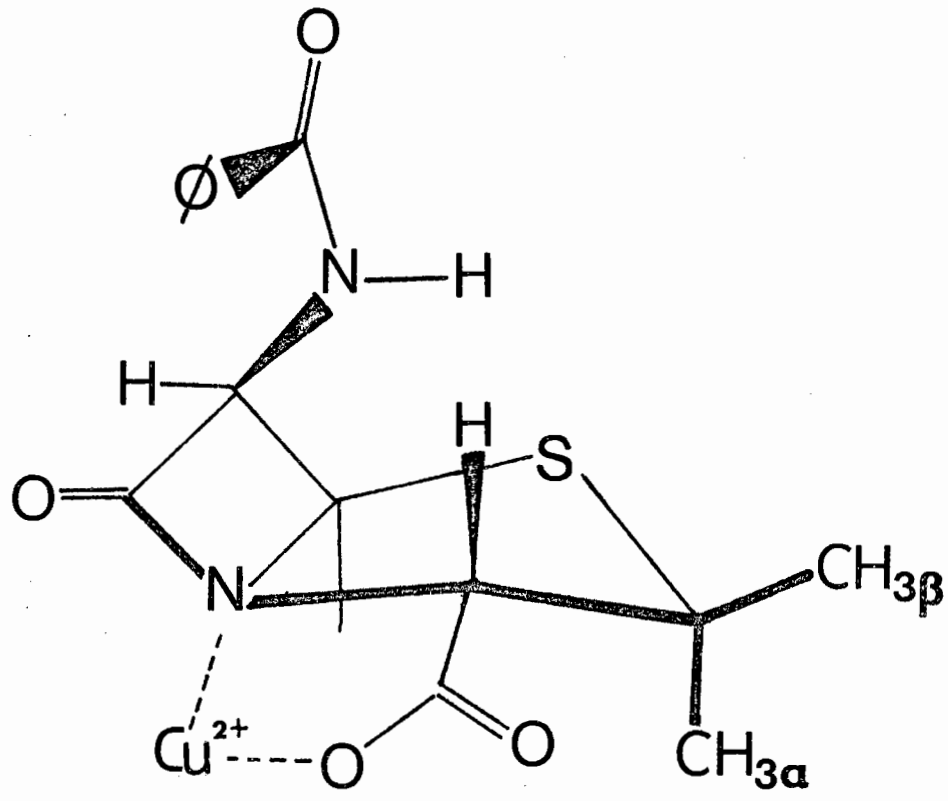


FIG. 2.13

Schematic drawing of the Cu(II)-benzylpenicillin complex in solution, showing the B conformation of the thiazolidine ring and the orientation of the side-chain.

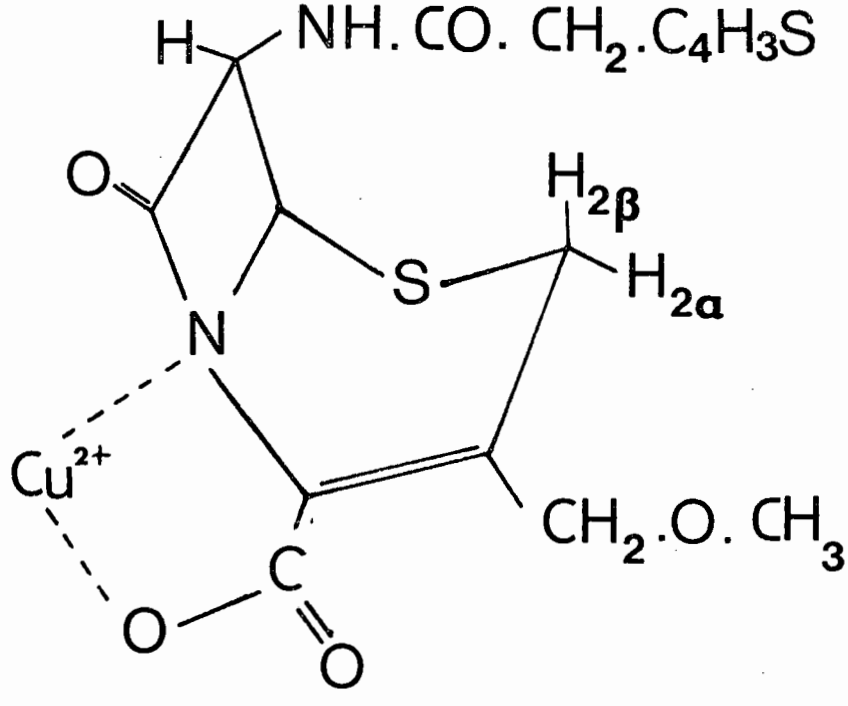


FIG. 2.14

Schematic drawing of the Cu(II)-cephalothin complex in solution, showing the B conformation of the dihydrothiazine ring.

A surprising result, in view of the fact that Cu(II) promotes the hydrolysis of penicillins, is the similarity between the results obtained using this metal-ion and Mn(II). Certainly the site and overall conformation of these two complexes are the same. Also, from the broadening of the side chain methylene protons, an upper limit of 0,01% can be put on coordination involving the side chain. This of course cannot preclude structure 1.3(a) from being the one responsible for the 'catalytic' hydrolysis of the penicillins.[13]

Qualitatively it can be seen from Fig. 2.9(b) that the higher field methyl group resonance broadens faster than the lower field one. Also from Table 2.4 it can also be seen that $CH_{3\alpha}$ is closer to the metal-ion than $CH_{3\beta}$. Hence the high field methyl resonance can be assigned to $CH_{3\alpha}$. This assignment is the same as that obtained using the nuclear Overhauser effect.[42]

As with thiaproline, an estimate of the rotational correlation time may be obtained using Stokes' Law. Using this and the slope of the graph, f vs. $1/T_{2p}$ (Fig. 2.11), an estimate of the metal-ion proton distance may be obtained. Using a value of $8.5A^{o}$ for the effective radius of the complex, values of $\tau_r = 6 \times 10^{-10}$ sec. and $r(M - H_3) = 5\ A^{o}$ are obtained. In view of the errors involved in the calculation the agreement between this value and the expected one of $3.4\ A^{o}$ is again satisfactory.

To investigate the effect of the side chain upon complex formation the Mn(II) - 6APA system was studied. The results of the metal broadening

which are given in Table 2.4 are consistent with binding at the thiazolidine ring. This is perhaps a little surprising in view of the work done on metal binding to peptides; where it was the terminal amino group which was found to be the most favourable coordination site. The deviation from this type of behaviour which 6APA shows may be significant to the hydrolysis of penicillins.

2.3.4 Cephalothin / Cu(II)

Much less is known about the interaction of cephalosporin antibiotics and metal-ions but because of their obvious similarity to the penicillins it was decided to compare the two systems. To do this cephalothin, one of the more common cephalosporins, was chosen.

The n.m.r. spectrum of cephalothin has previously been determined[43]. That the Cu(II) complex undergoes rapid chemical exchange at 23°C was shown by a variable temperature study. During the metal-ion titration, as with benzylpenicillin, the side chain (at C7) methylene is broadened very slowly. H_6 broadens fastest followed by H_7 indicating the same type of complex formation as with benzylpenicillin. The dihydrothiazine ring may exist in two conformations as shown in Fig.2.14, and the calculated internuclear distances for these two are given in Table 2.5. The experimental data shows an error of 10% which is quite high, but using any other site of coordination no meaningful fit of the data could be found. The dihydrothiazine ring must be flipping rapidly between the two conformations with an approximately equal lifetime for each.

It was possible to determine the time average conformation of the substituent of C_3 but since the energy barrier to rotation of this group is expected to be small, the information is not very meaningful.

Cephalothin provides another example in the use of paramagnetic relaxation agents in the assignment of n.m.r. spectra. The protons $H_{2\alpha}$ and $H_{2\beta}$ are not magnetically equivalent and give rise to an AB pattern. From the broadening of these peaks it is possible to assign the low field

resonances to $H_{2\alpha}$ and the high field ones to $H_{2\beta}$. This assignment is the same as that obtained using the nuclear Overhauser effect.[43]

TABLE 2.5

Comparison of measured and experimentally determined proton-metal distances for Cephalothin - Cu(II)

Proton	Measured distances (A^o) for two conformations[b] [A]	[B]	Experimental Distances[a] (A^o)
H_7	4.0	4.0	4.0
H_6	3.4	3.4	3.4
$H_{2\alpha}$	4.3	5.1	4.7
$H_{2\beta}$	5.4	5.1	5.2
CH_3	-	-	6.5

(a) Relative to H_6 (b) Conformation [A] has $H_{2\alpha}$ axial.

It was hoped that the effect of the side chain substituent might again be studied by observing the analogue of 6APA, 7-amino-cephalosporanic acid (7ACA), but the n.m.r. spectrum was not sufficiently well resolved, in aqueous solution, for meaningful results to be obtained.

2.3.5 Ampicillin and Cephalexin / Cu(II).

The two antibiotics ampicillin and cephalexin are interesting in that they have the same side chain which has a free amino group. The complex formed between Cu(II) and ampicillin is clearly of a different nature to that with benzylpenicillin as it is the side chain methine that is preferentially broadened. This proton was completely broadened after the addition of only 0.6μℓ of the Cu(II) stock solution while the other protons required the addition of ~50μℓ of this solution before they were appreciably broadened. Also H_6 broadens faster than H_5. Complexation then must be occurring at the side chain rather than on the ring.

Upon coordination at N_{10}, two five-membered chelate structures are possible by binding to N_8 or O_9. For both of these the metal-ion-H_{10} distance is ~3,7A°. Relative to this we observe a metal-ion-H_6 distance of ~5,6A°. If coordination occurs through N_{10} and N_9 then there is no possible conformation of the molecule in which the Cu-H_6 distance is greater than 4,4A°. If this were the case it would mean an error of 120% in the relative slopes, which is unacceptable. With coordination through N_{10} and O_9, however the observed position of H_6 can be accommodated. Hence it is this structure of the complex which is postulated to exist in solution.

The internuclear metal-proton distance to H_5 and H_6 is unaffected by flipping of the thiazolidine ring and hence using these protons it is possible to fix the time average conformation of the ring system in the complex (Fig.2.15). The measured and experimentally determined metal-proton

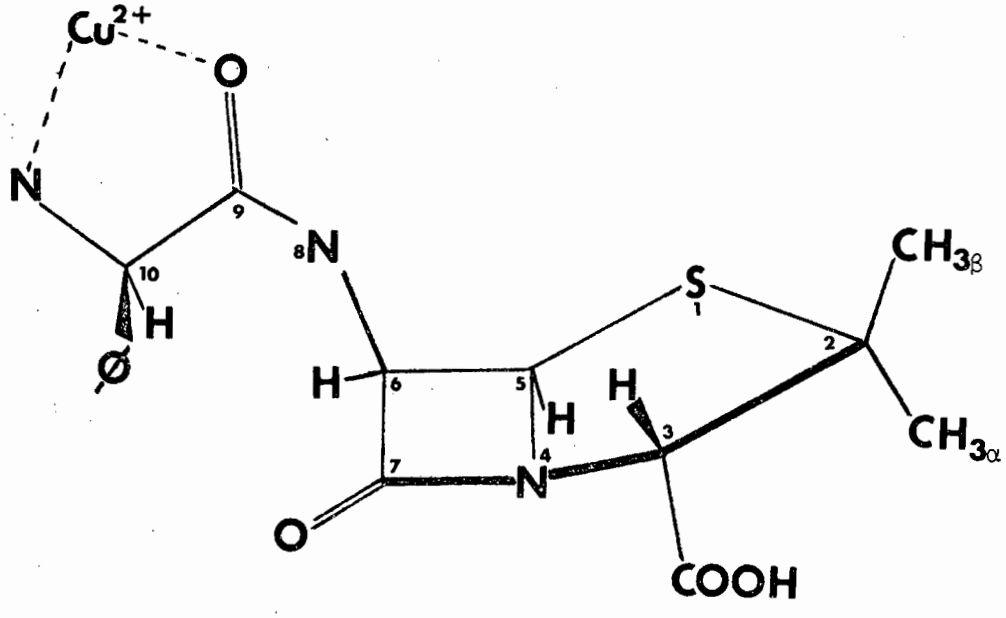


FIG. 2.15

Diagram showing the structure and conformation of the Cu(II)-ampicillin complex in solution. The thiazolidine ring is in the B conformation.

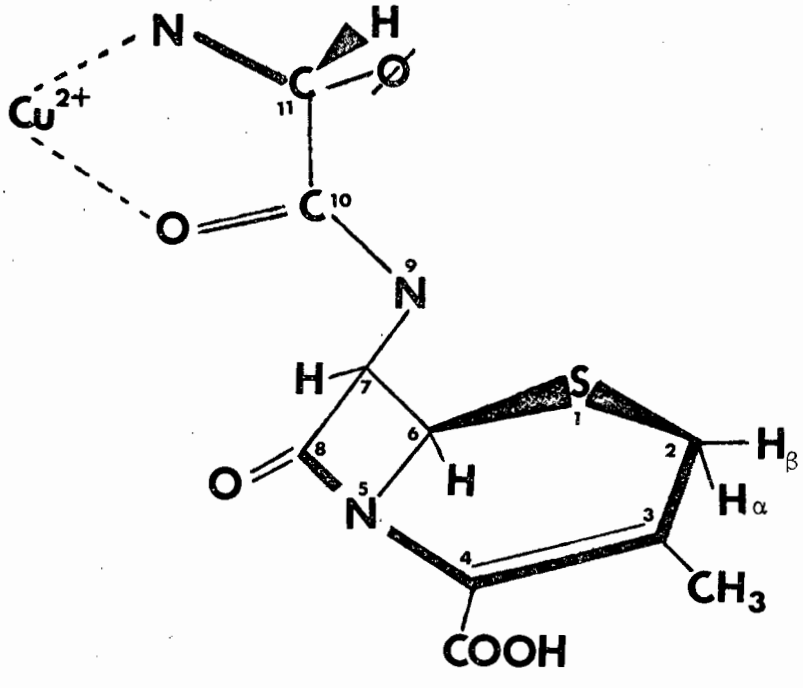


FIG. 2.16

Diagram showing the structure and conformation of the Cu(II)-cephalexin complex in solution. The dihydrothiazine ring is in the A conformation

distances for this conformation are given in Table 2.6. No preference for either of the conformations [A] or [B], can be established from our data.

TABLE 2.6

Comparison of measured and experimentally determined proton-metal distances for Ampicillin - Cu(II).

Proton	Measured distances (A^o) for two conformations[b] [A]	[B]	Experimental Distances[a] (A^o)
H_5	6.6	6.6	6.6
H_6	5.3	5.3	5.4
H_3	8.4	8.4	8.9
$CH_{3\alpha}$	8.2	8.8	8.6
$CH_{3\beta}$	6.1	8.1	7.9
H_{10}	3.4	3.4	3.1

(a) Relative to H_5. (b) Conformation [A] has $CH_{3\alpha}$ equatorial.

For ATP and metal ATP complexes it has been shown by x-ray studies[44] and M.O. calculations[45] that a folded structure has a higher stability than an extended one. It might have been possible that weak axial coordination could occur through the carboxylate group but this is prevented by steric considerations, in particular from the benzene ring.

Hence the open structure of Fig.2.15 is observed, very similar to that found in the crystal.[46]

Simlarly the interaction of Cu(II) with cephalexin was studied. By the same reasoning as for the Cu(II) ampicillin complex coordination through N_{11} and the peptide oxygen is proposed. An open structure is again observed though with a different orientation to that found for ampicillin (Fig.2.16). The measured and experimentally determined internuclear distances for this structure are given in Table 2.7.

TABLE 2.7

Comparison of measured and experimentally determined metal-proton distances for Cephalexin - Cu(II).

Proton	Measured distances (A^o) for two conformations[b] [A]	[B]	Experimental Distances[a](A^o)	Calculated Distance for 84%[A] + 16%[B]
H_{11}	3.7	3.7	3.7	3.7
H_6	7.2	7.2	7.2	7.2
H_7	5.4	5.4	5.6	5.6
$H_{2\beta}$	8.3	6.4	7.9	8.0
$H_{2\alpha}$	8.3	4.8	7.8	7.8
CH_3	8.6	7.8	8.3	8.4

(a) Relative to H_6. (b) Conformation [A] has $H_{2\alpha}$ axial.

The above determination was carried out at pH 8,5. At pH 5,5 the solubility of cephalexin is low and it was difficult to obtain quantitative data on the line broadening. However, it was observed that the side chain methine (H_{11}) was no longer preferentially broadened with respect to H_7. The pKa of the N_{11} amino group is 7,3 and so at pH 5,5 the metal-ion will have to compete with protons for this coordination site. The possibility is then, that as the pH of the solution is decreased the site of coordination changes from the side chain to the carboxylate group of the dihydrothiazine ring. The pKa of the carboxyl group is 5,3.[43] This type of behaviour follows that of peptides which in acid solution are coordinated through the terminal carboxylate group while in alkaline solution, through the terminal amino group.

2.4 General Discussion.

In general several interesting points arise from the n.m.r. study of the interaction of metal-ions and antibiotics. Firstly, the site of metal coordination to benzylpenicillin is seen to be at the carboxylate group and not between the β-lactam carbonyl and side chain amide nitrogen as proposed by Cressman.[13] Also this interaction is chelating and not merely an interaction between the carboxylate group and the metal-ion. A surprising result is the similarity between the Mn(II) and Cu(II), benzylpenicillin interaction since the Cu(II)ion 'catalysis' the hydrolysis of penicillins while Mn(II) does not.

The effect of the side chain can be seen from the study of 6APA. Here there is a free amino group which is expected to be the favourable site of metal coordination. For some reason, however, Mn(II) does not coordinate through this group but rather through the carboxate group as in benzylpenicillin. If this then, is the preferred site of coordination to 6APA, certainly it will be even more so in benzylpenicillin where the free amino group has been acylated. The reason for this anomalous coordination may be that chelation to the amine and ring carbonyl does not produce a planar ring which is shown by all other peptide complexes.[47]

Lastly, the study of cephalexin and ampicillin shows that, in the higher pH region at least, coordination is to the free amino group, and peptide oxygen. However, the normal mode of coordination of Cu(II) to peptides, in this pH region, is via the amino group and the deprotonated amide nitrogen and not the amide oxygen.[47] Also only in acid solution does coordination to peptides switch from the terminal amino group to

the terminal carboxylate group.[47] Since this has already occurred to an appreciable extent at pH 5,5 with cephalexin, there must be some form of stabilisation of the carboxylate complex. With peptides, chelation at the terminal carboxyl group can only occur with coordination to the adjacent deprotonated amide nitrogen. Deprotonation of this amide nitrogen only occurs at higher pH's. However, the penicillin and cephalosporin ring amide, being strained, does not exhibit the normal amide resonance and so coordination to this nitrogen does not result in a large decrease in the amide resonance stabilisation energy.

These general observations on the binding of metal-ions to the penicillin and cephalosporin antibiotics will be discussed more fully at a later stage when the results obtained using several other techniques have been discussed.

2.5 Experimental.

Pyrrole-2-carboxylic acid, thiaproline, sodium benzylpenicillin, cephalothin and cephalexin were all commercial products. 6APA and 7ACA were kindly donated by E.H. Flynn of Eli Lilly and Company and ampicillin by M.J. Soulal of Beecham Research laboratories. These were used without further purification except for freeze drying to reduce the water resonance.

Analar $Mn(ClO_4)_2 \cdot 6H_2O$ (8.10^{-3}M) and $Cu(ClO_4)_2 \cdot 6H_2O$ (4.10^{-2}M) solutions were prepared in D_2O and adjusted to pH 5.5 (pD 5.9) with NaOD or $DClO_4$. These solutions were used as the metal-ion source.

The metal-ion titrations were carried out on 0.1M solutions of the ligand in D_2O, small volumes of the metal-ion being added using a micro-syringe. In the benzylpenicillin - Cu(II) experiments the solutions were freshly prepared to minimise hydrolysis.

Spectra were recorded on a Varian XL-100 spectrometer at a probe temperature of 23°c. Care was taken to avoid saturation effects and samples were kept for 10 minutes to attain thermal equilibrium before spectra were recorded. In the case of the ampicillin/Cu(II) experiment a probe temperature of 50°c was used in order to shift the water resonance out of the way. Also because of solubility, the ampicillin and cephalexin experiments, were carried out of pH 8.5.

The transverse relaxation times, T_2s, were estimated from the line widths ($\Delta\nu$) at half peak light.

$$T_2^{-1} = \pi\Delta\nu$$

The analysis of the AB quartet of protons 5 and 6 of benzylpenicillin and 6APA was carried out by a least squares computer simulation of the observed curves. This was done on a Univac 1106 computer using a Fortran V program written especially for this purpose.

For methyl groups the calculated internuclear distance is

$$\frac{1}{r_{ave}^{6}} = \frac{1}{3}\left(\frac{1}{r_1^{6}} + \frac{1}{r_2^{6}} + \frac{1}{r_3^{6}}\right)$$

CHAPTER 3

POTENTIOMETRIC STUDY

3.1 Introduction.

In the study of metal-ligand interactions, it is of value to have some measure of the strength of the interaction. This is best provided by calorimetric determinations of ΔH^{o}, but stability constants may also be useful if treated with caution as indicated later. In recent years a large amount of work has been done in this field and several extensive compilations of the literature data exist.[48] A consideration of the available results allow certain broad generalisations as to the factors affecting the stability of a complex to be made. These are useful, especially when coupled with calorimetric measurements,in predicting both site and strength of coordination of unknown systems.

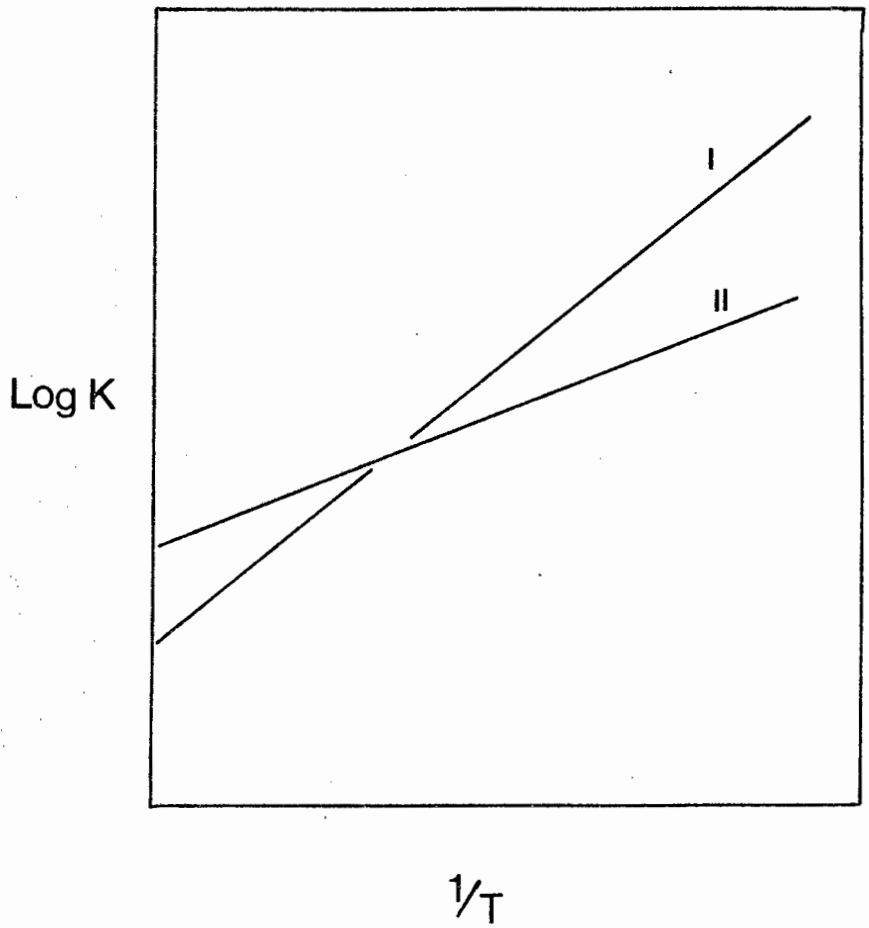
It is possible, by comparison with model systems, to draw certain inferences as to the site of coordination of the ligand being studied, the assumption being that if two ligands have similar donor groups and form complexes of similar stability, then the site of binding is the same. Lindskog and Nyman[49] were able to propose, by comparison with model ethylenediamine complexes, that the probable binding of zinc to carbonic anhydrase was through three nitrogen bases. Dennard and Williams[50], in a review article, cite a number of similar examples in the use of stability constants. Care must be taken, however, with this type of prediction since it may lead to the wrong conclusion.[51]

Fig. 3.1 shows the temperature dependence of log K. As shown here, in certain cases the graphs for two ligands may cross over, resulting in different interpretations of log K depending on the temperature. This

occurs when ΔH^{o} and ΔS^{o} oppose each other and so ΔG^{o} does not exhibit the expected trend. More correctly trends in ΔH^{o} and ΔS^{o} should be compared when assigning structure to a complex.

FIG. 3.1

HYPOTHETICAL TEMPERATURE DEPENDENCE OF LOG K FOR TWO LIGANDS.



One of the most successful attempts at rationalizing stability constant data is that of Hard and Soft Acids and Bases (HSAB).[52] This concept has many useful applications but the one most important here is that it helps predict which metal-ion will be found associated with which ligands or donor atoms. For example Na^+ and K^+ are hard acids, and since hard acids bind most strongly to hard bases, they will be strongly solvated in solution (H_2O is hard). Unfortunately, the metals of interest here Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) are all boarderline in that they can act as hard or soft acids. However, certain generalisations are possible as to the donor atoms preferred by these metal-ions. Hence Cu(II) prefers amines >> carboxylates, Zn(II) prefers -SH (cysteine) and imidazole, Ni(II) and Co(II) prefer amines and carboxylates and Mn(II) prefers carboxylates. This is illustrated by the coordination of transition metal-ions to nucleotides. The harder metals such as Mn(II) coordinate only through the phosphate oxygens while the relatively soft Cu(II) coordinates to both the phosphate and the nitrogen of the purine or pyrimidine base.[53]

While HSAB provides a good qualitative approach to bonding, quantitatively it is not as successful. The factors affecting the stability of the metal-ion complexes can, however, be divided into two groups, those depending on the nature of the metal-ion and those depending on the ligand. From an electrostatic approach to bonding the bond strength is given by:[54]

$$E = e^2 Z_M Z_L / r$$

The smaller the metal-ion or the more highly charged it is the stronger the metal ligand interaction. Thus Mg(II) should form stronger

bonds than Ca(II).

The inadequacies of the electrostatic approach are obvious, in that it does not take into account entropy solvation effects and any covalent bonding. Only for the very electropositive metal-ions and electronegative ligand donor atoms, where covalent bonding is small, does it account for certain of the observed trends.

