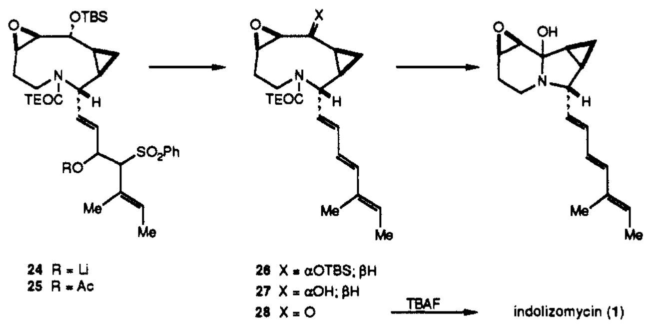
Scheme IV



which was reduced directly with sodium borohydride to give the crude enol ether **13** (Scheme III). Reaction of this material with 2-(trimethylsilyl)ethyl chloroformate (**14**, TEOC-Cl)[12] in benzene at room temperature afforded enone **15**[4a,b] in 30% overall yield from **12**. Nucleophilic epoxidation of **15** (30% H_2O_2; NaOH–methanol) afforded a mixture of epoxy ketones in 93% yield, differing only in the configuration at the oxide bond α to the keto group.[13] Treatment of the mixture with hydrazine[14] (methanol–cat. acetic acid; room temperature) afforded a single allylic alcohol, **17**.[4a,b] Epoxidation (*m*-chloroperbenzoic acid–methylene chloride)[15] afforded **18**[4a,b] which was protected as its TBS ether **19**[4a,b] (43% overall from **16**).

Oxidation of **19** (ozone, methylene chloride–methanol) followed by workup with dimethyl sulfide gave rise to aldehyde **20**,[4a,b] which was subjected to the action of (methoxymethylene)triphenylphosphorane. Photooxygenation of *E–Z* mixture **21**[16] followed by reduction of the resultant hydroperoxide with triphenylphosphine afforded enal **22**[4a] (53% overall from **19**). The setting for installation of the triene functionality was now at hand.

Reaction of lithio sulfone **23**[17] with aldehyde **22** (THF; −78 °C) was followed by direct acetylation of the presumed lithium alkoxide **24** (Scheme IV) with acetic anhydride. The mixture of acetoxy sulfone diastereomers **25**, when treated with 5% sodium amalgam, afforded triene **26**[4a] (77% yield from **22**). It was possible to selectively cleave the oxygen-bound silyl group of **26** (1 N periodic acid–THF)[18] to give alcohol **27**,[4a] which upon treatment with 1 equiv of tetra-*n*-propylammonium perruthenate[19] afforded keto urethane **28**[4a] (74% overall from **26**). Upon fluoride ion (TBAF) induced removal of the TEOC group, there was obtained, after two chromatographic purifications,[20] *dl*-indolizomycin in 29% yield. The ^1H NMR spectrum (500 MHz) was in agreement with the ^1H NMR spectrum (400 MHZ) of the natural product provided by Dr. Ikeda.[21] The structure was further confirmed by high-resolution and low-resolution mass spectroscopy as well as by ultraviolet measurements (λ_{max}^{MeOH} = 267 nm; reported value = 268 nm). The stereochemistry of both the allyl and epoxy groups has further been established by crystallographic determinations on congeners of the systems shown here.[22] Though a direct comparison with an authentic sample was not possible,[23] the claim of a total synthesis of indolizomycin can be asserted with complete confidence.[24]

Acknowledgment. This work was supported by NIH Grant CA 28824. NMR spectra were obtained through the auspices of the Northeast Regional NSF/NMR Facility at Yale University, which was supported by NSF Chemistry Division Grant CHE 7916210. We also thank Gayle Schulte of the Yale University Instrumentation Center for crystallographic determination on analogues of structures shown here.

Supplementary Material Available: Copies of NMR spectra for compounds **1** (natural and synthetic), **6–10**, **12**, **15–22**, and **25–28** (20 pages). Ordering information is given on any current masthead page.

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[12] It was important to use freshly prepared TEOC-Cl (from the reaction of 2-(trimethylsilyl)ethanol and 20% phosgene in toluene); cf.: Shute, R. E.; Rich, D. H. *Synthesis* **1987**, 346.

