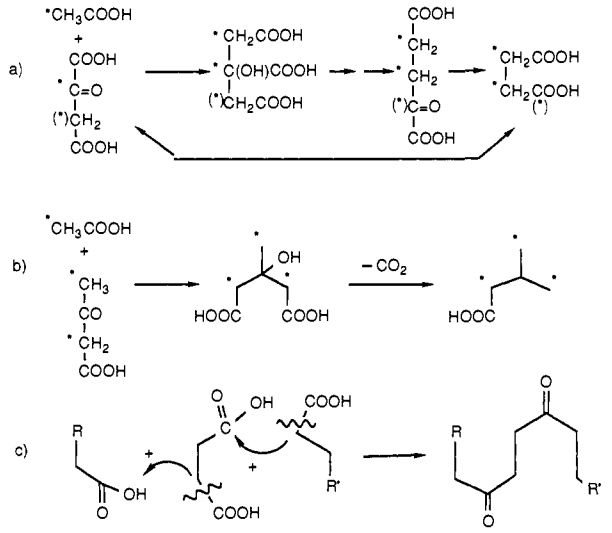
Scheme I



C22-C23, C25-C26, C28-C29, C33-C34, and C36-C37 come from acetate units. The best known examples of bond formation between acetate methyl and primarily methyl-derived carbons are a condensation with oxaloacetate to form citrate and a condensation with glyoxalate (which can be formed from isocitrate by the action of isocitrate lyase) to form L-malate. In both cases, the newly formed connectivity will appear in C2 and C3 of succinate (Scheme I, path a).[9] Thus it seems to be reasonable to assume that the three carbon fragments c, g, h, i, k, and l (Figure 1B) are derived from succinate directly or indirectly. Similarly the four-carbon unit, b, can be of α-keto glutarate, whose C2, C3, and C4 are derived from acetate methyl carbon. The connectivity seen with the four-carbon sequence C45-C13-C14-C15 may be explained by the condensation of propionate to acetate, since the labeling of the acetate methyl group often appears in all three carbons of a propionate-derived fragment.[10] Thus all the mysterious labeling patterns can be interpreted by asssuming the limited involvement of the organic acids metabolism. Regarding the other unusual building blocks a, f, and n, the labeling patterns obtained in acetate feeding experiment are in accordance with the formation of 3-hydroxyl-3-methylglutarate from acetate and acetoacetate, the first step in the isoprenoid biosynthesis (Scheme I, path b).

All these observations seem to suggest a new type of polyketide formation involving dicarboxylic acids, in which a Claisen-type condensation occurs to the α-position of the second carboxylic function with a loss of carboxyl group (Scheme I, path c).[11] The step involves very possibly activation with CoA binding and carboxylation as with the case of polyketide formation in general. Mixed polyketides are actually not new. However, the involvement of unusual fatty acids are mostly at the terminal units,[12] and inside the chains, the condensation is limited to the α-position of rather common monocarboxylic acids.

Attempts to prove the above hypothesis by feeding the putative precursors including [2,3-$^{13}C_2$]succinate have been so far unsuccessful. The failure is not surprising, because the rejection or lack of activation of certain exogenous metabolites by organisms, especially dinoflagellates, is very common and frequently experienced by the authors' group in biosynthetic studies with other dinoflagellates.

Acknowledgment. We are grateful to Professor Koji Nakanishi, Columbia University, for providing us with unpublished data and to Professor David Cane, Brown University, and Professor Heinz G. Floss, The Ohio State University, for precious suggestions. This research was supported by the National Institutes of Health, Grants GM-24425 and GM-28754, which is greatly appreciated. We are also thankful to the Yale University NMR Facility for NMR service.

Supplementary Material Available: Table of carbon-13 and proton chemical shifts and assignment for compound **2** and the 125-MHz ^{13}C NMR spectra of brevetoxin B derived from methyl- and carboxyl-^{13}C-labeled acetate (2 pages). Ordering information is given on any current masthead page.

