337. *Copper*(II), *Cobalt*(II), *and Nickel*(II) *Complexes of Various Antihistaminic Compounds: Their Stability and Thermodynamic Values.*

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A thermodynamic analysis of the complexes of a series of nine commercial antihistamines with copper(II), cobalt(II), and nickel(II) is presented. Results show that a stable complex is formed in each case. The free energy of formation of the complexes is suggested as a possible index of effectiveness for antihistaminic activity.

Until now, little attention has been given in the literature to the physicochemical properties of antihistaminic compounds. Therefore, a thermodynamic analysis of the complexes of a series of commercial antihistamines with copper(II), cobalt(II), and nickel(II) was undertaken. It is our opinion that the elucidation of the mechanism of antihistaminic activity may eventually lie in the physicochemical nature of the antihistamine molecule.

Recent thermodynamic analyses [1] of the complexes of histamine with these metals have shown that a stable chelate is formed in each case. It was believed that a similar study of the complexes of antihistamines with metal ions could provide valuable results, and possibly aid in evaluating the importance of complex formation in the mechanism of histaminic–antihistaminic activity.

Experimental

Procedure.—The potentiometric titration technique of Bjerrum,[2] as interpreted by Albert,[3] was employed. The titrations were carried out at 0° and 25° under an atmosphere of purified and presaturated nitrogen. A constant ionic strength of 0·06 was maintained with potassium chloride. Carbonate-free 0·1M-potassium hydroxide was used as the titrant. In cases where the complex formed was insoluble in aqueous solution, the titrations were carried out in 50% (vol.) dioxan in a manner similar to that of Van Uitert and Haas,[4] and of Holmes and Crimmin.[5] The response of the glass electrode to hydrogen ions in 50% dioxan was found to be linear, giving Holmes and Crimmin [5] correction factors of −0·17 and 0·03 at 0° and 25°, respectively.

A Leeds and Northrup pH meter, No. 7663-Al, capable of reproducing readings to within 0·02 pH unit, was used with Leeds and Northrup glass electrodes No. 1199—44 for the 0° and No. 1199—30 for the 25° titrations. Both were measured against a saturated calomel electrode.

Materials and Analysis.—Chemicals of analytical reagent grade were used throughout this work, with the exception of the antihistamines, which were supplied by various manufacturers as the best grade available.* Metal-ion concentrations were determined electrolytically, and all solutions were standardized gravimetrically by silver chloride precipitation.

Antistine, 2-(*N*-benzylanilinomethyl)imidazoline hydrochloride, was analyzed by forming the monopicrate: it was found to be 100% Antistine Hydrochloride. Benadryl, *NN*-dimethyl-2-diphenylmethoxyethylamine hydrochloride, was analyzed by potentiometric titration and found to be 99·22% Benadryl Hydrochloride. Pyribenzamine Hydrochloride, 2-[benzyl-(2-dimethylaminoethyl)amino]pyridine hydrochloride, was analyzed by forming the dipricrate and found to be 98·70% Pyribenzamine Hydrochloride. (−)-Adrenaline, (−)-1-(3,4-dihydroxyphenyl)-2-methylaminoethanol, certified to be >99% pure, and (−)-noradrenaline, (−)-2-amino-1-(3,4-dihydroxyphenyl)ethanol, were purchased from K. & K. Laboratories, Long Island City, N.Y. They were used without further purification. The noradrenaline was included in this study to evaluate the effect of the secondary amine group present in adrenaline.

* The antihistamines used in this work were graciously presented by the following manufacturers: Antistine and Pyribenzamine, Ciba Pharmaceutical Co.; Benadryl, Parke-Davis and Co.; Trimeton and Chlortrimeton, Schering Corp.; Neohetramine, Neparo Chemical Co.

[1] Mickel and Andrews, *J. Amer. Chem. Soc.*, 1955, **77**, 5291; Nicholas and Fernelius, *J. Phys. Chem.*, 1961, **65**, 1047.

[2] Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

[3] Albert, *Biochem. J.*, 1950, **47**, 531; 1952, **50**, 690.

[4] Van Uitert and Haas, *J. Amer. Chem. Soc.*, 1953, **75**, 451.

[5] Holmes and Crimmin, *J.*, 1955, 3467.

