addition of aqueous ammonia promotes the separation of $[Co(NH_2CH_2CH_2S)_3]$ from solution.

Acknowledgment.—The financial support of the National Institutes of Health is gratefully acknowledged. The help of Mr. Charles A. Root, who confirmed the synthetic procedures, is sincerely appreciated.

CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF ARIZONA, TUCSON, ARIZONA

The Influence of Metal Chelation on the Structure of Chelidamic Acid

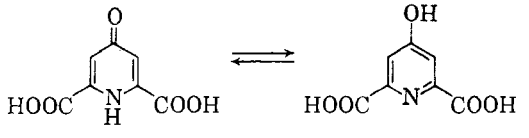
BY SASWATI P. BAG, QUINTUS FERNANDO, AND HENRY FREISER

Received April 9, 1962

The acid dissociation constants of 2,6-dicarboxy-4-hydroxypyridine (chelidamic acid) have been determined potentiometrically in water at 25°. Metal chelation was found to have an acid-strengthening effect on the 4-hydroxy group in chelidamic acid. The extent of this effect varied with the metal ion that was chelated. The pK_a of the hydroxy group in the metal chelate was found to increase in the order: Cu(II) < Co(II) < Zn(II) < Ni(II) < Mn(II).

The interest in the behavior of metal chelates has been centered largely around the effects that the ligand molecule, or various modifications of it, has on the chemistry of the metal ion. On the other hand the effect of metal ion chelation on the properties of the organic moiety should be of great interest, especially to biochemists.

It is well known that 2-hydroxypyridine and 4-hydroxypyridine in solution exist largely in their tautomeric forms as 2-pyridone and 4-pyridone, respectively, due to keto–enol tautomerism. In the present study, chelidamic acid, a substituted 4-pyridone with chelate-forming groups, was selected to determine whether chelation had any effect on this keto–enol tautomerism.



Keto form Enol form

Experimental

Purification of Chelidamic Acid and 2,6-Pyridinedicarboxylic Acid.—Chelidamic acid, obtained from K and K Laboratories, Inc., New York, N. Y., was purified by the following procedure: the crude acid was dissolved in 1 *M* NH_3 and warmed with animal charcoal, the solution was filtered, and the filtrate was neutralized with dilute HNO_3 to precipitate a pale yellow solid. This solid was redissolved in warm dilute HNO_3 and the solution was cooled and diluted, to yield a white precipitate. This precipitate was washed repeatedly with ice-cold water and finally recrystallized from ethanol. The compound obtained was colorless, m.p. 250°; lit.[1] m.p. 248°. The equivalent weight of the compound was determined by a potentiometric titration (calcd., 91.56; found, 92.20).

2,6-Pyridinedicarboxylic acid was obtained from K and K Laboratories, Inc., New York, N. Y. The compound, after recrystallizing several times from hot water and finally from ethanol, was colorless and melted with decomposition at 252° (lit.[2] 255°). The equivalent weight of the compound was determined by a potentiometric titration (calcd., 83.56; found, 83.80).

Acid Dissociation Constants.—The acid dissociation constants of chelidamic acid were determined potentiometrically as follows: a weighed quantity of the acid was dissolved in 100 ml. of water and titrated in a water-jacketed vessel at 25 ± 0.1° in an atmosphere of nitrogen with a solution of carbonate-free NaOH. A Beckman Model G pH meter, equipped with a glass–saturated calomel electrode pair and calibrated with buffer solutions at pH 4.00 and 7.00, was used for all pH measurements.

The acid dissociation constants of chelidamic acid also were determined spectrophotometrically by measuring the absorbances of a series of solutions of varying pH containing 4×10^{-5} *M* chelidamic acid. The ionic strength of each solution was maintained at 0.1 by the addition of $NaClO_4$.

The pH values of solutions in the extreme ranges (<2 or >11) were adjusted with perchloric acid or carbonate-free sodium hydroxide. In the intermediate ranges of pH, buffer solutions were used. The buffer components were CH_3CO_2H, $Na[O_2CCH_3]$, $NaBO_2$, K_2HPO_4, KH_2PO_4, and NH_3.

The ultraviolet spectra of all solutions were obtained with a Beckman Model DB or Cary Model 11 recording

(1) E. Riegel and M. C. Reinhard, *J. Am. Chem. Soc.*, **48**, 1334 (1926).

(2) M. Henze, *Ber.*, **67**, 751 (1934).

spectrophotometer in which stoppered 1-cm. quartz cells were used.

