TABLE II

DISSOCIATION CONSTANT OF $Co(NH_3)_6SO_4^+$

$10^4a = 10.15$, $K(a)$, $\mathring{a} = 3.8$ Å., $C = 0.70$; $K(b)$, $\mathring{a} = 6.2$ Å., $C = 0.39$, $1/(\epsilon_2 - \epsilon_1)$

10^2b	1.00	1.50	2.00	2.50	3.00	3.50
$D_1 - D_2$	0.304	0.332	0.354	0.3685	0.384	0.394
10^2y	0.52	1.1	1.85	2.7	3.75	4.95
10^2I	3.16	4.53	5.89	7.15	8.44	9.68
$10^3K(a)$	1.26	1.275	1.25	1.265	1.245	1.265
$10^2K(b)$	1.35	1.375	1.345	1.36	1.31	1.32

of K in Table II are (a) 0.00126 and (b) 0.001345. The 6.5% difference between these stresses the need to obtain measurements at very low ionic strengths for in these regions the $B\mathring{a}$ factor becomes less important and K can be found with greater precision. Our present answer is a little higher than our previous one.[4] This is chiefly due to the fact that for the latter $B\mathring{a}$ was taken as 1.0; if this were used for our present measurements, K would be 0.00120, *i.e.*, within 6% of the previous result. Accordingly the new measurements confirm the earlier ones, which were obtained at 248 and 250 mμ. It is difficult to indicate why we differ from Posey and Taube[1] since they have not given experimental details. Their method requires a straight line extrapolation and, judging by their Fig. 1, this was not too successful. These authors differ from us in that they used solutions containing 0.0001 *M* $HClO_4$. We repeated our measurements in the presence of 0.001 *M* acid, but this did not have any effect.

Another feature which also awaits an explanation is the large discrepancy between the conductance[3] and spectrophotometric values. It is possible that the new Fuoss–Onsager conductance treatment[10] may eventually provide an answer. Preliminary calculations with 1:1-valent salts show that this gives higher dissociation constants than do the older methods based on the limiting Onsager equation. This is in contrast to what happens when modifications are applied to the original Onsager treatment so that the effect of ion size can be examined. Davies, Otter and Prue[11] illustrate this in a discussion of the dissociation of cupric sulfate. The limiting law value of the dissociation constant in water at 25° is 0.0049, whereas for an ion-pair distance of 10 Å. it is 0.0040. The same type of trend was found by these authors by an analysis of cryoscopic and spectrophotometric data when increasing ion-pair distances are used. By the last of these methods they calculate K to be 0.0080 for an ion-pair distance of 4.3 Å. and with 10 Å., K = 0.0040. This is a much wider variation than we obtain with the present results on trying different ion sizes and is partly due to differences of experimental procedure. Davies, Otter and Prue balanced the optical densities of cupric sulfate against those of cupric perchlorate, and since they did not use constant ionic strengths, their interpretation involved using molar extinction coefficients which vary with the chosen ion-pair distance. Prue[12] can see no justification for taking a unique value of ϵ_2, although this contradicts the arguments leading to equation 3 unless the factor f_1f_2/f_3 does not remain constant at constant ionic strength. One way of examining this would be to obtain data leading to Table I with non-associating salts other than $NaClO_4$. An indirect test is to repeat the procedure at different constant ionic strengths. Judging by our work with the ion pair UO_2Cl^+, $\epsilon_2 - \epsilon_1$ does remain constant up to an ionic strength of about one but can change considerably in more concentrated media.

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[11] W. G. Davies, R. J. Otter and J. E. Prue, *Disc. Faraday Soc.* **24**, 103 (1957).

[12] J. E. Prue, ref. 11, p. 117.

[13] W. D. Bale, E. W. Davies and C. B. Monk, ref. 11, p. 94.

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[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY]

The Combination of Manganous and Cobaltous Ions with Imidazole[1]

BY R. BRUCE MARTIN AND JOHN T. EDSALL

RECEIVED MAY 15, 1958

Imidazole forms weak complexes with manganous ion, log k_1 = 1.65, log k_2 = 1.25 and with cobaltous ion, log k_1 = 2.42, log k_2 = 1.95, log k_3 = 1.58, log k_4 = 1.2 at 25° and ionic strength 0.16. The weakness of the binding does not permit a determination of the higher association constants at this ionic strength. A correlation between the binding of a series of metal ions with ammonia and with imidazole is described.

