THERMODYNAMICS OF THE INTERACTION OF DIVALENT MANGANESE WITH HISTAMINE AND CERTAIN ASSOCIATED SUBSTANCES

by

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TABLE OF CONTENTS

INTRODUCTION 1

EXPERIMENTAL 3

General Titration Procedure 3

Apparatus 4

Materials 5

General Composition of Titration Solutions 7

DISCUSSION 9

Ligand-Proton Association 9

Thermodynamics of Ligand-Proton Association 12

Manganese(II) Complexes with Nitrogen Bases 13

Concentration Effects 20

Histamine-Antihistamine Free Energy Comparisons 24

Complexing Data 31

ACKNOWLEDGMENT 39

REFERENCES 40

APPENDIX 43

Terms and Symbols 44

Derivation of Equations 45

INTRODUCTION

Many coordination complexes are known to form between nitrogen-containing molecules and metal ions, and such compounds have been extensively studied. The interaction of the manganese(II) ion with histamine, a nitrogen base, and with several associated substances forms the basis of this work.

The chemistry of manganese is quite complex; this may be attributed to the wide variety of oxidation states that manganese can assume. Potassium permanganate is familiar as one of the most common reagents in oxidimetry. Manganese dioxide, MnO_2, is one of the few stable quadrivalent manganese compounds. Less familiar perhaps, are the (III) and (VI) oxidation states, which may be obtained only under carefully controlled conditions. By far the most common manganese compounds are the stable Mn(II) salts.

The coordination chemistry of the Mn(II) ion has not been widely investigated. A number of oxygen-containing compounds have been shown to form complexes with Mn(II), but there is little mention in the literature of manganese complexes with nitrogen bases.

Cox, et al, (12) studied the manganese(II)-pyridine complex by x-ray methods; Bjerrum (6) reports stability constants for the Mn(II)-ethylenediamine complex; and Maley and Mellor (23) determined an overall stability constant for the histidine-Mn(II) reaction.

These data suggested the present study of the interactions of the manganous ion with various nitrogen bases. The objective has been to evaluate the stability constants associated with the complex formation

at several temperatures and to calculate the corresponding thermodynamic functions, ΔF, ΔH, and ΔS.

Histamine has long been known to be associated with the occurrence of allergic reactions. The familiar antihistamines are substances which are antagonists to histamine or which inhibit its action. The mechanism by which histamine and the antihistamines react in the body has not been clearly defined although many theories have been proposed to explain these interactions.

In 1952, Tolstoouhov (35) was one of the first to attempt a physical-chemical classification of the various antihistamines with regard to their antihistaminic activities. His classification related the ionization constants of eight antihistamines to their ability to neutralize the effects of histamine on the guinea pig ileum. In his conclusions he states:

> All of this demonstrates that the structural chemistry is not sufficient to give the clear-cut correlation between biological activity and the structure of the compound. Now, more than ever, we are convinced that only physical-chemical approaches to these problems can help solve them and provide us with answers to these questions.

In 1956, Lordi and Christian (20) made an extensive study to correlate physical properties of antihistamines with their physiological activities. No correlation was found to exist between ionization constants, solubility, or relative surface activity and physiological activity. Therefore, as late as 1956, no approach along Tolstoouhov's physical-chemical lines had been devised.

It has been proposed that histamine may react directly with body proteins to produce the physiological effects associated with allergic

reactions. However, Andrews and Lyons (3) observed no direct interaction when histamine was dialyzed against various proteins. It was shown, however, that an interaction could occur when certain metal ions were introduced into the system. Apparently then, the metal ion acts as a mediating agent between histamine and the protein. The complex formation between histamine and bovine plasma albumin has been shown to have a large negative Gibbs free energy change when copper is used as the mediating agent. It has also been shown by Andrews and Lyons (3) that antihistamines can interact with proteins in the same manner.

This indicates a possible mechanism for both histaminic and antihistaminic actions. By calculation of the Gibbs free energy change involved in histaminic and antihistaminic interactions with metal ions, it may be possible to classify the effectiveness of various antihistamines thermodynamically, when compared to the energy involved in similar histamine interactions.

Manganese is known to be present in the body in trace amounts, and it is involved in various enzymatic, biochemical reactions. Therefore, in this work Gibbs free energies of the reactions studied have been compared in the manner mentioned above.

EXPERIMENTAL

General Titration Procedure

A full description of the theory and derivation of equations pertinent to this work are to be found in the Appendix. A modified form of the potentiometric titration method of Bjerrum (6) was used.

Complex formation was followed by the observed decrease in basicity

of the complexing ligand in the presence of metal ions. The apparent reduction in basicity was in proportion to the extent of complex formation. To determine the successive formation constants it was necessary to calculate $\bar{n}$, the average number of ligands bound per metal ion, over a range of free ligand concentrations. This was accomplished by the titration of a standard ligand-metal ion solution in an acid medium with standard potassium hydroxide. A small volume of base was added from a micro-burette to a large volume of the acidified ligand-metal ion solution. This solution was made up in an excess of neutral electrolyte in order to maintain the ionic strength of the solution at a constant value throughout the titration.

The determination of the ligand dissociation constants was carried out prior to the complexing titrations. The same apparatus was used and the procedure was similar to that employed in the complexing reactions.

Apparatus

A 200 ml. Berzelius beaker was used as the titration cell. It was closed with a number eleven rubber stopper drilled to accommodate a saturated calomel reference electrode (Leeds and Northrup, 1199-19) and a glass electrode (Leeds and Northrup, 1199-30 for 25°C. and 45°C.; 1194-44 for 0°C.). A glass stirring screw was mounted in a silicone-greased glass bearing, and attached to a stirring motor by a rubber tube. The titrant, standard KOH, was added from a 5 ml. EXAX burette which was also inserted through the stopper into the cell. The cell was flushed with nitrogen which had been purified by passing first through alkaline pyrogallol to remove oxygen, and then through a $KClO_4$ pre-saturator. Both the pyrogallol and the pre-saturator were mounted in the constant temperature bath. A Leeds

and Northrup type 7663 universal pH meter was used to make the readings. This instrument is reported to have a limit of error of $\pm$ 0.05 pH unit and is reproducible to within 0.02 pH. A 0.05 M. potassium acid phthalate solution with pH 4.01 at 25°C. was used as the reference buffer.

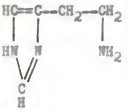
Materials

Histamine was purchased from both Eastman Organic Chemicals, Inc., and Fisher Scientific Company. The histamine from the Fisher Company was stated to be of "reagent grade". Samples from both suppliers gave consistent results. Histidine and imidazole were purchased from Eastman Organic Chemicals, Inc., and were used without further purification, as was ephedrine monohydrate which was purchased from K and K Laboratories. Benadryl was donated by Parke-Davis and Company and was stated to be of highest purity. All ligand solutions were standardized potentiometrically by titration with standard acid. The structural formulas of the ligands used are found in Table 1.

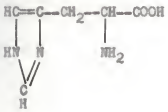
Manganous perchlorate hexahydrate was purchased from G. F. Smith Chemical Company. The stock solution was standardized by oxidation of the manganese to permanganate with KIO_4 followed by spectrophotometric analysis at 534 mμ, the wavelength of maximum absorption. A standard $KMnO_4$ Beer's Law curve was prepared in advance. The salt was found to be in excess of 99% purity.

The perchloric acid stock solution was prepared by dilution of the reagent grade 70% acid obtained from the G. F. Smith Chemical Company. It was standardized twice, once using sodium carbonate as the primary standard, and again by using the standard potassium hydroxide. Results from the two standardizations agreed well.

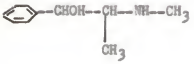
Table 1. Chemical structures of nitrogen bases studied.



Histamine

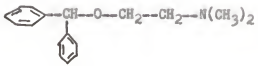


Histidine



Ephedrine

Imidazole



Benadryl

Carbonate-free potassium hydroxide was prepared by passing the solution through a hydroxide-saturated, IRA-400 Amberlite anion exchange column, and directly into a polyethylene bottle which was protected by an Ascarite tube. The resulting solution was standardized with potassium acid phthalate.

