heme,[14] iron(III) pheophytin,[14] iron(III) tetraphenylporphyrin,[14] copper(II) pheophytin,[14] and sodium tetraphenylporphyrin.[21] It is interesting that zinc phthalocyanine, a coplanar molecule in contrast to the preferred tetrahedral configuration of zinc, falls in this group, even although all of the other zinc chelate complexes studied to date appear to exchange rapidly in pyridine.

The apparent rapid complete exchange of all the other ("non-fused-ring") zinc complexes given in Table I is either actual or is a catalyzed exchange resulting from the addition of the chloroform–water mixture used in the separation. The former seems more likely, especially as the same separation method gives little or no induced exchange with the analogous copper(II) chelate complexes. It is interesting to compare these results for the zinc complexes with those for analogous copper(II) complexes[13] and nickel(II) complexes[22,23] at comparable concentrations.

For the 8-hydroxyquinoline complexes: nickel and zinc exchange rapidly, copper has not been run, magnesium[14] exchanges rapidly in water solution (2-phase system).

For salicylaldehyde (sal) derivatives: no exchange of sal-*o*-phenylenediimine found in the case of copper and nickel (3 and 48 hours, respectively), rapid for zinc. Slow exchange of sal-ethylenediimine observed for copper (2-hour half-time) and nickel (no exchange in 48 hours), rapid for zinc. Rapid exchange of sal-methylimine observed for copper and zinc, nickel not run.

For the acetylacetone-ethylenediimine complexes: copper (37-hour half-time) and nickel (no exchange in 48 hours) exchange slowly, zinc rapidly.

The order of lability is what is expected from the decreasing order of stability, Cu > Ni > Zn, with two exceptions. The order of exchange lability for the copper and nickel sal-ethylenediimine and acetylacetone-ethylenediimine is apparently the reverse of the stability order; this may be actual, or it may be due to use of a 7:3 pyridine–methyl cellosolve solvent in place of pyridine for the nickel studies.[24]

Of the zinc chelate complexes discussed in this paper only zinc phthalocyanine appears suitable, as far as its exchange properties are concerned, for use in Szilard–Chalmers concentration of radiozinc, assuming that the inorganic recoil zinc is separated by the pyridine–chloroform–water procedure used in the exchange separations.

LOS ANGELES 24, CALIFORNIA

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[21] J. B. Barnes and G. D. Dorough, quoted by M. D. Kamen, J. W. Kennedy and O. Myers, *J. chim. phys.*, **45**, 199 (1948).

[22] J. E. Johnson and N. F. Hall, THIS JOURNAL, **70**, 2344 (1948).

[23] N. F. Hall and B. R. Willeford, Jr., *ibid.*, **73**, 5419 (1951).

[24] Copper, nickel and presumably zinc form pyridine complexes with two, four and six pyridine molecules per gram-ion of metal, depending upon the relative concentrations [see, *e.g.*, J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941; P. C. Sinha and R. C. Ray, *J. Indian Chem. Soc.*, **25**, 247 (1948)]. It is possible that the greater pyridine concentration in the copper exchange studies resulted in formation of kinetically more active pyridine complexes.

[CONTRIBUTION FROM THE DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH, ARGONNE NATIONAL LABORATORY]

Stability of Alkaline Earth–Organic Acid Complexes Measured by Ion Exchange[1]

BY JACK SCHUBERT AND ARTHUR LINDENBAUM

RECEIVED DECEMBER 15, 1951

The formation constants for the complex ions formed between Ca^{++} and Sr^{++} and a series of mono-, di- and tricarboxylic acids have been measured in barbital buffered solutions at *p*H 7.2–7.3, $\mu = 0.16$, and $t = 25°$ by the equilibrium ion exchange technique. All the complex ions formed were of the 1:1 type. The degree of complex formation is found to be a function of the number, kind and position of the groupings within the organic molecule. Calculations of the formation constant under conditions where more than one complex ion is present in the solution and where the complex ion itself is taken up by the ion exchanger are made.

