GLC behavior. The stereocontrolled total synthesis of coriamyrtin is now completed through 15 steps from **5** and **6**, and all isomeric products arising in some synthetic steps were useful along the synthetic route. The information accumulated during the present synthesis will open the way to the synthesis of the other members of picrotoxanes possessing two vicinal oxirane rings.

Acknowledgment. We are grateful to Professor T. Okuda, Faculty of Pharmaceutical Sciences, Okayama University, for supplying an authentic sample of natural coriamyrtin.

Supplementary Material Available: Spectral data and physical constants for **1**, **4**, and **7-24** (6 pages). Ordering information is given on any current masthead page.

Enantiomeric Selectivity in Binding Tris(phenanthroline)zinc(II) to DNA

Jacqueline K. Barton,* J. J. Dannenberg, and Adrienne L. Raphael

City University of New York, Hunter College New York, New York 10021

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Intercalation into DNA, where planar aromatic cations insert between adjacent base pairs,[1] is an important mode of drug binding to nucleic acids and may be involved in protein-nucleic acid recognition.[2-4] Metallointercalation reagents have been proven as valuable probes of this binding mode.[5-7] X-ray fiber diffraction analysis[8] of a terpyridylplatinum(II) complex intercalated into DNA yielded evidence in strong support of neighbor-excluded drug binding at saturation and additionally, along with the single-crystal study of the terpyridylplatinum(II) complex bound to dCpG,[9] has been useful in establishing structural characteristics at the intercalation site. Again with use of square-planar platinum complexes, the stereochemical requirement for ligand planarity was nicely demonstrated in a comparative examination of the intercalators (phen)Pt(en)$^{2+}$ and (bipy)Pt(en)$^{2+}$ and the nonintercalative complex (py)$_2$Pt(en)$^{2+}$.[10] We find that the tetrahedral complexes (phen)ZnCl$_2$ and (phen)$_2$Zn^{2+} as well as the octahedrally coordinated (phen)$_3$Zn^{2+} also bind to DNA by intercalation (phen = 1,10-phenanthroline). Moreover since the tris(phenanthroline)zinc(II) complex contains a chiral center, we have examined and report here a stereoselective preference in its binding to the right-handed DNA helix.

Strong evidence for intercalation may be obtained by examining the effects of increasing drug concentrations on the mobility of supercoiled DNA in a gel electrophoresis experiment.[11] Duplex unwinding and lengthening are needed to accommodate intercalative stacking. Because of the topological constraints on a closed circle, this duplex unwinding yields a corresponding alteration in superhelicity and, in so doing, an amplified effect on the electrophoretic mobility of the DNA. A mixture of closed and nicked circular pM2 DNAs was electrophoresed through cylindrical 1% agarose gels containing increasing concentrations of either Zn(phen)Cl$_2$, Zn(phen)$_2^{2+}$, or Zn(phen)$_3^{2+}$.[12,13] Figure 1 shows a plot of the relative mobilities of nicked and closed circular DNAs after electrophoresis. Interestingly, a migration pattern that is reminiscent of ethidium intercalation is seen in the presence of each zinc complex. Initially, with low drug concentrations, the mobility of the supercoiled DNA diminishes. As the duplex unwinds to accommodate binding, the negative supercoils are released and the polymer becomes more flexible. At the minimum migration point, the nicked and closed circles comigrate since no supercoils remain. As the concentration of the reagent is increased further, the duplex unwinds more, leading to positive supercoiling and a more compact polymer of increased mobility. In contrast to those of covalent unwinding agents,[14] this interaction is reversible. DNA incubated with the zinc complexes for 2 h at 37 °C and electrophoresed through gels that do not contain these complexes show mobilities identical with DNA that had not been incubated with the zinc reagents. Also no detectable nicking as a function of incubation was evident.