Imidazole has been shown to be a useful model compound for the metal ion combining capacity of the histidyl group in proteins.[2] The study of its combination with metallic ions is here extended to the biologically important manganous ion and cobaltous ion.

Experimental

The imidazole was the same as that used previously,[3] cobaltous nitrate was Fisher Certified Reagent and manganous nitrate was Fisher Certified Reagent assayed solution. All solutions were CO_2 and O_2 free, prepared from distilled water which was passed through a mixed bed ion-exchange column and through which nitrogen was bubbled.

Standardized sodium hydroxide solution was added from a Gilmont ultramicroburet of 1.00-ml. capacity. The *p*H

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[1] This work was supported by grants from the National Science Foundation (G-3230) and from the United States Public Health Service (H-3169).

[2] F. R. N. Gurd and P. E. Wilcox, "Advances in Protein Chem.," Vol. XI, Academic Press, Inc., New York, N. Y., 1956, p. 311.

[3] J. T. Edsall, G. Felsenfeld, D. S. Goodman and F. R. N. Gurd, THIS JOURNAL, **76**, 3054 (1954); Y. Nozaki, F. R. N. Gurd, R. F. Chen and J. T. Edsall, *ibid.*, **79**, 2123 (1957).

was measured on a Beckman Model G *p*H meter. The ionic strength was 0.16 and the temperature 24–25° throughout the study.

Results

The results are tabulated in Tables I and II. In the third columns, *p*A is the negative logarithm of the free imidazole concentration, and in the fourth columns $\bar{\nu}$ is the average number of imidazole molecules bound per free metal ion.

TABLE I

TITRATION OF COBALT(II) NITRATE–IMIDAZOLE MIXTURES

Initial solution, 3.00 ml. of solution, 0.140 *M* in imidazolium nitrate and 0.00667 *M* in $Co(NO_3)_2$; titrated with 0.1511 *M* NaOH which was 0.16 *M* in $NaNO_3$

Base, ml.	*p*H	*p*A	$\bar{\nu}$
0.025	4.72	3.24	0.10
.050	4.97	3.00	.23
.075	5.13	2.85	.35
.100	5.28	2.71	.45
.150	5.50	2.51	.64
.200	5.65	2.36	.81
.300	5.89	2.15	1.11
.350	5.97	2.09	1.29
.450	6.12	1.97	1.56
.500	6.19	1.92	1.66
.550	6.26	1.86	1.72
.600	6.31	1.83	1.87
.700	6.42	1.75	2.00
.800	6.52	1.68	2.11
1.000	6.69	1.58	2.34
1.200	6.84	1.51	2.52
1.300	6.91	1.47	2.60
1.400	6.98	1.45	2.69
2.000	7.41	1.32	3.05

TABLE II

TITRATION OF MANGANESE(II) NITRATE–IMIDAZOLE MIXTURES

Initial solution, 3.07 ml. of solution, 0.137 *M* in imidazolium nitrate and 0.00710 *M* in $Mn(NO_3)_2$; titrated with 0.1511 *M* NaOH which was 0.16 *M* in $NaNO_3$.

Base ml.	*p*H	*p*A	$\bar{\nu}$
0.030	4.99	2.99	0.06
.080	5.45	2.55	.15
.130	5.70	2.31	.19
.230	5.95	2.09	.37
.330	6.13	1.94	.51
.430	6.29	1.81	.54
.530	6.39	1.74	.70
.830	6.67	1.56	.82
1.030	6.81	1.49	1.09
1.230	6.94	1.43	1.26

For the successive steps in the binding of a ligand A to a metallic ion M^{++}, we may write the equations

$$M^{++} + A \rightleftarrows MA^{++},\ MA^{++} + A \rightleftarrows MA_2^{++},\ \text{etc.},$$

and the related association constants, $k_1 = (MA^{++})/(M^{++})(A)$, $k_2 = (MA_2^{++})/(MA^{++})(A)$, etc., may be defined. Details of the evaluation of the association constants have been previously described.[3] The method of Scatchard for their evaluation was not used in this study, due to uncertainties in the effective coördination number and to the lesser accuracy of the data due to the weaker binding capacity. The values of the association constants were evaluated by drawing the best line through the points of a $\bar{\nu}$ *versus* *p*A plot and solving simultaneous equations by means of determinants. The values so derived were checked for accuracy by comparing the calculated curve with the plotted points.

