The barrier to the *sec*-butyl to *tert*-butyl cation rearrangement is high (E_a = 18 kcal mol^{-1}).[13] Nevertheless, the *tert*-butyl cation has been reported as a major byproduct in the reaction of *sec*-butyl halides with SbF_5 in solution at low temperature. Localized heating owing to ion formation has been suggested as the cause.[15] However, formation of the *sec*-butyl cation in the solid at temperatures below −85 °C results in only small amounts of the *tert*-butyl cation rearrangement product.[8]

The broad lines (150–500 Hz) of these carbonium ion spectra in solid SbF_5 preclude use of the line-width parameter as a measure of the exchange rate near the fast exchange limit.[16] An evaluation of the barrier for the hydride shift (eq 1) must rest on the detection of signals indicating slow exchange. The −190 °C spectrum shows no convincing evidence for a "static" *sec*-butyl cation. This result would not be surprising if solution-state barriers apply in the solid state. Thus, a recent estimate of $\Delta G^{\ddagger} < 2.4$ kcal mol^{-1} for the degenerate process shown in eq 1 is too low to permit detection of the static ion at −190 °C.[17] However, the rate of a chemical exchange process involving very small changes in atomic coordinates can be dramatically suppressed in the solid state,[18] and as noted above, the rate of carbon scrambling in the *sec*-butyl cation (eq 2) has been suppressed in solid SbF_5. If one can assume that this kind of suppression is operative for the degenerate hydride shift of the *sec*-butyl cation (eq 1), the barrier in solution is considerably less than 2.4 kcal mol^{-1}. Extension of these spectral measurements to lower temperatures and improvement in resolution may allow a more detailed structural characterization of the *sec*-butyl cation.[16,19]

Acknowledgment. The skilled assistance of R. D. Kendrick, A. J. Kalbin, and C. W. Rein in the design and construction of the apparatus is gratefully acknowledged. The help and encouragement of Dr. A. Diaz throughout the course of this work is appreciated. We are also pleased to acknowledge the National Science Foundation for support in the form of a Summer Faculty research stipend to P.C.M.

(15) Saunders, M.; Cox, D. W.; Ohlmstead, W. *J. Am. Chem. Soc.* **1973**, *95*, 3018.
(16) Some very recent results with much larger fluorine decoupling fields (~15 G) indicated that significant line narrowing can be achieved. Nevertheless, some residual line broadening may be anticipated owing to the nature of the sample. SbF_5 is believed to yield a variety of polyanions upon reaction with alkyl halides. Thus, solid-state samples of carbonium ion salts that we have prepared may lack the order characteristic of polycrystalline materials, and identical carbons in physically different sites would then yield a distribution of chemical shifts.[1]
(17) Saunders, M.; Kates, M. R. *J. Am. Chem. Soc.* **1978**, *100*, 7082.
(18) Miller, R. D.; Yannoni, C. S. *J. Am. Chem. Soc.* **1980**, *102*, 7396.
(19) For a recent discussion of structural representations of the *sec*-butyl cation, see: Schleyer, P. v. R.; Lenior, D.; Mison, P.; Liang, G.; Prakash, G. K. S.; Olah, G. A. *J. Am. Chem. Soc.* **1980**, *102*, 683.

Interaction of Metal Ions with Streptonigrin. 1. Formation of Copper(II) and Zinc(II) Complexes of the Antitumor Antibiotic

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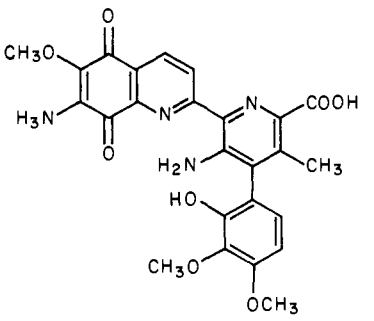
The aminoquinone antibiotic streptonigrin (I), a metabolite of *Streptomyces flocculus*,[1] is one of the most effective agents for the treatment of human cancers.[2] Though showing activity against lymphoma, melanoma, cancers of breast, cervix, head, and neck, as well as against viruses, streptonigrin has a number of undesirable side effects, including most severe bone marrow depression.[3] This high toxicity presently precludes the clinical use of the antibiotic.[4]



I

There is evidence that streptonigrin exerts its antitumor action via (1) interference with cell respiration and (2) disruption of cell replication.[4] Both mechanisms are thought to involve participation of metal ions[5] and require an electron source as well as oxygen. Although the chemistry of streptonigrin–metal complexes has not been delineated, Cu^{2+} and Fe^{2+} are known to accelerate streptonigrin-induced DNA scission, while Co^{2+} has been shown to be inhibitory in the same process.[6] Mechanistic elucidation of the metal complexes of streptonigrin is important not only for understanding the mode of action of the antibiotic but also for developing a rational approach to improve its chemotherapeutic properties.