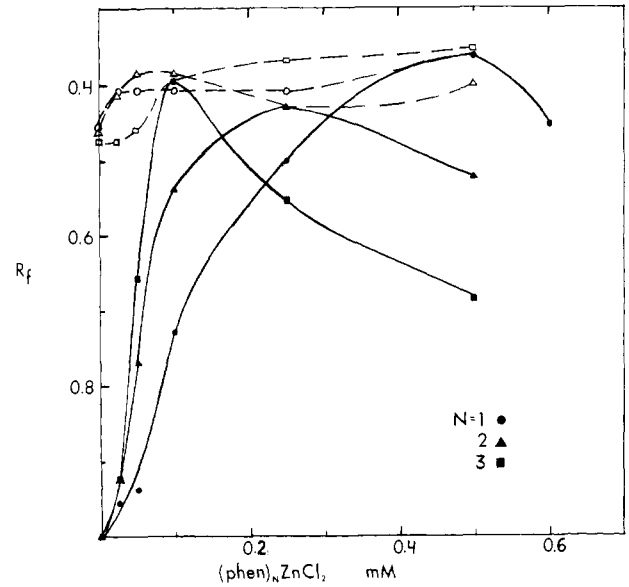

Figure 1. Relative mobilities (R_f) of closed (—) and nicked (- - -) circular pM2 DNAs in the presence of increasing concentrations of added Zn(phen)Cl$_2$ (●, ○), Zn(phen)$_2$Cl$_2$ (▲, △), and Zn(phen)$_3$Cl$_2$ (■, □), respectively. DNA samples (0.3 μg) were electrophoresed through 1% agarose cylinder gels containing 50 mM Tris acetate, 20 mM sodium acetate, 18 mM sodium chloride, as well as the added zinc reagent, at pH 7.0. Gels were stained with ethidium after electrophoresis at 30 V for 3 h, and mobilities were determined. Gel photographs are given in Figure 1S as supplementary material.

Some additional features of this experiment are noteworthy. In the presence of phenanthroline alone, at concentrations of 2.5-5.0 mM, there is only slight retardation of the superhelical DNA mobility. Since in this concentration range phenanthroline, which is neutral at pH 7.0, does not intercalate, the dissociation of a phenanthroline ligand from the metal center cannot lead to the unwinding observed. The unwinding effects also do not appear to be the result of direct metal coordination to the DNA. Coordination sites are not available in Zn(phen)$_3^{2+}$, yet with this reagent unwinding is apparent at low concentrations. Indeed, as can be seen in Figure 1, the comigration points of the nicked and

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(13) The zinc complexes were prepared in aqueous solution by stoichiometric addition and then isolated from high NaCl solution. The chloride salts were analyzed (percent) for zinc content as follows: (phen)ZnCl$_2$, Zn = 21.43, calcd = 20.65; (phen)$_2$ZnCl$_2$, Zn = 13.42, calcd = 13.16; (phen)$_3$ZnCl$_2$, Zn = 9.72, calcd = 9.66.
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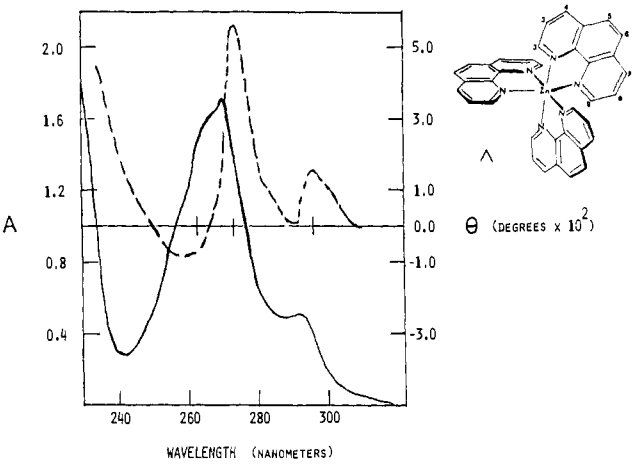

Figure 2. Absorption spectrum (—) of $Zn(phen)_3^{2+}$ prior to dialysis and circular dichroic spectrum (---) of a solution enriched in the Λ isomer as a result of dialysis against DNA. The concentration of the zinc complex in the diluted samples used for these spectra is 4.0×10^{-5} M. Equilibrium dialysis of calf thymus DNA (1 mM DNA–phosphate) with $Zn(phen)_3^{2+}$ (0.5–3 mM) was conducted in the electrophoresis buffer (see Figure 1) for 3 h at 4 °C. The circular dichroic spectrum represents that of the dialysate enriched in the unbound enantiomer. The maxima of the absorption and circular dichroic spectrum, after correction for shoulders, are seen to coincide at 263, 273, and 296 nm, as indicated.