Quantitative measurements of the stability of the complex ions formed between trace metals and the compounds involved in the tricarboxylic acid cycle[2] are of fundamental importance for the elucidation of many biological problems.[3] In this investigation, the formation constants between calcium and strontium and seventeen different organic acids have been determined under approximately physiological conditions, *i.e.*, ionic strength of 0.16 and *p*H 7.2–7.3.

All measurements were made by the equilibrium ion exchange method utilizing essentially carrier-free levels of radioactive cations.[4a–g] In a recent paper, Fronaeus,[5] has extended the ion exchange method to systems in which more than one complex is formed between the cation and ligand, *i.e.*, MA^+, MA_2 and MA_3^-, etc. In applying the ion exchange method for complex ion studies we have previously assumed that the complex ion itself is not taken up by the cation exchanger and that no other complex-forming ligands of any importance are present. An estimation of these effects is considered.

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[1] The material in this paper was presented at the 118th Meeting of the Am. Chem. Soc., Chicago, Ill., 1950 before the Division of Biological Chemistry, and the Symposium on Complex Ions and Polyelectrolytes, Division of Physical and Inorganic Chemistry and Division of Colloid Chemistry, Am. Chem. Soc., Ithaca, N. Y., June 18-20, 1951.

[2] H. G. Wood, *Physiol. Rev.*, **26**, 198 (1946).

[3] A. L. Lehninger, *ibid.*, **30**, 393 (1950).

[4] (a) J. Schubert, Manhattan Project Report, CN-2563, issued February, 1945; (b) J. Schubert, *J. Phys. Colloid Chem.*, **52**, 340 (1948); J. Schubert and J. W. Richter, *ibid.*, **52**, 350 (1948); (c) J. Schubert and J. W. Richter, THIS JOURNAL, **70**, 4259 (1948); (d) J. Schubert, E. R. Russell and L. S. Myers, Jr., *J. Biol. Chem.*, **185**, 387 (1950); (e) J. Schubert and A. Lindenbaum, *Nature*, **166**, 913 (1950); (f) J. Schubert, *Anal. Chem.*, **22**, 1359 (1950); (g) J. Schubert, *J. Phys. Chem.*, **56**, 113 (1952).

[5] S. Fronaeus, *Acta Chem. Scand.*, **5**, 859 (1951).

Experimental

Procedure.—The general procedure followed was exactly that described previously,[4c] *i.e.*, 100-ml. volumes of buffered solutions at constant ionic strength containing tracer were equilibrated with known weights of cation exchange resin (Dowex-50) in the presence of known concentrations of a given ligand.

Results

Formation constants, k_f, were calculated from the equation

$$k_f = \frac{(K_d^\circ/K_d) - 1}{(A)^n} \qquad (1)$$

where K_d° and K_d are the distribution coefficients of the divalent cation, M^{++}, between the resin and solution phases in the absence and presence of the ligand, A, respectively. From plots of $1/K_d$ *vs.* (A) it was found that all the complexes were of the 1:1 type, *i.e.*, $n = 1$. The results are summarized in Table I. Data for eight other complexes are included for comparative purposes. Reasonable agreement was usually obtained between the k_f values determined by the equilibrium ion exchange method and by methods such as e.m.f.,[6] the frog heart,[7] electrometric titration[8] and solubility.[9]

On the average, the K_d° (50 ml. of veronal buffer present) for Ca^{++} was 1.33, while that for Sr^{++} was 2.40. In all instances, the value of K_d° obtained by extrapolation of $1/K_d$ to zero concentration of the ligand was in agreement with the value measured directly. As a check on the time allowed for equilibrium (3 hours), the k_f for the Sr malate complex was determined after 1, 2, 3, 27 and 51 hours of continuous shaking at 25°. Within experimental error, no differences in the k_f or K_d° values were found.

