INVESTIGATION OF SELECTED THIOSEMICARBAZONES AND THEIR METAL COMPLEXES AS POTENTIAL ANTICANCER DRUGS

By

PAUL DAVID MOONEY

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1966

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
August, 1969

INVESTIGATION OF SELECTED THIOSEMICARBAZONES AND THEIR METAL COMPLEXES AS POTENTIAL ANTICANCER DRUGS

Thesis Approved:

Ernest M. Hodnett
Thesis Adviser

Neil Purdie

D. D. Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

I wish to express my gratitude to my wife, Sharon, for her patience and encouragement. I also wish to thank her for typing this thesis.

I wish to express my thanks to Dr. Ernest Hodnett for his invaluable guidance and counsel during the course of my graduate work and especially for help in the research leading to the production of this thesis.

A sincere expression of gratitude is extended to Dr. Neil Purdie for his consultations and for his availability and readiness to help and offer suggestions concerning portions of my work.

Thanks are due to the Oklahoma State University Department of Chemistry for financial assistance extended to me in the form of teaching assistantships each semester of my graduate enrollment except from June 1, 1968, to August 31, 1968, and I must express my appreciation to the Research Foundation and the National Science Foundation for a research grant extended to me during this period of time.

Finally, I would like to thank several of the other graduate students for helpful experimental hints and literature suggestions. Thanks are due to several students with similar research projects who have been quite helpful.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

The purpose of this investigation is to contribute some usable knowledge to the developing field of cancer chemotherapy. The intention is to elucidate a mechanism of action for one series of anticancer drugs. If a relationship between the physical or chemical properties of a series of organic compounds and their antitumor activities can be detected, then by preparing new drugs which optimize this trend or property new and, hopefully, more successful methods of treatment could be developed.

A class of compounds having known activities in various tumor systems was chosen for experimentation. Six thiosemicarbazones were selected which displayed activities from fair to excellent in various cancer systems of experimental animals. These were prepared from aldehydes of various N-heterocycles, specifically those of pyridine, pyrazine, quinoline and isoquinoline. Since these compounds have chemical properties conducive to chelation of metal ions, they made desirable subjects for a complexation study.

It was decided to study the complexation of these thiosemicarbazones with copper (II), cobalt (II), and nickel (II), and to determine the formation constants of the complexes in solution. By comparing the stability constants with the activities in tumor systems of the crystalline metal complexes which would be subsequently prepared, per-

haps a correlation could be detected between the complexing ability of a compound and its carcinostatic activity. When the roles played by trace metals in the normal metabolism of the human body are considered it seems possible that metal ions could also play a major role in the metabolism of abnormal cells. This role quite possibly could be related to complexing ability of the metals.

The method of choice for the determinations in this investigation is that of potentiometric titration.

Because of the relative insolubility of the thiosemicarbazones themselves and the greater degree of insolubility of the metal complexes, determination of the stability constants in a 75 per cent dioxane-water solution was first considered. Since no correlation between the mixed solvent system and aqueous biological media could be made, this determination was discarded in favor of determinations in a very dilute aqueous medium.

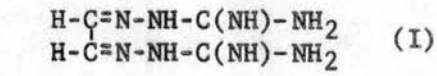
The determinations were performed and solid metal derivatives were prepared. These derivatives will be submitted for testing and after these results are obtained perhaps a correlation can be made.

CHAPTER II

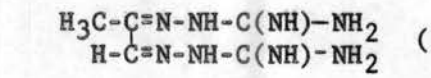
HISTORICAL

In most general texts on coordination or inorganic chemistry, reference is made to the development of prussian blue by Diesbach in Berlin as the eraliest recorded discovery of a metal complex. With the discovery of hexaammine cobalt (III) chloride, the real beginning of coordination chemistry is observed.[1,2]

The interest in thiosemicarbazones arose from an original interest in compounds displaying the characteristic azomethine structure, $R_2C{=}N-$ where R is either alkyl or hydrogen. Two compounds which exemplify this structure and which possess useful activity in chemotherapy are the guanylhydrazones of glyoxal and methylglyoxal (pyruvaldehyde). GAG (glyoxal bis (guanylhydrazone)) (I) and methyl-GAG (methylglyoxal bis(guanylhydrazone)) (II) are illustrated below showing the characteristic azomethine structure.



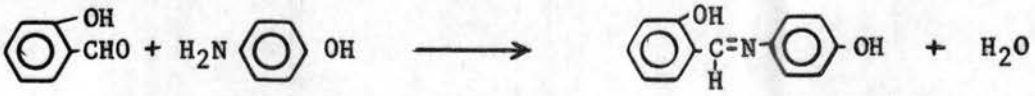
(I)



(II)

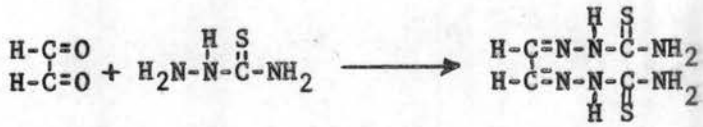
The research in this laboratory turned to schiff bases of aromatic aldehydes with various aromatic and non-aromatic primary amines. Salicylaldehyde enjoyed the most attention and a large number of compounds were prepared from this precursor. The activity of some of these compounds has been tested and reported.[3] A recently prepared Schiff base has demonstrated a high anti-leukemic activity in mouse leukemia L-1210.[4] This Schiff base was prepared by simply condensing

salicylaldehyde with p-aminophenol.



This compound is still being tested and the results have not been published yet. This condensation reaction between salicylaldehyde and p-aminophenol is typical of the reaction used in preparing the various compounds.

Reactions between aldehydes and thiosemicarbazide or thiosemicarbazide hydrochloride yield compounds containing the characteristic azomethine structure. An example of this is glyoxal bis (thiosemicarbazone).

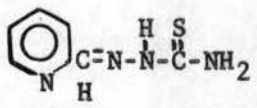


(III)

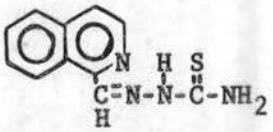
This compound when included in the diet inhibits the growth of certain tumors in mice.[5] This is not an isolated case of activity.

Isatin-3-thiosemicarbazone gives almost complete protection in mice which have been inoculated with vaccinia virus in dosages up to 100,000 times the usual lethal dose.[6,7] Of several compounds tested, the isatin structure seemed to give the best results. The thiosemicarbazone of 1-methylisatin has been used with some success in a smallpox epidemic. Thiosemicarbazones of the 2- and 5-thiazolecarboxaldehydes have shown good antiviral activity.[8] The carcinostatic activity of 2-keto-3-ethoxy-butyraldehyde (kethoxal) has been reported.[9] Besides the parent compound interest has been demonstrated in the bis (thiosemicarbazone) derivative of kethoxal.[9,10,11,12,13] In 1956 it was reported by Brockman et al. that the thiosemicarbazone of pyridine-2-carboxaldehyde (IV) demonstrated antileukemic activity in L-1210 leukemia.[14] This activity was verified by French and Blanz.[15] It was

noted by French and Blanz that the compound was active but was "treacherously and cumulatively toxic."[15] The activity of this compound inspired the preparation of thiosemicarbazones of some other heterocyclic aldehydes, notably that of isoquinoline-1-carboxaldehyde (V).[15]



(IV)



(V)

In mice bearing L-1210 leukemia this compound exhibited a lengthening of life up to 69 percent with an intraperitoneally injected dose of 80 mg. of compound per kilogram of animal which had been inoculated with the tumor. In Lewis lung carcinoma in mice at a dose level of 80 mg./kg. some toxicity was observed causing a few deaths but in the surviving mice 100 percent inhibition of the inoculated tumor was observed when the drug was administered either by intraperitoneal injection or by a stomach tube. In two other tumor systems the same compound exhibited high inhibitions by diet, injection, or stomach tube administration.

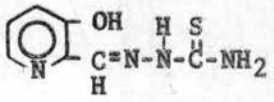
To follow up this promising start, 43 additional thiosemicarbazones were synthesized and tested in 4 different tumor systems.[16] Included in this list are the thiosemicarbazones of pyridine-2-carboxaldehyde and isoquinoline-1-carboxaldehyde (2-formylpyridine thiosemicarbazone and 1-formylisoquinoline thiosemicarbazone respectively). Several of these were found to display encouraging activities.

3-Hydroxy-2-formylpyridine thiosemicarbazone (VI) shows an 88 percent inhibition of tumor when tested in mice inoculated with transplanted Lewis lung carcinoma and a 79 percent increase in longevity in L-1210 mice.

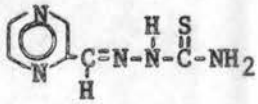
2-Formylpyrazine thiosemicarbazone (VII) showed inhibitions of 71 and 75 percent in Lewis lung carcinoma and a denosarcoma 755 respectively at a dose level of 75 mg./kg. in each case.

Also in the paper are the thiosemicarbazone derivatives of isoquinoline-1-carboxaldehyde and isoquinoline-3-carboxaldehyde, the latter of which does not display any significant carcinostatic activity.

In a private communication from Frederic A. French it was revealed that 5-hydroxy-2-formylpyridine thiosemicarbazone has exhibited activity in L-1210 leukemia which has caused come excitement.

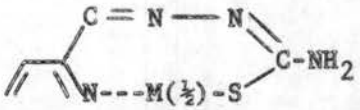


(VI)



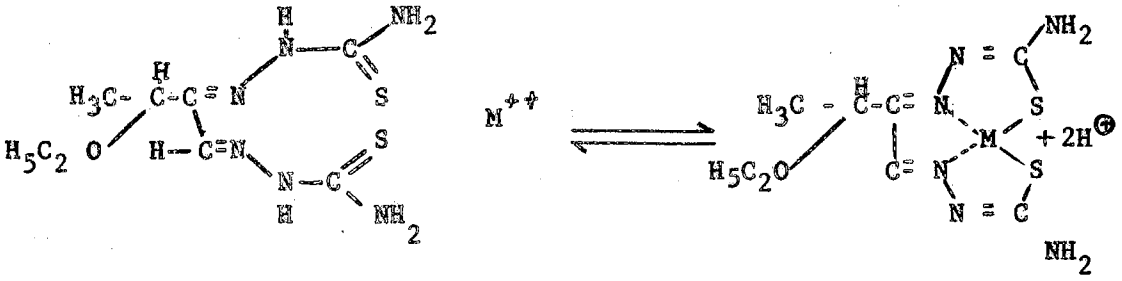
(VII)

It was suggested by French and Blanz[15,16] that these compounds might be acting at physiological pH values as tridentate ligands. They propose the formation of two five-membered chelate rings of partially conjugate character favoring an octahedral coordination of two ligands to one divalent metal ion which would yield an electrically neutral complex (VIII).



This is not the first suggestion that the activity of a drug might be related to its chelating abilities. As a matter of fact several compounds have been shown to have a dependence upon or have increased activities in the presence of metal ions. The antitumor activity of the copper chelate of pyruvaldehyde bis (thiosemicarbazone) is much greater than that of the ligand itself[17]. When the schiff base o-(N-phenyl-formimidoyl)phenol is complexed with cobalt its antitumor activity is increased.[18] The in vivo activity of kethoxal bis(thiosemicarbazone) against Walker 256 carcinosarcoma has been reported to

be enhanced by the presence of copper and zinc ions.[19,20] Responsibility for the activity of the drug both *in vivo* and *in vitro* has been laid to the copper chelate of the drug. The formation of the complex between kethoxal bis(thiosemicarbazone) and a divalent metal ion and the structure of the complex are reproduced below.



CHAPTER III

INTRODUCTION TO EXPERIMENTAL WORK

The compounds selected for this work were taken from the list of 43 thiosemicarbazones which were reported by French and Blanz.[16] Selection of the six compounds was done so by considering the activities in different tumor systems. It was desirable to select compounds of different activities so it could be ascertained whether the metal derivatives of those compounds could improve their activities and if so to what extent. It was desirable to determine if compounds of low activity could be made usefully active and whether those of high activity could be improved.

Perhaps a brief description of the term activity should be presented. The compounds tested were done so in four different tumor systems. Three of these, Sarcoma 180 (SAR), Lewis lung carcinoma (LLC), and adenocarcinoma 755 (ADN), are solid transplanted tumors and the fourth, leukemia L-1210 (L-1210), is a leukemia of mice induced by intraperitoneally injected leukemia cells. Treatment of each tumor system begins 24 hours after the transplant inoculation with one dose per day. In the case of the solid tumors, at the end of a specified number of days, the solid tumors are excised and weighed to the nearest milligram. At the same time tumors are excised from control mice which have received a similar tumor transplant at the same time but have received no treatment. For solid tumors evidence of

activity is a significant difference in tumor size in the test and the control animals. For the solid tumors the ratio T/C (test/control) is reported and the compound tested is considered to have activity meriting further tests if this ratio is less than or equal to 0.53 indicating that the treated tumor is only half the size of the untreated tumor. For the leukemia, since there is no solid tumor to measure, a lengthening of life is desired and the T/C ratio is a measure of time (days) from tumor transplant until death. Since a greater longevity is desired, A T/C value greater that 1.0 is desired and a value greater than or equal to 1.25 is considered active.

In addition to compounds V, VI, VII and VIII, two other compounds were selected for testing. These were 3-formylisoquinoline thiosemicarbazone (IX) and 5-hydroxy-2-formylpyridine thiosemicarbazone (X).

(IX)

(X)

The tumors, dose, and activities of these compounds are reported below.[16]

	L-1210		SAR		LLC		ADN	
Cmpd.	Dose (mg/kg)	T/C	Dose (mg/kg)	T/C	Dose (mg/kg)	T/C	Dose (mg/kg)	T/C
V	10	1.30	10	.60	10	.47	10	.71
VI	67	1.47	35	.76	67	.20	67	.10
VII	71	1.79	71	.49	75	.12	71	.32
VIII	75	1.36	75	.53	75	.29	75	.25
IX	150	1.13	100	.90	100	.72	75	1.01
X	---	----	---	---	---	---	---	----

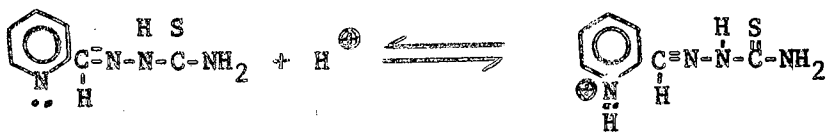
The statistics for (X) are not presently available but private communications indicated the activity is considerable.

Samples of these six compounds and a few others were obtained for testing from Frederic A. French of the Mount Zion Hospital and Medical Center, Chemotherapy Research Laboratory in Palo Alto, California.

If the structures of these compounds are considered it can be seen why they lend themselves well to chelation. The thoisemicarbazone structure may be considered to be a very weak acid due to the ionization of the tautomeric -SH form. $-SH \rightleftharpoons -S^{\ominus} + H^{\oplus}$ The hydrogen on the -SH, although only weakly acidic, should be titratable since the an ion resulting from the removal of the hydrogen would be stabilized by the conjugated structure, and a pKa value should be measureable.