The ionic strength was maintained at a constant value by the addition of a one-tenth molar solution of reagent grade potassium perchlorate.

General Composition of Titration Solutions

Several factors must be considered in preparation of the titration solutions. These are (1) the necessity of maintaining a nearly constant ionic strength, (2) prevention of precipitation of the metal, and (3) formulation of the solutions so that maximum binding of the metal can occur.

The ionic strength must necessarily be maintained at some constant, and preferably low, value in order to maintain constant activity coefficients. The thermodynamics can then be approximated from the "hybrid" equilibrium constants observed. A "hybrid" formation constant is one solved for in terms of stoichiometric reactant concentrations and experimentally measured hydrogen ion activities. They are neither true activity constants nor concentration constants. To calculate the corresponding activity constants, the correction equation of Debye-Hückel has been applied although there is some uncertainty in certain of its terms for large complex ions. An alternative procedure requires the determination of the stability constants at several ionic strengths and extrapolation of the values to zero ionic strength. The most common practice is to maintain the ionic strength at a constant value, and approximate the thermodynamic

functions at a constant value of the activity coefficient. This last method was used in this work.

The stability constants are known to decrease with increasing ionic strength, so it is imperative that conditions be the same for the formation of the species, Mli_{n-1}, as for formation of species Mli. The ionic strength must be as low as possible when measuring stability constants for weak complexes for the same reason.

It was found in solutions of high concentration, that the precipitation of the metal hydroxide was much more prevalent than in dilute solution work. Therefore, optimum conditions favored ligand solutions of the order of 2×10^{-3} M. with the metal ion concentration correspondingly less.

The ligand-to-metal concentration ratio was maintained at approximately four to one. In this way there was ample ligand present to allow complex formation to proceed to its fullest extent. A larger ratio was not needed because the monodentate ligands could never hope to satisfy the coordination number of six for manganese due to steric conditions.

The titration system had an original total volume of sixty ml. Complexing solutions were prepared as follows: 30 ml. of 0.10 M. $KClO_4$ were added to the titration cell from a 50 ml. burette, and 10 ml. each of 0.025 N. $HClO_4$, 0.01C M. ligand, and 0.0025 M. metal ion were added from pipettes. For a bidentate ligand, this provided enough acid for complete protonation. Acid concentrations were adjusted as needed in order to completely protonate the ligand in use. This system required approximately 3.0 ml. of 0.10 N. KOH for a complete titration. All complexing work was carried out in perchlorate media except the work with Benadryl·HCl, which was carried out in a chloride medium. The perchlorate ion was used

because it is generally accepted as a non-complexer (30). This ruled out any possibility of anion complex formation competing with the metal complex formation. The $HClO_4$ salt of Benadryl was found to be insoluble in water, while the HCl salt is very soluble. The contribution to the ionic strength of each component of the sample is shown in Table 2.

Table 2. Final electrolyte concentration and ionic strength of the titration solutions.

Electrolyte	Concentration(moles/liter)	$u = \frac{1}{2}\Sigma cz^2$
$Mn(ClO_4)_2$	0.00035	0.00105
$KClO_4$	0.05000	0.05000
$HClO_4$	0.00428	0.00428
	Total:	0.05533

Each complex was studied at three temperatures, 0°, 25°, and 45°C., at an ionic strength of 0.055. Values of the stability constants which were determined, and the associated thermodynamic quantities, are assembled in Tables 3 and 4 in the discussion. Complexing data are presented in Tables 8 and 9, following the discussion.

DISCUSSION

Ligand-Proton Association

The pK_{1H_n} values for the ligands studied have been reported in the past (32), (21), but they were redetermined under the conditions used in this work. In general, agreement with the previously reported values

was excellent.

Table 3. Thermodynamics of ligand-proton association.*

(ΔF and ΔH in Kcal. mole^{-1}, ΔS in cal. deg.$^{-1}$ mole^{-1})

Ligand	n	log $K_{LiH_n^+}$			$-\Delta F$			$-\Delta H$	$-\Delta S$
		0°	25°	45°	0°	25°	45°	0°- 45°	25°
Benadryl	1	9.67	9.12	8.64	12.1	12.4	12.6	12.3	-0.335
Ephedrine	1	10.45	9.60	8.95	13.1	13.1	13.1	13.2	0.335
Histamine	1	10.71	9.87	9.10	13.4	13.5	13.3	14.1	2.01
	2	6.62	6.14	5.63	8.3	8.4	8.2	8.6	0.671
Histidine	1	9.97	9.15	8.62	12.5	12.5	12.6	12.0	-1.68
	2	6.72	6.08	5.64	8.4	8.4	8.3	9.6	4.02
Imidazole	1	7.56	7.06	6.60	9.5	9.6	9.7	8.4	-4.02

* ligand-proton association reaction: $Li + H^+ = LiH^+$

The tertiary amine group of Benadryl has a pK value of 9.12 at 25°C. This value agrees well with that reported by Lyons (21), by Lordi and Christian (20), and by Smith (32). The fact that Benadryl presents only one coordination site indicates that it will form monodentate complexes, and this is substantiated by the magnitude of the stability constant of its manganese complex.

The most probable coordination site on the ephedrine molecule is a secondary nitrogen atom which is separated by two carbon atoms from a hydroxyl group. The pK value of the amine group is 9.60 at 25°C. The

hydroxyl group has a low pK, approximately 2.3. There is very little, if any, interaction between manganese and this hydroxyl group. Smith (32) also found the pK of ephedrine to be 9.60.

Histamine has been shown to form bidentate chelates with several metal ions, and manganese proved to be no exception. Chelation probably occurs through two nitrogen atoms, the primary amine attached to the side chain, and one nitrogen in the imidazole ring. The primary amine group has a pK of 9.87 at 25°C., while the imidazole nitrogen has a pK of 6.14. Smith reported 9.88 and 6.13 for histamine's pK values.

Histidine forms the strongest metal ion complexes of the ligands studied. The structure of histidine is similar in many respects to that of histamine, and the ring and side chain nitrogens gave pK values of 6.08 and 9.15 respectively, at 25°C. The pK of the carboxyl group is low and cannot be accurately determined potentiometrically. Other investigators report pK values of 6.08 and 9.20 at 20°C. (2), and 6.05 and 9.17 at 25°C. (19). Although, in general, complexing ability increases with the basic strength of the ligand, as mirrored in its pK values, histidine formed stronger metal ion complexes than did histamine, whose pK values are significantly higher. Histidine is capable of forming two ring structures with a metal ion,

and the possibility of coordination through the carboxyl group increases the degree of complex formation accordingly. The structure of the

histidine-metal chelate is not definitely known, but the effect of oxygen binding is surely felt.

The manganese-imidazole complex was studied to complete the picture of histamine's complexing properties. Imidazole forms a very weak complex with Mn(II); this is the result of the combined effect of a weakly complexing, monodentate ligand and a weakly complexing metal ion. The pK value of imidazole at 25°C. was found to be 7.06. Dedichen (13) published a value of 7.08, while Kirby and Neuberger (18) reported this to be 6.95, corrected to zero ionic strength.

Thermodynamics of Ligand-Proton Association

The Gibbs free energy, enthalpy, and entropy changes are assembled in Table 4. These quantities were calculated by means of the equations:

$$\Delta F_n = -2.303\ RT\ \log_{10} K_n$$

$$\log K = \frac{\Delta H}{2.303\ RT} + C$$

$$-\Delta S_n = \frac{\Delta F_n - \Delta H}{T}$$

ΔH was assumed to be the average enthalpy change over the temperature range, 0° to 45°C., and was calculated by a standard least-squares method (26) from the slopes of the best straight lines for the log K_n vs. $1/T$ plots. In calculating ΔS_n, the difference between the free energy change, ΔF_n, and the average enthalpy change was divided by the average temperature. Smith (32) reports thermodynamic values for these same ligands which are in excellent agreement with the values determined in this work. Mickel (27) reports values for histamine and imidazole over the temperature range, 0°

to 25°C. His ΔF_1 value for histamine at 25°C. is -13.5 Kcal./mole, and a value of -9.66 Kcal./mole is reported for imidazole. Mickel's ΔH and ΔS values aren't strictly comparable to those determined here since his ΔH was determined from the slope of a two point curve, and ΔS was calculated using the average temperature. Tanford and Wagner (34) calculated values of 7.5 Kcal. for ΔH, and -6.7 entropy units for the imidazolium ion dissociation.