In this communication we report that reaction between the antitumor antibiotic and copper(II) or zinc(II) halides leads to the corresponding divalent metal chelates, which exhibit *remarkably different spectroscopic, electrochemical, and chemical redox properties*. Thus, addition of anhydrous zinc(II) or copper(II) chloride to an acetonitrile solution of streptonigrin produces a deep-brown solution of the corresponding metal complex (eq 1).

$$\mathrm{HSN} + \mathrm{M^{2+}} \rightleftharpoons {}^{-}\mathrm{SN{\cdot}M^{2+}} + \mathrm{H^{+}} \qquad (1)$$
$$\mathrm{M^{2+}} = \mathrm{Cu^{2+}}, \mathrm{Zn^{2+}}$$

The spectra of the resulting metal derivatives differ from that of the antibiotic in a number of characteristic ways (Figure 1). For the zinc complex, the long-wavelength UV absorption is red shifted (375 → 400 nm) and its intensity enhanced in comparison with the spectrum of free streptonigrin. The 245-nm band of the parent antibiotic is split into a doublet, yielding a maximum at 235 nm and a shoulder at 255 nm, both decreased in intensity with respect to the absorption of the free ligand.

Complexation with copper(II) results in substantial broadening of the long-wavelength absorption (attributed to the quinoline quinone moiety) which is now centered at 415 nm. The pattern of the short-wavelength UV maxima remains quite similar to that of the parent antibiotic, except for a substantial decrease in the intensity of the 294-nm absorption band.

Formation of the complexes between streptonigrin and the metal ions is sensitive to the solvent and in aqueous media is strongly affected by the pH and the nature of the buffer.[7,8] Stepwise addition of $ZnCl_2$ to streptonigrin in 0.1 M Tris at pH 6.75 exhibits

(1) Rao, K. V.; Cullen, W. P. *Antibiot. Annu.* **1959**, 950.
(2) Davis, H. L.; VonHoff, D. D.; Henney, J. T.; Rozencweig, M. *Cancer Chemother. Pharmacol.* **1978**, *1*, 83.
(3) Wilson, W. L.; Labra, C.; Barrist, E. *Antibiot. Chemother. (Basel)* **1961**, *11*, 147.
(4) Lown, J. W. In "Bioorganic Chemistry"; Van Tamelen, E. E., Ed; Academic Press: New York, 1977; pp 95–121.
(5) Addition of chelating agents completely abolishes the biological activity of the drug. See: Bhuyan, B. K. In "Antibiotics"; Gottlieb, D.; Shaw, P. D., Eds.; Springer-Verlag: New York, 1967; p 175.
(6) Lown, J. W.; Sim, S. K. *Can. J. Chem.* **1976**, *54*, 2563–2572.
(7) For example, the dissociation constant of the streptonigrin–zinc complex in 0.1 M Tris, pH 7.0, was found to be 1.3 × 10^{-4} M, while in 0.0015 M citrate/0.015 M NaCl, pH 7.0, it is tenfold higher.
(8) Streptonigrin is most likely to be zwitterionic, at least in aqueous solution, yet considering the number of available protonation sites it is not clear where the H^+ is released when complexation takes place.