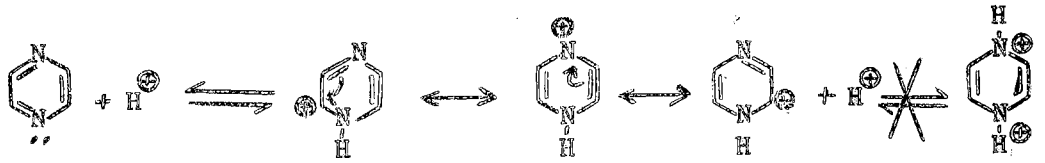
If a phenolic -OH is placed on the ring another source of hydrogen ions is present. The acid constant of this weakly acidic group should also be measureable. Using VI as an example this ionization may be demonstrated.

A third source of acidity may be found in the ring nitrogen atom of the pyridine structure. The protonation-ionization equilibrium may be measured and an acid constant calculated.

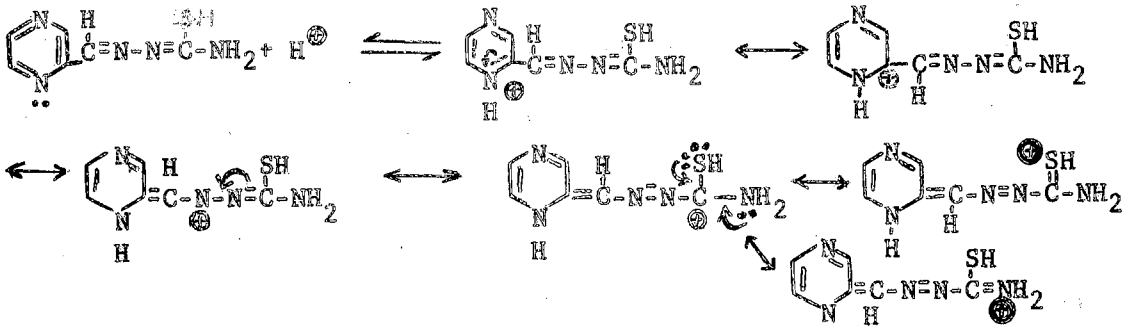


The charge resulting from the protonated ring may be shown by proper resonance structures to be delocalized thus stabilizing the ion.

If the pyrazine structure of compound VII is examined two nitrogen atoms are seen in the ring and the question may be raised whether one or both nitrogen atoms are protonated and if only one, which one? Pyrazine itself has only one reported pKa.[21] This is reasonable if one examines the molecule. Protonation of one nitrogen results in the introducation of a positive charge which is delocalized throughout the ring and, as the structures below show, to the greatest extent at the 2, 4, and 6 position. The second nitrogen is at the 4 position and the partially positive nature of this nitrogen makes a second protonation highly unlikely.



If a conjugated structure such as the thiosemicarbazone structure is substituted at the 2-position as in compound VII, it seems reasonable to assume that protonation occurs at the 1-position (ortho to the side group) so that not only the above delocalization results but an additional contribution is received from the conjugated side chain in the following suggested manner.



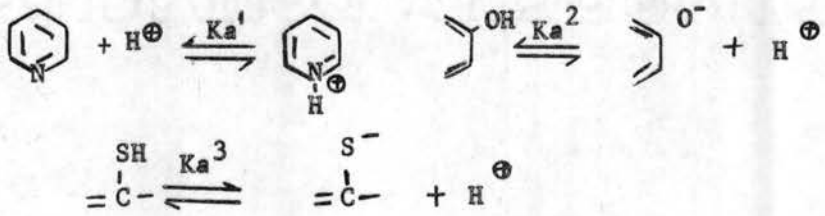
If protonation occurs on the nitrogen atom which is meta to the side chain this additional contribution cannot be demonstrated. It is thus suggested that for 2-formylpyrazine thiosemicarbazone (VII) only one nitrogen is protonated, a suggestion which is borne out by titration data and calculations; and this protonation occurs at the 1-position in preference to the 4-position so that the charge may be delocalized through the conjugated side chain which is ortho to the protonated ring position. In all other species it is assumed that the nitrogen atom is protonated and the charge delocalized where possible throughout a conjugated structure. This assumption is supported by the observation that the materials, all of which are insoluble at neutral pH are soluble at acid pH.

Since these compounds all have potentially titratable hydrogen atoms they may be treated as acidic ligands in complexation studies. Before any studies are made on complex formation by these compounds a measure of their acidity must be made.

It was desireable to make these measurements by potentiometric titration. However, the problem of solubility remained. All the compounds worked with were water insoluble. For this reason a mixed solvent system was considered. A 75% mixture of dioxane in distilled water seemed appealling since all the materials were soluble in this mixture and also there were ready references on this type of measurements done previously. However, since it was hoped to make some correlation between complexation and tumor activity, a non-aqueous medium and any properties measured in a non-aqueous medium would be useless since a biological medium is certainly not non-aqueous and a direct correlation could not be made. It was decided to return to aqueous

experiments.

It was observed that all the compounds tested, with the exception of the isoquinoline derivatives, were soluble in both acid and base, but precipitated in neutral solutions. In very dilute solutions though, the materials could be titrated smoothly through the neutral pH range (6.00-8.00) without the formation of a precipitate. It was interesting to note that when the solubility limits were exceeded a weighed sample dissolved in a measured amount of excess base would begin to precipitate shortly before sufficient acid was added to neutralize the excess base and the dissolved thiosemicarbazone. This precipitate could be redissolved by adding an amount of excess acid equivalent to the amount of compound present. If the acid was added slowly the amount of precipitate dissolved would be proportional to the amount of acid added. These solubilities serve to support the proposed ionization and protonation steps:



It was encouraging to note that a very dilute basic solution of the thiosemicarbazones could be neutralized without formation of a precipitate; now titrations could be done to measure the acid constants of the ligands. The constants are easily calculated after a titration according to some easily obtainable formulas. These formulas will now be derived for a triprotic acid and a diprotic acid of the forms $H_3A^{\oplus}$ and $H_2A^{\oplus}$ respectively.

For the case of a diprotic acid such as the fully protonated form of (IV), which for the sake of brevity is written as $H_2A^{\oplus}$, dissolved in base and the salt of which is being titrated with standard acid, the equations necessary for calculation of the acid dissociation constants are arrived at as follows:

$$H_2A^{\oplus} \underset{}{\overset{Ka^1}{\rightleftharpoons}} HA + H^{\oplus} \qquad Ka^1 = \frac{h \cdot [HA]}{[H_2A^{\oplus}] f_1}$$

$$HA \underset{}{\overset{Ka^2}{\rightleftharpoons}} A^{\ominus} + H^{\oplus} \qquad Ka^2 = \frac{h \cdot [A^-] f_2}{[HA]}$$

<u>For the first ionization</u>:

Total acid present = $a = [H_2A^{\oplus}] + [HA]$

Total ions present = $[H^{\oplus}] + [H_2A^{\oplus}] + [K^{\oplus}] + [Cl^-] + [OH^-]$

From electroneutrality: $[H^{\oplus}] + [H_2A^{\oplus}] + [K^{\oplus}] = [Cl^-] + [OH^-]$

$$[H_2A^{\oplus}] = [Cl^-] + [OH^-] - [K^{\oplus}] - [H^{\oplus}]$$

$$= [Cl^-] - [K^{\oplus}] - [H^{\oplus}] \text{ since } OH^- \text{ is negligible at low pH.}$$

$$[HA] = a - [H_2A^{\oplus}] = a - [Cl^-] + [K^{\oplus}] + [H^{\oplus}]$$

$$Ka^1 = \frac{h \cdot (a - [Cl^-] + [K^{\oplus}] + [H^{\oplus}])}{([Cl^-] - [K^{\oplus}] - [H^{\oplus}])} \cdot \frac{1}{f_1}$$

$$\log Ka^1 = \log h + \log Y - \log f_1$$

$$pKa^1 = pH - \log Y + \log f_1$$

where $Y = \dfrac{(a - [Cl^-] [K^+] + [H^+])}{([Cl^-] - [K^{\oplus}] - [H^{\oplus}])}$

<u>For the second ionization</u>:

Total acid = $a = [HA] + [A^-]$

Total ions present = $[H^{\oplus}] + [A^-] + [K^{\oplus}] + [OH^-] + [Cl^-]$

From electroneutrality: $[K^{\oplus}] + [H^{\oplus}] = [OH^-] + [A^-] + [Cl^-]$

$$[A^-] = [K^{\oplus}] + [H^{\oplus}] - [OH^-] - [Cl^-]$$

$$= [K^{\oplus}] - [OH^-] - [Cl^-]$$

since $[H^+]$ is negligible at high pH.

$HA = a - A^- = a - K \quad OH^- \quad Cl^-$

$$Ka^2 = \frac{h \cdot ([K^\oplus] - [OH^-] - [Cl^-]) \cdot f_1}{(a - [K^\oplus] + [OH^-] + [Cl^-])} = h \cdot Z \cdot f_1$$

$\log Ka^2 = \log h + \log Z + \log f_1$

$pKa^2 = pH - \log \quad - \log f_1$

Calculation of ionic strength (I) for the ionization

$H_2A^\oplus \rightleftharpoons HA + H^\oplus$

Charged species present: $K^\oplus + OH^- + H^\oplus + Cl^- + H_2A^\oplus$

$[H_2A^\oplus] + [K^\oplus] + [H^\oplus] = [Cl^-] + [OH^-]$

Ionic strength = $I = \frac{1}{2}\sum CiZi^2$ where Zi is the electronic charge on a particular ion and Ci is the concentration of that particular ion.[23]

$I = \frac{1}{2}([H_2A^\oplus] \cdot 1^2 + [K^\oplus] \cdot 1^2 + [Cl^-] \cdot 1^2 \quad [OH^-] \cdot 1^2)$

$= \frac{1}{2}(2[Cl^-] + 2[OH^-]) \cong [Cl^-]$ since $[OH^-]$ is negligible at low pH.

Calculation of f_1: Calculation of f_1 is possible by the relationship

$$-\log f_1 = .509\left\{\frac{\sqrt{I}}{1+\sqrt{I}} - 0.3I\right\}$$ Where I is ionic strength[24].

Calculation of ionic strength for the ionization

$HA \rightleftharpoons H^\oplus + A^-$:

Charged species present = $H^\oplus + Cl^- + K^\oplus + OH^- + A^-$

$[H^\oplus] + [K^\oplus] = [OH^-] + [A^-] + [Cl^-]$

$I = \frac{1}{2}\sum Ci Zi^2 = \frac{1}{2}([H^\oplus] + [K^\oplus] + [OH^-] + [A^-] + [Cl^-])$

$= \frac{1}{2}(2[H^\oplus] + 2[K^\oplus])$

$I \cong K^+$ since above pH 5 $[H^\oplus]$ is negligible as compared to $[K^\oplus]$.

Calculation of f_1 is the same as before.

For the case of the ionization of a triproticacid, a similar treatment yields for the first two ionizations, which are similar to those of a diprotic acid, equations for pKa^1 and pKa^2 as follows:

$$H_3A^+ \rightleftharpoons H_2A + H^+$$

$$K_1 = \frac{h \cdot [H_2A]}{[H_3A^\oplus]} \cdot \frac{1}{f_1} \qquad \text{If } [H_3A^\oplus] = [Cl^-] + [OH^-] - [H^\oplus] - [K^\oplus]$$

and $[H_2A] = a - [H_3A^\oplus]$ then with $[OH^-]$ dropping out at low pH, pKa^1 may be defined as:

$$pKa^1 = pH + \log X - \log f_1 \quad \text{where } X = \frac{[Cl^-] - [H^\oplus] - [K^\oplus]}{a - [H_3A^\oplus]}$$

and f_1 is calculated by knowing that the ionic strength is closely approximated as the chloride ion concentration ($I \cong [Cl^-]$).

$$pKa^2 = pH + \log Y + \log f_1$$

$$\text{where } Y = \frac{a - [H^\oplus] - [K^\oplus] + [Cl^-] + [OH^-]}{[H^\oplus] + [K^\oplus] - [Cl^-] - [OH^-]}$$

and f_1 is calculated by approximating ionic strength by the potassium ion concentration ($I \cong [K^\oplus]$).

Derivation of pKa^3

K_3 is derived as follows: $HA^- \rightleftharpoons H^\oplus + A^\ominus$; $K_3 = \frac{h \cdot [A^\ominus]}{[HA^-]} \cdot \frac{f_2}{f_1}$

$$HA^- \rightleftharpoons H^\oplus + A^= \qquad a = [HA^-] + [A^=]$$

Total ions present = $K^\oplus + OH^- + H^\oplus + Cl^- + HA^- + A^=$

$$[K^\oplus] + [H^\oplus] = [OH^-] + [Cl^-] + [HA^-] + 2[A^=]$$

$$[K^\oplus] \quad [H^\oplus] = [OH^-] + [Cl^-] + a + [A^=] + 2[A^=]$$

$[A^=] = [K^\oplus] - [Cl^-] - a$ with $[H^\oplus]$ and $[OH^-]$ considered negligible compared to $[K^\oplus]$ and $[Cl^-]$ at pH values less than 11.

$$[HA^-] = a - [A^=]$$

Calculation of ionic strength (I)

$$I = \tfrac{1}{2}\left([H^{\oplus}] + [Cl^{-}] + [K^{\oplus}] + [OH^{-}] + [HA^{-}] + 4[A^{=}] \right)$$

$$= \tfrac{1}{2}\left([H^{\oplus}] + [Cl^{-}] + [K^{\oplus}] + [OH^{-}] + a - [A^{=}] + 4[A^{=}] \right)$$

$$= \tfrac{1}{2}\left([H^{\oplus}] + [Cl^{-}] + [K^{\oplus}] + [OH^{-}] + a - 3[A^{=}] \right)$$

substitute for $A^{=}$

$$= \left([H^{\oplus}] + [K^{\oplus}] + [Cl^{-}] + [OH^{-}] + a + 3[K^{\oplus}] + 3[H^{\oplus}] - 3[OH^{-}] - 3[Cl^{-}] - 3a \right)$$

$$= \tfrac{1}{2}\left(4[H^{\oplus}] + 4[K^{\oplus}] - 2[Cl^{-}] - 2[OH^{-}] - 2a \right)$$

$$= 2[K^{\oplus}] - [Cl^{-}] - a$$ with $[H^{\oplus}]$ and $[OH^{-}]$ considered negligible compared to $[K^{\oplus}]$ and $[Cl^{-}]$ between pH = 3.0 to pH 11.0.

The values for f_1 and f_2 may now be calculated and with these values pKa^3 may be calculated as

$$pKa^3 = pH - \log \frac{[A^{=}]}{[HA^{-}]} - \log f_2 + \log f_1$$

$$-\log f_2 = (4.0) \cdot (.509) \left\{ \frac{\sqrt{I}}{1+\sqrt{I}} - 0.3\, I \right\}$$

The procedure chosen for the determination of the pKa is titration with standard acid of a sample dissolved in a known amount of excess standard potassium hydroxide. By knowing the amount of compound and amount of potassium hydroxide, the excess amount of base may be calculated. The amount of standard acid necessary to titrate the excess base may be calculated and added. Now the titration may be carried out as just a simple back-titration of an acid salt with standard acid.

