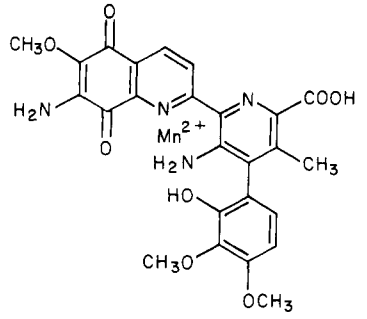
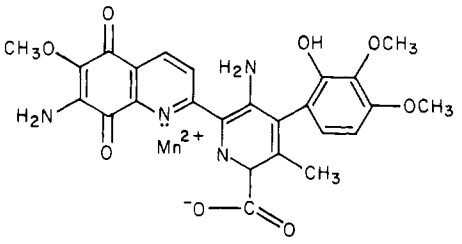


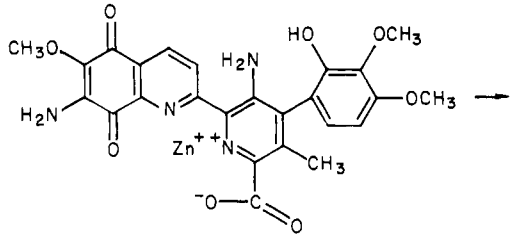
Ia



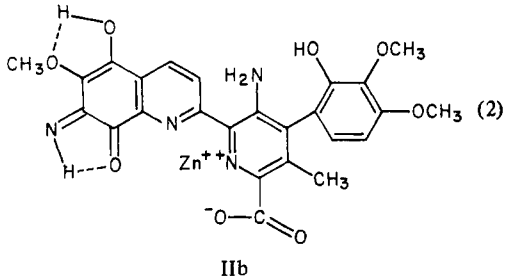
Ib

hard metal ions (such as Zn^{2+}), where charge–charge interactions are necessary to provide tight complexation.[10]

The observed differential catalytic roles exhibited by the complexing metal ions further imply that not only are the binding sites likely to be different but the structure of the redox-active aminoquinoline quinone moiety also appears to be perturbed. Previous studies have clearly indicated that the integrity of the *p*-quinone function is essential for effective biological activity of streptonigrin.[11] Thus, coordination of the quinone oxygen by an activating metal such as copper(II) could readily account for the enhanced rate of chemical reduction via stabilization of the developing negative charge. Similar interaction could explain the greater ease with which the electrochemical reduction of the copper complex takes place. The inhibitory effect of Zn^{2+} toward streptonigrin reduction on the other hand might be explicable in terms of a metal ion assisted tautomeric shift (eq 2), transforming

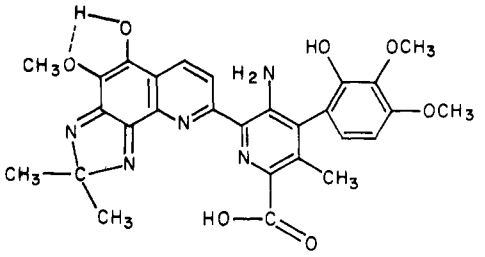


IIa



IIb

the *p*-quinone into an aza-substituted *o*-quinoid structure (IIb), isoelectronic with the biologically inactive isopropylideneazastreptonigrin III and its related *o*-quinoid analogues.[11] While



III

this as yet tentative assignment is certainly consistent with the spectral, chemical, and electrochemical reduction data obtained for the streptonigrin zinc complex, precedents for such tautomerism have been implied in previous investigations involving related aminoquinone derivatives.[12] Additional evidence in support of IIb comes from preliminary ^{13}C NMR experiments using the zinc complex of streptonigrin in dimethyl sulfoxide.[13a] We have observed that the carbon resonances which are shifted most on addition of excess zinc chloride are of $C_{2'}$ $C_{3'}$, COOH, C_6, and C_{10}[13b] sites which are likely to be involved in the structural changes suggested above.

The preparation and characterization of these and other streptonigrin–metal complexes present a new and exciting way to investigate and modify the chemical and biological properties of this interesting and potent antitumor antibiotic. Studies of complexes involving a series of other biologically occurring metal ions in conjunction with streptonigrin as well as with smaller model systems are currently under way in our laboratory.

