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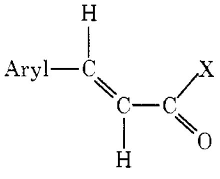
Lewis Acid Complexes Which Show Spectroscopic Similarities to an Alcohol Dehydrogenase Ternary Complex†

Charles T. Angelis, Michael F. Dunn,* David C. Muchmore,‡ and Richard M. Wing

ABSTRACT: *trans*-4-*N,N*-Dimethylaminocinnamaldehyde (II) (λ_{max}(H_2O) 398 nm, ϵ_{max} 3.15 × 10^4 M^{-1} cm^{-1}) reacts with the horse liver alcohol dehydrogenase–NADH complex to form a transient intermediate (III) (λ_{max} 464 nm, ϵ_{max} ~6.2 × 10^4 M^{-1} cm^{-1}) which breaks down to *trans*-4-*N,N*-dimethylaminocinnamyl alcohol and NAD^+. It was proposed that the intermediate has a dipolar quinodial structure that is stabilized by a coordination bond between the active site zinc ion and the carbonyl oxygen of II [Dunn, M. F., and Hutchison, J. S. (1973), *Biochemistry 12,* 4882]. In these studies, complexes of II or *trans*-4-*N,N*-dimethylaminocinnamaldehyde-2′-pyridinohydrazone (IV) with various Lewis acids in anhydrous aprotic solvents have been studied as spectroscopic and structural models for III. Both II and IV form complexes exhibiting red-shifted $\pi^* \leftarrow \pi$ spectral bands with a variety of Lewis acids. II forms complexes with $ZnCl_2$, $SnCl_4$, and $(C_2H_5)_3OBF_4$, and IV forms complexes with ZnI_2, $Zn(NO_3)_2$, $CoCl_2$, and $MnCl_2$. The magnitudes of the red shifts (39 to 110 nm) depend both on the chemical nature of the Lewis acid and on the properties of the solvent. The spectrum of the $ZnCl_2$ complex with II in diethyl ether (λ_{max} = 431 nm) is closely similar to the spectrum of III. The observed spectral red shifts are consistent with an inner sphere bonding interaction between the carbonyl oxygen of II, or the imine nitrogen of IV and the Lewis acid. The x-ray structure of the ZnI_2 complex of IV ($C_{26}H_{18}N_4ZnI_2$, mol wt 585.64 g/mol) which crystallizes in space group $P\bar{1}$ with unit cell dimensions of a = 13.47 (2) Å, b = 8.19 (1) Å, c = 9.69 (1) Å, α = 87.0 (1)°, β = 104.1 (1)°, and γ = 103.4 (1)° has been refined by full matrix least-squares to a final R of 0.092. An envelope conformation is observed for the five-membered ring created by chelation of zinc by the pyridine and imine nitrogens of the ligand, the coordination about zinc is distorted tetrahedral, and the π-bonding framework of the ligand suggests a quinoidal structure. The x-ray structure establishes that the spectral shift observed when zinc ion binds to IV is due to inner sphere coordination of the imine nitrogen. These studies support a structure for the enzyme-bound intermediate (III) involving the direct coordination of the carbonyl oxygen of II to the active site zinc ion.

The use of intense chromopheres in the study of enzyme catalytic mechanism can yield insight into the detailed chemical processes occurring at the enzyme site if the spectral changes are directly coupled to the chemical transformations(s). Substrate analogues based on the conjugated arylacryloyl moiety (I) have been particularly useful in the study of both proteases (Bernhard et al., 1965; Charney and Bernhard, 1967; Bernhard and Lau, 1971; Hinkle and Kirsch, 1970) and dehydrogenases (Malhotra and Bernhard, 1968, 1973; Dunn and Hutchison, 1973; Dunn et al., 1975). The spectra of these chromophores are highly sensitive to: (1) the chemical nature of the group X attached to the carbonyl carbon; (2) the electronic nature of substituents on the aryl group ortho and para to the acryloyl residue; and (3) to the polarity of the microenvironment (Dolter and Curran, 1960; Charney and Bernard, 1967; Bernhard and Lau, 1971; Dunn and Hutchison, 1973). Hence the spectral properties of the arylacryloyl



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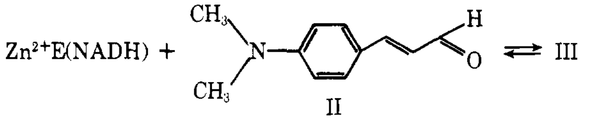
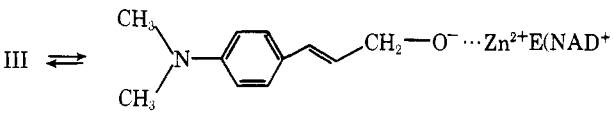
chromophore can provide specific information about both weak bonding interactions (i.e., hydrogen bonding, London dispersion forces, and Coulombic interactions) and strong bonding (covalent) interactions between site and substrate.

In previous papers on the horse liver alcohol dehydrogenase catalytic mechanism (Dunn and Hutchison, 1973; Dunn et al., 1975; Dunn, 1975), the kinetic and spectral properties of the intermediate formed during the reaction of the intense chromophore, *trans*-4-*N*,*N*-dimethylaminocinnamaldehyde (II), with the enzyme-NADH binary complex were described. These studies demonstrated that the enzyme-catalyzed reduction of II (λ_{max} 398 nm, ϵ_{max} 3.15 × 10^4 M^{-1} cm^{-1}) by NADH involves the transient formation of a chemical intermediate (III, λ_{max} 464 nm, ϵ_{max} $\simeq$6.2 × 10^4 M^{-1} cm^{-1}). Once formed, the intermediate decays to the expected products (alcohol and NAD^+) in a much slower, pH-dependent process that involves hydride transfer as shown in eq 1 to 4 (Dunn and Hutchison, 1973; Dunn and Morris, in preparation).



(1)



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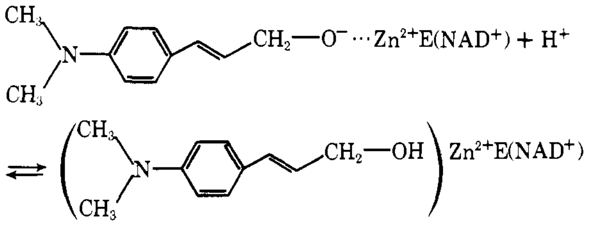

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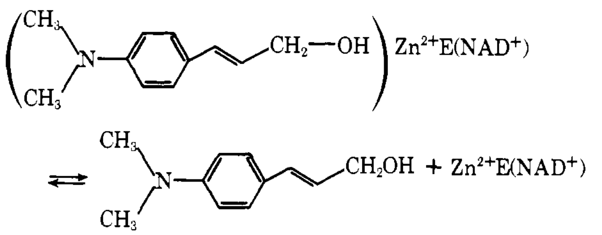
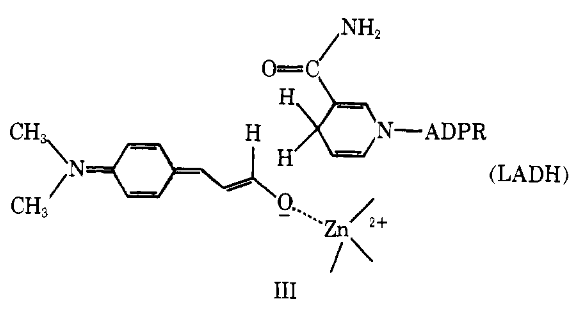


(4)

The formation of this intermediate was found to be a reversible, pH-independent process (eq 1). The spectral properties and the kinetic behavior of the intermediate led us to propose a structure for the intermediate which involves inner sphere coordination of the carbonyl oxygen of II to the active site zinc ion as depicted in Diagram I.