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[21] We thank Dr. Ikeda of the Institute of Microbiotic Chemistry in Tokyo for the ^1H NMR (400 MHz) and the ultraviolet spectra of indolizomycin.

[22] In addition to spectral agreement of synthetic and naturally derived indolizomycin, the structures were supported by key crystallographic measurements. X-ray crystal structures were obtained for the FMOC analogue of **15** and the methoxycarbonyl analogue of **18**. Thus the stereochemical relationship of the cyclopropane, epoxide, and allyl functionalities is fully established. The crystallographic data as well as all other supporting data and experimental procedures are found in the Ph.D. thesis of Guncheol Kim, Yale University, 1989, and will be described in due course.

[23] The fully synthetic material exhibited instability similar to that described for the natural product. Its decomposition does not lead to a well-defined product. After several hours at room temperature, under neutral conditions, substantial decomposition has occurred.

[24] We note that the synthesis per se does not establish the stereochemistry of the carbinolamine linkage. This matter has been previously considered.[1] Although the presumption is that the hydroxyl group is β in the antipode shown here, it has not been proven.

Spectroscopic Studies of the Mixed-Valent [Fe(II),Fe(III)] Forms of the Non-Heme Iron Protein Hemerythrin: Iron Coordination Differences Related to Reactivity

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Hemerythrin (Hr), the binuclear non-heme iron, oxygen transport protein,[1-3] can exist in the following three oxidation states: [Fe(III),Fe(III)] in oxy and met derivatives,[4-12] [Fe(II),

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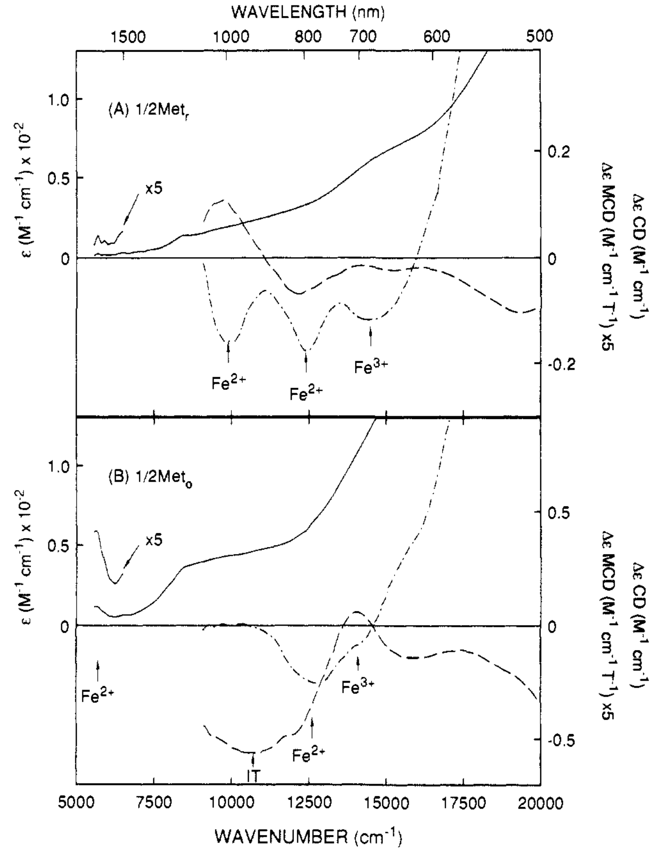

Figure 1. ABS (—), CD (- - -) and LTMCD (-·-) spectra of 1/2metHr. CD and ABS spectra were obtained at room temperature. LTMCD spectra were recorded at 4.2 K with a 5.0-T field in a 67% glycerine/buffer glass: (A) 1/2met$_r$ in 0.10 M HEPES, 0.30 M Na_2SO_4, pH 7.0, and (B) 1/2met$_o$ in 0.10 M MES, 0.30 M Na_2SO_4, pH 6.0. ε and Δε were calculated per dimer. For CD experiments, [Hr] ≃ 4 mM; for ABS, [Hr] ≃ 12 mM; and for MCD, the initial [Hr] ≃ 12 mM, but glycerol addition gave a final [Hr] ≃ 5 mM. The pH at which each 1/2met was examined was chosen to minimize further reactions.[15,16,38] Formation of 1/2met$_r$ and 1/2met$_o$ was quantitated by conversion to 1/2metN_3^-,[19] which has a prominent ABS peak at 470 nm (ε = 2400 M^{-1} cm^{-1}).[15f,19] In all cases, this method indicated >95% conversion to 1/2met.

