[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE UNIVERSITY OF CALIFORNIA]

Stability of Chelate Compounds

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Introduction

Although a great deal of work has been done in the preparation of chelate compounds and upon their structure,[2] no attempt has yet been made to determine even in a semi-quantitative manner how the structural factors of the organic residues, other than the simple geometry of ring formation, influence their tendency to form chelate compounds with heavy metal ions. The present paper is a report of a more or less quantitative survey to determine the influence of certain structural factors upon the stability of the chelate compounds of divalent copper in which the four atoms bound to the metal are all oxygen. The stability of the chelates must of course be defined with respect to a specific reaction. We have selected cupric ion as the metal component because of its great tendency to form chelate compounds which are not subject to oxidation as are those of Co^{++} and Fe^{++}, and have chosen to represent the stability in terms of the equilibrium

$$Cu^{++} + 2Ke^- \rightleftarrows CuKe_2 \qquad (I)$$

where Ke^- represents the anion of a β-diketone such as acetylacetone or an *o*-hydroxy aromatic aldehyde.

The method consists of determining the H^+ concentration of a solution containing known quantities of copper, chelating substance, acid and base. From the known acid dissociation constant of the chelating substance together with the prearranged concentrations of each of the above materials, it should be possible to calculate all the quantities required to determine an equilibrium constant. In order to get a wide variety of conditions in each solution the experiments were performed as titrations with alkali, starting with fixed amounts of copper ($T_{Cu^{++}}$), chelating agent (T_{HKe}) and excess acid (A).

Experimental

The titrations were made using a Beckman *p*H meter which gave values accurate to ±0.03 *p*H unit. Since most copper chelates are relatively insoluble in water, it was necessary to use a mixture of 50% by volume of dioxane and water. The dioxane was purified by refluxing for eight hours with 10% by weight of 1 *N* hydrochloric acid, standing over solid potassium hydroxide until two layers formed, removing the water layer and completing the drying with fresh potassium hydroxide. It was then distilled quickly over sodium and finally fractionated through a 30-plate column. It was necessary to calibrate the *p*H meter by titrating a water–dioxane mixture containing a little potassium chloride with 0.1 *N* perchloric acid. The results indicated that the actual *p*H meter readings were very nearly correct. In solutions more basic than *p*H 9 the glass electrode is in error even in water as a solvent. Since this error is reported to be dependent on the concentration and nature of the positive ions in the solution, an exact calibration in this region proved too difficult. Two water–dioxane mixtures containing different amounts of potassium chloride were titrated with 0.1 *N* sodium hydroxide giving two different curves, but both showed that above *p*H 9 the glass electrode makes the solutions appear more basic than they actually are. In the calculations the *p*H values in basic solution were not corrected; however, these affect the results for only a few of the compounds, and the qualitative conclusions would not be altered at all. A typical titration curve is shown in Fig. 1. In every case the initial volume of solution was 100 ml. and contained sufficient perchloric acid to give a *p*H of 1.7, $Cu(ClO_4)_2$ of the order of 10^{-3} *M*, and a 1.25-

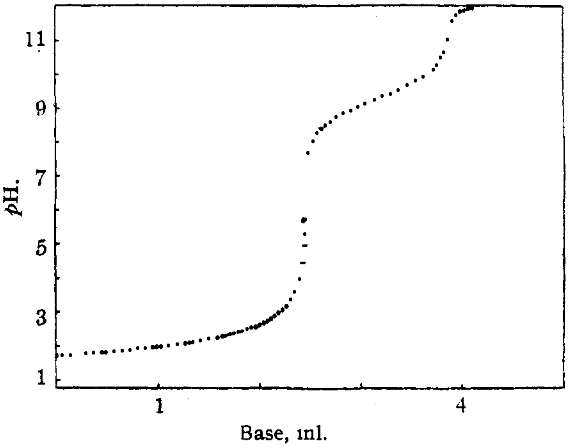

Fig. 1.—Furoylacetone: volume of solution, 100 cc.; $T_{HKe} = 0.02$ *N*; $T_{Cu^{++}} = 2.5 \times 10^{-3}$; $A = 0.02$ *N*; base = 1.025 *N* sodium hydroxide; temperature, 20°.