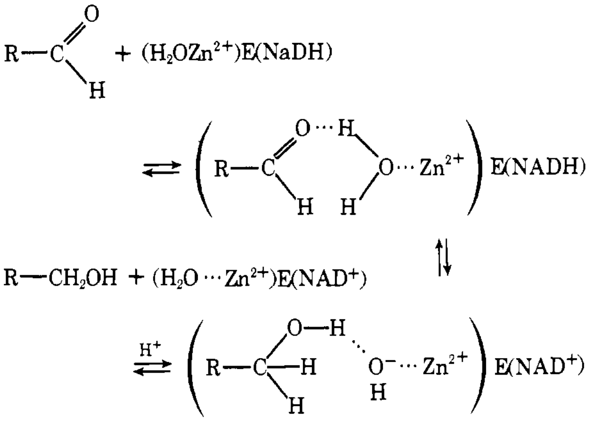
DIAGRAM I



III

Hence it was concluded that zinc ion functions as a Lewis acid catalyst in the activation of the aldehyde carbonyl for reduction via direct transfer of "hydride" (i.e., transfer of a proton and two electrons) from NADH as depicted in eq 2. Furthermore, it appears well established that zinc ion can function effectively as a Lewis acid catalyst in the promotion of aldehyde reduction by the reduced 1,4-dihydropyridine moiety in certain model systems (Creighton and Sigman, 1971; Schinkai and Bruice, 1972, 1973; Creighton et al., 1976).

Nevertheless, Sloan et al. (1975) have proposed an alternative role for zinc ion based on NMR[1] studies of cobalt(II)-substituted horse liver alcohol dehydrogenase. Analysis of the proton relaxation rates arising from the paramagnetic line broadening effects of the cobalt(II)-enzyme on ethanol and isobutyramide indicated that both ligands form ternary complexes with the enzyme and NADH in which the calculated closest approach distance between the active site metal ion and the ligand (~6 Å) is too large to allow inner sphere coordination to the metal ion. Since a water molecule has been found to occupy the fourth coordination site on the active site zinc ion (Brändén et al., 1976), it has been proposed by Sloan et al. (1975) that the metal ion coordinated water molecule is hydrogen bonded to, and positioned between, these ligands and the metal ion (see eq 5). They further propose that the zinc-bound water molecule functions as a protonic acid-base catalyst in the facilitation of hydride transfer as shown in eq 5-7;



(5)

(6)

(7)

Since the evidence in support of the structure proposed for the intermediate (III) (Diagram I) is circumstantial, we have undertaken the preparation of various zinc ion complexes which could serve as plausible spectroscopic models for III. We report UV-vis data for several zinc(II) model complexes involving inner sphere coordination of the carbonyl oxygen or imine nitrogen of the ligand to zinc ion. For one system, the mode of bonding to the metal ion has been unambiguously established via single crystal x-ray diffraction analysis.

Experimental Section

Materials. The commercially available compounds acetonitrile (Mallinckrodt), diethyl ether (Mallinckrodt), methylene chloride (Mallinckrodt), 2-hydrazinopyridine (Aldrich), *trans*-4-dimethylaminocinnamaldehyde (Aldrich), triethyloxonium fluoroborate (Alfa Products), zinc nitrate hexahydrate (Matheson Coleman and Bell), stannic chloride (Matheson Coleman and Bell), and zinc chloride (J.T. Baker) were the highest purity grades available and generally were used

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[1] Abbreviations used: NMR, nuclear magnetic resonance; UV-vis, ultraviolet-visible.

without further purification except as noted in the following text.

trans-4-N,N-Dimethylaminocinnamaldehyde-2′-pyridinohydrazone (IV). Equimolar amounts of 2-hydrazinopyridine, 1.09 g (10 mmol), and *trans*-4-*N*,*N*-dimethylaminocinnamaldehyde, 1.75 g (10 mmol), were dissolved in 150 mL of acetonitrile and warmed at 60 to 80 °C for 2 to 3 h. The resulting orange solution was then evaporated to dryness with the rotary evaporator, yielding IV as a mass of light yellow, microcrystalline material in near quantitative yield. The crude material was recrystallized twice from boiling acetonitrile, yielding long, light-yellow needles. Anal. Calcd for $C_{16}H_{18}N_4$: C, 72.15; H, 6.81; N, 21.04. Found: C, 72.07; H, 6.80; N, 21.08 (average of two determinations).

Diiodomono(trans-4-N,N-dimethylaminocinnamaldehyde-2′-pyridinohydrazone) Zinc(II). A solution containing 0.133 g (0.5 mmol) of IV and 0.148 g (0.5 mmol) of $Zn(NO_3)_2 \cdot 6H_2O$ in 1 mL of acetonitrile was prepared and mixed with a solution containing 0.150 g (1 mmol) of NaI in 1.2 mL of acetonitrile. Upon mixing, a white precipitate of $NaNO_3$ formed. The $NaNO_3$ precipitate was immediately removed by vacuum filtration. The filtrate on standing 1 to 2 min formed red crystals of the zinc complex in near quantitative yield. The crystals were collected and recrystallized from 20 mL of boiling acetonitrile.

Stoichiometry. The combining stoichiometry for the formation of the IV–zinc ion complex was determined according to the method of continuous variation (Job, 1928). The amount of complex formed was monitored at 450 nm as a function of the mole fraction of IV. The data yielded a theoretical stoichiometry of 1:1 as determined by the position of the maximum in the plot of ΔOD_{450} vs. ligand mole fraction. However, deviations in the plot at ligand mole fractions approaching 1.0 indicate the existence of complexes containing more than 1 mol of IV per g-atom of zinc ion.

Spectral Studies. The UV–vis spectra of the various Lewis acid complexes with IV and II were carried out in anhydrous solvents. Anhydrous dichloromethane was prepared via distillation from phosphorus pentoxide. Diethyl ether was distilled from lithium aluminum hydride. Acetonitrile was dried over molecular sieves. In those studies where the elimination of water from the system proved to be critical, the vessels used were protected from atmospheric moisture by serum stoppers and transfers were made by introducing aliquots by syringe. Anhydrous zinc chloride solutions were prepared by fusing zinc chloride under a stream of dry nitrogen. The anhydrous material was then dissolved in the desired solvent. UV–vis spectra were measured with either a Varian 635 spectrophotometer or a Cary 14 spectrophotometer.

