acceptors were examined to test their competence as oxidants for this enzymatic reaction. It was found that dichlorophenolindolphenol (DCPIP) was the most potent electron acceptor tested,[14] while flavin analogues show no effect on catalysis. The latter finding agrees well with the earlier assessment that the NADH oxidase activity is flavin independent. However, the revelation of ferricytochrome *c* and ferricyanide as effective mediators was most intriguing since both compounds are well-known one-electron oxidants. The stoichiometry of NADH oxidation versus ferricyanide reduction was consequently shown to be 1.98. These observations strongly suggest that the enzyme-catalyzed H_2O_2 formation is not a direct two-electron reduction of molecular oxygen but may instead be a one-electron reduction process followed by dismutation of the nascent superoxide ($2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$).

The NADH dependent $O_2^{\bullet-}$ generating activity was assessed by measuring the superoxide dismutase inhibitable reduction of ferricytochrome *c*.[15] The experimental results clearly show that one out of the five Fe(III) being reduced in this assay received its electron from $O_2^{\bullet-}$.[16] Although such single-electron transfer to and from O_2 accounts for only 20% of the total reduction flux, this may simply reflect its minimum contribution in facilitating electron egress in the redox process. Since the active enzyme is metal-free, the indisputable formation of $O_2^{\bullet-}$ as the proximate reducing intermediate suggests the participation of an enzyme-bound organic cofactor mediating the obligatory $2e^-/1e^-$ conversion as electrons pass on from NADH to O_2 in the catalysis.[17] Substantiating this proposition was the finding that the purified enzyme alone could accept two electrons from NADH stoichiometrically in the absence of any electron mediators. The observation of a characteristic free radical signal ($g = 2.002$) in the EPR spectrum obtained anaerobically with a sample of the enzyme and NADH at 8 K may also support the existence of an organic cofactor. Since the putative cofactor should be fully reduced by NADH under these conditions, the radical signal noted may be attributed to electron leakage from the reduced cofactor to residual oxygen in the frozen sample. In fact, the ratio of radical species to protein was estimated to be only 1%. Although the low abundance of this transient radical species was fully anticipated, ascertainment of the significance of such a low level of a radical intermediate must await further scrutiny. Since this enzyme is expected to operate via a single mechanism despite its dual functions as a NADH oxidase and a 3,4-glucoseen reductase, the unique $2e^-/1e^-$ switching capability found for this enzyme provides, for the first time, compelling evidence that it may operate through a radical mechanism.

Thus, the C-3 deoxygenation in the biosynthesis of ascarylose, and possibly the 3,6-dideoxyhexoses in general, may proceed with C–O bond disruption followed by stepwise $1e^-/1e^-$ reduction. The mechanistic revision of the reduction step from a hydride transfer to an electron-transfer process alleviates the fastidious constraint imposed on the guise of the reducing equivalent delivery from E_3 to E_1, since the maximum distance that an electron can move from a donor to an acceptor under physiological conditions is on the order of 10–20 Å.[18] Such long range communication can accommodate a much greater distance between E_3 and E_1 than previously surmised. Furthermore, a radical reduction mechanism could aptly explain the lack of direct hydride transfer from NADPH in the reduction of the 3,4-glucoseen-PMP complex **3** as well. The radical nature of this C-3 deoxygenation process is reminiscent of the well-known sugar deoxygenation reaction catalyzed by ribonucleotide reductase, albeit the mechanisms of these two deoxygenations are fundamentally distinct.[19,20] The answers for the remaining questions await the isolation of homogeneous E_1 and the complete structural characterization of the coenzyme. Work is continuing in these areas, and full details will be reported subsequently.

Acknowledgment. Financial support from NIH (GM 35906), Research Corporation, Eli Lilly & Company, and the donors of the Petroleum Research Fund, administered by the American Chemical Society, is greatly acknowledged. We thank Professor Maurice Kreevoy for helpful discussion and Professor Otto Lüderitz for the bacterial strain. H.W.L. also thanks the Camille & Henry Dreyfus Foundation for a grant awarded to Distinguished New Faculty in Chemistry and American Cancer Society for a Junior Faculty Research Award. The EPR spectra were measured with the help of Allen Orbille, Mark Harpel, and Professor John Lipscomb, to whom we are particularly indebted.

