Fe-O-Fe angle is calculated to be 127°. The close agreement of these values among B_2, metHrN$_3$, and the model compound suggests that B_2 also has the μ-oxobis(μ-carboxylato)diiron core; spectral similarities among these have also led others to suggest this common structure.[8,19] Histidine is probably coordinated to the cluster in B_2 as indicated by the low Z scatterers at 4.3 Å in the EXAFS spectrum and solvent-exchangeable features at 24 ppm in the NMR spectrum.[25] Shell 1B of B_2 is, however, 0.07 Å shorter than that of metHrN$_3$; this suggests that at least one of the terminal nitrogenous ligands of each iron of metHrN$_3$ is replaced by an oxyanion ligand, i.e., hydroxide, phenolate, or carboxylate, in B_2. Fe-O(anion) bonds are typically 0.1-0.2 Å shorter than Fe-N bonds in high-spin ferric complexes.[19-23] Differences in the coordination environments of B_2 and metHrN$_3$ are also suggested by a solvent-nonexchangeable feature at 19 ppm in the NMR spectrum of B_2[25] which is absent in metHrN$_3$.[26] Raman studies indicate the presence of coordinated hydroxide.[27] The replacement of a histidine at 2.15 Å with a hydroxide at 1.95 Å per iron would decrease the average bond length for shell 1B by 0.04 Å.

In summary, our EXAFS results indicate that the iron cluster in subunit B_2 of ribonucleotide reductase shares common structural units with that in metHrN$_3$; however, the two sites are not identical.

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Isobacteriochlorophyll *b* Analogues from Photoreduction of Metallochlorins

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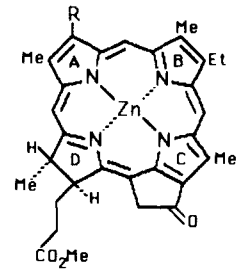
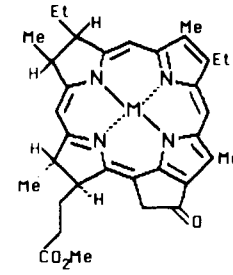
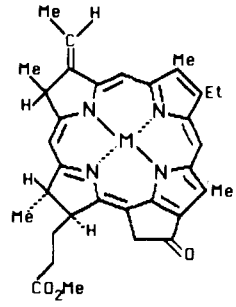
In light of the recent interest in the chemistry and synthesis of isobacteriochlorins, we have reinvestigated the photoreduction of zinc(II) methyl pyropheophorbide *a* (ZnPPheo, **1**) previously reported by Seely.[1] It has been known for some time that ascorbic acid photoreduction of zinc(II) phylloerythrin methyl ester with diazabicyclo[2.2.2]octane (DABCO) as a catalyst gives the thermodynamically least stable *cis*-pheophorbide (dihydroporphyrin).[2] However, no detailed structural or stereochemical information on further photoreduction of such dihydroporphyrins to isobacteriochlorins (tetrahydroporphyrins) appears in the literature. Seely proposed that when photoreduced with ascorbic acid and DABCO, [magnesium(II) or] zinc(II) PPheo (**1**) gives (magnesium or) zinc methyl 2-ethylisobacteriochlorin (ZnEIBC, **2**). This conclusion was primarily based on interpretation of absorption spectra of the EIBCs and of their oxidation products.[1] Herein we show that the initial photoreduction proceeds by way of simple cis reduction of a macrocyclic double bond to give a vinylisobacteriochlorin (VIBC) and that the double bond then migrates toward the macrocycle to give the first synthetic entry to a macrocyclic product bearing an ethylidene system similar to that found in bacteriochlorophylls (BChl) *b* and *g* and in the phycobilin chromophores. Demonstration of this fundamental chemistry suggests that the thermodynamically favorable sequence of macrocycle reduction, followed by "inward" vinyl double bond migration, is a viable alternative to the accepted direct vinyl reduction followed by "outward" macrocyclic double-bond migration to ethylidene in the biosynthesis of the BChl *b* and *g*, and the phycobilins.