Crystal Data. A lustrous red rhombohedral crystal 0.6 × 0.6 × 0.9 mm of $C_{16}H_{18}N_4ZnI_2$ (mol wt 585.64) was selected for the x-ray diffraction study. A triclinic crystal system was determined from precession and cone axis photographs. Refinement of the cell constants using 12 carefully centered reflections (Mo $K\alpha_1$ radiation, $\lambda = 0.70926$ Å) having $2\theta > 45°$ on a Picker Nuclear FACS II four circle diffractometer gave $a = 13.47$ (2) Å, $b = 8.19$ (1) Å, $c = 9.69$ (1) Å, $\alpha = 87.0$ (1)°, $\beta = 104.1$ (1)°, $\gamma = 103.4$ (1)° and a cell volume of 1010.9 Å^3. Based on a density measurement ($\rho = 1.92$ g/cm^3, determined via flotation in bromoform and dichloromethane), the unit cell contained two molecules per unit cell ($\rho_{\mathrm{calcd}} = 1.92$ g/cm^3). Since the diffraction symbol failed to yield a unique space group, $P\bar{1}$ was chosen and subsequently was shown to be correct by the solution of the structure.

A FACS II diffractometer with monochromatic Mo $K\bar{\alpha}$ radiation (via a graphite crystal in the diffracting position) was used to collect the intensity data at room temperature. The crystal was mounted so that the longest dimension was parallel to the goniometer axis. Sample to detector distance was 36.0 cm, and the window of the pulse height analyzer was adjusted to accept 90% of the diffracted intensity of the 003 reflection. Three standard reflections were remeasured every 50 reflections to monitor fluctuations in instrument stability and crystal decomposition (see paragraph concerning supplementary material at the end of this paper). The data were collected via 2θ scans of $1.8 + 0.692 \tan \theta$ (degrees) in two segments. The data in the range $\sin \theta/\lambda = 0.0369$ to 0.3050 were taken first. During measurement of the last 50 reflections in this segment, a diffractometer failure occurred which necessitated realignment of the crystal for collection of the second segment of data, causing a 15% increase in the standard intensity in the 0.5395 to 0.5959 range. This second segment was collected in order to accurately fix the atom coordinates. The standard reflections showed only a random fluctuation of 2.5% for both data segments. Ten-second backgrounds at the extremes of each scan were measured, and, of the 1265 reflections collected, 1149 were considered observed on the basis that their intensities $I = P - (t/20)B$ exceeded the background by 1.5σ ($\sigma(I) = [P + (t/20)^2B + (0.045I)^2]^{1/2}$, where P is the peak intensity, t is the scan time in seconds, and B is the background count). These data then were corrected for Lorentz and polarization effects, but not for absorption. The linear absorption coefficient was 43.4 cm^{-1}, and we were able to calculate a minimum transmission coefficient of 0.100 and a maximum of 0.145.

Structure Solution and Refinement. Solution of the structure was effected by the heavy atom method. An origin-removed Patterson[2] gave the positions of the two iodine atoms, and a subsequent Fourier map of the electron density revealed the remaining atoms. Full matrix refinement of the structure with isotropic temperature factors introduced only for the zinc and iodine atoms gave a final residual[3] of 0.092. The weighting scheme used throughout the refinement is given by $W = 4F^2/L^2\sigma(I)$ where L is the reciprocal Lorentz polarization correction. The weighted residual was 0.097 and χ was 2.68. A final difference Fourier map of F_{calcd} and F_{obsd} was too noisy to allow a realistic location of the hydrogen atoms ($\Delta\rho_{\mathrm{max}} = 1.47$).

Scattering powers[4] for all atoms and the anomalous dispersion corrections for zinc and iodine[5] were taken from standard sources.

Results

These model studies were designed to test the validity of the structure proposed for the intermediate observed during the

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[2] The calculations were initially carried out on a HP3000 computer using ISAS, an interactive set of crystallographic programs based on Zalkin's FORDAP for election density maps, and BDLSQ by Gantzel et al. for structure factor least-squares. The final calculations were done on an IBM 360/50 using FORDAP (ORFLS), Busing, Martin, and Levy's full matrix least-squares structure factor program (Oak Ridge National Laboratory Report No. TM-305), ORTEP, Johnson's drawing program (Oak Ridge National Laboratory Report No. TM-305), ORTEP, Johnson's drawing program (Oak Ridge National Laboratory Report No. 3794), and GEOM, Lin's program for distances, angles, and planes.

[3] The residual is defined to be $\Sigma[|F_o| - |F_c|/\Sigma|F_o|]$ and the weighed residual $[(\Sigma w|F_o| - |F_c|)^2/(\Sigma|wF_o|)^2]^{1/2}$.

[4] The scattering powers were taken from International Tables for X-ray Crystallography, Vol. III, Birmingham, England, Kynoch Press, 1962, p 202, Table 3.3.2B.

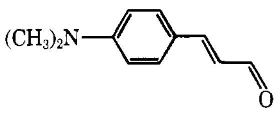
[5] The anomalous correction was made ((1962), International Tables for X-ray Crystallography, Vol. III, Birmingham, England, Kynoch Press, p 202, Table 3.3.1A).

TABLE I: Solvent Dependence of the Long Wavelength Absorption Bands of *trans*-4-*N*,*N*-Dimethylaminocinnamaldehyde (II) and *trans*-4-*N*,*N*-Dimethylaminocinnamaldehyde-2′-pyridinohydrazone (IV).

	II		IV	
Solvent (Z)[a] [ϵ][b]	λ_{max} (nm) (E_T)[f]	ϵ_{max} (M^{-1} cm^{-1}) × 10^{-4}	λ_{max} (nm) (E_T)[f]	ϵ_{max} (M^{-1} cm^{-1}) × 10^{-4}
10 M LiCl[e]	415 (68.8)	2.4	Insoluble	Insoluble
CF_3CH_2OH (~95)	407 (70.2)	3.3	375 (76.2)	3.4
4 M guanidinium sulfate[e]	406 (70.3)	3.0	Insoluble	Insoluble
8 M urea[e]	404 (70.7)	3.0	379 (75.3)	3.8
H_2O (94.6) [80][d]	398 (71.7)	3.1	372[e] (76.8)	3.7
Formamide (83.0) [111][d]	398 (71.7)	3.3	384 (74.3)	5.0
Me_2SO (70.4) [46.68][c]	388 (73.6)	3.4	384 (74.3)	5.5
Ethanol (79.6) [24.55][c]	388 (73.6)	3.3	374 (76.3)	5.4
$CHCl_3$ (63.2) [4.806][d]	386 (74.0)	3.1	379 (75.3)	4.9
DMF (68.0) [36.71][c]	383 (74.5)	3.3	380 (75.1)	5.3
CH_3CN (71.3) [37.5][c]	377 (75.7)	3.3	374 (76.3)	5.1
Acetone (~66) [20.70][c]	377 (75.7)	3.1	376 (76.0)	5.5
CCl_4 [2.238][d]	371 (77.0)	3.0	377 (75.7)	5.1
$(C_2H_5)_2O$ [4.335][d]	366 (78.0)	3.4	371 (77.0)	5.6
Dioxane (~62) [2.209][c]	360 (79.3)	3.1		
Cyclohexane (60.1) [2.023][c]	358 (79.8)	3.2	369 (77.4)	4.6

