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Isotopic Exchange Reactions of Zinc Chelate Complexes

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The exchange of radiozinc between zinc acetate and seven zinc chelate complexes in pyridine has been investigated at 25°. The data indicate little or no exchange with zinc phthalocyanine, a "fused-ring" chelate complex, within a 35-day reaction time, reactants *ca.* 0.002 *f* each. The remaining zinc complexes, all of the "non-fused-ring" chelate type, undergo complete exchange within a 0.5-minute reaction time, reactants *ca.* 0.01 *f* each; the possibility that this rapid exchange was induced by the chemical separation of the reactants was not eliminated. This behavior is in agreement with the predictions of Ruben, Kamen, Allen and Nahinsky for exchange lability of metallo-organic complexes based upon structural properties. In pyridine the exchange lability appears to be greater for zinc salicylaldehyde derivatives than for the corresponding copper(II) and nickel(II) chelate complexes.

Numerous investigations have been made of the properties and reactions of metal chelate complexes.[1] The relative stabilities of such complexes have been extensively studied; in particular the stability constants of metal complexes derived from 8-hydroxyquinoline, salicylaldehyde and acetylacetone have been investigated as a function of the central metal ion and of the structural and resonance properties of the complex, by more or less direct measurement of equilibrium constants[2-7] by polarographic methods,[8] and qualitatively by displacement reaction[9] and spectrophotometric[10] studies. In water-dioxane mixtures and in pyridine (and in some other solvents) the complexes derived from a given central metal atom and 8-hydroxyquinoline, acetylacetone, and salicylaldehyde decrease in stability, respectively, in that order; for each of these chelating substances (and for many others) the order of decreasing stability in terms of the central divalent metal atom is substantially the same: Pd > Cu > Ni > Pb > Co > Zn > Cd > Fe > Mn > Mg.

Although stability is not necessarily associated with slow rates of substitution or of isotopic exchange reactions,[11] within a series of closely related compounds, such as the complexes considered in this paper, there is often good correlation between stability and relative inertness toward isotopic exchange.[12] Perhaps the best instance of such a correlation is the work of Duffield and Calvin[13] on exchange between copper(II) acetate and copper(II) chelate derivatives of salicylaldehyde and acetylacetone in pyridine, which is apparently the only kinetic study of metal-chelate exchange of this type reported to date.

The investigations described in this paper were undertaken partly in order to compare the exchange lability of zinc chelate complexes with that of the corresponding copper(II) complexes studied by Duffield and Calvin, partly to ascertain which of such zinc complexes, if any, might be useful in the Szilard-Chalmers method of concentrating radioisotopes, and partly to provide a further test of the prediction of Ruben, Kamen, Allen and Nahinsky[14] that complexes in which the central metal atom is surrounded by a "fused-ring" structure (porphyrins, porphyrazines, phthalocyanines, etc.) should be extremely inert toward exchange with the metal ion in slightly acid solution. The first-stated objective represents a continuation of the work of Leventhal and Garner.[15]

Accordingly, we have examined the exchange of zinc acetate with seven zinc chelate complexes, one of the "fused-ring" type and the others of the "non-fused-ring" type, in pyridine under conditions analogous to those used by Duffield and Calvin.

Experimental Procedure

Radiozinc Tracer.—Active zinc acetate was prepared from a zinc chloride solution containing 250-day Zn^{65} prepared, purified and characterized as described by Leventhal and Garner.[15] The zinc was precipitated with potassium hydroxide, the precipitate centrifuged, washed with water, then dissolved in glacial acetic acid. The resulting solution was evaporated to dryness and heated to give anhydrous zinc acetate which was subsequently dissolved in anhydrous pyridine.

Chemicals.—Anhydrous zinc acetate was prepared by dehydration of C.P. zinc acetate dihydrate.

Crude zinc phthalocyanine, supplied through the courtesy of the E. I. du Pont de Nemours Co., Inc., Wilmington, Delaware, was purified by the method of Barrett, Dent and Linstead.[16] The purified product was sublimed at a pressure of 5 mm. giving brilliant blue crystals. The remaining zinc chelate complexes were made for the most part by the methods of Pfeiffer,[17] with modification in several instances. The purity of each complex was confirmed by chemical analysis for zinc, and in most cases for carbon and hydrogen as well.

Reagent-grade pyridine was distilled over C.P. potassium hydroxide. Reagent-grade chloroform was redistilled. All other chemicals were C.P. grade, used without further purification.

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[1] For a review see, *e.g.*, Diehl, *Chem. Revs.*, **21**, 39 (1937).

[2] M. Calvin and K. W. Wilson, THIS JOURNAL, **67**, 2003 (1945).