The values obtained in this study are for cobaltous ion, log k_1 = 2.42, log k_2 = 1.95, log k_3 = 1.58, log k_4 = 1.2 and for manganous ion, log k_1 = 1.65, log k_2 = 1.25. The limits of error for the log k_1 values are of the order of ±0.05 and are greater for the higher association constants. The error of the highest constant for each metal ion is at least ±0.1 due to lack of knowledge of the values of the succeeding constants. In order to determine the higher association constants accurately, it would be necessary to work at considerably greater ionic strengths.

Discussion

For both cobaltous and manganous ions four or six groups may associate with the metal ion. No direct determinations were made in this study of the maximum number of imidazole groups bound per metal ion. However, if the logarithms of the intrinsic association constants (denoted by log κ_1, log κ_2, etc., in the earlier papers from this Laboratory[3]) are computed for cobalt, the successive values are 1.82, 1.78, 1.76 and 1.82 if four groups are bound, and 1.64, 1.55, 1.45 and 1.35 if six are bound. The corresponding values for cobaltous ion and ammonia[4]—an interaction for which the coördination number is known to be 6—are 1.33, 1.23, 0.92, 0.89, 0.58 and 0.16. Since imidazole is a nitrogenous base, and in this respect is similar to ammonia, it may be inferred by analogy that cobaltous ion also binds six imidazole groups. Manganous ion has not been studied with ammonia and since only two constants are known from this study no conclusions may be drawn.

The association of cobaltous ion with histidine has been reported in two studies[5,6] with values of log k_1 and log k_2 of 7.3, 4.3 and 7.5, 6.3, respectively. These values are considerably greater than the values reported here for cobaltous ion and imidazole. Hence the view that cobalt is bound to histidine in a chelate complex is given further support.

The association of manganous ion with histidine also has been studied with a variety of values reported for log k_1, namely: 3.6, 4.2, 1.75, 2.1 (see references 7, 6, 8 and 9, respectively). The values vary more than the conditions under which they were studied and perhaps the last value is the most reliable. The increment in log k_1 for histidine as compared to imidazole is thus much less for manganous ion than for cobaltous ion, which is not unexpected if the association constants of the various metal ions with ethylenediamine are compared.

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(4) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, Denmark, 1941; *Chem. Revs.*, **46**, 381 (1950).

(5) J. Z. Hearon, D. Burk and A. L. Schade, *J. Natl. Cancer Inst.*, **9**, 337 (1949).

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(8) M. Cohn and J. Townsend, *Nature*, **173**, 1090 (1954).

(9) Reference 2 p. 335.

For ethylenediamine[2,10] and cobaltous ion log $k_1 = 5.89$; for manganous ion log $k_1 = 2.73$.

It is of interest to compare the values available for log k_1 of the various metal ions with ammonia and imidazole in Table III.

TABLE III

FIRST ASSOCIATION CONSTANTS (LOG k_1) FOR AMMONIA AND IMIDAZOLE WITH VARIOUS METALLIC IONS

	Cu^{++}	Ni^{++}	Cd^{++}	Zn^{++}	Co^{++}	Mn^{++}	Ca^{++}
Ammonia[a]	4.15	2.80	2.65	2.37	2.11	..	−0.2
Imidazole	4.33[b]	3.27[c]	2.80[d]	2.57[e]	2.42	1.65	0.08[f]

[a] Ref. 4. [b] Ref. 3. [c] Ref. 11. [d] C. Tanford and M. L. Wagner, THIS JOURNAL, **75**, 434 (1953). [e] Ref. 3. [f] J. Schubert, THIS JOURNAL **76**, 3442 (1954).