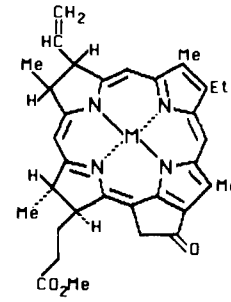
1 R = CH=CH$_2$
7 R = Et



2 M = Zn
8 M = 2H
9 M = Ni
} EIBC



3 M = Zn
5 M = 2H
} EdIBC



4 M = Zn
6 M = 2H
} VIBC

Seely's experiments were, by and large, carried out in spectrophotometry cuvettes. However, we have found that 100 mg of ZnPPheo (**1**) can be conveniently photoreduced in 8-10% ethanol in pyridine containing ascorbic acid and DABCO. Irradiation (fluorescent tubes, white light) of this mixture overnight produced 55 mg of reduced product. The visible absorption spectrum was consistent with that previously reported[1] for the zinc complex with a long-wavelength absorption maximum in pyridine at 626 nm and a shoulder (byproduct) around 608 nm (Figure 1A).

The proton NMR spectrum of the 626-nm product (in $CHCl_3$ and pyridine-d_5) was inconsistent with the expected product, ZnEIBC (**2**), but the meso proton chemical shifts (8.38, 7.56, 6.56 ppm) confirmed that ring A had been reduced. On the basis of extensive decoupling and nuclear Overhauser enhancement (NOE) studies, we conclude that the product is the zinc(II) ethylideneisobacteriochlorin (ZnEdIBC, **3**) rather than ZnEIBC (**2**). The network of NOE connectivities and chemical shifts in ring A is given in Figure 2. These same studies confirmed that, as with ring C in bacteriochlorophyll *b*,[3] the ethylidene group has the *E* configuration.[4]

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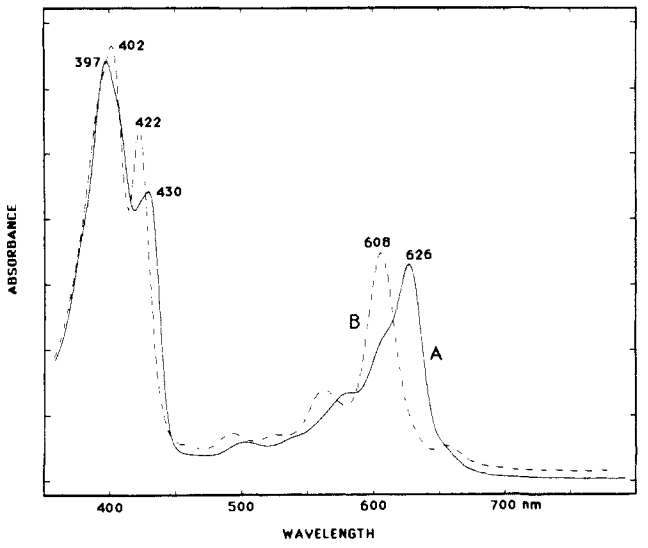

Figure 1. Electronic absorption spectra, in pyridine, of (A) predominantly ZnEdIBC (**3**) and (B) predominantly ZnVIBC (**4**).

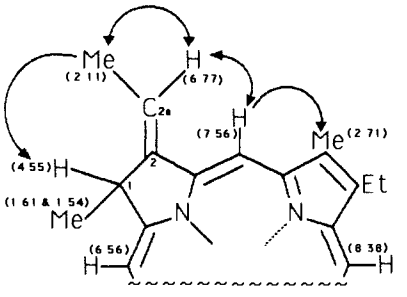

Figure 2. Proton NMR chemical shifts (in parentheses; solution in $CDCl_3$ + pyridine-d_5; Nicolet NT-360, 360 MHz) and network of NOE connectivities for ZnEdIBC (**3**). Coupling constants (Hz): $J_{H1\text{-}Me} = 7.3$; $J_{H1\text{-}H2a} = 1\text{-}2$; $J_{H2a\text{-}Me2a} = 7.1$ Hz.

When our compound **3** was treated with 2,3-dichloro-5,6-dicyanobenzoquinone, the optical and NMR spectra of the resulting oxidized product were identical with those of ZnPPheo (**1**), also confirming that the reduced product must still contain the 2-vinyl group.