Trimeton Maleate, *NN*-dimethyl-3-phenyl-3-2′-pyridylpropylamine maleate, was analyzed by potentiometric titration and found to be 97·70% Trimeton Maleate. Since the anion in this case is known to form complexes with metal ions [6] the maleate was exchanged with chloride on Amberlite IRA-400 resin that had previously been saturated with chloride. The final concentration of Trimeton Hydrochloride was determined spectrophotometrically at 262 mμ, the wavelength of maximum absorption for this compound. The extinction coefficient determined for the maleate was $4{\cdot}68 \times 10^3$ l. mole^{-1} cm.$^{-1}$. Chlortrimeton Maleate, *NN*-dimethyl-3-*p*-chlorophenyl-3-2′-pyridylpropylamine maleate, was analyzed by the techniques used for Trimeton Maleate. The wavelength of maximum absorption was found to be 261 mμ, with an extinction coefficient of $5{\cdot}90 \times 10^3$ l. mole^{-1} cm.$^{-1}$. Neohetramine Hydrochloride, 2-[(2-dimethylaminoethyl)-(4-methoxybenzyl)amino]pyrimidine hydrochloride, was analyzed by precipitation of the chloride as silver chloride and found to be 97·4% Neohetramine Hydrochloride. The material was used without further purification.

Discussion

Values for the base dissociation constants of the antihistamines, obtained from potentiometric titrations of the fully protonated antihistamine, together with the available literature values, are listed in Table 1. From the titration data, values of the average

Table 1.

Ionization constants of certain substances which inhibit the action of histamine.

	p*K* for Hli		p*K* for H_2li		p*K* for H_3li			p*K* for Hli	
Compound	at 0°	at 25°	at 0°	at 25°	at 0°	at 25°	Compound	at 0°	at 25°
(−)-Adrenaline ...	10·98	10·02 9·90 [a]	9·61	8·78 8·71 [a]	2·75	2·58	Chlortrimeton	9·48	9·13 9·16,[b] 9·07 [c]
(−)-Noradrenaline	10·69	9·98 9·78 [a]	9·34	8·82 8·73 [a]		3·30	Neohetramine	9·66	8·94 8·84,[b] 8·70 [c]
(+)-Adrenaline ...	10·57	9·96	9·17	8·64			Trimeton	9·40	8·83 9·29,[b] 9·30 [c]
Antistine	11·09	10·13 10·06,[b] 8·26 [c]	2·37	2·45 3·94 [b]			Pyribenzamine	8·70	8·15 8·96,[b] 8·70 [c]
Benadryl	9·67	9·12 9·00,[b] 7·44 [c]							

[a] Lewis, *Brit. J. Pharmacol.*, 1954, **9**, 488. [b] Ref. 7. [c] Tolstoouhov, *Trans. N.Y. Acad. Sci.* 1952, **14**, 260.

number of protons bound per ligand are determined and plotted against the pH. Evaluating the pH at the 1/2 and 3/2 equivalence points yields the corresponding values. Generally, good agreement is found between our values and those of Lordi and Christian,[7] except for Pyribenzamine. This difference may be due to the solvent (dioxan) effect on the ionization of Pyribenzamine.

Graphical methods were used for the evaluation of the stability constants of the complexes. Assuming stepwise formation of a bis-complex, Albert [3] has shown that reliable values for the first and second stepwise stability constants, K_1 and K_2, are obtained from $K = 1/(\text{li})_f$, when the average number of ligands bound per central metal ion ($\bar{n}$) in the system is 0·5 and 1·5, respectively. Similarly, reliable values for K_{st}, the overall stability constant, are obtained from $K_{st} = 1/(\text{li})_f^2$, when $\bar{n}$ is unity. In these equations, $(\text{li})_f$ represents the molar concentration of unbound ligand.

With dibasic ligands, if we assume stepwise formation reactions resulting in complexes with two ligand molecules bound to each metal ion, formation curves can be obtained by plotting $\bar{n}$ against $p(\text{li})_f$, by means of the equations:

$$\bar{n} = [(\text{li})_t - (\text{li})_f\alpha]/(\text{M})_t;$$
$$(\text{li})_f = [(\text{HA})_t - (\text{KOH}) - (\text{H}^+)]/\beta;$$
$$\alpha = 1 + [(\text{H}^+)/K'] + [(\text{H}^+)^2/K'K''];$$
$$\beta = [(\text{H}^+)/K'] + [2(\text{H}^+)^2/K'K''].$$

[6] Martell and Calvin, "Chemistry of the Metal Chelate Compounds," Prentice-Hall, Inc., New York, 1952, p. 517.
[7] Lordi and Christian, *J. Amer. Pharmaceut. Assoc.* (*Sci. Edn.*), 1956, **45**, 300.