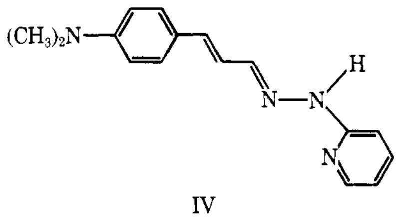
[a] Z is a measure of solvent polarity derived from the position of the charge-transfer band of 1-ethyl-4-carbomethoxypyridinium iodide. See Kosower (1958a–c). [b] Solvent dielectric constant, values taken from Riddick and Burger (1970). [c] At 25 °C. [d] At 20 °C. [e] pH 8 Tris-HCl buffer. [f] Transition energies (kcal/mol radiation).

reaction of II with the alcohol dehydrogenase-NADH complex (Dunn and Hutchison, 1973; Dunn et al., 1975; see Diagram I). Two classes of metalloorganic complexes were chosen as possible spectroscopic models for the enzyme-bound intermediate (III). The first class is derived from ligands in which the *trans*-4-*N*,*N*-dimethylaminocinnamyl moiety (IV) has been incorporated into a bidentate divalent metal ion ligand. The second class consists of complexes derived from the direct complexation of II with several different Lewis acids.



$+ M^{2+} \rightleftharpoons$ complex (8)

II



$+ M^{2+} \rightleftharpoons$ complex

IV

(9)

M^{2+} = divalent metal ion

Compound IV was chosen for these studies because (as will be shown) the bidentate character of the ligand conveys a high affinity for the various divalent metal ions under study. The properties of the zinc complex have made possible both an unambiguous determination of the coordination state and the identification of the specific donor atoms involved in inner sphere coordination to the metal ion via x-ray diffraction analysis of the crystalline complex.

The data presented in Table I demonstrate that the position of the absorption maximum for the long wavelength $\pi^* \leftarrow \pi$ electronic transition of II is highly sensitive to the polarity of the medium. The position of the absorption maximum ranges from 358 nm in cyclohexane to 415 nm in aqueous 10 M LiCl (pH 8). Note also that the energy of the transition gives a reasonably linear correlation with both the solvent dielectric constant and Z (the solvent-dependent position of the charge-transfer band of 1-ethyl-4-carbomethoxypyridinium iodide).[6] The position of the long wavelength electronic transition exhibited by IV (Table I) shows a similar, although reduced, sensitivity to the polarity of the medium. This red shift is fully consistent with our assignment of this band as a $\pi^* \leftarrow \pi$ transition (Suzuki, 1967). Note that the relatively polar solvents (formamide, trifluoroethanol, dimethylformamide) yield transitions of lower energy (longer wavelength) than do the relatively nonpolar solvents (ether, cyclohexane) for both II and IV. These data show that the position of the absorption maximum depends (critically) on both the polarity and the specific hydrogen bonding characteristics of the medium.

When IV ($\lambda_{max}(CH_3CN)$ 374 nm, ϵ_{max} 5.11 × 10^4 M^{-1} cm^{-1}) and $Zn(NO_3)_2$ are mixed in acetonitrile, there occurs the rapid, reversible formation of a new chromophoric species ($\lambda_{max}(CH_3CN)$ 426 nm, ϵ_{max} 4.47 × 10^4 M^{-1} cm^{-1}), as illustrated in Figure 1. The presence of a sharp isosbestic point in the spectra (Figure 1a) strongly indicates that the system contains only two (interconvertible) chromophores, the free ligand (IV) and the zinc ion complex (V). Stoichiometry measurements using Job's method of continuous variations (Job, 1928) yielded a ligand to metal ion stoichiometry of 1:1.

The data given in Table II summarize UV-vis spectral data for the reaction of select divalent metal ions with several structural homologues of IV. These data demonstrate that: (1) the magnitude of the observed red shift in the spectrum is relatively insensitive to the nature of the divalent metal ion; (2) the magnitude of the spectral shift is strongly dependent on the chemical nature of the chromophore (compare IV with

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[6] Kosower (1958a–c), Kosower et al. (1961), and Kosower and Wu (1961) have shown that the position of the charge transfer absorption band of 1-ethyl-4-carbomethoxypyridinium iodide provides a measure of solvent polarity, and they defined the transition energy for the band in a particular solvent as the Z value for that solvent. These elegant studies have demonstrated the usefulness of Z values both in the assignment of transitions and the semiquantitation of solvent effects on electronic spectra, and the interested reader is referred to these works.

TABLE II: Spectral Properties of the Lewis Acid Complexes of *trans*-4-*N,N*-Dimethylaminocinnamaldehyde (II) and *trans*-4-*N,N*-Dimethylaminocinnamaldehyde-2′-pyridinohydrazone (IV) and Homologues of IV.

			Ligand		Complex		
Ligand	Lewis Acid	Solvent	λ_{max} (nm)	ϵ_{max} (M^{-1} cm^{-1}) × 10^{-4}	λ_{max} (nm)	ϵ_{max} (M^{-1} cm^{-1}) × 10^{-4}	$\Delta\lambda_{max}$ (nm)[d]
IV	$Zn(NO_3)_2$	CH_3CN	374	5.11	426	4.47	52
IV	$Zn(I)_2$	CH_3CN	374	5.11	428	4.47	54
IV	$CoCl_2$	CH_3CN	374	5.11	430	3.57	56
IV	$MnCl_2$	CH_3CN	374	5.11	415		41
IV	$NiCl_2$	CH_3CN	374	5.11	413		39
VI[a]	$Zn(NO_3)_2$	CH_3CN	348	4.47	375	3.58	27
VII[b]	$Zn(NO_3)_2$	CH_3CN	324		342		18
VIII[c]	$Zn(NO_3)_2$	CH_3CN	284		286		2
II	$ZnCl_2$	$(C_2H_5)_2O$	367		431	~4.5	64
II	$SnCl_4$	$(C_2H_5)_2O$	367		452		85
II	$SnCl_4$	CH_2Cl_2	385		476		91
II	$(C_2H_5)_3OBF_4$	CH_2Cl_2	385		495		110
II	LADH	H_2O	398		464	~6.2	66

[a] *trans*-4-Methoxycinnamaldehyde-2′-pyridinohydrazone. [b] Benzaldehyde-2′-pyridinohydrazone. [c] Acetaldehyde-2′-pyridinohydrazone. [d] λ_{max}(complex) − λ_{max}(ligand) = $\Delta\lambda_{max}$.