For a polyprotic acid the amount of acid necessary to titrate the first equivalent was calculated and this was considered as titrating a single proton. For the second equivalent the titration was again considered as titration of the $HA \rightleftharpoons H^{\oplus} + A^{-}$ equilibrium, and so on for each proton on the acid. As long as the pKa values of

each ionization are different from each other by a value greater than or equal to 2.0 each proton titrated can be considered as a separate titration and the simple pKa's calculated by using the formulas already discussed. If the pKa's differ by values less than 2.0 they are considered to be overlapping and special calculations bust be used. This case was not encountered so will not be discussed.

To calculate the formation constants of the various ligands, once again the potentiometric method was chosen. Any substance capable of binding a proton can bind a positively charged metal instead and the resulting hydrogen-ion concentration can be used as a measure of complexing. Being completely general and allowing a ligand of the type HL (HA) where the ligand has only one potential anionic form, an example of chelation may be written as:

$$HL + M^{++} \underset{}{\overset{K_1}{\rightleftharpoons}} ML^{\oplus} + H^{\oplus} \qquad ML^{\oplus} + HL \overset{K_2}{\rightleftharpoons} ML_2 + H^{\oplus}$$

and a new term B_2 is introduced and defined as $B_2 = K_1K_2$ or the overall formation constant.

The ligands used in this investigation all have nitrogens in an aromatic ring which are capable of being protonated. The titrations were done so that the calculated amount of acid was added sufficient to protonate all anions and then protonate 1 ring nitrogen. The ligand was then treated as $H_2A^{\oplus}$ or simply H_2A. The equations presented by Albert and Serjeant[21] were then revised and used to determine the formation or stability constants. The ligands were titrated in the presence of a known amount of metal perchlorate with standard potassium hydroxide.

To begin developing the equations used the concentration of free and combined hydrogen ions is expressed two ways:

$$Ca = [Lo] \cdot f_1 - [KOH] \quad \text{equation 1}$$

where $[Lo]$ is the concentration of ligand originally added and corrected for any dilution, and KOH is concentration of titrant added.

$$[Ho] = h + [HL] + 2\,[H_2L^{\oplus}] \cdot f_1 - oh \quad \text{equation 2}$$

where h and oh are the activities of the hydrogen and hydroxyl ions respectively.

Since $Ca = [Ho]$,

$$2\,[H_2L^{\oplus}] \cdot f_1 + HL = 2\,[Lo] \cdot f_1 - [KOH] - h + oh \quad \text{equation 3}$$

By the laws of mass action the ionizations of the ligands may be expressed as:

$$H_2L^{\oplus} \rightleftharpoons HL \; H^{\oplus} \qquad K_1 = \frac{[HL] \cdot h}{[H_2L^{\oplus}]} \cdot \frac{1}{f_1}$$

$$[H_2L^{\oplus}] = \frac{[HL] \cdot h}{K_1 f_1} \quad \text{equation 4}$$

$$HL \rightleftharpoons H^{\oplus} + L^{-} \qquad K_2 = \frac{h \cdot [L^{-}] \cdot f_1}{[HL]} \qquad [HL] = \frac{h \cdot [L^{-}] \cdot f_1}{K_2}$$

equation 5

Substituting (5) into (4) the values for $[H_2L^{\oplus}]$ and $[HL]$ are found as $\frac{h^2 \cdot [L^{-}]}{K_1 K_2}$ and $\frac{h \cdot [L^{-}] \cdot f_1}{K_2}$ respectively.

By substituting equations 4 and 5 into 3, equation 6 may be arrived at.

$$\frac{2\,h^2\,[L^{-}]}{K_1 K_2} + \frac{h \cdot [L^{-}] \cdot f_1}{K_2} = 2\,[Lo] \cdot f_1 - [KOH] - h + oh$$

$$[L^{-}] \left(\frac{2\,h^2}{K_1 K_2} + \frac{h f_1}{K_2}\right) = 2 \cdot [Lo] \cdot f_1 - [KOH] - h + oh$$

$$L^{-} = \frac{2\,[Lo] \cdot f_1 - [KOH] - h + oh}{P} \quad \text{equation 6}$$

Where P is defined as $\left(\frac{2\,h^2}{K_1 K_2} + \frac{h\,f_1}{K_2}\right)$

The total activity of ligand Lo may be expressed as:

$$[Lo] = [L^-] \cdot f_1 + [HL] + [H_2L^{\oplus}] \cdot f_1 + [LM^{\oplus}] f_1 + 2[L_2M]$$

equation 8

If $\bar{n}$ is defined as the average number of liqands bound by one atom of metal the following relationships are obtained.

$$\bar{n} = \frac{[LM^{\oplus}] \cdot f_1 + 2[L_2M]}{Mo \quad f_2}$$ equation 9

$$\bar{n}[Mo] \cdot f_2 = [LM^{\oplus}] \cdot f_1 + 2[L_2M]$$ equation 10

Substitute equation 10 into equation 8.

$$[Lo] = [L^-] + [HL] + [H_2L^{\oplus}] \cdot f_1 + \bar{n}[Mo] \cdot f_2$$ equation 11

Substitute equations 4 and 5 into equation 11.

$$[Lo] = [L^-]\left(f_1 + \frac{h \cdot [L^-] \cdot f_1}{K_2} + \frac{h^2[L^-] \cdot f_1}{K_1 K_2}\right) + \bar{n}[Mo] \cdot f_2$$

$$[Lo] - [L^-] \cdot f_1 \left(1 + \frac{h}{K_2} + \frac{h^2}{K_1 K_2}\right) = \bar{n}[Mo] \cdot f_2$$

$$\bar{n} = \frac{[Lo] - [L^-] \cdot f_1 \cdot Q}{[Mo] \cdot f_2}$$

where $Q = \left(1 + \frac{h}{K_2} + \frac{h^2}{K_1 K_2}\right)$

Assuming a 2 to 1 complex as suggested by French and Blanz [15] the J.Bjerrum equation [21] is summed over n for values of n = o to 2.

$$\sum_{n=0}^{2} (\bar{n} - n) \cdot Bn [L^-]^n = 0$$

Irving and Rossotti[25] express this summation as:

$$\bar{n} + (\bar{n} - 1) \cdot K_1 \cdot [L^-] + (\bar{n} - 2) \cdot K_1 K_2 \cdot [L^-]^2 = 0$$

which can be rewritten with activity coefficients as:

$$\frac{\bar{n}}{(\bar{n} - 1)[L^-] f_1} = \frac{(2 - \bar{n})[L^-] f_1}{(\bar{n} - 1)} \beta_2 - [K_1]$$ equation 12

This equation is in the form of a straight line for which the slope is B_2 and the intercept is $-K_1$. The constants are evaluated by the method of least squares.

Values of $(\bar{n}/\ \bar{n} - 1) \cdot [A^=]$ are calculated for each pH reading, are termed Y and are added to give $\sum Y$. All values of $(2 - \bar{n}) \cdot [A^-] / (\bar{n}-1)$ are tabulated, are called X and added to give $\sum X$. Values of X^2 and XY are tabulated for each titration reading and added to give $\sum X^2$ and $\sum XY$ respectively. These sums are used to solve the standard simultaneous equation for least squares which are:

$$\sum Y = na + b \sum X$$

$$\sum XY = a \sum X + b \sum X^2$$

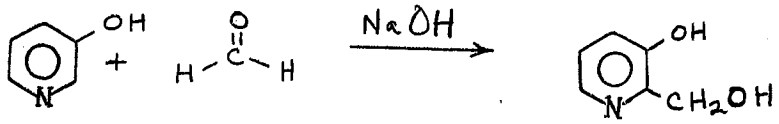
where $a = -K_1$, $b = B_2$ and n = the number of observations. The solution of these equations gives mean values for $-K_1$ and B_2. Since $B_2 = K_1K_2$, K_2 may be arrived at by $K_2 = B_2/K_1$. Individual values of B_2 and K_1 may be obtained by assuming that the mean values of K_1 and B_2 are correct then substituting them into equation 12. Substituting the mean value of B_2 allows calculation of K_1 and vice versa using the mean value of K_1.

All the above calculations were performed routinely on an IBM 360 computer using the programs duplicated below. The stability constant program puts out a data card for each point in the titration on which is punched values for Y, X, W (a weighting factor which equals 1.0) and volume of potassium hydroxide. These are then used as data for the least squares treatment.

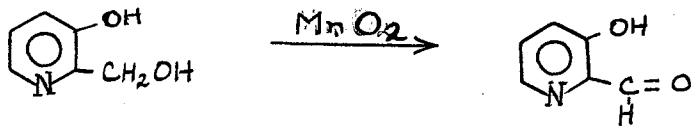
The stability constant experiments were performed for cobalt (II), copper (II), and nickel (II) for each of ligands (IV), (VI), (VII), and (X). Compounds (V) and (IX) which were selected for testing could not be dissolved in either acid or base so could not be titrated. It was now desirable to prepare solid metal chelates using these same metals. More of the ligands had to be prepared before derivatives could be made. Because of ease of preparation the two selected for derivatization were (IV) and (VI), 2-formylpyridine thiosemicarbazone and 3-hydroxy-2-formylpyridine thiosemicarbazone respectively.

Compound (IV) was prepared simply by condensing pyridine-2-carboxaldehyde with thiosemicarbazide to give the thiosemicarbazone derivative.

Compound (VI) required a more detailed procedure. First 3-hydroxypyridine was hydroxymethylated at the 2-position using sodium hydroxide and a 40 percent aqueous solution of formaldehyde.



This 2-hydroxymethyl-3-hydroxypyridine was then oxidized by amorphous manganese dioxide which was prepared by heating manganous carbonate at 400 degrees for 24 hours, to 3-hydroxypyridine-2-carboxaldehyde.



This aldehyde was then condensed with thiosemicarbazide to yield the desired thiosemicarbazone.

The metal derivatives were prepared by simply introducing a measured amount of the proper metal salt dissolved in distilled water into an acidic aqueous solution of the ligand. This solution was then adjusted with sodium bicarbonate to pH 5.0-6.0, as measured with indicator paper. The metal complexes precipitated out as extremely fine granules which were too hard and slow to filter so separation was effected by centrifugation. The precipitates were washed 5-6 times with water to remove excess metal ions and then twice with hot ethanol to remove any unreacted ligand, with centrifugation after each washing.

These derivatives were prepared and will be submitted for testing of anti-tumor activity after metal analysis on them is complete.

CHAPTER IV

EXPERIMENTAL

Apparatus

All the potentiometric measurements in this work were performed using a Beckman Research pH Meter which allows pH reading accuracy to 3 decimal places. The samples were titrated at constant temperature in a glass cup around which was a water jacket. A Haake heating circulater and Brinkman Thermocool cooling unit were used in conjunction with each other to circulate water, whose temperature was maintained at 25 degrees, through the water jacket around the titration vessel. Stirring was accomplished magnetically by use of a Teflon-coated stirring bar and a Mag-Mix magnetic stirrer from Precision Instruments Co. Titrations were performed using pyrex burettes fitted with Teflon stopcocks. The burettes had a 10-milliliter volume with 0.05 ml-graduations. A rubber bung was secured over the top of the titration vessel after the sample had been dissolved. The burette, electrodes and a small tube which delivered a stream of nitrogen were inserted through openings in the rubber bung. Thus the titrations could be performed over a period of thirty minutes to an hour under a nitrogen atmosphere.

Chemicals

As mentioned before, the thiosemicarbazones tested in this investigation were secured from Frederic A. French of Palo Alto, California. All of them appeared as powders of varying shades of yellow.

The potassium hydroxide solution used as titrant was carbonate-free potassium hydroxide prepared as described by Albert and Serjeant.[21] Briefly, it was prepared, with all manipulations performed under nitrogen, by dissolving Baker reagent grade potassium hydroxide pellets in doubly distilled water. Barium hydroxide was added and the barium carbonate allowed to precipitate overnight. The supernatant was passed through an ion-exchange column packed with Dowex 50W-X8 cation exchange resin in the potassium form. The eluted carbonate-free potassium hydroxide was stored in a 2-liter polyethylene bottle fitted with a polyethylene sample withdrawal tube, on the end of which was placed a piece of surgical tubing with a pinch-clamp. To allow air to enter the container each time a sample is withdrawn, a glass tube was fitted through the rubber stopper in the top of the bottle and a soda-lime-filled drying tube was placed on the end of the air vent tube. Thus any air admitted to the interior of the container is essentially free of carbon dioxide. The potassium hydroxide thus prepared was standardized against reagent grade potassium acid phthalate dried under vacuum for 24 hours. After standardization a volume of water was added in sufficient quantity to make the base approximately 0.10 normal. The base was again standardized with potassium hydrogen phthalate.

The acid used was Fisher reagent grade 0.10 normal standard Hydrochloric acid which was standardized for four-decimal-point

accuracy against the prepared carbonate-free standard potassium hydroxide.

The metal salts were prepared from cobalt, nickel and copper perchlorates which were acquired in salt form from the G. Frederick Smith Chemical Company of Columbus, Ohio. The perchlorate salts were used to avoid additional complexing by the anion since the perchlorate ion is not a good ligand. Solutions of each salt were made by dissolving sufficient amounts of the respective salts in a volume of doubly distilled water and then adding enough water to make 500 ml. of approximately 0.01 M solutions. The salt solutions were analyzed by cation exchange chromatography. Triplicate aliquots of the solutions were placed separately on a column of Dowex 50W-X8 100-200 mesh which was in the hydrogen form. The samples were eluted with distilled water until the eluants tested neutral with indicator paper. The collected eluants were titrated with standard base to determine the milliequivalents of acid and thus the milliequivalents of metal could be calculated.

General Titration Procedures

A sample of thiosemicarbazone was weighed out and the number of millimoles calculated. The amount of sample weighed out was necessarily kept low because of solubility considerations. The sample was dissolved in 3.0 ml. of standard potassium hydroxide. By knowing the weight of the sample and the number of protons to be removed by the base (-SH, or -SH and-OH) the amount of base neutralized by the sample could be calculated and thus the amount of excess base was known. The amount of acid necessary to neutralize this excess base was calculated and added to the sample and then 40.0 ml. of water was added to give a

sufficiently dilute solution to prevent precipitation. Now the amount of acid is calculated which is necessary to titrate the -SH group present, the -OH group it there is one, and also to protonate the nitrogen in the ring. The sample was then titrated with this amount of hydrochloric acid. The pH values were recorded after each addition and thus the pKa values could be calculated. At this point the ligand could be considered as either H_2A^+ or H_3A^+ depending on the structure. Now the amount of metal salt solution was calculated which would put enough metal ions in solution to give an approximate 2 to 1 ligand to metal molar ratio. This volume of salt solution was mixed in the sample and the sample was titrated with just enough potassium hydroxide (a calculated amount) to remove every proton just placed on the ligand. This data was used to calculate the stability constant of the metal-ligand complex. It should be added that upon the formation of any trace of a precipitate the titration must be stopped because any further data will be useless. This is true of both types of titrations -- pKa and stability constant.

pKa Titration of 2-Formylpyridine Thiosemicarbazone (V)

A sample of compound IV was measured out which weighed 0.0280 g. The molecular weight of IV is 180.214 so this sample is 0.1554 millimoles. This was dissolved in 3.0 ml. of 0.1054 N potassium hydroxide. Since there is only one proton to be removed (that of the -SH group) only 0.1554 millimoles of the potassium hydroxide is neutralized and this leaves 0.1608 millequivalents of excess base. This excess base required 1.448 ml. of 0.1110 N acid. To protonate the ring nitrogen requires an additional 1.400 ml. of 0.1110 N hydrochloric acid was added to neutralize the excess base then 40.0 ml. of water was

added by a volumetric pipette and the sample was routinely titrated. All calculations involving the data were done on an IBM 360 computer. Since the pKa is theoretically that pH at half neutralization, the values calculated near the middle of each titration were averaged and those on each end thrown out. This gave values of $pKa^1 = 3.723 \pm .072$ and $pKa^2 = 10.871 \pm .071$ for this compound.

pKa Titration Data for 2-Formylpyridine Thiosemicarbazone

Vol. HCl	pH	Vol. HCl	pH
0.05	11.131	0.05	7.093
0.15	11.094	0.15	6.130
0.25	11.013	0.25	5.147
0.35	10.944	0.35	4.628
0.45	10.875	0.45	4.430
0.55	10.804	0.55	4.272
0.65	10.735	0.65	4.141
0.75	10.659	0.75	4.015
0.85	10.564	0.85	3.892
0.95	10.457	0.95	3.792
1.05	10.296	1.05	3.722
1.15	10.116	1.15	3.629
1.25	9.886	1.25	3.555
1.35	9.373	1.35	3.464
1.38	8.615		

Preparation of 0.01 M Cobalt Perchlorate Solution

1.83 grams of cobalt perchlorate hexahydrate were weighed and then dissolved in 50 ml. of doubly distilled water and this solution diluted to 500.0 ml. in a 500 ml. volumetric flask.