It has been known for many years that the usual order of stability of the first row transition metal complexes is:

$$Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II)$$

This general trend, known as the Irving-Williams[55] order of stability, is followed by the vast majority of ligands. If a particular ligand, be it a metalloenzyme or not, deviates from this order it must be accounted for in the mechanistic scheme. For example K_1 for Cu(II) EDTA is only slightly greater than for Ni(II).[56] This is because Cu(II) is unable to coordinate all six ligand donors strongly while Ni(II) can.

The Irving-Williams stability order is in fact a manifestation of the crystal field stabilization energy (CFSE).[57] This is a gain in stability brought about by the electronic configuration of the metal and the nature of the ligand. Amine donor groups give rise to a large splitting of the eg and t_{2g} metal d-orbitals and so the order of stabilities is determined largely by the enthalpy ΔH of reaction. Carboxylate donors, on the other hand do not give rise to such large crystal field splitting and are in fact slightly endothermic. The driving force for the reaction is the entropy change ΔS. The negatively charged carboxylate group is highly

solvated in solution and in coordination these water molecules are released, resulting in a positive entropy change.

Because of the different behaviour of these two types of donors it is often valuable to prepare a series of metal complexes with varying transition metal-ion. An inspection of the enthalpies and entropies of reaction for each of the metal-ions may allow certain conclusions as to the donor atoms to be made.

One of the influences that the nature of the ligand has on the stability of metal complexes has already been illustrated, i.e. their effect on the CFSE. As with the metal-ions other correlations exist between the nature of the ligand and the stability of the complex. One of the most successful correlations is that between the basicity (pKa) of the ligand and the stability of its complex. The reasoning is simply that the stronger a ligand binds a proton i.e. the more basic it is, the stronger it will bind a metal-ion. Such a correlation is shown for the reaction between Cu(II) and several carboxylic acids in Fig. 3.2.[58]

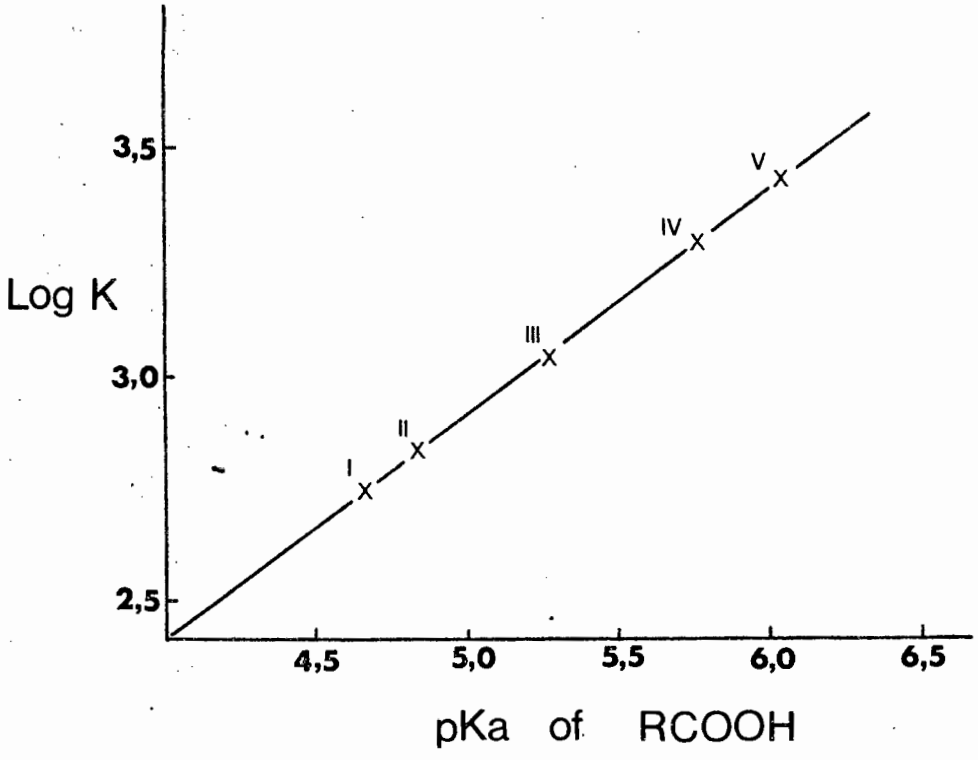
No correlation exists between the ligand pKa and log K for complexes where the ligands have different donor atoms. This is not surprising in view of the previous discussion of the preferences metals show for different donor atoms.

Such plots of pKa vs. log K have their uses (see discussion) in establishing the coordination of additional groups of the ligand. HO CH_2COO^- and $CH_3CH_2SCH_2COO^-$ both form much more stable complexes with Cu(II) than their pKa's would suggest, using the above correlation.[59]

This has been explained in terms of further coordination of the hydroxyl and sulphur atoms. Mn(II) and Zn(II), being harder metals do not coordinate the sulphur atom and so do not have this anomalous stability.

FIG. 3.2

CORRELATION BETWEEN LOG K AND pK FOR A SERIES OF CARBOXYLIC ACIDS: WHERE R = I $p\text{-}NO_2C_6H_4^-$, II $m\text{-}ClC_6H_4^-$, III $C_6H_5^-$, IV CH_3^-, V $p\text{-}CH_3C_6H_4^-$[58].



Experimentally it has been observed that chelating ligands form more stable complexes than comparable monodentate ligands.[60] Thus for ethylenediamine (en) and ammonia we have:[47]

$$Ni(NH_3)_2^{2+} + en \rightleftharpoons Ni(en)^{2+} + 2NH_3$$

$$\log K = 2.5$$

It is only valid to compare ligands whose donor groups form complexes of similar stabilities. Ethylenediamine and ammonia have similar pKa values and so from the previous discussion should form complexes of similar stability.

The existance of this chelate effect can be rationalized in terms of the entropy change accompanying the reaction, three products being formed from two reactants. The magnitude of ΔS^{o} however, does depend on the standard state used in its evaluation.[61]

The chelate effect can also be rationalized from a statistical view point. If the bidentate and monodentate ligands are comparable the probabilities of forming the complexes M-A-A and M-B are the same. A-A and B represent the bidentate and monodentate ligands respectively. The probability of coordination of the second donor group, however, is much less for the monodentate ligand than the bidentate one, since the second donor atom of the bidentate ligand is held in close proximity to the metal. This can be seen from the reaction kinetics.

$$M - A\text{-}A \underset{k\text{-}1}{\overset{k_1}{\rightleftharpoons}} M\langle^{A}_{A}) \quad .... \ 1$$

$$MB + B \underset{k\text{-}1}{\overset{k_1}{\rightleftharpoons}} MB_2 \quad .... \ 2$$

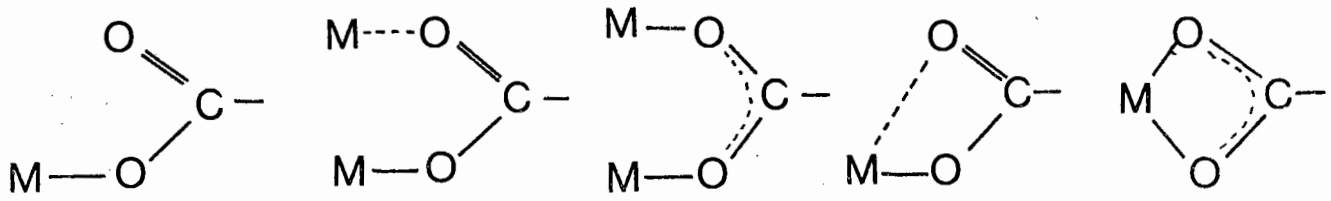
In reaction 1 and 2, if there is no ring strain, since the ligands have comparable donor groups k_{-1} will be the same for both reactions. k_1 for the first reaction however, will be much greater than k_1 for the second

reaction. The equilibrium constant $K = {}^{k_1}/_{k_{-1}}$ will therefore be greater for the chelate complex. This also accounts for the difference in stability as the ring size changes, ring closure being faster for five-membered rings than six-membered rings etc. Rings smaller than five-membered are strained and so k_{-1} is greater.

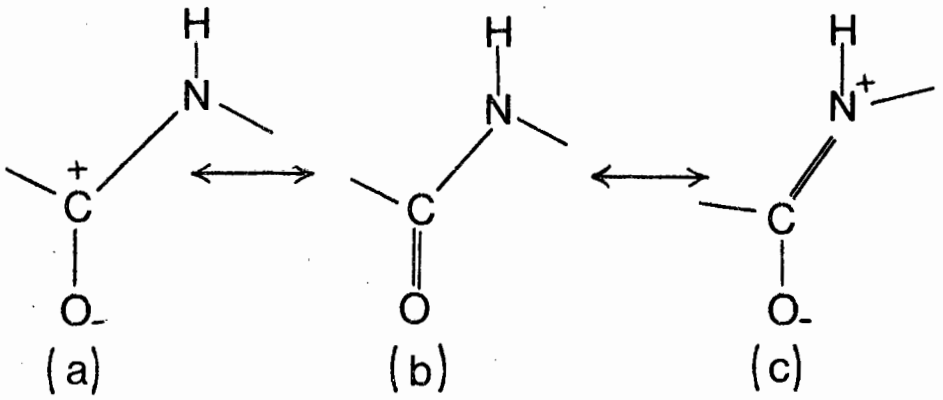
Since penicillin can be regarded as a substituted dipeptide it is of interest to look at the metal complexes of these compounds. The main difference between peptides and penicillins is the lack of a terminal amino group. Since this is the most favoured coordination site of metals to peptides, certain differences in the stability of the two ligands must be expected. However, valuable information as to the binding of metals to the peptide nitrogen and oxygen, and the terminal carboxyl group can still be obtained.[62]

The terminal carboxyl groups of peptides are usually found co-ordinated to a metal-ion only when they are able to complete a chelate ring. This they are normally able to do in α and β amino acids but not in peptides. The carboxyl group of peptides are only really favourably orientated for chelation if the terminal residue is histidine.[63,64] Otherwise the metal carboxyl interaction is either non-chelating or absent. The hard metal-ions such as Ag(I) have the highest probability of forming such complexes.[65]

There are several types of metal carboxyl interactions which have been found using n.m.r. and crystalography. These are shown below:[62]



Similarly the metal peptide oxygen interactions are usually chelating - very few non-chelating interactions being known. Since metal binding is normally to the terminal amino group a five-membered chelate ring can be formed between this and the adjacent oxygen or nitrogen of the peptide group. This peptide group must remain planar in order to preserve the amide resonance.



Coordination involving the peptide oxygen would tend to stabilise the (a) and (c) canonical forms. The peptide nitrogen can only coordinate if the peptide proton dissociates,[66] otherwise coordination would result in a change of the nitrogen hybridisation from SP^2 to SP^3, with loss of planarity and resonance stabilisation. Thus under conditions where the peptide hydrogen is not labilised chelation is completed by coordination to the peptide oxygen. Many examples of this exist.[62]

Examples where the peptide proton has been labilised resulting in coordination to the peptide nitrogen rather than the oxygen are limited to metals which can have a large CFSE.[62] The resulting CFSE stabilisation on binding to a nitrogen rather than an oxygen is the driving force for the reaction.

The peptide group cannot be regarded as acidic since in the pH range 1-14 its proton is not lost. Upon metal coordination, however, this proton is far more labile. The pKa for the Cu(II) and Ni(II) complexes of triglycine are ~ 6 and 8 respectively.[67,68] This reflects the difference in stability of the Cu(II) and Ni(II) complexes.

Coordination of the peptide nitrogen stabilises the (c) canonical form since the electron shift to the metal-ion is smaller than that to the proton. This can be seen from the change in bond lengths of the C = O and C-N bonds.[62] C = O bond length changes from 1.24A^{O} to 1.26A^{O} and the C-N bond length from 1.33A^{O} to 1.30A^{O} upon coordination to Cu(II).

These preliminary observations of the metal bonding of peptides can be useful in predicting the structure of the metal penicillin complexes. This will be discussed more fully in the discussion of the metal penicillin interactions.

At the start of this investigation, there were several questions which we hoped to answer:

(i) What is the strength of the metal penicillin interaction?

(ii) What is the structure of the complex formed and

(iii) How does complexation affect the stability of the penicillin?

By determining the stability constants the first question is easily answered. Using model compounds some insight into the second problem can be gained from potentiometry. Correlation of the results gained using a variety of techniques may lead to an answer to the final question.

3.2 Theory.

The general theory of formation constants, and the potentiometric determination thereof, is dealt with in the literature.[69,70] Only a broad outline, and an explanation of the notation, will therefore be given here.

The formation of an equilibrium in solution is characterised by its equilibrium constant. Where this refers to the formation of a metal complex the term formation or stability constant is usually used. The stepwise formation of a complex can be described by a set of equilibrium constants.

$$M + L \rightleftharpoons ML \qquad K_1 = [ML]/[M][L]$$

$$ML + L \rightleftharpoons ML_2 \qquad K_2 = [ML_2]/[ML][L]$$

These are stoicheiometric stability constants.

By convention the equilibria are written as 'formations' rather than 'dissociations', the equilibrium constants then being the formation constants. This is opposite to the symbol K_a, which is the equilibrium constant for dissociation of an acid.

Strictly speaking the stability constants should be expressed in terms of activities rather than concentrations. It is possible to measure the stability constants over a wide range of ionic strengths, enabling an extrapolation to zero ionic strength where the activities will be represented

by the concentrations. It is more common, however, to carry out measurements at constant ionic strength, using a support electrolyte. Comparisons between systems at the same ionic strength are then meaningful.

The above set of equilibria can also be represented by a set of overall or cumulative stability constants, denoted by β.

$$M + L \rightleftharpoons ML \qquad \beta_1 = K_1 = [ML]/[M][L]$$

$$M + 2L \rightleftharpoons ML_2 \qquad \beta_2 = K_1K_2 = [ML_2]/[M][L]^2$$

In general we can write for β_n.

$$\beta_n = \prod_{i=1}^{n} K_i$$

More specifically account can be taken of the possibility of the formation of protonated, hydroxo or oligonuclear complexes by the subscripts of β. The order of these subscripts varies from author to author. In the present study the order used is ligand, metal, proton e.g. the formation constant for the species $L_pM_qH_r$, is given by:

$$\beta_{pqr} = \frac{[L_pM_qH_r]}{[L]^p[M]^q[H]^r} \qquad \ldots\ldots\ldots (3.1)$$

When r = -1 this refers to a proton removed to a water molecule or to a hydroxide ligand added.

At any given point in the titration the total concentration of a

component, T_L say, is given by the sum of the concentrations of the individual species, including the free component concentration:

$$T_L = [L] + [ML] + 2[ML_2] + \ldots\ldots$$

$$= [L] + \sum_{r=0}^{R} \sum_{q=1}^{Q} \sum_{p=1}^{P} p[L]^p [M]^q [H]^r \beta_{pqr} \quad \ldots\ldots\ldots (3.2)$$

In a potentiometric study of these equilibra, using a hydrogen responsive electrode, it is the free hydrogen ion concentration which is known at each point in the titration. This is given by equation 3.3 where the term F_N replaces 2.303/RT in the normal Nernstian equation. This takes into account any deviation from Nernstian behaviour.

$$E = E_o + F_N \log [H^+] \quad \ldots\ldots\ldots (3.3)$$

Since the total component concentrations are known from the analytical concentrations of the solutions used, it is the β's and free metal and ligand concentrations which are unknown. If there are n_p titration points and $n_{M.B.E.}$ mass balance equations at each point, there will be a total of $n_p \times n_{M.B.E.}$ free concentration. Since the free hydrogen ion concentration is known at each titration point there will be $n_c = n_p(n_{M.B.E.}-1)$ unknown free concentration. If there are n unknown β's there will be a total of $n_c + n$ parameters to be determined.

In the computer program MINIQUAD,[71] which was used here, all these paramenters are considered as independent variables. With SCOGS,[72] however, only the β's are refined, the free component concentrations being

calculated from the appropriate mass balance equation (i.e. equation 3.2). The method of least-squares refinement used in these programs is unimportant, save that the function chosen for minimisation in MINIQUAD is:

$$U = \sum_{1=1}^{N} (T_i^{calc} - T_i^{obs})^2 \qquad \ldots\ldots\ldots (3.4)$$

each component being given the same weighting.

A useful concept in the interpretation of the experimental data is $\bar{z}$ (or $\bar{n}$), the average number of ligands bound to the metal.

$$\bar{z} = \frac{T_L - [L]}{T_M} = \frac{\Sigma\, i\, \beta_i [L]^i}{1 + \Sigma\, \beta_i [L]^i} \qquad \ldots\ldots\ldots (3.5)$$

This definition of $\bar{z}$ applies to the formation of mononuclear binary complexes only. A plot of $\bar{z}$ vs pL (-log [L]), called a formation curve or Z plot, gives a pictoral representation of the equilibria occurring. It is characteristic of the different species present in solution, which in turn depend on the experimental conditions. If only mononuclear binary complexes are formed in solution, $\bar{z}$ is independent of the total component concentrations. This means all the formation curves should overlap. Any deviation of $\bar{z}$ from this general pattern can be useful in predicting what additional species may be present, protonated and hydroxo species giving the formation curves a 'fan' like appearance.[73] If the major species present in solution are simple binary complexes an estimate of the formation constants can be obtained from the pL values corresponding to half integral $\bar{z}$ values[70] i.e. $^1/_2$, $^3/_2$, $^5/_2$.

Since the formation curve is characteristic of the equilibria

existing in solution, a final test of the proposed model (species present in solution) and refined β's, is to generate a set of titration data using these β's and the relevant experimental conditions, and then construct a Z plot using this data. This is termed pseudoplotting,[74] and computer programs, PSEUDOPLOT[74] and SCOUTS, exist whereby this can easily be done. The program SCOUTS has been written in these laboratories and its pseudoplotting facilities checked against PSEUDOPLOT. If the model is valid the theoretical and experimental formation curves should superimpose.

The 'goodness of fit' between experimental and calculated data may also be determined from the statistical output of MINIQUAD. This is in the form of the crystalographic R factor.[74]

$$R = \sqrt{\frac{\sum_{1}^{3N} (f_i^{calc} - f_i^{obs})^2}{\sum_{1}^{3N} (f_i^{obs})^2}}$$

Where the f_i^{calc}'s are the total component concentrations defined by equation 3.2, and N is the number of data points. It is possible to calculate, from estimates of the errors in the analytical concentrations and the rules for propargation of errors, a limiting value of R, R_{lim}. If then, $R < R_{lim}$ the fit of the model with the experimental data can be regarded as satisfactory.

As pointed out by Vacca, Sabitini and Gristina[74], it is often possible to refine a chemical model of the system which gives better agreement (lower R) than a previous model. However, the new model may not be significantly (in the statistical sense) better. They have

3.3 Results and Interpretations.

3.3.1 Thiaproline.

The stepwise protonation constants of all the ligands studied here are given in Table 3.1. The first protonation is assigned, by analogy with other amino acid systems,[75] to the secondary amine nitrogen, while the second is assigned to the carboxyl group. The weak basic character of thiaproline (log β_{101} = 6.1) as compared to other amino acids, alanine (log β_{101} = 9.9) and cysteine (log β_{101} = 8.2), is due to the inductive effect of the sulphur.[76] In cysteine the sulphur is two carbon atoms removed from the amino group and results in a lowering of the basicity by ~2pK units as compared to alanine. In thiaproline the sulphur is only one carbon away from the amino group and so the inductuve effect can be expected to be markedly greater.

In a similar fashion the sulphur substituent in thiaproline gives rise to the increased acidity of the carboxyl group. The difference in the log β's of proline (1.99) and thiaproline (1.5) is of the same order of magnitude as that found between cysteine (1.8) and alanine (2.34). The rather high standard deviation in log β for the carboxyl group protonation was a result of the non-linear response of the glass electrode below pH 2.[77] Hence titration data could not be collected below this point, and the maximum formation percentage of the LH_2^+ species in solution is low. This can be seen from the formation curve (Fig. 3.3).

The metal complex stability constants are given in Table 3.1 and the formation curves in Figs. 3.4 - 3.7. These show several interesting

TABLE 3.1

Log β for the species $L_p M_q H_r$ at 25°C and I = 0.150 M $NaClO_4$. L = Ligand, M = Metal^{2+} ion, H = H^+, σ = standard deviation in log constant, n = number of titration readings for each series, R the crystalographic R-factor.

L	M	p q r	log β	σ	n	R	χ^2
THIAPROLINATE$^-$		1 0 1	6.109	0.003	88	0.0075	140
		1 0 2	7.616	0.017			
	Cu	1 1 0	6.02	0.02	270	0.0037	27.4
		2 1 0	11.22	0.03			
		1 1 1	7.85	0.09			
	Co	1 1 0	3.025	0.0075	245	0.0013	41.9
		2 1 0	5.354	0.0054			
	Zn	1 1 0	3.103	0.006	343	0.002	20.5
		2 1 0	5.629	0.005			

....Continued/...

TABLE 3.1 - CONTINUED:

L	M	p q r	log β	σ	n	R	χ^2
	Ni	1 1 0	3.70	0.02	245	0.0075	380
		2 1 0	6.767	0.02			
		3 1 0	8.193	0.08			
		1 1 -1	-4.5	0.3			
$HIPPURATE^-$		1 0 1	3.475	0.003	191	0.0095	59
	Cu	1 1 0	2.28	0.01	265	0.0035	139
	Cu	1 1 0	1.99	0.03	265	0.003	117
		2 1 0	2.8	0.26			
$HIPPURATE^-$	Ni	1 1 0	1.25	0.026	138	0.01	604
$PENICILLINATE^-$		1 0 1	2.73	0.01	62	0.026	24.1
	Ni	1 1 0	1.74	0.01	59	0.005	83.5
PENICILLOATE		1 0 1	5.19	0.005	122	0.0097	40.2
		1 0 2	7.49	0.015			
		1 0 3	9.25	0.025			

....Continued/...

TABLE 3.1 - CONTINUED:

L	M	p q r	log β	σ	n	R	χ^2
	Cu	1 1 0	6.70	0.02	77	0.014	40.0
	Ni	1 1 0	3.07	0.02	90	0.015	137.4

features. The first point to notice is the general overlap of each set of formation curves, independent of the component concentrations. This is in striking contrast to the 'fan' like appearance of the Ni - allopurinol formation curves,[78] and indicates mononuclear stepwise complex formation.[79]

The copper formation curves Fig. 3.4 are seen to rise to a limiting value of 2. This suggests the most coordinated species is the CuL_2 complex, which for Jahn-Teller reasons is square planar, the thiaprolinate ion acting as a bidentate ligand involving the carboxyl and amino groups in coordination. This would give rise to an energetically favoured five-membered ring.

The value of R_{lim} calculated from the experimental conditions used in this study does not vary much from system to system and is taken as 0.01 throughout. Since R for the Cu(II) - thiaprolinate system is less than this the agreement between calculated and experimental data is good. If the protonated species, postulated in our model, was omitted from the calculation the R factor obtained was 0,007 which is also less than R_{lim}. That the one model was significantly better than the other was tested for by using the Hamilton test:

$$R_1/R_2 = 2.3 > R_{543,267,0\cdot05} = 1.84$$

That the one model described the experimental data better could also be seen from the value of χ^2 (chi - squared) for the two models. These values for the two models, with and without the protonated species,

are 27 and 417 respectively.

From the Z plot of the Co(II) - thiaprolinate system (Fig.3.5) it can be seen that only ML_1 and ML_2 species have been formed under the experimental conditions used. Unlike Cu(II) these curves do not level off, indicating that the lack of an ML_3 species is due, rather to the component concentration than to the structure of the complex. The limiting factor here was the solubility of the thiaproline which did not allow the use of higher ligand concentration.

Zinc (II) was found to behave very similarly to Co(II) giving similar formation curves (Fig. 3.6) and stability constants. The same type of behaviour is shown by several other amino acid systems. The Zn(II) - thiaprolinate formation curves follow those of Zn(II) - glutaminate, serinate[80] and tryptophanate[79] in being more stable than those of Co(II). The Zn(II) and Co(II) histidinate systems behave in a reverse fashion.[79]

Like the other metals, Ni(II) formed simple mononuclear binary complexes. The formation curves (Fig. 3.7) also show, however, a certain amount of forward fanning. This was found to be extremely sensitive to systematic error in the total hydrogen ion concentrations and could be eliminated by altering the hydrogen ion concentration by an amount well within its experimental uncertainty. This is in accord with Resotti and Resotti[70] who have shown that the ends of the titration curve are extremely error sensitive.

The back-bending shown by one of the formation curves and simulated in the pseudoplot is due to the hydroxo species $Ni_4(OH)_4$[81] which

was included in the model. It was possible to refine the hydroxo species ML(OH). This gave an improvement in R which was only on the boarderline of being statistically significant at the 0.05 confidence level. $R_{1/R_2} = 1.43$, $R_{494,241,005} = 1.845$. The improvement in χ^2 is more marked, being from 3650 to 380. The latter value is still high but no additional species could be found.

Overall the log β's obey the Irving-Williams stability series Mn < Co < Cu > Zn. This is to be expected as thiaproline follows closely the behaviour of other amino acids.