(14) The same result was first observed by Rubenstein and Strominger.[2d] With DCPIP as the electron acceptor, this enzyme exhibits a K_m of 53.7 mM for NADH and a V_{max} of 128 nmol·min^{-1} mg^{-1}.

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(16) It is also possible that $O_2^{\bullet-}$ is the obligatory transient intermediate, but only 20% of the $O_2^{\bullet-}$ generated could be trapped by dismutase.

(17) A likely candidate for this organic cofactor is a quinone type coenzyme which is known to have the capability of serving as a $2e^-/1e^-$ switch and, in general, has a UV absorption at ca. 270–300 nm. Furthermore, the NADH oxidase activity can be inactivated by treatment with hydride reducing reagents and phenylhydrazines.

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(20) The possible intermediacy of a 3,4-glucoseen-PMP radical in the deoxygenation process has precedent since a pyridoxal phosphate stabilized aziridine radical has been suggested as the central intermediate in the reaction catalyzed by lysine 2,3-aminomutase (Moss, M.; Frey, P. A. *J. Biol. Chem.* **1987**, *262*, 14859).

Polyaza Cavity Shaped Molecules. 15. Stereocontrol in the Formation of Binuclear Complexes

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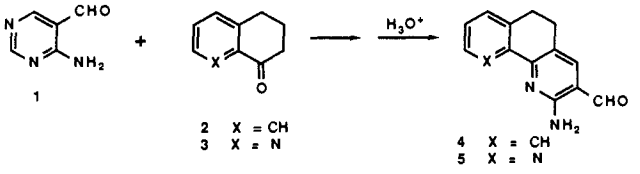
Received August 10, 1988

There has been considerable recent interest in the study of macrocycles containing polypyridyl subunits and their metal complexes.[1-8] While cyclic systems are often quite effective in chelating a single metal atom, they do not often lend themselves to the incorporation of more than one metal in a stereocontrolled fashion. As an extension of our work on the conformational properties of monoannelated bipyridine and bis-annelated terpyridine type systems, we herein report the preparation of larger polyaza cavities which show utility in binuclear coordination.

Caluwe and co-workers have demonstrated the usefulness of 4-aminopyrimidine-5-carboxaldehyde (**1**) as a synthon for the



stereochemically controlled introduction of 1,8-naphthyridine units

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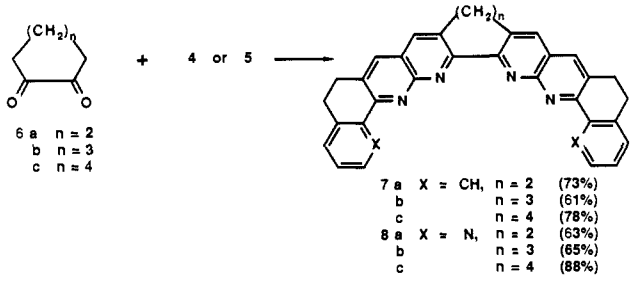
into polycondensed systems.[9] The condensation of **1** with α-tetralone provides 5,6-dihydropyrimido[4,5-*b*]-1-phenanthridine which may be hydrolyzed with aqueous acid to give the 2-amino-3-carboxaldehyde derivative **4**. Similar treatment of 5,6,7,8-tetrahydro-8-quinolone (**3**) produces 2-amino-5,6-dihydro-1,10-phenanthroline-3-carboxaldehyde (**5**) in 65% overall yield. The Friedländer condensation of **4** or **5** with ketones **2** and **3** leads to bis-annelated 2,7-diaryl-1,8-naphthyridines containing two to four sp^2 nitrogens in the cavity.[10] If we condense 2 equiv of the aminoaldehydes **4** or **5** with 1,2-cycloalkanediones **6**, the



nonacyclic systems **7** and **8** may be prepared in the yields indicated. Cycloheptanone and cyclooctanone analogues of **2** and **3** are available so that in principle all three bridges of **7** and **8** can be varied from two to four carbons in length. In the present work we have chosen to hold the two outermost ethano bridges constant since they help define a 5,6-dihydro-1,10-phenanthroline chelating subunit.