The acid dissociation constants of the phenolic group in a series of metal chelidamates were determined potentiometrically as described above. The solution of metal perchlorate–chelidamic acid in 1:2 ratio was titrated potentiometrically at 25° in an atmosphere of nitrogen with a carbonate-free NaOH solution. These titrations were repeated after replacing chelidamic acid with 2,6-pyridinedicarboxylic acid, the metal:ligand ratios being kept constant at 1:2. The titration curves showed that after the carboxylic acid protons were neutralized, no further proton release occurred. A competitive titration was carried out in which equimolar quantities of chelidamic acid and 2,6-pyridinedicarboxylic acid, together with sufficient copper(II) perchlorate to give a metal:ligand ratio of 1:2, were titrated potentiometrically with NaOH. The titration curve showed that proton release occurred after all the carboxylic acid protons were neutralized, thereby indicating that chelidamic acid rather than the 2,6-pyridinedicarboxylic acid formed a chelate.

Results

The first two acid dissociation constants, K_1 and K_2 of chelidamic acid, corresponding to the loss of the two protons from the two carboxylic acid groups, were evaluated from the potentiometric titration data by means of the equation

$$\frac{(H^+)^2S}{(S-2)} + (H^+)K_1\frac{(S-1)}{(S-2)} + K_1K_2 = 0$$

where $S = (H^+ + Na^+ - OH^-)/T_a$ and T_a is the total concentration of chelidamic acid. A plot of $(H^+)^2S/(S-2)$ *vs.* $(H^+)(S-1)/(S-2)$ gave a straight line having a slope equal to K_1 and intercept equal to K_1K_2.

The acid dissociation constant of the 4-hydroxy group in chelidamic acid, K_3, was determined by substituting the potentiometric titration data in the equation

$$K_3 = \frac{(H^+)(S' - 2T_a)}{(3T_a - S')}$$

where $S' = (Na^+ + H^+ - OH^-)$ and T_a is the total concentration of chelidamic acid.

TABLE I

ACID DISSOCIATION CONSTANTS OF CHELIDAMIC ACID IN WATER AT 25 ± 0.1°

Method	pK_1	pK_2	pK_3
Potentiometric	<2	3.47 ± 0.01 Ionic strength ≈ 0.005	11.4 ± 0.1 Ionic strength ≈ 0.01
Spectrophotometric	...	3.2 ± 0.1 Ionic strength = 0.1	10.8 ± 0.1 Ionic strength = 0.1

Table I gives the results obtained from three separate potentiometric titrations. The pK_3 value corresponding to the dissociation of the hydroxyl group is larger than the pK values of the carboxylic acid groups by about 8. This is therefore an ideal situation in which the influence of metal chelate formation (with the pyridine nitrogen and the carboxylic acid groups) on the acid dissociation constant of the hydroxy group can be investigated.

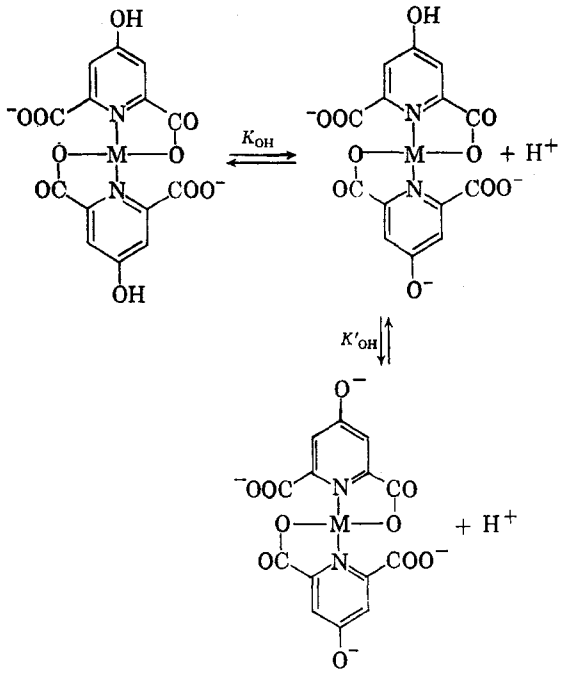
The ultraviolet spectra of chelidamic acid, in a series of solutions of varying pH and constant ionic strength of 0.1, were examined to verify the pK values determined potentiometrically, as well as to identify, if possible, the various ionic species of the acid that were present in solution.

