The organosilane–F^- reagent system allows us to study the stability and reactivity of metal-free carbanion species which are otherwise labile even at low temperatures.

(18) Protodesilylation of (polyfluorovinyl)silanes with the aid of KF in aqueous dimethyl sulfoxide is recently disclosed: Martin, S.; Sauvetre, R.; Normant, J. F. *Tetrahedron Lett.* **1983**, *24*, 5615.
(19) Hiyama, T.; Obayashi, M. *Tetrahedron Lett.* **1983**, *24*, 4113.

Iron-57 Nuclear Magnetic Resonance Spectroscopic Study of Carbonmonoxymyoglobin†

Hee Cheon Lee, Janice Koles Gard, Theodore L. Brown, and Eric Oldfield*‡

School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Received February 25, 1985

Iron-57 has a very large nuclear magnetic resonance (NMR) chemical shift range[1,2] and is expected to be a sensitive probe of heme–ligand coordination, or electronic structure, in heme proteins. Unfortunately, it is not a sensitive nucleus ($I = {}^1/_2$, 2.2% natural abundance, ν_L = 11.7 MHz at 8.45 T (360-MHz ^{1}H resonance frequency)), so to date no iron-57 NMR spectra of proteins have been reported. In this paper, we report the first observation of iron-57 NMR spectra of a metalloprotein, carbonmonoxymyoglobin ($M_r \sim 18\,000$ daltons), which has been made possible by combined use of isotopic enrichment and a sensitive "home-built" NMR spectrometer, equipped with a 20-mm sideways-spinning probe.[3] Sensitivity is adequate to record partially relaxed spectra for T_1 measurements, and we show that combined T_1 and T_2 determinations yield values for the anisotropy of the chemical shielding tensor and the rotational correlation time, τ_R, of the protein, the latter values being in accord with previous carbon-13 NMR determinations.[4] Our results indicate that the iron-57 isotropic chemical shifts (σ_i) and chemical shift anisotropies ($|\sigma_\parallel - \sigma_\perp|$) of a variety of other proteins (e.g. hemoglobin, chloroperoxidase, cytochrome P450) should be accessible via iron-57 enrichment and high-field large-sample operation, yielding potentially useful information on the nature of iron–ligand interactions in such systems.

We show in Figure 1A the natural-abundance iron-57 NMR spectrum of ferrocene ($(\pi\text{-}C_5H_5)_2Fe$, 0.8 M in C_6H_6) obtained in a 20-mm sideways-spinning probe at 8.45 T (corresponding to an ^{57}Fe resonance frequency of 11.7 MHz). This spectrum (S/N ~9, total acquisition time = 30 min) compares favorably with results presented previously by others.[2,5-7] On the basis of previous work, we assign ferrocene a chemical shift of 1531 ppm downfield from $Fe(CO)_5$.[5] The line width is ~2.5 ± 0.5 Hz, in accord with the 2.6-Hz value (at 9.7 MHz) observed by Nozawa et al.[7]

In contrast to the narrow line spectrum of ferrocene, we show in Figure 1B the iron-57 NMR spectrum of [^{57}Fe]carbonmonoxymyoglobin (MbCO, 15 mM in 50 mM phosphate buffer, pH 7.1, at 23 °C). The chemical shift observed is 8227 ppm downfield from $Fe(CO)_5$, slightly more deshielded than the 8211 ppm value found for Fe(PP-IX)(CO)(py) (PP-IX = protoporphyrin-IX) 0.05 M in pyridine,[1] and the line width (55 ± 5 Hz) is considerably greater than the ~2.5-Hz value found for ferrocene, due to the increased rotational correlation time of the protein. The protein sample appeared to contain exclusively MbCO, as determined by visible absorption spectrophotometry and by natural abundance carbon-13 NMR spectroscopy (data not shown), shortly after iron-57 NMR data acquisition. A second set of iron-57 NMR experiments (inset in Figure 1B) showed no NMR signals from a natural-abundance sample of MbCO but again contained a well-resolved signal at 8227 ppm from the enriched material.