Acknowledgment. We thank Dr. Steven J. Gould of the University of Connecticut and Dr. Steven M. Weinreb of Pennsylvania State University for stimulating discussions, Dr. Michael J. Clarke of Boston College for access to his electrochemical apparatus, and Dr. John D. Douros of Drug Research and Development, Chemotherapy, NCI, for a generous gift of streptonigrin. This work was supported by the Research Corporation and by Grant No. 1424-C-1 of the American Cancer Society, Massachusetts Chapter.

(10) The complexation pattern following the Irving–Williams series appears to be relevant here, particularly considering the observed difference in binding constants obtained for the Zn(II) and Cu(II) complexes of ethylenediamine and glycine, respectively. Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201. For more detailed discussion, see: Angelici, R. J. In "Inorganic Biochemistry"; Eichhorn, G. L., Ed; Elsevier: New York, 1973; pp 63–101.

(11) (a) Kremer, W. B.; Laszlo, J. *Cancer Chemother. Rep.* **1967**, *19*, 51. (b) Driscoll, J. S.; Hazard, G. F.; Wood, H. H.; Goldin, A. *Ibid.* **1974**, *4*, 1–362.

(12) Liao, T. K.; Nyberg, W. H.; Cheng, C. C. *J. Heterocycl. Chem.* **1976**, *13*, 1063–1065 and references therein. These authors were the first to propose explicitly that the aminoquinone moiety of the streptonigrin A ring is subject to acid-catalyzed tautomerism, leading to a structure analogous to the quinoline quinone moiety in IIb.

(13) (a) We have reproduced the ^{13}C NMR spectra reported by Gould et al. [Gould, S. J.; Chang, C. C. *J. Am. Chem. Soc.* **1978**, *100*, 1624–1626] under identical conditions. (b) The C_{10} is the quaternary carbon, ortho to the carbonyl which is adjacent to the methoxy group. The ^{13}C NMR spectra of the ligand and the complex were obtained in collaboration with Dr. D. J. Sardella of our department and will be published separately.

Resonance Enhanced Raman Identification of the Zinc–Oxygen Bond in a Horse Liver Alcohol Dehydrogenase–Nicotinamide Adenine Dinucleotide–Aldehyde Transient Chemical Intermediate

Paul W. Jagodzinski* and Warner L. Peticolas

Department of Chemistry and Institute of Molecular Biology
University of Oregon, Eugene, Oregon 97403

Received August 8, 1980

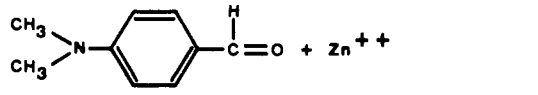
Aromatic aldehydes have been recently used by several investigators as model substrates for horse liver alcohol dehydrogenase (LADH, EC 1.1.1.1.).[1-6] We have found that the

(1) Bernhard, S. A.; Dunn, M. F.; Luisi, P. L.; Schack, P. *Biochemistry* **1970**, *9*, 185–192.

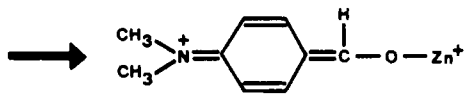
0002-7863/81/1503-234$01.00/0

aldehyde (dimethylamino)benzaldehyde (DABA) is readily converted into (dimethylamino)benzyl alcohol in the presence of LADH and the coenzyme nicotinamide adenine dinucleotide (NADH) in the pH range 7.0–9.0. We have also found that DABA in pH 9.6 0.1 M pyrophosphate buffer forms a stable LADH/NADH/DABA transient chemical intermediate (TCI) as has been shown by earlier work on the substrate (dimethylamino)cinnamaldehyde (DACA).[2] The ultraviolet electronic absorption spectrum of DABA alone in aqueous solution has an absorption maximum at 352 nm which shifts to 380 nm in the stable TCI complex. However, the red trailing edge of the absorption band extends into the 400–430-nm region. This ternary complex gives an excellent, highly structured, preresonant Raman spectrum when excited by the 488.0-nm line of an argon ion laser with the following concentrations: 1×10^{-3} N LADH, 1×10^{-3} M NADH, and 0.7×10^{-3} M DABA.