(9) After one round through TCA cycle, C3 of oxaloacetate should carry one-half of the labeling from the incorporated acetate. Thus one may expect a 2:1 isotope ratio in C2 and C3 of succinate formed in the next round. The rather even enrichment observed in the spectrum can be explained by the quickly declining uptake and utilization of acetate by the organism during the incubation.

(10) The information was provided by Professor David Cane.

(11) An alternative explanation may be the condensation to C3 of pyruvate as seen in the biosynthesis of chlorothricin (Lee, J. J.; Lee, J. P.; Keller, P. J.; Cottrell, C. E.; Chang, C. J.; Zahner, H.; Floss, H. G. *J. Antibiot.* **1986**, *39*, 1123-1134.). If that is the case, pyruvate has to come from the oxidative decarboxylation of malate to account for the labeling pattern.

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Characterization of Six-Coordinate Ferryl Protoheme by Resonance Raman and Optical Absorption Spectroscopy

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Ferryl species (Fe^{IV}=O) have been postulated in the catalytic cycle of cytochrome *c* oxidase,[1] as the oxygen donating species in cytochrome P-450,[2] and as intermediates in the reactions of catalases and peroxidases.[3] Given the diverse chemistry catalyzed by these various enzymes, heme pocket modulation of the chemical reactivity of the Fe^{IV}=O unit seems likely. Resonance Raman detection of the ν(Fe=O) in various protein species and model compounds supports this notion. In a comparison of ferryl peroxidase species,[4] ferryl myoglobin,[5] and five- and six-coordinate heme model compounds,[6,7] the frequency of ν(Fe=O) varies by $\sim$85 cm^{-1} (see Table I). This is in strong contrast to ν(Fe—O_2) which varies by only $\sim$10 cm^{-1} in protein species and heme model compounds.[8] In addition, there are distinct differences in the optical spectra of the various ferryl protein species.[9-11] Previously

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Table I. Comparison of ν(Fe=O) for Various Ferryl Species

species[a]	ν(Fe=O)[b]	ref	species[a]	ν(Fe=O)[b]	ref
HRP, II, pH 6.0	776	4c,e	Fe=O(OEP)	852	6
HRP II, pH 11.0	787	4b,c	(THF)Fe=O(TpivPP)	829	7
CCP ES	767	4d	(NMI)Fe=O(TpivPP)	807	7
ferryl Mb	797	5	(NMI)Fe=O(PPIXDME)	820	*c*
Fe=O(TMP)	843	21	(NMI)Fe=O(TPP)	820	*c*
Fe=O(TPP)	852	6	(NMI)Fe=O(OEP)	820	*c*

[a] Abbreviations: HRP II, horseradish peroxidase compound II; CCP ES, cytochrome c peroxidase compound ES; Mb, myoglobin; TMP, tetramesitylporphyrin; TPP, tetraphenylporphyrin; OEP, octaethylporphyrin; THF, tetrahydrofuran; NMI, 1-methylimidazole; TpivPP, "picket fence" porphyrin [tetrakis(*o*-pivaloylphenyl)porphyrin]; PPIXDME, protoporphyrin IX dimethyl ester. [b] Frequency in cm^{-1}. [c] This work and ref 15.