Compound **8** may be considered as being comprised of three 2,2′-bipyridine (bpy) subunits, each bridged at the 3,3′-position and fused to its neighbor through a common 5,6-bond. In earlier

work we have carefully examined the conformational properties of similar bpy species.[11] They exist as a pair of conformational enantiomers whose interconversion is a function of the length of the polymethylene bridge. One can calculate the dihedral angle (α) defined by the planes of the two covalently bound pyridines to be 20° (n = 2), 48° (n = 3), and 61° (n = 4).[12] Since each bpy represents a chiral center, there are eight possible stereoisomers for each compound **7** and **8**. When the torsion angles about the bridged subunits all have the same sign, the ligand **8c** is found to be decidedly helical in structure with a total twist angle of 101°.

Treatment of **8a** with 2.5 equiv of $Ru(bpy)_2Cl_2$ provides a metal complex which shows the incorporation of one $Ru(bpy)_2^{2+}$ unit by FAB mass spectrometry. Two equivalent bidentate sites are available in **8a**. Both sites are 5,6-dihydro-1,10-phenanthrolino moieties: a central one and two distal ones. The ^1H NMR spectrum of this material shows a complex aromatic region accounting for 26 nonequivalent protons, while the aliphatic region is also complex and integrates for 12 protons. The ^{13}C NMR shows more than three types of aliphatic carbons which further supports an unsymmetrical structure having the $Ru(bpy)_2$ chelated at a distal site. To support this conclusion we treated **7a** with $Ru(bpy)_2Cl_2$ under identical conditions and observed no reaction. In **7a** we have only a central 1,4-bidentate site where coordination would lead to a species having C_2 symmetry. Apparently this site is too sterically congested to chelate with $Ru(bpy)_2^{2+}$, and similar behavior from **8a** is not unexpected. Treatment of the higher homologs **8b** and **8c** with 1 equiv of $Ru(bpy)_2Cl_2$ resulted in the formation of analogous mononuclear complexes.

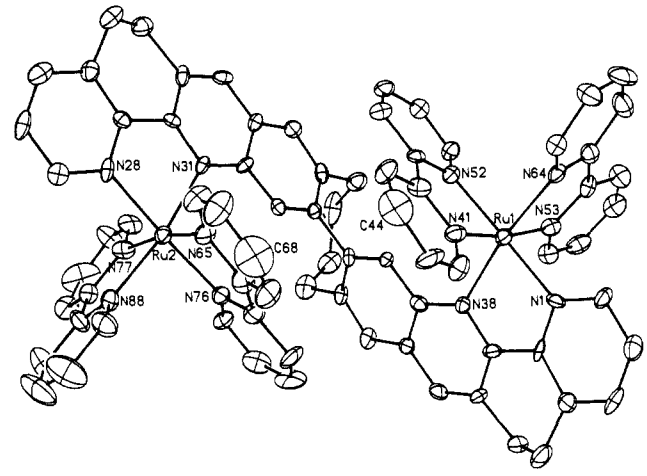


Figure 1. View of the $(bpy)_2Ru(\mathbf{8c})Ru(bpy)_2^{4+}$ cation, with labeling of key atoms. The thermal ellipsoids are 20% equiprobability envelopes, and hydrogens have been omitted for clarity.

Another important subunit of **8** is the 3,3′-dimethylene-2′-pyridyl-1,8-naphthyridine (pynap-2) moiety, and earlier work has explored the properties of complexes such as $Ru(pynap\text{-}2)(bpy)_2^{2+}$.[13] Treatment of **8c** with 2.5 equiv of $Ru(bpy)_2Cl_2$ leads to the formation of $(bpy)_2Ru(\mathbf{8c})Ru(bpy)_2^{4+}$ as determined by ^1H NMR and FAB mass spectroscopy. A single-crystal X-ray analysis was carried out, and an ORTEP plot of the complex is shown in Figure 1.[14] It is evident that **8c** has adopted a helical conformation. The two $Ru(bpy)_2^{2+}$ moieties are held very close to one another such that the Ru1–Ru2 distance is 8.12 Å and the C_4 carbons of the interior bpys (C44, C68) are separated by only 3.23 Å. This congestion is also manifested by considerable nonplanarity in the N41 and N65 pyridine rings. The dihedral angle around the bridge connecting the two pynap-2 units has increased to 74° further reflecting the crowded nature of the complex. In contrast, the dihedral angle of the two distal 5,6-dihydro-1,10-phenanthroline moieties has decreased to about 10° as a result of coordination.