Manganese(II) Complexes with Nitrogen Bases

Basolo and Pearson (4) note that among the characteristics of the ligand which are generally recognized as influencing the stability of complexes are (1) basicity of the ligand, (2) the number of metal chelate rings per ligand, (3) the size of the chelate ring, (4) steric effects, (5) resonance effects, and (6) the ligand atom. Basicity is one of the major factors influencing the stability of coordination compounds. This is to be expected since the role played by the hydrogen ion and metal ions is essentially the same, so that the ligand with a strong affinity for a proton (one that is strongly basic) may well show the same behavior toward metal ions. Plate I shows the correlation between the basicity of the ligands studied and the stability constants of the manganese(II) complexes with the ligands. Note that if K_1 for the histamine-manganese interaction is halved to put it on a monodentate basis, as are the other ligands, the value of log K_1 falls on the curve. If the K_1 for the Benadryl-manganese complex is corrected to eliminate the chloride binding effects, then the log K_1 also falls right on the curve. Thus a good linear correlation is seen between log K_n and the basicity of the ligand. Histidine does not show this correlation because the carboxyl group tends to lower the $K_1 iH_n^+$ values,

while it increases the degree of binding of metal ions. This moves the histidine point above and to the left of its expected place on the log K_n vs. log K_{11H^+} curve in Plate I.

The formation constants and thermodynamic quantities for the manganese(II) complex with the various ligands studied are assembled in Table 4.

Table 4. Thermodynamics of ligand-manganese(II) complex formation.

(ΔF and ΔH in Kcal. mole^{-1}, ΔS in cal. deg.$^{-1}$ mole^{-1})

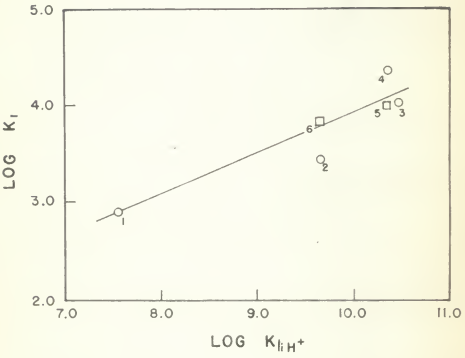
Ligand	n	log $K_{(li)_nM}$			-ΔF			-ΔH	-ΔS
		0°	25°	45°	0°	25°	45°	0°-45°	25°
Benadryl	1	3.44	3.23	3.12	4.30	4.41	4.26	2.85	-5.27
	2	3.20	3.14		4.02	4.29		0.89	-11.41
Ephedrine	1	4.01	3.45	3.25	5.03	4.72	4.74	6.37	5.53
	2	3.45	3.26		4.33	4.45		2.83	-5.50
Histamine	1	4.35	3.82	3.34	5.44	5.23	4.88	8.84	12.10
	2	3.80	3.65	3.42	4.77	5.00	5.00	3.27	-5.81
	3	3.70			4.63				
Histidine	1	5.30	4.43	3.88	6.64	6.07	5.67	12.57	21.80
	2	3.90	3.66	3.27	4.88	5.01	4.78	5.41	1.34
	3	3.50	3.49	3.37	4.38	4.78	4.93	1.08	-12.41
Imidazole	1	2.93			3.68				
	2	2.92			3.66				

EXPLANATION OF PLATE I

Mn(II) Complex stability, log K_1 as a function of ligand basicity, log K_{11H^+}

1. Imidazole
2. Benadryl·HCl
3. Ephedrine
4. Histamine
5. Histamine, $K_{1/2}$
6. Benadryl·$HClO_4$

PLATE I



In general, manganese forms the weakest complexes of the divalent metal ions in the series Mn<Fe<Co<Ni<Cu>Zn (4). There is little information in the literature concerning Mn(II) complexes with nitrogen bases, although a number of amino acid and other carboxyl complexes have been reported.

The outer electronic configuration of Mn(II) is $3s^2 3p^6 3d^5$. It usually forms labile octahedral complexes of the sp^3d^2 type, using the 4d orbitals. Exceptions to the rule are the manganese carbonyls which form d^2sp^3 complexes; these are much more stable than the labile, sp^3d^2 type (4). The magnitudes of the stability constants determined in this investigation are consistent with an sp^3d^2 complex configuration, since the complexes are relatively unstable.

The only ligands which achieved the maximum coordination number of six with manganese were histidine and histamine; these two ligands also formed the strongest complexes. This is an example of the chelate effect, i. e., the increase in complex stability with the dentate character of the ligand (4). There is a great deal of quantitative data in support of the observation that the greater the number of points of attachment of each ligand to the central metal ion, the greater the stability of the complex (4).

It is felt that steric hindrance was a major factor controlling complex formation between ephedrine and Benadryl with manganese, since only two molecules of each antihistamine appear to be bound by the manganese and these are bulky molecules of high molecular weight. This factor was probably operative in the imidazole-Mn(II) complex also. Four ligand molecules were bound to each manganous ion at 0°C., but only the first two consecutive formation constants are reported. It is felt that the data for the other

two constants is in error because of concentration effects which will be discussed later. No evidence of complexing was indicated between Mn(II) and imidazole at the two higher temperatures.

These three ligands, Benadryl, ephedrine, and imidazole, are most probably monodentate in character. There is an adjacent hydroxyl group in ephedrine which, with the secondary nitrogen, could possibly form a bidentate chelate with manganese, yielding a five membered ring. This contribution is felt to be slight since comparison data for the ethylamine and ethanolamine-silver complexes show almost equal consecutive formation constants (9). Ephedrine is slightly more basic than Benadryl; this enhances its complexing ability and may explain why ephedrine complexes are more stable (by 730cal/mole at 0°C.) than those of Benadryl.

The histidine-manganese complex is the only one studied which has previously been reported in the literature. Maley and Mellor (23) found the log K_1K_2 for histidine at 25° to be 7.76, but no experimental conditions were specified. Albert (2) reports the first consecutive stability constant to be less than 4.0 at 20°C. in a chloride medium.

Complex data for manganese with other, similar nitrogen bases is included here for comparison purposes. Ethylenediamine forms a Mn(II) complex having log K_1 = 2.73 and log K_2 = 2.06 at 30°C. in 1M. KCl (6). The logarithms of the glycine-Mn(II) formation constants are reported to be 3.2 and 2.3 for K_1 and K_2 at 20°C. with the ionic strength equal to 0.01 (1). From these data it is indicated, at least qualitatively, that the carboxyl group of glycine tends to increase the stability of the complex. This same effect is noted in the Mn(II) complexes with histamine and histidine where the same structural relationships exist. The effect of carboxyl

binding is more prominent in the K_1 values than in the other two consecutive formation constants; compare log K_1(histidine) = 4.43 with log K_1(histamine) = 3.82, while log K_2(histidine) = 3.66 and log K_2(histamine) = 3.65 at 25°C. This sort of comparison indicates that the first molecule of histidine may bind to manganese through the primary amine nitrogen and through the carboxyl oxygen forming a five membered ring. The other two molecules of histidine which are bound appear to coordinate through the two nitrogen atoms as postulated for histamine, forming a six membered ring, and satisfying the coordination number of six for manganese.

All of the complexes studied were found to decrease in stability as the temperature increased from 0° to 45°C. The Gibbs free energy change for each complex was negative, and all were within the range of 4.0 to 6.6 Kcal/mole. Each enthalpy change was found to be negative also, with values ranging from ΔH_2 = -0.89 Kcal./mole for Benadryl-Mn(II) to ΔH_1 = -12.57 Kcal./mole for histidine-Mn(II).