TABLE I

TITRATION OF 50% DIOXANE–WATER SOLUTIONS OF $Cu(ClO_4)_2$, HKe(0.02*M*) AND $HClO_4$(0.02*M*) WITH AQUEOUS NaOH(1.025*N*)

For Series A: $T_{Cu} = 2.5 \times 10^{-3}M$; for Series B: $T_{Cu} = 1.0 \times 10^{-3}M$.

3-*n*-Propyl salicylaldehyde $pK_D = 9.6$			2-Hydroxy-1 naphthaldehyde[a] $pK_D = 8.4$			Trifluoroacetyl acetone[b] $pK_D = 6.7$		
ml. NaOH	*p*H	$\bar{n}$	ml. NaOH	*p*H	$\bar{n}$	ml. $Cu(ClO_4)_2$	*p*H	$\bar{n}$
			Series A					
2.034	4.40	0.52	1.976	2.97	0.56	5.000	2.42	0.58
2.118	4.93	0.86	2.073	3.35	0.76	3.010	2.52	0.78
2.157	5.22	1.01	2.126	3.59	0.90	2.001	2.60	0.99
2.198	5.52	1.17	2.180	3.88	1.07	1.602	2.65	1.10
2.283	6.12	1.51	2.283	4.57	1.44	0.859	2.79	1.50
			Series B					
1.931	3.97	0.31	1.921	3.22	0.50			
1.976	4.63	0.69	1.976	3.57	0.74			
2.017	5.33	1.09	2.028	4.13	1.08			
2.060	6.03	1.51	2.080	4.88	1.54			

[a] In both A and B, $T_{HKe} = 0.01$ *M*. [b] This was done by titrating the diketone with aqueous $Cu(ClO_4)_2$(0.125*M*), $T_{HKe} = 0.01M$.

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(1) Abstracted from the thesis of K. W. Wilson, submitted in partial fulfillment for the degree of M.S. at the University of California, Berkeley, California, June, 1945.

(2) (a) P. Pfeiffer, *Angew. Chem.*, **53**, 93 (1940); (b) S. A. Voznesensky, *Uspekhi Khim.*, **3**, 531 (1934); (c) H. Diehl, *Chem. Rev.*, **21**, 39 (1937).

to 5-fold excess of ketone. The first flat portion of the curve represents the formation of the copper chelate; the sharp rise indicates that chelate formation is complete, and the last flat portion represents the titration of the excess ketone. The H^+ at the calculated midpoint of this final flat portion was taken as the acid dissociation constant of the ketone. In all calculations the concentrations were corrected for the change in volume produced by the addition of the NaOH. The results on three representative compounds are given in Table I.

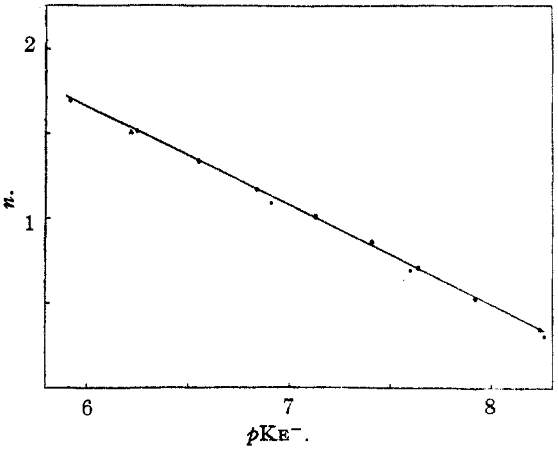

Fig. 2.—3-*n*-Propylsalicylaldehyde: $pK_D = 9.6$:

	T_{HKe}	$T_{Cu^{++}}$	Temp.,° C.	NaOH, *N*
○	0.02	2.5×10^{-3}	30	1.025
△	0.02	1.0×10^{-3}	30	1.025

Results

In the first attempt at calculation of an equilibrium constant we assumed that all the copper was present as Cu^{++} and $CuKe_2$ and calculated values for $K_f = (CuKe_2)/(Cu^{++})(Ke^-)^2$. The values obtained for salicylaldehyde were

*p*H	4.25	4.00	3.75	3.50
log K_f	13.9	14.3	14.6	14.9

Similar calculations were made for 8 other compounds and this same variation of K_f with *p*H also observed. This indicates that the equilibrium is not simply as represented by (I).

