to the CEPI solutions to obtain solutions with the same ionic strength as those used for the measurement of k_d. The further addition of 0.1 M HTFMS had no measurable effect. It was not possible to directly measure Z values for solvent mixtures containing more than 50% v/v water. For such mixtures, Z was estimated from extrapolation of a plot of Z vs. mole fraction of the organic component.

Factors affecting the rate constants for redox reactions of reduced thionine are of particular interest because of the possibility of using thionine in the construction of a photogalvanic cell.[19-22] Further work will be necessary to determine whether the rate constants for other reactions in the iron-thionine system (e.g., the oxidation of $TH_2^{\cdot+}$ and TH_3^+ by Fe^{3+}) are also dependent on solvent Z values.

Acknowledgment. This research was supported by the National Science Foundation's Research Applied to National Needs program under Grant SE/AER 72-03597.

References and Notes

(1) Indicated structures are arbitrary canonical forms.
(2) V. Balzani and V. Carassite, "Photochemistry of Coordination Compounds", Academic Press, New York, N.Y., 1970, pp 156-158, and references cited therein.
(3) R. Havemann and K. G. Reimer, *Z. Phys. Chem. (Leipzig)*, **216**, 334 (1961), and earlier papers of this series.
(4) C. G. Hatchard and C. A. Parker, *Trans. Faraday Soc.*, **54**, 1093 (1961).
(5) R. Hardwick, *J. Am. Chem. Soc.*, **80**, 5667 (1958).
(6) S. Ainsworth, *J. Phys. Chem.*, **64**, 715 (1960).
(7) J. Schlag, *Z. Phys. Chem. (Frankfurt am Main)*, **20**, 53 (1959).
(8) E. Rabinowitch, *J. Chem. Phys.*, **8**, 551 (1940).
(9) Present work.
(10) E. M. Kosower, "An Introduction to Physical Organic Chemistry", Wiley, New York, N.Y., 1968, and references cited therein.
(11) Solvent Z values cited in Figure 1 are for 0.1 M ionic strength.
(12) The possibility of such a correlation has been suggested by Kosower[13] for a similar system.
(13) E. M. Kosower, A. Teuerstein, and A. J. Swallow, *J. Am. Chem. Soc.*, **95**, 6127 (1973).
(14) M. Mohammad and E. M. Kosower, *J. Am. Chem. Soc.*, **93**, 2709 (1971).
(15) M. Mohammad and E. M. Kosower, *J. Am. Chem. Soc.*, **93**, 2713 (1971).
(16) A referee has called attention to the fact that the data of Figure 1 correspond to $\Delta\Delta G^{\ddagger}/0.5\Delta Z \sim 0.5$, a value considerably smaller than the 0.7-0.8 reported[15] for the electron-transfer reaction between 1-ethyl-4-carboxymethylpyridinyl radical (Py) and 4-nitrobenzyl halides. Reaction 2 is different in charge type from the latter reaction (which is identical with the charge type of the process which defines Z) in a way such that the observed smaller sensitivity is to be expected.
(17) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions", Butterworths, London, 1959, p 458.
(18) M. Mohammad and E. M. Kosower, *J. Phys. Chem.*, **74**, 1153 (1970).
(19) E. Rabinowitch, *J. Chem. Phys.*, **8**, 360 (1940).
(20) A. E. Potter and L. H. Thaler, *Sol. Energy*, **3**, 1 (1957).
(21) K. G. Mathai and E. Rabinowitch, *J. Phys. Chem.*, **66**, 663 (1962).
(22) L. J. Miller, *U.S. Dep. Commer., Off. Tech. Serv.*, Report AD 282, 878 (1962).

Peter D. Wildes, Norman N. Lichtin*
Morton Z. Hoffman
Department of Chemistry, Boston University
Boston, Massachusetts 02215
Received October 19, 1974

Interpretation of Electron Spin Resonance Copper(II) Isotropic Hyperfine Splittings

Sir:

In a recent communication Zink and Drago[1] proposed that the predominant mechanism affecting the ESR nuclear isotropic hyperfine splitting in Cu^{2+} systems is a change in the energy separation between the ligand and the copper atomic orbitals. They offered this mechanism as an alternative to my earlier one[2] which consisted of a covalent and 4s dependence for the isotropic A values.

To review the problem briefly, the experimental isotropic ESR A values[3] predict a covalency dependence which is the direct opposite of the dependence obtained from the anisotropic ESR A values.[2] Since the trend predicted from the anisotropic ESR A values was consistent with other experimental data concerning covalency,[3] the anisotropic ESR results were accepted by me as correct, and the isotropic ESR theory was assumed to be incorrect.