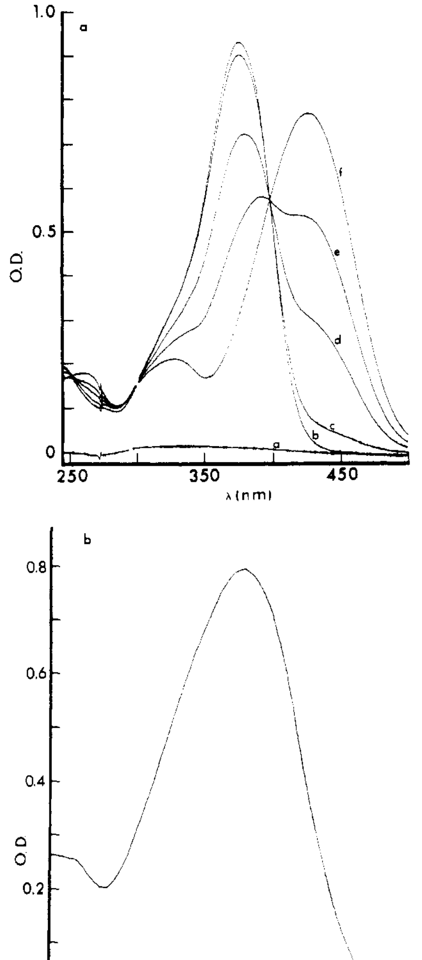


FIGURE 1: (a) Comparison of the spectrum of 18 μM IV (trace b) with the spectra obtained on the addition of $Zn(NO_3)_2$ to IV in acetonitrile at 25 °C (traces c–f). $Zn(NO_3)_2$ concentrations: trace b, 0; trace c, 3.27 μM; trace d, 6.50 μM; trace e, 9.65 μM; trace f, 15.8 μM. Trace a: baseline (anhydrous acetonitrile). Path length: 1.00 cm. (b) The spectrum of diiodomono(*trans*-4-*N,N*-dimethylaminocinnamaldehyde-2′-pyridinohydrazone) zinc(II) in anhydrous acetonitrile. The spectrum was obtained by dissolving a crystal of the diiodo salt in anhydrous acetonitrile (approximate concentration: 15.3 μM).

the *p*-methoxycinnamaldehyde, benzaldehyde, and acetaldehyde homologues, VI, VII, and VIII); (3) the 2′-aminopyridyl portion of the chromophore (as expected on chemical grounds) does not contribute appreciably to the red shift (viz., the small, 2 nm, shift observed with the acetaldehyde derivative, VIII).

The second class of complexes (those derived from the coordination of II with a Lewis acid) have proven to be much more difficult to prepare and characterize due to their diminished stabilities. Previous studies (Dunn and Hutchison, 1973; M. F. Dunn, unpublished results) have shown that *in aqueous milieu* protonation of II results in a large blue shift of the absorption maximum; therefore protonation must occur at the dimethylamino nitrogen rather than at the carbonyl oxygen. In contrast, the spectra presented in Figures 2a and 2b and Table II show that the reaction of II with a variety of Lewis acids ($ZnCl_2$, $SnCl_4$, triethyloxonium fluoroborate) *in anhydrous aprotic media* results in the reversible formation of a new chromophoric species exhibiting spectra which are significantly red shifted (64 to 110 nm) relative to the spectrum of II in the same solvent. The spectral properties of these systems are summarized in Table II. These data demonstrate that the magnitude of the observed red shift depends both on the chemical nature of the Lewis acid and on the properties of the solvent. More importantly (as will be discussed in the following paragraphs) these shifts are much greater than the shifts attributable to solvent polarity and/or specific hydrogen bonding interactions (compare Tables I and II).

Tables III and IV and Figure 3a,b summarize the structural data obtained from the x-ray diffraction study. Table III lists the atom coordinates and corresponding thermal parameters for the refined structure. Bond lengths (Table IV), angles, and best planes were derived from this data.

The coordination about zinc was found to be distorted tetrahedral, Figure 3a,b. Equal bond lengths were found for the iodine–zinc bonds [2.540 (5) Å and 2.547 (5) Å] and for the nitrogen–zinc bonds [2.06 (2) Å and 2.11 (1) Å]. The metal atom–nitrogen–nitrogen plane (N13–Zn–N16) and the metal atom–iodine–iodine plane (I–Zn–I) are nearly perpendicular (90.7°) as required for a tetrahedral ligand array. However, angular distortions from the tetrahedral value (109.5°) were found both for the nitrogen–zinc–nitrogen angle [80 (1)°] and for the iodine–zinc–iodine angle [116.5 (4)°]. The five-

TABLE III: Positional and Thermal Parameters in Diiodomono(*trans*-4-*N*,*N*-dimethylaminocinnamaldehyde-2′-pyridinohydrazone) Zinc(II).

	X^a	Y	Z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
I1	0.0894 (1)	0.1621 (2)	0.1788 (2)	0.0102 (2)	0.0168 (4)	0.0147 (4)	0.0018 (2)	0.0010 (2)	−0.0052 (3)
I2	0.3265 (1)	0.0332 (2)	0.5283 (2)	0.0131 (2)	0.0113 (3)	0.0257 (5)	0.0038 (2)	0.0003 (3)	−0.0018 (3)
Zn	0.2111 (2)	0.2359 (3)	0.4239 (3)	0.0063 (2)	0.0091 (4)	0.0120 (5)	0.0007 (3)	0.0032 (3)	−0.0022 (4)
C1	0.821 (2)	0.3[illegible]5 (3)	−0.077 (3)	5.1 (6)					
C2	0.718 (2)	0.060 (5)	−0.023 (3)	10.1 (11)					
N3	0.736 (2)	0.241 (4)	−0.020 (3)	8.1 (7)					
C4	0.679 (2)	0.323 (4)	0.0446 (2)	5.3 (7)					
C5	0.597 (2)	0.219 (3)	0.109 (3)	4.2 (6)					
C6	0.542 (2)	0.296 (3)	0.186 (2)	3.2 (5)					
C7	0.642 (2)	0.579 (3)	0.130 (2)	3.7 (5)					
C8	0.699 (2)	0.503 (3)	0.066 (2)	3.4 (5)					
C9	0.560 (2)	0.470 (4)	0.196 (2)	4.1 (6)					
C10	0.498 (2)	0.558 (3)	0.260 (2)	3.7 (5)					
C11	0.422 (2)	0.464 (3)	0.321 (2)	4.0 (5)					
C12	0.373 (2)	0.566 (3)	0.384 (2)	5.0 (6)					
N13	0.291 (1)	0.490 (2)	0.433 (2)	3.4 (4)					
N14	0.237 (1)	0.575 (2)	0.501 (2)	4.0 (4)					
C15	0.163 (2)	0.487 (4)	0.570 (3)	3.9 (5)					
N16	0.142 (1)	0.326 (3)	0.563 (2)	3.8 (5)					
C17	0.068 (2)	0.223 (3)	0.629 (3)	4.4 (6)					
C18	0.019 (2)	0.307 (4)	0.707 (3)	5.8 (7)					
C19	0.043 (1)	0.484 (4)	0.719 (3)	4.8 (6)					
C20	0.112 (2)	0.580 (3)	0.644 (2)	3.76 (5)					

[a] Number in parentheses represents the standard deviation of the last significant digit.

TABLE IV: Summary of Interatomic Bond Lengths for Diiodomono(*trans*-4-*N*,*N*-dimethylaminocinnamaldehyde-2′-pyridinohydrazone Zinc(II) Derived from the Atomic Coordinates of the Refined Structure.