We then assumed that the reaction went in steps and that the copper was present as Cu^{++}, $CuKe^+$, and $CuKe_2$. To make the calculations we turned to the method of J. Bjerrum.[3] We calculate the successive constants $K_1 = (CuKe^+)/(Cu^{++})(Ke^-)$ and $K_2 = (CuKe_2)/(CuKe^+)(Ke^-)$ from a knowledge of $\bar{n}$, the average number of Ke^- bound to a Cu^{++}. To find $\bar{n}$ we need only calculate the total amount of Ke^- which is bound and divide by the total amount of copper present. The total bound ketone is calculated from the equations

$$T_{Cu^{++}} = Cu^{++} + CuKe^+ + CuKe_2$$
$$T_{HKe} = HKe + Ke^- + CuKe^+ + 2CuKe_2$$
$$CuK_2^+ + 2Cu^{++} + Na^+ + H^+ = ClO_4^- + OH^- + Ke^-$$
$$K_D = (H^+)(Ke^-)/(HKe)$$
$$ClO_4^- = A + 2T_{Cu^{++}}$$

Using these, we arrive at the final results: Total bound ketone $= CuKe^+ + 2\ CuKe_2 = Na^+ - A + H^+ - \frac{10^{-14*}}{H^+} - \frac{K_D}{H^+}\left(T_{HKe} + A - Na^+ - H^+ + \frac{10^{-14}}{H^+}\right)$. The values of $\bar{n}$ are plotted against pKe^-, and the formation constants determined from the curve. Then $1/Ke^-$ at $\bar{n} = 0.5$ and 1.5 gives K_1 and K_2, respectively, to a first approximation, and at $\bar{n} = 1.0$ gives an average constant such that $(K_{av.})^2 = K_1K_2$. A typical curve is shown in Fig. 2.

For several of the compounds the curves from series A and B did not coincide but gave separate parallel lines as shown in Fig. 3. In all cases the upper curve was obtained from the titration in which the total copper concentration was the least. The equation for $\bar{n}$ may be written as $\bar{n} = (Na^+ - A + H^+)/T_{Cu^{++}}$ the other terms being negligible. If the values we used for Na^+ and H^+ were too high, or if our value for A were too low, then $\bar{n}$ would be too high. If this error in Na^+, H^+, or A were the same in both series, then the error in $\bar{n}$ would be greatest for the titration with the least total copper. It can be calculated that an error of 0.5×10^{-3} in the numerator of the expression for $\bar{n}$ would produce a difference of 0.3 in $\bar{n}$ for the two series. Since in the last stages of preparation of the ketones strong acids were used, it seems possible that an acid impurity would make our value of A too low and produce the effect described above. Since T_{HKe} is usually 0.02 *M* this means that 2½ mole per cent. of acid impurity, something less than 1% by weight, could account for this deviation.

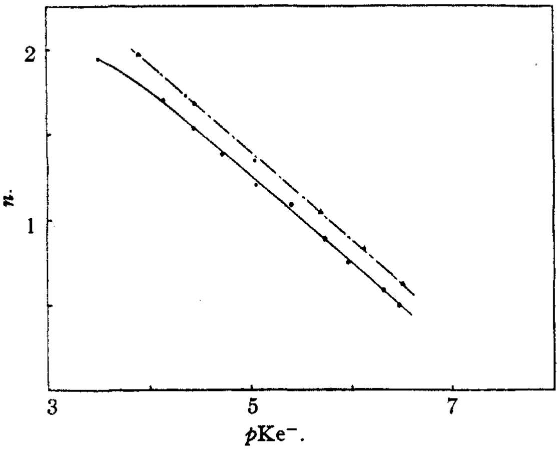

Fig. 3.—3-Chlorosalicylaldehyde: $pK_D = 7.8$:

	T_{HKe}	$T_{Cu^{++}}$	Temp., °C.	NaOH, *N*
⊙	0.02	2.5×10^{-3}	26	1.025
△	0.02	1.0×10^{-3}	27	1.025

The results so calculated are given in Table II,

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

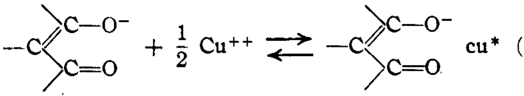
* Although 10^{-14} is certainly not the correct value to use for K_w in the present circumstances, the terms in which it appears are of negligible value in all of the cases herein measured.

together with K_D, the apparent acid dissociation constant for the chelating molecule under the present conditions of solvent and temperature.