Chelidamic acid has two principal regions of absorption, ~210 mμ and 260–280 mμ. In acid solutions, below a pH of 3.5, the two bands are located at 205 and 265 mμ. At pH values between 3.5 and 10, both bands undergo a red shift to ~210 and 280 mμ. Three regions of absorption are present in alkaline solutions (pH > 11), a high intensity band at 220 mμ, a lower intensity band at 260 mμ, and a shoulder at ~290 mμ. The ~260 mμ band was selected for absorbance *vs.* pH measurements. The pK values obtained are shown in Table I.

The ultraviolet spectra of aqueous solutions containing a metal:chelidamic acid ratio of 1:2 were examined in the pH range 4.5 to 11.2. In all the metal chelates two regions of absorption, ~280 and ~220 mμ, were present throughout the pH range examined. The intensities of the two bands showed small variations with pH.

The ultraviolet spectra of 2,6-pyridinedicarboxylic acid showed four principal bands, ~220, ~260, ~270, and 280 mμ, in acid solutions (pH = 1.0) and alkaline solutions (pH = 12.0). The positions of the bands did not shift appreciably with pH. Aqueous solutions containing metal:2,6-pyridinedicarboxylic acid ratios of 1:2 had similar absorption bands in the ultraviolet.

The presence of Cu(II), Ni(II), Co(II), Zn(II), and Mn(II) in a solution containing the ligand gives rise to an inflection in the titration curve when 4 moles of base per mole of metal ion have been added. This is caused by the chelation of 1 mole of metal ion with 2 moles of ligand. A second region of proton release in the titration curves of the chelates appears after 2 moles of base per mole of metal ion have been added. This is caused by the release of two protons from the two hydroxyl groups in the metal chelate molecule.



These dissociations are characterized by pK_a values which are relatively close together. However, approximate pK_a values for the dissociation of each of the two hydroxyl groups in the metal chelate molecule can be calculated from the half-neutralization points on these titration curves. The results are given in Table II.

TABLE II

ACID DISSOCIATION CONSTANTS OF THE HYDROXY GROUPS[a] IN METAL CHELIDAMATES IN WATER AT 25 ± 0.1°

Metal ion in chelate	pK_{OH}	pK'_{OH}
Cu^{++}	5.0	6.9
Co^{++}	5.5	7.7
Mn^{++}	6.4	8.9
Ni^{++}	5.7	8.0
Zn^{++}	5.7	7.8

[a] pK_{OH} refers to the dissociation of the proton from one of the coördinated ligands and pK'_{OH} refers to that from the other.

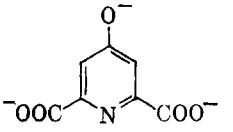
The titration curve for the Cu(II) chelate has an inflection point which is somewhat greater than 6 moles of base per mole of metal ion, probably because of the hydrolysis of the Cu(II) chelate.

Potentiometric titrations of solutions containing 2,6-pyridinedicarboxylic acid as well as solutions containing this ligand and the metal ions, in the ratio 1:2 were carried out. The protons from the carboxylic acid groups were titrated and no further proton release was observed. This shows that no hydroxy complexes of the metal ions were formed between pH 5 and 8.

Equimolar quantities of chelidamic acid and 2,6-pyridinedicarboxylic acid were added to a solution containing Cu(II). Each of the ligands was present in sufficient amount to form a 2:1 complex with the metal ion. The potentiometric titration curve showed that the chelidamic acid had formed a complex with the metal ion, since the protons from the hydroxy groups were released at relatively low pH values, just as in the Cu(II)–chelidamic acid titration.

Discussion

The fact that pK values of chelidamic acid are lower than the corresponding pK values of 2,6-pyridinedicarboxylic acid[3] might be taken as an indication of the predominace of the pyridone tautomer in chelidamic acid, since the C=O group in this tautomer has a strong electron-withdrawing action. In addition, the spectrum of chelidamic acid shows a strong absorption band at 280 mμ, and in very alkaline solutions, in which the triply charged anion is present, this band practically disappears (probably because of a shift in its position). The spectrum of 2,6-pyridinedicarboxylic acid, on the other hand, does not show this behavior. It is possible that the 280 mμ band in chelidamic acid arises from a π–π^* transition in the pyridone tautomer. The disappearance of this band in solutions of high alkalinity suggests that the triply charged anion is



