was ambiguous. The chemical shifts for all methyl groups were comparable to those of **1**. The arguments previously used for proposing the C-13 configuration of **1**, when applied to **2**, favor the β geometry of the side chain, a result that correlates well with Sharpless' structural proposals for related cyclization products.[13] Finally, we reinvestigated and confirmed structure **3** proposed by van Tamelen for the cyclization product of all *trans*-2,3-oxidosqualene under acidic conditions ($SnCl_4$, benzene).[14]

Confirmation of the configuration at C-13 in **1** and **2** as well as the determination of that of C-14 in **1** remains a necessary step in understanding the enzymic mechanism. If **1** and **2** really have different stereochemistry at C-13, a detailed discussion of the possible geometry of the transition state would shed new light on the enzyme-substrate relationship. However, the unambiguous demonstration of the formation of the 6-6-5 squeleton underlines the crucial role of the entire side chain in the cyclization process (see preceding paper).

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Protein Nitrogen Coordination to the FeMo Center of Nitrogenase from *Clostridium Pasteurianum*

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Nitrogenase is a two-component enzyme system consisting of an iron (Fe) and an iron-molybdenum (FeMo) protein.[1] Together these proteins catalyze the ATP-driven six-electron reduction of N_2 to ammonia with concomitant evolution of H_2. In its resting state the FeMo protein exhibits a unique EPR spectrum that is associated with the FeMo cofactor (FeMoco or M center) which is believed to be a key part of the active site for N_2 fixation.[2,3] The EPR signal arises from an $S = {}^3/_2$ center consisting of 6-8 iron atoms and 8-10 sulfur atoms per molybdenum atom.[3-5] Information concerning the cofactor structure has been obtained from X-ray absorption,[6] EXAFS,[6,7] Mössbauer,[8] and EPR[9] data and, more recently, from ^1H, ^{57}Fe, ^{95}Mo, and ^{33}S ENDOR studies.[10,11] Indirect evidence for nitrogen[12] or oxygen donor ligands to Mo[6,7a] and, somewhat less definitively,[7c] to Fe was previously suggested in the analysis of EXAFS data. However, no conclusive evidence for the type of ligand nor its definition as part of FeMoco or as part of the protein was presented. In this communication we report electron spin echo (ESE) experiments on the resting state of FeMo protein isolated from *Clostridium pasteurianum* and of FeMoco isolated in *N*-methyl formamide (NMF) from that protein. The ESE modulation spectrum of the protein contains lines characteristic of nuclear quadrupole transitions for nitrogen coordinated to the paramagnetic metal center. These frequencies are clearly absent from the spectrum of FeMoco isolated in NMF suggesting that the FeMo cofactor is coordinated

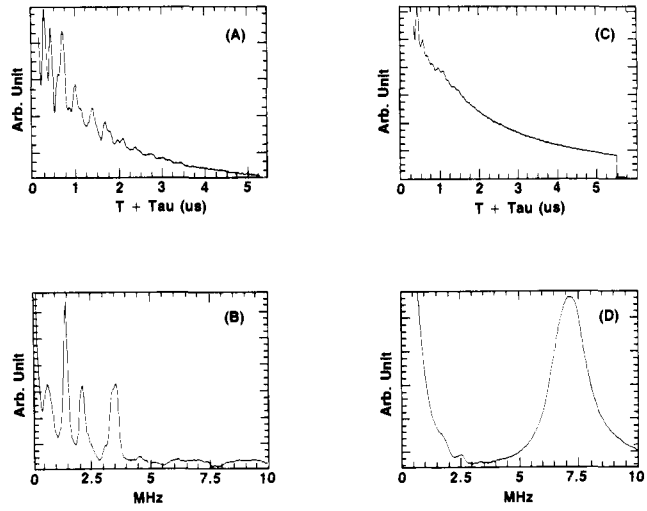

Figure 1. The three pulse stimulated echo envelope waveform is shown for the FeMo protein in (A) and its Fourier transform in (B) and for the FeMo cofactor in (C) and its Fourier transform in (D). The time axis in (A) and (C) is $\tau + t_1$. Conditions are $\tau = 0.12$ μs, B_0, 1740 G, microwave frequency, 9.0595 GHz; temperature, 4.2 K; $\pi/2$ pulse width, 0.02 μs. Fourier transformation was facilitated by using a procedure described by Mims.[21]

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(11) ^{14}N couplings were not observed in recent ENDOR studies on the FeMo protein reported by Hoffman, Orme-Johnson, and co-workers. Two possibilities exist to account for the observation of ^{14}N couplings in the present ESE experiments but not in the ENDOR experiment. First, the pure quadrupole transitions, $\Delta M_I = \pm 1$, 2, observed in the ESE experiment are forbidden in ENDOR while the allowed ENDOR transitions, $\Delta M_S = \pm 1$, $\Delta M_I = \pm 1$, may be too broad to be detected. Second, the magnitude of the ENDOR enhancement depends on a delicate balance between nuclear and electronic spin relaxation rates. The ratio of these relaxation rates may not be favorable for ENDOR at low temperatures.

(12) Preliminary two-pulse electron spin echo data, in which low-frequency modulation was observed, was sited as an unpublished result in ref 10.

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to the protein through at least one nitrogen ligand.