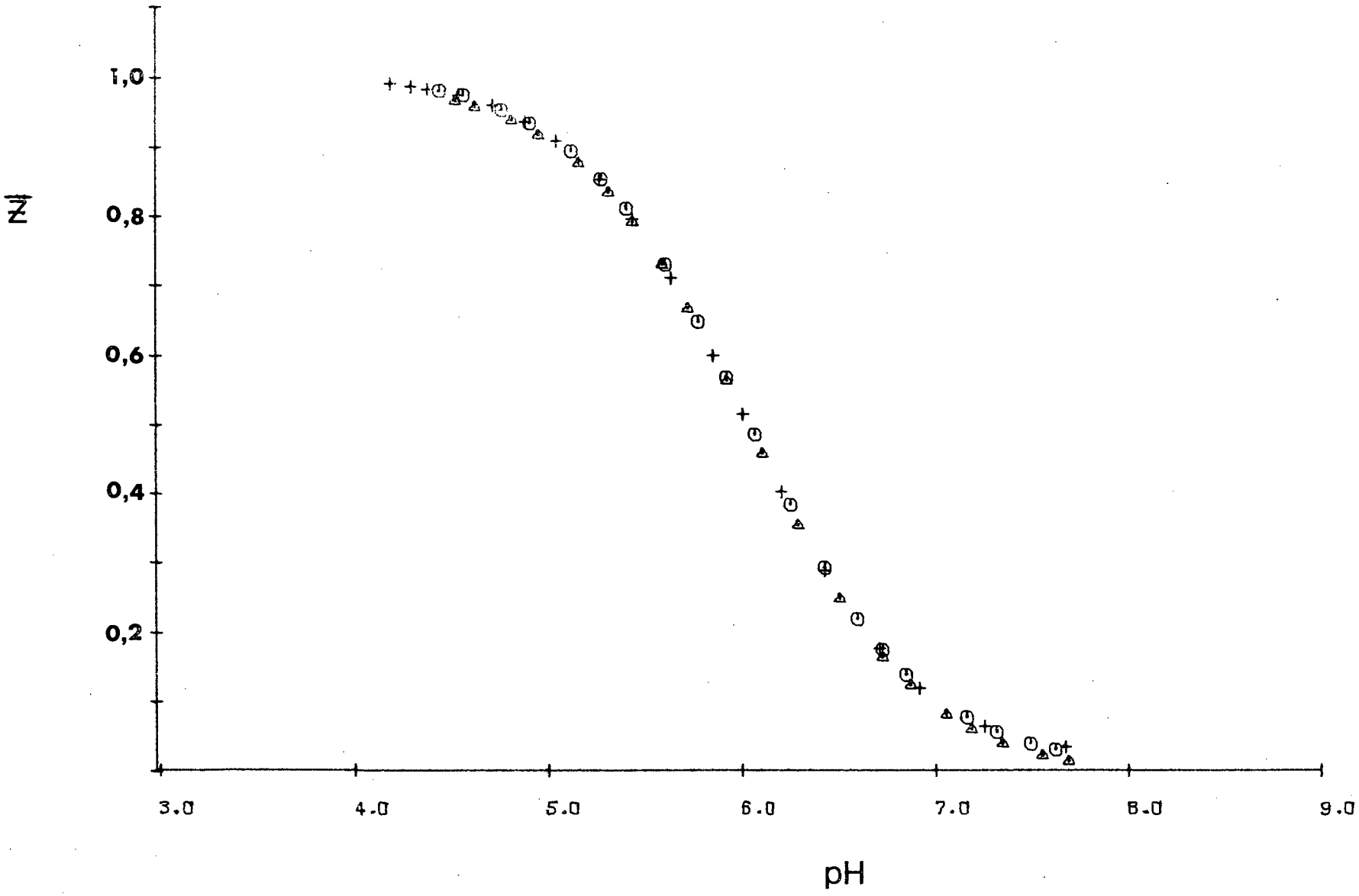


FIG. 3.3

Experimental Z plot points for the protonation of thiaproline at 25°C, μ = 150 mM ($NaClO_4$).

The symbols represent different total ligand concentrations.

FIG. 3.4

Metal complex formation curve for the Cu(II)-thiaprolinate system at 25°C and μ = 150 mM($NaClO_4$). The symbols represent different combinations of total ligand and total metal concentrations. The full line represents a pseudoplot of the "best" β values for the system.

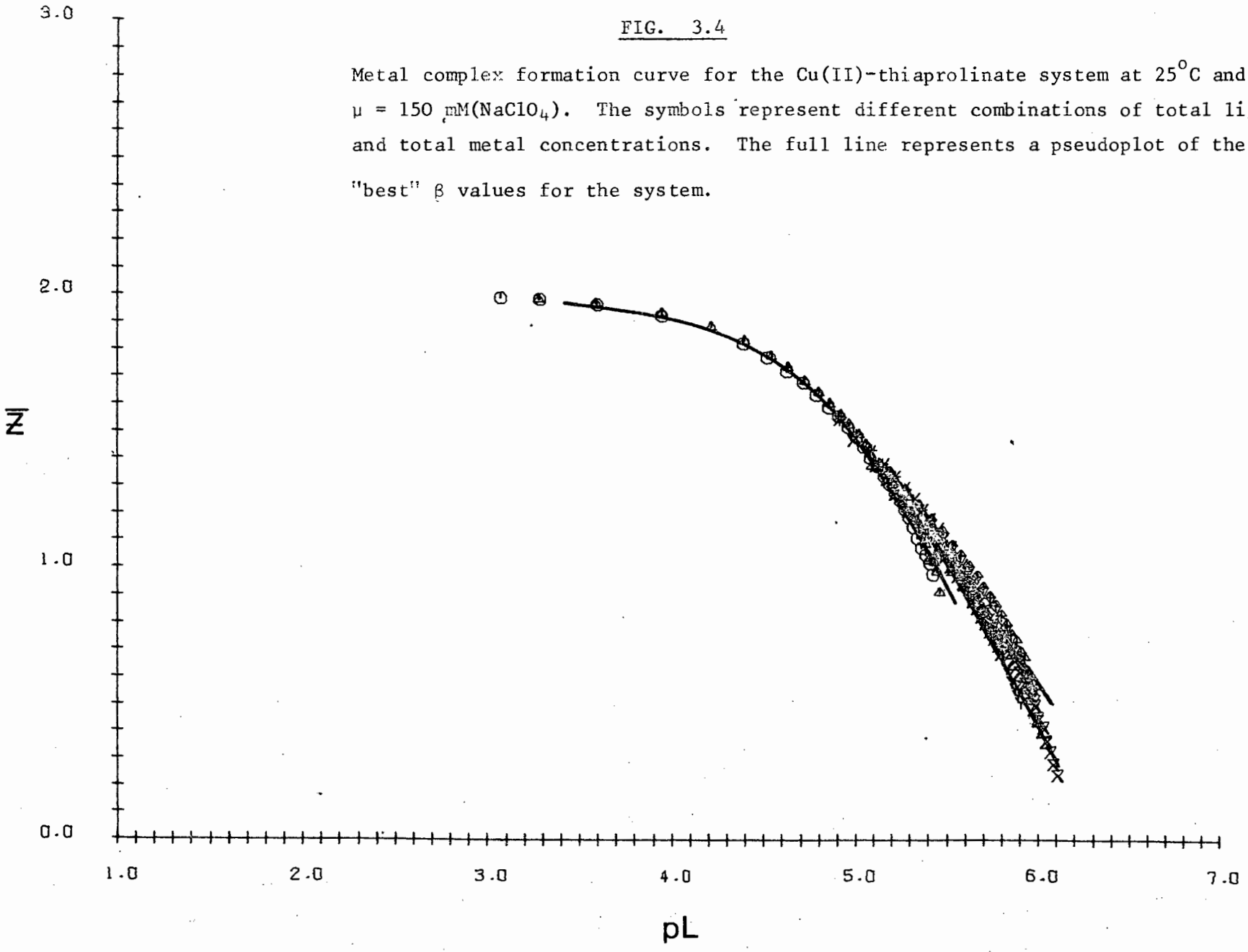
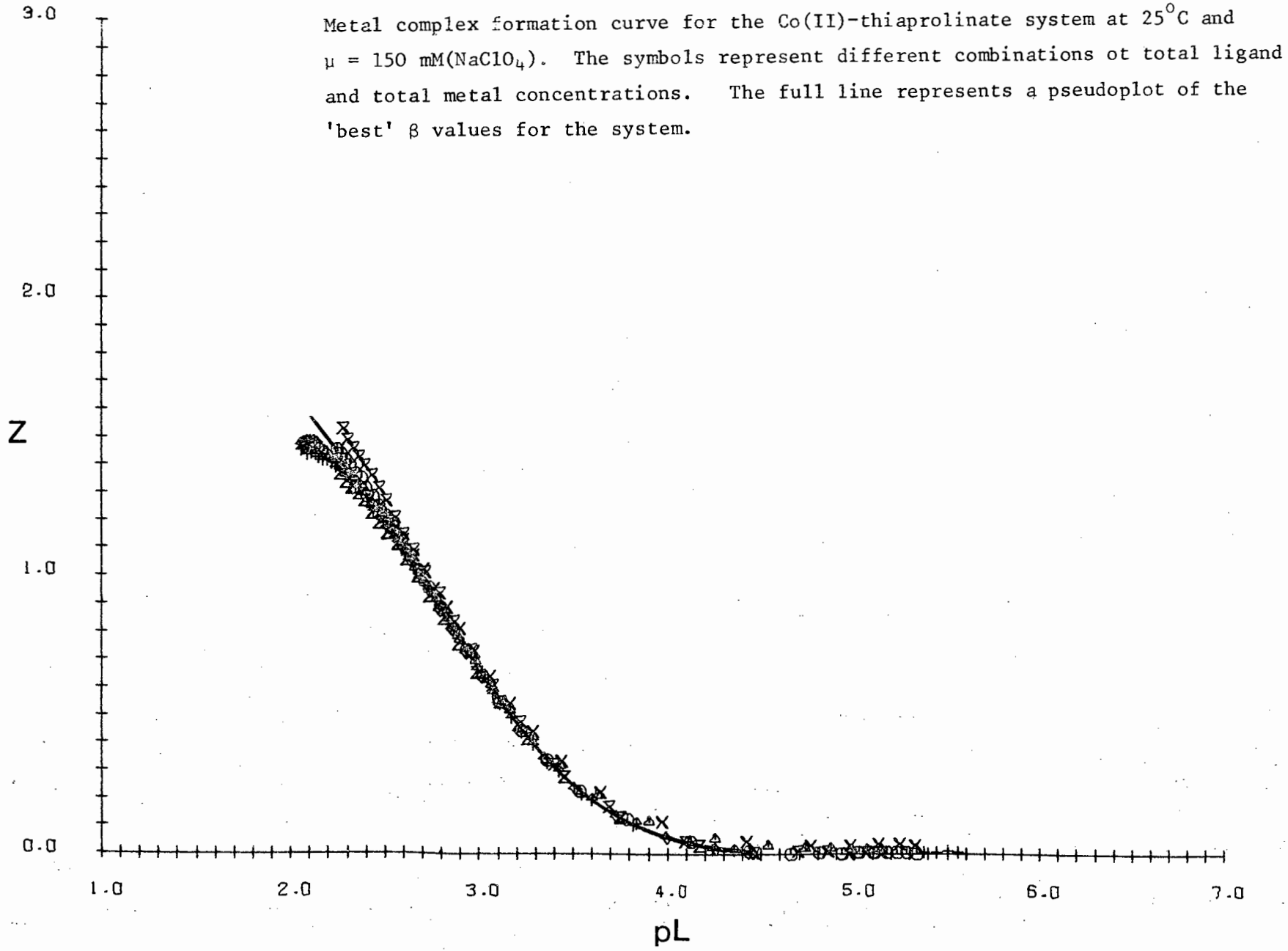


FIG. 3.5



Metal complex formation curve for the Co(II)-thiaprolinate system at 25°C and μ = 150 mM($NaClO_4$). The symbols represent different combinations ot total ligand and total metal concentrations. The full line represents a pseudoplot of the 'best' β values for the system.

FIG. 3.6

Metal complex formation curve for the Zn(II)-thiaprolinate system at 25°C and μ = 150 mM($NaClO_4$). The symbols represent different combinations of total ligand and total metal concentrations. The full line represents a pseudoplot of the "best" β values for the system.

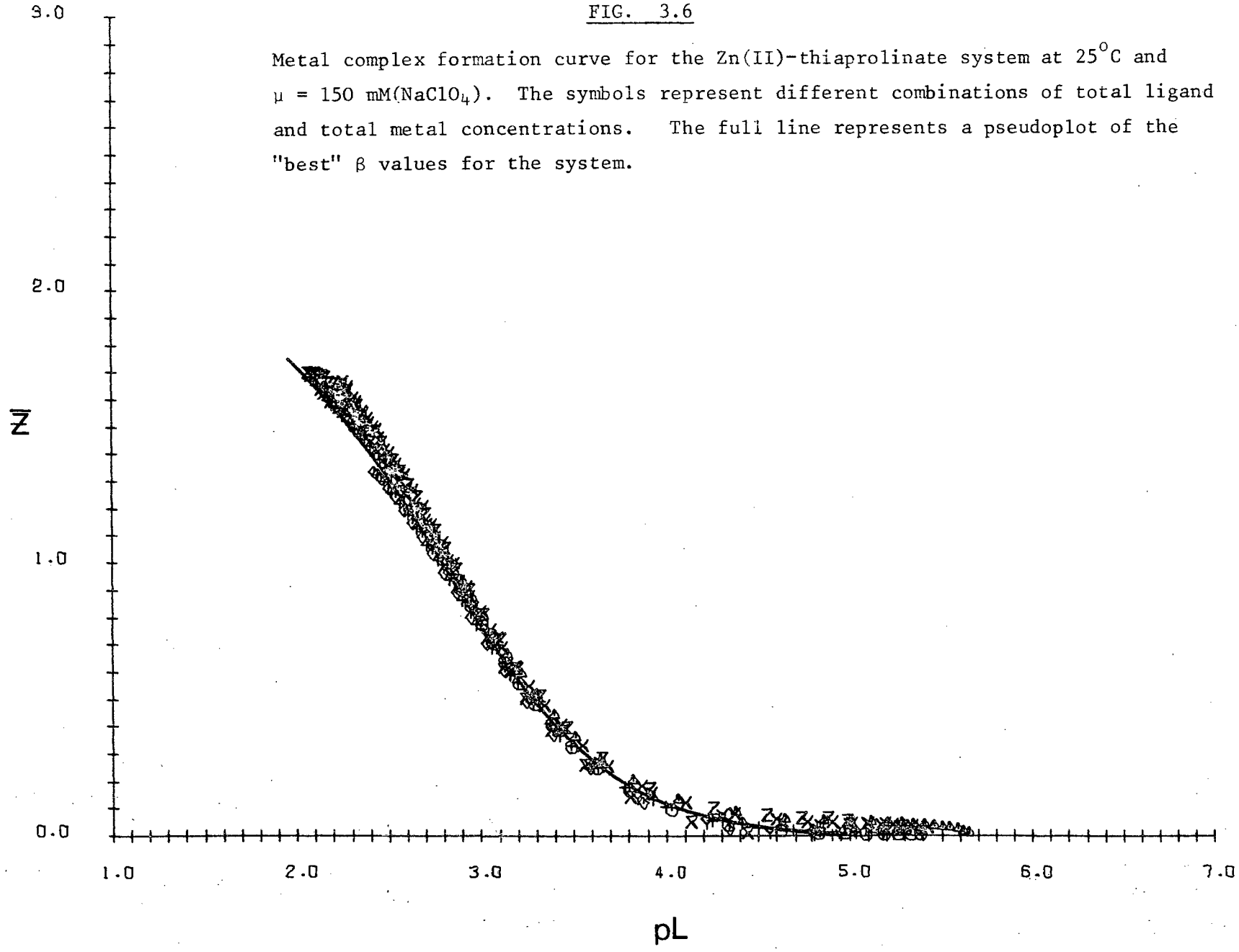
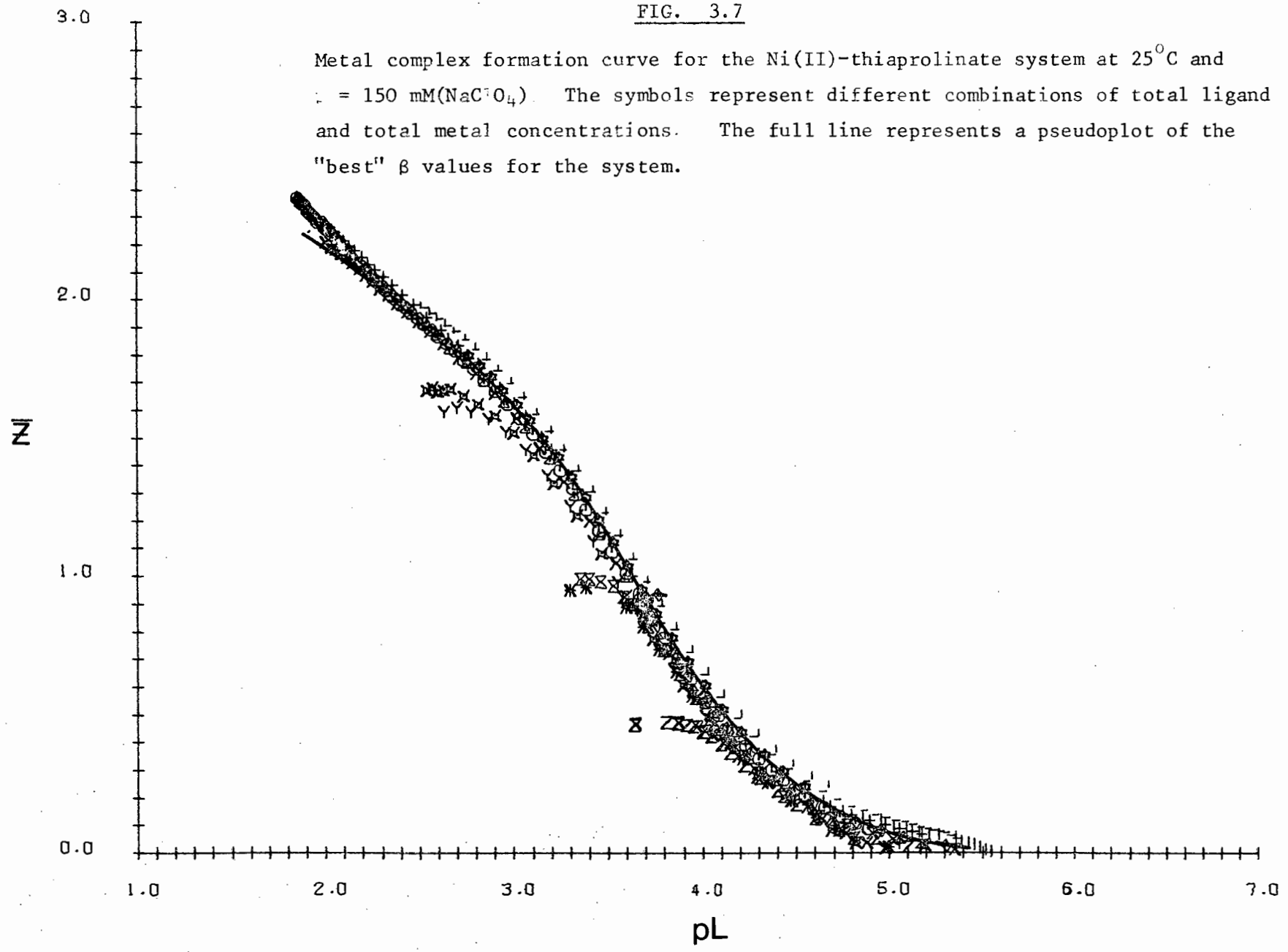


FIG. 3.7

Metal complex formation curve for the Ni(II)-thiaprolinate system at 25°C and μ = 150 mM($NaClO_4$). The symbols represent different combinations of total ligand and total metal concentrations. The full line represents a pseudoplot of the "best" β values for the system.



3.3.2 Hippuric Acid.

TABLE 3.2

Log β_{110} values for Copper (II) complexes of hippuric acid and several related ligands.

Ligand	Log β_{110}	Conditions	Reference
Hippuric acid	2.03	0.15 M $NaClO_4$, 25°C	Present work
Acetic Acid	1.8	0.1 M, 25°C	81
N-acetylglycine	2.14	0.015 M, 20°C	84
N-acetylglycylglycine	2.07	0.015 M, 20°C	84
N-hippurylthiazolidine-4-carboxylic acid	1.8	0.1 M KCl, 22°C	23
N-hippurylpipecolinic acid	2.1	0.1 M KCl, 22°C	23
N-benzoyl-2-methyl-glutamic acid	2.2	0.1 M, 25°C	94
N-acetylglutamic acid	2.2	0.1 M, 25°C	94

The thiaprolinate system was a fortunate starting point in this potentiometric study in that it was fairly well behaved, being reasonably soluble and forming stable complexes. Hippuric acid on the other hand was not very soluble and formed only weak complexes. The result of this is shown in the poor quality of the formation curves. (Figs. 3.9 and 3.10)

The formation constants of hippuric acid are given together with literature data for related compounds in Tables 3.1 and 3.2. The acid was found to behave similarly to other peptides in that the amide nitrogen was found to be non-basic. Thus only the carboxyl group is involved in protonation. The high acidity of amino acids in general, eg. glycine pK 2.35[48] as opposed to 4.53 for acetic acid,[82] is due to the inductive effect of the positively charged nitrogen. Acetylation of this amino group abolishes this positive charge and introduces instead the electronegative amide substituent. The effect of this on glycine is to increase pK by ~1.3 units. (acetylglycine pK = 3.6[48]). If this, is also the case with hippuric acid,the benzene ring not playing a part as it is too far removed from the carboxyl group (cf substituted carboxylic acids[83]), the expected pK value is 3.6. The value of 3.5 is in good agreement with this.

The analogy between hippuric acid and peptides stops when metal complexes are considered because metal binding to peptides is via the terminal amino group and the N or O of the amide linkage. Since hippuric acid does not have this terminal amino group, metal binding must be via the carboxyl group. The formation constants of Cu(II) and Ni(II) hippurate are very similar to those of acetic acid,[82] $\log \beta_{110}$ (Ni) = 1.8, $\log \beta_{110}$ (Cu) = 2.2 indicating that the ligand is monodentate and that the

amide group is not involved in binding.

Although the formation curves are (Figs. 3.9,3.10) not of good quality we have confidence in our conclusion because of:

(i) the agreement between the theoretically predicted and experimentally determined β_H values in our work;

(ii) the comparison of our system with several other similar systems to be found in the literature. These are shown in Table 3.2. It can be seen that in each case the metal binding is weak and that there is a good correlation between these and our results.

(iii) The crystal structure of a Cu(II) - hippurate complex has been determined.[84] This has the hippurate ions coordinated through the carboxyl group only. In fact the complex is a dimer, $Cu_2\ Hipp_4 \cdot 4H_2O$, the copper being pentacoordinate with two of the hippurates bridging. Potentiometry cannot, of course, distinguish between ML_2 and M_2L_4, the formation constant of the one merely being the square root of the other.

(iv) Although cases are known of complexation facilitating the loss of a proton from the amide nitrogen in peptides,[85] the possibility of this occurring with hippuric acid was thoroughly tested for by computer analysis. Also an n.m.r. pH titration was carried out to see if the amide nitrogen could be induced to release its proton at very high pH. The results are shown in Fig. 3.11. It can be seen that there is no detectable shift in the methylene proton resonance above pH_2. Similar results have been obtained for acetylglycine[86] where it was found that the rate of exchange of the amide nitrogen proton increased with pH and metal complexation

but was never significantly ionised. Presumably this is because, although complexation may promote the loss of a proton, the metal binding is weak, and metal hydroxides are precipitated before it can occur.

It was possible to refine two different models for the Cu(II) - hippurate system, as shown in Table 3.1. Although both these models gave R values less than R_{lim} they are not statistically different from each other. The improvement in χ^2 is also very small in going from the one model to the other. As a consequence the simplest model should be chosen.

The value of R for the Ni(II) - hippurate system is $\simeq R_{lim}$ but χ^2 is high indicating that our model may not represent an adequate description of the experimental data.

FIG. 3.8

Experimental Z plot points for the protonation of hippuric acid at 25°C, μ = 150 mM($NaClO_4$). The symbols represent different total ligand concentrations.

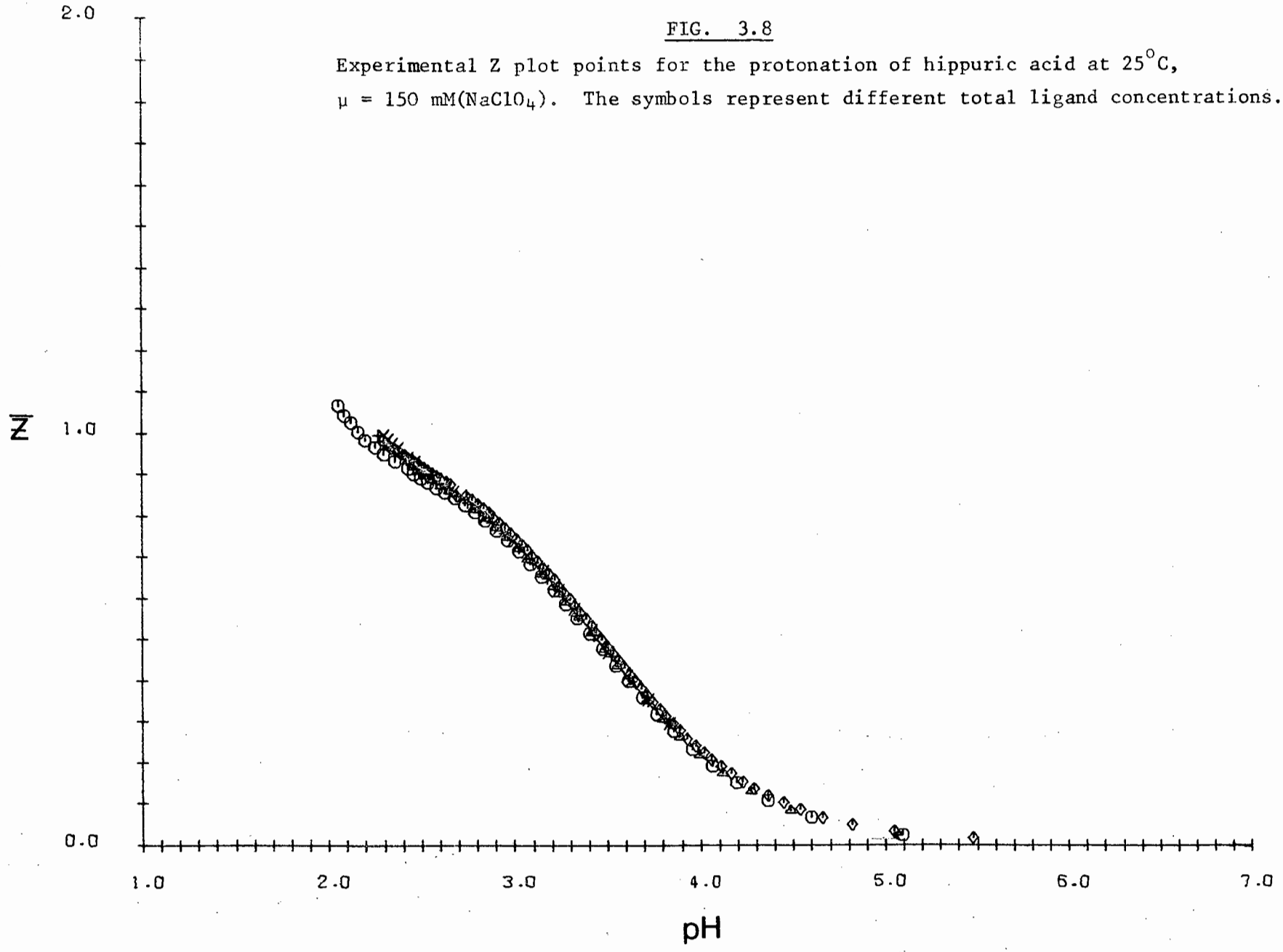


FIG. 3.9

Metal complex formation curve for the Ni(II)-hippurate system at 25°C and μ = 150 mM($NaClO_4$). The symbols represent different combinations of total ligand and total metal concentrations. The full line represents a pseudoplot of the "best" β values for the system.