closed circular DNAs appear at decreasing concentrations of 0.5, 0.25, and 0.10 mM mono-, bis-, and tris(phenanthroline)zinc(II), respectively. It is likely that increasing phenanthroline substitution yields an increase in binding affinity. These results together indicate that the cationic complexes reversibly unwind the duplex through partial insertion of the phenanthroline ligands between the base pairs.[15]

The finding that $Zn(phen)_3^{2+}$ binds to DNA led us to consider the effects of the chirality of the complex on binding to a right-handed helical duplex. A calf thymus DNA solution (1 mM nucleotide) was dialyzed at 4 °C against a racemic mixture of the tris(phenanthroline)zinc(II) species (0.5–3 mM). After equilibration the circular dichroic spectrum of the dialysate was recorded and is given in Figure 2. The dialysate was optically enriched as a result of the preferential binding of one enantiomer to the DNA. The close correspondence between the absorption spectrum, also shown, and the circular dichroic spectrum is apparent. Peaks centered at 296, 273, and 263 nm can be observed in both spectra. Also no contribution from degraded DNA, the only other source of optical activity, is evident. This result is consistent with our observations that binding does not lead to DNA cleavage even upon incubation at 37 °C.

The circular dichroic spectrum is strikingly similar to those of the corresponding Cr(III), Fe(II), Co(III), and Ni(II) enantiomeric phenanthroline complexes that have been resolved.[16] Tris(phenanthroline) complexes of Cr(II), Fe(II), and Co(II) racemize more rapidly than they exchange their ligands; k_{rac} = 0.1, 6.5×10^{-4}, and 6.9 s^{-1}, respectively, in aqueous solution at 25 °C. The Ni(II) species in contrast exchanges with a half-life of 1 day at 25 °C, which is comparable to its ligand dissociation rate. It has been proposed[17] that the phenanthroline complexes of Cr(II), Co(II), and Fe(II) racemize via an intramolecular twist mechanism involving inner-sphere expansion as a result of electronic excitation to empty e_g^* orbitals. The $Ni(phen)_3^{2+}$ cation cannot accommodate a similar excitation and therefore can only racemize by a slower dissociative mechanism. We find the half-life for racemization of $Zn(phen)_3^{2+}$ to be 10 days at 4 °C in our buffer system. The $Zn(phen)_3^{2+}$ cation, then, does not appear to be exceedingly labile optically, which is contrary to previous suggestions.[18] Our data indicate that a similar slow dissociation of the rigid phenanthroline ligands is required in the case of zinc(II) complexes. Simply on the basis of ligand dissociation rates,[19] however, it is not clear why this racemization is not faster. The tightly packed and hydrophobic character of the cation may limit both ligand substitution and dissociation, especially in our buffer system.

Inspection of CPK space-filling models of the enantiomeric pair of metal complexes bound to right-handed double helical DNA has led to the assignment of absolute configuration as is given in Figure 2. On the basis of steric considerations, it appears that it is the Δ isomer that binds preferentially to the right-handed duplex.[20] When one phenanthroline ligand of the Δ isomer is partially inserted between the base pairs, the remaining ligands, which also have a right-handedness, fit closely along the helical groove. In contrast, the nonintercalated ligands of the Λ isomer, with a left-handed stepwise screw, interfere with the phosphate backbone above and below the plane of the intercalated ligand. When the Λ isomer is bound, the H3–H8′ distance of 10.4 Å must be normal to the phosphate backbone. This leads to nonbonded repulsion between these phenanthroline hydrogen atoms and the phosphate oxygen atoms; the closest nonbonded internuclear distance of phosphate oxygen groups in B-DNA is 11.7 Å.[21] When the Δ isomer is bound to the DNA, in contrast, it is the shorter H3–H3′ distance of 6.8 Å that is normal to the phosphate backbone and intercalation is facile.