As a model compound for this TCI complex the procedure of Angelis and co-workers has been followed, and we have studied the interaction of DABA with $ZnCl_2$ in anhydrous methylene chloride.[7] Under these conditions the Zn(II) appears to react with the DABA to form a zinc–oxygen bond with the absorption maximum of DABA shifting from 337 to 385 nm upon complexation. This reaction may be written schematically as



DABA



DABA–Zn Complex

In this schematic reaction the zinc is given a nominal charge of 2+ in order to indicate the transfer of positive charge from the zinc to the nitrogen when the complex is formed. However, the actual charge on the zinc will depend upon its coordination to the available anions. Upon complexing with the zinc in methylene chloride, the DABA exhibits a rather marked change in its resonant Raman spectrum. Particularly striking is the complete disappearance of the carbonyl stretching line at 1664 cm^{-1}. Furthermore the DABA–Zn complex in methylene chloride shows the same resonance Raman lines as does the LADH/NADH/DABA TCI complex in aqueous buffer described above. Since these Raman spectra exhibit a very large number of sharp lines between 200 and 1800 cm^{-1}, it is not possible to reproduce them in this brief communication. However, in Table I we list the strongest and most significant Raman lines in these spectra.

Due to the disappearance of the carbonyl stretching line in the model complex, we feel that an inner-sphere complex has been formed. The similarity of the spectra of the protein and model systems indicates inner-sphere complexation in the protein. In addition, this model complex exhibits a line at 386 cm^{-1} which is absent in the spectrum of the DABA alone and may be assigned to the Zn–O stretching vibration since it is well-known to occur in this frequency region.[8] Indeed, this line falls within the region predicted for $Zn(H_2O)_6^{2+}$ (390 ± 5 cm^{-1}).[8] A plausible alternative assignment would have this new line arising from a low-frequency bending mode. However, based on the rest of the lines observed in this Raman spectrum of the complex, we feel that the origin of this line is the Zn–O stretching mode. The low-frequency line observed at 354 cm^{-1} probably shifts to 340 cm^{-1} and not to 386 cm^{-1} in the spectrum of the complex.

Table I. Major Raman Lines (cm^{-1}) for DABA, the DABA-Zn Complex, and the DABA/NADH/LADH Ternary Complex[a]

DABA[b]	DABA-Zn[b]	DABA-NADH-LADH[c]	tentative assignments
1664 (49)[d]		1663 (100)[e]	C=O stretching
-----	1626 (82)	---1623 (35)	ring, C=C stretching, C=N stretching
1601 (100)		1606 (8)	ring
-----	1588 (65)	--- 1579 (58)	C=N stretching
1558 (34)			
-----	1545 (40)	---1547 (24)	
1534 (11)			C=N stretching[f]
1440 (19)	1444 (35)	1445 (58)	
	1418 (24)	~1420 (8)	
1397 (15)	1401 (100)	1396 (55)	C–N stretching
1375 (8)	1379 (45)	~1380 (20)	
	1356 (24)		
1246 (15)			
1170 (62)	1180 (20)	1174 (14)	
		1021 (35)	LADH (tryptophan)
		1004 (72)	LADH (phenylalanine), buffer
	997 (13)		C–O stretching
948 (3)	944 (9)	944 (15)	
836 (17)	846 (69)	852 (35)	
	386 (26)	368 (13)	Zn–O stretching
354 (21)	340 (12)		

[a] DABA, (dimethylamino)benzaldehyde; NADH, nicotinamide adenine dinucleotide, reduced; LADH, liver alcohol dehydrogenase. [b] Methylene chloride solvent. [c] Pyrophosphate buffer, pH 9.6. [d] Relative intensities in parentheses. [e] Contributions from LADH and resonance enhanced NADH (Rodgers and Peticolas, unpublished results) make this intensity anomalously high. [f] Possibly due to delocalization in the ground state of DABA.