Analysis of 0.01 M Cobalt Perchlorate Solution

A slurry of Dowex 50W-X8 cation exchange resin was prepared in doubly distilled water. This slurry was poured into a column which had a loosely packed glass wool plug in the bottom and the resin allowed to settle until a bed of 1 cm. by 15 cm. was obtained. One

hundred mls of 1 M hydrochloric acid were eluted through the column to insure that the resin was in the acid form. The resin was washed with doubly-distilled water until the eluant produced no precipitate with an aqueous 5% silver nitrate solution. An aliquot of 1.99 ml. of metal solution was introduced in the column and eluted with water until a droplet of the eluant tested neutral to pH paper. This eluant was then titrated with standard base. Duplicate experiments showed the concentration of the cobalt (II) solution to be 0.0140 M. Solutions of copper perchlorate and nickel perchlorate were also prepared and analyzed in this manner.

Stability Constant Titration of 2-Formylpyrazine Thiosemicarbazone

A sample of 2-formylpyrazine thiosemicarbazone weighing 0.0104 grams was measured out and was calculated to be 0.9573 millimoles. This sample was dissolved in 3.0 ml. of 0.1203 N potassium hydroxide and 40.00 ml. of water was added. 3.87 ml. of 0.1082 N hydrochloric acid were added which was calculated to be sufficient to neutralize all excess potassium hydroxide then protonate the $-S^-$ ion and also one ring nitrogen. A volume of copper perchlorate solution was added sufficient to give a 2 to 1 ligand to metal ratio in the solution. This volume calculates to be 2.804 ml. of 0.0102 M copper perchlorate. The sample was titrated with 0.95 ml. of 0.1203 N potassium hydroxide which is the amount required to remove two protons from 0.0573 millimoles of dibasic acid.

Potassium Hydroxide Added (ml.)	pH	Potassium Hydroxide Added (ml.)	pH
0.02	2.867	0.52	3.394
0.07	2.898	0.57	3.517
0.12	2.937	0.62	3.691
0.17	2.973	0.67	3.959
0.22	3.010	0.72	4.489
0.27	3.057	0.77	5.659
0.32	3.104	0.82	6.390
0.37	3.163	0.87	6.974
0.42	3.228	0.92	7.545

These data were used for the stability constant program reproduced elsewhere in this theses. The IBM 360 computer output gave values for X, Y, $\bar{n}$, L^- and various other values calculated during the course of the computations. Also the computer punched out one data card for each point on the titration curve. On this card were punched values for X, Y, volume of OH^-, and a weighting factor. These cards are used with a program for a least squares straight line fit for the best values of K_1 and B_2. Any cards coinciding with a point on the curve which yielded calculated negative values of $\bar{n}$ or L^- were thrown out and not included in the curve fit.

Submittal of Data for Least Squares Straight Line Fit

In front of the complete mass of data, a card must be inserted on which is punched the total number of data sets to be analyzed. Now the complete deck of data is placed behind this card. The first card of the individual data sets is the title card as punched out by the stability constant program. After each of these a card which is hand punched must be placed which gives the number of data points in that individual titration, and the number of terms in the equation to be fit --in this case 2. Next is placed the card which was punched by

the computer on which is such information as volume of titrant, pH, molecular weight, etc. Finally the individual data cards, which punched by the computer, corresponding to that respective titration. In each ensuing set of titration data the one hand-punched card giving number of points and points in the equation to be fit (2) must be placed as just described.

Synthesis of 2-Formylpyridine Thiosemicarbazone:

In a 3-neck 500-ml. round-bottom flask fitted with a separatory funnel with standard taper joint, a condenser for reflux, and a standard-taper glass stopper, was placed a mixture of 27.3 grams of thiosemicarbazide, 117 ml. of water and 3.0 ml. of glacial acetic acid. The mixture was warmed with a heating mantle and stirred magnetically until solution was effected. In the separatory funnel was placed a warm solution of 32.13 grams of pyridine-2-carboxaldehyde dissolved in 120 ml. of denatured 95% ethanol. The solution in the funnel was added slowly with stirring and heating to the solution in the flask. The solution in the flask turned a greenish-yellow color and a cream-colored crystalline precipitate quickly formed. This precipitate was filtered off, washed three times with 25 ml. portions of denatured 95% ethanol, recrystallized from denatured 95% ethanol, dried and then weighed. The yield was 46.5 grams (86.1% yield) of 2-formylpyridine thiosemicarbazone which melted at 215-216° C. It was interesting to note that the product when recrystallized from ethanol was a cream-colored needle crystal. Upon drying while exposed to light the crystals turned a bright yellow in color. Material dried and then stored for a period of six months in a brown glass bottle stayed cream colored. Upon exposure to light

these too turned yellow.

Preparation of Nickel Derivative of IV

In a 400 ml. beaker 9.0 grams (0.05 moles) of crystalline 2-formylpyridine thiosemicarbazone was dissolved in 100 ml. of cool 1 N. sodium hydroxide. In a 100 ml. beaker was dissolved 6.23 grams (0.025 moles) of nickel acetate tetrahydrate. The metal solution was poured into the solution of IV which was being stirred magnetically. A precipitate formed immediately. The mixture was adjusted with hydrochloric acid to a pH of 8 as indicated by Accutint pH 1-12 wide range indicator paper. A precipitate the color of cocoa settled as a fluffy grannular material with an extremely pale yellow supernatant. The granules of the precipitate were too fine to be separated with maximum recovery by filtration (the pores of a sintered glass filter funnel were plugged up by the precipitate). The residue was centrifuged in a Fisher Scientific Co. International Clinical Centrifuge. The precipitate was washed twice with distilled water (to remove any uncomplexed nickel (II) and centrifuged between each washing. It was then washed twice with boiling ethanol to remove any uncomplexed ligand with centrifugation after each washing. The precipitate was dried overnight over calcium chloride in a vacuum dissicator. Yield was 9.5 grams of a dark tan powder which did not melt below 300°.

Qualitative Tests on Nickel Complex of IV

To insure that a complex of thiosemicarbazide was not the product recovered, the above procedure was duplicated substituting thiosemicarbazide for IV and the precipitate could not be duplicated.

A sample of the dry precipitate was ignited and a residue was left indicating the presence of a metal.

Since nickel hydroxide ($Ni(OH)_2$) is a transluscent pale green jelly-like precipitate it was extremely doubtful that the tan precipitate was some type of nickel hydroxide material. To prove the presence of the ligand sodium fusion was performed on a sample of the tan precipitate and positive tests yielding prussian blue and lead sulfide indicated the presence of nitrogen and sulfur respectively, thus confirming the presence of the ligand.

Synthesis of 3-Hydroxy-2-formylpyridine thiosemicarbazone was similar to the procedure of Heinert and Martell.[22]

3-Hydroxy-2-hydroxymethylpyridine hydrochloride

To a solution of 3-hydroxypyridine (47.5 g., 150 moles) and sodium hydroxide (20 g.) in water (200 mls), Merck 40% formaldehyde solution (40 mls, .5 moles) was added. The clear mixture was warmed for five hours at 95° then cooled to room temperature and acetic acid (30 g., .5 moles) was added. The water was removed under reduced pressure, the remaining viscous oil was stirred with Baker reagent grade acetone (.5 liters) and the precipitated sodium acetate was filtered off. The solid was extracted with warm acetone (three 250 ml. portions) and the combined extracts were further diluted with 750 ml. of acetone. The additional precipitate was removed by filtration and the solution was concentrated under reduced pressure

to 1 liter. Hydrogen chloride gas was introduced at 0° whereupon a colorless crystalline precipitate immediately formed. As soon as formation of the precipitate ceased it was filtered off, washed with cold acetone (100 ml.) and stirred with a saturated solution of hydrogen chloride in absolute ethanol at 0° to dissolve unreacted pyridol. Filtration and washing yielded a nearly colorless solid. This solid was dissolved in a minimum of hot water and 2 grams of Nuchar C-115-N activated carbon were stirred in. The solution was warmed and stirred for 20 minutes whereupon the carbon was filtered off and a large volume (1 liter) of acetone was added to the clear filtrate. Filtration and drying gave about 21% yield (17.2 grams) of a white solid which darkened above 180° and turned black around 200°.

3-Hydroxpyridine-2-carboxaldehyde thiosemicarbazone and its copper (II) complex

3-hydroxy-2-hydroxymethylpyridine hydrochloride (16.2 g., 0.1 mole) and amorphous manganese dioxide (8.7 g., 0.1 mole), prepared by heating manganous carbonate for 24 hours at 400°, were suspended in absolute ethanol (200 ml.), heated with stirring to reflux temperature and 96% sulfuric acid (10.2 g., 0.1 mole) in 50 ml. of ethanol was added over a period of 30 minutes. After additional heating under reflux for 1 hour the black solid turned brown and the pH rose to 6 as tested with indicator paper. The reaction mixture was cooled to 40° and filtered. The dark yellow solution was diluted with water (200 ml.) and manganous carbonate was precipitated by adding excess sodium bicarbonate. The filtrate was extracted with ether (one 400 ml. and two 150 ml. portions) and the combined ether

extracts were extracted with 3.7% hydrochloric acid (four 25 ml. portions, containing 0.1 mole of hydrochloric acid). The acidic extracts were freed from ethanol *in vacuo* and adjusted to pH 7 with sodium bicarbonate. Ether was added until the aqueous material was saturated and two layers appeared. This mixture was then extracted with ether (three 100 ml. portions).

At this point the procedure deviated from that of Heinert and Martell. The ether extracts were combined and a sample was tested with a reagent of 2,4-dinitrophenylhydrazine in methanol and hydrochloric acid. One ml. of the 2,4-DNP reagent was added to three ml. of the ether extract. An orange powder precipitated which, when separated, washed and dried, decomposed at 250-255°. This agrees with the melting point (dec.) reported by Heinert and Martell[22] for the 2,4-dinitrophenylhydrazone hydrochloride deriviatve of 3-hydroxypyridine-2-carboxaldehyde. This solution was allowed to sit overnight whereupon two layers formed. The top layer was an ether layer and the bottom dark brown was aqueous. A sample of the brown aqueous layer was tested with 2,4-dinitrophenylhydrazine and the orange powder recovered was the previously discussed 2,4-DNP derivative indicating the presence of the desired aldehyde. Two samples of the brown aqueous layer (5 ml. each) were refluxed for periods of 1 hour and 15 hours in solutions of water (50 ml.), glacial acetic acid (10 ml.) and thiosemicarbazide (5 g.).

The solution refluxed for 1 hour yielded light yellow crystals overnight whose melting point and infrared spectrum were identical with thiosemicarbazide. These were filtered off. To a 5 ml. aliquot of the liquor was added 1 ml. of 5% copper (II) nitrate solution.

When sufficient sodium bicarbonate was added to make the medium slightly basic an olive-green precipitate was formed. An aqueous solution of thiosemicarbazide, when treated in the same way, did not produce this precipitate. The remainder of the liquor was put in a beaker and to this was added with stirring a solution of copper (II) nitrate (5 grams) and water (15 ml.), and sodium bicarbonate to bring the pH to 7.0-7.5. When effervescence ceased the same gray-green precipitate formed. This precipitate was centrifuged and washed five times with distilled water, centrifuging after each washing. This procedure yielded a dry dark gray-green powder which does not melt below 300°. Sodium fusion was performed on a sample of this powder and the presence of the ligand was indicated by the formation of lead sulfide and prussian blue. Ignition left a residue indicating presence of a metal. Two or three milligrams decomposed with five drops of concentrated nitric acid and diluted with 2 ml. of water gave a light blue-green solution which turned deep blue when neutralized with concentrated ammonium hydroxide thus indicating the presence of copper.

The solution which refluxed 15 hours was allowed to sit overnight and no crystals were observed to settle out. Sodium chloride 5 grams) was added and the solution was allowed to sit for five days whereupon a suspension of fine crystals formed. On the bottom of the flask were several score of large dark orange crystals in the shape of parallelograms and star-like clusters of these parallelograms. Two of these crystals were removed, blotted, dried, and then crushed. A melting point range of 230-235 (dec.) was found which agrees with the 225-235 (dec.) reported [22] for the thiosemicarbazone derivative

of 3-hydroxypyridine-2-carboxaldehyde.

Cobalt (II) and Nickel (II) Complexes of 3-Hydroxy-2-formylpyridine TSC

Having thus shown that 3-hydroxy-2-formylpyridine thiosemicarbazone has been formed the cobalt (II) and nickel (II) derivatives were prepared by substituting cobalt (II) nitrate and nickel (II) acetate for copper (II) nitrate. The cobalt complex is a very dark greenish-brown material which does not melt below 300°. The nickel complex is a tan powder which does not melt below 300°.

Preparation of Copper (II) and Cobalt (II) Complexes of IV

Copper acetate and cobalt nitrate were used and the procedure used to prepare the nickel (II) derivative of IV was followed.

The copper (II) complex is an olive-green powder which melts at 210-211.5°. The cobalt (II) complex is a powder the color of cocoa with an extremely faint reddish tinge. It does not melt below 300°.