The values for ammonia are at 30° and ionic strength 2 except for calcium ion which is at 23°. However, the temperature coefficient of log k_1 for the calcium complex is probably small.[11] The values for imidazole are all at 25° and ionic strength 0.16. Comparison of the values of log k_1 at different temperatures and ionic strengths is valid because the same correction term probably would be applied in all cases.

The average difference in log k_1 between imidazole and ammonia for all the metals is 0.3 ± 0.1 and is amazingly constant over a 10^4 range in binding capacity. Thus a value of log k_1 of 1.4 may be predicted with some assurance for the combination of manganous ion with ammonia. This would be difficult to determine directly due to the weak binding and the tendency of manganous ion to combine with hydroxide ion. An analysis similar to that previously given[3] indicates that no significant hydroxide formation occurs under the conditions of this study with imidazole and either cobaltous or manganous ion. The only "metal" ion investigated that does not fit the above table is hydrogen ion where log k_1 is 9.28 for ammonia and 7.11 for imidazole under the conditions used in each case. Imidazole forms metal complexes more avidly than one would predict from comparison of its acid ionization constant with that of ammonia. A relatively constant difference between other association or intrinsic constants may also be formulated with similar or slightly less success than described above with log k_1.

Just as the correspondence of the log k_1 values for imidazole and ammonia with various metal ions allows a prediction of the value for manganous ion and ammonia, so the known values of log k_1 for other metal ions with ammonia may be used to estimate a value of log k_1 for the same metal ion with imidazole by adding 0.3 to the log k_1 value for ammonia. This is particularly valuable in the study of proteins because many metals have been studied with ammonia[4] and the value of log k_1 is all that is needed as higher complexes are unlikely with proteins.

If the histidyl group is part of the active site of enzymes such as α-chymotrypsin a correspondence might be expected between the log k_1 values of the metal ions with imidazole and the inhibitory effects of the same metal ions on the enzyme catalyzed reactions.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE GEORGE WASHINGTON UNIVERSITY]

The Fluoroplatinates. IV. Preparation, Density and Solubility of the Fluoroplatinates of Magnesium and the Alkaline Earth Metals

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RECEIVED MAY 1, 1958

The fluoroplatinates of magnesium, calcium and strontium were prepared by the reaction of fluoroplatinic acid with the oxide, hydroxide and carbonate of the respective metals. The barium fluoroplatinate was prepared by the reaction of lanthanum fluoroplatinate with barium chloride. The densities varied from 2.65 g./cm.³ for the magnesium fluoroplatinate to 6.04 g./cm.³ for the barium salt. The solubilities varied from 104.9 g./100 ml. of solution for calcium fluoroplatinate to 0.171 g./100 ml. of solution for the barium salt.

The chemical literature contains no data on the preparation, composition, density or solubility of the fluoroplatinates of magnesium, calcium, strontium or barium. The barium fluoroplatinate was prepared by Klemm[2] presumably by heating a mixture of a barium salt and platinum in a stream of fluorine, although no details of the method were published. Cox[3] reported that Sharpe has prepared the strontium fluoroplatinate, but the procedure was not described. The present work was undertaken to prepare the fluoroplatinates of magnesium and the alkaline earths and to study some of their properties.

Experimental

Preparation of Reagents. Lanthanum Fluoride.—A slight excess of 48% hydrofluoric acid was added to a hot solution of lanthanum ammonium nitrate in a polyethylene beaker. After the precipitate of lanthanum fluoride had settled, it was filtered and washed, first with water and then with acetone. It was dried overnight at 110° and for 0.5 hr. at 400°.

Platinum Powder.—Platinum foil (>99.9% pure) (purchased from The American Platinum Works) was dissolved in boiling aqua regia and the resulting solution was diluted to ten times its volume and filtered. Hydrazine was added in small amounts until all of the platinum had precipitated. It then was filtered off, washed with water followed by acetone and dried in a furnace at 500–600°.

(1) Abstracted from a portion of the dissertation submitted by Marriner K. Norr in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) W. Klemm, *Angew. Chem.*, **66**, 468 (1954).

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