Discussion

It is possible to make approximate corrections for the presence of adsorbable complex ions and complex formation between M^{++} and ligands other than A. For calcium citrate, for example, it is of interest to calculate the magnitude of the factor needed to have k_f refer to a "standard" state, 0.16 *M* NaCl at 25°. In this case the solution contains a mixture of $(CaCit)^-$ and $(CaB)^+$ where (B) represents the molar concentration of the monovalent buffer components, acetate ($k_f = 4.2$) and diethyl barbiturate ($k_f = 4.5$) which are considered to act as a single ligand, B^-, having a formation constant of

$$k_f^{B^-} = \sqrt{4.2 \times 4.5} = 4.3.$$

The correction term is derived by including in the distribution coefficient as many species as seems reasonable under given experimental conditions. Thus, in the calcium citrate example we have

$$K_d^\circ = \frac{(CaR_2) + (CaB)R}{(Ca^{++}) + (CaB)^+};$$

$$K_d = \frac{(CaR_2) + (CaB)R}{(Ca^{++}) + (CaB)^+ + (CaCit)^-} \qquad (2)$$

By following our previous procedure[4d] the correction term, *i.e.*, the term which multiplies the right-hand side of equation (1), is

$$(1 + NB)/(1 + NB - BK_d^\circ k_f^{B^-}/K_d^{Ca^{++}}) \qquad (3)$$

where $N = K_d^{CaB^+}k_f^{B^-}/K_d^{Ca^{++}}$.

In the experiment, in which 100 ml. of solution, 100 mg. of sodium resin and 50 ml. of buffer were used, $B = (0.02856 \times 2 \times 50)/100 = 0.02856$ *M*. In pure 0.16 *M* NaCl the value of $K_d^{Ca^{++}}$ is 1.5, while $K_d^\circ = 1.33$. In the expression for N the term $K_d^{CaB^+}$ must be evaluated. This is difficult to do experimentally but a reasonable estimation can be made by assuming that the exchange affinity of CaB^+ is equivalent to Na^+. At worst, this assumption is uncertain by a factor of about two. Since the resin contains 0.39 meq. of Na^+, and the solution 16 meq., $K_d^{CaB^+} = 0.39/16 \times 100/100 = 0.024$. Consequently $N = (0.024 \times 4.3)/1.5 = 0.069$, and $NB = 0.069 \times 0.02856 = 2.0 \times 10^{-3}$. By substitution of the numerical terms in the correction factor (3) we find that it is equal to 1.12, hence an increase of about 12% in the value of k_f for the calcium citrate complex when 50 ml. of buffer is present yields the value of k_f in pure NaCl.

In similar manner when a positively charged complex ion such as calcium gluconate only (CaG^+) is formed the correction term is equal to

$$1/(1 - K_d^{CaG^+}/K_d) \qquad (4)$$

from which the correction term is readily estimated to be about 2%. The correction (*i.e.*, standardization) factors for the formation constants have not been included in Table I because, under the restricted solution concentrations employed, these factors usually fall within the range of experimental uncertainty in the value of the formation constant itself.

From Table I can be deduced a measure of the effect of the number and position of groups such as the –OH, –COOH and double bonds on k_f. The conclusions drawn are somewhat tentative since at $\mu = 0.16$ the activity coefficient corrections may reverse an apparent order of stability when comparisons are made at $\mu = 0$. Of particular interest is the 5-fold increase in bonding affinity following a shift of the –OH group in isocitric acid from the alpha carbon atom to the center carbon atom to give citric acid. An increase in chain length by one $-CH_2$ group results in about a 2-fold change in the formation constant for all the types of organic acids listed in Table I. The relations of binding affinity to configuration in most of the other compounds listed in Table I have been commented upon by several investigators.[4e–g,6,8–11]

It is worth noting that the differences in stability of the Ca and Sr complexes of the various organic acids might be utilized to effect their chromatographic ion exchange separations.

Acknowledgment.—We wish to thank Dr. L. S. Myers, Jr., for stimulating discussions, particularly on points involving the development of the various equations. The technical assistance of Mr. Roman C. Lesko is gratefully acknowledged. We are indebted to Dr. H. B. Vickery, Connecticut Agricul-

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(6) N. R. Joseph, *J. Biol. Chem.*, **164**, 529 (1946).

(7) A. B. Hastings, F. S. McLean, L. Eichelberger, J. L. Hall and E. DaCosta, *ibid.*, **107**, 351 (1934).