Table I. Theoretical Molecular Orbital Data for $Cu(O-CR_1-CH-CR_2-O)_2$

R_1	R_2	R_1[a]	R_2[a]	Energy[d] of $d_{x^2-y^2}$ MO	α[e]	α'[f]
F	F	F	F	−75.5881	0.9408	0.0704
H	F	F	F	−75.3716	0.9293	0.0685
H	F	H[b]	F[b]	−75.1629	0.9186	0.0673
H	F	H[c]	F[c]	−75.1622	0.9185	0.0666
H	H	F	F	−75.0699	0.9124	0.0676
H	H	H	F	−74.8640	0.9026	0.0662
H	H	H	H	−74.5814	0.8880	0.0655

[a] R_1 and R_2 of the second ligand. [b] The fluorines are trans to each other. [c] The fluorines are cis to each other. [d] In units of 1000 cm^{-1}. [e] The coefficient of the $d_{x^2-y^2}$ atomic orbital in the singly occupied molecular orbital. [f] The coefficient of the 4s atomic orbital in the molecular orbital which is predominantly d_{z^2} in character.

Zink and Drago[1] make the opposite assumption. They assume that the trend predicted by the isotropic ESR A values is correct, and, therefore, the anisotropic theory must be incorrect (although they do not make the second part of this statement it is implicit from their paper).

The basis of their mechanism is that electron withdrawing groups should decrease the energy difference between the metal $d_{x^2-y^2}$ atomic orbital and the ligand σ orbital. The decrease would then result in a greater covalency for this particular molecular orbital. Since the overall accepted effect of electron withdrawing groups is to decrease the covalency, they postulate that the above increase is more than offset by changes in the other occupied molecular orbitals.

The basic question is the following. What is the effect of electron withdrawing groups on the singly (electron) occupied molecular orbital? To determine this behavior I have extended the molecular orbital calculations of Cotton, Harris, and Wise[4] for $Cu(O-CR_1-CH-CR_2-O)_2$ to cases where R_1 and/or R_2 are fluorines. The pertinent results are given in Table I. They are completely consistent with my earlier conclusions and, also, in agreement with the trend in substituent effects on energy levels as determined from ionization potential data.[5,6]

One does not have to rely on theoretical calculations to test these two alternate models. Ligand hyperfine splitting data can be used to give a more direct indication of unpaired electron delocalization. The limited data of this type that were available to Zink and Drago were considered by them to be inconclusive on this point due to the possibility of hybridization changes. Fortunately, a very complete single-crystal ESR study of the influences of different host lattices upon the ESR parameters of a Cu(II) complex has been published.[7] The anisotropic metal hyperfine splittings and the ligand nitrogen splittings gave a covalency trend which is in the opposite direction of the trend given by the isotropic metal hyperfine values. In addition the isotropic term *did correlate with the amount of orthorhombic distortion* (expected if 4s mixing is the predominant reason for changes in the isotropic hyperfine term).

If the above arguments concerning the incorrectness of Zink and Drago's model are accepted, one is still left with the question of what assumption or part of their model is not applicable. This question appears to be answered by the X-ray structure analysis of copper bisacetylacetonate-

quinoline adduct.[8] Zink and Drago's model was originally proposed to explain base adduct behavior, but in their model they did not consider the possibility that the Cu(II) would be moved out of the equatorial plane by the base adduct (0.21 Å in the quinoline case). Thus, the effect of the more favorable relative energy positions of the metal and ligand atomic orbitals is opposed by the longer Cu-ligand distance and the less favorable overlaps of the orbitals. This point is discussed in more detail by Wayland and Garito.[9]

In conclusion the expectation that the $1/r^3$ dipolar anisotropic term gives a more straightforward indication of the covalency than the isotropic (Fermi contact) term is upheld. As reviewed elsewhere[10] there are still deficiencies in the theory of obtaining bonding parameters of transition metal complexes from ESR data, but Zink and Drago's mechanism does not appear to be the answer.

Acknowledgment. This research was supported by the National Science Foundation through Grant GP-9485.

References and Notes

(1) J. I. Zink and R. S. Drago, *J. Am. Chem. Soc.*, **94**, 4550 (1972).
(2) H. A. Kuska, M. T. Rogers, and R. E. Drullinger, *J. Phys. Chem.*, **71**, 109 (1967).
(3) H. A. Kuska and M. T. Rogers, *J. Chem. Phys.*, **43**, 1744 (1965).
(4) F. A. Cotton, C. B. Harris, and J. J. Wise, *Inorg. Chem*, **6**, 909 (1967).
(5) H. F. Holtzclaw, Jr., L. Lintvedt, H. E. Baumgarten, R. G. Parker, M. M. Bursey, and P. F. Royerson, *J. Am. Chem. Soc.*, **91**, 3774 (1969).
(6) B. W. Levitt and L. S. Levitt, *J. Coord. Chem.*, **3**, 187 (1973).
(7) J. Ammeter, G. Rist, and Hs. H. Gunthard, *J. Chem. Phys.*, **57**, 3853 (1973).
(8) S. Ooi and Q. Fernando, *Chem. Commun.*, 532 (1967).
(9) B. B. Wayland and A. F. Garito, *Inorg. Chem.*, **8**, 182 (1969).
(10) H. A. Kuska and M. T. Rogers, "Spectroscopy in Inorganic Chemistry," Vol. II, Academic Press, New York, N.Y. 1971, pp. 175–196.