The fact that no further proton release was observed with 2,6-pyridinedicarboxylic acid chelates satisfactorily rules out the possibility that in the chelidamic acid case metal hydrolysis, rather than proton loss from the reagent, was observed. The competitive titration of the metal in the presence of both compounds yielded the striking result that the chelidamic acid chelates were more stable than the 2,6-pyridinedicarboxylic acid chelates. Normally, in such closely related reagents, it would be expected that the more basic reagent would give the more stable chelate. This apparent anomaly can be explained by the assumption that the hydroxypyridine structure rather than the pyridone structure is present in the chelate. The electron-releasing property of the

(3) R. M. Tichane and W. E. Bennett, *J. Am. Chem. Soc.*, **79**, 1293 (1957).

hydroxyl group would account for a higher basicity in the pyridine tautomer than would be found in 2,6-pyridinedicarboxylic acid. If this is the case, then chelation has promoted a profound change in the chelidamic acid structure. The remarkable drop of from 4 to 6 in the pK value of the hydroxy group (pK_3) also may be explained by the presence of the pyridine tautomer in the chelates. The observed pK_3 of chelidamic acid does not represent a true dissociation constant of the phenolic hydroxy group, since it involves the tautomeric equilibrium constant as well. If the chelate is in the pyridine tautomer, then dissociation of the hydroxy group can be accomplished much more readily. The variation in the pK_3 of the chelates from metal to metal represents a smaller but significant effect of the variation in the electron-withdrawing ability of the metal ions. It is interesting to note in this regard that the order observed, Cu(II) > Co(II) > Zn(II) > Ni(II) > Mn(II), parallels the acid strengthening effect that metals have on coordinated water molecules, *i.e.*, on the ease of metal hydrolysis. This suggests the possibility for the metal to transmit its electronegative influence on an oxygen atom, through an aromatic ring. This ability of metal ions to exert an electronic influence over a distance certainly would have important biochemical implications. At the present time, a series of compounds which will serve to further test this hypothesis is under study.

Acknowledgment.—The authors are grateful to the National Institutes of Health for financial support.

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Metal Derivatives of Arylazopyrazolone Compounds. IV. Molarity Quotients of Azopyrazolone Compounds Containing *o*-Carboxymethoxy and *o*-Carboxythiomethoxy Groups

BY FRED A. SNAVELY AND GLENN C. CRAVER[1]

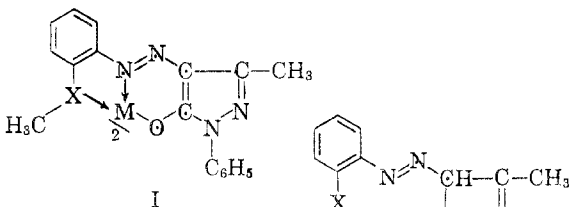
Received July 1, 1961

The relative stabilities of the metal derivatives of tetradentate azopyrazolone compounds containing an *o*-carboxymethoxy or an *o*-carboxythiomethoxy group have been measured potentiometrically in 75 volume % dioxane. Divalent copper, nickel, cobalt, and cadmium show a stronger affinity for sulfur than for oxygen. Divalent zinc, manganese, magnesium, calcium, strontium, barium, and the uranyl ion bond stronger to oxygen than to sulfur.

Introduction

Recently we reported[2] that arylazo compounds (I) which contained either an *o*-methoxy or an *o*-thiomethoxy group coördinated to metal ions as terdentate ligands. Divalent copper, nickel, cobalt, and cadmium formed more stable derivatives with the thiomethoxy compound, while zinc formed a more stable derivative with the methoxy compound.

Pfitzner[3] reports the possible coördination of an ether-like oxygen to copper(II) where the group *o*-substituted to the azo linkage is carboxymethoxy. Two compounds of this type, 1-phenyl-3-methyl-4-(2-carboxymethoxybenzeneazo)-5-pyrazolone (IIa) and 1-phenyl-3-methyl-4-(2-carboxythiomethoxybenzeneazo)-5-pyrazolone (IIb), were prepared in order to study the relative affinities



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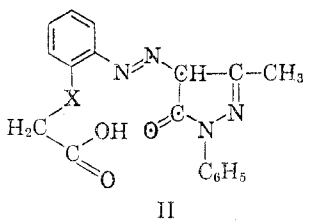

II

(1) Summer Research Student (1958).
(2) F. A. Snavely, B. D. Krecker, and C. G. Clark, *J. Am. Chem. Soc.*, **81**, 2337 (1959).
(3) H. Pfitzner, *Angew. Chem.*, **62**, 245 (1950).