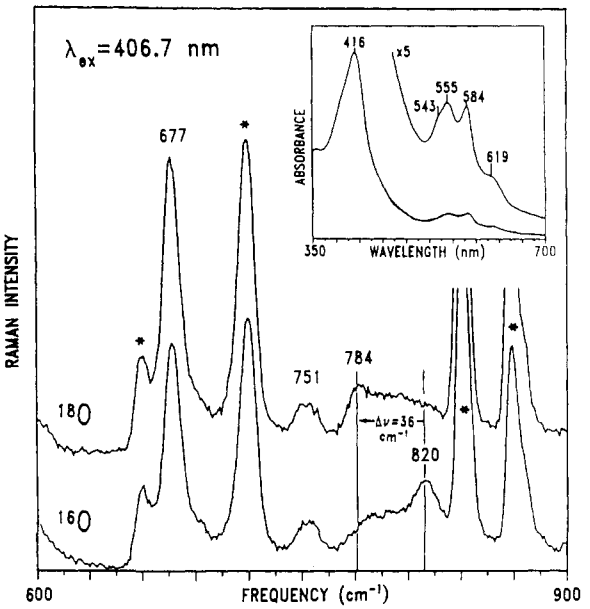

Figure 1. Resonance Raman spectra of [(NMI)Fe^{IV}=^{18}O(PPIXDME)] (upper trace) and [(NMI)Fe^{IV}=^{16}O(PPIXDME)] (lower trace). Vertical line denotes ν(Fe=O); * denotes solvent peak. Spectra were obtained in toluene-d_8 at −130 °C with ~15 mW (406.7 nm) incident on the sample. (Inset) Optical absorption spectrum of [(NMI)Fe^{IV}=O(PPIXDME)] in toluene at −90 °C. The shoulder at 619 nm is due primarily to μ-oxo dimer contamination [(PPIXDME)Fe^{III}]$_2$O.

reported ferryl model compounds have given insight into the factors affecting the ν(Fe=O) frequency, but since they were made on non-physiologically active hemes, they do not give specific information about the optical spectra or the other Raman-active vibrations of the protoheme-containing ferryl protein species. To address these points, we present here optical and Raman data for a six-coordinate, imidazole-ligated, ferryl protoheme model compound.

The (1-methylimidazole)ferryl protoporphyrin IX dimethyl ester, [(NMI)Fe^{IV}=O(PPIXDME)], was prepared according to ref 12. The optical absorption spectrum[13a] is inset in Figure 1. The shoulder at 619 nm is due primarily to μ-oxo dimer contamination. The peak positions compare favorably with those of ferryl hemoglobin[9] and ferryl leghemoglobin[10] (~418, 545, and 575 nm) but are most similar in both wavelengths and relative intensities to those of ferryl myoglobin (~420, 550, and 580 nm at pH 6.8).[11] The spectrum of [(NMI)Fe^{IV}=O(PPIXDME)], like the ferryl globin species, is distinct from that of horseradish peroxidase compound II (~418, 527, and 555 nm).[14] In Figure 1 we present the intermediate frequency region of the Raman spectrum[13b] of [(NMI)Fe^{IV}=O(PPIXDME)]. The peak at 820 cm^{-1} shifts to 784 cm^{-1} upon substitution of ^{16}O by ^{18}O and is assigned to the Fe=O stretching vibration. The 36-cm^{-1} shift is expected for a ferryl structure. The ν(Fe=O) frequency observed for [(NMI)Fe^{IV}=O(PPIXDME)] does not vary with temperature over the range −90 to −190 °C or with porphyrin ring substituents (TPP or OEP).[15] This frequency is compared with the ν(Fe=O) frequencies of other ferryl species in Table I. The high-frequency region of [(NMI)Fe^{IV}=O(PPIXDME)] contains features of both HRP II[16] and ferryl myoglobin[5] yet it is not identical with either.[15] The similarity of the optical spectrum of ferryl myoglobin with that of [(NMI)Fe^{IV}=O(PPIXDME)] indicates that the two are electronically very similar. The difference in the ν(Fe=O) frequencies suggests environmental perturbations. Differences in the ν(Fe=O) frequency between the peroxidase species and ferryl myoglobin have been discussed in terms of hydrogen bonding effects[4c,e] and out-of-plane iron effects.[5] It has been suggested that π-charge donation from the protein into the porphyrin may be important in the mechanism of horseradish peroxidase,[17] but it is not known whether this has any affect on the ν(Fe=O) frequency. Comparison of these systems with the the model compounds gives further insights into the variables which affect the ν(Fe=O) frequency.