(8) R. K. Cannan and A. Kibrick, THIS JOURNAL, **60**, 2314 (1938).

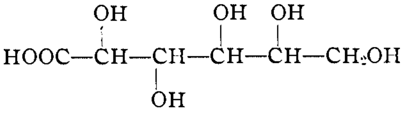
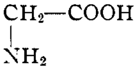
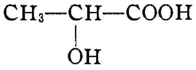
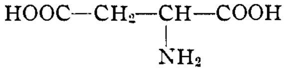
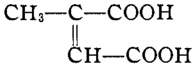
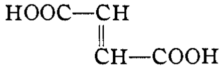
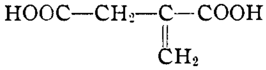
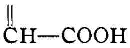
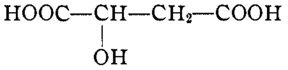
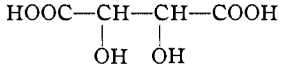
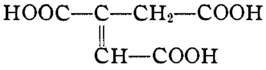
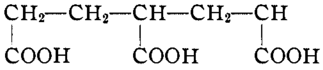
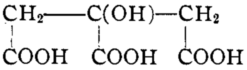
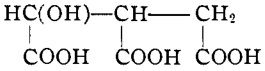
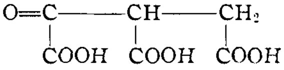
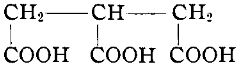
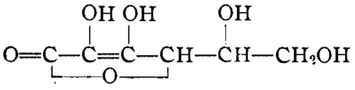
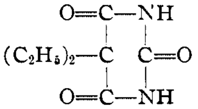
(9) J. Muus and H. Lebel, *K. Danske Vidensk. Selsk., Math.-fys. Medd.*, **13**, No. 19 (1936).

(10) N. E. Topp and C. W. Davies, *J. Chem. Soc.*, 87 (1940).

(11) I. Greenwald, *J. Biol. Chem.*, **124**, 437 (1938).

TABLE I

FORMATION CONSTANTS BETWEEN Ca^{++} AND Sr^{++} AND ORGANIC ANIONS AT pH 7.2–7.3, μ = 0.16, AND t = 25°[a]

Parent acid	Structural formula	Concn. range of parent acid (moles/liter)	log k_f Ca	log k_f Sr	log k_f as reported in literature for similar μ[b] Ca	log k_f as reported in literature for similar μ[b] Sr
Acetic	CH_3-COOH	0.016–0.15	(0.62)	(0.10)	0.53,[6] 0.53[8]	0.43[8]
Gluconic	 $HOOC-CH(OH)-CH(OH)-CH(OH)-CH(OH)-CH_2OH$	.008– .08	1.22	1.01	1.21[8]	1.00[8]
Glycine	 $CH_2(NH_2)-COOH$	.04– .12	(0)	(0.6)		
Lactic	 $CH_3-CH(OH)-COOH$	.04– .08	(0.8)	(0.5)	0.82,[6] 1.07[8]	0.70[8]
Pyruvic	$CH_3-CO-COOH$	.04– .08	(0.8)	(0.5)		
Aspartic[4g]	 $HOOC-CH_2-CH(NH_2)-COOH$	.015– .12	0.44	0.9		
Citraconic	 $CH_3-C(COOH)=CH-COOH$	.006– .025	1.3	1.3		
Fumaric[4f]	 $HOOC-CH=CH-COOH$	.01– .05	(0.48)	(0.54)		
Glutaric	$HOOC-CH_2-CH_2-CH_2-COOH$	.02– .04	(0.55)	(0.6)	1.06[8]	
Itaconic	 $HOOC-CH_2-C(=CH_2)-COOH$	.01– .04	1.2	0.96		
α-Ketoglutaric	$HOOC-CH_2-CH_2-CO-COOH$	.005– .04	1.29	1.14		
Maleic[4f]	 $CH(COOH)=CH-COOH$	.004– .04	1.1	1.1		
Malic[4g]	 $HOOC-CH(OH)-CH_2-COOH$	.005– .04	2.06	1.45		
		.004– .04	..	1.42	1.80[8]	1.45[8]
Malonic	$HOOC-CH_2-COOH$	.01– .04	1.36	1.27	1.46[8]	1.25[8]
Oxalacetic[c]	$HOOC-CH_2-CO-COOH$	.007– .04	1.60	..		
Succinic[4g]	$HOOC-CH_2-CH_2-COOH$	.005– .04	1	0.9	1.16,[6] 1.20[8]	1.06[8]
Tartaric[4f]	 $HOOC-CH(OH)-CH(OH)-COOH$	.009– .03	1.78	1.59	1.80[8]	1.65,[8] 1.94[6]
Aconitic (*trans*)	 $HOOC-C(=CH-COOH)-CH_2-COOH$	.02– .005	1.50	1.51		
α-Carboxypimelic	 $CH_2(COOH)-CH_2-CH(COOH)-CH_2-CH(COOH)$	.001– .02	1.59	1.54		
Citric[d,4e,4g]	 $CH_2(COOH)-C(OH)(COOH)-CH_2(COOH)$	.0002–.004	3.15	2.82	3.17,[6] 3.22[7]	2.92,[6] 2.70[7]
		.0008–.002	3.16	2.88	3.21[9]	
Isocitric	 $HC(OH)(COOH)-CH(COOH)-CH_2(COOH)$	.002– .01	2.47	2.02		
Oxalosuccinic[e]	 $O=C(COOH)-CH(COOH)-CH_2(COOH)$	.002– .01	(1.5)	..		
Tricarballylic[4e]	 $CH_2(COOH)-CH(COOH)-CH_2(COOH)$	.002– .02	1.82	1.68	(1.61–1.87)[9]	
Ascorbic		.02– .08	(0.19)	(0.35)		
Diethylbarbituric		.016– .15	(0.66)	(0.48)		