[3] M. Calvin and N. C. Melchior, *ibid.*, **70**, 3270 (1948).

[4] D. P. Mellor and L. Maley, *Nature*, **159**, 370 (1947); **161**, 436 (1948).

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[7] L. G. Van Uitert, W. C. Fernelius and B. E. Douglas, "Studies on the Relative Stabilities of Coördination Complexes of β-Diketones with Metal Ions" (paper presented at the Symposium on Equilibrium and Rate Behavior of Complex Ions in Solution), Chicago, Ill., February 22, 1951.

[8] M. Calvin and R. H. Bailes, THIS JOURNAL, **68**, 949 (1946).

[9] P. Pfeiffer, H. Thielert and H. Glaser, *J. prakt. Chem.*, **152**, 145 (1939).

[10] K. Yamasaki and K. Sone, *J. Chem. Soc. Japan*, **69**, 70 (1948); *Nature*, **166**, 998 (1950).

[11] This has been recently well documented by H. Taube, *Chem. Revs.*, **50**, 69 (1952).

[12] A survey of this and related problems has been given by C. S. Garner, "Applications of Exchange Reactions in Studying Lability" (paper presented at the Symposium on Equilibrium and Rate Behavior of Complex Ions in Solution), Chicago, Ill., February 21, 1951.

[13] R. B. Duffield and M. Calvin, THIS JOURNAL, **68**, 557 (1946).

[14] S. Ruben, M. D. Kamen, M. B. Allen and P. Nahinsky, *ibid.*, **64**, 2297 (1942).

[15] L. Leventhal and C. S. Garner, *ibid.*, **71**, 371 (1949).

[16] P. A. Barrett, C. E. Dent and R. P. Linstead, *J. Chem. Soc.*, 1719 (1936).

[17] P. Pfeiffer, E. Breith, E. Lübbe and T. Tsumaki, *ibid.*, **503**, 84 (1933). For a summary see P. Pfeiffer, *Angew. Chem.*, **53**, 93 (1940).

Exchange Procedure.—Exchange mixtures were synthesized volumetrically from gravimetrically standardized stock solutions of the complex compounds and of radiozinc acetate in anhydrous pyridine. Twenty ml. each of the chelate solution and the radiozinc acetate solution were placed in separate compartments of a reaction flask having a partitioned bottom, brought to the temperature of the thermostated bath, and the reaction started by shaking the flask. The reaction time was considered as the time interval between shaking the flask and adding the separating mixture, shaken immediately upon addition. The separation procedure was essentially that of Duffield and Calvin.[13] A mixture of 20 ml. each of chloroform and distilled water was added to the reaction vessel and shaken, stopping the reaction. Two phases are formed, most or all of the chelate compound extracting into the chloroform–pyridine layer, and essentially all of the zinc acetate extracting into the water–pyridine layer. After the layers were separated, the chloroform–pyridine layer was shaken with 20 ml. of water, and the water–pyridine layer with 20 ml. of chloroform. The chloroform layers were combined, and the water layers separately united. To each was added 1 g. of ammonium acetate to provide buffering action, the chloroform evaporated off, and hydrogen sulfide passed in for 15 minutes at 65° to decompose the chelate and precipitate the zinc as the sulfide. Each mixture was boiled, cooled to room temperature, then centrifuged; the supernatant solution was always tested for radioactivity and found to be inactive. The washed precipitates were quantitatively transferred to weighed crucibles and ignited in air at 820° to convert the zinc sulfide to the oxide for weighing, inasmuch as we found attempts to dry and weigh the sulfide led to errors arising from uncontrolled partial oxidation of the sulfide. The resulting zinc oxide samples were slurried with water, transferred onto small filter-paper discs, dried with acetone under suction, then lightly lacquered in preparation for determination of the specific radioactivity. Recovery of the total zinc (*ca.* 65 mg. as ZnO) and total activity (*ca.* 1500–2000 counts per minute) in a given exchange experiment usually fell within the range 98–100% and 95–105%, respectively.

Because of the lower solubility of zinc phthalocyanine in pyridine and the fact that this complex is not decomposed by the hydrogen sulfide treatment, the above procedure was modified for this compound. Following the separation as given above, the chloroform was evaporated off the combined chelate-containing layers which were then fumed with concentrated nitric acid to decompose the phthalocyanine. In all but one series of experiments, additional zinc carrier was added at this point inasmuch as the weight of zinc was only *ca.* 3 mg. in each fraction. In all but one of the phthalocyanine runs the resulting solutions were counted directly with a dipping Geiger counter. The recovery of total activity in these exchanges was 98–100%.