A trans ligand effect is seen very clearly in the model compounds (see Table I). The five-coordinate models display the highest ν(Fe=O) frequency while the strong ligand (NMI), six-coordinate samples display the lowest frequency. Electron density from the sixth ligand, along the *z* axis (normal to the heme plane), may compete with the ferryl oxygen for σ-bonding[18] and weaken the Fe=O bond. Alternately, if the iron is initially displaced toward the oxygen in the five-coordinate species, the presence of a strong sixth ligand may pull the iron into plane causing greater π-interaction between iron and porphyrin orbitals and a weakening of the Fe=O π-bond.[5] The difference in the ν(Fe=O) frequency of our models (in toluene) vs. the TpivPP[7] species (in THF) appears to result from solvent effects since temperature and ring substituent effects were ruled out above. This may reflect stronger imidazole binding in the more polar and nonaromatic THF. Although hydrogen bonding to the distal histidine could be involved, the lower ν(Fe=O) frequency of ferryl myoglobin may indicate stronger imidazole ligation in myoglobin than in the model compounds. This seems reasonable since the imidazole is protein bound in myoglobin and may not easily move or rotate to less strongly ligating configurations available to the free solution models. Similar trans ligand effects may contribute to the difference in ν(Fe=O) frequencies observed for HRP II and ferryl myoglobin. For the respective five-coordinate ferrous enzymes,

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(13) (a) Optical absorption spectra were obtained by using a house-built optical Dewer mounted in a Perkin-Elmer Lambda 5. The sample was contained in an EPR tube which was cooled to −90 °C by flowing cold nitrogen gas. (b) Raman spectra were obtained with a Spex 1401 scanning monochromator (with PMT detection) by using 15-mW incident power at 406.7 nm (Spectra-Physics Model 164 Kr ion) in a backscattering geometry. The samples, contained in EPR tubes, were spun continuously in a Dewer while the desired temperature was maintained by flowing cold nitrogen gas.
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the higher $\nu(Fe^{II}$–imidazole) frequency for HRP indicates stronger imidazole ligation.[19,20] For the oxy complexes, $\nu(Fe^{II}$–imidazole) is also higher for HRP than for myoglobin but its $\nu(Fe-O_2)$[8c] is lower. A similar inverse relationship between $\nu(Fe^{II}$–CO) and trans ligand strength has been reported for monomeric insect hemoglobins[18a] and heme model compounds.[18b]

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3-Lithio-1,5-dimethoxypentane. Prognostication, Preparation, and Some Properties of a Dimeric Alkyllithium

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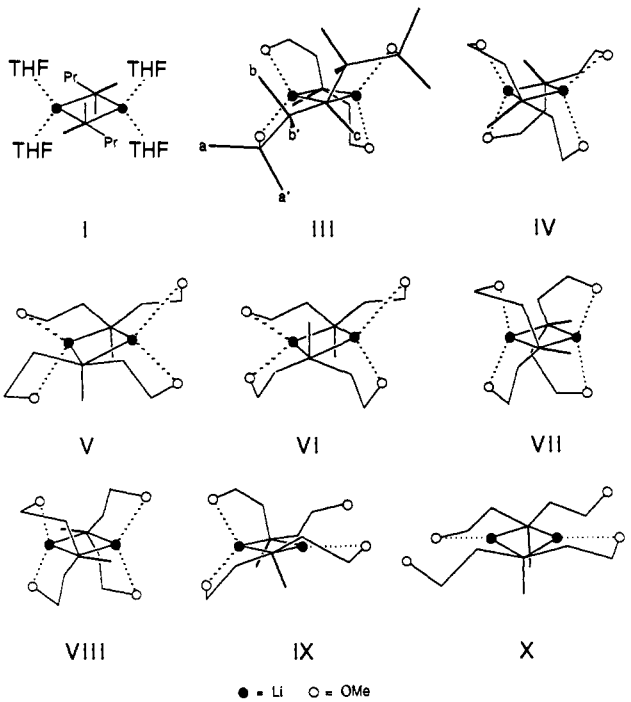
Tetracoordination of lithium is an important principle of organolithium aggregation and complexation.[1] Accordingly, the dimer of *n*-BuLi, recently shown to be present in THF solutions, was assigned a structure of type I, in which two molecules of THF are coordinated to each lithium atom.[2,3] Due to its ephemerality,[3,4] chances for isolation of I are dim. However, closer scrutiny of its properties would seem desirable. Many reactions of tetrameric alkyllithiums in ether solvents involve deaggregated species as the reactive intermediates.[5] In view of the advantages gained by coordinating the required alkoxy groups *intramolecularly*, we expected the dimer of 3-lithio-1,5-dimethoxypentane (II) to be stable in hydrocarbon solutions and amenable to the usual methods of examination.[6]