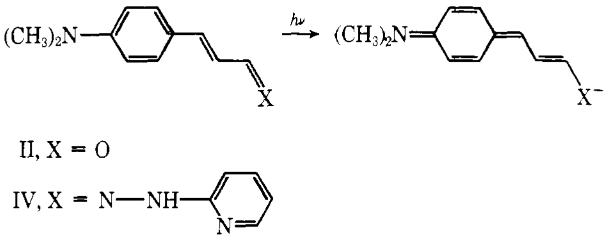
Bond	Length (Å)	Bond	Length (Å)
I1-Zn	2.547 (5)	C9-C10	1.47 (4)
I2-Zn	2.540 (5)	C10-C11	1.37 (4)
Zn-N13	2.11 (1)	C11-C12	1.42 (4)
Zn-N16	2.06 (2)	C12-N13	1.32 (3)
C1-N3	1.44 (4)	N13-N14	1.40 (3)
C2-N3	1.45 (5)	N14-C15	1.38 (3)
N3-C4	1.39 (5)	C15-N16	1.29 (4)
C4-C5	1.48 (4)	N16-C17	1.41 (3)
C5-C6	1.43 (4)	C17-C18	1.40 (4)
C6-C9	1.40 (4)	C18-C19	1.42 (4)
C4-C8	1.45 (4)	C19-C20	1.40 (4)
C8-C7	1.37 (4)	C20-C15	1.46 (4)
C7-C9	1.50 (4)		

membered ring formed by the zinc atom and the atoms, N13, N14, C15, and N16, was found to be an envelope form with the zinc [0.074 (3) Å], N14 [0.07 (1) Å], and C15 [0.03 (2) Å] lying above the calculated plane and N13 [−0.09 (2) Å] and N16 [−0.08 (2) Å] below.

Table IV summarizes the bond lengths for the ligand. Alternating bond lengths were noted for members of the aniline ring: bonds C7-C8 [1.37 (4) Å] and C5-C6 [1.43 (4) Å] are shorter than bonds C4-C5 [1.48 (4) Å], C4-C8 [1.45 (4) Å], and C7-C9 [1.50 (4) Å]. Also the N3-C4 [1.39 (5) Å] bond was somewhat shorter than a carbon-nitrogen single bond. Calculation of the least-squares planes for the aniline ring and the dimethylamino group showed these to be coplanar within 6°. The bridge atoms deviate from the plane of the aniline ring by 0.12 Å (C10), 0.11 Å (C11), and 0.16 Å (C12).

Discussion and Conclusions

The long wavelength electronic transitions which characterize both compound II and compound IV may be represented by the charge rearrangement shown below:



(10)

The transition energy for such a process is lowered by the placement of an electron acceptor (i.e., a Lewis acid or a properly oriented dipolar molecule) in close proximity to X and, consequently, results in a shift of the absorption maximum to longer wavelengths. This is true because the greater electron density on X in the excited state makes possible a stronger (stabilizing) interaction between X and the Lewis acid (or the dipole) in the excited state than in the ground state. Therefore, the position of the long wavelength absorption maximum will be red-shifted in solvents of high dielectric constant.[7] These predictions are verified by the data presented in Table I. The respective long wavelength absorption bands exhibited by II and IV both are shifted to longer wavelengths as the dielectric constant of the solvent increases.

The data presented in Table II and Figures 1 and 2 show that, when an electrophile is in close proximity to the oxygen

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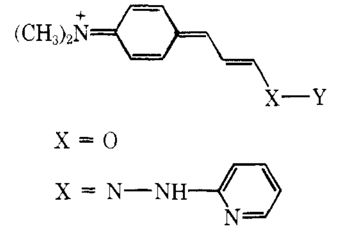
[7] Such $\pi^* \leftarrow \pi$ electronic transitions in other systems (Jaffé et al., 1964a,b; Brauman et al., 1968) have been found to yield excited states in which the pK_a of the conjugate acid is increased as much as 5 to 20 pK_a units. For example, the pK_a of the conjugate acid of azobenzene is shifted some 21.8 pK_a units in the excited state (Jaffé et al., 1964a) relative to the pK_a of the ground state.

or nitrogen to which electron density is transferred by the excitation process, the absorption maximum is displaced to even longer wavelengths. Note that the above considerations predict that the stronger the electron withdrawing (accepting) character of the electrophile, the larger the displacement will be. In the extreme, localization of a nonbonding pair of electrons (from the carboxyl oxygen of II or the imine nitrogen of IV) via σ bond formation to the electrophile (Y), as shown below, will generate a ground state containing a formal positive charge which is delocalized over the π-bonding framework.[8,9]



Such a ground state bonding arrangement should provide an approximate upper limit (ignoring solvent effects) on the magnitude of the spectral shift in the absorption maximum. Ethylation of II (viz. Table I and Figure 2b) undoubtedly involves localization of a nonbonding electron pair from the carbonyl oxygen into a σ bond.

The data in Table I show that the absorption of II is considerably red-shifted in strongly hydrogen bonding milieu relative to polar aprotic solvents (compare formamide, 398 nm, and dimethylformamide, 383 nm). Indeed, it is clear that solvents which are capable of forming strong hydrogen bonds give rise to relatively low energy electronic transitions. Nevertheless, the magnitude of these red shifts is much smaller than those observed for the Lewis acid complexes.

The choice of IV as a spectroscopic model for the interaction of the alcohol dehydrogenase active site zinc ion with II is based on the following three suppositions: (1) coordination of IV involves a metal–nitrogen bond to the imine nitrogen (N13); (2) the 2′-aminopyridyl moiety does not contribute appreciably to the long wavelength $\pi^* \leftarrow \pi$ transition of the *trans-N,N*-dimethylaminocinnamyl chromophore; (3) the $\pi^* \leftarrow \pi$ transitions of II and IV are similar in energy and exhibit qualitatively similar electron density distributions in both the ground state and the excited state.

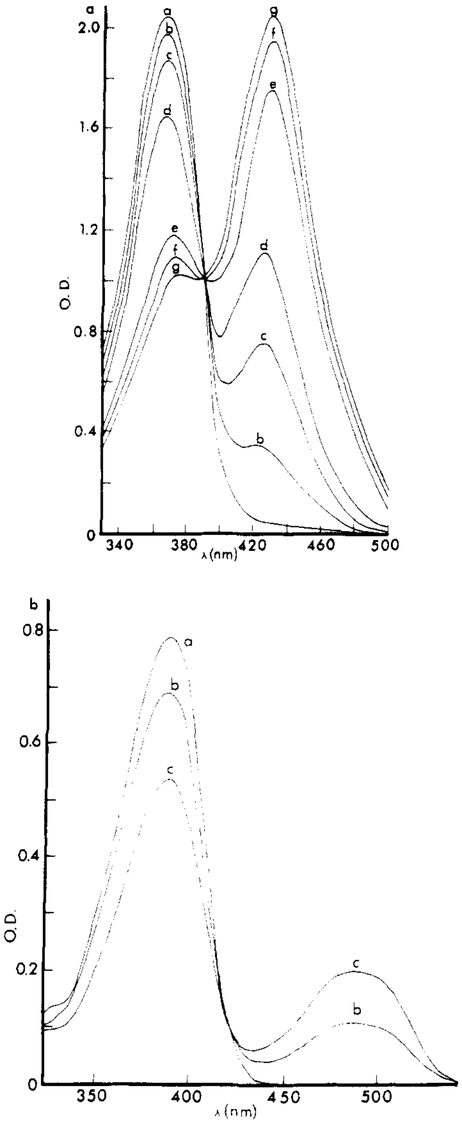


FIGURE 2: (a) Comparison of the spectrum of 61 μM *trans*-4-*N,N*-dimethylaminocinnamaldehyde (II) (trace a) with the spectra obtained on the addition of anhydrous $ZnCl_2$ to II in anhydrous ethyl ether at 25 °C (traces b–g). $ZnCl_2$ concentrations: trace b, ~4 mM; trace c, ~28 mM; trace g, ~32 mM. The 1-cm pathlength cuvette was equipped with a serum cap and additions were made via a microliter Hamilton syringe (see Experimental Section). (b) Comparison of the spectrum of 0.25 mM II (trace a) with the spectra obtained on reaction of II with triethyloxonium fluoroborate in anhydrous methylene chloride. Triethyloxonium fluoroborate concentrations: trace a, 0; trace b, ~0.2 mM; trace c, ~5 mM. Spectra were taken by transfer of 0.15-mL aliquots to demountable 0.10-mm pathlength cuvettes.