FeMo protein from *C. pasteurianum*[13,14] and FeMoco[3,4,15] were isolated as previously described. ESE experiments were performed on a spectrometer described in detail elsewhere.[16] Data were collected by using a two-dimensional three-pulse sequence. The echo intensity is sampled at time τ following the third $\pi/2$ pulse in a $\pi/2$-τ-$\pi/2$-t_1-$\pi/2$-τ pulse sequence. The echo envelope is recorded for the evolution time t_1 for constant τ at a fixed magnetic field setting. Ligand hyperfine and quadrupolar couplings are observed as a modulation of the echo envelope. This modulation arises from forbidden transitions along the coupled electron-nuclear spin eigenstates which have been coherently excited by the strong microwave pulses.[17]

The time domain echo envelope pattern recorded at the g = 3.7 position in the EPR spectrum for the FeMo protein and FeMo cofactor are shown in Figure 1 (parts A and C, respectively). The cosine Fourier transform spectra, Figure 1 (parts B and D), facilitate identification of the couplings. Four transitions at 0.66, 1.45, 2.09, and 3.5 MHz are clearly identified in the spectrum of the protein. These frequencies arise from transitions among the nuclear quadrupole levels of an ^{14}N nucleus coordinated to the paramagnetic center. As seen in Figure 1D, these nitrogen quadrupole transitions are not observed in the spectrum of FeMoco isolated in NMF. The spectrum of FeMo protein prepared by using a phosphate buffer was identical with that obtained by using TRIS buffer, establishing that the transitions do not arise from a nitrogen atom in the buffer. Furthermore, allowing the FeMo protein to turnover under $^{15}N_2$ (with MgATP, Fe protein, and reductant present) did not change the modulation, eliminating an ephemeral albeit mechanistically significant nitrogen atom in the FeMoco unit as responsible for the nitrogen coupling. Finally, numerical simulations of the ESE spectrum and the frequency shifts of the peaks in the spectra recorded at a variety of different g values indicate that the Fermi-contact hyperfine interaction is 1.6 ± 0.1 MHz.[18] A non-zero Fermi contact coupling can only arise from a covalently coordinated ligand. We conclude that the FeMo center is covalently bound to the protein by at least one nitrogenous ligand whose signature is clearly seen in the ESE experiment.

Mims and co-workers have tabulated ^{14}N coupling frequencies for some heme iron proteins and model iron complexes.[19] In contrast to Cu(II) complexes where directly coordinated nitrogen atoms do not contribute to the echo envelope modulation,[20] quadrupole couplings from directly coordinated nitrogen atoms are observed in low-spin heme Fe(III) complexes. The lowest three frequencies in Figure 1B are not far from the three frequencies observed for the N_1 nitrogen in imidazole–heme–mercaptoethanol [or for the amine N in propylamine–heme–mercaptoethanol]. Moreover, these three frequencies are substantially different from those attributed to the remote N_3 nitrogen of imidazole ligands in either Cu(II) or low-spin Fe(III) heme complexes. These observations suggest that the spectrum in Figure 1B arises from the quadrupole transitions of a nitrogen atom directly coordinated to the FeMo center. It must be recognized, however, that in the absence of a chemical model for the system we are not able to exclude categorically the possibility that the modulation is due to a more remote nitrogen on a covalently coordinated nitrogeneous ligand. This alternative interpretation is particularly important to consider since the details of the electronic structure of the FeMo center as well as the effects of other, as yet, unidentified ligands on the nitrogen couplings are not yet known. We are presently investigating a variety of nitrogenous ligands on FeMoco as well as on model FeMo compounds to determine which ligand reproduces the experimental FeMo protein modulation spectrum.

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An Acyl–Iminium Ion Cyclization Route to a Novel Conformationally Restricted Dipeptide Mimic: Applications to Angiotensin-Converting Enzyme Inhibition

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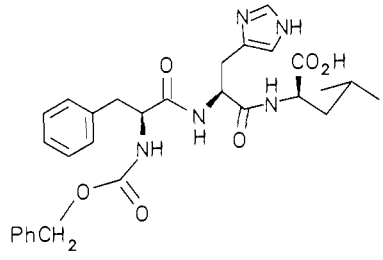
The utility of inhibitors of angiotensin-converting enzyme[1,2] as antihypertensive agents[3,4] has been demonstrated clinically.[5] Our approach to the design of ACE inhibitors was to mimic closely the three carboxy-terminus amino acids of the natural substrate, angiotensin I. The tripeptide fragment (*N*-benzyloxycarbonyl-Phe-His-Leu-OH; **1**) itself has modest affinity for ACE with a $K_M = 1 \times 10^{-5}$ M.[6] Appropriate analyses led to the design of the tricyclic compound **2** as a lipophilic, conformationally restricted mimic of tripeptide **1**. Computer modeling confirmed a unique



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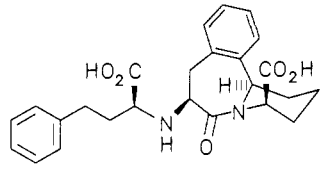

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miminum-energy conformation for **2** where the fused phenyl ring is oriented in close proximity to the terminal carboxyl group. Evaluation of inhibitor **2** containing this rigid tricyclic fragment provided useful insights into the binding requirements of ACE. The chemistry developed for the synthesis of **2** is generally useful for the synthesis of other conformationally restricted peptides. The desired optically active form of key tricyclic dipeptide intermediate **8** was prepared by using L-phenylalanine as the chiral template (Scheme I). *N*-Phthaloyl-L-phenylalanine **3** was coupled to racemic unsaturated amine **4**[7] in 92% yield (EEDQ, CH_2Cl_2). Ozonolysis[8] of the resulting diastereomeric amides **5** (O_3, CH_2Cl_2,

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