After treating *tert*-butyl(1,5-dimethoxy-3-pentyl)mercury with 1 equiv of *t*-BuLi in pentane at −15 °C and removing volatile materials (pentane, $(t\text{-Bu})_2Hg$, etc.) by bulb-to-bulb distillation (20 °C, 10^{-5} torr), we extracted II from the residue with pentane and purified it by sublimation (20 °C, 10^{-5} torr) to give colorless crystals melting around 30 °C. Reaction with C_2H_5OD gave 1,5-dimethoxypentane-*3*-d_1. The degree of association of II was found to be 2 (isothermal distillation, 0.004–0.02 M pentane, 28.4 °C).[7,8]

The ^1H and ^{13}C NMR spectra of $(II)_2$ at ambient and low temperature are given in Table I. At −110 °C the signals of H_b and $H_{b'}$ are extremely broad. During these temperature variations the signal of H_c remains essentially unchanged. At 71 °C (solvent C_6D_6) all proton signals are broadened, that of H_c approximating the shape of a quintet. The ^7Li NMR spectrum consisted of a singlet at 1.34 ($\Delta\nu_{1/2}$ = 4.6 Hz, C_6D_6, 20 °C) or 1.40 ppm ($\Delta\nu_{1/2}$ = 14 Hz, cyclopentane, −100 °C).[9]

The 1:1 pairs of similar signals in the low-temperature ^1H and ^{13}C NMR spectra of $(II)_2$ suggest that in the predominating species the CH_2CH_2OMe moieties are present in two different environments. These are averaged by transpositions of chelate rings that are fast on the NMR time scales above ca. −100 °C. Structure III [⇌ III′ (mirror image)] fits the NMR data. It contains two types of CH_2CH_2OMe groups, while the presence of only one type of H_c and lithium atom, respectively, is in accord with the observation of a single signal for H_c and one (though broadened at −100 °C) ^7Li resonance at all temperatures studied. However, stereoisomers with two types of lithium atoms [e.g., IV (⇌ IV′)] with equal or only slightly different chemical shifts cannot be excluded. The remaining possible structures for $(II)_2$ with fully coordinated lithium atoms, V–VIII, are not in agreement with the −90 °C ^1H NMR data.



The coalescence temperature of the methoxy proton signals (−91 ± 2 °C) shows that $\Delta G^{\ddagger}$ for the chelate ring transpositions has a value of 9.3 ± 0.2 kcal/mol, which is lower than those ($\Delta G^{\ddagger}$ = 12–15 kcal/mol) found for similar transpositions (CH_2OMe,[10] CH_2NMe_2[6b]) in intramolecularly coordinated alkyllithium tetramers. We suppose that the breaking of Li–O coordinative bonds

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(9) Chemical shift of ^7Li relative to external 1 M dry LiBr in THF [δ (50% LiBr in H_2O) −1.04 and δ (2-lithiobutane in C_6D_6) 0.77. Not corrected for volume magnetic susceptibility.
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