The byproduct absorbing at 608 nm also oxidized to ZnPPheo (**1**), but at a slower rate. Logic suggested that the blue-shifted material should be zinc(II) vinylisobacteriochlorin (ZnVIBC, **4**), an isobacteriochlorin similar to that reported by Seely to be the major product. Catalytic hydrogenation of the ethylidene/vinyl mixture gave only one isobacteriochlorin (ZnEIBC, **2**), absorbing at 600 nm in CH_2Cl_2.

Careful spectrophotometric monitoring of the photoreduction showed that the 608-nm product was a precursor of the ethylidene compound **3**. Depending on the reaction conditions, the relative proportions of the two reduced products **3** and **4** was usually 50–90% in favor of the ethylidene compound. Milligram quantities of free bases **5** and **6** [obtained by treatment of the zinc(II) ethylidene/vinyl mixture with trifluoroacetic acid] were separated for NMR by reversed-phase HPLC. Formation of the ethylidene compound **3** from its vinyl precursor **4** is merely an allylic shift, probably a consequence of the basic reaction conditions. Also isolated from the reduction reaction was a small amount of zinc(II) methyl mesopyropheophorbide *a* (**7**), presumably produced by further rearrangement of the ethylidene material to chlorin.

Photoreduction to give predominantly ZnVIBC (**4**) was achieved using benzene as the solvent in place of pyridine and by cutting down the excess of DABCO. In this way, reduced products greater than 95% enriched in **4** were isolated on a large scale. The optical spectum of **4** obtained in this way is shown in Figure 1B; in benzene the long-wavelength absorption appears at 600 nm, while it is at 608 nm in pyridine.

Isolation of pure isobacteriochlorin also allowed an investigation of the stereochemistry of the photoreduction. The vinyl group was readily reduced with hydrogen and Pd/C to give ZnEIBC (**2**), which could be demetalated to EIBC (**8**). The best separations were accomplished with NiEIBC (**9**), obtained by nickel(II) insertion into EIBC (**8**). This nickel complex was separated by reversed-phase HPLC into three peaks, two of which (80% of product) were identical with the *cis*-NiEIBC (**9**),[5] and the other 20% we assign to the trans product. (One cis and one trans isomer have coincidental retention times.) The stereochemistry was confirmed by NMR spectroscopy. The two major resonances for each meso proton had chemical shifts identical with the corresponding meso protons of *cis*-NiEIBC (**9**), thus confirming the stereochemistry of the newly reduced ring as mainly cis. The stereochemistry of the isobacteriochlorin obtained by hydrogenation of the ethylidene/vinyl mixture was determined to be 70% trans and 30% cis in the same manner.

Apart from providing a simple synthetic route to ethylidene-containing macrocycles, the chemical ease with which the ethylidene compound **3** can be synthesized may have important biosynthetic implications. The ethylidene function is found in BChl *b* and *g* and in the chromophores from the phycobilins. Reduction of the carbon–carbon double bond in the pyrrole subunit followed by migration of the vinyl double bond to the ethylidene position is at least as likely as vinyl reduction to ethyl, followed by migration of the pyrrole carbon–carbon double bond to the ethylidene site; indeed, this last step would be thermodynamically unfavorable owing to loss of aromaticity in an equilibrium process.

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Pressure Dissociation of a Protein–Protein Electron-Transfer Complex

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There has been intense recent interest in the molecular mechanisms of electron transfer between physiological redox transfer partners and the mechanisms by which such proteins recognize and form chemically competent complexes with the observed high degree of specificity. We report herein the first determination of the partial specific volume change attending the formation of a protein–protein complex between the physiological redox transfer partners cytochrome b_5 and hepatic cytochrome P-450$_{LM2}$.

Cytochrome P-450 isozyme LM_2 and cytochrome b_5 solubilized from the hepatic endoplasmic reticulum have been shown to form a tight 1:1 complex in solution.[1,2,9] Formation of this complex is readily monitored by a large perturbation of the P-450$_{LM2}$ optical spectrum that is attributed to a change in the spin state of the

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[‡] University of Connecticut Health Science Center.
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