This is to our knowledge the first example of enantiomeric selectivity in DNA intercalation.[22] The stereospecificity that we find serves as a simple illustration of how asymmetric drugs might bind to the asymmetric DNA helix. Our results reflect quite clearly the importance of the nonintercalative groups on drug binding to the duplex. The chirality of the pair of nonintercalating phenanthroline ligands determines the relative affinities for the enantiomers. It will be interesting to examine whether the Λ enantiomer of $Zn(phen)_3^{2+}$ or some derivative thereof binds preferentially to a left-handed DNA helix.[23] It is evident that this feature of helical symmetry can be important to drug recognition.[24] Certainly the enantiomeric selectivity in binding tris(phenanthroline) metal complexes to DNA will be useful to the design of probes that distinguish the different DNA forms.

Acknowledgment. The support of the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the City University of New York PSC-BHE Research Award Program are gratefully acknowledged, as well as is the NSF

(15) These data show that neither dissociated phenanthroline nor even the monosubstituted zinc complex serves as the primary intercalator in these solutions. Thus far, however, we cannot rule out the possibility that in the experiments using mono- and bis(phenanthroline)zinc(II) the solution equilibrium favors the formation of $Zn(phen)_3^{2+}$, and it is this chiral species that binds intercalatively to DNA. In solution $Zn(phen)_2^{2+}$ may also be six-coordinate and chiral. Studies of optical enrichment after dialysis against the mono- and bis-substituted species should clarify these points.

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Undergraduate Research Program (A.L.R.).

Registry No. $Zn(phen)Cl_2$, 14049-94-6; $Zn(phen)_2^{2+}$, 16561-56-1; Δ-$Zn(phen)_3^{2+}$, 82660-10-4; Λ-$Zn(phen)_3^{2+}$, 82660-11-5.

Supplementary Material Available: Photographs (Figure 1S) for the gel electrophoretic data given in Figure 1 (2 pages). Ordering information is given on any current masthead page.

Biosynthesis of the Antitumor Antibiotic Naphthyridinomycin

Milton J. Zmijewski, Jr.,* and Marcia Mikolajczak

Department of Medicinal Chemistry, College of Pharmacy University of Utah, Salt Lake City, Utah 84112

Venkatappa Viswanatha and Victor J. Hruby

Department of Chemistry and Biochemistry University of Arizona, Tucson, Arizona 85721

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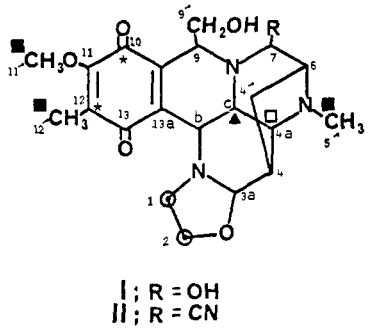
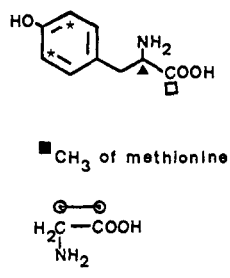
Naphthyridinomycin (I), a novel heterocyclic quinone antibiotic,



I; R = OH
II; R = CN



■ CH_3 of methionine

is produced in liquid shake culture by *Streptomyces lusitanus* NRRL 8034.[1,2] The structure of naphthyridinomycin was established by X-ray crystallography.[3] This compound has been shown to be a potent antimicrobial and antitumor agent.[4] Mechanism of action studies have shown that in bacteria naphthyridinomycin primarily inhibits DNA synthesis,[5] due to the binding of the antibiotic to dG·dC base pairs of the DNA.[6] A major impediment to our biosynthetic studies was the instability of naphthyridinomycin in concentrated organic or aqueous solvents. This problem was resolved by converting naphthyridinomycin to cyanonaphthyridinomycin (II).[4] This latter compound is a stable crystalline derivative suitable for chemical or spectral studies. Cyanonapthyridinomycin, therefore, was utilized to demonstrate that tyrosine, the *S*-methyl group of methionine, glycine, and ornithine are efficiently incorporated into the antibiotic.

Radioisotope-labeled substrates were fed to cultures of *S. lusitanus* growing in 100 mL of a complex production medium in stoppered 500-mL Erlenmeyer flasks as described previously.[6] After 72 h of growth at 25 °C, the labeled substrates were added, and the culture was incubated an additional 24 h.[7] The filtered broth from the 96-h culture was treated with sodium cyanide to generate cyanonaphthyridinomycin and the antibiotic extracted with methylene chloride. The bright orange compound was successively purified as described in the legend of Table I to yield ~20 mg/L.