In addition to the Zn–O vibration, there are a number of lines present in the spectrum of the DABA–Zn complex which are missing in the spectrum of the DABA alone. These include very strong, sharp lines at 1626, 1588, and 1545 cm^{-1}. Furthermore, drastic intensity changes are noted for the lines at 1401, 1379, and 846 cm^{-1}. In addition to the line at 1664 cm^{-1}, Raman lines at 1601 and 1558 cm^{-1} are missing in the spectrum of the DABA–Zn complex. These remarkable changes in the vibrational spectrum are consistent with the change in electronic structure for DABA upon complexation with zinc.

As can be seen in Table I, the LADH/NADH/DABA TCI complex exhibits all of the strong Raman lines of the DABA–Zn model compound, including that which we have assigned to the Zn–O bond. This vibration, at 368 cm^{-1}, is lower in frequency than in the model compound and higher than the low-frequency mode at 354 cm^{-1} for DABA alone. This indicates that the Zn–O bond in the TCI complex is not quite as strong as in the model compound or that in the protein–substrate complex we are observing a mixture of free and bound DABA. The very strong sharp lines at 1623, 1579, 1547, and 1396 cm^{-1} show clearly that the electronic structure of the DABA in the protein complex is identical with that in the model compound and that the molecular force field of the DABA has changed markedly.

The resonance Raman spectrum of the intermediate supports the conclusion that the zinc withdraws electron density from the aldehyde oxygen, forming a strong zinc–oxygen coordinate covalent bond, leading to a positive charge on the carbon of the aldehyde moiety which can accept hydride transfer from the coenzyme NADH. The structure of DABA is such that the zinc complex is sufficiently stable due to internal electronic rearrangement to a quinoidlike structure so that it can even be prepared in organic solvents such as CH_2Cl_2. However, with other substrates the protein portion of the LADH probably plays an important role in stabilizing the complex.

(2) Dunn, M. F.; Hutchison, J. S. *Biochemistry* **1973**, *12*, 4882–4892.
(3) Dunn, M. F.; Biellmann, J.-F.; Branlant, G. *Biochemistry* **1975**, *14*, 3176–3182.
(4) Morris, R. G.; Saliman, G.; Dunn, M. F. *Biochemistry* **1980**, *19*, 725–731.
(5) Koerber, S. C.; Schack, P.; Au, A. M.-J.; Dunn, M. F. *Biochemistry* **1980**, *19*, 731–738.
(6) Anderson, D. Ph.D. Dissertation, University of Oregon, Eugene, OR, 1980.
(7) Angelis, C. T.; Dunn, M. F.; Muchmore, D. C.; Wing, R. M. *Biochemistry* **1977**, *16*, 2922–2931.
(8) Kanno, H.; Hiraishi, J. *J. Raman Spectrosc.* **1980**, *9*, 85 and references therein.

Although resonance Raman spectroscopy has been shown to be useful in the characterization of both enzymes and enzyme–substrate complexes, we believe that we have now been able to identify a metal–substrate bond in a protein–substrate complex. This result supports a protein–substrate structure with the carbonyl oxygen bound directly to the catalytic zinc in LADH as championed by Brändén and Eklund[9] and Dunn.[1-5] However, the evidence for the formation of the Zn–O bond and loss of the carbonyl moiety is much stronger.

Acknowledgment. Support of this work by NSF Grant PCM76-82222 and PHS Grant GM15547 is gratefully acknowledged.

(9) Brändén, C.-I.; Eklund, H. In "Molecular Interactions and Activity in Proteins"; *Ciba Found. Symp. Excerpta Med.* **1978**, *60*, 63–80.