CHAPTER V

RESULTS

1. 2-Formylpyridine thiosemicarbazone cobalt (II) complex:

   Chocolate-brown powder, m.p. 300°

2. 2-Formylpyridine thiosemicarbazone copper (II) complex:

   Olive-green powder, m.p. 210-211.5°

3. 2-Formylpyridine thiosemicarbazone nickel (II) complex:

   Light brown powder m.p. 300°

4. 3-Hydroxy-2-formylpyridine thiosemicarbazone cobalt (II) complex:

   Dark brown powder m.p. 300°

5. 3-Hydroxy-2-formylpyridine thiosemicarbazone copper (II) complex:

   Dark olive powder m.p. 300°

6. 3-Hydroxy-2-formylpyridine thiosemicarbazone nickel (II) complex:

   Tan powder m.p. 300°

CHAPTER VI

DISCUSSION OF RESULTS

The main problem encountered in this work was that of solubility. The pKa titrations, because of low solubility of the compounds, were necessarily done in dilute solutions i.e. concentrations around 10^{-3} molar. No problem was encountered in basic or acidic regions but in the neutral region of pH 6-8, if the solution were not dilute enough, the compound in question would precipitate out thus preventing any further measurements from being taken. If the solutions were sufficiently dilute, however, the titrations could be carried smoothly through the neutral region of pH 6-8.

In analysis of the data from the pKa titrations a value for the pKa could, of course, be obtained from each point in the titration. It was found that occasionally the individual calculated pKa values would vary noticeably from one another. To find an average the values near each end of the titration were disregarded and if any value near the middle was grossly out of proportion to those around it, that value would also be discarded. The remainder of the values were used to find a simple arithmetic average pKa. The variation in each average pKa treated this way would be of the nature of $\pm$ .05 to $\pm$ .3. However, the reproduceability of the average pKa from one experiment to the next was quite good. Average pKa values from repeated titrations of the same compound were used to find what is reported in this

thesis as the "mean average pKa", or a number average of the averages. Reproduceability was such that variation in these mean averages were generally on the order of $\pm$ .05 to $\pm$ .12.

The problem of solubility was a problem in the stability constant calculations but, as before, if the solution was kept sufficiently dilute, the titrations could be carried to completion even up to pH values as high as 10 without precipitation.

The main problem faced in determination of the stability constants was the calculations themselves. The completion of the computer analysis yielded values which, in every case except two, were negative for K_1, positive for K_2, and negative for β_2 (the product K_1K_2). It was interesting to note, however, that the values thus obtained demonstrated some degree of reproduceability (Table 2). The calculations were them performed without activity corrections to obtain conditional constants at constant ionic strength. The ionic strength is approximated closely by the sum of the chloride ion concentration and twice the perchlorate ion concentration. Representative ionic strengths are on the order of .01 molar throughout the titration with minor changes in the fourth decimal place. Values obtained by these calculations for the most part became positive but in nearly every case reproduceability was gone (Table 3).

Several suggestions may be offered in an attempt to explain the negative values.

First, of course, the equations might not describe the proper complexation reaction. Perhaps the reaction $H_2A^{\oplus} + M^{++} \underset{}{\overset{K_1}{\rightleftharpoons}} MA^{\oplus} + 2H^{\oplus}$ is not the complexing form of the ligand. If only one proton is replaced by the metal ion i.e. the form of the ligand is HA rather than A^{-},

then the derivations presented in this thesis are invalid for calculation of the formation constants and other treatments should be applied.

A second suggestion is that the data are faulty. This appears unlikely because of the reproduceability of the values obtained.

A third alternative must be considered and that is decomposition. In one or two of the experiments the faint odor of hydrogen sulfide was noticed indicating decomposition of the sulfur portion of the molecule. In a private communication F.A. French, from whom the original samples were obtained, recently disclosed that he has found some evidence of decomposition of these materials. If decomposition does occur, it evidently is not in either acid or basic solution because it would have been noticed in the pKa determinations and this was not the case. Perhaps with more evidence, such as might be obtained from ultraviolet absorption studies, a metal-catalyzed decomposition could be investigated.

It is regrettable that the isoquinoline compounds could not be dissolved thus no information of any kind could be obtained.

It is also regrettable that thus far the stability constants are not determined because they would have provided interesting speculation when compared with the anti-cancer activities of the solid metal chelates which will be submitted for physiological testing upon completion of metal analysis.

TABLE I

INDIVIDUAL pKa VALUES

Compound	Mean Average pKa^1	Mean Average pKa^2	Mean Average pKa^3
IV	3.723 - .072	10.871 - .071	
VI	4.035 - .039	7.988 - .094	11.228 - .093
X	3.411 - .128	6.076 - .125	9.792 - .080
VII	3.257 - .193	10.571 - .065	

TABLE II

THERMODYNAMIC STABILITY CONSTANT RESULTS

COMPOUND	K_1 Co	K_2 Co	B_2 Co	K_1 Cu	K_2 Cu	B_2 Cu	K_1 Ni	K_2 Ni	B_2 Ni
IV	$-.673\times10^{12}$	$.116\times10^{5}$	$-.783\times10^{16}$	$-.519\times10^{11}$	$-.137\times10^{3}$	$.713\times10^{13}$			
IV	$-.384\times10^{13}$	$.198\times10^{4}$	$-.764\times10^{16}$	$-.765\times10^{12}$	$.251\times10^{5}$	$-.192\times10^{17}$	$-.126\times10^{13}$	$.628\times10^{9}$	$-.795\times10^{21}$
IV	$-.207\times10^{12}$	$.258\times10^{4}$	$-.535\times10^{15}$	$-.103\times10^{13}$	$.309\times10^{5}$	$-.319\times10^{17}$	$-.174\times10^{13}$	$.582\times10^{8}$	$-.101\times10^{21}$
IV	$-.195\times10^{13}$	$.109\times10^{4}$	$-.214\times10^{16}$	$-.154\times10^{13}$	$.288\times10^{6}$	$-.444\times10^{18}$	$-.100\times10^{14}$	$.697\times10^{10}$	$-.702\times10^{23}$
IV	$-.120\times10^{13}$	$.124\times10^{5}$	$-.149\times10^{17}$	$-.289\times10^{13}$	$.542\times10^{9}$	$-.157\times10^{22}$	$-.641\times10^{13}$	$.759\times10^{10}$	$-.487\times10^{23}$
IV	$-.634\times10^{12}$	$.729\times10^{4}$	$-.462\times10^{16}$						
IV	$-.855\times10^{13}$	$.310\times10^{4}$	$-.265\times10^{17}$						
VII	$.853\times10^{10}$	$.989\times10^{3}$	$.843\times10^{13}$	$-.625\times10^{12}$	$.377\times10^{6}$	$-.235\times10^{18}$	$-.169\times10^{13}$	$.198\times10^{8}$	$-.335\times10^{20}$
VII	$-.113\times10^{12}$	$.857\times10^{3}$	$-.974\times10^{14}$	$-.352\times10^{12}$	$.520\times10^{5}$	$-.183\times10^{17}$	$.175\times10^{16}$	$.171\times10^{8}$	$.300\times10^{23}$
X	$-.337\times10^{11}$	$.340\times10^{3}$	$-.114\times10^{14}$	$-.328\times10^{11}$	$.579\times10^{6}$	$-.190\times10^{17}$	$-.262\times10^{10}$	$.532\times10^{5}$	$-.140\times10^{15}$
X	$-.797\times10^{12}$	$.149\times10^{8}$	$-.119\times10^{20}$	$-.237\times10^{12}$	$.478\times10^{8}$	$-.113\times10^{20}$	$-.760\times10^{12}$	$.132\times10^{5}$	$-.101\times10^{17}$
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
VI	$-.588\times10^{12}$	$.247\times10^{4}$	$-.145\times10^{16}$	$-.605\times10^{13}$	$.310\times10^{11}$	$-.188\times10^{24}$	$-.687\times10^{13}$	$.110\times10^{9}$	$-.757\times10^{21}$
VI	$-.659\times10^{13}$	$.542\times10^{4}$	$-.357\times10^{17}$	$-.877\times10^{13}$	$.104\times10^{11}$	$-.916\times10^{23}$	$-.847\times10^{13}$	$.961\times10^{8}$	$-.814\times10^{21}$
VI	$.787\times10^{10}$	$.872\times10^{3}$	$.686\times10^{13}$	$-.341\times10^{14}$	$.444\times10^{10}$	$-.1515\times10^{24}$	$-.103\times10^{13}$	1195×10^{6}	$-.202\times10^{18}$
VI				$-.309\times10^{12}$	$.417\times10^{10}$	$-.129\times10^{22}$			

TABLE III

STABILITY CONSTANT CALCULATIONS

Compound	B_2	K_1	K_2	Log B_2	Log K_1	Log K_2
IV Co(II)	-2.1678×10^{11}	2.3132×10^{8}	-9.37×10^{2}	-------	8.3642	-------
IV Co(II)	-6.54×10^{11}	5.31×10^{8}	-1.23×10^{3}	-------	8.7348	-------
IV Co(II)	-5.57×10^{16}	-4.38×10^{11}	1.28×10^{5}	-------	-------	5.1046
IV Co(II)	-8.00×10^{15}	-3.30×10^{11}	2.42×10^{4}	-------	-------	-------
IV Cu(II)	1.14×10^{9}	8.10×10^{6}	1.41×10^{2}	9.0574	6.9084	2.1490
IV Cu(II)	7.22×10^{18}	2.53×10^{10}	2.86×10^{8}	18.8583	10.4023	8.4560
IV Ni(II)	-2.42×10^{23}	-5.02×10^{12}	4.82×10^{10}	-------	-------	10.6831
IV Ni(II)	-1.37×10^{24}	-2.01×10^{13}	6.80×10^{10}	-------	-------	10.8325
						10.7578 - .0747
VI Co(II)	3.72×10^{17}	4.82×10^{7}	7.71×10^{9}	17.5705	7.6833	9.8872
VI Co(II)	1.05×10^{14}	4.66×10^{8}	2.26×10^{5}	14.0222	8.6679	5.3543
VI Co(II)	4.78×10^{22}	1.57×10^{12}	3.05×10^{10}	22.6793	12.1956	10.4836
VI Cu(II)	5.98×10^{18}	1.94×10^{9}	3.08×10^{9}	18.7769	9.2879	9.4889
VI Cu(II)	9.17×10^{20}	1.41×10^{12}	6.48×10^{10}	22.9623	12.1504	10.8119
VI Cu(II)	2.84×10^{18}	2.31×10^{10}	1.23×10^{9}	20.4527	10.3643	10.0883
VI Cu(II)	6.46×10^{18}	1.79×10^{9}	3.61×10^{9}	18.8104	9.2529	9.5575
				19.35 - 1.11	9.64 - .73	9.71 - .38
VI Ni(II)	-9.71×10^{23}	-4.57×10^{12}	2.13×10^{11}	-------	-------	11.3277
VI Ni(II)	1.87×10^{19}	4.47×10^{9}	4.17×10^{9}	19.2708	9.6502	9.6204
---------					9.6502	9.6204

TABLE III Continued

Compound	B_2	K_1	K_2	Log B_2	Log K_1	Log K_2
X Co(II)	9.42×10^{15}	8.96×10^{8}	1.05×10^{7}	15.9741	8,9521	7.0219
X Cu(II)	-5.19×10^{22}	-1.64×10^{12}	3.17×10^{10}	-------	-------	10.5014
X Cu(II)	2.85×10^{15}	4.14×10^{8}	6.88×10^{6}	15.4541	8,6167	6.8374
X Ni (II)	8.58×10^{18}	3.28×10^{10}	2.61×10^{8}	18.9333	10.5160	8.4173
X Ni (II)	3.87×10^{15}	8.84×10^{7}	4.38×10^{7}	15.5878	7.9464	7.6414
				17.26 - 1.67	9.23 - 1.28	8.03 - .39
VII Co(II)	-4.79×10^{11}	9.82×10^{9}	$-4.87 \times 10_{2}$	-------	9.9923	-------
VII Co(II)	2.03×10^{13}	2.78×10^{10}	7.31×10^{2}	13.3077	10.4437	2.8641
VII Cu(II)	4.00×10^{16}	2.15×10^{10}	1.86×10^{6}	16.6018	10.3328	6.2691
VII Cu(II)	6.82×10^{17}	2.90×10^{10}	2.35×10^{7}	17.8335	10.4620	7.3715
				17.22 - .62	10.40 - .06	6.82 - .55
VII Ni(II)	3.61×10^{18}	3.24×10^{9}	1.11×10^{9}	18.5570	9.5106	9.0465
VII Ni(II)	5.42×10^{18}	2.13×10^{9}	2.54×10^{9}	18.7337	9.3275	9.4062
				18.65 - .09	9.42 - .09	9.23 - .18

BIBLIOGRAPHY

(1) Basolo and Johnson, Coordination Chemistry, New York, Benjamin and Co., 1964.

(2) Jones, Mark M., Elementary Coordination Chemistry, Englewood Cliffs, N.J., Prentice Hall, Inc., 1964.

(3) Hodnett, Ernest M., and Willie, Winfred, Proc. Okla. Acad. Sci. 46, 107-111, 1966.

(4) Hodnett, Ernest M., and Moore, Charles H., (unpublished results).

(5) French, F.A. and Freedlander, B.L., Cancer Res., 18, 1290-1300, 1958.

(6) Bauer, D.J., Brit. J. Exp. Path., 36, 105, 1955.

(7) Bauer, D.J., and Sadler, P.W., Brit. J. Pharmacol, 15, 101-110, 1960.

(8) Campaigne, E., Thompson, R.L., and Van Werth, J.E., J. Med. Chem., 1, 577-599, 1959.

(9) French, F.A., and Freedlander, B.L., Cancer Res., 18, 172-175, 1958.

(10) Petering, H.G., Buskirk, H.H., and Underwood, G.E., Cancer Res., 24, 367-372, 1964.

(11) Mihich, E., and Nichol, C.A., Cancer Res., 25, 1410-1426, 1965.

(12) Petering, H.G., Van Giessen, G.J., Crim, J.A., and Buskirk, H.H., J. Med. Chem., 9,

(13) French, F.A., and Freedlander, B.L., Cancer Res., 20, 519, 1960.

(14) Brockman, R.W., Thomson, J.R., Bell, M.J., and Skipper, H.E., Cancer Res., 16, 167-170, 1956.

(15) French, F.A., and Blanz, E.J., Cancer Res., 25, 1454-1458, 1965.

(16) French, F.A., and Blanz, E.J., J. Med. Chem., 9, 585-589, 1966.

(17) Cappuccino, J.G., Proc. Am. Assoc. Cancer Res., 41, 1966.

(18) Hodnett, E.M. and Willie, W., Proc. Okla. Acad. Sci., 46, 107-111, 1966.

(19) Petering, H.G., Buskirk, H.H., Crim, J.A., and Van Giessen, G.J., Pharmacologist, 5, 271, 1963.

(20) Petering, H.G., Buskirk, H.H., and Kupiecki, F.P., Fed. Proc., 24, 454, 1965.

(21) Albert and Seijeant, Ionization Constants of Acids and Bases, New York, John Wiley & Sons, Inc., 1962.

(22) Heinert, Dietrich, and Martell, Arthur E., Tetrahedron, 3, 49-61, 1958.

(23) Davies, C.W., Ion Association, London, Butterworth & Co. Ltd., 1962, pp. 41.

(24) Laitinen, Herbert A., Chemical Analysis, New York, McGraw-Hill Book Co., Inc., 1960, pp. 9.

(25) Irving, H., and Rossotti, (Mrs.) H.S., J. Chem. Soc., 3397, 1953.