The unfavorable entropy term for the manganese complexes with histamine, histidine, and ephedrine is not the major factor controlling complex stability; in each case a large $-\Delta H$ term completely offsets the $T\Delta S_1$ contribution. Spike and Parry (32) have noted that the chelate effect, operative in each of these cases to a greater or lesser extent, is partially an enthalpy effect. Cotton and Harris (11) have shown that Cu(II) and Ni(II) form stronger complexes with ethylenediamine than with 1,3-propanediamine, even though the latter is the stronger base, and that the increased stability is not chiefly due to the entropy, but rather to the enthalpy of formation.

It is generally believed that saturated six-membered ring complexes

are less stable than those with five membered rings (24). Both histidine and histamine form six membered rings while ephedrine, if it acts as a bidentate ligand at all, would form a five membered ring. The entropy terms for histidine and histamine are -21.80 and -12.10 entropy units, respectively, while the entropy term for ephedrine is only -5.53 entropy units.

The less favorable ΔS terms for the histidine and histamine interactions may be a result of steric strain in the six membered ring (4), or as Irving and coworkers have suggested (17), from some steric interaction between hydrogen atoms. Fernelius and coworkers (22) have attributed the negative ΔS term for the silver chelate with 1,3-propanediamine to a strained ring structure. Six membered rings usually assume a puckered configuration which could allow proton interaction; this would lead to an increase in the order of the system, and to a decrease in ΔS. Five membered rings are more nearly planar, so no proton interaction would be expected to occur. The smaller negative entropy term observed for the ephedrine-manganese interaction could then result from (1) the possibility of the formation of a five membered ring, or (2) exclusively monodentate interaction. Benadryl can form only monodentate complexes, and the expected positive ΔS term is obtained for its manganese complex.

Concentration Effects

The formation curves for these weak complexes do not conform to the standard S-shaped curves which are obtained for strong complexes, such as the cobalt-histamine complex. The idealized formation curves should converge to a maximum value of $\bar{n}$ at high free ligand concentrations (small values of $p(ll)^f$). The curves obtained in this investigation gave a nearly

constant value of $p(li)_f$ for large values of $\bar{n}$, and $p(li)_f$ even increased in some cases for large values of $\bar{n}$. This happened in the case of manganese-imidazole, and the third and fourth consecutive formation constants were therefore greater in magnitude than the first two. Of course, this behavior is unnatural, so these data were considered to be incorrect.

The source of trouble was not immediately apparent. A four to one ligand to metal ratio had been used, which should have provided ample excess ligand to allow $p(li)_f$ to decrease as the maximum degree of complexing occurred.

Some exploratory work was done with the total ligand concentration increased by a factor of ten, and the ligand to metal ratio maintained at four to one. This allowed the free ligand concentration to increase by more than a factor of ten. A sample calculation will illustrate this point:

$(li)_t$	$(M)_t$	$(li)_f$ at $\bar{n} = 3$	$p(li)_f$
1.40×10^{-3}	3.00×10^{-4}	1.10×10^{-3}	2.95
2.00×10^{-2}	5.00×10^{-3}	1.50×10^{-2}	1.82

The formation curve for the histidine-manganese complex at 25°C. using two different ligand concentrations is shown in Plate II. The curve on the left results from a total ligand concentration equal to 2.0×10^{-2}M.; the curve on the right results from $(li)_t$ equal to 1.4×10^{-3}M. The ionic strength in the former was 0.064, while in the latter it was 0.055. The shift in the formation curve is startling.

The consecutive formation constants are determined as follows: $\log K_1 = p(li)_f$ at $\bar{n} = 0.5$; $\log K_2 = p(li)_f$ at $\bar{n} = 1.5$; $\log K_3 = p(li)_f$ at $\bar{n} = 2.5$

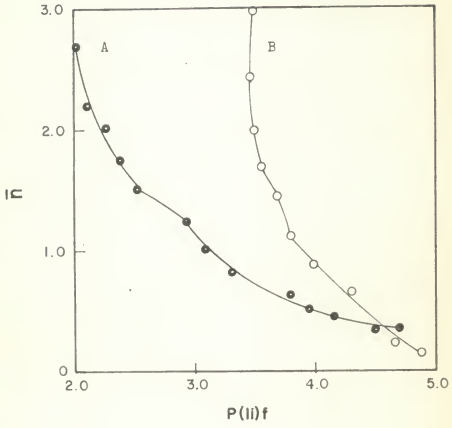
EXPLANATION OF PLATE II

The effect of the ligand concentration on the formation curve: histidine-Mn(II) complex at 25°C.

A = Histidine concentration, 2.0×10^{-2} M.

B = Histidine concentration, 1.4×10^{-3} M.

PLATE II



(see Appendix for derivation). The values obtained from the two curves are assembled in Table 5.

Table 5. Histidine-manganese stability constants at 25°C.

$(li)_t = 2.00 \times 10^{-2}$ M.			$(li)_t = 1.40 \times 10^{-3}$ M.		
	log			log	
K_1	K_2	K_3	K_1	K_2	K_3
4.06	2.59	1.85	4.43	3.66	3.49

The decrease in K_1 with increased ligand concentration is slight, and might be expected from the increase in ionic strength. The differences in the second and third consecutive formation constants are rather large; however, the smaller values resulting from the system with the greater ligand concentration are probably the more accurate.

In summary it can be said that the total ligand concentration is a critical factor in the determination of the stability constants for these weak complexes, and optimum ligand and metal ion concentrations must be experimentally determined prior to the stability constant determination.*

Histamine-Antihistamine Free Energy Comparisons

The Gibbs free energy changes per bond formed in the ligand-metal complex reaction at 25°C. are assembled in Table 6.

* This effect was first noted by Mr. I. D. Chawla and was brought to the author's attention by him in a private communication.

Table 6. $-\Delta F$ per bond formed in ligand-metal complex formation, (Kcal./mole).

	Mn^{++}		Fe^{++}[1]	Fe^{+++}[1]	Hg^{++}[1]	Zn^{++}[1]	Co^{++}	Cu^{++}
Ligand	0°	25°	25°	25°	25°	25°	25°	25°
Benadryl	8.32	8.70	13.3	10.1	10.3	10.7	14.3[3]	9.0[3]
Ephedrine	9.36	9.17	13.6	9.3	11.0	12.8		
Histamine	5.44	5.23	7.9	5.1	8.2	7.3	13.0[2]	9.4[2]
Histidine	6.64	6.07	8.0	5.5	9.9	9.3		
Imidazole	7.34		8.7		9.5	8.3		7.3[2]

[1]Data from dissertation of Smith (32)
[2]Data from dissertation of Mickel (27)
[3]Data from dissertation of Lyons (21)

Since histamine is a bidendate ligand, and Benadryl and ephedrine are monodentates, the ΔF_1 for histamine has been compared to $\Delta F_1 + \Delta F_2$ for the antihistamines. Histidine and imidazole data are included for comparison.

It will be noted that, per bond formed, both antihistamines form stronger manganese complexes than does histamine. This conclusion supports the theory of competitive binding, i.e., the theory that there is a competition between histamine and the antihistamine for the same binding site on the tissue (the metal ion). If the antihistamine is bound in preference to histamine, then the allergic reaction may be alleviated. The free energy changes appear to support this theory because the antihistamine-manganese reaction is more favored than is the histamine-manganese reaction.

Data from the dissertations of Mickel, Smith, and Lyons (27), (32), (21)

are also included in Table 6 to present a more complete case for the competitive binding theory. In every instance, except that of the copper-Benadryl complex, the negative free energy change of the metal-antihistamine reaction is greater than that of the corresponding histamine reaction.

The ratios of the free energy change of the antihistamine-metal complex to that of the respective histamine-metal complex are tabulated in Table 7. Data from Smith, Mickel, and Lyons are included. Ratios greater than unity may represent the preferential formation of the antihistamine complex. Some fairly well defined groups are seen to emerge; Tolstoouhov's idea of a physical-chemical antihistamine classification may have been confirmed. When data become available for other metal complexes with these ligands, the divisions will be more clearly defined and a true thermodynamic classification of the effectiveness of various antihistamines will be possible.

Table 7. Ratios of free energy changes of antihistamine complexes to histamine complexes.