Fe(II)] in deoxy derivatives,[4,10b,11b-d,13] and [Fe(III),Fe(II)] in the semi- or 1/2met derivatives.[14] X-ray crystallographic studies on metHr at pH < 7 have shown one Fe to be six coordinate and the other to be five coordinate; exogenous ligands bind to the latter, making it six coordinate.[10a] The mixed-valent 1/2met has been intensely studied,[14-20] as it differs, depending upon the method

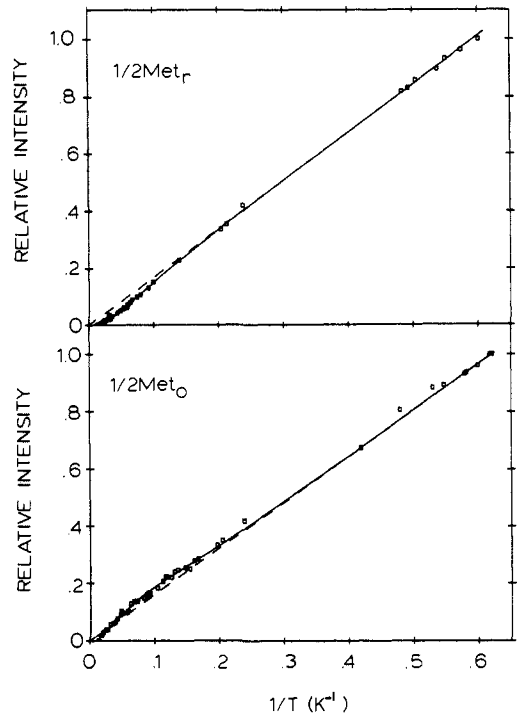

Figure 2. Temperature dependence of 1/2metHr MCD. Measured intensity with a 2.0-T field (□), predicted Curie law behavior from the ground doublet (- - -), and the fit (—) for 1/2met$_r$ at 500 nm (top) and 1/2met$_o$ at 800 nm (bottom). Note: The error bars on any individual point are smaller than the symbol size used.

of preparation. 1/2Met$_r$, prepared by reduction of met,[14] is quickly oxidized, but reduced slowly,[15d,f,16b,19] and has a rhombic EPR.[15e] Oxidation of deoxy gives 1/2met$_o$,[15d] which is quickly reduced, but is slowly oxidized,[15d,f,16e] and exhibits an axial EPR spectrum.[15e] In this study, excited-state spectral probes (CD, absorption (ABS), low-temperature MCD (LTMCD)) are used to determine the coordination geometry of each Fe, and variable-temperature MCD (VTMCD) is used to probe the ground-state magnetic structure, thus bridging ligation. It is found that 1/2met$_r$ and 1/2met$_o$ have very different spectral features, which reflect ligation differences of the ferrous center. This appears to play a key role in the different redox behaviors of the two forms.

OxyHr was isolated from *Phascolopsis gouldii*;[21] met and deoxy were prepared from oxy.[9,13] 1/2Met was prepared by adding 1 equiv of $K_3Fe(CN)_6$ or $Na_2S_2O_4$ to deoxy or met, respectively, under anaerobic conditions. Absorption spectra to 820 nm were taken on an HP8452A spectrometer with extension to 1800 nm (in D_2O) on a Cary 17 instrument. CD spectra were obtained with a JASCO J500C spectropolarimeter equipped with an Oxford SM4-6T magnet for MCD.[22]