Convenient Thermal Sources of β-Cyanoalkyl Radicals from α-Hydroxydiazenes

Avtar S. Nazran and John Warkentin*

Department of Chemistry, McMaster University Hamilton, Ontario, L8S 4M1, Canada

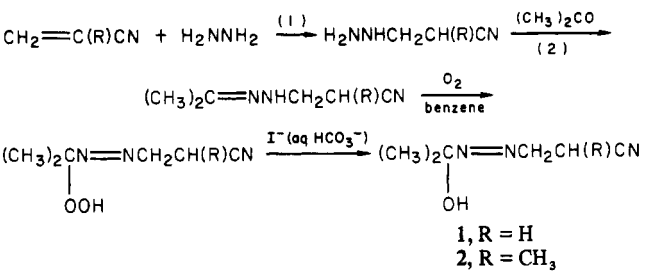
Received July 22, 1980

A general effect of the structural change $RCH_2H \rightarrow RCH_2X$, where X is a substitutent, is to lower the bond dissociation energy of the α C–H bonds.[1-3] A common consequence of this feature is that homolytic abstractions α to X, rather than β or further removed from X, are kinetically favored.[4,5] Well-known examples include the strong preference for radical substitution on ethylbenzene, ethanol, and propionitrile to form, respectively, 1-phenylethyl, 1-hydroxyethyl, and 1-cyanoethyl radicals instead of 2-phenylethyl, 2-hydroxyethyl, and 2-cyanoethyl radicals. Precursors for relatively inaccessible radicals of the latter types are therefore of considerable interest, especially if they afford those radicals uncontaminated by their normally favored isomers under mild conditions. We wish to report convenient thermal sources of 2-cyanoethyl and 2-cyanopropyl radicals and a preliminary synthetic application of one of those radicals.

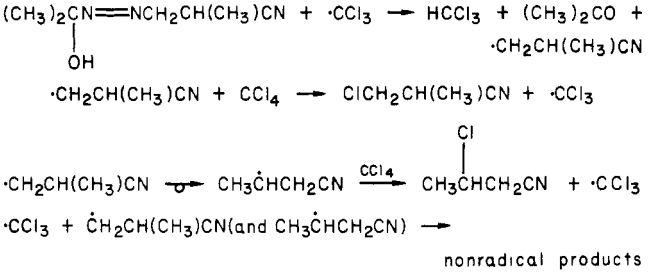
α-Hydroxydiazenes **1**, **2** (also known as azocarbinols) were synthesized by the route shown in Scheme I.[6] The alkylhydrazines from the first step (70–80% yield) were distilled (4 torr, pot temperature 130 °C) to remove products of overalkylation. Alkylhydrazones (ca. 80% yields) from the second step were distilled under the same conditions. Omission of these purification steps led to induction periods of hours or days for the subsequent autoxidation step.

Hydroperoxide intermediates were not isolated, except in small quantities for ^{1}H NMR spectra,[7] but were decomposed[8] directly

Scheme I



Scheme II



after concentration of the solutions with a rotary evaporator (**Caution**: do not heat or remove all solvent). Azocarbinols **1** and **2** were identified from their thermolysis chemistry, infrared spectra (CN and OH), and ^{1}H NMR spectra. **1**: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.45 (s, 6 H), 2.83 (t, 2 H, J = 6 Hz), 4.18 (t, 2 H, J = 6 Hz), 4.33 (s, 1 H). **2**: δ 1.43 (d, 3 H, J = 7 Hz), 1.48 (s, 6 H), 3.25 (m, 1 H), 4.00 (d, 2 H, J = 7 Hz), 4.28 (s, 1 H). A trace of acetone, from spontaneous decomposition of the azocarbinols, could be detected from the ^{1}H NMR spectra of freshly prepared **1** and **2**.

In degassed CCl_4, in a sealed tube, **1** and **2** decompose slowly at room temperature. Decomposition is first order in azocarbinol with rate constant $k_{35}(\mathbf{2}) = 1.4 \times 10^{-5}$ s^{-1}. Major products from **1** are acetone (84%), chloroform, and 3-chloropropionitrile (84%). Similarly, **2** affords acetone (81%), chloroform (87%), 3-chloro-2-methylpropionitrile (~46%), and 3-chlorobutyronitrile (~46%). The latter is an expected product from rearrangement of the primary 2-cyano-1-propyl radical to the 1-methyl-2-cyanoethyl radical.[9,10]

Decompositions of **1** and **2** in degassed benzene are about 3-fold slower than those in CCl_4 and produce cyanoalkanes and acetone as major products. Inhibition of the decomposition of **2**, in benzene, by added free radicals diphenylpicrylhydrazyl (DPPH) or 2,2,6,6-tetramethylpiperidinyl-1-oxy (TMPO) indicates that a chain mechanism is involved. Thus, decomposition of **2** (0.271 M) at 35 °C, in the presence of DPPH (1.07×10^{-2} M), had an induction period of 110 min. Similarly, with **2** (0.361 M) and TMPO (3.97×10^{-2} M) at 80 °C, decomposition did not begin until 30 min had elapsed. Corresponding samples not containing added free radical started smoothly without detectable induction periods.