Table I. Incorporation of ^{14}C-Labeled Substrates into Cyanonaphthyridinomycin

substrate	% incorporation[a]
[U-^{14}C]-L-tyrosine	1.8[b]
[1-^{14}C]-L-tyrosine	1.2
[*methyl*-^{14}C]-L-methionine	2.5
[3-^{14}C]-DL-dihydroxyphenylalanine	<0.1
[2-^{14}C]glycine	2.1
[1-^{14}C]-DL-ornithine	1.5
[1-^{14}C]-DL-glutamate	<0.1
[U-^{14}C]-D-glucose	<0.1
[1-^{14}C]acetate	<0.1
[2-^{14}C]acetate	<0.1

[a] Per 2 mg produced in 100 mL of broth. [b] Each antibiotic extract was purified successively on an Alumina V column (80:20 benzene:chloroform), silica gel preparative plates (60:20:30 acetone:isopropanol:dichloroethane) and ODS reverse-phase plates (80:20 methanol:water) or until constant specific activity was reached.

Of the potential precursors tested, [U-^{14}C]- and [1-^{14}C]-L-tyrosine, [*methyl*-^{14}C]-L-methionine, [2-^{14}C]glycine, and [1-^{14}C]-DL-ornithine (Table I) were well incorporated into cyanonaphthyridinomycin. To obtain further information on the incorporation of tyrosine and methionine, we degraded cyanonaphthyridinomycin samples labeled from [U-^{14}C]-L-tyrosine and [*methyl*-^{14}C]-L-methionine by Kuhn–Roth oxidation and Schmidt degradation[8] to yield carbons 12 and 12′ of the antibiotic. Approximately one-ninth (12%) of the label that [U-^{14}C]-L-tyrosine contributes to the antiobiotic was found at C-12, suggesting that all nine carbons of tyrosine were incorporated. The methyl group from methionine labeled C-12′ (37%) and presumably the other two-thirds of the label was incorporated at the *O*- and *N*-methyl positions. [3-^{14}C]-DL-dihydroxylphenylalanine (DOPA) was not detectably incorporated into the antibiotic, suggesting that the oxygen functionality at C-10 was being introduced in a subsequent step in the biosynthesis. None of the other substrates listed in Table I were detectably incorporated.

To confirm the incorporation of tyrosine and methionine and to examine the role of glycine in the biosynthesis, in additional studies we utilized ^{13}C-enriched precursors and ^{13}C NMR. The ^{13}C NMR spectrum of cyanonaphthyridinomycin (Table II) was assigned in part on the basis of the previous assignments of the ^{13}C NMR spectrum of the saframycins[10,11] and in part by utilizing established NMR pulsing techniques to discriminate quaternary, methine, methylene, and methyl carbons.[12] Assignments of the protons in the ^1H NMR spectrum of cyanonaphthyridinomycin and 9′-acetylcyanonaphthyridinomycin were completed, and this information was used to assign the carbon resonance signals of the proton-bearing carbons.[9]

As expected from the degradation studies, [*methyl*-^{13}C]-DL-methionine enriched only the *O*-, *N*-, and *C*-methyl groups of the antibiotic (Table II). [1-^{13}C]- [2-^{13}C]-DL-tyrosine enriched carbons 4a and 13c, respectively. The quinone ring of cyano-

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(9) ^1H and ^{13}C NMR spectra were recorded on a JEOL FX-270 NMR spectrometer at 270 and 67.8 MHz, respectively. The ^{13}C NMR spectrum of cyanonaphthyridinomycin consisted of 21 resolved resonances. Acetylation at C-9′ revealed that C-9′ and C-2 were overlapping in the original spectrum. Even in the acetylated antibiotic, these carbons were split by less than 0.1 ppm. The proton signals in the ^1H NMR spectrum of the acetylated compound were resolved well enough to carry out selective homonuclear decoupling. This aided in the assignment of the proton spectrum. Selective ^1H–^{13}C decoupling was employed to assign the carbon signals in the ^{13}C NMR spectrum of the acetylated compound.
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