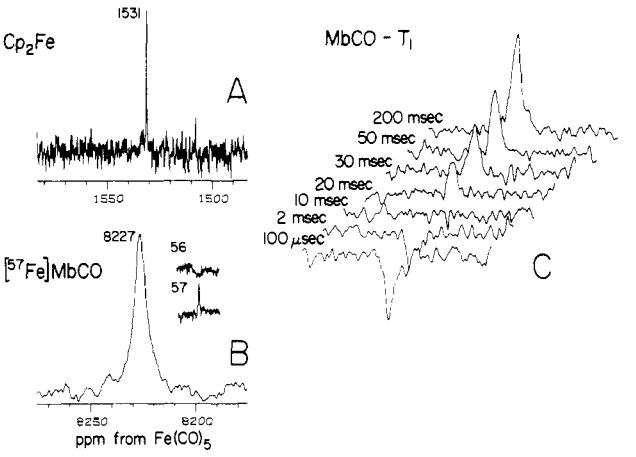

Figure 1. Iron-57 NMR spectra of ferrocene and [^{57}Fe]carbonmonoxymyoglobin at 8.45 T (corresponding to an ^{57}Fe resonance frequency of about 11.6 MHz or a ^{1}H resonance frequency of 360 MHz) and partially relaxed Fourier transform (PRFT) data set for MbCO. (a) 0.8 M Cp_2Fe in C_6H_6, 1040 scans, 2-s recycle time, 35-μs 90°-pulse excitation, 2-Hz line broadening; (B) [^{57}Fe]MbCO (15 mM in 50 mM phosphate buffer, pH 7.1, 23 °C) 120 001 scans, 500-ms recycle time, 35-μs pulse excitation, 20-Hz line broadening; (inset) spectra of natural abundance MbCO and [^{57}Fe]MbCO recorded under same sample and spectrometer condition as in (B), except only 27 400 scans per spectrum; (C) PRFT data set using the basic conditions noted in (B), the τ values are given on the Figure.

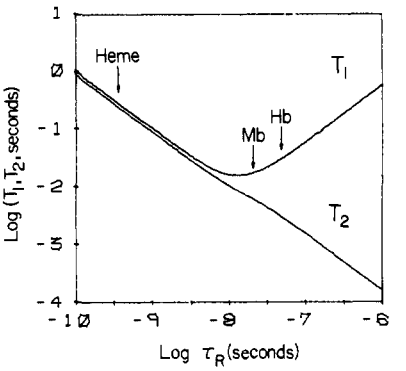

Figure 2. log–log plots of T_1 and T_2 vs. τ_R (both in seconds) for iron-57 relaxation via a chemical shift anisotropy mechanism at 8.45 T and assuming $|\sigma_\parallel - \sigma_\perp|$ = 3600 ppm. T_1/τ_R combinations appropriate for Fe(PP-IX)(CO)(py), MbCO, and HbCO are indicated.

We now consider the relaxation of iron in proteins. We show in Figure 1C inversion-recovery (180°–τ–90°, ref 8) partially relaxed Fourier transform (PRFT) data for [^{57}Fe]MbCO, at 8.45 T. Analysis of the results in Figure 1C yields T_1 = 17 ± 3 ms, at 23 °C. Since there are no protons particularly close by, it seems reasonable to believe that relaxation will be dominated by the chemical shift anisotropy mechanism,[1,8] in which case[8]

$$1/T_1 = {}^1/_{15}\gamma^2 H_0^2(\sigma_\parallel - \sigma_\perp)^2 \frac{2\tau_R}{1 + \omega^2\tau_R^2} \qquad (1)$$