The column headed $K_{av.}$ (cor.) is the value obtained by adjusting the values at the two different copper concentrations on the basis of the possible errors discussed above.

Discussion

The value of $K_{av.}$ can be taken in a first approximation to represent the equilibrium constant for the general reaction



(II)

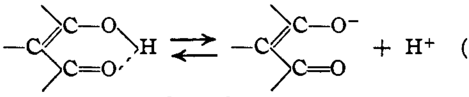
irrespective of whether the copper has another chelating group attached or not. The accuracy of the data as yet does not warrant any discussion of the difference between the first (K_1) and second (K_2) constants other than to mention that the purely statistical effect should make $\log \frac{K_1}{K_2} = \log \frac{4}{1/2} = 0.9$, which is the order of magnitude observed (Table II). Another limitation imposed by the character of the data is on a discussion of the absolute values of the constants themselves. Until the temperature coefficients are determined and by that the heats and entropies of the reactions, we will concern ourselves only with the relative values of the constants, *i. e.*, with the changes in the constants produced by structural changes in the organic residues. The differences so obtained can reasonably be expected to be largely in the heats of the reactions since the ionic type of the reaction remains the same throughout the series and the sizes of the organic anions do not vary a great deal, so that the entropy of the reactions should be fairly constant through the series.[4]

TABLE II[a]

Compound	Higher $T_{Cu^{++}}$ K_1	K_2	$\log \frac{K_1}{K_2}$	$K_{av.}$	Lower $T_{Cu^{++}}$ $K_{av.}$	$K_{av.}$ (cor.)	K_D
Salicylaldehyde	$10^{7.5}$	$10^{5.8}$	1.7	$10^{6.5}$	$10^{5.8}$	$10^{5.5}$	$10^{-9.5}$
3-*n*-Propyl[b]	$10^{8.0}$	$10^{6.3}$	1.7	$10^{7.1}$	$10^{7.1}$	$10^{7.1}$	$10^{-9.6}$
5-Methyl[c]	$10^{7.7}$	$10^{6.0}$	1.7	$10^{6.8}$	$10^{6.95}$	$10^{5.7}$	$10^{-9.7}$
4,6-Dimethyl[c]	$10^{8.3}$	$10^{6.7}$	1.6	$10^{7.5}$	$10^{7.9}$	$10^{7.2}$	$10^{-10.4}$
3-Ethoxy[c]	$10^{7.95}$	$10^{5.85}$	1.9	$10^{7.1}$	$10^{7.1}$	$10^{7.1}$	$10^{-9.4}$
3-Methoxy[c]	$10^{7.5}$	$10^{5.8}$	1.7	$10^{5.55}$	$10^{6.75}$	$10^{5.5}$	$10^{-9.2}$
4-Methoxy[c]	$10^{7.75}$	$10^{6.35}$	1.4	$10^{7.1}$	$10^{7.4}$	$10^{6.9}$	$10^{-9.3}$
3-Nitro[d]	$10^{4.9}$	$10^{3.4}$	1.5	$10^{4.2}$	$10^{4.4}$	$10^{4.1}$	$10^{-6.0}$
4-Nitro[c]	$10^{5.6}$	$10^{4.2}$	1.4	$10^{4.9}$	$10^{5.3}$	$10^{4.6}$	$10^{-7.4}$
5-Nitro[c]	$10^{4.7}$	$10^{4.0}$	0.7	$10^{4.4}$	$10^{4.4}$	$10^{4.4}$	$10^{-5.9}$
3-Fluoro[d]	$10^{6.6}$	$10^{4.9}$	1.7	$10^{5.8}$	$10^{5.9}$	$10^{5.7}$	$10^{-7.8}$
3-Chloro[c]	$10^{6.5}$	$10^{4.5}$	2.0	$10^{5.5}$	$10^{5.8}$	$10^{5.3}$	$10^{-7.8}$
5-Chloro	$10^{6.65}$			$10^{5.95}$	$10^{6.4}$	$10^{5.7}$	$10^{-8.6}$
2,1-Naph[f]	$10^{7.6}$	$10^{6.0}$	1.6	$10^{6.8}$	$10^{6.6}$	$10^{7.0}$	$10^{-8.4}$
2,3-Naph[f]						est $10^{6.8}$	$10^{-9.9}$
Acetyl-Ac	$10^{9.0}$	$10^{8.1}$	0.9	$10^{8.75}$	$10^{8.7}$	$10^{8.7}$	$10^{-9.7}$
Tri-F Ac[d]	$10^{5.3}$	$10^{5.9}$	0.4	$10^{6.1}$		$10^{6.1}$	$10^{-6.7}$
Furoyl Ac[e]	$10^{8.7}$	$10^{8.2}$	0.5	$10^{8.5}$	$10^{8.7}$	$10^{8.4}$	$10^{-9.3}$
Benzoyl Ac						$10^{9.0}$	$10^{-9.8}$
C-Methyl Ac[e]	$10^{8.5}$	$10^{7.3}$	1.2	$10^{7.7}$	$10^{8.3}$	$10^{7.3}$	$10^{-11.8}$
Acetoacetic	$10^{8.4}$	$10^{6.5}$	1.9	$10^{7.5}$	$10^{8.1}$	$10^{7.1}$	$10^{-11.2}$