APPENDIX

PROGRAM FOR THREE pKa CALCULATIONS

```
      $JOB 2211-50011,KP=26          PAUL D. MOONEY
      C     THIS PROGRAM COMPUTES THE THERMODYNAMIC ACID CONSTANTS--%PKA1,PKA2
      C     ,PKA3<--FOR A TRIPROTIC ACID WHOSE FULLY PROTONATED FORM POSSESSES
      C     A PLUS ONE %&< CHARGE--H3A%&<.
      C     THE DATA IS OBTAINED BY DISSOLVING THE ACID IN AN EXCESS
      C     OF STANDARD KOH, ADDING A CALCULATED VOLUME OF STANDARD HCL TO
      C     NEUTRALIZE THE EXCESS KOH, AND THEN TITRATING THIS SALT SOLUTION
      C     WITH HCL.  AFTER NEUTRALIZING THE EXCESS KOH, THE SOLUTION IS
      C     SIMPLY ONE OF KCL AND THE ACID SALT %A<K2 WHICH IS THEN TITRATED.
      1      DIMENSIONA(19),VOLHCL(98),PH(98),TOTALA(98),CONK1(98),CONCL(98),
     1CLMIN(98),TOTK1(98),A2MIN(98),HAMIN(98),SALTS(98),HACTY(98),
     2IONSTR(98),SQROOT(98),MINLG1(98),MINLG2(98),PKA3(98),FONE(98),
     3FTWO(98),TOTCL(98),POH(98),CONOH(98),CONH2A(98),PKA2(98),
     4CONH3A(98),PKA1(98)
      2      REAL INVOL,MOLWT,NORHCL,NOROH,INITCL,IONSTR,MINLG1,MINLG2
      3     40 FORMAT(10X,6HPKAX #,F4.1,13X,17HINIT VOL OF HCL #,F7.3//<
      4     50 FORMAT(2X,19A4<
      5     51 FORMAT(1H1<
      6     52 FORMAT(10X,19A4<
      7    100 FORMAT(I2,F6.2,F7.2,2F7.4,F5.2,F7.4,F4.1,F7.3<
      8    101 FORMAT(F5.2,F7.3<
      9    200 FORMAT(///10X,15HNO OF POINTS # ,I2,3X,21HINIT VOL OF LIQUID # ,
     1F6.2,3X,9HMOL WT # ,F7.2,3X,12HSAMPLE WT # ,F7.4,3X,12HN OF ACID #
     2 ,F7.4///<
     10    202 FORMAT(2X,124H*************************************************
     1*************************************************************
     2******<
     11    205 FORMAT(4X,F6.2,4X,F7.3,2X,E12.5,3X,E12.5,5X,E12.5,3X,F8.5,4X,F8.5,
     17X,E12.5,6X,F7.3<
     12    211 FORMAT(5X,6HVOLHCL,5X,2HPH,7X,6HIONSTR,10X,4HF%1<,13X,4HF%2<,9X,
     17HCONCH3A,5X,7HCONCH2A,10X,5HSALTS,12X,4HPKA1<
     13    212 FORMAT(5X,6HVOLHCL,5X,2HPH,7X,6HIONSTR,10X,4HF%1<,13X,4HF%2<,9X,
     17HCONCH2A,5X,7HHAMINUS,10X,5HSALTS,12X,4HPKA2<
     14    213 FORMAT(5X,6HVOLHCL,5X,2HPH,7X,6HIONSTR,10X,4HF%1<,13X,4HF%2<,9X,
     17HA2MINUS,5X,7HHAMINUS,10X,5HSALTS,12X,4HPKA3<
     15     10 READ(5,50)(A(J),J=1,19)
     16        WRITE(6,51)
     17        WRITE(6,52)(A(J),J=1,19)
     18        READ(5,100)NOPTS,INVOL,MOLWT,SAMWT,NORHCL,VOLKOH,NOROH,PKAX,INITCL
      C     VOLKOH IS THE VOLUME OF STANDARD KOH IN WHICH THE ACID SAMPLE
      C     WAS DISSOLVED.
      C     INITCL IS THE VOLUME OF STANDARD HCL ADDED TO NEUTRALIZE THE
      C     EXCESS KOH IN WHICH THE SAMPLE IS DISSOLVED.  A PORTION OF THE KOH
      C     IS NEUTRALIZED BY THE  ACID SAMPLE ITSELF LEAVING AN EXCESS AMOUNT
      C     OF KOH TO BE NEUTRALIZED BY STANDARD HCL IN ORDER TO START THE
      C     TITRATION AT THE STOICHIOMETRIC POINT OF KCL PLUS THE POTASSIUM
      C     SALT OF THE ACID.
      C     INITCL IS THIS AMOUNT OF HCL ADDED TO NEUTRALIZE THE EXCESS KOH
      C     PLUS ANY HCL TITRANT IN A PREVIOUS PORTION OF THE TITRATION--IF
      C     ANY.
      C     PKAX IS THE PKA VALUE TO BE CALCULATED--PKA1,PKA2,PKA3--AND HAS
      C     ONE OF THE VALUES 1.0, 2.0, OR 3.0.  IT IS FORMATTED F4.1.
     19        IF(NOPTS.EQ.99)GO TO 999
     20        WRITE(6,200)NOPTS,INVOL,MOLWT,SAMWT,NORHCL
     21        WRITE(6,40)PKAX,INITCL
     22        IF(PKAX.EQ.1.)GO TO 111
     23        IF(PKAX.EQ.2.)GO TO 222
     24        WRITE(6,213)
     25        WRITE(6,202)
      C     THIS PORTION OF THE PROGRAM COMPUTES PKA3.
     26        I=0
     27        DO334 I=1,NOPTS
     28        READ(5,101)VOLHCL(I),PH(I)
     29        TOTALA(I)=(SAMWT*1000.0)/(MOLWT*(INVOL+VOLHCL(I)))
     30        CONK1(I)=2.*TOTALA(I)
     31        CONCL(I)=VOLHCL(I)*NORHCL/(INVOL+VOLHCL(I))
     32        CLMIN(I)=((INITCL+VOLHCL(I))*NORHCL)/(INVOL+VOLHCL(I))
     33        TOTK1(I)=(VOLKOH*NOROH)/(INVOL+VOLHCL(I))
     34        A2MIN(I)=TOTK1(I)-CLMIN(I)-TOTALA(I)
     35        HAMIN(I)=TOTALA(I)-A2MIN(I)
     36        SALTS(I)=A2MIN(I)/HAMIN(I)
     37        HACTY(I)=EXP(-2.30258*PH(I))
     38        IONSTR(I)=2.*TOTK1(I)-CLMIN(I)-TOTALA(I)
     39        SQROOT(I)=(SQRT(IONSTR(I)))/(1.+SQRT(IONSTR(I)))
     40        MINLG1(I)=.509*(SQROOT(I)+0.3*IONSTR(I))
     41        MINLG2(I)=4.*MINLG1(I)
     42        PKA3(I)=PH(I)-ALOG10(SALTS(I))+MINLG2(I)-MINLG1(I)
     43        FONE(I)=EXP(-2.30258*MINLG1(I))
     44        FTWO(I)=EXP(-2.30258*MINLG2(I))
     45        WRITE(6,205)VOLHCL(I),PH(I),IONSTR(I),FONE(I),FTWO(I),HAMIN(I),
     1A2MIN(I),SALTS(I),PKA3(I)
     46    334 CONTINUE
     47        WRITE(6,202)
     48        GO TO 10
     49    222 CONTINUE
      C     THIS PORTION OF THE PROGRAM COMPUTES PKA2.
     50        WRITE(6,212)
     51        WRITE(6,202)
     52        I=0
     53        DO298 I=1,NOPTS
     54        READ(5,101)VOLHCL(I),PH(I)
     55        TOTK1(I)=(VOLKOH*NOROH)/(INVOL+VOLHCL(I))
     56        IONSTR(I)=TOTK1(I)
     57        SQROOT(I)=(SQRT(IONSTR(I)))/(1.+SQRT(IONSTR(I)))
     58        MINLG1(I)=.509*(SQROOT(I)+0.3*IONSTR(I))
     59        MINLG2(I)=4.*MINLG1(I)
     60        FONE(I)=EXP(-2.30258*MINLG1(I))
     61        FTWO(I)=EXP(-2.30258*MINLG2(I))
     62        TOTALA(I)=(SAMWT*1000.)/(MOLWT*(INVOL+VOLHCL(I)))
     63        HACTY(I)=EXP(-2.30258*PH(I))
     64        TOTCL(I)=((INITCL+VOLHCL(I))*NORHCL)/(INVOL+VOLHCL(I))
     65        POH(I)=(13.997-PH(I))
     66        CONOH(I)=(EXP(-2.30258*POH(I)))/FONE(I)
     67        HAMIN(I)=HACTY(I)+TOTK1(I)-CONOH(I)-TOTCL(I)
     68        CONH2A(I)=TOTALA(I)-TOTK1(I)+CONOH(I)+TOTCL(I)
     69        SALTS(I)=HAMIN(I)/CONH2A(I)
     70        PKA2(I)=PH(I)-ALOG10(SALTS(I))+MINLG1(I)
     71        WRITE(6,205)VOLHCL(I),PH(I),IONSTR(I),FONE(I),FTWO(I),CONH2A(I),
     1HAMIN(I),SALTS(I),PKA2(I)
     72    298 CONTINUE
     73        WRITE(6,202)
     74        GO TO 10
     75    111 CONTINUE
     76        WRITE(6,211)
     77        WRITE(6,202)
      C     THIS PORTION OF THE PROGRAM COMPUTES PKA1.
     78        I=0
     79        DO398 I=1,NOPTS
     80        READ(5,101)VOLHCL(I),PH(I)
     81        TOTK1(I)=(VOLKOH*NOROH)/(INVOL+VOLHCL(I))
```

Continued

```
      TOTCL(I)=(INITCL+VOLHCL(I))*NORHCL/(INVOL+VOLHCL(I))
      IONSTR(I)=TOTCL(I)
      SQROOT(I)=(SQRT(IONSTR(I)))/(1.+SQRT(IONSTR(I)))
      MINLG1(I)=.509*(SQROOT(I)+.3*IONSTR(I))
      MINLG2(I)=4.*MINLG1(I)
      FONE(I)=EXP(-2.30258*MINLG1(I))
      FTWO(I)=EXP(-2.30258*MINLG2(I))
      TOTALA(I)=(SAMWT*1000.)/(MOLWT*(INVOL+VOLHCL(I)))
      HACTY(I)=EXP(-2.30258*PH(I))
      CONH3A(I)=TOTCL(I)-TOTK1(I)
      CONH2A(I)=TOTALA(I)-CONH3A(I)
      SALTS(I)=CONH2A(I)/CONH3A(I)
      PKA1(I)=PH(I)-ALOG10(SALTS(I))-MINLG1(I)
      WRITE(6,205)VOLHCL(I),PH(I),IONSTR(I),FONE(I),FTWO(I),CONH2A(I),
     1CONH3A(I),SALTS(I),PKA1(I)
  398 CONTINUE
      WRITE(6,202)
      GO TO 10
  999 CONTINUE
      STOP
      END

$ENTRY
```

SAMPLE pKa CALCULATION OUTPUT

3-OH-2-FPTSC PKA1 NB%II< P. 46%BOTTOM< P. D. MOONEY

NO OF POINTS # 13 INIT VOL OF LIQUID # 46.29 MOL WT # 196.21 SAMPLE WT # 0.0126 N OF ACID # 0.0997

PKAX # 1.0 INIT VOL OF HCL # 3.290

VOLHCL	PH	IONSTR	F%1<	F%2<	CONCH3A	CONCH2A	SALTS	PKA1
0.06	5.890	0.72059E-02	0.91007E 00	0.68595E 00	0.00127	0.00011	0.11362E 02	4.794
0.10	5.231	0.72857E-02	0.90962E 00	0.68459E 00	0.00119	0.00020	0.59932E 01	4.412
0.15	4.684	0.73852E-02	0.90906E 00	0.68292E 00	0.00108	0.00031	0.35326E 01	4.094
0.20	4.410	0.74845E-02	0.90851E 00	0.68126E 00	0.00097	0.00041	0.23529E 01	3.997
0.25	4.233	0.75835E-02	0.90796E 00	0.67963E 00	0.00086	0.00052	0.16604E 01	3.971
0.31	4.073	0.77021E-02	0.90731E 00	0.67769E 00	0.00073	0.00065	0.11320E 01	3.977
0.35	3.967	0.77810E-02	0.90689E 00	0.67641E 00	0.00065	0.00073	0.88276E 00	3.979
0.40	3.850	0.78795E-02	0.90636E 00	0.67483E 00	0.00054	0.00084	0.64267E 00	3.999
0.45	3.756	0.79777E-02	0.90583E 00	0.67327E 00	0.00043	0.00094	0.45695E 00	4.053
0.51	3.658	0.80953E-02	0.90521E 00	0.67141E 00	0.00030	0.00107	0.28280E 00	4.163
0.55	3.578	0.81735E-02	0.90479E 00	0.67019E 00	0.00022	0.00115	0.18915E 00	4.260
0.60	3.509	0.82711E-02	0.90428E 00	0.66868E 00	0.00011	0.00126	0.87820E-01	4.522
0.63	3.461	0.83296E-02	0.90398E 00	0.66778E 00	0.00005	0.00132	0.35361E-01	4.369

3-OH-2-FPTSC PKA2 NB%I< P. 68 P. D. MOONEY

NO OF POINTS # 12 INIT VOL OF LIQUID # 46.20 MOL WT # 196.21 SAMPLE WT # 0.0347 N OF ACID # 0.1110

PKAX # 2.0 INIT VOL OF HCL # 2.200

VOLHCL	PH	IONSTR	F%1<	F%2<	CONCH2A	HAMINUS	SALTS	PKA2
0.05	9.427	0.91157E-02	0.90001E 00	0.65612E 00	0.00014	0.00369	0.26700E 02	5.046
0.10	9.153	0.91058E-02	0.90006E 00	0.65626E 00	0.00024	0.00358	0.14667E 02	8.032
0.20	8.694	0.90862E-02	0.90015E 00	0.65655E 00	0.00047	0.00334	0.70725E 01	7.990
0.30	8.476	0.90667E-02	0.90025E 00	0.65683E 00	0.00071	0.00310	0.43743E 01	7.881
0.40	8.339	0.90472E-02	0.90035E 00	0.65711E 00	0.00094	0.00285	0.30225E 01	7.904
0.50	8.186	0.90278E-02	0.90044E 00	0.65739E 00	0.00118	0.00261	0.22136E 01	7.886
0.60	8.026	0.90085E-02	0.90054E 00	0.65767E 00	0.00141	0.00237	0.16752E 01	7.847
0.70	7.898	0.89893E-02	0.90063E 00	0.65795E 00	0.00165	0.00212	0.12910E 01	7.833
0.80	7.807	0.89702E-02	0.90073E 00	0.65822E 00	0.00188	0.00188	0.10032E 01	7.851
0.90	7.729	0.89512E-02	0.90082E 00	0.65850E 00	0.00211	0.00164	0.77956E 00	7.883
1.00	7.635	0.89322E-02	0.90092E 00	0.65878E 00	0.00234	0.00141	0.60095E 00	7.902
1.10	7.525	0.89133E-02	0.90101E 00	0.65905E 00	0.00257	0.00117	0.45475E 00	7.912