Here α and β are constants dependent on the unbound hydrogen-ion concentration and on the first (K') and second (K'') base dissociation constants of the ligand. Parentheses ( ) are used to indicate molar concentration of the species. M denotes the metal. The subscript t refers to total bound plus unbound species, and $(HA)_t$ represents the concentration of protons contributed from all sources except the solvent.

TABLE 2.

Stepwise stability constants of certain metal–antihistamine complexes.

		log K_1		log K_2		log K_{st} (log K_1K_2)		log K_{st} (-2 log $(li)_f$)	
Ligand	M++	at 0°	at 25°	at 0°	at 25°	at 0°	at 25°	at 0°	at 25°
Antistine.....	Ni	3·88	4·02	3·52	3·75	7·40	7·77	7·14	7·70
	Co	3·69	3·96	3·41	3·69	7·10	7·65	6·90	7·64
	Cu	6·82	6·14	6·16	5·85	12·98	11·99	13·14	12·30
Benadryl.....	Ni	3·66	3·30	(3·36) [a]	(3·23) [a]	(7·02) [a]	(6·53) [a]	6·94	6·52
	Co	3·47	3·32	(3·30) [a]	(3·23) [a]	(6·77) [a]	(6·55) [a]	6·66	6·54
	Cu	5·68	5·36	5·26	4·78	10·94	10·14	11·02	10·50
Neohetramine ...	Ni	3·94	4·41	3·50	4·00	7·44	8·41	7·24	8·32
	Co	3·46	3·15	(3·25) [a]	(3·15) [a]	(6·71) [a]	(6·30) [a]	6·60	6·30
	Cu	†	†	†	†	†	†	†	†
(−)-Noradrenaline	Ni	5·76	5·28	3·75	(3·50) [a]	9·51	(8·78) [a]	9·50	8·00
	Co	5·32	4·82	3·62	(3·50) [a]	8·94	(8·32) [a]	8·64	7·36
	Cu	10·23	9·13	6·50	5·87	16·73	15·00	18·36	16·32
(+)-Adrenaline ...	Ni	6·17	6·22	3·58	3·66	9·75	9·88	9·00	9·26
	Co	5·68	5·76	4·07	4·29	9·75	10·05	9·60	10·06
	Cu	11·42	10·70	8·93	6·70	20·35	17·40	20·30	18·44
Chlortrimeton ...	Ni	*	*	*	*	*	*	*	*
	Co	*	*	*	*	*	*	*	*
	Cu	6·43	5·40	5·32	4·51	11·75	9·91	11·14	9·26
Pyribenzamine ...	Ni	3·38	3·76	2·74	3·22	6·12	6·98	5·84	6·94
	Co	3·17	3·42	2·87	3·07	6·04	6·49	5·84	6·34
	Cu	4·67	4·53	4·30	4·05	8·97	8·58	8·98	8·80
Trimeton.....	Ni	*	*	*	*	*	*	*	*
	Co	*	*	*	*	*	*	*	*
	Cu	5·39	5·17	5·02	4·90	10·41	10·07	11·14	10·04
(−)-Adrenaline ...	Ni	6·17	5·65	3·71	3·52	9·88	9·17	8·90	8·40
	Co	6·09	5·42	4·19	3·80	10·28	9·22	10·30	8·94
	Cu	10·96	10·50	8·60	7·90	19·56	18·40	19·06	17·72

( ) [a] Indicates values derived from an extrapolation of the formation curve to higher $\bar{n}$ values.
* No stepwise complex formed. † Insoluble complex formed.

The stability constants are determined by evaluating $p(li)_f$ at the appropriate points on the formation curve. Table 2 lists values of the stepwise stability constants and provides a comparison of the overall stability constants, evaluated graphically at $\bar{n} = 1$, with those obtained from the product of the two stepwise constants. Values obtained at $\bar{n} = 1$ are considered more reliable. Evaluation of the stepwise constants assumes that the concentration of the bis-complex is negligible at $\bar{n} = 0{\cdot}5$, and that the concentration of the free metal ion is negligible at $\bar{n} = 1{\cdot}5$. No such assumption is needed for the overall stability constants at $\bar{n} = 1$. These overall constants and the calculated values of the associated thermodynamic functions are given in Table 3. It is estimated that the values for these functions are correct to within $\pm 1{\cdot}0$ kcal./mole for ΔG and ΔH, and to within $\pm 0{\cdot}5$ e.u. for ΔS.