Heating a solution of **1** (0.282 g, 2.00 mmol) and azobenzene (1.46 g, 8.02 mmol) in benzene (5.0 mL) at 75 °C for 10 h

(1) Benson, S. W. "Thermochemical Kinetics", 2nd ed; Wiley: New York, 1976; p 309, for example.

(2) "Handbook of Chemistry and Physics", 59th ed; Weast, R. C., Ed.; CRC Press: Boca Raton, FL, 1978–1979; pp F-237–F-241.

(3) Notable exceptions are the fluoro and trifluoromethyl substituents which raise bond dissociation energies of α C–H bonds slightly or else leave them essentially unchanged.[2]

(4) Since there is no strong connection between thermodynamics and kinetics, there are many exceptions arising from dominance of polar and/or steric effects, over bond enthalpy effects, on the free energies of transition states.

(5) For a recent discussion of the effects of substituents on the kinetics of H abstraction from substituted toluenes, see: Fisher, T. H.; Meierhoefer, A. W. *J. Org. Chem.* **1978**, *43*, 224 and references cited there.

(6) Autoxidation of hydrazones has been extensively studied. See, for example: (a) Pausacker, K. H. *J. Chem. Soc.* **1950**, 3478. (b) Criegee, R.; Lohaus, G. *Chem. Ber.* **1951**, *84*, 219. (c) Belamy, A. J.; Guthrie, R. D. *J. Chem. Soc.* **1965**, 2788. (d) Taylor, W. F.; Weiss, H. A.; Wallace, T. J. *J. Org. Chem.* **1969**, *34*, 1759. (e) Schulz, M.; Missol, U. *Z. Chem.* **1974**, *14*, 265.

(7) ^{1}H NMR spectra (90 MHz, $CDCl_3$) of the hydroperoxides and their precursors: $(CH_3)_2C(OOH)N{=}NCH_2CH_2CN$—δ 1.47 (s, 6 H), 2.83 (t, 2 H, J = 6 Hz), 4.18 (t, 2 H, J = 6 Hz), 9.08 (s br, 1 H); $(CH_3)_2C(OOH)N{=}NCH_2$ $CH(CH_3)CN$—δ 1.38 (d, 3 H, J = 7 Hz), 1.50 (s, 6 H), 3.25 (m, 1 H), 4.00 (d, 2 H, J = 7 Hz), 9.05 (s, 1 H); $(CH_3)_2C{=}NNHCH_2CH_2CN$—δ 1.88 (s, 3 H), 1.95 (s, 3 H), 2.65 (t, 2 H, J = 6 Hz), 3.43 (t, 2 H, J = 6 Hz), 4.75 (s br, 1 H); $(CH_3)_2C{=}NNHCH_2CH(CH_3)CN$—δ 1.30 (d, 3 H, J = 7 Hz), 1.75 (s, 3 H), 1.93 (s, 3 H), 3.05 (m, 1 H), 3.25 (d, 2 H, J = 7 Hz), 4.85 (s br, 1 H).

(8) Triphenylphosphine is an alternative reagent for converting hydroperoxides to alcohols under mild conditions.[6e]

(9) Intramolecular radical additions to the nitrile function are well-known[10] and homopropargyl radicals, which are close analogues of 2-cyanopropyl radicals, rearrange with ease: Ingold, K. U.; Warkentin, J. *Can. J. Chem.* **1980**, *58*, 348.

(10) Beckwith, A. L. J.; Ingold, K. U. In "Rearrangements in Ground and Excited States"; de Mayo, P., Ed.; Academic Press: in press.

0002-7863/81/1503-236$01.00/0