[a] The first thirteen compounds are salicylaldehyde derivatives, the hydroxyl group being the 2 position. The fourteenth and fifteenth compounds are derivatives of β-naphtol. The next five compounds are derivatives of acetylacetone while the last is acetoacetic ester. [b] Prepared by Dr. W. G. Miller. [c] Prepared by Dr. L. N. Ferguson. [d] Prepared by Dr. J. C. Reid. [e] Prepared by Students in the Chemistry 101 Classes. [f] Prepared by N. C. Melchior.

These considerations hold fairly well for a formally very similar reaction; namely, the dissociation of organic acids. The reaction may be written for the chelating substances, as



(III)

with $K_D = \frac{[H^+][Ke^-]}{[HKe]}$.[5] This parallels reaction II in the reverse direction. If the factors influencing the bonding of hydrogen have a similar influence on the bonding of copper, the two constants, K_D and $K_{av.}$, should have a linear relation to each other. A plot of log K_D against log $K_{av.}$ is shown in Fig. 4. It is apparent that the compounds fall into at least two and possibly four classes. Within each class the linear relationship is approximately followed, and the slopes of the two lines obtained are very nearly the same. Thus it appears that the forces responsible for holding the copper in the chelate are made up of at least two different components, one which is of the same character for both copper and hydrogen, and the other which is quite different for copper from what it is for hydrogen. Presumably anything that may be said about the nature of the bonding of the acidic hydrogen[6] may be said about the first component of the bonding forces of copper. It is in this component that the ionic or coulombic effects will be found, *i. e.*, the effect of the charge and charge distribution of the anion, and the charge and radius of the cation.

In order to learn something of the nature of the second component, let us examine the essential structural changes which are responsible for the four different groups of compounds which we have studied. They may be represented by the four ions shown.

It becomes apparent that the most profound change is in the nature of the double bond be-

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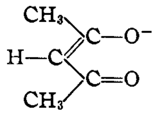
* The symbol cu is used here to represent 1 equivalent of copper. Furthermore, the representation of the bonding between the copper and the organic residue is omitted at this stage since this is the point under discussion.

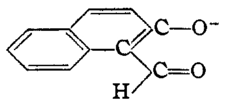
(4) However, one should not lose sight of the possibility that in individual cases wide departures from this assumption might obtain.

(5) K_D as written here represents the acid strength of the enol form as such. This, however, is not quite the constant which we have measured and called K_D. The measured value represents the apparent dissociation constant and includes the constant for the keto-enol tautomerism, in the following manner: $K_{enol} = K_{apparent}(1 + K^1)$ where $K^1 = [keto]/[enol]$. It is clear that for the aromatic compounds $K^1 \cong 0$, so that $K_{enol} \cong K_{apparent} = K_D$. For the aliphatic compounds, however, K^1 will have an appreciable value, thus making K_D for these compounds a lower limit for the acidity of the enol.

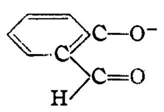
(6) Branch and Calvin, "The Theory of Organic Chemistry," Chap. VI, Prentice-Hall, Inc., New York. N. Y., 1941.