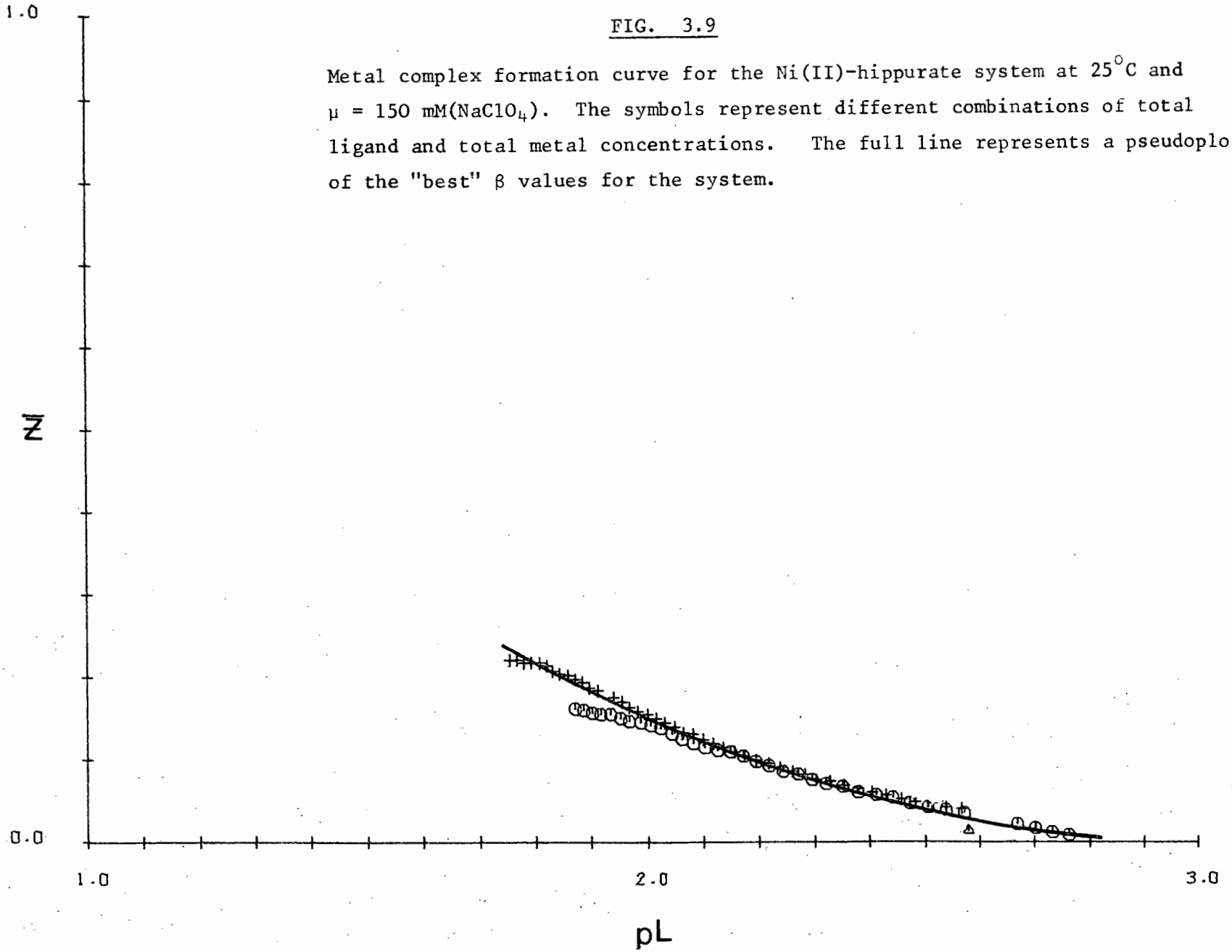
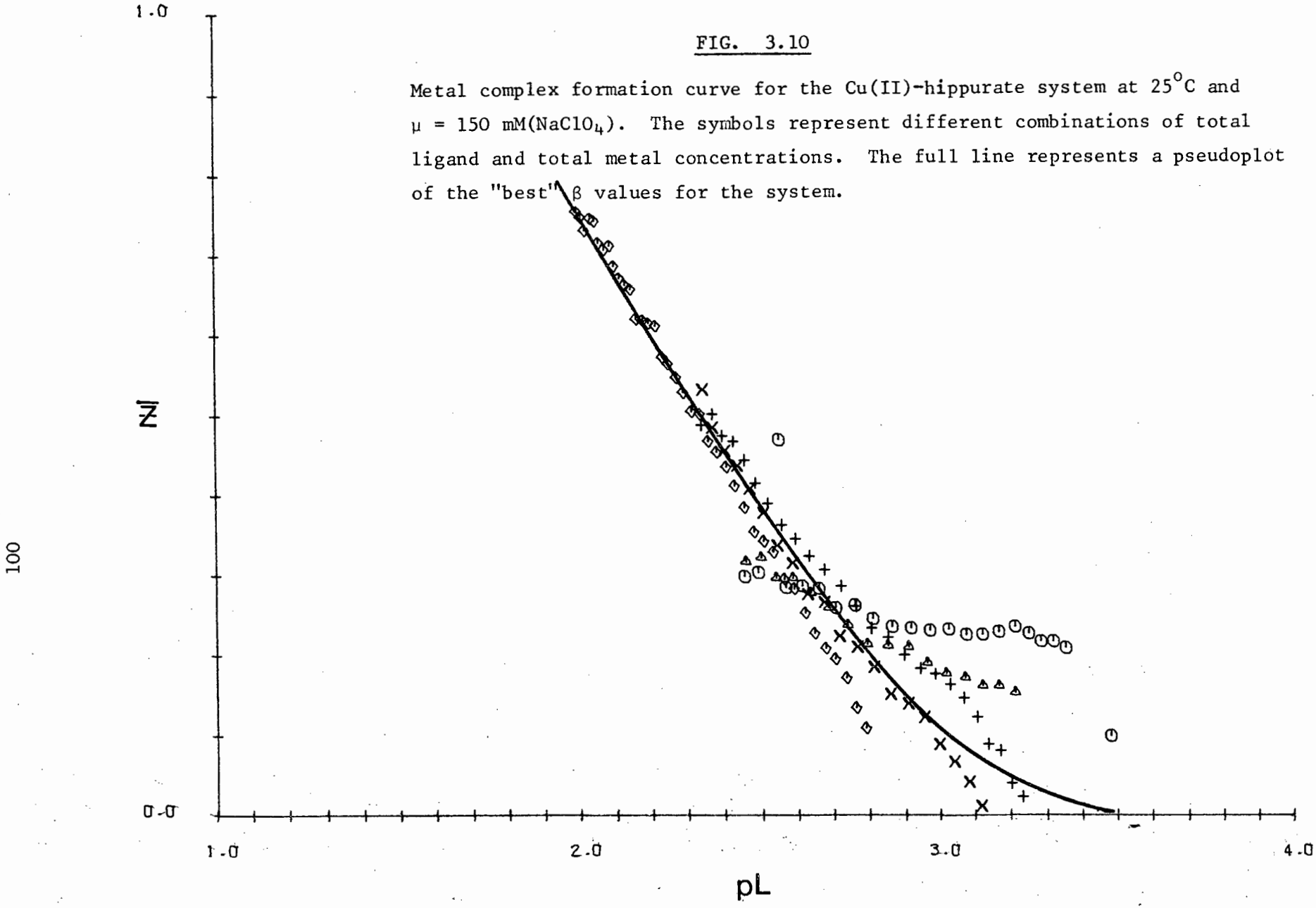


FIG. 3.10

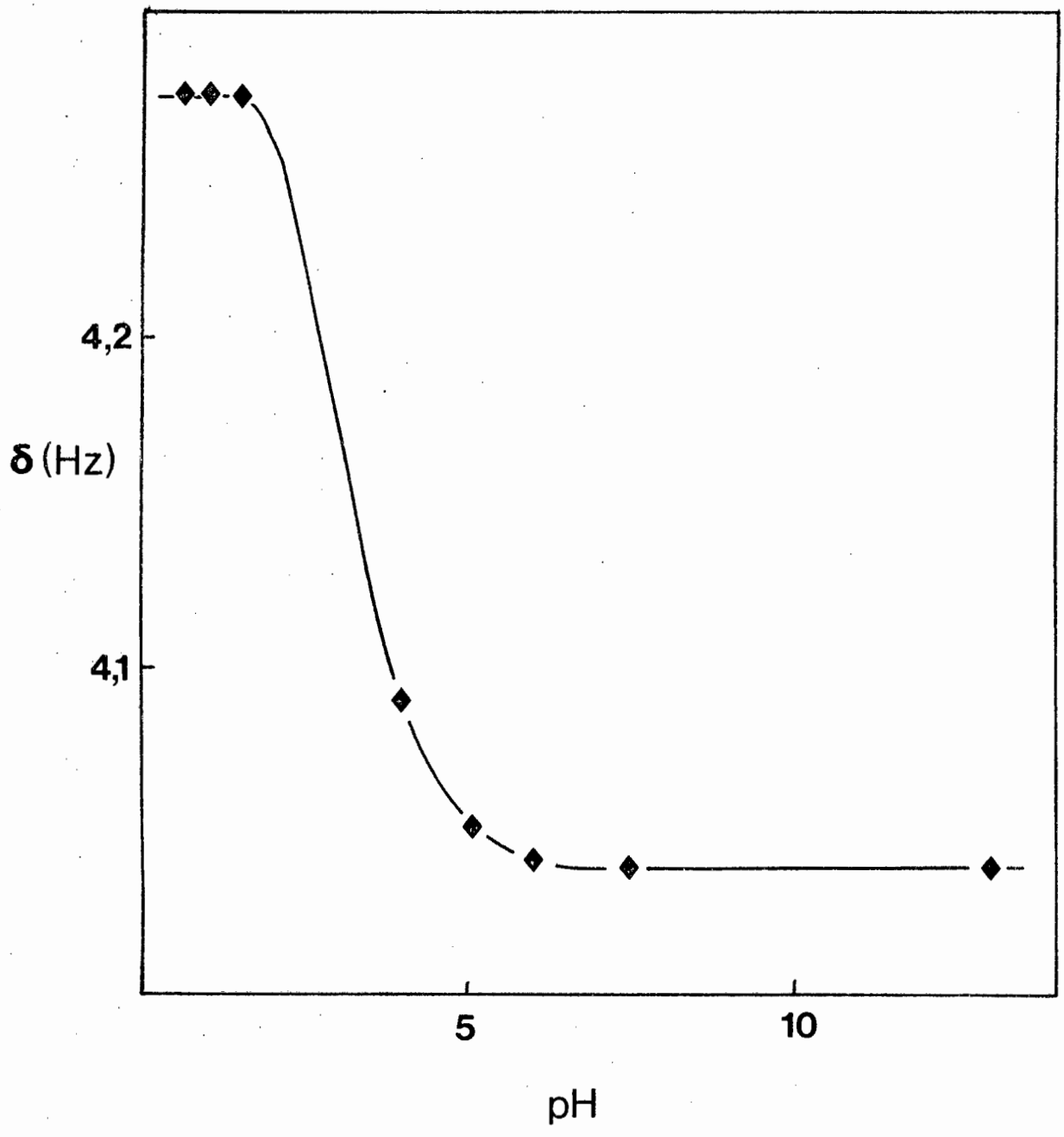
Metal complex formation curve for the Cu(II)-hippurate system at 25°C and μ = 150 mM($NaClO_4$). The symbols represent different combinations of total ligand and total metal concentrations. The full line represents a pseudoplot of the "best" β values for the system.



100

FIG. 3.11

pH dependence of chemical shift of CH_2 resonance of hippuric acid.



3.3.3 Penicillin.

Weiss et al[23] claim to have determined potentiometrically the formation constant for Cu-penicillin. Cressman et al[87] in their potentiometric study found the titration curve to be time dependent. Under the conditions of the present study a variation of ~ 10 mV/min was observed, which is quite unacceptable for stability constant determination. The reason for this continual pH drift is hydrolysis of the penicillin to penicilloic acid. Cressman et al[13] have used the time dependence of the pH to determine both the rate of hydrolysis and the formation constant of the Cu(II) - penicillin complex.

The interaction of Ni(II) with penicillin does not show this marked time dependence and could be studied potentiometrically. The protonation of penicillin is shown in Fig. 3.12. This shows that there is only one ionizable proton under the experimental condition used. It was in fact the lack of basicity of the ring nitrogen that lead to the acceptance of the β-lactam structure for penicillin over the oxazolone-thiazolidine structure.[76] The protonation constant obtained from this curve agrees well with the literature, and can also be rationalised on the basis of the inductive effects of the amide and ring sulphur.[76]

As already stated it was possible to study the Ni(II) penicillin interaction. The formation curves for this system are shown in Fig. 3.13. Once again they did not rise to a large $\bar{z}$ value indicating only weak complexation. This is to be expected from the previous work on hippuric acid.

A comparison of the β's for penicillin and hippuric acid yield an interesting phenominum. $\Delta p\beta_{101}$ (Hipp - Pen) = + 0.75 whereas $\Delta p\beta_{110}$ (NiHipp - NiPen) = - 0.49. If we accept the association constant for Cu-penicillin determined by Cressman[13] et al we get $\Delta p\beta_{110}$ (CuHipp - CuPen) = - 0.35. Williams[80] has suggested that this phenominum of stability constant inversion may be due to amide involvement in coordination. That the amide nitrogen of penicillin should be involved in binding while that of hippuric acid is not can be explained by the decrease in amide resonance in the strained β-lactam system.[88] Normally it is the existance of this resonance which makes the amide nitrogen such a poor ligand.

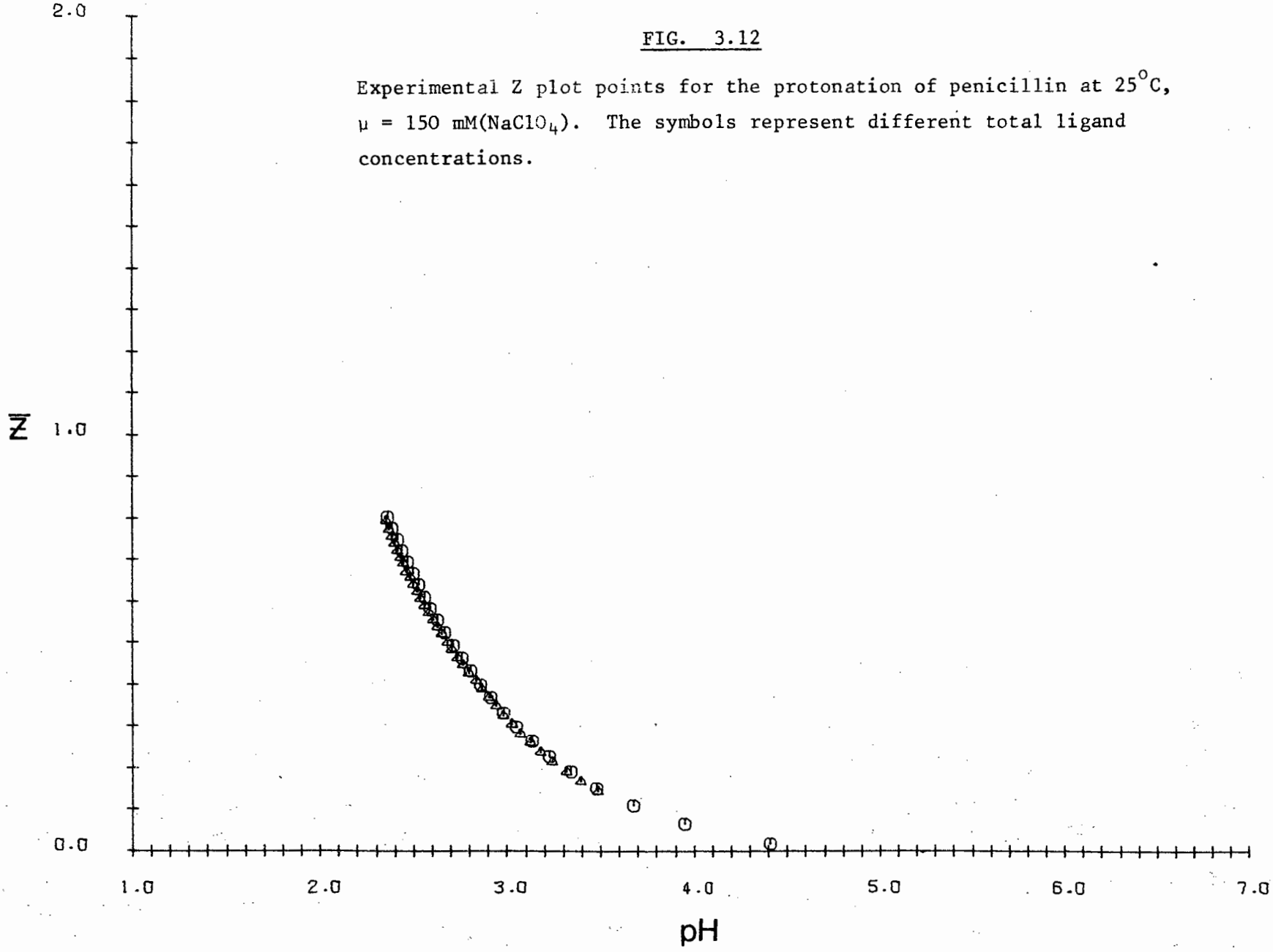
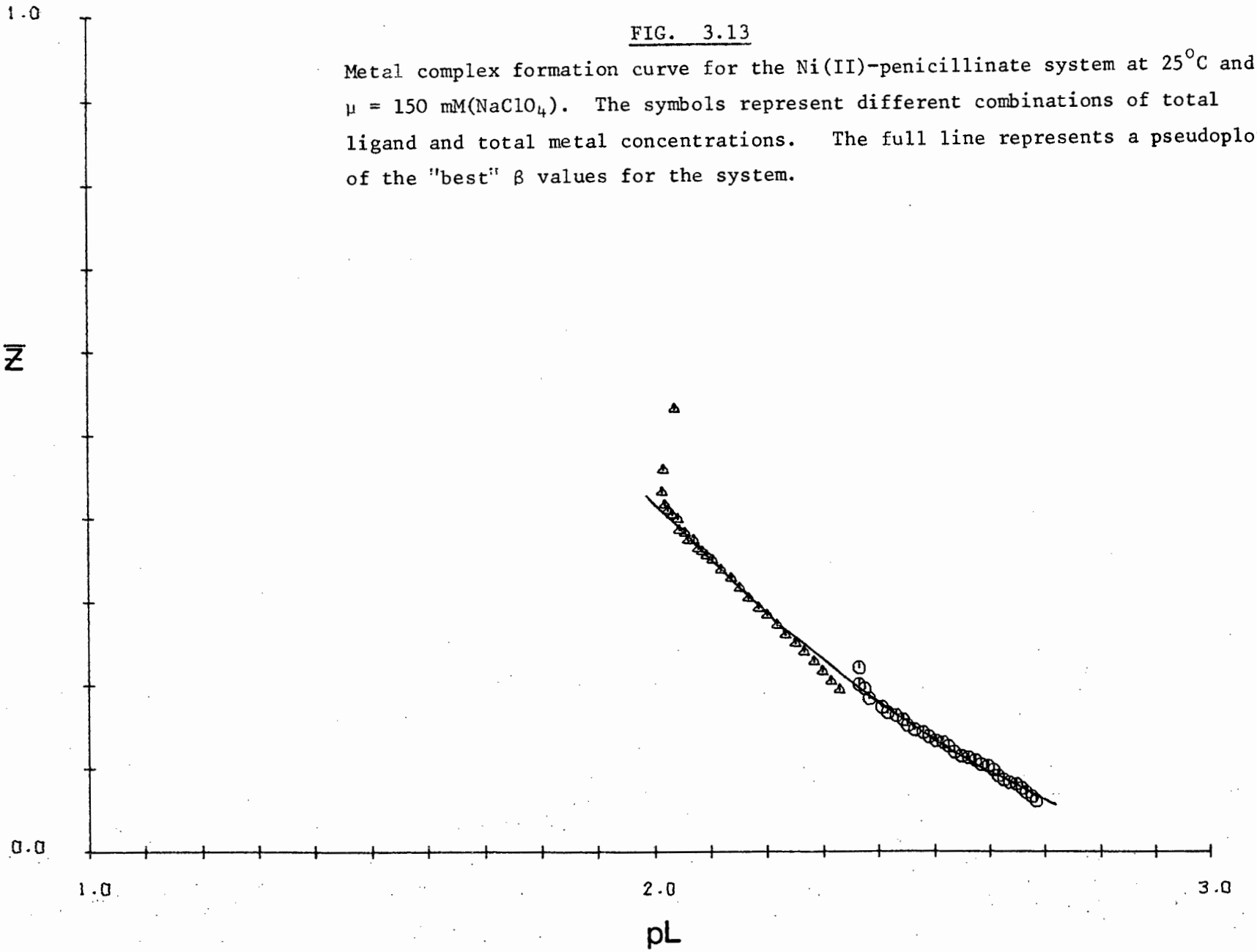


FIG. 3.12

Experimental Z plot points for the protonation of penicillin at 25°C, μ = 150 mM($NaClO_4$). The symbols represent different total ligand concentrations.

FIG. 3.13

Metal complex formation curve for the Ni(II)-penicillinate system at 25°C and μ = 150 mM($NaClO_4$). The symbols represent different combinations of total ligand and total metal concentrations. The full line represents a pseudoplot of the "best" β values for the system.



3.3.4 Penicilloic Acid.

Finally the H^+, Ni(II) and Cu(II) binding to penicilloic acid has been investigated. The protonation curve given in Fig. 3.14 show the presence of 3 ionisable protons. The refined constant (see Table 3.1) are in good agreement with literature values.[76,89] The assignment of β_{101} to the amino group is generally agreed upon, but the assignment of the subsequent protonations is not altogether unambiguous. From the structure of penicilloic acid it would be expected that two carboxyl protonations would be obtained, having values close to those of hippuric acid and thiaproline. The effect of a thiazolidine substituent on the pK of hippuric acid, and visa versa, should be small. Hence the pK values of 2.30 and 1.76 which were obtained are assigned to the hippuric acid and thiaproline type carboxyls respectively. These values do deviate slightly from those expected, the 2.3 being too low and the 1.76 too high, and could be due to a compensation effect taking place during the computer analysis.

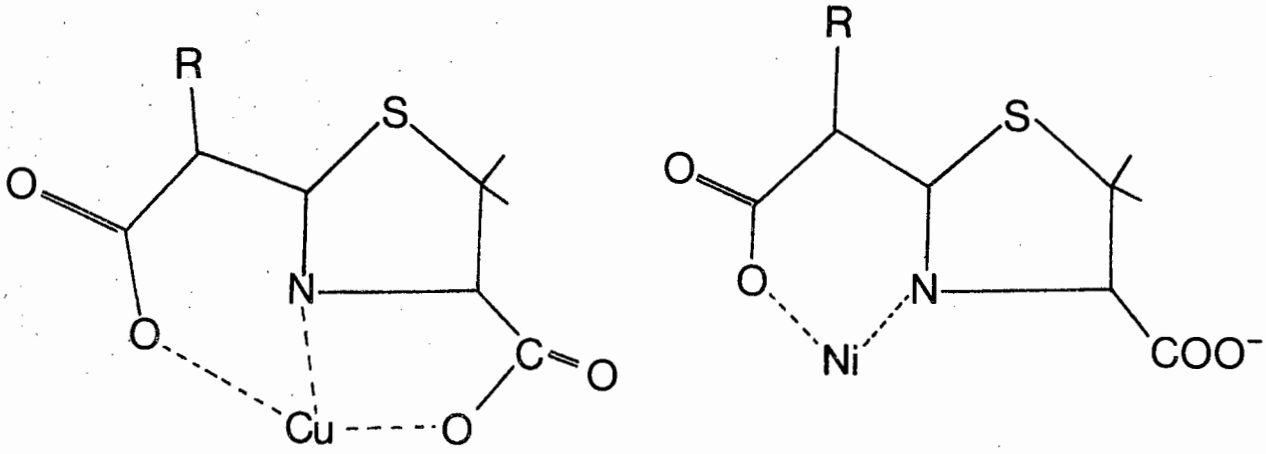
The interaction of Cu(II) and penicilloic acid yielded a bright blue colour in the 2.0 - 5.5 pH range. At the higher pH limit there was a sharp change in pH accompanied by a change in colour. First the solution turned a light green which darkened on standing, until a dark wine red colour developed. It was not possible to carry out any potentiometric determinations in this region as there was a continuous pH drift, probably due to degradation of the penicilloic acid.

Solid complexes could be isolated from both these coloured regions.

Bright blue xtals were obtained from the pH 2 - 3.5 region, which analysed as Cu-Penicilloate $\cdot H_2O$. This water molecule could not be removed without decomposition of the complex.[90] In the higher pH range a pale grey precipitate was obtained which analysed as Cu-Penilloic acid. The penilloic acid could be regenerated from the complex by the action of H_2S, CuS_2 being precipitated.[90] It appears then, that in the higher pH range the penicilloic acid is decarboxylated to penilloic acid.

The Cu(II) and Ni(II) penicilloic acid formation curves are given in Figs. 3.15 and 3.16, and the formation constants in Table 3.1. Once again we find an inversion of β_{101} and β_{110} for penicilloic acid and thiaproline. $\Delta p\beta_{101} = 0.92$ (thiaproline-penicilloic acid) whereas $\Delta p\beta_{110} = -0.68$ (CuThia - CuPoic) and + 0.62(NiThia - NiPoic). From the analysis of the solid Cu·Penicilloic acid complex it appears that the penicilloic acid must be acting as a tridentate ligand with a water molecule occupying the fourth coordination site. This indicates that in fact, the inversion of $\Delta p\beta$ does reflect coordination of a further group. This adds confidence to the suggestion of penicillin being bidentate.

The Ni(II) penicilloic acid complex does not give this inversion, suggesting that only two of the potential binding sites are coordinated - the thiaproline ring nitrogen and one of the carboxyl groups. Binding to either of the carboxyl groups would give rise to a stable five-membered ring but from the proton binding the hippuric acid proton would be expected to form a stronger bond. Thus the proposed structures are:



Overall it can be seen that the complexes of penicillin (and indeed the same is expected for the cephalosporins) are weak. The consequence of this will be discussed in Chapter 5.

FIG. 3.14

Experimental Z plot points for the protonation of penicilloic acid at 25°C, μ = 150 mM($NaClO_4$). The symbols represent different total ligand concentrations.