† This work was supported in part by the US National Institutes of Health (Grant HL-19481).
‡ USPHS Research Career Development Awardee (1979–1984) supported in part by Grant CA-00595.
(1) Baltzer, L.; Becker, E. D.; Averill, B. A.; Hutchinson, J. M; Gansow, O. *J. Am. Chem. Soc.* **1984**, *106*, 2444.
(2) Nozawa, T.; Sato, M.; Hatano, M.; Kobayashi, N.; Osa, T. *Chem. Lett.* **1983**, 1289.
(3) Oldfield, E.; Meadows, M. *J. Magn. Reson.* **1978**, *31*, 327.
(4) Oldfield, E.; Norton, R. S.; Allerhand, A. *J. Biol. Chem.* **1975**, *250*, 6368.
(5) Jenny, T.; Von Philipsborn, W.; Kronenbitter, J.; Schwenk, A. *J. Organomet. Chem.* **1981**, *205*, 211.
(6) Haslinger, E.; Robien, W.; Schlogl, K.; Weissensteiner, W. *J. Organomet. Chem.* **1981**, *218*, C11.
(7) Nozawa, T.; Hatano, M.; Sato, M.; Toida, Y.; Batholdi, E. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3837.

0002-7863/85/1507-4087$01.50/0

and

$$1/T_2 = {}^1/_{90}\gamma^2 H_0^2(\sigma_\parallel - \sigma_\perp)^2 \left\{ \frac{6\tau_R}{1 + \omega^2\tau_R{}^2} + 8\tau_R \right\} \quad (2)$$

where the symbols have their usual meanings.[8] For the planar heme moiety, the chemical shift tensor is likely to be axially symmetric, as indicated in eq 1 and 2.

There are thus two unknowns, $|\sigma_\parallel - \sigma_\perp|$ and τ_R, but also two observables, T_1 and T_2. Thus, both $|\sigma_\parallel - \sigma_\perp|$ and τ_R can be determined, at least if we assume T_2 can be obtained from the line width. Alternatively, we can use the known τ_R for MbCO under these conditions (τ_R = 20 ns, ref 4) to obtain $|\sigma_\parallel - \sigma_\perp|$ from eq 1 and then *predict* the CSA contribution to the line width from eq 2.

For τ_R = 20 ns, eq 1 yields $|\sigma_\parallel - \sigma_\perp|$ = 3600 ppm, and eq 2 predicts a line width, W, of 48.6 Hz, which is close to the observed line width of 55 ± 5 Hz (Figure 1B). Alternatively, we can use the observed T_1 (17 ms) and line width (55 Hz) and eq 1 and 2, as shown in Figure 2, to predict $|\sigma_\parallel - \sigma_\perp|$ = 3680 ppm and τ_R = 22 ns. Iron–hydrogen dipolar contributions to the iron-57 relaxation are minimal since the nearest hydrogens are at least ~3 Å away (on the proximal histidine residue) and yield T_1^{-1} and T_2^{-1} contributions of 0.003 and 0.009 s^{-1}, respectively.

A second source of relaxation could be via ^{57}Fe–^{14}N scalar coupling of the second kind, to the four heme nitrogens directly coordinated to iron. On the basis of the ^{14}N quadrupole coupling constants for pyrrole and a series of metal-coordinated pyridines[9-11] we estimate τ_s for ^{14}N to be ~2×10^{-5} s (assuming τ_R = 20 ns). Since $^1J_{Fe-N}$ ~6 Hz,[2,12] we compute scalar contributions of $T_1^s = 2 \times 10^8$ s and T_2^s = 50 s, which are both quite negligible. Thus, as observed experimentally, there is no resolvable Fe–N J coupling, and the ~50-Hz line widths predicted from the T_1 and τ_R data are in excellent agreement with observation.

We now consider the prognosis for iron-57 NMR studies of proteins. The results of Figure 2 show, at least for the case $|\sigma_\parallel - \sigma_\perp|$ = 3600 ppm, that for small proteins characterized by τ_R ~20 ns (such as cytochrome c and myoglobin, ref 4), T_1 values will be at or close to the minimum value possible (15.8 ms at τ_R = 14 ns), favorable circumstance for rapid data acquisition. In future studies using optimum recycle/pulse width combinations, it is clear from the results of Figures 1B (inset, 27 400 scans) and 2 that acceptable signal-to-noise ratio spectra should be achievable in ~$27\,400 \times 2T_1 \approx 15$ min of data acquisition.