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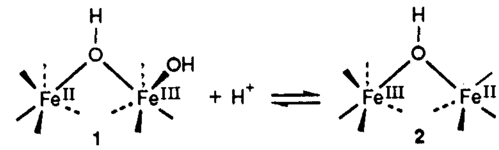
The CD, ABS, and LTMCD spectra for 1/2met$_r$ and 1/2met$_o$ are shown in Figure 1. 1/2Met$_r$ (Figure 1A) shows no ABS below 7000 cm^{-1}. The ABS intensity increases to higher energy with shoulders at 10000 and 14900 cm^{-1}.[23] The transitions in this region can be resolved, using CD and LTMCD, into bands at ~9900 and ~12300 cm^{-1}, which do not change significantly with exogenous ligand binding, as well as a peak at ~14700 cm^{-1}, which is perturbed by ligand binding. Since exogenous ligands are known to bind to the ferric center in 1/2met,[9,18b,24] the ~14700-cm^{-1} band must be a transition of the Fe^{3+}. Its energy and intensity are consistent with a $^6A_1 \rightarrow {}^4T_2$ transition as found in octahedral mononuclear complexes.[25,26] The MCD intensities of the lower energy bands are too large for Fe^{3+},[26] and their insensitivity to ligand perturbation indicates that they are associated with the ferrous center. Their energies are consistent with the $^5T_2 \rightarrow {}^5E$ transitions observed in other octahedral Fe^{2+} complexes,[17,27,28] with a splitting of the ^5E state (Δ^5E) of 2400 cm^{-1}.

1/2Met$_o$ shows a new ABS band peaking below 6000 cm^{-1} (Figure 1B)[29] and a broad, intense (~2 times that of 1/2met$_r$) absorbance feature in the 10000-cm^{-1} region. Coinciding with the ~10000-cm^{-1} ABS is an intense, negative CD band at 10700 cm^{-1} which lacks LTMCD intensity. The LTMCD shows a peak at 12600 cm^{-1} with a shoulder at 14200 cm^{-1}, which also appears in CD. The intense ABS/CD feature at 10700 cm^{-1} can be assigned, based on its lack of LTMCD,[30] as an intervalence transfer (IT) transition. Fe^{3+} in metHrs has no transitions below 8000 cm^{-1};[9] therefore, the <6000-cm^{-1} transition must be due to the ferrous center. Further, the high intensity and low energy of the 12500-cm^{-1} band indicate that it must also be due to the ferrous center,[13,25-28] giving Δ^5E of at least 7600 cm^{-1}. As Fe^{2+} has no higher energy spin-allowed bands,[31] the 14200-cm^{-1} band can be assigned as a $^6A_1 \rightarrow {}^4T_2$ transition of the ferric center. The large Δ^5E of the Fe^{2+} in 1/2met$_o$ indicates that it must be five coordinate on the basis of observed Δ^5E for Fe^{2+} complexes[27,28] (e.g., in deoxyHr:[13] six-coordinate Fe^{2+}, Δ^5E = 1600 cm^{-1}; five-coordinate Fe^{2+}, Δ^5E > 7200 cm^{-1}). Again, the energy of the Fe^{3+} transition is consistent with six coordination.[25,26]

The VTMCD data for 1/2met$_r$ and 1/2met$_o$ are given in Figure 2. The data for both show deviations from Curie law behavior, indicating thermal population of excited states. Note that these deviations are of opposite sign. The data for each have been fit to a Boltzmann population of levels[13,32] to give the first excited state energy, E_1. Using the spin Hamiltonian (eq 1)[13,33] with the experimental E_1 from VTMCD, and g_{eff} from EPR for each 1/2met, best fits were obtained for the parameters $D_{\text{Fe}^{2+}}$, $E_{\text{Fe}^{2+}}$, $g_{z\text{Fe}^{2+}}$, and the exchange parameter, J.[34,35] This treatment involves complete diagonalization of the spin Hamiltonian matrix, instead of a perturbation treatment,[36] and is required when $D_{\text{Fe}^{2+}}$ and J are comparable.[13]