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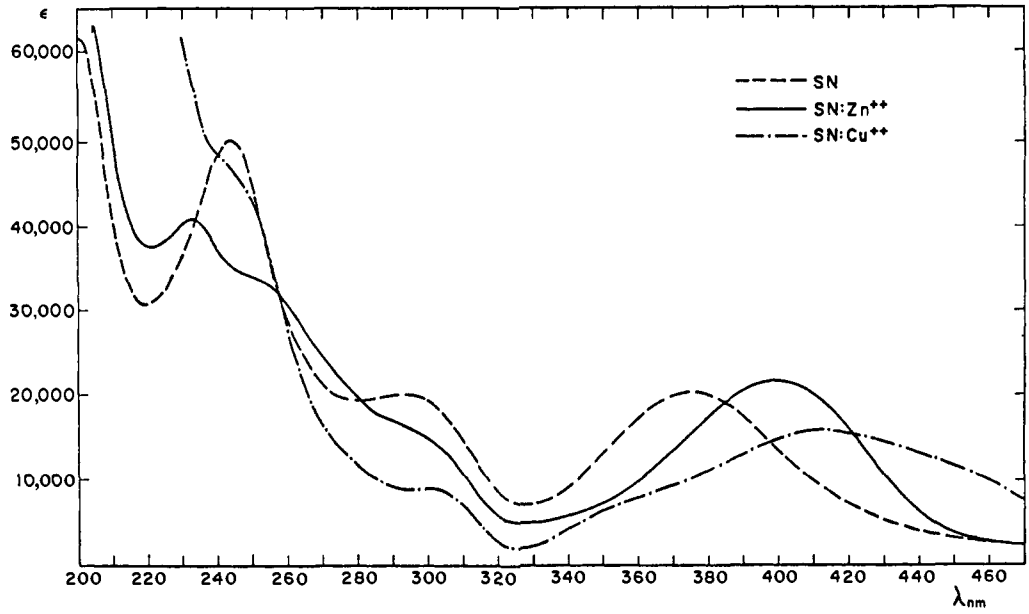


Figure 1. The spectra of streptonigrin and its copper(II) and zinc(II) chloride complexes in acetonitrile at 25 °C. The absorption of both complexes was determined with respect to reference solutions containing the same concentration of the corresponding metal halide.

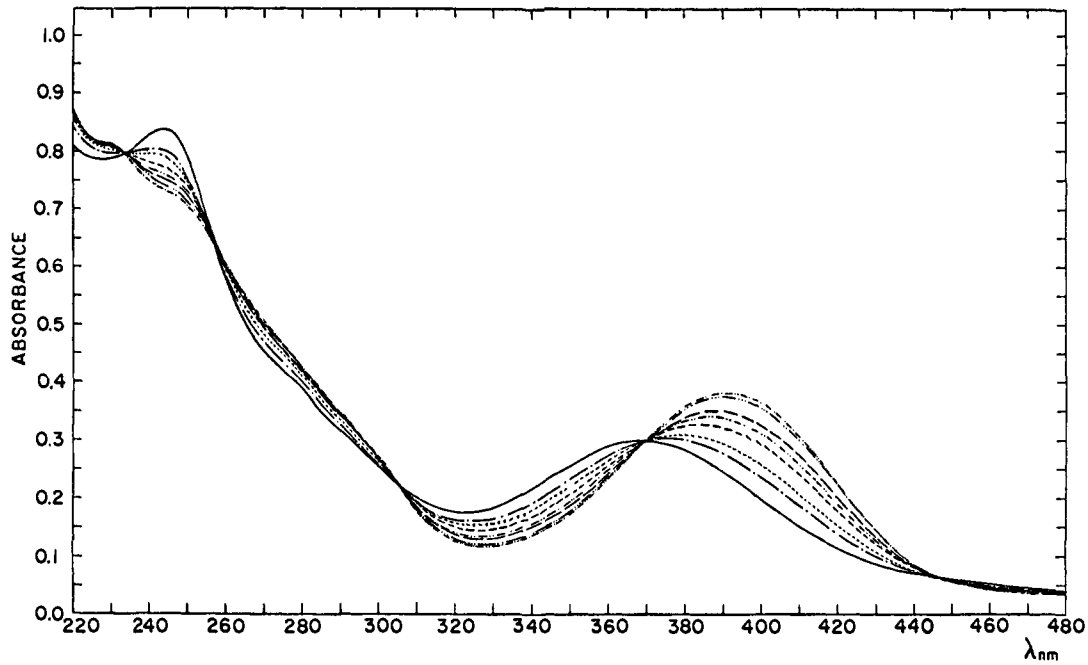


Figure 2. Complexation between streptonigrin and zinc chloride in 0.1 M Tris of pH 6.76 containing 8% acetonitrile. The curves correspond to (——) 2.1×10^{-5} M, (- - -) 5.6×10^{-5} M, (– – –) 1.4×10^{-4} M, (·····) 2.2×10^{-4} M, (— — —) 3.6×10^{-4} M, (·····) 7.2×10^{-4} M, and (·····) 1.1×10^{-3} M concentrations of $ZnCl_2$ in the solution.

five sharp isosbestic points (Figure 2). Potentiometric titration in 50% aqueous dioxane reveals that addition of 1 equiv of metal ion releases 1 mol of protons/mol of streptonigrin. The pK_a of the free ligand (determined to be 6.5 in 1:1 dioxane–water) is lowered by 2.3 and 3.3 pK_a units in the presence of 1 equiv of Zn^{2+} and Cu^{2+}, respectively.