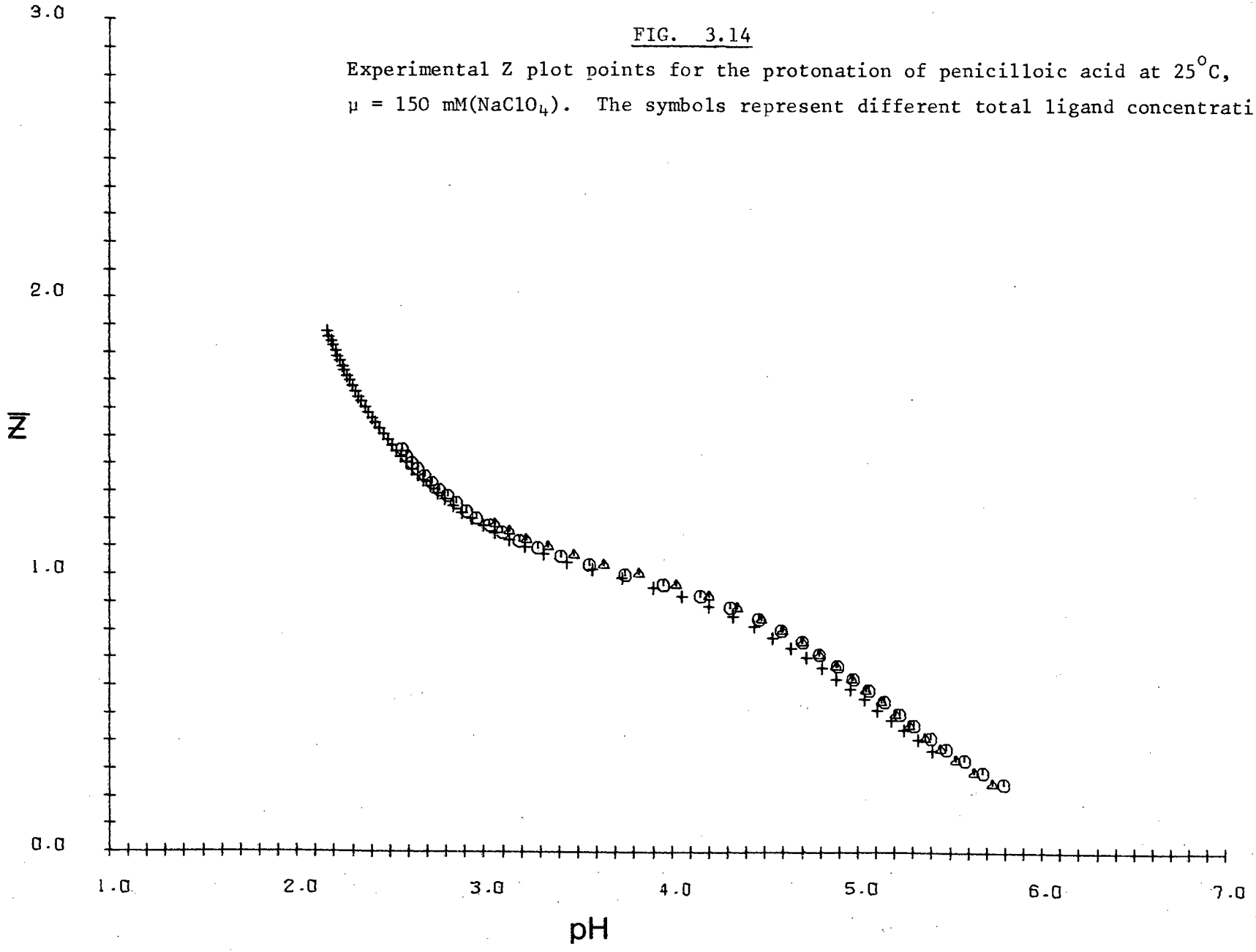


FIG. 3.15

Metal complex formation curve for the Cu(II)-penicilloate system at 25°C and μ = 150 mM($NaClO_4$). The symbols represent different combinations of total ligand and total metal concentrations. The full line represents a pseudoplot of the "best" β values for the system.

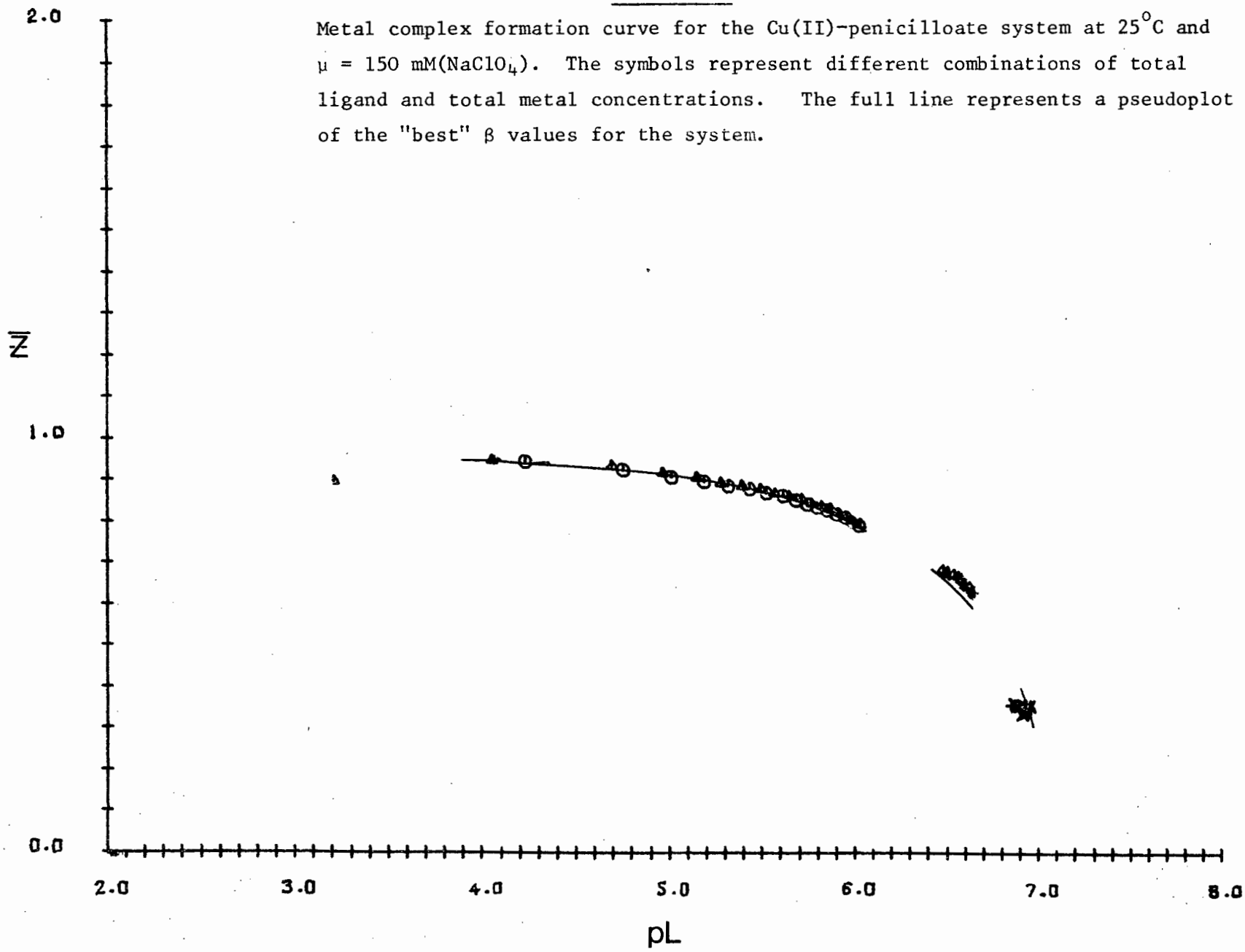
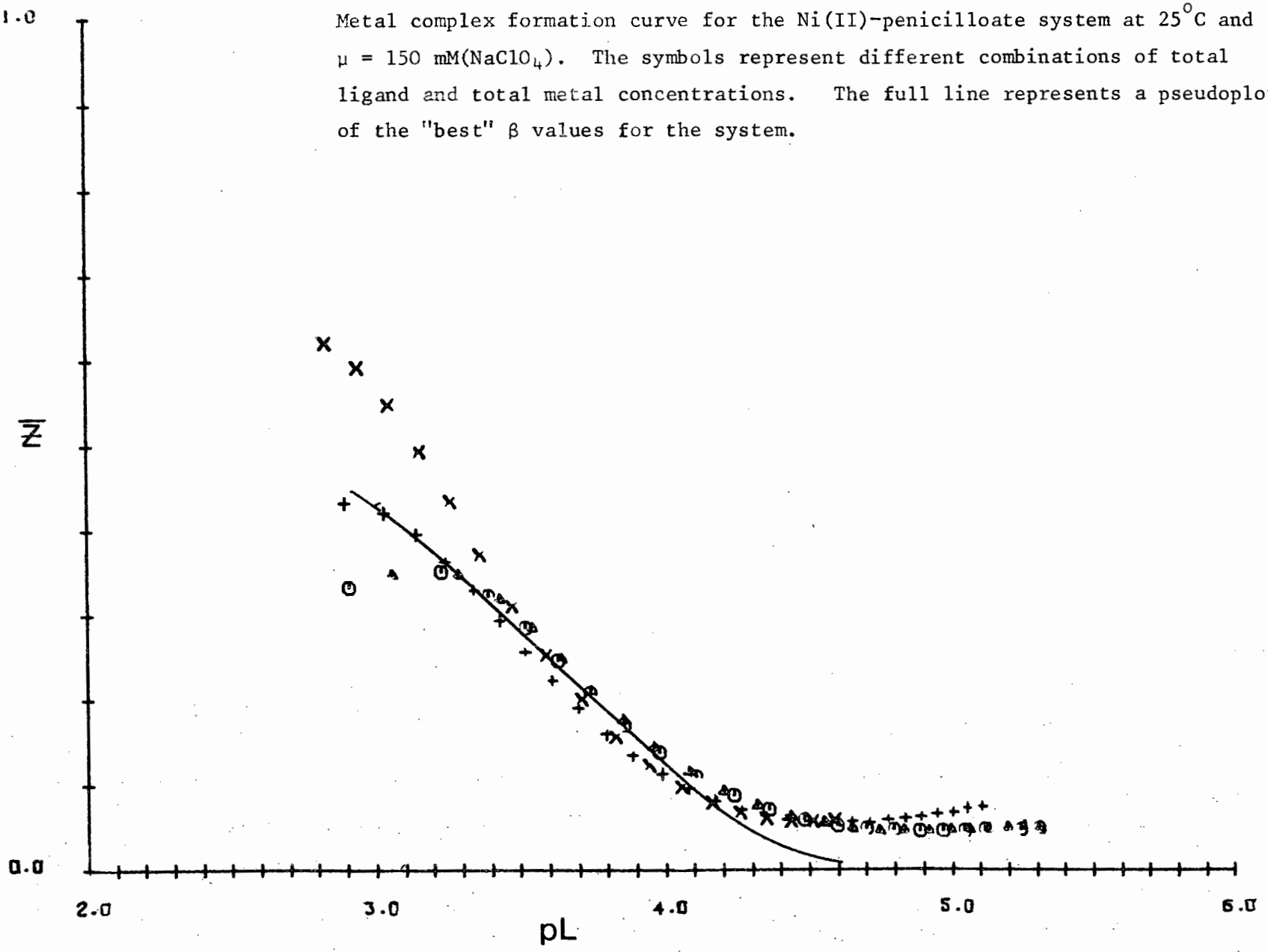


FIG. 3.16

Metal complex formation curve for the Ni(II)-penicilloate system at 25°C and μ = 150 mM($NaClO_4$). The symbols represent different combinations of total ligand and total metal concentrations. The full line represents a pseudoplot of the "best" β values for the system.



3.4 Experimental.

The experimental proceedure used in this potentiometric study is similar to that used by Williams[80] and other workers in this field. In outline the proceedure involves the potentiometric titration of several solutions containing different metal : ligand ratios and concentrations. The protonation constants are determined from solutions containing no metal-ions. The data obtained (ml,mV) is then analysed using the published computer programs MINIQUAD[71] and SCOGS[72] to yield the best β's. Different models can be tried and the final one checked by 'pseudoplotting'. This is shown in Figs. 3.4 - 3.16.

Titrations were carried out in a Metrohm (cat.No.EA876-20) titrating vessel under an oxygen free, nitrogen atmosphere. The high purity nitrogen was in fact further purified by bubbling through Fiesers[91] solution to remove any residual oxygen, washed twice with distilled water and finally passed through a solution of the support electrolyte at the correct concentration. All the wash bottles were placed in a thermostated water bath so that the nitrogen was at the correct temperature when entering the reaction vessel. Back diffusion along the nitrogen outlet was prevented by having this under water.

The metal perchlorates were all of analytical reagent grade and supplied by K and K Laboratories. Stock solutions of these were prepared and standardised by EDTA titration.[92] The $NaClO_4$, supplied by Merick, was further purified by filtration first through an 8.0μ Sartorius membrane filter. This filter was found to remove a considerable amount of 'crud'

penicilloic acid and then back titrating the excess alkali. The effective molecular weight was found to be 370. The difference between this and the theoretical molecular weight of 358 is probably due to absorved water - the compound being fairly hygroscopic.

The penicilloic acid was prepared by the method of Rapson and Bird[89] in which benzylpenicillin is hydrolysed at pH 12 for 15 minutes at room temperature. The resulting penicilloic acid solution was adjusted to pH 6.5 and either used or discarded with 4 hours of preparation. The acid was standardised by potentiometric titration, the amine protonation being detected using a Gran plot.[70] The carboxylic acid dissociation could not be used since this was masked by the excess acid, the two giving overlapping inflection. This was why penicillin could not be standardised by direct alkaline titration.

The experimental proceedure followed the general course of dissolution of the ligand in 20ml of 0.01 M $HClO_4$ and then titration using 0.1M NaOH. The metal-ion was added from a stock solution using a micro-pipette. Generally the titration was carried out in both directions, using first 0.1M NaOH and then 0.1M $HClO_4$. These were added to the reaction vessel using a Metrohm (cat.No.E274) burette which had previously been calibrated. The burettes could be read with a precision of ±0.005ml. The whole apparatus was thermostated at $2.5^{\circ} \pm 0.1^{\circ}C$ using a Heidolph thermostat.

The reaction was followed using a combination of a Russel pH Ltd. glass electrode (SF 75/C14) and a calomel electrode in which the KCl had been replaced by NaCl. The calomel electrode and reaction vessel were

connected by means of a $NaClO_4$ bridge. The EMF readings were taken on a Radiometer PHM 64 digital pH-meter. This gave a stability of 0.1 mV. A diagram of the complete apparatus is shown in Fig. 3.17.

The electrodes were calibrated against acetic acid using an iterative technique developed in this laboratory.[94] The mathematical analysis allows the refinement of both E^{o} and F_N of equation 3.2, from the titration data and an approximately known β_H. This calibration is valid only in the region ± 2pH units of the β_H of acetic acid. In the higher pH range the electrodes were calibrated against imidazole. The inadvisability of using a strong acid strong base titration to calibrate the electrodes is shown in Fig. 3.18, where it can be seen that even at low pH's there is a marked curvature in the graph of emf against pH. Fig. 3.18 also shows the acetic acid and imidazole calibration curves.

FIG. 3.17

Diagram of apparatus used in the determination of formation constants. The nitrogen bubbler and thermometer are not shown.

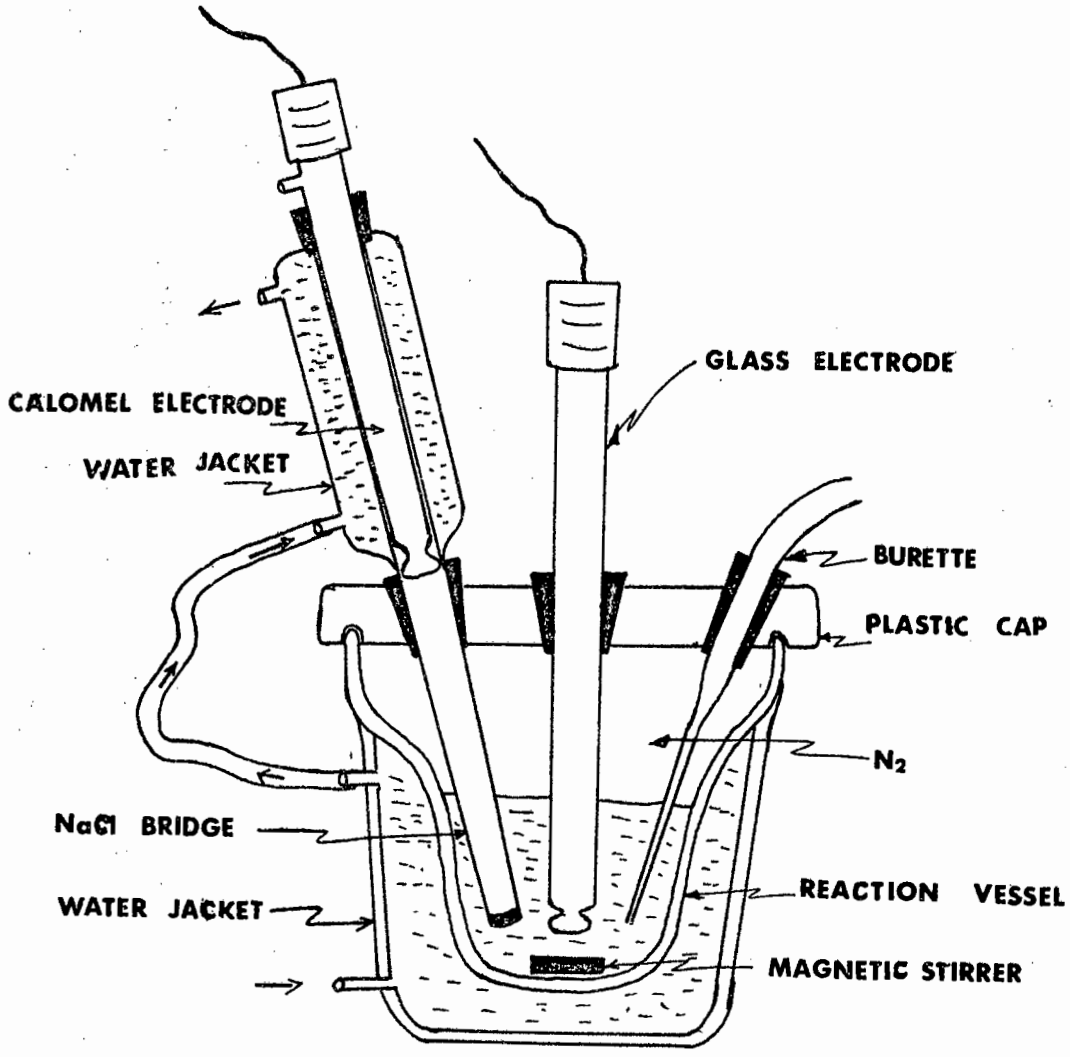
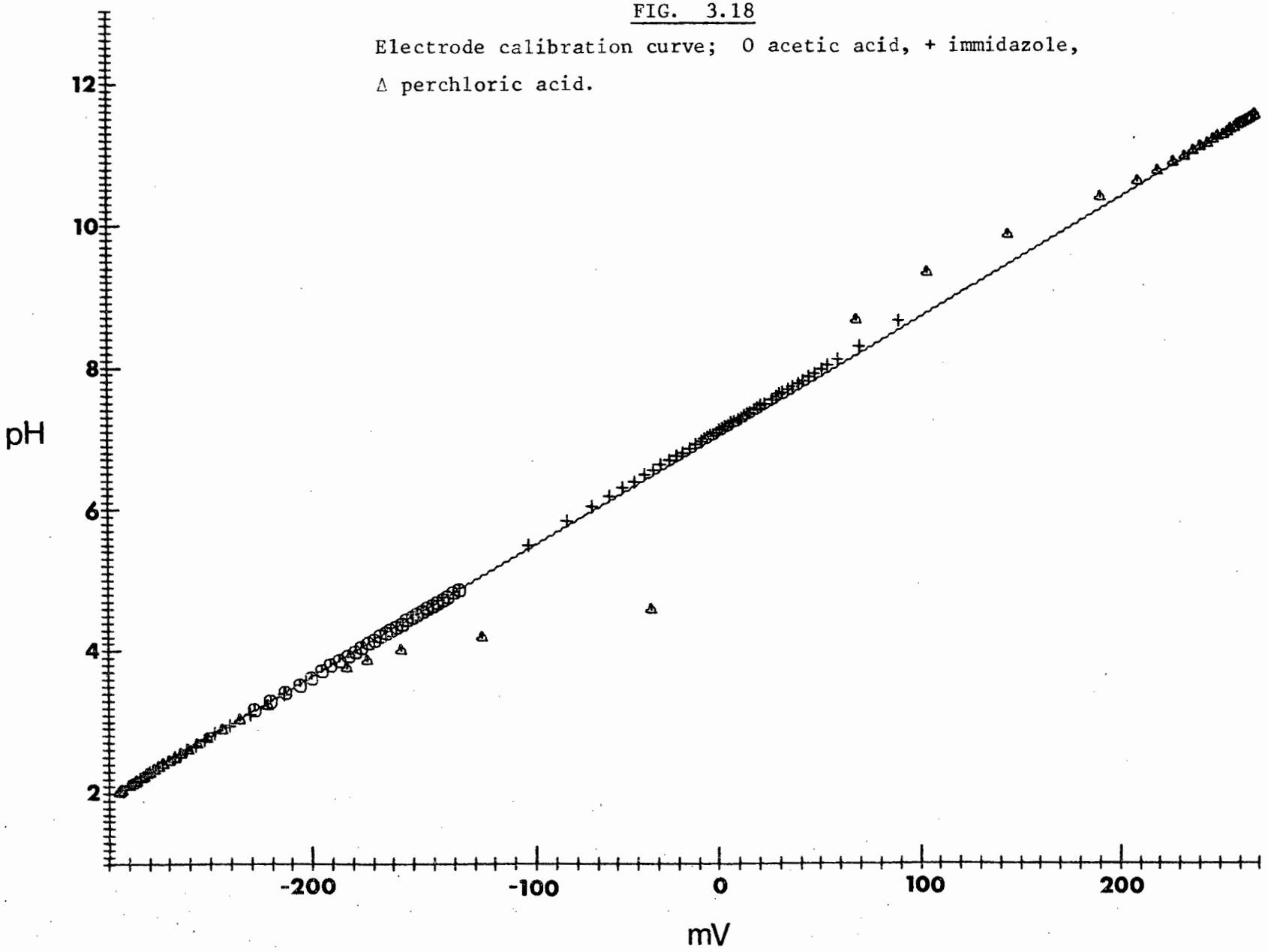


FIG. 3.18

Electrode calibration curve; O acetic acid, + immidazole, Δ perchloric acid.



CHAPTER 4

KINETIC STUDY

Introduction to Kinetics of Ni(II) Substitution Reactions.

Most of the early kinetic studies on metal substitution reactions were carried out on the inert Co(III) and Cr(III) complexes since these could be easily followed using conventional methods, and also the structure of the complexes had been well characterised. The study of the more labile, divalent transition metal-ions had to await the advent of flow and relaxation techniques. (Review articles on these techniques exist in the literature.[96,97]) In 1952, Taube[98] reviewed all the observations (mostly qualitative) that had been made up until that time and was able to relate reactivity to the electronic configuration of the complex ion. This review gave a certain impetus to the field and soon further studies were able to establish the order of reactivity as; V(II) < Ni(II) < Co(II) < Fe(II) < Mn(II) < Zn(II) < Cr(II) < Cu(II). This is the same order, with the exception of Cr(II) and Cu(II), that is expected on the basis of crystal field theory for a six coordinate ion going to a five coordinate transition state.[99] The extremely rapid reaction rates of Cr(II) and Cu(II) were attributed to Jahn-Teller effects, the axial water molecules being more labile than those occupying equatorial positions. This was later confirmed by Swift and Connick.[38]

Of the divalent, first row, transition metal-ions Ni(II) has received by far the most attention. This is because it is relatively inert and hence more amenable to study. Also its +2 oxidation state is the only one exhibited and its complexes have been well characterised.[100,48] As for V(II), which reacts more slowly and should therefore be just as easy or even easier to study, it can be readily oxidised and the +2 state is oxygen sensitive.

Substitution reactions can proceed via either an SN1 or an SN2 mechanism. The evidence to date has been overwhelmingly in favour of the SN1 mechanism since the rate constants appear independent of the nature of the ligand and only depend on its charge. With the dissociative mechanism there are two main possibilities:

(i) a limiting SN1 mechanism in which a five coordinate intermediate is formed which has a sufficient lifetime to discriminate between different nucleophiles.

(ii) The Eigen-Tamm[101] mechanism in which an outer-sphere complex or ion-pair is rapidly formed between the hydrated metal-ion and the substituting ligand. This is followed by loss of a coordinated water molecule in the rate determining step and formation of the inner-sphere complex. This second mechanism explains the main body of experimental results nicely since differences in rate constants of species of differing charge can be assigned to changes in the equilibrium constants of the outer-sphere complex. The overall reaction scheme is:

$$M(H_2O)_6 + L \underset{}{\overset{K_{os}}{\rightleftharpoons}} M(H_2O)_6 \cdot L \xrightarrow{k_o} ML(H_2O)_5 + H_2O$$

for which $$d[ML]/dt = \frac{k_o K_{os}[M(H_2O)_6][L]}{1 + K_{os}[L]}$$

Since the outer-sphere complex associations are usually weak and the ligand concentrations low $K_{os}[L]$ is normally << 1 and so

$$k_f = K_{os} k_o$$

Where K_{os} is the diffusion-controlled equilibrium constant for the formation of the outer-sphere complex.

Support for the Eigen-Tamm mechanism comes from cases where k_o could be measured directly using relaxation techniques.[102] These values were found to be in good agreement with the first-order rate constant determined by Swift and Connick for water exchange. Alternatively it is possible to obtain reasonable estimates of K_{os} from two functions derived independently by Fuoss[103] and Eigen[104] i.e.:

$$K_{os} = \frac{4\pi\, Na^3}{3000} e^{-U(a)/kT}$$

where $$U(a) = \frac{Z_M Z_L e^2}{aD} - \frac{Z_M Z_L e^2}{D(1 + \chi a)} K$$

and $$\chi^2 = \frac{8\Pi\, Ne^2}{1000DkT} \mu$$

These calculated values of K_{os} can then be used to calculate k_o from k_f. Once again there is good agreement between k_o and the rate of water exchange.