Antihistamine	Mn^{+2}	Fe^{+2}	Fe^{+3}	Zn^{+2}	Hg^{+2}	Cu^{+2}	Co^{+2}	Ni^{+2}
Benadryl	1.7	1.7	1.9	1.5	1.3	1.1	1.2	1.0
Ephedrine	1.7	1.7	1.8	1.8	1.3			

	Mn^{+2}	Fe^{+2}	Fe^{+3}	Zn^{+2}	Hg^{+2}	Cu^{+2}	Co^{+2}	Ni^{+2}
Antistine		1.1	1.0	1.1	1.1	0.7	0.7	0.6
Neohetramine		1.0	0.8	0.8	0.6		0.6	0.6

EXPLANATION OF PLATE III

Complex formation curves

A: Histamine - Mn(II)

○ - 0°C

□ - 25°C

● - 45°C

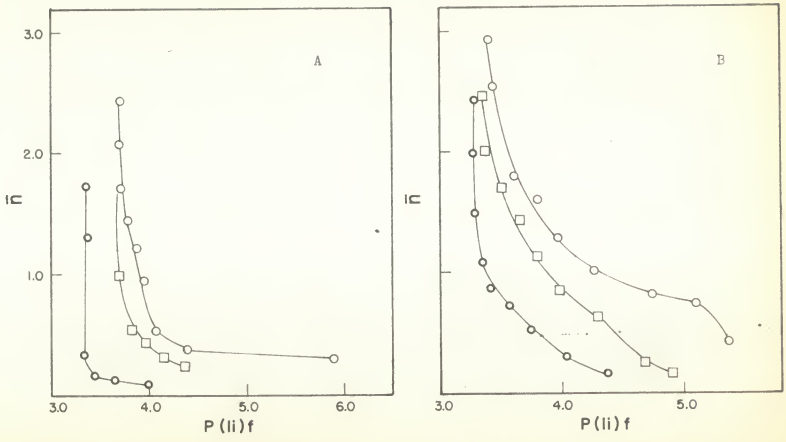
B: Histidine - Mn(II)

○ - 0°C

□ - 25°C

● - 45°C

PLATE III

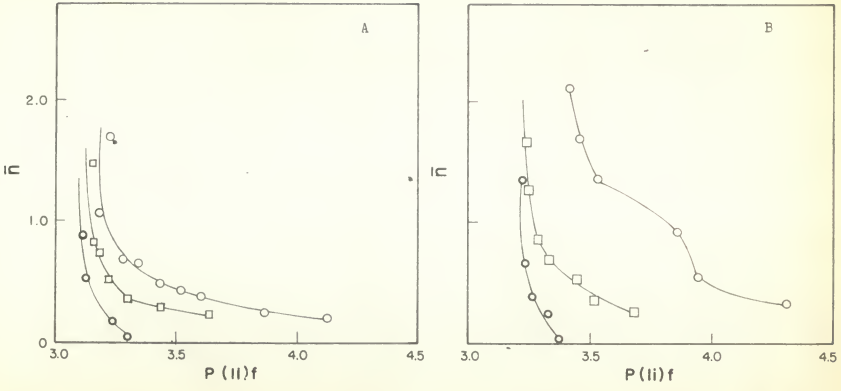


EXPLANATION OF PLATE IV

Complex formation curves

A: Ephedrine - Mn(II)	B: Benadryl - Mn(II)
○ - 0°C	○ - 0°C
□ - 25°C	□ - 25°C
● - 45°C	● - 45°C

PLATE IV



Complexing Data

Complexing data for the systems studied are assembled in Table 8. The concentration of reactants used in each titration system are listed separately in Table 9. The titration systems can be identified by matching the letter associated with each set of data in Table 8 with the same letter in Table 9.

Table 8. A. Histamine-manganese complexing data at 0°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
0.80	5.82	5.6606×10^5	1.0543×10^5	8.59	0.228
0.90	6.22	1.0863×10^5	1.8624×10^5	7.88	0.286
1.10	6.79	1.3962×10^4	1.9571×10^4	7.00	0.385
1.20	7.22	3.8801×10^3	4.6550×10^3	6.44	0.311
1.32	8.67	1.1177×10^2	1.1175×10^2	4.90	0.352
1.35	9.21	3.2833×10^1	3.1914×10^1	4.38	0.454
1.40	9.60	1.3951×10^1	1.2964×10^1	4.04	0.649
1.45	9.73	1.0595×10^1	9.6024×10^0	3.95	0.937
1.50	9.87	7.9110×10^0	6.9148×10^0	3.87	1.213
1.55	10.01	6.0381×10^0	5.0402×10^0	3.79	1.471
1.65	10.23	4.0316×10^0	3.0323×10^0	3.72	2.083
1.70	10.32	3.4644×10^0	2.4649×10^0	3.73	2.441

Table 8. B. Histamine-manganese complexing data at 25°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
2.35	8.52	2.3462×10^1	2.2553×10^1	4.384	0.226
2.40	8.84	1.1683×10^1	1.0704×10^1	4.149	0.320
2.45	9.03	7.9131×10^0	6.9212×10^0	3.947	0.433
2.50	9.18	5.8922×10^0	4.8963×10^0	3.839	0.550
2.65	9.47	3.5051×10^0	2.5064×10^0	3.713	1.038
2.80	9.69	2.5112×10^0	1.5112×10^0	3.756	1.711

Table 8. C. Histamine-manganese complexing data at 45°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
2.30	8.01	1.081×10^{1}	9.851×10^{0}	3.991	0.104
2.40	8.43	4.716×10^{0}	3.722×10^{0}	3.638	0.149
2.50	8.72	2.901×10^{0}	1.903×10^{0}	3.429	0.167
2.60	8.90	2.250×10^{0}	1.251×10^{0}	3.349	0.363
2.70	9.04	1.977×10^{0}	6.602×10^{-1}	3.345	0.760
2.80	9.18	1.662×10^{0}	9.851×10^{-1}	3.396	1.323
2.90	9.28	1.524×10^{0}	5.242×10^{-1}	3.646	2.244

Table 8. D. Histidine-manganese complexing data at 0°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
1.40	5.71	2.0244×10^{5}	3.8675×10^{5}	8.152	0.368
1.50	6.07	4.3454×10^{4}	7.8953×10^{4}	7.501	0.537
1.60	6.35	1.3919×10^{4}	2.3668×10^{4}	7.022	0.674
1.70	6.62	5.0336×10^{3}	7.8316×10^{3}	6.593	0.767
1.80	6.95	1.6604×10^{3}	2.2721×10^{3}	6.114	0.770
1.90	7.49	3.5400×10^{2}	4.0413×10^{2}	5.450	0.780
2.02	8.46	3.3923×10^{1}	3.3510×10^{1}	4.431	0.895
2.10	8.88	1.3327×10^{1}	1.2411×10^{1}	4.083	1.272
2.25	9.32	5.4784×10^{0}	4.4896×10^{0}	3.834	2.090
2.30	9.50	3.9582×10^{0}	2.9631×10^{0}	3.733	2.331
2.35	9.60	3.3488×10^{0}	2.3519×10^{0}	3.740	2.650

Table 8. E. Histidine-manganese complexing data at 25°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
2.35	7.02	1.362×10^{2}	1.354×10^{2}	4.910	0.169
2.40	7.29	7.386×10^{1}	7.330×10^{1}	4.670	0.274
2.50	7.73	2.790×10^{1}	2.749×10^{1}	4.293	0.649
2.60	8.08	1.287×10^{1}	1.199×10^{1}	3.982	0.875
2.70	8.31	7.957×10^{0}	6.998×10^{0}	3.803	1.162
2.80	8.50	5.486×10^{0}	4.503×10^{0}	3.681	1.449
2.90	8.68	3.949×10^{0}	2.956×10^{0}	3.572	1.725
3.00	8.86	2.954×10^{0}	1.957×10^{0}	3.494	2.003
3.10	8.99	2.445×10^{0}	1.447×10^{0}	3.484	2.430
3.20	9.12	2.073×10^{0}	1.074×10^{0}	3.520	2.950