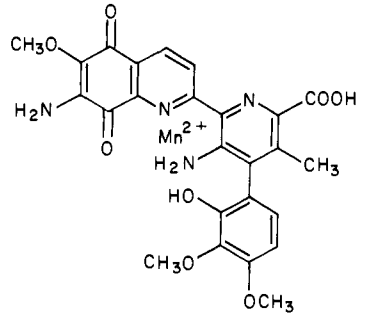
Electrochemical reduction of streptonigrin in acetonitrile (with 0.1 M tetrabutylammonium perchlorate as supporting electrolyte) yields a double wave, corresponding to two successive one-electron reductions. For the copper complex a similar type of cyclic voltammogram is obtained with the first reduction peak shifted 0.28 V toward less negative potentials. Complexation with zinc on the other hand results in a completely irreversible reduction pattern shifted by −0.7 V to more negative reduction potentials.

Chemical reduction of the streptonigrin complexes by *N*-alkyldihydropyridines in acetonitrile exhibits a similar pattern. The copper(II) complex of streptonigrin is reduced by *N*-benzyldihydronicotinamide more than 10^3 times faster[9] than the metal-free antibiotic. Zn^{2+}, on the other hand, inhibits the reaction. Since the reductant is stable in the presence of 1×10^{-4} M $CuCl_2$ for several hours, the reduction does not seem to involve the $Cu^{2+} + e^- \rightarrow Cu^+$ path.

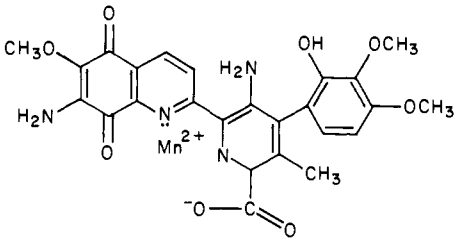
The spectroscopic data as well as the kinetic and electrochemical redox properties clearly indicate that the zinc and copper complexes of streptonigrin are structurally different. On the basis of these observations, we propose that the two different metal derivatives are likely to arise from complexation involving two different streptonigrin conformers, Ia and Ib.

For metal ions which are known to function as strong Lewis acids, including Cu^{2+}, the amine nitrogen of the pyridine C ring may serve as the donor atom, while the alternative binding site, including the picolinic acid moiety, may be functional in anchoring

(9) When 5×10^{-5} M *N*-benzyldihydronicotinamide is used, under pseudo-first-order conditions (with excess reductant), the reaction is too fast to be measured by conventional spectrophotometry.



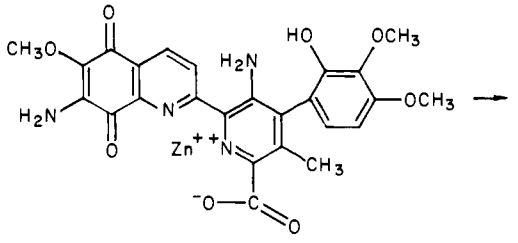
Ia



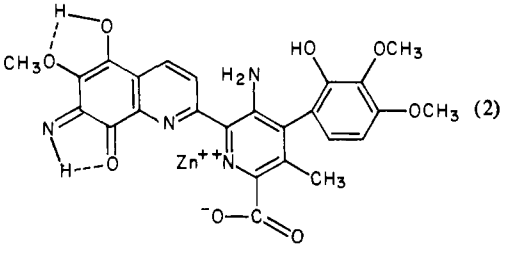
Ib

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

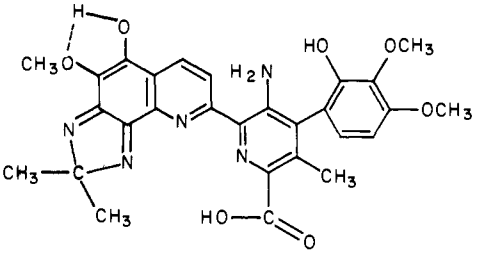


IIa



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

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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.

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