[a] Numbers in parentheses are approximate because of large experimental errors. [b] The values listed for reference (8) were determined at about μ = 0.2. [c] Measurements made at 3°. [d] The preliminary value for the k_f of the calcium citrate complex as reported in reference 4e was found to be inaccurate. [e] Acid assayed at 95% purity. However, when dissolved gave yellow color.

tural Experiment Station, P. O. Box 1106, New Haven, Conn., for furnishing us with the dimethyl ester of isocitric lactone and the directions by which we converted it to the free acid. Dr. B. Vennesland of the Department of Biochemistry, University of Chicago, very kindly gave us a specially purified sample of oxalacetic acid.

CHICAGO, ILLINOIS

[CONTRIBUTION FROM THE PURDUE UNIVERSITY DEPARTMENT OF CHEMISTRY]

Studies on Some Ferrous Complexes of Substituted 1,10-Phenanthrolines[1]

BY WARREN W. BRANDT AND DELORA K. GULLSTROM

RECEIVED JANUARY 2, 1952

The stabilities of some 5-substituted 1,10-phenanthroline–iron(II) complexes were determined using rate data and equilibrium studies. The stability decreases in this order: 5-methyl, 1,10-phenanthroline itself, 5-phenyl, 5-chloro, 5-nitro. The stability of these complexes appears to be dependent only upon the acid dissociation constant for the organic molecule. A correlation was shown to exist between K_a, K_d and the formal potential of the $[Fe(Phen)_3]^{++}$–$[Fe(Phen)_3]^{+++}$ half-cell reaction, and calculations were made of the stability of the ferric complex utilizing this relationship. The change of formal potential with hydrogen ion concentration tends to support the 1:1 combination of $[Fe(Phen)_3]^{+++}$ and hydrogen ion postulated by Lee, Kolthoff and Leussing. A relationship was observed between stability and wave length of maximum absorption in the case of the ferric complexes, but not the ferrous.

Introduction

The factors affecting the stability of complex ions have been of interest for many years. Most of the early work was only qualitative or semi-quantitative in nature. In recent years, however, workers have attempted to control the variables and to measure in a quantitative manner the effect of changing the metal or substituents in the complexing group. Generalizations have been made upon complex stability of certain metals.