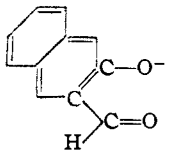
Enolate ion of acetyl acetone
A



Naphtholate ion of 2-Hydroxynaphthaldehyde-1
B



Phenolate ion of Salicylaldehyde
C



Naphtholate ion of 2-Hydroxynaphthaldehyde-3
D

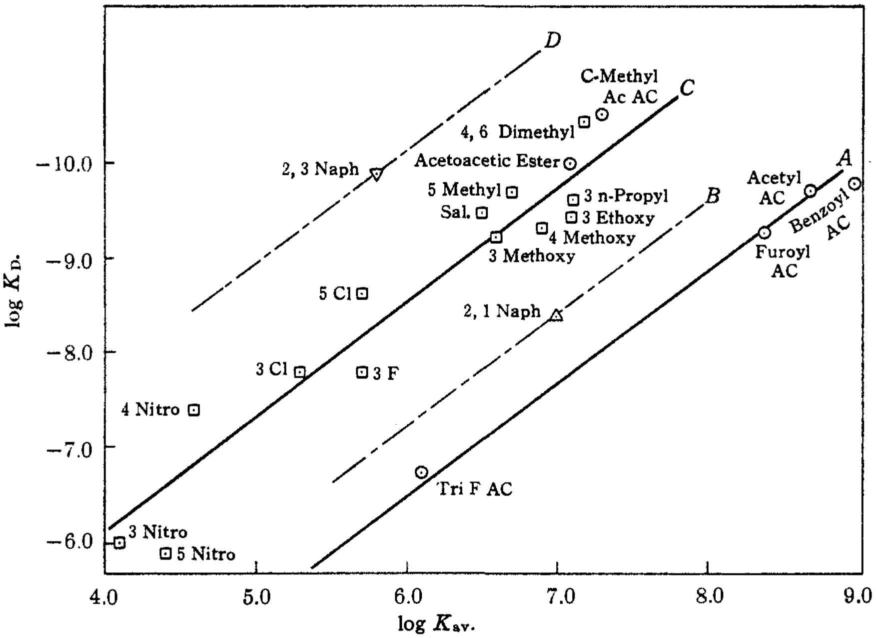
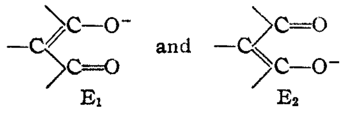


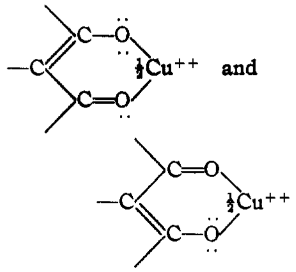
Fig. 4.—□, Line C, substituted salicylaldehydes; ⊙, line A, β-diketones and β-keto ester; △, line B, 2-hydroxynaphthaldehyde-1; ▽, line D, 2-hydroxynaphthaldehyde-3.

tween the two carbon atoms of the three carbon system forming the conjugated chain between the two oxygen atoms. One method of expressing this change would be to assign a numerical "bond order"[7] to it, *i. e.*, a number to express the degree of "double-bondedness" of the bond, as it is determined by any resonance system in which it takes part other than that of the enolate ion, as expressed by the forms

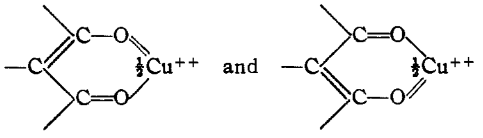


If this is done in the usual fashion,[7] we obtain for A, 2; for B, 1.67; for C, 1.5; and for D, 1.33.

We see that this is just the order of decreasing stability of the copper compounds at a constant acidity of the chelating agent. Furthermore, this is just the order of decreasing effectiveness of the enolate resonance (E_1 and E_2) since for these to play a part the double bond must be free to shift and this freedom is inhibited the more as it is involved in other resonances as measured by its "bond order." We are thus led to the conclusion that *the enolate resonance (E_1 and E_2) plays a different and far greater part in the bonding of copper than it does in the bonding of hydrogen.* The question of how this comes about is one which cannot yet be answered with certainty. There are at least two possible (and related) suggestions. It is well known that the transition elements are most easily introduced into chelate compounds, *i. e.*, those elements whose ions have low-lying vacant orbitals, which are capable of accepting electron pairs to form homopolar bonds. For Cu^{++} these might be 3d^{1}4s^{1}4p^2 or 4s^{1}4p^3. If an electron pair from each oxygen is allowed to form a homopolar bond with Cu^{++} thus



it is conceivable that the rearrangement of the formal charges on the oxygen atoms as shown would greatly enhance the energy contribution of the enolate resonance. The second and somewhat more satisfying point of view would be to carry this homopolar bonding one step further and write as an important contributor a completely conjugated six-membered ring analogous to benzene, pyridine or the pyrylium salts



Such a structure, however, raises questions as to the availability and geometry of orbitals of the metal, the discussion of which we will reserve for a future paper involving the differences in the chelating ability of the elements.