The x-ray structure of V (Figure 3a,b) demonstrates that the abovementioned UV-vis spectral shift which occurs when zinc ion binds to IV has its origins in the formation of an inner sphere coordination bond between zinc ion and the imine nitrogen (N13). The close similarity of the spectrum of the zinc complex to the spectra of the cobalt(II), nickel(II), and manganese(II) complexes strongly indicates similar modes of bonding in these complexes (viz., Table II).

The distorted tetrahedral arrangement of the donor atoms in the complex (viz., Figure 3a,b) and the chemical nature of the donor atoms in many respects are similar to the environment found for the alcohol dehydrogenase active site zinc ion (Brändén et al., 1973). The enzyme-bound active site zinc ion is four-coordinate and the four ligands are arranged in a distorted tetrahedral array about the metal ion. Three of the ligands are derived from amino acid side chains (two cysteinyl sulfhydryls and one histidyl imidazolyl group). The fourth ligand in the native enzyme appears to be a water molecule derived from aqueous milieu. Note that this ligand field involves

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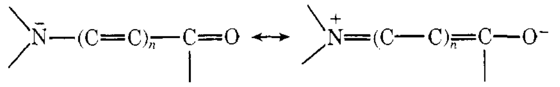
[8] Davidson and Jencks (1969) have pointed out that conjugated systems such as II exhibit chemical and electronic properties which are similar to amides and therefore can be considered to be "vinylogous amides", i.e., compounds . . . "in which the carbonyl and amino groups of the amide are separated by a conjugated system of double bonds, through which the normal amide resonance can occur":



$$>\overset{-}{N}-(C{=}C)_n-\underset{|}{C}{=}O \longleftrightarrow >\overset{+}{N}{=}(C-C)_n{=}\underset{|}{C}-O^-$$

[9] Note that in aqueous media, the localized Coulombic charge field produced as a consequence of protonation or alkylation of the dimethylamino nitrogen of II can be dissipated via ion–dipole interactions involving ordered water molecules in the first and second solvent shells about the ion. No such dissipation of charge is possible in methylene chloride. Hence, alkylation at the carbonyl oxygen of II becomes the energetically more favorable process in this solvent because the resulting charge is extensively delocalized over the conjugated system. Similar solvent-dependent changes in apparent basicity have been noted for other systems (Jencks, 1969; Hine, 1962; Ingold, 1969).

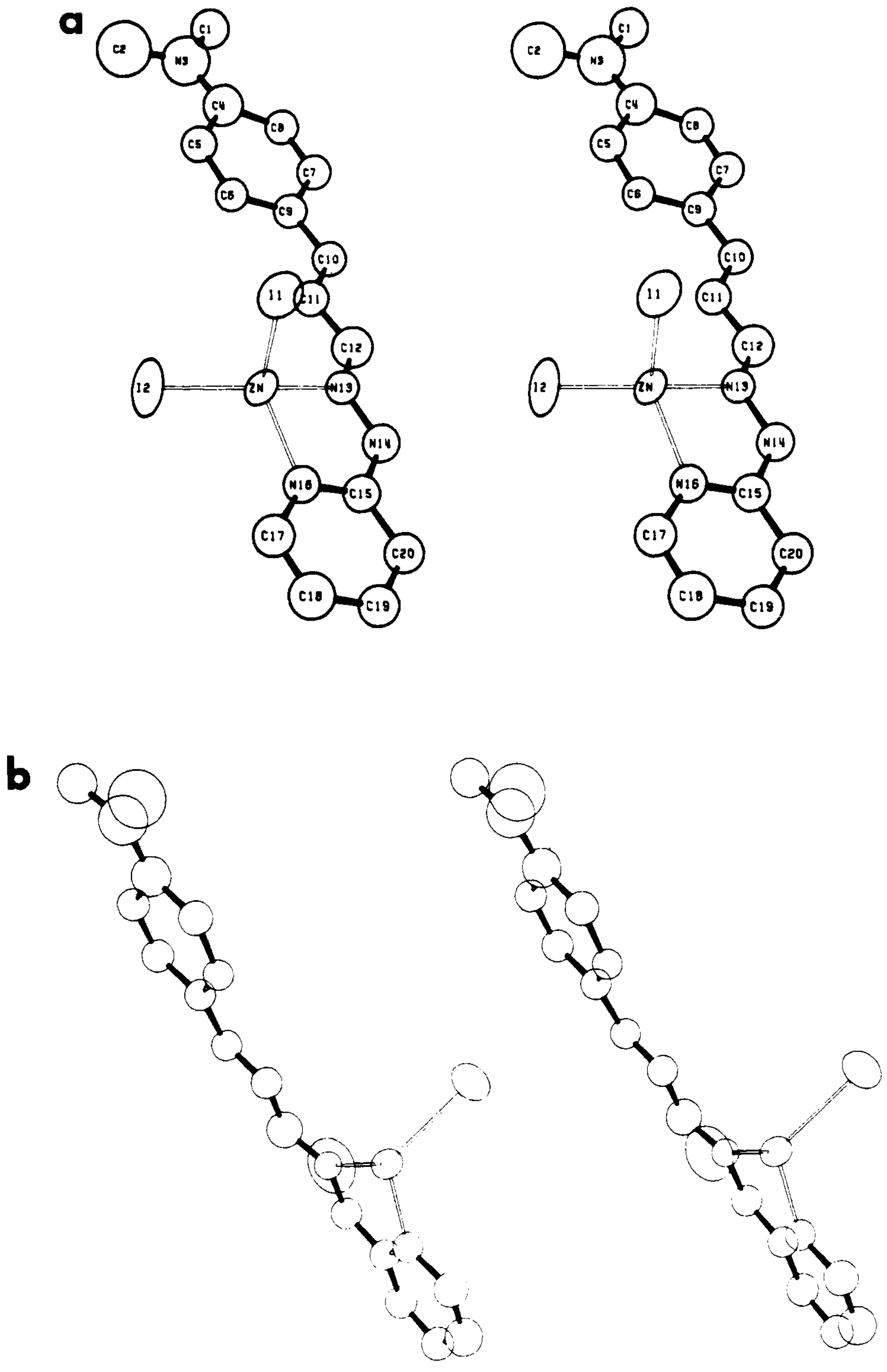


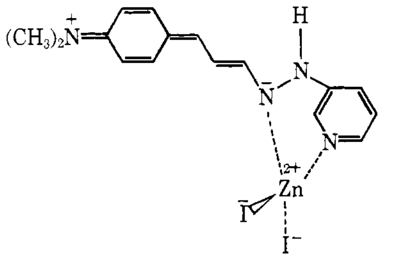
FIGURE 3: (a) Stereo drawing of V viewed perpendicular to the plane of the pyridine ring. (b) Stereo drawing viewed parallel to the plane of the pyridine ring. These structures were computer drawn using the coordinates listed in Table III.