H. A. Kuska
Department of Chemistry, University of Akron
Akron, Ohio 44325

Received November 20, 1974

Active Site Directed Inhibition of Enzymes Utilizing Deaminatively Produced Carbonium Ions. Application to Chymotrypsin

Sir:

Carbonium ions generated from diazonium ions in the deamination of aliphatic amines are exceptionally reactive species.

$$RNH_2 \longrightarrow RN_2^+ \longrightarrow R^+ + N_2 \qquad (1)$$

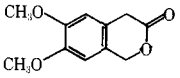
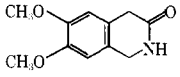
For example, they are capable of alkylating alcoholic and aromatic compounds and of abstracting halide ions from solvents such as chloroform.[1] Carbonium ions of this type if released at the active site of an enzyme should alkylate amide and other functional groups at the site, thus "labeling" them. We now report an application of this type of labeling to α-chymotrypsin.

Chymotrypsin catalyzes the hydrolysis of derivatives of carboxylic acid (proteins, amides, esters, etc.).[2] It is generally agreed that serine-195 of chymotrypsin attacks the carbonyl group of the substrate, displacing a leaving group X and generating a modified enzyme in which the serine hydroxyl group has been acylated.

$$RC(=O)X + HOEnz \longrightarrow HX + RC(=O)OEnz \xrightarrow{H_2O} RCO_2H + HOEnz \qquad (2)$$

Hydrolysis of the acyl group then regenerates the enzyme (eq 2).[3]

Table I. Effect of Inhibitors and Related Compounds on Chymotrypsin Activity

Inhibitor added[a]	% enzymatic activity[b]
None (control)[c]	100
Nitrosolactam III	6
Nitrosolactam III + hydrocinnamic acid[d]	12
Decomposition products of nitrosolactam III[e]	95
	101
	99
$NaNO_2$	100

[a] The compounds tested were added in 110-fold molar excess (final inhibitor concn = 2.5×10^{-3} *M* and chymotrypsin concn = 2.3×10^{-5} *M*). The enzyme–inhibitor solutions contained 9% acetonitrile in 0.08 *M* Tris buffer, 0.1 *M* in $CaCl_2$ at pH 7.8; they were incubated for 2 hr at 0°. See text for alternative conditions. [b] Determined by a rate assay using the *N*-benzoyl-L-tyrosine ethyl ester (BTEE) method.[11a] Enzyme concentrations in some of the runs were measured by titration with cinnamoylimidazole.[11b] [c] Acetonitrile (9%) added. [d] [Hydrocinnamic acid] = 1.2×10^{-2} *M* in the enzyme + III solution. [e] A completely decomposed solution (24 hr at 25°) of nitrosolactam (III) in the Tris buffer, pH 7.8. The chymotrypsin sample exposed to these products showed no incorporation of ^{14}C.

Utilizing the concepts outlined above, we have synthesized and tested as an irreversible inhibitor of chymotrypsin the *N*-nitroso-*N*-benzylamide of *N'*-isobutyrylphenylalanine (I).[4] As a derivative of the aromatic amino acid phenylalanine, this compound should be readily attacked by chymotrypsin, especially since a "good" leaving group (II) is formed.[5]

$$C_6H_5CH_2CH(NHCOCH(CH_3)_2)C(=O)N(N=O)CH_2C_6H_5 \xrightarrow{EnzOH} \qquad (3)$$

I

$$C_6H_5CH_2C(HO)(NHCOCH(CH_3)_2)COEnz + N(N=O)CH_2C_6H_5 \longleftrightarrow N(N-O^-)CH_2C_6H_5$$

II

HOEnz = enzyme

The leaving group in this case is designed to yield carbonium ions (eq 4).[6] It is believed that the steps illustrated are

$$II \xrightarrow{H_2O} NH(NO)CH_2C_6H_5 \longrightarrow C_6H_5CH_2N_2^+ \longrightarrow C_6H_5CH_2^+ \qquad (4)$$

fast processes,[7] and therefore the carbonium ion should be formed at or near the active site of the attacking enzyme molecule—in a position to alkylate basic functional groups (HB) on the enzyme.

$$C_6H_5CH_2OH \xleftarrow{H_2O} C_6H_5CH_2^+ \xrightarrow{Hb-Enz} C_6H_5CH_2B-Enz \qquad (5)$$

Compound I is, in fact, attacked by α-chymotrypsin. The rate of decomposition of a 5.7×10^{-4} *M* solution of I is accelerated (ca. six times) by 6.7×10^{-7} *M* enzyme in Tris buffer,[8] and, in addition, the enzyme becomes irreversibly inhibited to the extent of ~20%. The products of the reaction are benzyl alcohol and *N*-isobutyrylphenylalanine. Neither these products nor the *N*-benzylamide of isobutyrylphenylalanine irreversibly inhibit the enzyme; the amide, further, is only slowly hydrolyzed by the enzyme.[5]