Radioactivity Measurements.—Both fractions from a given exchange experiment were usually counted as solid samples mounted reproducibly under a glass-wall Geiger counter used with a scale-of-64 circuit, the 0.45- to 1.14-Mev. γ-radiation associated with the decay of Zn^{65} being counted through a compound absorber to take out positron and X-radiations. In some cases solutions were counted with a glass-wall dipping counter, the densities of the solutions being kept constant so that scattering and absorption corrections were unnecessary. Corrections for background (12–20 counts/min.) and coincidence loss were applied, as well as for decay and change in counter efficiency (by use of standard Zn^{65} aliquots). Samples were counted to a standard deviation of 1–2%.

Results

The results are given in Table I, some of the results of Leventhal and Garner[15] being included to facilitate comparison. The per cent. exchange was calculated from the relationship

$$\%\ \text{exchange} = 100\, S_{(c)}/S_{(c)\infty} \qquad (1)$$

where $S_{(c)}$ is the specific activity of the initially inactive chelate fraction at time t and $S_{(c)\infty}$ is the equilibrium specific activity of the chelate fraction (and of the zinc acetate fraction). The latter was taken as the average specific activity of the exchange mixture. Each value of the per cent. exchange given in Table I is the average of two or more determinations.

Discussion

The zinc phthalocyanine exchange results show an apparent reproducible zero-time exchange of *ca.* 20%, arising from separation-induced exchange or incomplete separation.[18] Because of the relatively low concentrations involved in the phthalocyanine experiments it was not possible with the analytical procedure used to determine the extent of separation of the reactants to better than *ca.* 20%. A tendency was noted for the acetate-containing and phthalocyanine-containing layers not to separate completely. It seems likely, therefore, that the apparent zero-time exchange resulted from incomplete separation. The data clearly show that the per cent. exchange does not increase with reaction time up to the longest time tried, 35 days, and that after correction for the apparent zero-time exchange the phthalocyanine is highly inert toward exchange in pyridine. This lack of exchange lability is in agreement with the postulate of Ruben, Kamen, Allen and Nahinsky[14] that "fused-ring" chelate complexes should not exhibit metallic exchange in slightly acid solution, which they suggest may be because all four metal-nitrogen bonds must be broken simultaneously for exchange to occur. All "fused-ring" chelates for which exchange studies have been reported so far show this inertness toward metallic exchange, namely, chlorophyll-a and -b,[19] iron(III) hemoglobin,[14,20] iron(III)

Table I

Exchange between Zinc Acetate and Zinc Chelate Complexes in Pyridine at 25°

Zinc chelate complex	Chelate concn., *f*	Zinc acetate concn., *f*	Exchange time, *t*	Exch., %
Phthalocyanine	0.0026	0.00099	0.5 min.	18[a]
Phthalocyanine	.0025	.0019	.5 min.	18[a]
Phthalocyanine	.0025	.0019	2 days	18[a]
Phthalocyanine	.0025	.0019	10 days	20[a]
Phthalocyanine	.0025	.0019	35 days	23
Diphenylthiocarbazone	.0100	.00995	.5 min.	103
Diphenylthiocarbazone	.0100	.00925	.5 min.	98[b]
8-Hydroxyquinolate	.0100	.00995	.5 min.	102
Anthranilate	.0100	.00995	.5 min.	110
Salicylaldehyde-ethylenediimine	.0100	.00935	.5 min.	100
Salicylaldehyde-*o*-phenylenediimine	.00935	.00750	.5 min.	99
Salicylaldehyde-*o*-phenylenediimine	.00094	.00100	.5 min.	90[a,c]
Salicylaldehyde-methylimine	.0100	.00930	.5 min.	97
Salicylaldehyde-methylimine	.0100	.00995	2 days	96
Acetylacetonate	.0100	.0100	.5 min.	97[d]
Acetylacetone-ethylenediimine	.0100	.0100	.5 min.	98[c,d]
Benzoylacetonate	.0104	.0100	.5 min.	93[c,d]
Nicotinylacetonate	.0034	.0034	.5 min.	101[d]

[a] Zn carrier added to separated fractions. [b] Chloroform solvent. [c] Exchange at 0°. [d] Results of Leventhal and Garner.[15]

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(18) For a discussion of zero-time exchange factors see R. J. Prestwood and A. C. Wahl, This Journal, **71**, 3137 (1949).

(19) S. Ruben, A. W. Frenkel and M. D. Kamen, *J. Phys. Chem.* **46**, 710 (1942).

(20) P. F. Hahn, W. F. Bale, J. F. Ross, R. A. Hettig and G. H. Whipple, *Science*, **92**, 131 (1940).

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.

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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.

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

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

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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).