For larger species, the results of Figure 2 indicate longer T_1 values and broader line widths. For hemoglobin (τ_R ~44 ns, ref 4, corrected for r_{CH} = 1.10 Å), we predict T_1 ~28 ms and a line width of ~92 Hz—not greatly different from the results with MbCO. For much larger proteins (M_r ~200 000, τ_R ~0.1 μs), T_1 values will be ~60 ms and line widths ~200 Hz, and again such species should be accessible, although of course the inherent dilution expected will increase data acquisition periods considerably.

Note for a system the size of hemoglobin, or larger, eq 1 reduces to

$$1/T_1 = 2(\sigma_\parallel - \sigma_\perp)^2/15\tau_R \quad (3)$$

so that T_1 values are independent of magnetic field strength (and the gyromagnetic ratio of the nucleus in question). However, the full sensitivity gains expected from high-field operation will be partially offset by the quadratic increases in line width. Indeed, we find for MbCO at 11.7 T T_1 = 15 ms and W = 110 Hz, which compares favorably with the predicted values of 14.6 ms and 98 Hz, respectively.

Note Added in Proof. The ^{57}Fe chemical shift of the isopropyl isocyanide adduct of (^{57}Fe)Mb is at 9256 ppm, over 1000 ppm from the carbonmonoxy species.

(8) Farrar, T. C.; Becker, E. D. "Pulse and Fourier Transform NMR"; Academic Press: New York, 1971.
(9) Schempp, E.; Bray, P. J. *J. Chem. Phys.* **1968**, *48*, 2380.
(10) Nygaard, L.; Nielsen, J. T.; Kirchheiner, J.; Maltensen, G.; Rastrup-Andersen, J.; Sørensen, G. O. *J. Mol. Struct.* **1969**, *3*, 491.
(11) Rubenacker, G. V.; Brown, T. L. *Inorg. Chem.* **1980**, *19*, 392.
(12) Kidd, R. G.; Goodfellow, R. J. In "NMR and the Periodic Table"; Harris, R. K., Mann, B. E., Eds.; Academic Press: New York, 1978; pp 195-278.

Asymmetric Synthesis via Chiral Sulfinylallyl Anion. Total Synthesis of (+)-Hirsutene: Facile Ring Closure Involving Enol Thioether and Enol Acetate Moieties

Duy H. Hua,* Gurudas Sinai-Zingde, and S. Venkataraman

Department of Chemistry, Kansas State University
Manhattan, Kansas 66506

Received January 28, 1985

Asymmetric induction reactions involving sulfoxides possessing chiral sulfur have received increasing attention in the past decade. The reactions of chiral α-sulfinyl anions with a variety of electrophiles often proceed with substantial asymmetric inductions at the newly formed chiral centers.[1] However, asymmetric induction in reactions involving anions of allylic sulfoxides has only been observed with benzaldehydes.[2] Generally, facile racemization at the sulfur atom occurs via a reversible [2,3] sigmatropic process.[3,4] We now report the regio- and stereochemical aspects of reactions of chiral sulfinylallyl anions with various cyclic enones[5] and the utilization of these reactions in the asymmetric synthesis of (+)-hirsutene (**1**), one of a variety of sesquiterpenoids isolated from the extract of *Coriolus consors*[6] and presumed to be the biogenetic precursor of coriolin,[7] hirsutic acid,[8] and complicatic acid.[9]

Treatment of (+)-(*R*)-allyl *p*-tolyl sulfoxide (**2**)[3] with lithium diisopropylamide (LDA) in THF at −78 °C for 1 h followed by 1 equiv of HMPA and 1 equiv of 2-cyclopentenone at −78 °C for 5 min provided the 1,4-adduct **3**[10] in 90% yield with 96% ee