Using the Eigen-Tamm mechanism it is easy to see why the reaction rate is independent of the ligand and depends only on the metal-ion solvent exchange rate. The charge of the ligand will be important in determining K_{os}. Multidentate ligands, on the otherhand, have more complex mechanisms because of the multiplicity of reaction steps. The Eigen-Tamm mechanism can, however, be extended to cover this. The reaction scheme would then be:

$$Ni(H_2O)_6 + L\text{-}L \overset{K_{os}}{\rightleftharpoons} Ni(H_2O)_6 \cdot L\text{-}L \underset{K_{-2}}{\overset{k_2}{\rightleftharpoons}} (H_2O)_5\, Ni\text{-}L\text{-}L + H_2O$$

$$(H_2O)_5\ Ni\text{-}L\text{-}L \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} (H_2O)_4\ Ni\begin{smallmatrix}L\\L\end{smallmatrix}) + H_2O$$

If the outer-sphere complex equilibrium is established very rapidly, in comparison to the other steps, and also if the stationary state condition for the intermediates is applied, then the rate equation is:

$$d[NiL_2]dt = k_f\,[Ni(H_2O)_6^{2+}]\,[L\text{-}L] - k_d[NiL_2]$$

where $k_f = K_{os}\,k_2k_3/(k_{-2} + k_3)$

$k_d = k_{-2}k_{-3}/(k_{-2} + k_3)$

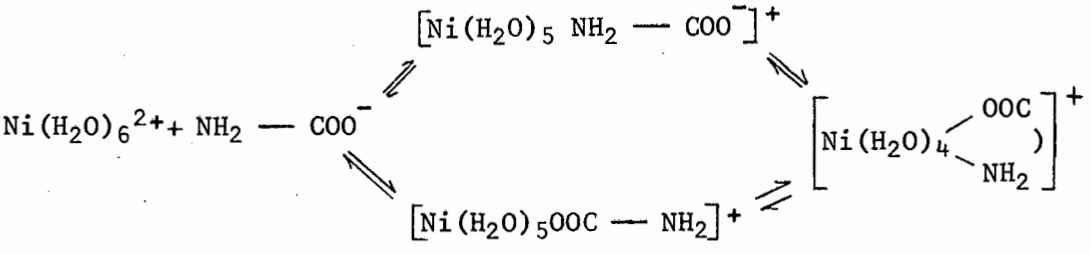
Depending on the relative magnitude of k_{-2} and k_3 two limiting cases are obtained. It depends, in fact, on these two rates, whether chelation is controlled by the rate of ring closure, or the rate of solvent exchange.

If $k_3 >> k_{-2}$ then $k_f = K_{os}k_2$ and the overall rate of chelation will depend on the rate of formation of the first metal-ligand bond. This means that the rate will be the same as for a monodentate ligand. This appears to be the case for the majority of strongly binding bidentate ligands with Ni(II)[105,106] e.g. the reaction of bipy and py with Ni(II) proceed at similar rates indicating that there is no inhibition to ring closure.[107]

If $k_3 << k_{-2}$ then $k_f = K_{os}k_2k_3/k_{-2}$ and the rate determining step will shift from water loss to ring closure. The term 'sterically controlled substitution' (SCS), as opposed to 'normal' substitution, has been coined for this type of behaviour.[108]

It is easy to see why, with the nitrogen chelates, normal substitution holds since the first bond formed is relatively strong and so is not broken before ring closure can take place. With the more labile carboxylates, however, this may not always be the case e.g. for malonate and other dicarboxylic acids, $k_3 \simeq k_{-2}$.[109] Thus there will be frequent first bond formation and rupture before ring closure can take place i.e. SCS.

The amino acids and their amides provide an interesting case in that they have both nitrogen and oxygen donors. In this case there are two possible pathways for chelation to occur, depending on whether first bond formation is to the nitrogen or oxygen.



Experimentally the formation rate constants of glycine and other amino acids, have been found to be the same as other mononegative ligands, indicating that the M-N bond is formed first and that there is no inhibition to ring closure.[110] If the M-O bond had formed first, slower rates might have been expected, as with the dicarboxylic acids. Consistent with this observation is the similarity of k_f for glycinamide and the di, tri and tetraglycines.[110]

Ring opening on the otherhand takes place via the carboxyl group. This is shown by the pH dependence of k_d.[107] k_d is independent of pH

above pH3 which indicates that the breaking of the bond between the carboxyl group and metal-ion is rate determining.

Although with Ni(II), chelation occurs at the 'normal' rate, there is a certain amount of steric control since the rate decreases as the ring size increases [110,111] e.g. $NH_2CH_2COO^-$ (4×10^4), $NH_2CH(C_2H_5)COO^-$ (1×10^4), and $NH_2CH(CH_3)CH_2COO^-$ (4×10^3). Also both β-alanine[107] and β-aminobutyric[111] acid which form six-membered chelate rings react more slowly than their α counterparts.

Protonation of one of the coordination sites of a bidentate ligand might be expected to slow down the reaction rate by decreasing the rate of ring closure. The reaction of Ni(II) with enH^+, however, proceeds at the same rate as $N^+(CH_3)_3CH_2CH_2NH_2$ which is unidentate.[107,110] The rate is also that expected for a monoprotonated ligand. This indicates that the effect of the proton is merely to decrease K_{os}.

If on the otherhand the proton can effectively block the initial site of coordination, the reaction rates are abnormally slow. This is the case with $bipyH^+$ and $phenH^+$ which react abnormally slowly with Ni(II).[113] This cannot be interpreted in terms of K_{os}, but rather represents the difficulty the metal has in getting to the protonated site.

The most striking examples of unreactivity of monoprotonated ligands are given by the amino acids and their amides. These react 10^4 times slower with Ni(II) when they are protonated, and in fact their unreactivity may represent an extreme SCS.[110,114] The reason for this unreactivity is that since the amino groups is protonated, first attachment of the ligand

must be via the very labile carboxyl group. This leads to sterically controlled substitution. Strong molecular interactions, which are present in the zwitterion may also be responsible for some of the unreactivity, since these would have to be broken before chelation could occur.

Finally since the 'normal' formation rate constants are independent of the ligand it is the dissociative rate constants, k_d, which govern the stability of the complex. These will then vary according to the basicity of the ligand. Similar relationships have been found between k_d and the protonation constants of amines[115] and carboxylic acids.[109]

It might be thought that the differentiation between the two substitution mechanisms i.e. 'normal' and SCS would be relatively simple and just depend on whether the formation rate constant, k_f, was 'normal' or not. This however, is not always the case as is shown by hydroxyproline[116] and 3,4-dihydroxyphenylalanine.[117] From Table 4.1 it can be seen that the k_f values for Cu(II), Ni(II) and Co(II) with these ligands are significantly less than their unsubstituted counterparts. Either this decreased reactivity represents a shift to a different rate determining step, such as ring closure, or else the hydroxyl groups are interfering with the reaction by say hydrogen bonding and so misorientating the ligand for coordination. Since however, the effect of the hydroxyl substituents appear the same for all three metal-ions, it is suggested that the latter explanation holds.

TABLE 4.1

Typical Formation Rate Constants for Ni(II), Cu(II) and Co(II).

Ligand	Charge	k_f	Ref.
(a) Normal Substitution			
Ni(II) - Malonic Acid	-2	7×10^4	119
L-Cysteine	-1	1.5×10^4	112
α alanine	-1	2.0×10^4	108
glycyglycine	-1	2.1×10^4	120
proline	-1	3.4×10^4	116
Hydroxyproline	-1	1.2×10^4	116
3,4 dihydroxyphenyl-alanine	-1	2.2×10^3	121
L-tyrosine	-1	1.4×10^4	121
Ammonia	0	3.3×10^3	122
1,10-phenanthroline	0	3.9×10^3	113
Cu(II) - proline	-1	2.5×10^9	116
hydroxyproline	-1	7.4×10^8	116
3,4 Dihydroxyphenylalanine	-1	1.1×10^8	121
L-tyrosine	-1	1.1×10^9	121
Co(II) - proline	-1	3.5×10^5	116
hydroxyproline	-1	7.0×10^4	116
3,4 dihydroxyphenylalanine	-1	4.3×10^5	121
L-tyrosine	-1	1.3×10^6	121
(b) Sterically Controlled Substitution.			
Ni(II) β-alanine	-1	1×10^4	108
β-aminobutyric acid	-1	4×10^3	111

With these considerations in mind a kinetic investigation of the penicillins was undertaken. Previous kinetic studies of the penicillins dealt exclusively with the rate of hydrolysis of the β-lactam ring and its potentiation by Cu(II).[13] The rate of reaction with the metal being fast compared to any of the subsequent steps. Using the data obtained from a number of structurally related compounds these workers were able to propose a site for the intermediate metal penicillin complex. Our objective in this research program was to, firstly obtain some of the reaction parameters of the first step of their reaction sequence, and to see if these could lead to an assignment of structure for the metal complex. This was intended as a confirmation of the n.m.r. and potentiometric studies. The metal chosen for study was Ni(II). While this was not an ideal choice since Cu(II) and Mn(II) had been used in the n.m.r. study and Cu(II) 'catalysis' the hydrolysis of penicillins while Ni(II) does not, it was the only metal-ion whose kinetics was slow enough to be studied using stopped flow, temperature jump and pressure jump not being available in this laboratory. However, it was felt that useful deductions might still be obtained using only this metal and a variety of ligands.

As has been stated previously, the metal substitution reaction rates are invariably independent of the ligand. Only where the reaction mechanism is not 'normal' i.e. SCS, do the ligand characteristics play a part. However, the two most likely chelation sites of metal-ions to penicillin occur at either end of the molecule (see Fig. 1.3). Chelation as in structure I involves coordination to two neutral donors, while in structure II coordination is to a negative ligand. Wilkins et al have

shown in their study of 4,7-diphenyl - 1,10-phenantholine disulphonate[118] that a charge located far from the coordination site has little or no effect on the kinetics. Hence it was envisaged that coordination at either of these two sites would be reflected in the reaction rate. Similarly the site of coordination of ampicillin could possible be determined.

4.2 Results and Interpretations.

4.2.1 Penicilloic Acid, Ampicillin and Thiaproline.

The reactions between Ni(II) and penicilloic acid, ampicillin and thiaproline were followed using a stopped-flow reactor, the absorbance change accompanying reaction being monitored. In the case of thiaproline and ampicillin these absorbance changes were extremely small and so high noise levels were encountered. These limited the accuracy with which the reactions could be studied. The experimental conditions were chosen such that only mononuclear complexes were formed. In each case the reaction was shown to be first-order with respect to the ligand concentration by the exponential optical density vs. time traces that were obtained, the metal always being in at least 10 fold excess. The exponentiality of the traces was confirmed by semilogarithmic plots of $|A_\infty - At|$ against time which were linear over several half lives. From the gradients of these lines the experimental first-order rate constants were obtained since:

$$t = -\frac{1}{k_{OBS}} \ln|A_\infty - At| + \text{constant} \qquad \ldots\ldots\ldots (4.1)$$

The dependence of the rate constant upon the metal-ion concentration was investigated by varying the metal concentration, while keeping the pH constant. The reaction was found to be first-order with respect to this component. Secondly the pH dependence of the reaction rates were investigated. Thiaproline, penicilloic acid and ampicillin were found to exhibit the

same behaviour towards Ni(II) as other amino acids[110] in that, if the Ni(II) concentration is held constant and the pH varied, k_{OBS} increases sharply with increasing pH. This pH effect indicates that more than one species is reactive towards the metal and that the concentration of the more reactive species increases with increasing pH. In this case it is the deprotonation of the zwitterion that occurs as the pH is raised. By analogy then, with other amino acid systems, the reaction mechanism proposed is the same as that of Cassatt and Wilkins,[110] viz:

$$M + L \xrightarrow{k_1} ML$$

$$M + LH \xrightarrow{k_2} ML + H^+$$

$$LH \underset{}{\overset{K_a}{\rightleftharpoons}} L + H^+$$

where $L = L^-$, L^- and $L^=$ for the three ligands thiaproline, ampicillin and penicilloic acid respectively. For simplicity the charge on the metal-ion has been omitted. K_a is the acid dissociation constant, which was determined potentiometrically (see Chapter 3) for thiaproline(pKa 6.1) and penicilloic acid (pKa 5.19), and obtained from the literature for ampicillin[89] (pKa 7.25).

The rate equation resulting from this mechanism is (see appendix for derivation):

$$k_{OBS} = [M] \frac{k_2[H^+] + k_1K_a}{K_a + [H^+]} \qquad \ldots\ldots\ldots (4.2)$$

This equation has the expected linear dependence on the metal-ion concentration, for a first-order reaction. Hence plots of k_{OBS} against $[M]$, at constant $[H^+]$, should be linear with gradient:

$$\text{slope} = \frac{k_2[H^+] + k_1K_a}{K_a + [H^+]} \qquad \ldots\ldots\ldots (4.3)$$

These plots are shown in Figs. 4.1 - 4.3 for the three ligands studied.

Equation 4.3 can be rearranged to give:

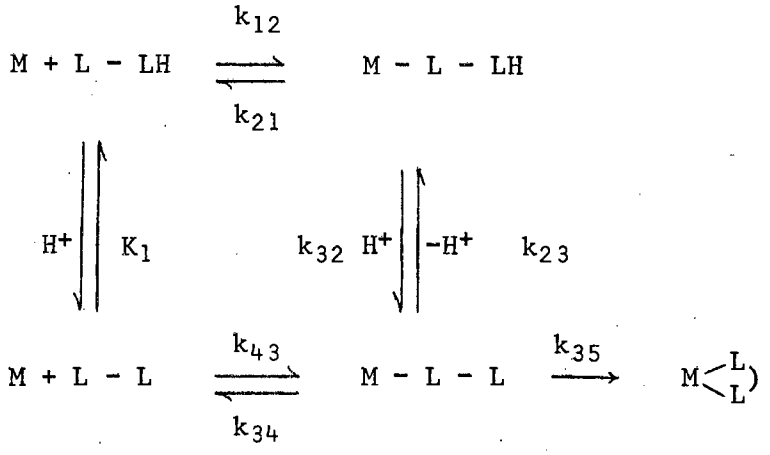
$$\text{slope}\ \frac{(K_a + [H^+])}{[H^+]} = k_2 + \frac{k_1K_a}{[H^+]} \qquad \ldots\ldots\ldots (4.4)$$

Hence a graph of $\text{slope}(K_a + [H^+])/[H^+]$ against $1/[H^+]$ should be linear with intercept k_2 and gradient k_1K_a. These graphs are shown in Figs.4.4-4.6 and the resolved rate constants are given in Table 4.3.

The satisfactory fit of the data to the rate equation increases our confidence in the proposed mechanism. Several points must, however, be made. In the analysis the reverse reactions of k_1 and k_2 were not considered. If this mechanism had been proposed it would not have affected the analysis of the forward rate constants in any way. The dissociative rate constants would appear as intercepts in the k_{OBS} vs. $[M]$ plots (equation 4.2). These intercepts, however, could not be determined with a sufficient accuracy to allow any meaningful analysis to be carried out on them. This was because of the error involved in the analysis of the oscibliscope reaction traces, which had a high noise level. Also the

dissociative rate constants are small which means that a large emphasis has to be placed on small differences in the intercepts.

Secondly a more complex ring closure mechanism could have been proposed.[113]



If stationary state concentrations are assumed for all non-chelating structures, then the rate equation 4.5 is obtained:

$$k_{OBS}\ \frac{(K_1 + [H^+])}{[H^+]} = \frac{k_{12}k_{23}k_{35} + k_{23}k_{35}k_{43}K_1[H^+]^{-1}}{k_{21}k_{32}[H^+] + k_{23}(k_{35} + k_{34})} \quad \ldots (4.5)$$

If $k_{21}k_{32}[H^+] < k_{23}(k_{35} + k_{34})$, which is most likely in the pH range at which this study was undertaken, then:

$$k_{OBS}\ \frac{(K_1 + [H^+])}{[H^+]} = \frac{k_{12}k_{35}}{k_{35} + k_{34}} + \frac{k_{35}k_{43}}{(k_{35} + k_{34})}\ \frac{K_1}{[H^+]} \quad \ldots\ldots (4.6)$$

which is the form of equation 4.4 with $k_1 = k_{35}k_{43}/(k_{35} + k_{34})$ and

$k_2 = k_{12}k_{35}/(k_{35} + k_{34})$. Hence the analysis as carried out cannot distinguish between these two mechanisms. However, if $k_{21}k_{32}[H^+] \simeq k_{23}(k_{35} + k_{34})$, then a linear relationship between $k_{OBS}(K_1 + [H^+])/[H^+]$ and $1/[H^+]$ no longer exists. This may account for the small deviation from linearity shown by penicilloic acid, where excellent oscilliscope traces were obtained and a better fit may have been expected. The fit of the data for the other two ligands, however, is well within the experimental error.

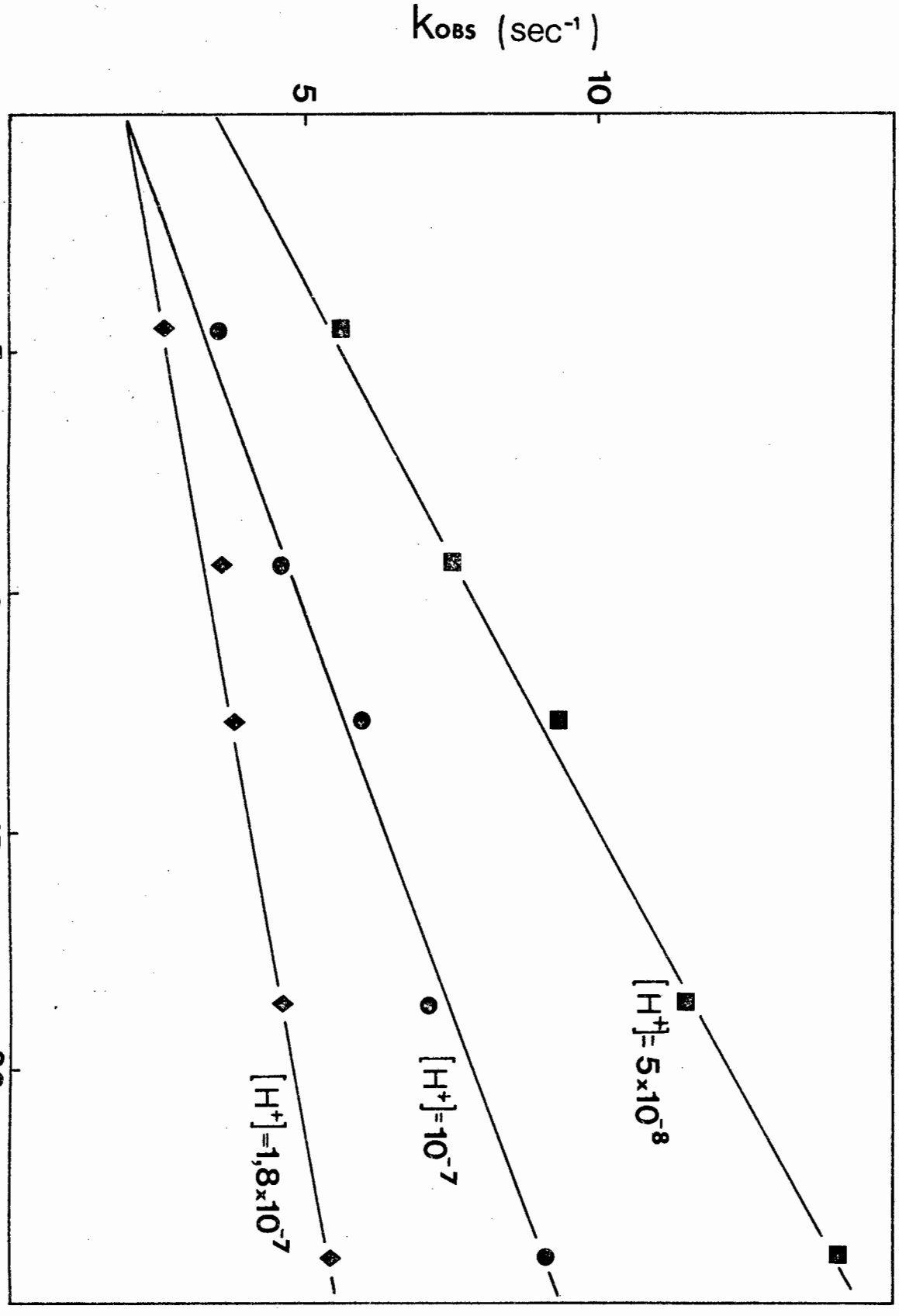


FIG. 4.1

k_{OBS} vs $[Ni^{2+}]$ at constant $[H^+]$ for ampicillin.

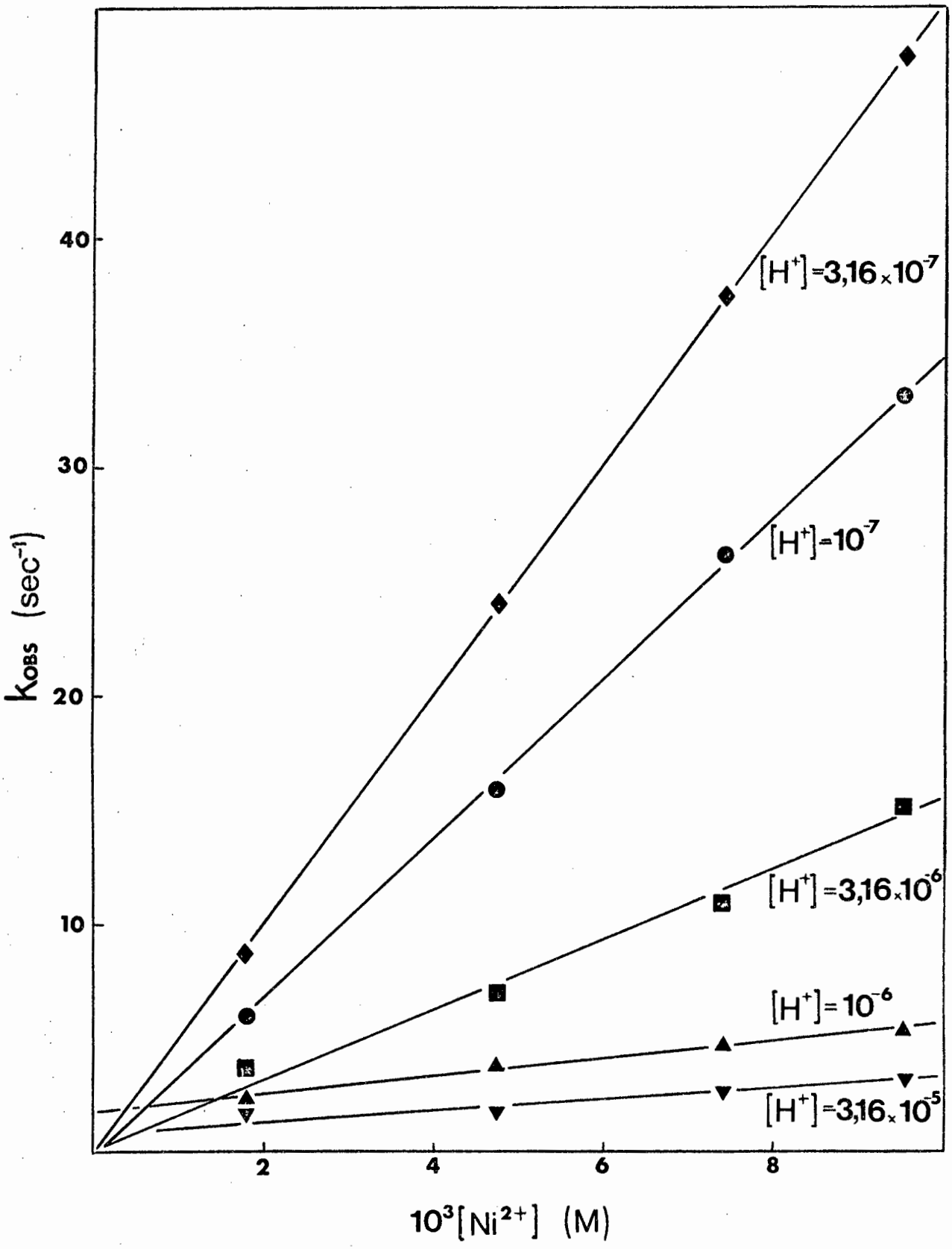


FIG. 4.2

k_{OBS} vs $[Ni^{2+}]$ at constant $[H^+]$ for penicilloic acid.