PROGRAM FOR TWO pKa CALCULATIONS

```
      $JOB 2211-50011,KP=26          PAUL D. MOONEY
      C     THIS PROGRAM COMPUTES THE THERMODYNAMIC PKA VALUES FOR A DIBASIC
      C     ACID WHICH ,IN ITS FULLY PROTONATED FORM, HAS A %&< CHARGE.
      C     I.E. H2A%&<.  THE DATA IS OBTAINED BY TITRATING THE ACID SALT---
      C     THE SOLUTION RESULTING FROM DISSOLVING THE ACID IN STANDARD KOH---
      C     WITH STANDARD HCL.
    1    49 FORMAT(10X,6HPKAX #,F4.1,13X,17HINIT VOL OF HCL #,F7.3//<
    2    50 FORMAT(2X,19A4<
    3    51 FORMAT(1H1<
    4    52 FORMAT(20X,19A4<
    5   100 FORMAT(I2,F6.2,F7.2,2F7.4,F5.2,F7.4,F4.1,F7.3<
    6   200 FORMAT(///10X,15HNO OF POINTS # ,I2,3X,21HINIT VOL OF LIQUID # ,
           1F6.2,3X,9HMOL WT # ,F7.2,3X,12HSAMPLE WT # ,F7.4,3X,12HN OF ACID #
           2 ,F7.4///<
    7   101 FORMAT(F5.2,F7.3<
    8   201 FORMAT(5X,6HVOLHCL,5X,2HPH,7X,6HIONSTR,10X,4HF%1<,13X,6HAMINUS,7X,
           15HHACID,10X,5HSALTS,18X,4HPKA2<
    9   202 FORMAT(2X,115H*****************************************************
           1*************************************************<
   10   205 FORMAT(6X,F5.2,3X,F6.3,1X,E14.6,2X,E14.6,5X,F8.5,4X,F8.5,6X,E14.6,
           110X,F7.3<
   11   203 FORMAT(5X,6HVOLHCL,5X,2HPH,7X,6HIONSTR,10X,4HF%1<,13X,5HCONHA,
           17X,6HCONH2A,10X,5HSALTS,18X,4HPKA1<
   12       REALINVOL,MOLWT,NORHCL,NOROH,INITCL,IONSTR,MINLG
   13       DIMENSIONVOLHCL(98),PH(98),A(19),CONK1(98),IONSTR(98),SQROOT(98),
           1MINLG(98),FONE(98),CONLH(98),HACTY(98),CONCL(98),POH(98),CONOH(98)
           2,AMINUS(98),HACID(98),SALTS(98),PKA2(98),CHION(98),CONLH2(98),
           3CONH2A(98),CONHA(98),PKA1(98),CLMIN(98),TOTK1(98)
   14    10 READ(5,50)(A(J),J=1,19)
   15       WRITE(6,51)
   16       WRITE(6,52)(A(J),J=1,19)
   17       READ(5,100)NOPTS,INVOL,MOLWT,SAMWT,NORHCL,VOLKOH,NOROH,PKAX,INITCL
      C     PKAX IS THE PKA VALUE BEING CALCULATED AND HAS THE VALUE OF &1.0
      C     OR &2.0.  IT IS FORMATTED F4.1.
      C     INITCL IS THE VOLUME OF CHLORIDE ION PREVIOUSLY ADDED IN THE
      C     FORM OF HCL ADDED EITHER TO NEUTRALIZE EXCESS KOH OR AS TITRANT
      C     IN A PREVIOUS PORTION OF THE TITRATION.
   18       IF(NOPTS.EQ.99)GO TO 999
   19       WRITE(6,200)NOPTS,INVOL,MOLWT,SAMWT,NORHCL
   20       WRITE(6,49)PKAX,INITCL
   21       IF(PKAX.EQ.I.)GO TO 500
   22       WRITE(6,201)
   23       WRITE(6,202)
      C     THIS PORTION OF THE PROGRAM COMPUTES PKA2.
   24       I=0
   25       DO 499 I=1,NOPTS
   26       READ(5,101)VOLHCL(I),PH(I)
   27       TOTK1(I)=VOLKOH*NOROH/(INVOL+VOLHCL(I))
      C     IN THIS CASE THE IONIC STRENGTH IS MEASURED DIRECTLY AS THE
      C     POTASSIUM ION CONCENTRATION.
   28       IONSTR(I)=TOTK1(I)
   29       SQROOT(I)=(SQRT(IONSTR(I)))/(1.+SQRT(IONSTR(I)))
   30       MINLG(I)=.509*(SQROOT(I)-0.3*IONSTR(I))
   31       FONE(I)=EXP(-2.30258*MINLG(I))
   32       CONLH(I)=(SAMWT*1000.)/(MOLWT*(INVOL+VOLHCL(I)))
   33       CONK1(I)=CONLH(I)
   34       HACTY(I)=EXP(-2.30258*PH(I))
      C     THE VALUE REPRESENTED BY THE VARIABLE NAME CLMIN%I< IS THE TOTAL
      C     CHLORIDE CONCENTRATION.
      C     THE VALUE REPRESENTED BY THE VARIABLE NAME CONCL%I< IS THE
      C     CHLORIDE CONTRATION ADDED IN THE FORM OF HYDROCHLORIC ACID %HCL<.
   35       CLMIN(I)=(INITCL+VOLHCL(I))*NORHCL/(INVOL+VOLHCL(I))
   36       CONCL(I)=(VOLHCL(I)*NORHCL)/(INVOL+VOLHCL(I))
   37       POH(I)=(13.997-PH(I))
   38       CONOH(I)=(EXP(-2.30258*POH(I)))/FONE(I)
   39       AMINUS(I)=HACTY(I)+CONK1(I)-CONOH(I)-CONCL(I)
   40       HACID(I)=CONLH(I)-CONK1(I)+CONOH(I)+CONCL(I)
   41       SALTS(I)=AMINUS(I)/HACID(I)
   42       PKA2(I)=PH(I)-ALOG10(SALTS(I))+MINLG(I)
   43       WRITE(6,205)VOLHCL(I),PH(I),IONSTR(I),FONE(I),AMINUS(I),HACID(I),
           1SALTS(I),PKA2(I)
  499 CONTINUE
   44       WRITE(6,202)
   45       GO TO 10
   46    500 CONTINUE
   47    THIS PORTION OF THE PROGRAM COMPUTES PKA1.
   48       WRITE(6,203)
   49       WRITE(6,202)
   50       I=0
   51       DO998 I=1,NOPTS
   52       READ(5,101)VOLHCL(I),PH(I)
   53       TOTK1(I)=(VOLKOH*NOROH)/(INVOL+VOLHCL(I))
   54       CLMIN(I)=(INITCL+VOLHCL(I))*NORHCL/(INVOL+VOLHCL(I))
   55       CONCL(I)=(VOLHCL(I)*NORHCL)/(INVOL+VOLHCL(I))
      C     IN THIS CASE IONIC STRENGTH IS MEASURED DIRECTLY AS TOTAL CHLORIDE
      C     ION CONCENTRATION.
      C     TOTAL CHLORIDE ION CONCENTRATION IS INDICATED BY THE VARIABLE NAME
      C     CLMIN%I<.  THE VARIABLE NAME CONCL%I< IS A MEASURE OF CHLORIDE ION
      C     ADDED IN THE FORM OF HYDROCHLORIC ACID %HCL<.
   56       IONSTR(I)=CLMIN(I)
   57       SQROOT(I)=(SQRT(IONSTR(I)))/(1.+SQRT(IONSTR(I)))
   58       MINLG(I)=.509*(SQROOT(I)-0.3*IONSTR(I))
   59       FONE(I)=EXP(-2.30258*MINLG(I))
   60       HACTY(I)=EXP(-2.30258*PH(I))
   61       CHION(I)=HACTY(I)/FONE(I)
   62       CONLH2(I)=(SAMWT*1000.)/(MOLWT*(INVOL+VOLHCL(I)))
      C     CONLH2 IS A MISNOMER.  IT INDICATES THE FULLY PROTONATED FORM OF
      C     THE LIGAND WHEN IN ACTUALITY IT IS THE K%&< SALT FORM OF THE
      C     TOTAL ACID.
   63       CONK1(I)=CONLH2(I)
   64       CONH2A(I)=CLMIN(I)-TOTK1(I)
   65       CONHA(I)=CONLH2(I)-CONH2A(I)
   66       SALTS(I)=CONHA(I)/CONH2A(I)
   67       PKA1(I)=PH(I)-ALOG10(SALTS(I))-MINLG(I)
   68       WRITE(6,205)VOLHCL(I),PH(I),IONSTR(I),FONE(I),CONHA(I),CONH2A(I),
           1SALTS(I),PKA1(I)
  998 CONTINUE
   69       WRITE(6,202)
   70       GO TO 10
   71    999 CONTINUE
   72       STOP
   73       END
   74
```

PROGRAM FOR STABILITY CONSTANT CALCULATIONS

```
FORTRAN IV G LEVEL 1, MOD 3            MAIN            DATE = 69107        07/19/22

C     THIS PROGRAM COMPUTES THERMODYNAMIC FORMATION %STABILITY<
C     CONSTANTS FOR A DIVALENT METAL AND A BIDENTATE ACIDIC LIGAND.
C     THE PROGRAM ALSO PUNCHES OUT DATA WHICH IS READY FOR ANALYSIS BY
C     A LEAST SQUARES STRAIGHT LINE FIT TO DETERMINE THE BEST K%1< AND
C     K%2< VALUES IN ADDITION TO THE PRODUCT K%1<*K%2< WHICH IS
C     EXPRESSED AS THE TERM B%2<.
0001        DIMENSION A(19),VOLOH(98),PH(98),CONME(98),CONKOH(98),CONLH(98),
           1POH(98),CLO4(98),CONCL(98),IONSTR(98),SQROOT(98),KA2(98),P(98),
           2Q(98),OHACTY(98),CONL(98),NBAR(98),Y(98),X(98),KA1(98),MINLG1(98),
           3MINLG2(98),HACTY(98),FONE(98),FTWO(98),CONOH(98)
0002        REAL INVOL,MWLH,NOROH,MOLME,INITCL,NORHCL,IONSTR,MINLG1,MINLG2,
           1KA1,KA2,NBAR
0003         1 FORMAT(1H1)
0004         7 FORMAT(2X,19A4)
0005         2 FORMAT(I2,F6.2,F7.2,F7.4,2F9.6,F6.2,3F7.3,F7.4)
0006        20 FORMAT(I2,F5.2,F6.2,F6.4,2F8.6,F5.2,2F6.3)
0007         3 FORMAT(/2X,17HNUMBER OF POINTS#,I2,3X,16HMOL WT OF COMPD#,F7.2,
           13X,5HPKA1#,F7.3,3X,5HPKA2#,F7.3)
0008         8 FORMAT(2X,13HINIT VOL H2O#,F5.2,3X,10HSAMPLE WT#,F7.4,3X,
           110HN OF BASE#,F9.6,3X,16HM OF METAL SOLN#,F9.6,3X,18HVOL OF METAL
           2SOLN#,F5.2//)
0009         4 FORMAT(2X,8HVOL BASE,4X,2HPH,7X,5HCONME,5X,5HCONLH,5X,5HHACTY,
           19X,5HCONOH,6X,4HCONL,6X,4HNBAR,7X,1HY,9X,1HX,11X,4HF%1<,9X,
           24HF%2<)
0010       202 FORMAT(1X,130H************************************************************
           1************************************************************
           2************)
0011         5 FORMAT(F6.2,F7.3)
0012         6 FORMAT(2X,F6.2,3X,F7.3,2(2X,F9.5),2X,E10.3,1X,E10.3,1X,4(E9.2,1X),
           1E11.4,2X,F11.4)
0013         9 FORMAT(3E14.8,F6.2)
0014           W=1.0
C     IN THIS PROGRAM THE TERM W IS MEANINGLESS.  IN A CERTAIN
C     LEAST SQUARES FIT IT IS NECESSARY AS A WEIGHTING TERM WHICH
C     SPECIFIES EQUAL WEIGHT FOR ALL DATA.  IN THIS PROGRAM A WEIGHTING
C     FACTOR OF 1.0 IS ASSUMED BUT THIS VALUE OF 1.0 IS STATED AND
C     PRINTED OUT FOR USE IF THE PROGRAM REFERRED TO IS EVER USED.
0015        10 READ(5,7)(A(J),J=1,19)
0016           READ(5,2)NOPTS,INVOL,MWLH,SWLH,NOROH,MOLME,VOLME,PKA1,PKA2,INITCL,
           1NORHCL
0017           IF(NOPTS.EQ.99)GO TO 999
0018           WRITE(6,1)
0019           WRITE(6,7)(A(J),J=1,19)
0020           WRITE(7,7)(A(J),J=1,19)
0021           WRITE(6,3)NOPTS,MWLH,PKA1,PKA2
0022           WRITE(6,8)INVOL,SWLH,NOROH,MOLME,VOLME
0023           WRITE(7,20)NOPTS,INVOL,MWLH,SWLH,NOROH,MOLME,VOLME,PKA1,PKA2
0024           WRITE(6,4)
0025           WRITE(6,202)
0026           I=0
0027           DO11 I=1,NOPTS
0028           READ(5,5)VOLOH(I),PH(I)
0029           CONME(I)=(MOLME*VOLME)/(INVOL+VOLME+VOLOH(I))
0030           CONKOH(I)=(NOROH*VOLOH(I))/(INVOL+VOLME+VOLOH(I))
0031           CONLH(I)=(SWLH*1000.)/(MWLH*(INVOL+VOLME+VOLOH(I)))
0032           POH(I)=14.167-PH(I)
0033           CLO4(I)=2.*CONME(I)
0034           CONCL(I)=((INITCL*NORHCL)/(INVOL+VOLME+VOLOH(I))

FORTRAN IV G LEVEL 1, MOD 3            MAIN            DATE = 69107        07/19/22

0035           IONSTR(I)=CONCL(I)+2.*CLO4(I)
0036           SQROOT(I)=(SQRT(IONSTR(I)))/(1.+SQRT(IONSTR(I)))
0037           MINLG1(I)=.509*(SQROOT(I)-0.3*IONSTR(I))
0038           MINLG2(I)=4.0*MINLG1(I)
0039           FONE(I)=EXP(-2.30258*MINLG1(I))
0040           FTWO(I)=EXP(-2.30258*MINLG2(I))
0041           HACTY(I)=EXP(-2.30258*PH(I))
0042           KA1(I)=EXP(-2.30258*PKA1)
0043           KA2(I)=EXP(-2.30258*PKA2)
0044           P(I)=((2.*HACTY(I)**2)/(KA1(I)*KA2(I)))+(HACTY(I)/KA2(I))
0045           Q(I)=(HACTY(I)**2/(KA1(I)*KA2(I)))+HACTY(I)/KA2(I)+1.
0046           OHACTY(I)=EXP(-2.30258*POH(I))
0047           CONOH(I)=OHACTY(I)
0048           CONL(I)=(CONLH(I)*FONE(I)-CONKOH(I)-HACTY(I)+OHACTY(I))/P(I)
0049           NBAR(I)=(CONLH(I)*FONE(I)-CONL(I)*FONE(I)*Q(I))/(CONME(I)*FTWO(I))
0050           Y(I)=NBAR(I)/((NBAR(I)-1.)*CONL(I))
0051           X(I)=((2.-NBAR(I))*CONL(I))/(NBAR(I)-1.)
0052           WRITE(6,6)VOLOH(I),PH(I),CONME(I),CONLH(I),HACTY(I),CONOH(I),CONL(
           1I),NBAR(I),Y(I),X(I),FONE(I),FTWO(I)
0053           WRITE(7,9)Y(I),X(I),W,VOLOH(I)
0054        11 CONTINUE
0055           WRITE(6,202)
0056           GO TO 10
0057       999 CONTINUE
0058           STOP
```