Table 8. F. Histidine-manganese complexing data at 45°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
2.90	7.12	3.376×10^{1}	3.380×10^{1}	4.382	0.197
3.00	7.52	6.935×10^{0}	5.971×10^{0}	3.741	0.577
3.10	7.85	6.935×10^{0}	5.971×10^{0}	3.743	0.577
3.20	8.09	4.414×10^{0}	3.426×10^{0}	3.574	0.753
3.30	8.32	3.004×10^{0}	2.008×10^{0}	3.452	0.921
3.40	8.49	2.351×10^{0}	1.351×10^{0}	3.342	1.101
3.50	8.70	1.833×10^{0}	8.334×10^{-1}	3.284	1.563
3.60	8.87	1.560×10^{0}	5.606×10^{-1}	3.292	2.061
3.70	9.06	1.364×10^{0}	3.647×10^{-1}	3.384	2.554

Table 8. G. Benadryl-manganese complexing data at 0°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
0.10	8.37	2.100×10^{1}	2.000×10^{1}	4.12	0.202
0.15	8.66	1.123×10^{1}	1.023×10^{1}	3.86	0.246
0.25	8.96	6.117×10^{0}	5.117×10^{0}	3.61	0.375
0.30	9.08	4.901×10^{0}	3.901×10^{0}	3.52	0.434
0.35	9.19	4.028×10^{0}	3.028×10^{0}	3.44	0.482
0.45	9.36	3.047×10^{0}	2.047×10^{0}	3.34	0.645
0.50	9.46	2.624×10^{0}	1.624×10^{0}	3.28	0.674
0.70	9.77	1.793×10^{0}	7.934×10^{-1}	3.18	1.110
0.80	9.86	1.648×10^{0}	6.479×10^{-1}	3.25	1.720

Table 8. H. Benadryl-manganese complexing data at 25°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
0.20	8.36	6.752×10^{0}	5.752×10^{0}	3.64	0.23
0.30	8.61	4.232×10^{0}	3.232×10^{0}	3.44	0.29
0.40	8.81	3.032×10^{0}	2.032×10^{0}	3.30	0.35
0.50	8.97	2.412×10^{0}	1.412×10^{0}	3.22	0.49
0.60	9.10	2.047×10^{0}	1.047×10^{0}	3.19	0.74
0.65	9.18	1.871×10^{0}	8.707×10^{-1}	3.16	0.83
0.70	9.24	1.759×10^{0}	7.586×10^{-1}	3.16	1.45
0.80	9.39	1.537×10^{0}	5.369×10^{-1}	3.17	1.46

Table 8. I. Benadryl-manganese complexing data at 45°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
2.10	8.31	3.1354×10^{0}	2.1354×10^{0}	3.30	0.045
2.15	8.42	2.6594×10^{0}	1.6594×10^{0}	3.24	0.162
2.28	8.70	1.8690×10^{0}	8.6900×10^{-1}	3.13	0.526
2.30	8.75	1.7729×10^{0}	7.7292×10^{-1}	3.11	0.521
2.35	8.84	1.6288×10^{0}	6.2880×10^{-1}	3.12	0.900
2.40	8.93	1.5109×10^{0}	5.1091×10^{-1}	3.18	1.454

Table 8. J. Ephedrine-manganese complexing data at 0°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
1.40	8.97	3.122×10^1	3.022×10^1	4.31	0.343
1.50	9.38	1.275×10^1	1.175×10^1	3.94	0.532
1.60	9.52	9.531×10^0	8.531×10^0	3.86	0.933
1.70	9.88	4.700×10^0	3.700×10^0	3.56	0.993
1.80	9.99	3.881×10^0	2.881×10^0	3.53	1.37
1.90	10.15	2.997×10^0	1.997×10^0	3.46	1.67
2.00	10.26	2.551×10^0	1.551×10^0	3.42	2.12
2.10	10.35	2.259×10^0	1.259×10^0	3.52	2.69

Table 8. K. Ephedrine-manganese complexing data at 25°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
1.30	8.78	7.574×10^0	6.574×10^0	3.69	0.265
1.35	8.99	5.064×10^0	4.064×10^0	3.52	0.336
1.40	9.11	4.092×10^0	3.092×10^0	3.45	0.522
1.45	9.22	3.398×10^0	2.398×10^0	3.39	0.714
1.50	9.38	2.657×10^0	1.657×10^0	3.29	0.840
1.58	9.51	2.231×10^0	1.231×10^0	3.28	1.24
1.60	9.55	2.119×10^0	1.119×10^0	3.27	1.34
1.65	9.65	1.888×10^0	8.884×10^{-1}	3.27	1.67
1.70	9.71	1.773×10^0	7.729×10^{-1}	3.33	2.21

Table 8. L. Ephedrine-manganese complexing data at 45°C.

ml. KOH	pH	α	β	$p(11)_f$	$\bar{n}$
1.40	8.51	3.759×10^0	2.759×10^0	3.37	0.032
1.45	8.59	3.295×10^0	2.295×10^0	3.33	0.241
1.50	8.71	2.732×10^0	1.732×10^0	3.26	0.400
1.58	8.79	2.446×10^0	1.446×10^0	3.24	0.678
1.65	8.94	2.018×10^0	1.018×10^0	3.25	1.37

Table 8. M. Imidazole-manganese complexing data at 0°C.

ml. KOH	pH	α	β	$p(ii)_f$	$\bar{n}$
1.67	7.47	2.229×10^{0}	1.229×10^{0}	3.06	0.109
1.80	7.63	1.851×10^{0}	8.509×10^{-1}	2.99	0.283
1.90	7.78	1.600×10^{0}	6.000×10^{-1}	2.93	0.295
2.00	7.91	1.447×10^{0}	4.473×10^{-1}	2.92	0.629
2.10	8.12	1.276×10^{0}	2.756×10^{-1}	2.86	0.558
2.20	8.29	1.186×10^{0}	1.862×10^{-1}	2.92	1.552
2.30	8.57	1.098×10^{0}	9.782×10^{-2}	3.19	3.500

Table 9. Composition of titration systems.

Histamine-manganese system.

A.	Histamine	10 ml.	9.2757×10^{-3}M.
	$Mn(ClO_4)_2$	10	2.2080×10^{-3}
	$HClO_4$	10	2.7490×10^{-2}
	$KClO_4$	30	1.0000×10^{-1}
	KOH		1.4860×10^{-1}
B.	Histamine	10 ml.	6.5390×10^{-3}M.
	$Mn(ClO_4)_2$	10	2.2058×10^{-3}
	$HClO_4$	10	2.7450×10^{-2}
	$KClO_4$	30	1.0000×10^{-1}
	KOH		9.2100×10^{-2}
C.	Histamine	10 ml.	7.1072×10^{-3}M.
	$Mn(ClO_4)_2$	10	2.2058×10^{-3}
	$HClO_4$	10	2.7450×10^{-2}
	$KClO_4$	30	1.0000×10^{-1}
	KOH		9.2100×10^{-2}

Histidine-manganese system.

D.	Histidine	10 ml.	9.7061×10^{-3}M.
	$Mn(ClO_4)_2$	10	2.2080×10^{-3}
	$HClO_4$	10	2.8400×10^{-2}
	$KClO_4$	30	1.0000×10^{-1}
	KOH		1.5080×10^{-1}
E.	Histidine	10 ml.	1.0430×10^{-2}M.
	$Mn(ClO_4)_2$	10	2.2058×10^{-3}
	$HClO_4$	10	2.2870×10^{-2}
	$KClO_4$	30	1.0000×10^{-1}
	KOH		9.7730×10^{-2}
F.	Histidine	10 ml.	9.2098×10^{-3}M.
	$Mn(ClO_4)_2$	10	2.2058×10^{-3}
	$HClO_4$	10	2.7920×10^{-2}
	$KClO_4$	30	1.0000×10^{-1}
	KOH		9.7730×10^{-2}

Benadryl-manganese system.