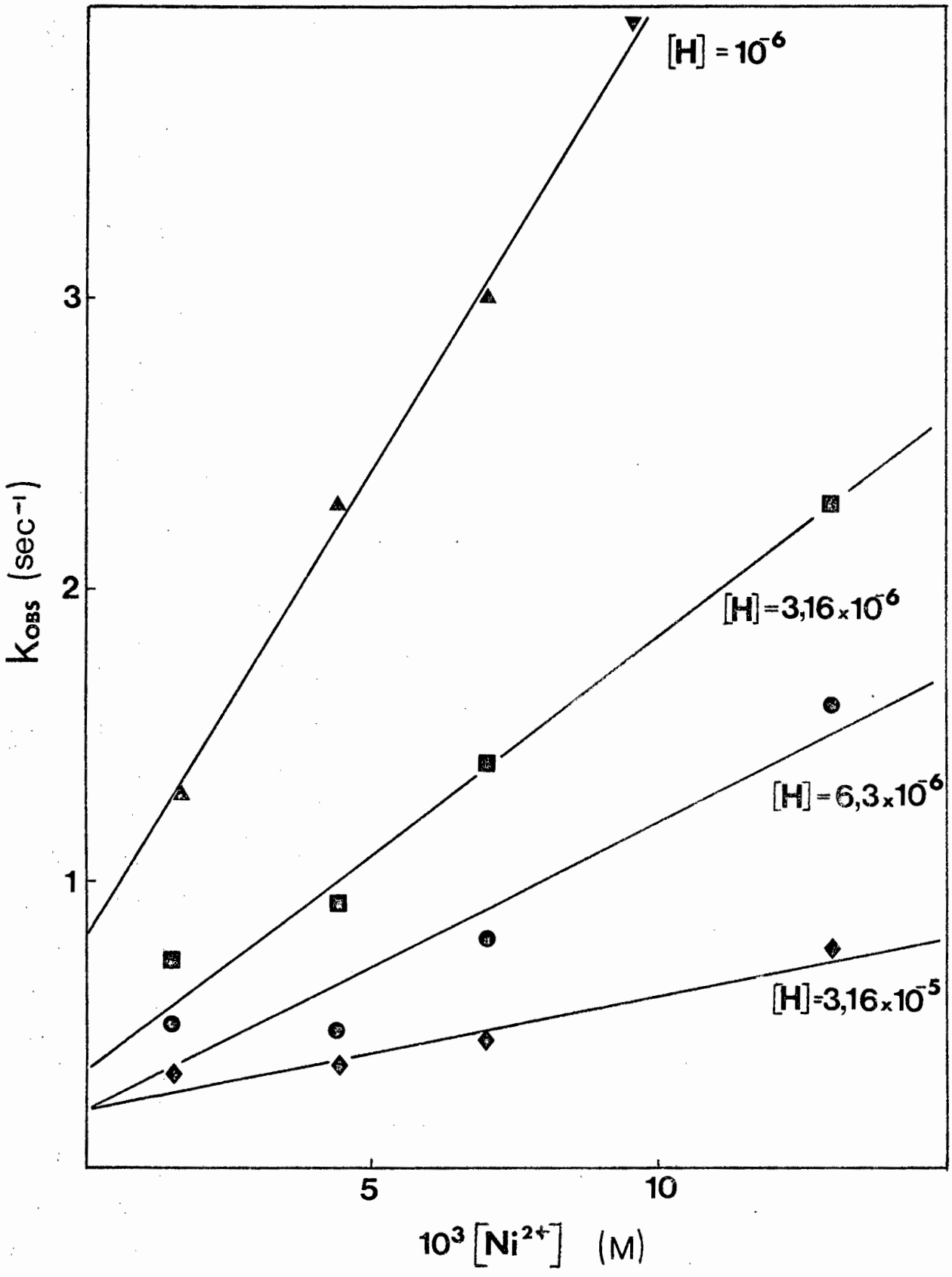
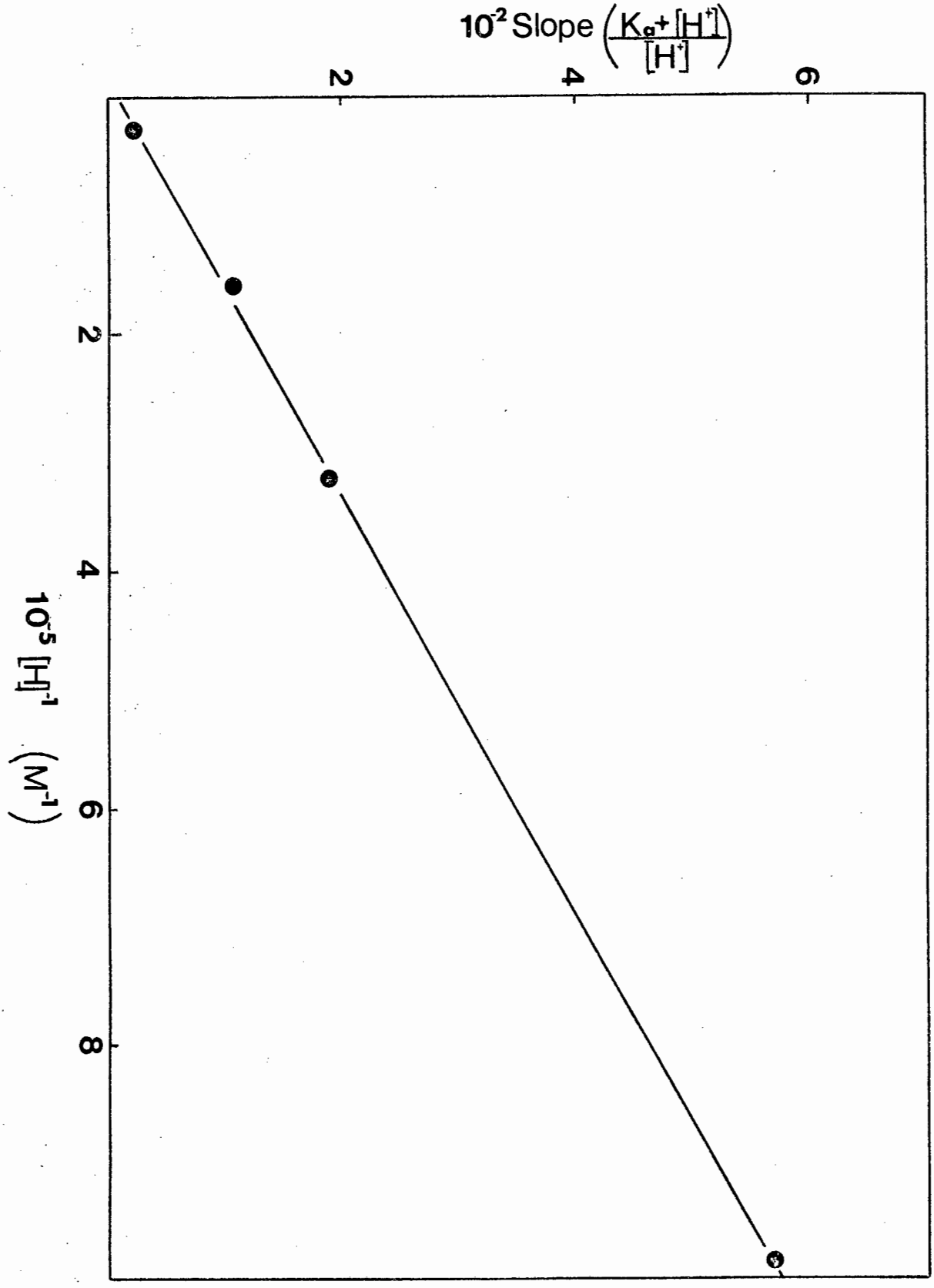


FIG. 4.3

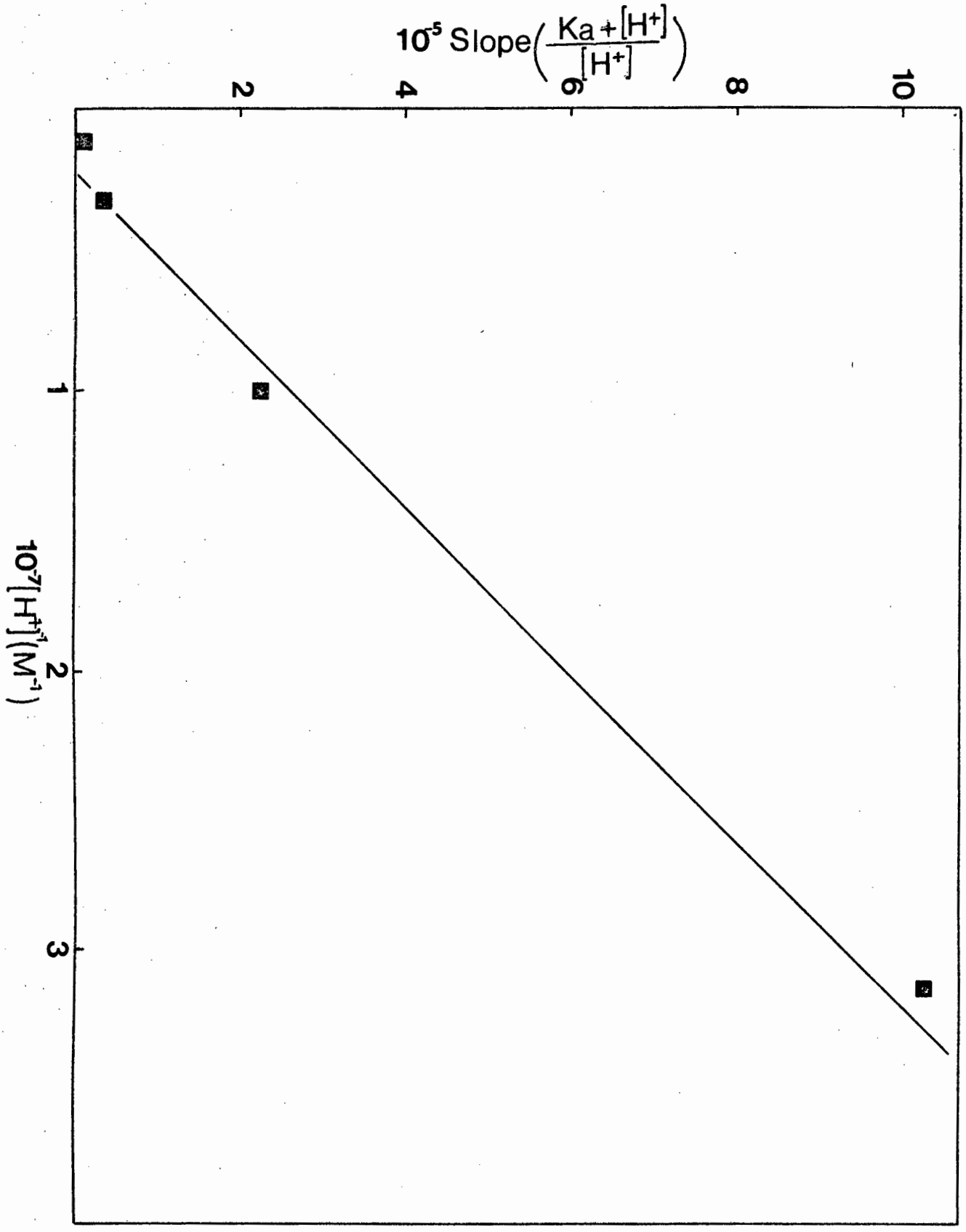
k_{OBS} $[Ni^{2+}]$ at constant $[H^+]$ for thiaproline.

FIG. 4.4



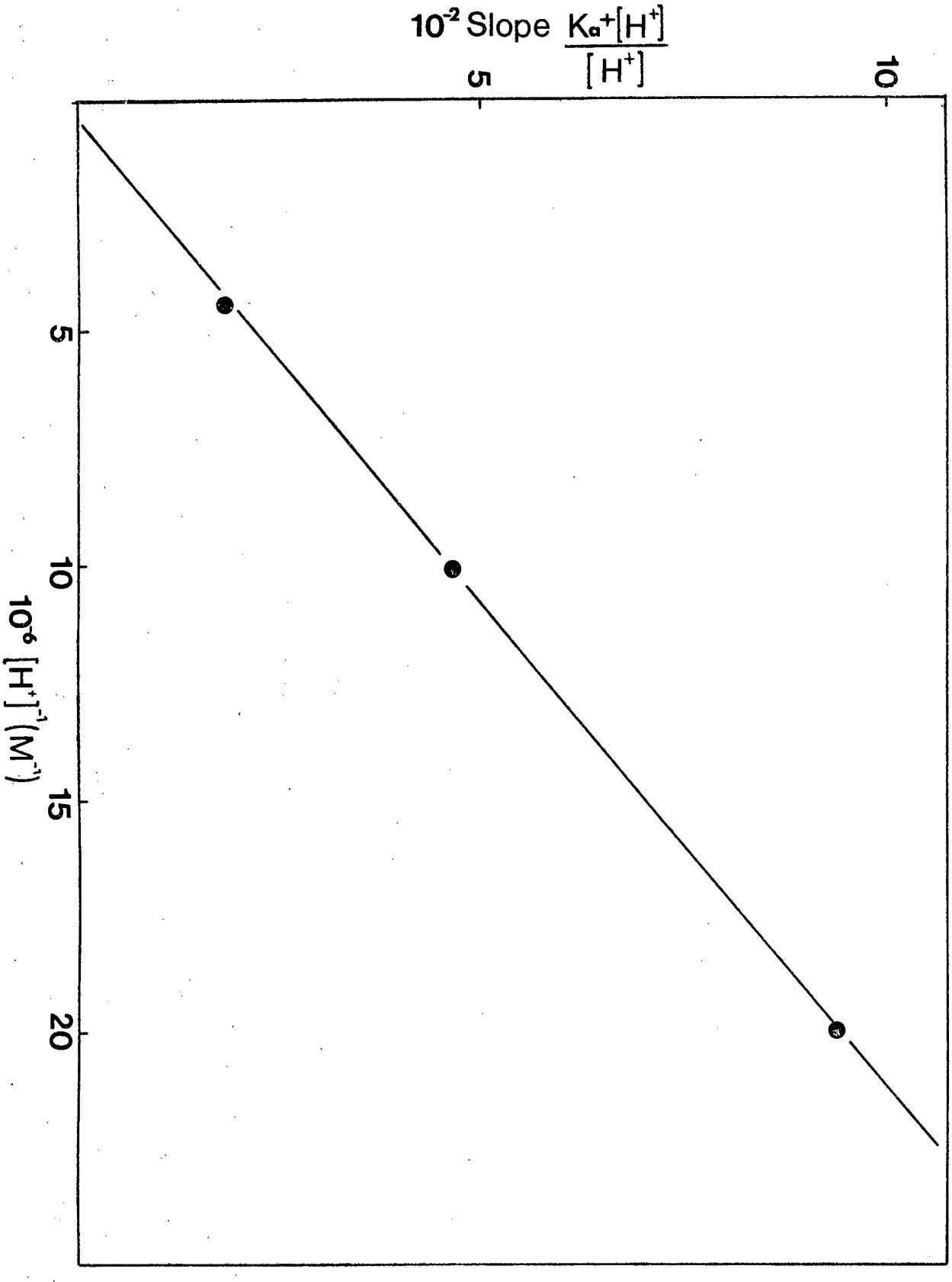
Slope $\left(\frac{Ka + [H^+]}{[H^+]}\right)$ vs $^{1}/[H^+]$ for thiaproline.

FIG. 4.5



Slope $\left(\frac{Ka + [H^+]}{[H^+]}\right)$ vs $1/[H^+]$ for penicilloic acid.

FIG. 4.6



Slope $\left(\frac{Ka + [H^+]}{[H^+]}\right)$ vs $^{1}/_{[H^+]}$ for ampicillin.

4.2.2 Benzylpenicillin.

The reaction between benzylpenicillin and Ni(II) was also followed using a stopped-flow reactor. Once again the absorbance changes accompanying reaction were small, resulting in a high noise level. The reaction was found to be first order with respect to the ligand concentration, the metal-ion concentration being in at least 10 fold excess. The reaction was also shown to be first-order with respect to the metal-ion concentration by the linearity of the k_{OBS} vs. $[Ni^{2+}]$ plot (Fig. 4.7). The $[H^+]$ dependence of the rate equation was also investigated in the pH range 6.1 to 7.8, and found to be independent of $[H^+]$. This is shown in Table 4.2.

TABLE 4.2

Experimental Rate constants for the Reaction between Benzylpenicillin and Ni(II) at constant metal-ion concentration.

Temp. 25°C ; μ = 0.15 M ; $[Ni^{2+}]$ = 7.5×10^{-3} M

pH	6.1	6.6	6.8	7.1	7.3	7.7	7.8
k_{OBS}	34.4	43.3	34.3	36.2	43.8	41.9	40.1

The mechanism proposed for the reaction, is therefore:

$$Ni^{2+} + L^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} NiL^+$$

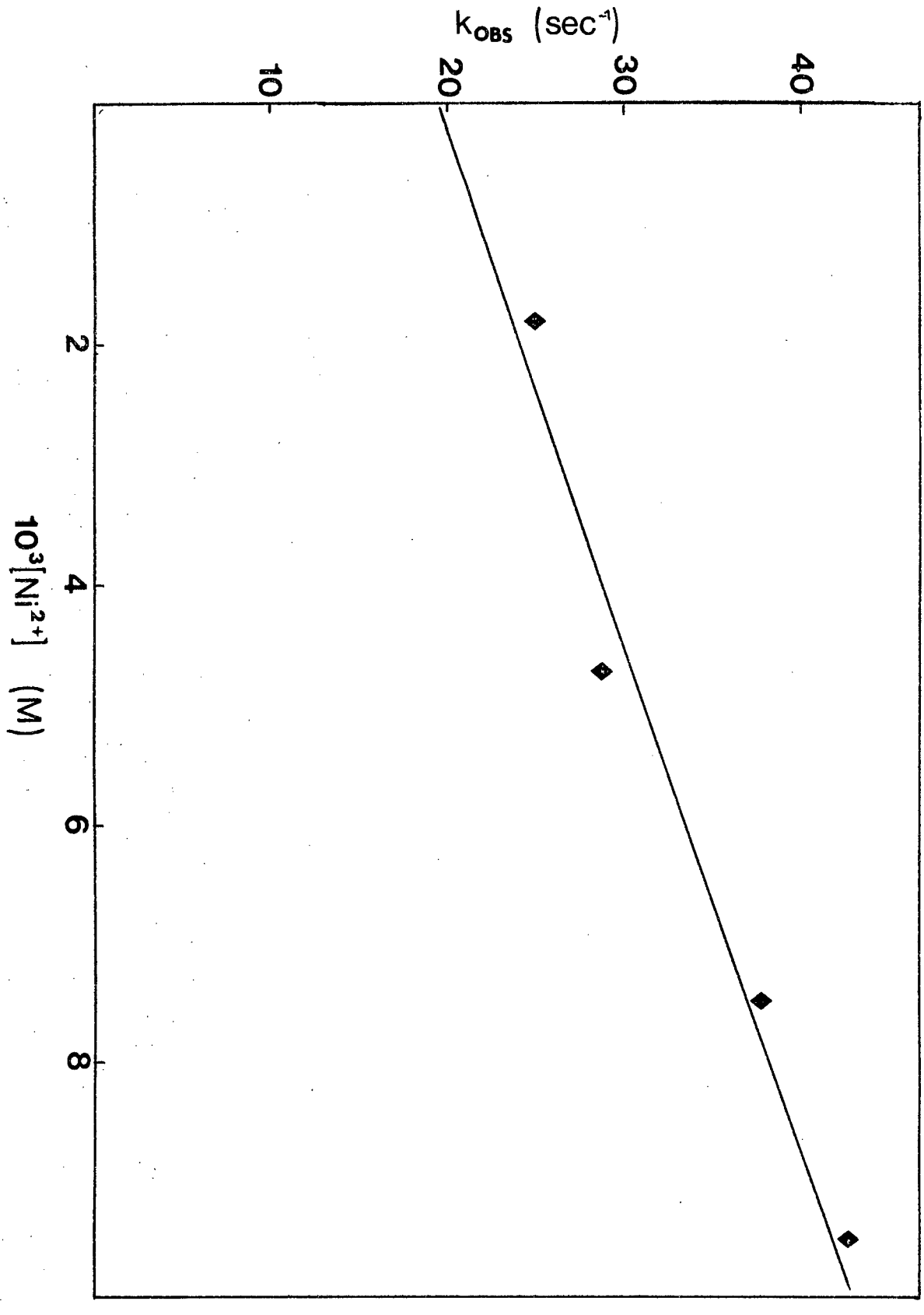
for which the rate equation 4.7 is obtained:

$$k_{OBS} = k_1 [Ni^{2+}] + k_2 \quad \ldots\ldots\ldots (4.7)$$

The pathway via the protonated species has been omitted because the study was conducted well above the pKa of this species. From the graph of k_{OBS} vs. $[Ni^{2+}]$ (Fig. 4.7) the rate constants k_1 and k_2 were obtained as gradient and intercept respectively. These are tabulated in Table 4.3 along with the rate constants of the other ligands investigated.

That the reaction rate was independent of $[H^+]$ was not surprising in view of the potentiometric investigation of this ligand which showed the absence of a basic nitrogen centre. An attempt was made to determine the reaction rates of the protonated species by working in the pH range 2.5 - 3.5. In this region, however, the extent of reaction and the absorbance changes accompanying reaction were prohibitive.

FIG. 4.7



k_{OBS} vs $[Ni^{2+}]$ for benzylpenicillin.

4.3 Discussion.

As was stated at the beginning of this section the objective of this study was firstly to obtain kinetic information about the Ni(II) - antibiotic coordination reaction and secondly to see if this information could lead to an assignment of structure for the complex in solution.

TABLE 4.3

Rate Constants for the Formation (k_f) and Dissociation (k_d) of Ni(II) complexes at 25°C and ionic strength 0.15 M

LIGAND	Reacting form of Ligand	k_f M^{-1} sec^{-1}	k_d sec^{-1}
Thiaproline	L^-	$7,5 \times 10^2$	$0,15^a$
Penicilloic Acid	$L^=$	$5,2 \times 10^3$	$4,3^a$
Ampicillin	L^-	$6,9 \times 10^2$	-
Penicillin	L^-	$2,3 \times 10^3$	19,3

(a) Calculated using $k_d = k_f/K^{116}$

The most striking feature of the reaction kinetics is the unreactivity of the protonated ligand which was experimentally not distinguishable from zero. This is in sharp contrast to the rate constant of enH^+ (6×10^2). This decreased reactivity of protonated amino acids has been explained in

terms of an extreme sterically controlled substitution (S.C.S.) mechanism, since upon protonation first bond formation must switch from the amino group to the carboxylic group. Here the rate of ring closure is comparable with, or smaller than, the rate at which the first bond formed is broken. Undoubtedly a certain contribution to the unreactivity must arise from strong intramolecular interactions which are present in the zwitterion, and have to be broken before coordination can take place.

The rate constant for thiaproline is much lower than that expected for a 'normal' substitution mechanism. There are two possible explanations for this; either there has been a shift to another rate determining step i.e. S.C.S. or else, hydrogen bonding between the sulphur and coordinated water molecules is occurring. This would misorientate the ligand with respect to metal coordination, thus lowering the rate constant. Comparison with proline and hydroxyproline suggest that it is the former which occurs.[116] The addition of an hydroxyl substituent onto the proline ring was found to decrease the forward rate constant from $3,4 \times 10^4$ to $1,2 \times 10^4$. Since this effect was found to be the same for Cu(II) and Co(II) it was concluded that a shift to another rate determining step had not taken place, but rather, that hydrogen bonding was misorientating the ligand. Our value for k_f is still an order of magnitude less than that of hydroxyproline, and since hydrogen bonding via the sulphur atom of thiaproline is expected to be less than that of the hydroxyl group of hydroxyproline, a shift to another rate determining step is postulated. Unfortunately the rate constants of Cu(II) and Co(II) with this ligand, are outside the range of the stopped flow reactor, and so the postulate could not be confirmed.

It is not unreasonable, though, since the inductive effect of the sulphur is known to reduce the basicity of the nitrogen by c.a. 4 log β units. This will increase the rate at which the first bond formed is broken, perhaps to a value comparable with the rate of ring closure. This would mean a shift to another rate determining step. That the inductive effect of the sulphur weakens the metal-nitrogen bond can be seen from the dissociative rate constant (0.15) which is higher than that of proline (0.024) and hydroxyproline (0.014). The slower rate for hydroxyproline relative to that of proline is attributed to hydrogen bonding inhibiting the release of the ligand. Hence if this does occur with thiaproline it is outweighed by the inductive effect of the sulphur.

The forward rate constant of benzylpenicillin is also lower than that expected for a 'normal' substitution mechanism. However, the rate is higher than that of thiaproline. One possible explanation for this is that, firstly a S.C.S. mechanism is operative which decreases the rate constant while hydrogen bonding to the β-lactam carbonyl orientates the molecule for coordination, increasing the rate constant in an internal conjugate base type mechanism.[122] These two opposing mechanisms would result in a rate constant higher than that of thiaproline but lower than 'normal'. From the discussion of thiaproline the possibility of hydrogen bonding decreasing the reaction rate was discounted, since this would lead to a rate constant lower than that of thiaproline.

The other possible explanation of the rate constant is that coordination does not involve the carboxyl group but rather occurs between the β-lactam carbonyl and side chain amide nitrogen (structure I Fig. 1.3).

Coordination here would involve a neutral species for which the 'normal' substitution rate is ~3 x 10^3. The value of 2,3 x 10^3 for benzylpenicillin is in good agreement with this. However, this value for 'normal' substitution was obtained from the rate constants of strong nitrogen chelates. If, on the otherhand, comparisons with acetylacetonate were made, the agreement would not be so good. The enol form of acetyacetonate, has a rate constant of 5,0.[123] This is because a S.C.S. mechanism is operative. For the same reason that a S.C.S. mechanism applies to acetylacetonate, so it would be expected to apply to coordination at the penicillin side chain, and a rate constant substantially less than 10^3 would be expected. For this reason, and also by comparison with penicilloic acid, the former postulate is favoured.

The rate of reaction between Ni(II) and penicilloic acid is 5,2 x 10^3. The problem is whether this represents coordination with a bidentate or tridentate ligand. From the potentiometric study we suspect that it is acting as a bidentate ligand, the one carboxyl group not being involved in chelation. If this is the case a rate constant of ~2 x 10^4 is expected for 'normal' substitution. The actual value is less than this and close to that for penicillin. Thus we conclude that the same mechanism is operative in both cases, and again the increased dissociative rate constant reflects the basicity of the nitrogen.

The reaction between Ni(II) and ampicillin differs from the other three ligands in that coordination involves the side chain amino group, which is present in this molecule. Thus the ligand acts as a neutral species, the charge being located far from the site of coordination, and a

rate constant of 3-4 x 10^3 is unexpected for 'normal' substitution. The actual value of 6,9 x 10^2 is lower than this. A S.C.S. mechanism is not favoured here as coordination involves a nitrogen of pKa 7.25, and there is no ring strain. Hence the most probable reason for the decreased reactivity is hydrogen bonding.

4.4 Experimental.

The reaction between $Ni(ClO_4)_2$ and ampicillin (Beecham Research Laboratories), thiaproline (Sigma Chemical Co.), benzylpenicillin (Glaxo-Allenburys (Pty) Ltd.) and penicilloic acid were followed spectrophotometrically on a Durrum D-110 stopped-flow reactor. The U.V. spectra of the ligands alone and in the presence of $Ni(ClO_4)_2$ were recorded, on a Beckman DK-2A spectrophotometer, in order to determine the wavelengths at which the maximum absorbance change accompanied reaction. These had to be varied slightly in order to optimise the signal to noise ratio of the stopped-flow reactor. The wavelengths were 220 nm for thiaproline, 225 nm for ampicillin, 250 nm for penicilloic acid and 220 nm for benzylpenicillin. The penicilloic acid was prepared from benzylpenicillin by the method of Rapson and Bird,[89] and the $Ni(ClO_4)$ from $NiCO_3$ and $HClO_4$.

All reactions were studied at $25^{\circ} \pm 0.5^{\circ}C$ and an ionic strength of 0,15 M ($NaClO_4$), a 0.02 M borax/manitol buffer being used to maintain the pH. The pH measurements were made on a Metrohm E 300 B pH meter using a combination of a Russel pH Ltd. glass electrode (SF 75/C14) and a calomel electrode in which the KCl had been replaced by NaCl to prevent the precipitation of $KClO_4$ in the porous membrane. The meter was calibrated with Beckman buffers of pH 4 and 7. The pH of each solution was adjusted by dropwise addition of concentrated $HClO_4$ or NaOH.

The reactions were studied under pseudo-first-order conditions with the nickel concentration in at least 10 fold excess of the ligand. The ligand concentration was 10^{-4} M. k_{OBS} could then be obtained from semilogarithmic plots of t against $|A_{\infty} - At|$.

CHAPTER 5

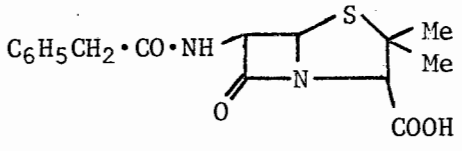
GENERAL DISCUSSION.