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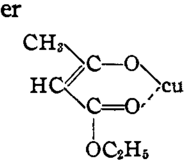
[7] Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1939; Branch and Calvin, "The Theory of Organic Chemistry," p. 113, Prentice-Hall, Inc., New York, N. Y., 1941.

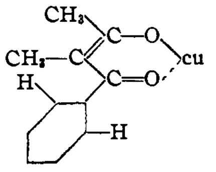
It is of interest now to comment on the two very wide deviations from the line A of Fig. 4, namely, acetoacetic ester and C-methylbenzoylacetone. Part of the deviation is undoubtedly due to the value of K_D which, for these two compounds, having small amounts of enol relative to the other compounds falling near line A, must be considerably smaller than the true value for the enol form. The rest of it, probably about half, must be attributed to other causes.

When we write the structure of the chelate of acetoacetic ester



we see that the enolate (or benzenoid) resonance involving the copper is greatly interfered with by the participation of one of the C=O groups in the very strong ester resonance, thus accounting for the very large decrease in the stability of the chelate.

When the structure of the C-methyl benzoylacetone chelate is examined



using what information we have concerning C to C bond distances and angles, it becomes apparent that, for the completely coplanar structure which would be required for the effective contribution of the enolate (or benzenoid) resonance involving the copper, there should be quite an appreciable interference between the C-methyl group and its neighbors on either side, *i. e.*, the phenyl hydrogen atom and the end methyl group. This interference would tend to distort the coplanar molecule and thus decrease the contribution of the enolate (or benzenoid) resonance and hence decrease the stability of the chelate.

There are other smaller or second order effects which would influence this enolate (or benzenoid) resonance, such as the simple dissymmetry caused by any one-sided substitution, the interaction of the phenyl and carbonyl groups in benzoylacetone, the resonance interaction of a nitro group in salicylaldehyde, etc. It is very likely that these effects contribute to the scattering of the points from the lines in Fig. 4. But the essential nature of the conclusions cannot be changed by these smaller effects.

Summary

1. The stability against dissociation into their component ions, of twenty-one chelate compounds of copper, has been determined.

2. It has been shown that in addition to their character as bases, a resonance effect involving the copper plays a very important role in determining the stability of these compounds.

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Studies on Protein Denaturation. I. Electrophoretic Study Kinetics at Neutrality of Heat Denaturation of β-Lactoglobulin[1]

BY DAVID R. BRIGGS AND ROBERT HULL

In the course of some orientation experiments preliminary to a study of the electroviscous properties of heat denatured β-lactoglobulin, a Tiselius electrophoresis diagram was made on a sample of this protein which had been titrated to *p*H 7.0 (no foreign salts present), heated for forty minutes at 80°, then dialyzed at 6°, against successive amounts of phosphate buffer, *p*H 6.9, ionic strength 0.1, and the electrophoresis carried out under the final equilibrated buffer. While the native protein was an electrophoretically homogeneous material, the heat denatured protein was found to consist of two distinct components, each nearly electrophoretically homogeneous, but differing from each other in mobility by a ratio of approximately 2:3. The slow component had a mobility very near to that of the native protein. By repeating this experiment, varying only the time of heating, it was found that the ratios of the two components varied with time of heating, the fast fraction increasing progressively in amount at the expense of the slow fraction as time of heating was increased (see patterns D_{10}, D_{11} and D_{22}, Fig. 1).

During the denaturation process, conducted as it was at a *p*H removed from the isoelectric point of the native protein (I. E. P. = *p*H 5.2), little change in the appearance of the protein solution took place. A slight opalescence developed which seemed roughly to parallel in degree the amount of fast fraction formed. Qualitatively it was observed that the rate of conversion of slow to fast form during the heating was a function of *p*H of the solution, ionic strength of the solution, time of heating and temperature of heating. The mobilities (after dialysis to standard conditions)

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