SAMPLE STABILITY CONSTANT OUTPUT

CU COMPLEX OF GLYCINE ALBERT AND SERJEANT DATA P. D. MOONEY

NUMBER OF POINTS#19 MOL WT OF COMPD# 75.07 PKA1# 2.220 PKA2# 9.860
INIT VOL H2O# 46.00 SAMPLE WT# 0.0375 N OF BASE# 0.100000 M OF METAL SOLN# 0.062800 VOL OF METAL SOLN# 4.00

VOL BASE	PH	CONME	CONLH	HACTY	CONOH	CONL	NBAR	Y	X	F%1<	F%2<
0.25	3.300	0.004999	0.009941	0.501E-03	0.136E-10	0.18E-08	0.86E 00	-0.33E 10	-0.14E-07	0.8710E 00	0.5754E 00
0.50	3.380	0.004974	0.009892	0.417E-03	0.163E-10	0.21E-08	0.95E 00	-0.88E 10	-0.43E-07	0.8712E 00	0.5761E 00
0.85	3.510	0.004940	0.009824	0.309E-03	0.220E-10	0.27E-08	0.11E 01	0.48E 10	0.29E-07	0.8715E 00	0.5770E 00
1.00	3.580	0.004925	0.009795	0.263E-03	0.259E-10	0.30E-08	0.11E 01	0.26E 10	0.18E-07	0.8717E 00	0.5773E 00
1.25	3.700	0.004901	0.009747	0.200E-03	0.341E-10	0.38E-08	0.13E 01	0.13E 10	0.11E-07	0.8719E 00	0.5780E 00
1.50	3.830	0.004878	0.009700	0.148E-03	0.460E-10	0.48E-08	0.14E 01	0.77E 09	0.82E-08	0.8722E 00	0.5786E 00
1.75	3.960	0.004854	0.009653	0.110E-03	0.621E-10	0.60E-08	0.15E 01	0.51E 09	0.62E-08	0.8724E 00	0.5792E 00
2.00	4.120	0.004831	0.009606	0.759E-04	0.897E-10	0.79E-08	0.16E 01	0.33E 09	0.49E-08	0.8726E 00	0.5799E 00
2.25	4.280	0.004808	0.009560	0.525E-04	0.130E-09	0.10E-07	0.18E 01	0.23E 09	0.34E-08	0.8729E 00	0.5805E 00
2.50	4.470	0.004785	0.009515	0.339E-04	0.201E-09	0.14E-07	0.19E 01	0.15E 09	0.17E-08	0.8731E 00	0.5811E 00
2.75	4.660	0.004762	0.009470	0.219E-04	0.311E-09	0.19E-07	0.20E 01	0.10E 09	-0.59E-09	0.8733E 00	0.5817E 00
3.00	4.850	0.004740	0.009425	0.141E-04	0.482E-09	0.25E-07	0.22E 01	0.74E 08	-0.37E-08	0.8736E 00	0.5823E 00
3.25	5.050	0.004717	0.009381	0.891E-05	0.764E-09	0.32E-07	0.23E 01	0.55E 08	-0.78E-08	0.8738E 00	0.5829E 00
3.50	5.270	0.004695	0.009337	0.537E-05	0.127E-08	0.41E-07	0.25E 01	0.41E 08	-0.13E-07	0.8740E 00	0.5836E 00
3.75	5.480	0.004673	0.009294	0.331E-05	0.206E-08	0.48E-07	0.26E 01	0.34E 08	-0.18E-07	0.8742E 00	0.5842E 00
4.00	5.720	0.004652	0.009251	0.191E-05	0.357E-08	0.49E-07	0.28E 01	0.32E 08	-0.21E-07	0.8745E 00	0.5848E 00
4.25	6.010	0.004630	0.009208	0.977E-06	0.697E-08	0.31E-07	0.29E 01	0.49E 08	-0.15E-07	0.8747E 00	0.5854E 00
4.50	6.280	0.004609	0.009166	0.525E-06	0.130E-07	-0.63E-07	0.30E 01	-0.24E 08	0.32E-07	0.8749E 00	0.5859E 00
4.99	8.960	0.004558	0.009084	0.110E-08	0.621E-05	-0.14E-03	0.34E 01	-0.10E 05	0.81E-04	0.8753E 00	0.5871E 00

HASP-II JOB STATISTICS -- 113 CARDS READ -- 213 LINES PRINTED -- 21 CARDS PUNCHED -- 2.55 MINUTES EXECUTION TIME

PROGRAM FOR LEAST SQUARES TREATMENT

```
      $JOB 2211-50011,KP=26          PAUL D. MOONEY
      C     THIS PROGRAM FITS POINTS TO THE BEST STRAIGHT LINE AND REPORTS
      C     THE INTERCEPT AS K%1< AND THE SLOPE AS B%2< WHICH IS DEFINED AS
      C     THE PRODUCT K%1<*K%2<.
    1   100 FORMAT(20A4<
    2   101 FORMAT(1H1,25X,20A4<
    3   200 FORMAT(I2,F5.2,F6.2,F6.4,2F8.6,F5.2,2F6.3<
    4   201 FORMAT(/2X,17HNUMBER OF POINTS#,I2,3X,16HMOL WT OF COMPD#,F6.2,
           13X,5HPKA1#,F6.3,3X,5HPKA2#,F6.3<
    5   202 FORMAT(2X,13HINIT VOL H2O#,F6.2,3X,10HSAMPLE WT#,F7.4,3X,
           11OHN OF BASE#,F9.6,3X,16HM OF METAL SOLN#,F9.6,3X,18HVOL OF METAL
           2SOLN#,F6.2//<
    6   301 FORMAT(3E14.8,F6.2<
    7   400 FORMAT(/////5X,11HVOL OF BASE,18X,4HX%I<,18X,4HY%I<,18X,
           15HXY%I<,18X,5HXX%I<<
    8   401 FORMAT(7X,F6.2,16X,E15.8,8X,E15.8,8X,E15.8,8X,E15.8<
    9   402 FORMAT(2X,115H*****************************************************
           1*************************************************************<
   10   403 FORMAT(/23X,6HSUMX #,E15.8,3X,6HSUMY #,E15.8,2X,7HSUMXY #,E15.8,2X
           1,7HSUMXX #,E15.8/////<
   11   700 FORMAT(20X,6HK%1< #,E15.8<
   12   701 FORMAT(20X,6HK%2< #,E15.8<
   13   702 FORMAT(20X,6HB%2< #,E15.8<
   14   520 FORMAT(///20X,11HLOG B%2< # ,F9.5<
   15   600 FORMAT(///20X,53HLOG B%2< # ---B%2< IS NEGATIVE SO NO LOG IS POSSI
           1BLE.<
   16   601 FORMAT(20X,11HLOG K%1< # ,F9.5<
   17   602 FORMAT(20X,53HLOG K%1< # ---K%1< IS NEGATIVE SO NO LOG IS POSSIBLE
           1.<
   18   603 FORMAT(20X,11HLOG K%2< # ,F9.5<
   19   604 FORMAT(20X,53HLOG K%2< # ---K%2< IS NEGATIVE SO NO LOG IS POSSIBLE
           1.<
   20        REAL INVOL,MWLH,NOROH,MOLME,KONE,KTWO
   21        DIMENSION X(98),Y(98),TITLE(20),W(98),VOLOH(98),XY(98),XX(98)
   22     10 READ(5,100)TITLE
   23        WRITE(6,101)TITLE
   24        READ(5,200)NOPTS,INVOL,MWLH,SWLH,NOROH,MOLME,VOLME,PKA1,PKA2
   25        IF(NOPTS.EQ.99) GO TO 999
   26        WRITE(6,201)NOPTS,MWLH,PKA1,PKA2
   27        WRITE(6,202)INVOL,SWLH,NOROH,MOLME,VOLME
   28        SUMX=0.000
   29        SUMXX=0.000
   30        SUMXY=0.000
   31        SUMY=0.000
   32        I=1
      C     W%1< HAS NO MEANING IN THIS PROGRAM.  IT IS OUTPUT FROM THE
      C     STABILITY CONSTANT PROGRAM AND IS NECESSARY IN ANOTHER LEAST
      C     SQUARES FIT PROGRAM.
   33        DO 300 I=1,NOPTS
   34        READ(5,301)Y(I),X(I),W(I),VOLOH(I)
   35        SUMX=SUMX+X(I)
   36        SUMY=SUMY+Y(I)
   37        SUMXY=SUMXY+X(I)*Y(I)
   38        SUMXX=SUMXX+X(I)*X(I)
   39        XY(I)=X(I)*Y(I)
   40        XX(I)=X(I)*X(I)
   41   300 CONTINUE
   42        WRITE(6,400)
   43        WRITE(6,402)
   44        WRITE(6,401)(VOLOH(I),X(I),Y(I),XY(I),XX(I),I=1,NOPTS)
   45        WRITE(6,402)
   46        WRITE(6,403)SUMX,SUMY,SUMXY,SUMXX
   47        PTS=FLOAT(NOPTS)
   48        BETA2=(SUMY*SUMX-PTS*SUMXY)/(SUMX*SUMX-PTS*SUMXX)
   49        KONE=(SUMX*SUMXY-SUMY*SUMXX)/(PTS*SUMXX-SUMX*SUMX)
      C     THE EXPRESSION FOR KONE WHICH IS THE Y-INTERCEPT HAS THE
      C     NUMERATOR MULTIPLIED BY %-1< SO THE EXPRESSION IS NOT THAT
      C     OBTAINED BY SOLVING THE SIMULTANEOUS EQUATION.  THIS IS DONE
      C     BECAUSE ALBERT AND SERJEANT REPORT THE DERIVATION YIELDS -KI
      C     AS THE INTERCEPT.
   50        KTWO=BETA2/KONE
   51        WRITE(6,700)KONE
   52        WRITE(6,701)KTWO
   53        WRITE(6,702)BETA2
   54        IF(BETA2.LT.0.0)GO TO 501
   55        XLOGB2=ALOG10(BETA2)
   56        WRITE(6,520)XLOGB2
   57        GO TO 502
   58   501 WRITE(6,600)
   59   502 IF(KONE.LT.0.0)GOTO503
   60        XLOGK1=ALOG10(KONE)
   61        WRITE(6,601)XLOGK1
   62        GO TO 504
   63   503 WRITE(6,602)
   64   504 IF(KTWO.LT.0.0)GO TO 505
   65        XLOGK2=ALOG10(KTWO)
   66        WRITE(6,603)XLOGK2
   67        GO TO 506
   68   505 WRITE(6,604)
   69   506 GO TO 10
   70   999 CONTINUE
   71        STOP
   72        END

      $ENTRY
```

SAMPLE LEAST SQUARES OUTPUT

CU COMPLEX OF GLYCINE ALBERT AND SERJEANT DATA P. D. MOONEY

NUMBER OF POINTS#19 MOL WT OF COMPD# 75.07 PKA1# 2.220 PKA2# 9.860
INIT VOL H2O# 46.00 SAMPLE WT# 0.0375 N OF BASE# 0.100000 M OF METAL SOLN# 0.062800 VOL OF METAL SOLN# 4.00

VOL OF BASE	X%I<	Y%I<	XY%I<	XX%I<
0.25	-0.14387980E-07	-0.32920870E 10	0.47366500E 02	0.20701420E-15
0.50	-0.42728440E-07	-0.87582100E 10	0.37422460E 03	0.18257200E-14
0.85	0.28648260E-07	0.47799450E 10	0.13693710E 03	0.82072320E-15
1.00	0.18022350E-07	0.25964650E 10	0.46794430E 02	0.32480530E-15
1.25	0.11266180E-07	0.13051540E 10	0.14704100E 02	0.12692680E-15
1.50	0.82474540E-08	0.77388770E 09	0.63826030E 01	0.68020490E-16
1.75	0.61757670E-08	0.50618080E 09	0.31260540E 01	0.38140090E-16
2.00	0.48526190E-08	0.32997290E 09	0.16012320E 01	0.23547910E-16
2.25	0.33607030E-08	0.22570590E 09	0.75853060E 00	0.11294330E-16
2.50	0.17265890E-08	0.14999670E 09	0.25898270E 00	0.29811120E-17
2.75	-0.59014030E-09	0.10355840E 09	-0.61113990E-01	0.34826560E-18
3.00	-0.37081830E-08	0.74364890E 08	-0.27575860E 00	0.13750620E-16
3.25	-0.77917010E-08	0.54604800E 08	-0.42546420E 00	0.60710600E-16
3.50	-0.13124600E-07	0.40654440E 08	-0.53357360E 00	0.17225530E-15
3.75	-0.18057020E-07	0.34006600E 08	-0.61405830E 00	0.32605620E-15
4.00	-0.21185350E-07	0.31881420E 08	-0.67541900E 00	0.44891900E-15
4.25	-0.14661280E-07	0.49329580E 08	-0.72323520E 00	0.21495330E-15
4.50	0.32026050E-07	-0.23768670E 08	-0.76121670E 00	0.10256670E-14
4.99	0.81377100E-04	-0.10110170E 05	-0.82273700E 00	0.66222290E-08

SUMX # 0.81355180E-04 SUMY #-0.10183630E 10 SUMXY # 0.62725970E 03 SUMXX # 0.66222330E-08

K%1< # 0.57002160E 08
K%2< # 0.13946840E 05
B%2< # 0.79500030E 12

LOG B%2< # 11.90037
LOG K%1< # 7.75589
LOG K%2< # 4.14447

VITA 2

Paul David Mooney

Candidate for the Degree of

Master of Science

Thesis: INVESTIGATION OF SELECTED THIOSEMICARBAZONES AND THEIR METAL COMPLEXES AS POTENTIAL ANTICANCER DRUGS.

Major Field: Chemistry

Biographical:

Personal Data: The author was born in Stroud, Oklahoma, on August 10, 1943, the son of John William and Alma Payne Mooney. On July 8, 1966, he married Sharon Lynn Smith.

Education: The author graduated from C.E. Donart High School in Stillwater, Oklahoma in May, 1961. He entered Oklahoma State University in June,1961, and in January, 1966, completed the requirements for the degree of Bachelor of Science in the field of Chemistry. He was admitted to the Graduate School at Oklahoma State University, Stillwater, Oklahoma where he completed the requirements for Master of Science degree August, 1969.

Professional experience: The author served as a graduate teaching assistant from the Summer of 1966, to the Summer of 1969. He received a National Science Foundation Research Assistantship through the Oklahoma State University Research Foundation for the period of June 1, 1968, to August 31, 1968.

Membership in Professional Societies: The author is a member of Phi Lambda Upsilon national honorary chemistry fraternity.