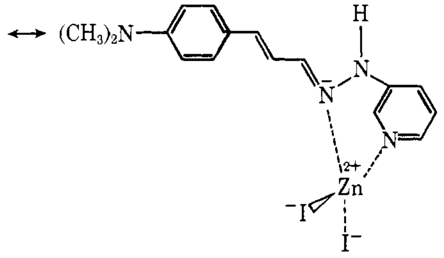
two highly polarizable thiolate anions and one sp^2-hybridized nitrogen, while the zinc ion complex of IV contains two highly polarizable iodide ions and one sp^2-hybridized nitrogen (N16) in addition to the metal-nitrogen bond to "substrate" at the fourth coordination site (via Figure 3a,b).

The supposition that contributions of the 2′-aminopyridyl group to the long wavelength $\pi^* \leftarrow \pi$ transitions of IV in the zinc complex are negligible is supported by two observations: (1) the spectrum of the hydrazone derived from acetaldehyde (compound VIII, see Table II) exhibits only a small (2 nm) red-shift when complexed to zinc ion; (2) the interatomic distances for the zinc complex of IV indicate that the N13–N14 nitrogen-nitrogen bond (1.40 (3) Å) is essentially a single bond (Liminga and Olovsson, 1964). Therefore (as expected on chemical grounds), the conjugated π-bonding framework of the *N,N*-dimethylaminocinnamyl group is insulated from the π-bonding framework of the 2′-aminopyridyl system by this single bond.

The x-ray structure provides a partial explanation for the spectroscopic properties. The interatomic distances for N3–

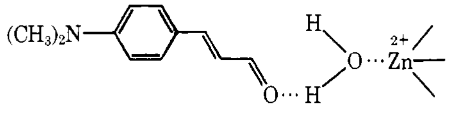
C4,[10] C5–C6, and C7–C8 indicate a localization of double-bond character. Also note that the dimethylamino group is nearly coplanar with the ring. Similar bonding interactions have been noted for other aniline derivatives where extensive delocalization of the nonbonding electrons on nitrogen occurs (Trueblood et al., 1961; Wuok and Trotter, 1965; Bachechi and Zambonelli, 1973; Hlavatá, 1971). Thus the x-ray results suggest that a quinodial "resonance" form (IX) is an important contributor to the electronic ground state of the zinc complex.


IX

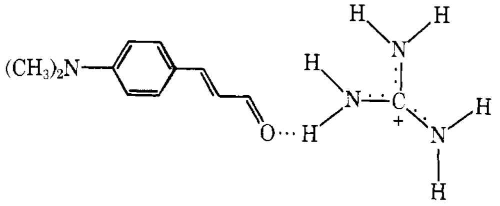

X

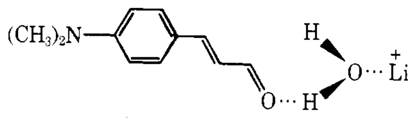
The reaction of *trans*-4-*N*,*N*-dimethylaminocinnamaldehyde (II) with the horse liver alcohol dehydrogenase–NADH complex proceeds via the rapid, reversible, pH-independent formation of a chemical intermediate (λ_{max} 464 nm). Although the presence of bound NADH is essential to the formation of the intermediate, and II is bound within van der Waal's contact distance of the nicotinamide moiety, the coenzyme is not chemically altered in this step (Dunn and Hutchison, 1973; Dunn et al., 1975).

The pH-independent nature of both the spectrum of the intermediate and the kinetics of intermediate formation virtually eliminates any explanation for the intermediate based on the protonation of II (Dunn and Hutchison, 1973). Consequently, it was proposed that coordination of the carbonyl oxygen of II to the active site zinc ion (as depicted in Diagram I) is the only reasonable structure for the intermediate that also explains the above-cited chemical and physical properties. Nevertheless, this structural assignment, in the absence of a high resolution x-ray structure for the enzyme complex, is based primarily on circumstantial evidence. The work presented in this study effectively rules out an alternative explanation for the spectrum of the intermediate not previously considered in detail, a structure involving a strong hydrogen bond to the carbonyl oxygen of II. The mechanism proposed for alcohol dehydrogenase catalysis by Sloan et al. (1975) involves the zinc-bound water molecule as a general acid catalyst in the facilitation of aldehyde reduction (see eq 5–7). This proposal suggests an alternative structure for the intermediate in which the spectral red shift arises from a strong hydrogen bond between the carbonyl oxygen of II and the zinc-coordinated water molecule as depicted below:


XI

Note, however, that strongly hydrogen bonding solvents such as trifluoroethanol, 8 M urea, water, and formamide do not bring about a sufficient shift in the absorption maximum of II to account for the spectrum of the intermediate via this hypothesis. Furthermore, the shifts observed in solvent systems which include both a strong hydrogen bonding capability and a proximal positive charge (e.g., 4 M guanidinium sulfate and 10 M LiCl), and which presumably interact with II as shown below, do not differ significantly from those found for the nonionic systems (e.g., trifluoroethanol, 8 M urea, water, and formamide). For these reasons, we conclude that a structure such as XI cannot account for the spectral properties of the intermediate.


XII


XIII

The UV-vis spectral studies of the two model systems discussed above together with the x-ray structure of the zinc iodide complex of IV constitute strong additional evidence for a structure for the enzyme-bound intermediate (III) involving inner sphere coordination of the carbonyl oxygen of II to the active site zinc ion.

Supplementary Material Available

Structure factor tables (8 pages). Ordering information is available on any current masthead page.

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[10] Comparison of amino nitrogen–carbon bond lengths for nitroanilines shows a bond length of 1.371 Å for *p*-nitroaniline (Trueblood et al., 1961) and a bond length of 1.358 Å for *p*-nitro-*N*,*N*-dimethylaniline (Wuok and Trotter, 1965), whereas Palm (1966) reported a C–N bond length of 1.40 Å for *p*-chloroaniline, Dattagupta and Saha (1973) reported a C–N bond length of 1.436 Å for the C–N bond of the *p*-*N*,*N*-dimethylaminobenzaldehyde hydrobromide salt, and Bachechi and Zambonelli (1973) have reported a C–N bond length of 1.380 Å for the dimethylamino nitrogen–carbon bond of *p*-*N*,*N*-dimethylaminobenzaldehyde oxime. Note that these bond lengths are shorter than a normal C–N single bond (1.47 Å). The decrease in bond length undoubtedly is due to the sp^2 character of nitrogen in these aromatic systems (Trueblood et al., 1961).

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