(1) (a) Tsuchihashi, G.; Iriuchijima, S.; Ishibashi, M. *Tetrahedron Lett.* **1972**, 4605-4608. (b) Tsuchihashi, G.; Iriuchijima, S.; Maniwa, K. *Tetrahedron Lett.* **1973**, 3389-3392. (c) Johnson, C. R.; Schroeck, C. W.; Shanklin, J. R. *J. Am. Chem. Soc.* **1973**, *95*, 7424-7431. (d) Kunieda, N.; Nokami, J.; Kinoshita, M. *Tetrahedron Lett.* **1974**, 3997-4000. (e) Biellmann, J. F.; Vicens, J. J. *Tetrahedron Lett.* **1974**, 2915-2918. (f) Tsuchihashi, G.; Mitamura, S.; Ogura, K. *Tetrahedron Lett.* **1976**, 855-858. (g) Nishihata, K.; Nishio, M. *Tetrahedron Lett.* **1976**, 1695-1698. (h) Mioskowski, C.; Solladie, G. *Chem. Commun.* **1977**, 162-163. (i) Chassaing, G.; Lett, R.; Marquet, A. *Tetrahedron Lett.* **1978**, 471-474. (j) Mioskowski, C.; Solladie, G. *Tetrahedron* **1980**, *36*, 227-236. (k) Posner, G. H.; Malame, J. P.; Miura, K. *J. Am. Chem. Soc.* **1981**, *103*, 3886-3888. (l) Corey, E. J.; Weigel, L. O.; Chamberlin, A. R.; Cho, H.; Hua, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 6613-6615. (m) Solladie, G. *Synthesis* **1981**, 185-196.
(2) Ridley, D. D.; Smal, M. A. *Aust. J. Chem.* **1983**, *36*, 1049-1055 and ref 1 cited therein.
(3) Bickart, P.; Carson, F. W.; Jacobus, J.; Miller, E. G.; Mislow, K. *J. Am. Chem. Soc.* **1968**, *90*, 4869-4876.
(4) Allyl sulfoxide/allyl sulfenate rearrangements in organic synthesis have been studied. For the reviews, see: (a) Hoffman, R. W. "Organic Sulfur Chemistry"; Freidlina, R. K., Skorova, A. E., Eds.; Pergamon Press: New York, 1981; pp 69-80. (b) Evans, D. A.; Andrews, G. A. *Acc. Chem. Res.* **1974**, 147-155.
(5) The regioselectivity of addition to α-enones has been reviewed: Lefour, M. M.; Loupy, A. *Tetrahedron* **1978**, *34*, 2597-2605.
(6) (a) Nozoe, S.; Furukawa, J.; Sankawa, U.; Shibata, S. *Tetrahedron Lett.* **1976**, 195-198. (b) Feline, T. C.; Mellows, G.; Jones, R. B.; Phillips, L. *Chem. Commun.* **1974**, 63-64.
(7) Nakamura, H.; Takita, T.; Umezawa, H.; Kunishima, M.; Nakayama, Y.; Iitaka, Y. *J. Antibiot.* **1974**, *27*, 301-302 and earlier reports cited therein.
(8) Comer, F. W.; McCapra, F.; Qureshi, I. H.; Scott, A. I. *Tetrahedron* **1967**, *23*, 4761-4768.
(9) Mellows, G.; Mantle, P. B.; Feline, T. C.; Williams, D. J. *Phytochemistry* **1973**, *12*, 2717-2720.
(10) Binns, M. R.; Hayne, R. K.; Houston, T. L. *Aust. J. Chem.* **1981**, *34*, 2465-2467. All enantiomers are being depicted with absolute stereochemistry as indicated. All new compounds displayed satisfactory ^1H NMR (400 MHz), ^{13}C NMR (100 MHz), UV, IR, and low-resolution mass spectra and satisfactory elemental analysis or chemical ionization MS.

0002-7863/85/1507-4088$01.50/0