The electronic absorption spectra of the mono- and binuclear complexes of **8c** are quite similar to that of $Ru(pynap\text{-}2)(bpy)_2^{2+}$, evidencing characteristic MLCT bands at 450 and 520 nm. The shorter wavelength band corresponds to a d-π_1* transition from the metal to a bpy ligand, and the longer wavelength band corresponds to a d-π_2* transition involving the bridging ligand **8c**. The intensity of the absorptions for the binuclear complex are about twice those of the mononuclear. For the binuclear species d-π_2* is somewhat less intense relative to d-π_1*, implying some interaction between these two halves of the complex. In accord with this observation, the d-π_2* intensity is even more diminished for the binuclear complex derived from **8b** where greater interaction would be expected.

The redox properties of $Ru(bpy)_2(\mathbf{8c})^{2+}$ and $Ru(bpy)_2(pynap\text{-}2)^{2+}$ are similar with both showing a reversible first reduction at −0.99 V (vs SCE) and a reversible oxidation at 1.22 V. The second and third reduction potentials show increasing irreversible behavior for $Ru(bpy)_2(\mathbf{8c})^{2+}$. A single quasi-reversible oxidation wave is observed for $(bpy)_2Ru(\mathbf{8c})Ru(bpy)_2^{4+}$ at 1.28 V, and the first reduction splits into two waves at −0.95 and −1.05 V. Addition of an electron to the Ru(I)–Ru(I) species affords a molecule which evidences strong absorption onto the electrode surface. Clearly the two Ru centers in the binuclear complex are close enough to influence one another, unlike a related ethano-tethered system which enjoys much greater conformational mobility.[15]

It is interesting to note that both metal centers depicted in Figure 1 have the Δ configuration. Examination of a molecular model of the binuclear complex of **8c** indicates that the Δ,Δ form

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would experience even greater interaction of the interior bpy ligands. In earlier work we have observed similar diastereoselectivity in the coordination of 3,3'-tetramethylene-2,2'-bipyridine.[16] Since the crystal is centrosymmetric, the enantiomeric Λ,Λ form is present in equal amount. Recent studies on chiral ruthenium tris-diimine complexes have shown that the Λ form binds preferentially to the left-handed helical form of DNA and upon irradiation becomes an A-conformation-specific DNA cleaver.[17] The implications for complexes such as the one prepared in this study are under consideration.

Acknowledgment. We are indebted to Dr. James Korp for assistance with the X-ray determination. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, the Robert A. Welch Foundation, and the National Science Foundation (CHE-8607935) for the support of this research. The NMR spectrometer was partially funded by NSF (CHE-866352). C.H. would also like to thank Elf Aquitaine and the French Ministry of Foreign Affaires for a Bourse Lavoisier.

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M43 Antibiotics: Methylated Vancomycins and Unrearranged CDP-I Analogues

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Received July 27, 1988

Vancomycin is a clinically important antibiotic discovered in 1956.[1] One of the three original vancomycin-producing strains of *Amycolatopsis orientalis*[2] (previously designated *Nocardia orientalis* and *Streptomyces orientalis*) numbered M43-05865 (NRRL 2450), produced a new antibiotic designated M43A as the major product. The ratio of M43A to vancomycin produced by strain M43A-05865 is about 2.5:1. Among the minor metabolites produced by this strain are several previously described compounds and include A51568A and traces of A51568B,[3] desvancosamine A51568A, agluco A51568A, desvancosamine vancomycin, aglucovancomycin, desvancosamine M43A (also named M43C), and agluco M43A.[4] This strain also produced small amounts of M43D and trace quantities of M43B. Antibiotic M43A is a tri-*N*-methylleucine analogue of vancomycin, while M43B is the desamido derivative of M43A. The minor metabolite, M43D, is the di-*N*-methylleucine analogue of vancomycin.