Scheme I



$$\begin{aligned}\hat{H} = {} & -2J\hat{S}_{\text{Fe}^{3+}}\hat{S}_{\text{Fe}^{2+}} + D_{\text{Fe}^{2+}}(\hat{S}^2_{z\text{Fe}^{2+}} - 2) + \\ & E_{\text{Fe}^{2+}}(\hat{S}^2_{x\text{Fe}^{2+}} - \hat{S}^2_{y\text{Fe}^{2+}}) + D_{\text{Fe}^{3+}}(\hat{S}^2_{z\text{Fe}^{3+}} - 35/12) + \\ & g_{z\text{Fe}^{2+}}\beta H_z\hat{S}_{z\text{Fe}^{2+}} + g_{x\text{Fe}^{2+}}\beta H_x\hat{S}_{x\text{Fe}^{2+}} + g_{y\text{Fe}^{2+}}\beta H_y\hat{S}_{y\text{Fe}^{2+}} + \\ & g_{z\text{Fe}^{3+}}\beta H_z\hat{S}_{z\text{Fe}^{3+}} + g_{x\text{Fe}^{3+}}\beta H_x\hat{S}_{x\text{Fe}^{3+}} + g_{y\text{Fe}^{3+}}\beta H_y\hat{S}_{y\text{Fe}^{3+}} \quad (1)\end{aligned}$$

For 1/2met$_r$ ($E_1 = 21 \pm 4$ cm^{-1}; g_{eff} = 1.94, 1.86, 1.65): $D_{\text{Fe}^{2+}} = -7 \pm 1$ cm^{-1}; $|E_{\text{Fe}^{2+}}/D_{\text{Fe}^{2+}}| = 0.14 \pm 0.04$; $g_{z\text{Fe}^{2+}} = 2.16 \pm 0.01$; and $J = -9 \pm 1$ cm^{-1}. For 1/2met$_o$ ($E_1 = 17 \pm 3$ cm^{-1}; g_{eff} = 1.95, 1.70, 1.70): $D_{\text{Fe}^{2+}} = +3 \pm 1$ cm^{-1}; $|E_{\text{Fe}^{2+}}/D_{\text{Fe}^{2+}}| = 0$; $g_{z\text{Fe}^{2+}} = 2.063 \pm 0.005$; and $J = -7 \pm 1$ cm^{-1}.

The analysis of the VTMCD and EPR data demonstrates that 1/2met$_r$ and 1/2met$_o$ have similar values of J, indicating that the antiferromagnetic pathway is similar for both, while the magnitude of J is consistent with a bridging OH$^-$, as has been found for 1/2metN$_3^-$.[18,19] The major difference in the ground-state properties of 1/2met$_r$ and 1/2met$_o$ is the large variation in spin Hamiltonian parameters of the Fe^{2+}. This ground-state difference is consistent with the large excited-state spectral differences (vide supra), which shows that in 1/2met$_r$ both irons are six coordinate, while in 1/2met$_o$ the Fe^{2+} is five coordinate and the Fe^{3+} is six coordinate. As the VTMCD shows no change in bridging ligation, the difference between 1/2met$_r$ and 1/2met$_o$ must be an exogenous ligand bound in 1/2met$_r$, but not in 1/2met$_o$. Only H$_2$O derived ligands are accessible, and since H$_2$O does not bind in either met[9,10] or deoxy,[10b,13] the exogenous ligand in 1/2met$_r$ is likely OH$^-$.[37,38]

The redox reactivities of 1/2met$_r$ and 1/2met$_o$ are thus dependent on the presence of bound exogenous OH$^-$. 1/2Met$_o$ can be rapidly reduced as no large structural rearrangement is necessary to reach deoxy, which contains six- and five-coordinate ferrous centers with no exogenous ligand and an endogenous hydroxo bridge.[13] Alternatively, the rapid oxidation of 1/2met$_r$ suggests that the exogenous OH$^-$ is required for rapid deprotonation of the bridging OH$^-$, to reach the oxo-bridged met. These results for the interconversion of 1/2met$_r$ (**1**) and 1/2met$_o$ (**2**) are summarized in Scheme I. Note that in Scheme I the electron shifts between the irons,[19] which is a ligand-induced intramolecular electron transfer process.

Acknowledgment. We thank the NSF (Grant DMB 8716199) for support of this research and Dr. Richard Reem for developing the spin Hamiltonian program package and for helpful discussions.

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