There are several possible sites of coordination of metal-ions to penicillin. The complex may be monodentate, involving the carboxyl group or ring sulphur, or bidentate, involving the carboxyl group and ring nitrogen or the β-lactam carbonyl and side chain amide nitrogen. It is not possible for both amide nitrogens to be coordinated to the same metal-ion, or for chelation between the β-lactam carbonyl and nitrogen to occur. Of the possible sites, that of the ring sulphur can be eliminated by comparison of the stability constant of the Cu(II)-penicillin complex with the stability constants of several related compounds, determined by Weiss et al.[23] These are given in Table 5.1. The similarity between the formation constants of the compounds with and without a ring sulphur allows this possible site of coordination to be eliminated. This leaves three possibilities, one monodentate and two bidentate.

TABLE 5.1

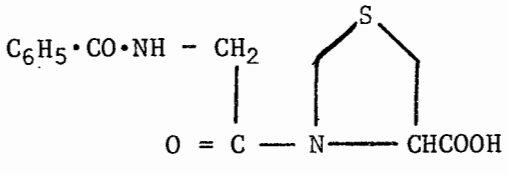
Formation constants of Cu(II) complexes of compounds structurally related to penicillin.

Benzylpenicillin



log K = 2.63[13]

N-hippuryl-thiazolidine-4-carboxylic acid.

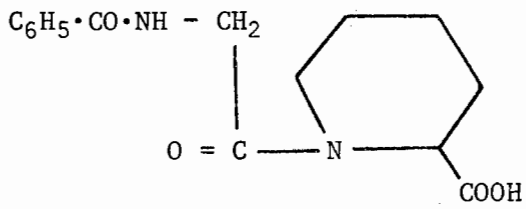


log K = 1.8[23]

(...Continued...)

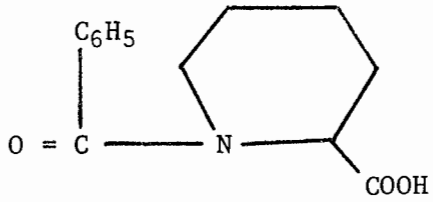
(Table 5.1 - continued)

N-hippurylpipecolinic acid



log K = 2.1[23]

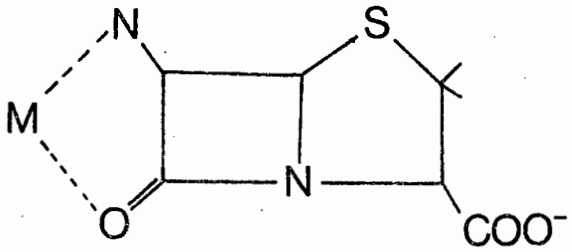
N-benzoylpipecolinic acid



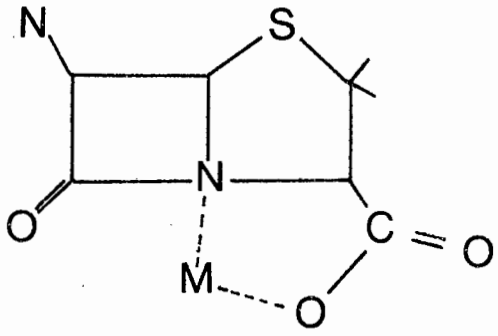
log K = 1.8[23]

Cressman et al,[13] in an attempt to determine the mechanism and site of complexation, studied the rate of hydrolysis of various penicillins and related compounds in the presence and absence of Cu(II). Based on their reaction scheme, they were able to measure the formation constants of the Cu(II) complexes, at various temperatures. This led to a knowledge of ΔH and ΔS, which these workers claimed, suggested a chelate type structure for the complex. However, the accuracy of this type of ΔH and ΔS determination is questionable. Notwithstanding this, it is highly likely that the Cu(II)-penicillin complex is chelating, otherwise, if the metal-ion did not interact directly with the β-lactam it would be extremely difficult to rationalise the 10^6 effect that Cu(II) has on the rate of hydrolysis. Also infra-red studies on solid metal complexes of penicillin, indicate that the β-lactam is involved

in coordination. Therefore, the two most likely sites of coordination are:



I



II

In an attempt to differentiate between these two possible structures Cressman et al[13] studied the rate of hydrolysis of 6-amino-penicillanic acid (6APA), 6-bromopenicillanic acid and penicillanic acid. The hydrolysis rates obtained for these compounds in the presence of Cu(II) are given in Table 5.2.

It can be seen immediately that, in the presence of Cu(II), the substituent at the 6 position has a considerable effect on the rate of hydrolysis of the β-lactam ring. This led Cressman et al[13] to propose that chelation occurred at site I. However, these workers failed to report the rate of hydrolysis of these compounds, in the absence of Cu(II). Comparisons can, however, be made with other β-lactam systems.

Table 5.3 indicates the rate of hydrolysis of everal monocyclic and thiazolidine-β-lactams. Compound I, II and III differ only in the type

of substituent at the 3-position of the 2-azetidinone nucleus, and there is a considerable difference in their rates of hydrolysis. This may be due to steric inhibition of attack at the carbonyl group during hydrolysis, as has been shown to be the case for phenyl substituents in the α-position of ethylesters of aliphatic acids.[124] The other possibility is that the phenyl groups have a polarising effect on the β-lactam carbonyl.

TABLE 5.2

Half-life of hydrolysis reaction of various penicillins at pH 5.5 and 30°C.[13]

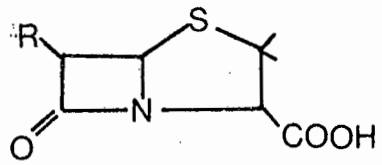
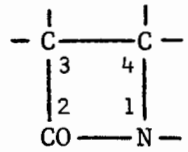
		$t_{\frac{1}{2}}$
penicillanic acid	R = H	>> 20 min
6 - bromopenicillanic acid	R = Br	23 min
6 - aminopenicillanic acid	R = NH_2	40-50 sec.
benzylpenicillin		20-30 sec.

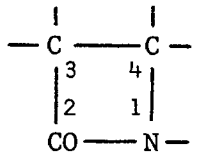
TABLE 5.3

Rates of hydrolysis of some β-lactams.[125]

		Conditions of hydrolysis	Extent of hydrolysis
I	1,3,3,4-tetraphenyl-	0.5 M KOH in MeOH, reflux 25 hrs.	very slight
II	1,4-diphenyl-3,3-dimethyl-	0.5 M KOH in MeOH, reflux 25 hrs.	34%
III	1,4-diphenyl-	1.0 M KOH in MeOH, reflux 1 hr.	85%
IV	1-benzyl-3 methyl-4-phenyl	0.12 M NaOH in EtOH, 76°, 29 hrs.	63%
V	1-cyclohexanemethyl-3-methyl-4-cyclohexyl-	0.12 M NaOH in EtOH, 76°, 29 hrs.	No change
VI	1-phenyl-3-phenylacetamido-	1M NaOH, 100°, 90 min	Extensive hydrolysis
VII	1-cyclohexyl-3-cyclohexane-acetamido-	1M NaOH, 100°, 15 min	Extensive hydrolysis
VIII	3-phenylacetamido-1-α-D-isovaleric acid	NaOBu in BuOH, 100°, 1 min	rapid hydrolysis
IX	2,α,α-triphenyl-2-thiazolidineacetic acid	0.1M NaOH, 90°, 3 hrs	No change

...continued ...

TABLE 5.3 - continued:

		Conditions of hydrolysis	Extent of hydrolysis
X	2-phenyl-2-thiazolidine-α-isobutyric acid	0.1M NaOH, refluxed 5 hrs.	No Change
XI	benzylpenicillin	0.1M NaOH, room temp, 1 hr.	extensive hydrolysis

Compounds IV and V differ only in the degree of saturation of the carboxylic ring and hence there must be some effect, other than steric inhibition, which explains the difference in hydrolysis rates. In this regard, it has been shown[126] that the rates of hydrolysis of amides increases with increasing strength of the parent acid. Hence, since phenylacetic acid (pKa 4.25) is a stronger acid than cyclohexaneacetic acid (pKa 4.63), IV is expected to undergo hydrolysis at a faster rate than V, which it is observed to do. The parent amine strengths also differ to some extent and account should be taken of this.

The last three monocyclic β-lactams contain acylamino groups α to the β-lactam carbonyl, and hydrolyse at a rate which approaches that of penicillin. This is compatable with the observation that α-acylamino acids are far stronger than the corresponding aliphatic acids.[124] Hence, by virtue of the above observation that the stronger the parent acid the faster the rate of hydrolysis, β-lactams derived from the α-acylamino acids should hydrolyse at a far faster rate.

The thiazolidine β-lactams, IX and X, are much less reactive than penicillin, neither being appreciably hydrolysed after 3 hours at 90°C in 0.1M NaOH. This may be due to the absence of a carboxyl group in the 4-position, or the lack of an acylamino group α to the β-lactam carbonyl. In view of the above discussion of monocyclic β-lactams it seems likely that the reactivity of penicillin is due, rather to the acylamino group, than to the carboxyl group.

Based on the results obtained for the monocyclic and bicyclic β-lactams it would have been surprising if penicillanic acid, in which the

parent acid is acetic acid (pKa 4.75), had undergone any hydrolysis after 20 min . Glycine (pKa 2.34) and bromoacetic acid (pKa 2.87) are far more acidic, and their amides are therefore, expected to hydrolyse far more rapidly. Hence it can be seen, that while the conclusion made by Cressman et al,[13] that the side chain has a considerable effect on the rate of hydrolysis of the β-lactam, their conclusion that this is due to chelation of the Cu(II) at the side chain is not necessarily correct.

So far all the results obtained in this laboratory support structure II. The n.m.r results are by far the most conclusive as they give a direct picture of the structure of the complex in solution. Qualitatively these results indicated coordination at the carboxyl group, while the quantitative results fitted structure II. It must be pointed out though, that n.m.r only gives the time average structure of the complex and so while structure II is the most predominent species the possibility of other species coexisting in solution cannot be excluded. However, an upper limit of 0.1% could be placed on coordination of the side chain of penicillin. Besides indicating that coordination involved the carboxyl group, the n.m.r gave several surprising results.

Firstly, identical results were obtained for penicillin using both Cu(II) and Mn(II). This was surprising since there is little doubt that it is the coordination of the metal-ion to penicillin which increases the rate of hydrolysis, and it is known that Cu(II) has a marked effect on the rate of hydrolysis, while Mn(II) does not. The most probable explanation of this lies in the degree of metal-nitrogen bonding which

takes place in the complex. Cu(II), being a relatively H.S.A.B soft metal-ion shows a large preference for nitrogen coordination. In this regard Cu(II) is known to coordinate to peptide nitrogens in preference to peptide oxygens.[46] Less is known of Mn(II) peptide binding but the difference in the strength of interaction can be seen from the pKa of the Cu(II) and Ni(II) peptide complexes, which are 6 and 8 respectively. Hence it is postulated that the difference between the heavy metal-ion interactions of penicillin is one of degree - Cu(II) causes the penicillin to be hydrolysed faster than Ni(II), Mn(II) etc., and that this difference in hydrolysis rate is due to the amount of lone pair donation, by the β-lactam nitrogen, to the metal-ion. The more the β-lactam nitrogen donates its lone pair to the metal-ion, the more labile the amide bond will be.

The above hypothesis, while explaining the difference in reactivity of the Cu(II) and Ni(II) penicillin complexes, does not account for the high rate of degradation produced by Zn(II), since this metal-ion is even harder (H.S.A.B) than Ni(II). Besides noting that Zn(II) rapidly inactivates penicillin, little work has been done on the mechanism and degradation products of this hydrolysis. Ayim and Rapson,[127] in a preliminary publication, noted that while Zn(II) and Cu(II) 'catalyse' the alcoholysis of penicillin their mechanisms differ. Perhaps then, this is the reason for the difference in reactivity of Ni(II) and Zn(II).

The second interesting observation, to emerge from the n.m.r study, was the site of complexation of Mn(II) to 6APA. This ligand has a free amino group and complexation is expected to occur here. However, this is not

observed to be so, chelation occurring as in penicillin. This behaviour is anomalous, since only in very acid solution, does the site of coordination of the first row transition metal-ions to peptides, switch from the terminal amino group to the terminal carboxyl group.[46] The metal peptide complexes are, however chelating, a five-membered ring being formed upon coordination to the adjacent peptide nitrogen or oxygen. In all cases this five-membered ring is planar, while with 6APA coordination to the amino and β-lactam carbonyl groups cannot produce a planar five-membered ring. It is this lack of planarity which is seen as preventing chelation between these two groups. Since, then, chelation cannot occur to the amino group, coordination occurs preferentially to the carboxyl group. An interesting point to note is that, if coordination does not occur to the amino group of 6-APA it is even less likely to occur at the side chain N of penicillin.

Finally, an interesting feature of coordination was observed with ampicillin and cephalexin. Both these antibiotics have an amino substituent on the side chain, and above pH7 the metal-ion coordinates to this group. A chelate structure is obtained by metal coordination to the peptide bond oxygen (Fig. 2.15). What is surprising about the metal ampicillin and cephalexin interaction is that, below pH5 coordination does not occur exclusively to the side chain amino group. While quantitative results could not be obtained in this pH region, the observed spectral broadening indicated that a certain amount of complexation was occurring to the carboxyl group as in penicillin and cephalothin. This result is surprising in view of the known behaviour of other peptide systems[46] and is taken as an indication of the favourability of the carboxyl site.

While n.m.r gave the conformation of the Cu(II) and Mn(II)-penicillin complexes in solution, it was felt that these results should be confirmed, and extended, using other techniques. Hence a potentiometric investigation of the penicillin system was undertaken. While these results are not definite, they are certainly indicative of coordination involving the carboxyl group. Comparison of the results obtained in this study for Ni(II)-penicillin with those obtained by Cressman et al[13] for Cu(II)-penicillin, and Weiss et al[23] for substituted thiazolidines and piperidines, indicates that coordination is to the carboxyl group. If coordination occurred at the side chain, as suggested by Cressman, similar results would not be expected for N-benzoylpipecolinic acid and N-hippurylpipecolinic acid. Also, since there would be no ring strain involved in coordination to the side chain of N-hippuryl-thiazolidine-4-carboxylic acid, this complex would be expected to be more stable than the analogous penicillin complex. This increased stability is not observed. The only other groups common to all these compounds are the carboxyl and ring nitrogen, and so the conclusion is that coordination is to these two groups. The greater stability of the Cu(II)-penicillin complex, compared with the Cu(II)-N-hippuryl-thiazolidine-4-carboxylic acid complex may be due to the decreased 'amidicity' of the penicillin nitrogen over that of the N-hippuryl-thiazolidine-4-carboxylic acid nitrogen.

In the present study the stability constant of the Ni(II)-penicillin complex was determined. This showed an anomalous stability when compared with the pKa of the ligand, and was taken as being indicative of coordination to the β-lactam nitrogen. This was supported by the results obtained for Ni(II) and Cu(II) with penicilloic acid. Using the pKa correlation,

penicilloic acid was suggested to be tridentate when coordinated to Cu(II). This was confirmed by analysis of the solid metal complex, which could be isolated.

Finally the kinetics of the Ni(II) penicillin interaction was studied. The reaction was found to proceed at a rate much slower than 'normal', and an S.C.S mechanism had to be invoked to explain the results. From the rate of the reaction the conclusion was reached that, either the reaction represented coordination of a neutral, monodentate ligand or a bidentate, mononegative ligand in which a S.C.S mechanism was operative. The reaction could not have been to a mononegative, monodentate ligand (i.e. monodentate to carboxyl group) as the reaction rate was far too slow. Similarly, the reaction could not have involved a neutral bidentate ligand (i.e. coordination to side chain amide and β-lactam carbonyl) as the reaction rate was far too high. Coordination to the β-lactam carbonyl, in a monodentate fashion, could not be eliminated on the basis of the reaction rate, as it was of the right order of magnitude, for coordination to such a centre. However, comparisons with thiaproline and penicilloic acid, which do not have a β-lactam carbonyl, allowed this possibility to be eliminated. Hence, from the kinetic study the conclusion was reached, that with Ni(II),coordination to penicillin is as in structure II.

In the preceeding discussion of the results obtained in this study, it was shown how they all support structure II as representing the metal penicillin complex. Based on a knowledge of the structure of penicillin, and the interaction of metals and peptides the relative merits

of the two sites can be discussed. Site I involves a carbonyl oxygen and an amide nitrogen, while site II involves a carboxyl group and an amide nitrogen. Amide nitrogens are not good ligands because metal coordination results in a loss of the amide resonance. Hence coordination to this group does not occur without loss of the amide proton, which restores the sp^2 character of the nitrogen. In penicillin, the β-lactam nitrogen is distorted from a planar sp^2 structure, and has a large amount of sp^3 character. This can be seen from Table 5.4, which lists the distance of the nitrogen above the plane of its three substituents. For a completely sp^2 hybridised nitrogen there is no distortion from planarity, while for an sp^3 nitrogen (represented by trimethylamine in Table 5.4) the nitrogen is 0.56 A^o above the plane of its three substituents. The nitrogen of penicillin is 0.4 A^o above this plane and so has a considerable amount of sp^3 character, which results in a decrease in the amide resonance. The β-lactam nitrogen of cephaloglycine is not as displaced from planarity as that of penicillin but an enamine resonance with the Δ^3 double bond also reduces the amide resonance. This accounts for the lability and antibiotic activity of the penicillins and Δ^3 cephalosporins. Since the β-lactam nitrogen does not have the same 'amidicity' as the side chain amide nitrogen it is expected to be a better ligand. Also carboxyl groups are known to coordinate first row transition metals in preference to carbonyl groups, so in this regard coordination at site II is expected to be stronger than site I.

Another important factor in the relative stability of the two sites is the geometry of the coordination centres. Table 5.5 shows the molecular parameters obtained for the penicillin and cephalosporin Cu(II)

complex, for the two possible coordination sites, assuming typical metal to nitrogen and oxygen bond lengths and angles. Since the β-lactam carbonyl is sp^2 hybridised its lone pairs will lie in the plane of the β-lactam ring, and at an angle of 120° to the β-lactam carbonyl. If the metal-ion is assumed to coordinate to this oxygen it can be seen that the side chain amide nitrogen coordination geometry is highly strained. The metal-ion does not lie in the plane of the β-lactam ring, as predicted by the SP^2 character of O_8, but lies at an angle of 35° to this plane. Also the resulting metal-nitrogen bond length is 3.4 A° as opposed to the expected value of 1.9 A°. These molecular parameters apply equally well to Cu(II) complexation to cephalosporin at site I.

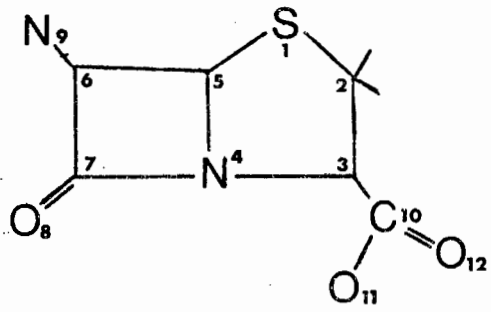
TABLE 5.4

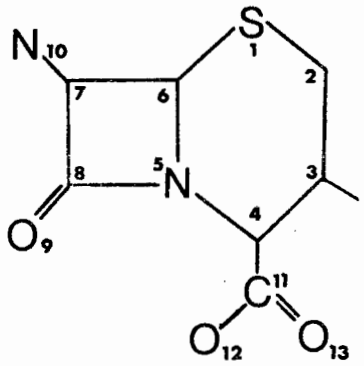
Distance of nitrogen from plane for some Penicillin and Cephalosporin antibiotics.

Compound	Distance of N from plane A°	Reference
trimethylamine	0.56	128
penicillin G	0.4	129
cephaloridine	0.24	8
cephaloglycine	0.22	8
Δ^2-cephalosporin	0.065	8
unfused β-lactams	0	130

TABLE 5.5

Molecular Parameters obtained from Drieding models of Cu(II) - penicillin and cephalosporin complexes.





Site I assuming typical bond angles and lengths for M-O bond.

Parameter	Observed value	Typical value
CuO_8	2 A^{o}	2 A^{o}
$C_7\widehat{O_8}Cu$	120^{o}	
CuN_9	3.4 A^{o}	1.9 A^{o}
$C_6\widehat{C_7O_8}Cu$	35^{o}	0^{o}
$N_9\widehat{CuO_8}$	80^{o}	90^{o}

Penicillin: Site II assuming typical bond angles and lengths for M-O bond.

Parameter	Observed value	Typical value
CuO_{11}	2 A°	2 A°
$Cu\widehat{O_{11}C_{10}}$	109°	109°
CuN_4 ([A] conformation)	2.3 A°	1.9 A°
CuN_4 ([B] conformation)	2 A°	1.9 A°
Deviation of M-N bond from tetrahedral axis		3° ([A] conformation) 5° ([B] conformation)

Cephalosporin: Site II assuming typical bond lengths and angles for for the M-O bond.

Parameter	Observed value	Typical value
CuO_{12}	2 A°	2 A°
$Cu\widehat{O_{12}C_{11}}$	120°	120°
Cu N_5 ([A] conf.)	3.3 A°	1.9 A°
Cu N_5 ([B] conf.)	2.5 A°	1.9 A°
Deviation of M-N bond from tetrahedral axis		45° ([A] conf.) 10° ([B] conf.)

On the other hand, complexation at site II of penicillin and cephalosporin, does not result in such a strained system. This can be seen from Table 5.5 when coordination to the carboxylic oxygen at the tetrahedral angle and a bond length of $2A^{o}$ has been assumed. In the case of penicillin the metal-ion is only distorted 3^{o}-5^{o} from the tetrahedral axis of the nitrogen, depending on the conformation of the thiazolidine ring. Also the metal-nitrogen bond length is 2-2.3 A^{o}, which compares well with the 'normal' bond length of 1.9 A^{o}. The case for cephalosporin is not as ideal, the metal nitrogen bond length being longer and the deviation from the tetrahedral axis greater. There is a marked difference between the parameters for the two dihydrothiazine ring conformations, with conformation [B] being the more favourable. From the n.m.r results it was not possible to distinguish any preference for either of these two conformations. This was not surprising since none of the metal-proton distances were sensitive to this conformational change.

This theoretical comparison of sites I and II shows a definite preference for site II, which is the site favoured by the results in this study. Also, if coordination is at site II, the cephalosporin complex is seen to be less stable than the penicillin complex, both from the geometry of the complex and also the 'amidicity' of the β-lactam nitrogen. If, on the other hand, coordination is at site I the stabilities of the two complexes should be similar. Since cephalosporin can be purified by precipitation as the metal complex,[20] while penicillin is rapidly degraded by certain metal-ions, it seems that structure II must be the correct one.

A P P E N D I X

Derivation of the Rate Equation for the Reaction of Ni(II) with Thiaproline, Penicilloic acid and Ampicillin.

The proposed mechanism is:

$$M + L \xrightarrow{k_1} ML$$

$$M + LH \xrightarrow{k_2} ML + H^+$$

$$LH \underset{}{\overset{K_a}{\rightleftharpoons}} L + H^+$$

where $L = L^-$, L^- and $L^=$ for the three ligands thiaproline, ampicillin and penicilloic acid respectively. The charge on the metal-ion has been omitted.

The rate law for the formation of the product ML, can be written:

$$\frac{d[ML]}{dt} = k_1 [M][L] + k_2 [M][LH] \quad \ldots\ldots\ldots\ldots (1)$$

The total concentration of the ligand at time t, $[L]t$, is:

$$[L]t = [L] + [LH] = [L] + \frac{[L][H^+]}{Ka} \quad \ldots\ldots\ldots\ldots (2)$$

Hence:

$$[L] = [L]t \frac{K_a}{Ka + [H^+]} \quad \ldots\ldots\ldots\ldots (3)$$

The concentration of the product at time t will be:

$$[ML] = [L]_o - [L]t \quad \ldots\ldots\ldots\ldots (4)$$

Where $[L]o$ is the initial total ligand concentration.

Substituting for $[L]t$ equation (4) becomes:

$$[ML] = [L]o - [L]\ \frac{Ka + [H^+]}{Ka} \qquad \ldots\ldots\ldots\ldots (5)$$

which on rearrangement gives:

$$[L] = ([L]o - [ML])\ \frac{Ka}{Ka + [H^+]} \qquad \ldots\ldots\ldots\ldots (6)$$

Sutstituting equation (6) into equation (1) gives:

$$\frac{d[ML]}{dt} = [L]o\ \frac{Ka}{Ka + [H^+]}\left(k_1[M] + \frac{k_2[M][H^+]}{Ka}\right)$$

$$- [ML]\ \frac{Ka}{Ka + [H^+]}\left(k_1[M] + \frac{k_2[M][H^+]}{Ka}\right) \qquad \ldots\ldots\ldots\ldots (7)$$

For the sake of brevity, let the coefficient of $[L]o$ be 'a' and that of $[ML]$ be 'b'.

i.e. $$\frac{d[ML]}{dt} = a[L]o - b[ML] \qquad \ldots\ldots\ldots\ldots (8)$$

Taking integrals:

$$\int_{[ML]=0}^{[ML]} \frac{d[P]}{a[L]o - b[ML]} = \int_{t=0}^{t} dt \qquad \ldots\ldots\ldots\ldots (9)$$

Since $[H^+]$ is constant during a kinetic run, and $[M]$ is in such large excess over $[L]o$ as to be effectively constant, 'a' and 'b' will be constant.

Thus:

$$\int_{t=0}^{t} dt = t$$

$$= \int_{[ML]=0}^{[ML]} \frac{d[ML]}{a[L]o - b[ML]}$$

$$= \left[-\frac{1}{b} \ln (a[L]o - b[ML]) \right]_{o}^{[ML]}$$

$$= -\frac{1}{b} \operatorname{Ln} \frac{a[L]o - b[ML]}{a[L]o} \qquad \ldots\ldots\ldots\ldots (10)$$

When the system has reached equilibrium:

$$\frac{d[ML]_e}{dt} = 0$$

Where $[ML]_e$ is the equilibrium concentration of the metal complex. Hence, from equations (4) and (8):

$$a[L]o = b[ML]_e$$

$$= b[L]o - b[L]_e \qquad \ldots\ldots\ldots\ldots (11)$$

Substituting (11) into (10):

$$bt = -\ln \frac{b[L]t - b[L]_e}{b[L]_o - b[L]_e}$$

$$= \ln \frac{[L]_o - [L]_e}{[L]t - [L]_e}$$

Clearly, since for a reversible first-order reaction:

$$k_{OBS}\, t = \ln \frac{[L]_o - [L]_e}{[L]t - [L]_e}$$

$$k_{OBS} = b = [M]\left(\frac{k_2[H^+] + k_1 Ka}{Ka + [H^+]}\right)$$

(equation 4.2)

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