The filtered broth of culture *Amycolatopsis orientalis* M43-05865 was purified on the cation resin Dowex 50W-X4, and the M43 complex was obtained on lyophilization of the eluates. The individual M43 factors were obtained by chromatographic purification on RP-18 reversed phase and then desalted by using a Diaion HP20 column.

The HPLC retention times of M43 factors D and A are different from those of vancomycin. M43D is less polar than vancomycin, and M43A is less polar than both M43D and vancomycin.

(1) McCormick, M. H.; Stark, W. M.; Pittenger, G. E.; Pittenger, R. C.; McGuire, J. M. *Antibiot. Annu.* **1955-56**, 606-611.
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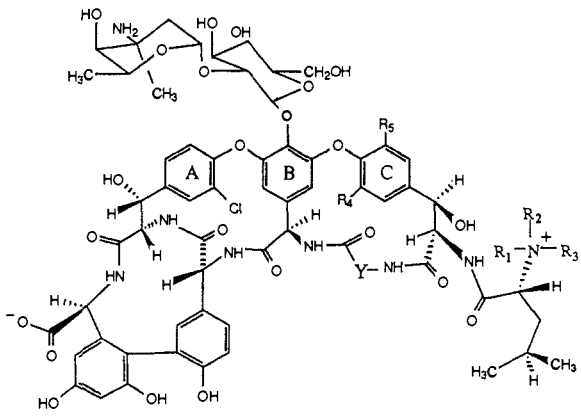
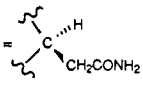
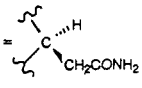
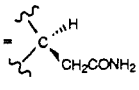
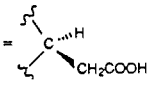
Chart I

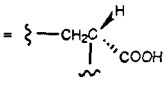


1. Vancomycin: R_1= R_2= R_4= H; R_3= CH_3; R_5= Cl; Y = 

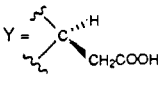
2. M43D: R_1= R_4= H; R_2= R_3= CH_3; R_5= Cl; Y = 

3. M43A: R_1= R_2= R_3= CH_3; R_4=H; R_5= Cl; Y = 

4. M43B: R_1= R_2= R_3= CH_3; R_4= H; R_5 = Cl; Y = 

5. CDP-1: R_1= R_2= R_5= H; R_3= CH_3; R_4= Cl; Y = 

6. R_1= R_2= R_3= CH_3; R_4= Cl; R_5= H; Y = 

8. M43F: R_1= R_2= R_4=H; R_3= CH_3; R_5= Cl; Y = 

The FABMS of M43D shows that it is 14 mass units higher than vancomycin and that M43A is 28 mass units higher than vancomycin. The FABMS also shows cleavages of vancosaminyl and vancosaminyl-*O*-glucosyl fragments from both M43D and M43A, thereby suggesting that the additional mass of 14 and 28 units in M43D and M43A, respectively, are present in the aglucone moieties of the two antibiotics and not in the sugar residues.

The ^{1}H NMR spectra of M43D and M43A are similar to those of vancomycin, except for the signals due to the N-methylated leucine portion of the metabolites (see Table I, Supplementary Material). The intensities of the *N*-methyl signals at 2.13 ppm for M43D and 3.20 ppm for M43A were higher than those due to vancomycin at 2.34 ppm. The above mass spectral and ^{1}H NMR data suggested that M43D contained *N,N*-dimethylleucine and M43A included *N,N,N*-trimethylleucine, and these compounds were assigned structures **2** and **3**, respectively.

This structural assignment of M43A was confirmed by X-ray analysis of a crystalline derivative **6** of M43A. A 100-mg sample of M43A hydrochloride was dissolved in 2 mL of water. The resulting solution at pH 4.2 was heated at 65 °C without stirring for 24 h, when crystals of the rearranged M43A derivative **6** were deposited. Under similar conditions, vancomycin yields CDP-I[5] **5** (crystalline degradation product I), and its X-ray structure determination represented a major step in the structure elucidation of vancomycin.[6] Later revisions of vancomycin structure[7,8] have

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