All the antihistamines studied form stable complexes. The stability constants of the copper(II) complexes are decidedly greater than those for the corresponding complexes with cobalt(II) and nickel(II). With a particular metal ion the antihistamines form complexes of essentially the same order of stability; however, (+)- and (−)-adrenaline and (−)-noradrenaline form somewhat more stable complexes. Recognized experimental difficulties are inherent in the study of complexes of adrenaline-type molecules in aqueous

TABLE 3.

Thermodynamic quantities of certain substances which inhibit the action of histamine at 0°c and 25°c.

	M^{++}	Temp.	log K_{st}	$-\Delta G$ (kcal./mole)	ΔS (e.u.)	ΔH (kcal./mole)
Antistine	Ni	25°	7·70	10·6	66·0	9·1
	Ni	0	7·14	8·9		
	Co	25	7·64	10·4	72·0	11·0
	Co	0	6·90	8·6		
	Cu	25	12·30	16·8	14·4	−12·5
	Cu	0	13·14	16·4		
Benadryl	Ni	25	6·52	8·9	8·8	−6·3
	Ni	0	6·94	8·7		
	Co	25	6·54	8·9	24·0	−1·8
	Co	0	6·66	8·3		
	Cu	25	10·50	14·3	22·0	−7·8
	Cu	0	11·02	13·8		
Neohetramine	Ni	25	8·32	11·4	92·4	16·2
	Ni	0	7·24	9·1		
	Co	25	6·30	8·6	14·0	−4·4
	Co	0	6·60	8·3		
	Cu	25	†	†		
	Cu	0	†	†		
(−)-Noradrenaline	Ni	25	8·00	10·9	−38·4	−22·4
	Ni	0	9·50	11·9		
	Co	25	7·36	10·1	−30·0	−19·0
	Co	0	8·64	10·8		
	Cu	25	16·32	22·3	−26·8	−30·3
	Cu	0	18·36	23·0		
(+)-Adrenaline	Ni	25	9·26	12·6	55·6	3·9
	Ni	0	9·00	11·3		
	Co	25	10·06	13·7	69·2	6·9
	Co	0	9·60	12·0		
	Cu	25	18·44	25·2	−8·4	−27·7
	Cu	0	20·30	25·4		
Chlortrimeton	Ni	25	*	*		
	Ni	0	*	*		
	Co	25	*	*		
	Co	0	*	*		
	Cu	25	9·26	12·6	−52·0	−24·8
	Cu	0	11·14	13·9		
Pyribenzamine	Ni	25	6·94	9·5	86·8	16·4
	Ni	0	5·84	7·3		
	Co	25	6·34	8·7	54·0	7·5
	Co	0	5·84	7·3		
	Cu	25	8·80	12·0	32·0	−2·5
	Cu	0	8·98	11·2		
Trimeton	Ni	25	*	*		
	Ni	0	*	*		
	Co	25	*	*		
	Co	0	*	*		
	Cu	25	10·04	13·7	−9·2	−16·4
	Cu	0	11·14	13·9		
(−)-Adrenaline	Ni	25	8·40	11·5	13·6	−7·4
	Ni	0	8·90	11·1		
	Co	25	8·94	12·2	−26·8	−20·2
	Co	0	10·30	12·9		
	Cu	25	17·72	24·2	14·4	−19·9
	Cu	0	19·06	23·8		

* No stepwise complex formed. † Insoluble complex formed.

solution. These difficulties will usually result in an uncertainty in the quantitative evaluation of the associated thermodynamic properties. However, the increased stability exhibited by these complexes, over other types of antihistaminic complexes, is of a sufficient size to be clearly demonstrated. Adrenaline and noradrenaline are among the

most basic of the antihistaminics studied, and are capable of forming five-membered chelate rings. Because of the similarity in stabilities of the adrenaline and noradrenaline complexes the phenolic hydroxyl groups may contribute appreciably in the resultant mechanism of complex formation. Entropy changes for the complex-forming reaction are exceptionally high in some cases, indicating perhaps that complete chelation extensively alters solvation of the various species present.

A current theory of antihistaminic activity centres around the concept of competitive binding,[8] in which the antihistamines are in competition with the histamine for receptor sites. In addition, it is suggested here that these receptor sites may be metal ions. The presence in the antihistamine molecule of groups situated in such a position as to allow the formation of a strain-free chelate ring (or, less preferably, a co-ordinate bond) may be involved in, or possibly even required for, superior antihistaminic activity. It seems reasonable that the free energy of formation for the complexes involving a given metal ion could be used as an index of the effectiveness of the various antihistamines.

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[8] Wells, *Ann. N.Y. Acad. Sci.*, 1950, **50**, 1202.