G.	Benadryl·HCl	10 ml.	1.0000×10^{-2}M.
	$Mn(ClO_4)_2$	10	2.5870×10^{-3}
	KCl	30	1.0000×10^{-1}
	H_2O	10	
	KOH		9.7730×10^{-2}
H.	Benadryl·HCl	10 ml.	1.0000×10^{-2}M.
	$Mn(ClO_4)_2$	10	2.5870×10^{-3}
	KCl	30	1.0000×10^{-1}
	H_2O	10	
	KOH		9.7730×10^{-2}
I.	Benadryl·HCl	10 ml.	1.0000×10^{-2}M.
	$Mn(ClO_4)_2$	10	2.5870×10^{-3}
	KCl	30	1.0000×10^{-1}
	HCl	10	2.7490×10^{-2}
	KOH		1.4360×10^{-1}

Ephedrine-manganese system.

J.	Ephedrine	10 ml.	1.0140×10^{-2}M.
	$Mn(ClO_4)_2$	10	2.2058×10^{-3}
	$HClO_4$	10	2.2870×10^{-2}
	$KClO_4$	30	1.0000×10^{-1}
	KOH		9.7730×10^{-2}
K.	Ephedrine	10 ml.	1.0000×10^{-2}M.
	$Mn(ClO_4)_2$	10	2.2058×10^{-3}
	$KClO_4$	30	1.0000×10^{-1}
	$HClO_4$	10	2.7490×10^{-2}
	KOH		1.4860×10^{-1}
L.	Ephedrine	10 ml.	1.0400×10^{-2}M.
	$Mn(ClO_4)_2$	10	2.2058×10^{-3}
	$HClO_4$	10	2.8400×10^{-2}
	$KClO_4$	30	1.0000×10^{-1}
	KOH		1.5080×10^{-1}

Imidazole-manganese system.

M.	Imidazole	10 ml.	1.2120×10^{-2}M.
	$Mn(ClO_4)_2$	10	2.2058×10^{-3}
	$HClO_4$	10	2.2370×10^{-2}
	$KClO_4$	30	1.0000×10^{-1}
	KOH		9.7730×10^{-2}

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REFERENCES

1. Albert, A.
Quantitative studies of the avidity of naturally occurring substances for trace metals. 3. Pteridines, riboflavin and purines. Biochem. J. 54: 646. 1953.

2. Albert, A.
Quantitative studies of the avidity of naturally occurring substances for trace metals. Biochem. J. 50: 690-697. 1952.

3. Andrews, A. C., and T. D. Lyons.
Binding of histamine and antihistamine to bovine serum albumin by mediation with $Cu(II)$. Science. 126: No. 3273, 561-562. 1957.

4. Basolo, Fred, and Ralph G. Pearson.
Mechanisms of inorganic reactions. New York: John Wiley and Sons, Inc., 1958.

5. Bertsch, Charles R., W. Conrad Fernelius, and B. P. Block.
A thermodynamic study of some complexes of metal ions with polyamines. J. Phys. Chem. 62: 444-450. 1958.

6. Bjerrum, Jannik.
Metal ammine formation in aqueous solution. Copenhagen: P. Haase and Son., 1941.

7. Bjerrum, Jannik.
The tendency of metal ions toward complex formation. Chem. Revs. 46: 381-401. 1950.

8. Bjerrum, Jannik, Gerold Schwarzenbach, and Lars Gunnar Sillen.
Stability constants. London: The Chemical Society, 1957.

9. Bruehlman, R. J., and F. H. Verhoek.
The basic strengths of amines as measured by the stabilities of their complexes with silver ions. J. Am. Chem. Soc. 70: 1401. 1948.

10. Calvin, M., and K. W. Wilson.
Stability of chelate compounds. J. Am. Chem. Soc. 67: 2003-2007. 1945.

11. Cotton, F. A., and F. E. Harris
The thermodynamics of chelate formation. I. Experimental determination of enthalpies and entropies in diamine-metal ion systems. J. Phys. Chem. 59: 1203. 1945.

12. Cox, E. G., A. J. Shorter, W. Wardlaw, and W. J. R. Way.
The stereochemistry of quadrivalent atoms: cobalt and manganese. J. Chem. Soc. 1937: 1556.

13. Dedichen, G.
Affinitats grossen einiger cyclischer Basen. Ber. 39: 1831-1856. 1906.

14. Goldberg, David E., and W. Conrad Fernelius.
A thermodynamic study of some coordination complexes of metal ions with diprotic nitrogen compounds containing one heterocyclic nitrogen atom. J. Phys. Chem. 63: 1246. 1959.

15. Hofmann, Klaus.
Imidazole and its derivatives. New York: Interscience Publishers. 1953.

16. Idson, Bernard.
Antihistamine drugs. Chem. Revs. 47: 307-527. 1950.

17. Irving, H., R. J. P. Williams, D. J. Ferrett, and A. E. Williams.
The influence of ring size upon the stability of metal chelates. J. Chem. Soc. 1954: 3494-3504.

18. Kirby, A. H. M., and Neuberger, A.
Glyoxalines- determination of their pK values and the use of their solutions as buffers. Biochem. J. 32: 1146-1151. 1938.

19. Li, N. D., and Manning, R. A.
Some metal complexes of sulfur-containing amino acids. J. Am. Chem. Soc. 77: 5225-5228. 1955.

20. Lordi, N. G., and J. E. Christian.
Physical properties and pharmaceutical activity of antihistamines. J. Am. Pharm. Assoc. 45: 300-305. 1956.

21. Lyons, T. D.
Thermodynamic properties of complexes associated with protein-histamine-antihistamine interactions. Ph. D. Dissertation. Kansas State College. 1957.

22. McIntyre, G. H., Jr., R. P. Block, and W. Conrad Fernelius.
A thermodynamic study of some metal-amine coordination compounds. J. Am. Chem. Soc. 81: 529. 1959.

23. Maley, L. E., and D. P. Mellor.
Stability of some metal complexes of histidine. Nature. 165: 453. 1950.

24. Mann, F. G.
Tetrachloro(triaminopropane-Y-monohydrochloride)platinum, and optically active complex salt of a new type. J. Chem. Soc. 1927: 1224-1232.

25. Martell, Arthur E., and Melvin Calvin.
Chemistry of the metal chelate compounds. New York: Prentice Hall, 1952.

26. Mellor, J. W.
Higher mathematics for students of chemistry and physics. Dover Publications, Inc., 1955. 326 p.

27. Mickel, B. L.
Cation complexes of histamine and imidazole. Ph. D. Dissertation. Kansas State College, 1955.

28. Mickel, B. L. and A. C. Andrews.
Complexes of histamine with Co(II), Ni(II), and Cu(II). J. Am. Chem. Soc. 77: 323. 1955.

29. Mickel, B. L., and A. C. Andrews.
Stability of the histamine chelates. J. Am. Chem. Soc. 77: 5291-5292. 1955.

30. Moller, Therald.
Inorganic chemistry. New York: John Wiley and Sons, Inc., 1952. 237 p.

31. Rocha e Silva, M.
Histamine, its role in anaphylaxis and allergy. Springfield: Charles C. Thomas, 1955.

32. Smith, Ivan C.
Thermodynamic stabilities of the metal ion complexes of allergically associated molecules. Ph. D. Dissertation. Kansas State University, 1960.

33. Spike, Clark G., and Robert W. Parry.
Thermodynamics of chelation. II. Bond energy effects in chelate ring formation. J. Am. Chem. Soc. 75: 2770-2772. 1953.

34. Tanford, Charles, and Myron L. Wagner.
The consecutive constants for the association of cadmium with imidazole. J. Am. Chem. Soc. 75: 434-435. 1953.

35. Tolstoouhov, A. V.
The physical-chemical approach to the problem of chemotherapy. Trans. N. Y. Acad. Sci. 14: 260-269. 1952.

APPENDIX

Terms and Symbols

Certain terms and symbols are used frequently in the text as well as in the derivation that follows: They are listed here so that no misunderstanding will occur in the terminology used.

Complex	the product which results when a metal ion combines with an electron donor.
Chelate	the complex which is formed by the combination of a metal ion with a substance having two or more donor groups, such that a ring structure is produced.
Ligand	a molecule or ion which is bound, in a complex, to a central ion.
Bidentate	a chelate formed by the combination of a metal ion with a ligand which contributes two donor groups to the formation of one ring.
M	central metal ion.
li	ligand.
Mli_n	complex or chelate formed by the addition of the n^{th} ligand to Mli_{n-1}.
$\bar{n}$	average number of ligands bound per metal ion.
$\bar{n}_H$	average number of protons bound per molecule of uncomplexed ligand.
$(li)_t$	total stoichiometric concentration of added complexing ligand.
$(li)_f$	concentration of free ligand (unprotonated and not complexed by metal).
$p(li)_f$	$-\log(li)_f$
$(M)_t$	total stoichiometric concentration of added metal ion.
$(HA)_t$	total stoichiometric concentration of added standard acid.