Because the tris-1,10-phenanthroline iron(II) ion is of considerable analytical interest, a study of this system might give further impetus to a theory which would enable the analytical chemist to predict in advance the properties of a given chelate. With data of this nature, he could order a tailor-made complex for a particular use.

Investigations have been carried out on the stability of the tris-1,10-phenanthroline–iron(II) complex itself by Lee, Kolthoff and Leussing.[2] They also determined the kinetics of complex formation and decomposition, the effect of acid concentration and the stabilities of the corresponding ferric complex. It would then appear to be of general and analytical interest to examine these same properties for a number of substituted phenanthrolines. From such an investigation information could be obtained as to the effects of substitution upon the properties of the complex and insight could be gained in predicting what changes in the molecule might enhance its use for a particular purpose.

The compounds studied were four substituted 1,10-phenanthrolines as well as the parent compound. The substituents were in every case in the 5-position.

Experimental

Materials Used.—1,10-Phenanthroline (ortho phenanthroline) monohydrate, 5-nitro-1,10-phenanthroline and 5-methyl-1,10-phenanthroline were obtained from the G. Frederick Smith Chemical Company. The 5-phenyl-1,10-phenanthroline was furnished by Professor Francis Case, Temple University, and the 5-chloro-1,10-phenanthroline was furnished by Professor G. Frederick Smith, University of Illinois.

Standard solutions of the following compounds were prepared from analytical reagent chemicals and standardized by accepted procedures: sulfuric acid, hydrochloric acid and ceric sulfate.

Spectrophotometric Data.—A Beckman DU spectrophotometer was used to obtain spectrophotometric curves of the various complexes studied. Values for the molar absorbancy index were based on the amount of iron present.

Determination of K_a.—K_a, the acid dissociation constant of the organic molecule, was determined by measuring the hydrogen ion activity in a solution containing 25 mg. of the substituted 1,10-phenanthroline and enough hydrochloric acid to react with approximately three-fourths of the phenanthroline present. Because of the insolubility of the substituted compounds it was necessary to determine the apparent *p*H in water–dioxane mixtures. The total volume used in all cases was 50 ml. 1,10-Phenanthroline itself was measured in solutions containing from 0 to 14 ml. of dioxane.

Determination of $k_{\mathrm{diss.}}$.—Suitable amounts of ferrous ammonium sulfate and the substituted phenanthroline to be studied were weighed out and mixed in a 100-ml. volumetric flask. From 5 to 50 ml. of 2 *M* sulfuric acid was added and the solution made up to volume using iron-free distilled water. Zero time was taken when half of the acid had been added. At intervals the amount of complex present was determined by measurements of the absorbancy at the previously determined wave length of maximum absorbancy. These measurements were made on a Beckman DU spectrophotometer.

Rates of Formation.—Reaction mixtures were prepared by mixing together sulfuric acid of the desired molarity and an appropriate amount of ferrous ammonium sulfate in a 100-ml. volumetric flask. A weighed amount of the substituted phenanthroline dissolved in a known amount of sulfuric acid was added and the solution was made up to volume. After various reaction periods, the mixtures were analyzed spectrophotometrically for the amount of complex formed. In the determination of the rate of formation of the complexes, the concentrations of all the reactants were kept large relative to the amount of complex formed.

Determination of $k_{\mathrm{diss.}}$ from Equilibrium Data.—Equilibrium values were obtained on iron(II)–phenanthroline solutions by taking spectrophotometric readings on solutions of the complex until the value was constant for 12 hours.

Oxidation–Reduction Potentials.—Potentiometric curves were run on a Leeds–Northrup student-type potentiometer using a calomel reference electrode and a platinum indicator electrode. Ceric sulfate was used as the titrant in all cases and all solutions were 1 *F* in sulfuric acid.

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(1) An abstract of a thesis submitted by Miss Delora K. Gullstrom to the Graduate School of Purdue University, 1951, in partial fulfillment of the requirements for the Degree of Master of Science.

(2) (a) T. S. Lee, I. M. Kolthoff and D. L. Leussing, THIS JOURNAL, **70**, 2348 (1948); (b) **70**, 3596 (1948).