$(KOH)_t$	total stoichiometric concentration of added standard base.
K_n	consecutive formation constant for the complexing reaction, $MLi_{n-1} + Li = MLi_n$.
K_{st}	stability constant for the complex reaction; equal to the continued product of the consecutive constants.
$k_{LiH^+} = k'$	proton dissociation constant or equilibrium constant for the reaction, $LiH^+ = Li + H^+$.
$k_{LiH_2^{++}} = k''$	proton dissociation constant or equilibrium constant for the reaction, $LiH_2^{++} = LiH^+ + H^+$.
pk_{LiH^+}	$-\log k_{LiH^+}$
$pk_{LiH_2^{++}}$	$-\log k_{LiH_2^{++}}$

Derivation of Equations

The method used to calculate the stability constants was developed by Jannik Bjerrum (6). This method was modified in the last step of the calculation; the values of log K_n were read directly from the formation curve in which $\bar{n}$ was plotted against $p(Li)_f$, instead of being calculated by the method of approximations used by Bjerrum. The logarithms of the consecutive formation constants are equal to the values of $p(Li)_f$ at $\bar{n}$ equal to 0.5, 1.5, and 2.5, etc.

The complexing reactions are:

$$Li + M \rightleftharpoons LiM \tag{1}$$

$$LiM + Li \rightleftharpoons Li_2M \tag{2}$$

$$Li_2M + Li \rightleftharpoons Li_3M \tag{3}$$

The consecutive formation constants for reactions (1) to (3) are:

$$K_1 = (11M)/(11)_f(M)_f \tag{4}$$

$$K_2 = (11_2M)/(11)_f(11M) \tag{5}$$

$$K_3 = (11_3M)/(11)_f(11_2M) \tag{6}$$

The overall stability constant for the complexing reaction is

$$K_{st.} = K_1K_2K_3 \tag{7}$$

When values of $p(11)_f$ are known as a function of $\bar{n}$, then the consecutive formation constants can be calculated from equations (4) through (6). In each case $K = 1/(11)_f$ since at $\bar{n} = 0.5$, $(11M) = (M)_f$; at $\bar{n} = 1.5$, $(11_2M) = (11M)$; and at $\bar{n} = 2.5$, $(11_3M) = (11_2M)$. These terms, $\bar{n}$ and $p(11)_f$, are obtained as follows:

$$\bar{n} = \left[(11M) + 2(11_2M) + 3(11_3M)\right]/(M)_t \tag{8}$$

$$(11)_t = (11)_f + (11H^+) + (11H_2^{++}) + (11M) + 2(11_2M) + 3(11_3M) \tag{9}$$

Solving (9) for $(11M)+2(11_2M)+3(11_3M)$ and substituting into (3):

$$\bar{n} = \left[(11)_t - (11)_f - (11H^+) - (11H_2^{++})\right] / (M)_t \tag{10}$$

The ionization constants for the ligand may be expressed as:

$$k_{11H^+} = (11)_f(H^+)_f/(11H^+) = k' \tag{11}$$

$$k_{11H_2^{++}} = (11H^+)(H^+)_f/(11H_2^{++}) = k'' \tag{12}$$

Solving equations (11) and (12) for the acid form of the ligand, and substituting into (10):

$$\bar{n} = \frac{(11)_t - (11)_f\left[1 + (H^+)/k' + (H^+)^2/k'k''\right]}{(M)_t} \tag{13}$$

The term in brackets is designated as α in the tables of complexing data.

All terms in equation (13) can be measured or calculated except $(1\bar{1})_f$. An expression for $(1\bar{1})_f$ may be obtained by considering two equivalent expressions for the total hydrogen ion concentration, $(H^+)_t$.

$$(H^+)_t = (H^+)_f + (1\bar{1}H^+) + 2(1\bar{1}H_2^{++}) \tag{14}$$

$$(H^+)_t = (HA)_t - (KOH)_t \tag{15}$$

Equating (14) and (15):

$$(1\bar{1}H^+) + 2(1\bar{1}H_2^{++}) = (HA)_t - (KOH)_t - (H^+)_f \tag{16}$$

Substituting for $(1\bar{1}H)$ and $2(1\bar{1}H_2^{++})$ from (11) and (12) and rearranging:

$$(1\bar{1})_f = \frac{(HA)_t - (H^+)_f - (KOH)_t}{(H^+)/k' + 2(H^+)^2/k'k''} \tag{17}$$

The denominator in (17) is designated as β in the tables of complexing data.

Equations (13) and (17) are used to calculate the $\bar{n}$ and $(1\bar{1})_f$ terms. Each term in these two equations is either known or experimentally measured.

THERMODYNAMICS OF THE INTERACTION OF DIVALENT MANGANESE WITH HISTAMINE AND CERTAIN ASSOCIATED SUBSTANCES

by

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The primary objective of this investigation has been to study thermodynamically the complexes formed between divalent manganese and several nitrogen bases. There is little information in the literature about such complexes, although Mn(II) is known to form complexes with amino acids and with carboxyl compounds.

Histidine was found to form the strongest complexes with manganese, and the influence of carboxyl binding was seen in the K_{11M} value, which was significantly greater than K_{11M} for the histamine-Mn(II) complex. A comparison of the K_{11_2M} and K_{11_3M} values for the manganese complexes with these two ligands shows that the corresponding constants are nearly identical. Thus, binding through the two nitrogen atoms is indicated, with the formation of a six membered ring, in both histamine and histidine.

The Gibbs free energy changes for all of the complexes studied were negative, as were the ΔH terms. The ΔS terms for histamine, histidine, and ephedrine complexes were negative, indicating an increase in the order of the system upon complex formation. Various investigators have attributed this negative entropy term to the formation of a strained ring structure, or to the interaction of adjacent hydrogen atoms in the chelate ring. However, these unfavorable $T\Delta S$ terms are completely offset by the large negative enthalpy changes, so that favorable ΔF terms result in each case. The other complexes showed positive ΔS terms, which may be accounted for by an increase in the number of species present after complex formation due to the release of water of hydration.

The formation curves from which the stability constants are calculated were found to deviate markedly from the ideal S - shaped curve for these and other weak complexes. It was found during the course

of this investigation that the shape of the formation curve approaches the ideal S shape much more closely if a relatively high concentration of ligand is used. The use of a high ligand concentration causes a marked decrease in the second and succeeding consecutive formation constants, while the first remains almost the same as the one determined at lower ligand concentrations. Cognisance of this concentration effect is felt to be of importance because it is now realized that many weak complexes can be studied by the potentiometric method as accurately as are the often reported strong complexes.

The compounds which were studied are biologically active: histamine is known to be intimately involved in many allergic disorders, and Benadryl and ephedrine are both antihistamines, or antagonists to histamine. Histidine and imidazole complexes were studied because of their close structural relationships to histamine.

It has previously been shown that histamine and several antihistamines do not bind directly to body tissues, but that if a metal ion is introduced into the system, binding does occur, with the metal ion acting as a mediating agent. Thus, the theory of competitive binding of histamine and antihistamines to various metal ions has been proposed; if the antihistamine is more strongly bound to the metal than is histamine, then it should be effective in alleviating the allergic reaction. The Gibbs free energies of complex formation were calculated, and comparison shows that both Benadryl and ephedrine complexes with Mn(II) have larger negative free energies than does the histamine complex. The ratio of ΔF(antihistamine complex)/ΔF(histamine complex) was tabulated for several metal ion complexes with various antihistamines. The magnitude of this ratio may indicate the

relative effectiveness of the various antihistamines. Some fairly well defined groups emerge, and when more complexing data become available, a more complete thermodynamic antihistamine classification may be possible.