**Table I.** Crystal Parameters for Antibiotic A23187

| | |
|---|---|
| $a$ | 15.759 (4) Å |
| $b$ | 10.377 (4) Å |
| $c$ | 8.592 (3) Å |
| $\beta$ | 95.97 (2)° |
| Space group | $P2_1$ |
| Molecules/cell | 2 |
| Obsd density | 1.264 g cm$^{-3}$ |
| Calcd density | 1.244 g cm$^{-3}$ |

The structure was solved by direct methods using the program MULTAN[3] and was refined by the least-squares method. The final R factor, using anisotropic temperature factors for all heavy atoms and isotropic temperature factors for all hydrogen atoms (at assumed locations), was 0.063. The refined atomic coordinates for the heavy atoms are given in Table II.[4] The conformation of the molecule in the crystal is shown in Figure 1.

The structure consists of three basic units, $\alpha$-ketopyrrole, a substituted benzoxazole, and a spiro ring system similar to those found in the polyether antibiotics—monensin,[5] nigericin,[6] grisorixin,[7] dianemycin,[8] X-206,[9] and A204A.[10] In the polyether antibiotics, the spiro ring systems consist of a five- and a six-membered ring, whereas A23187 contains two six-membered rings. Comparison of the spiro moieties in the polyethers and in A23187 shows that conformationally, they are very similar; in each case, the ring ether oxygen atoms are in axial or pseudoaxial conformations, and the points of attachment of the rest of the molecular chain are equatorial or pseudoequatorial.

Because the molecule contains no atom with strong anomalous X-ray scattering, we have been unable to determine experimentally the absolute configuration. However, in Figure 1, the chiralities of the asymmetric centers in each of the two six-membered rings of the spiro group are the same as those found in all the polyethers which contain a spiro six-membered ring.[11] It seems probable, therefore, that the absolute configuration shown is correct.

In the crystalline, free acid form of A23187, there are three internal hydrogen bonds, as shown by the dotted lines in Figure 1. The hydrogen bond between the pyrrole nitrogen atom and one of the carboxyl oxygen atoms holds the ends of the molecule in close proximity,

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(10) N. D. Jones, M. O. Chaney, J. W. Chamberlin, R. L. Hamill, and S. Chen, *J. Amer. Chem. Soc.*, **95**, 3399 (1973).

(11) Dianemycin (ref 8) contains two spiro ring systems. The one closest to the carboxyl end of the molecule has the same chiralities found in the polyether antibiotics mentioned above, but the other spiro moiety has several asymmetric centers with reversed chirality.

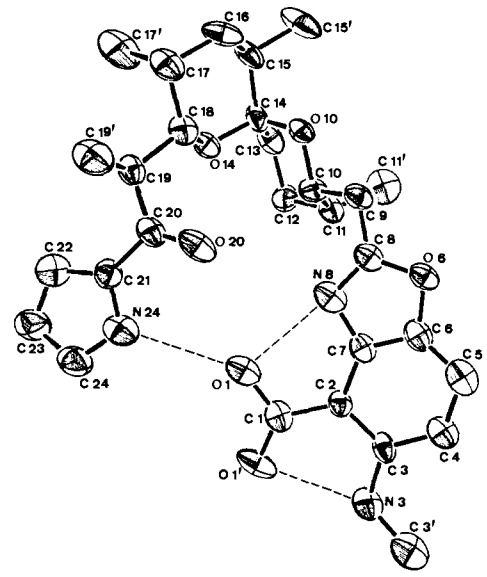


**Figure 1.** Conformation and proposed absolute configuration of A23187 in the crystal. The thermal ellipsoids are drawn at the 50% probability level.

a feature usually seen in the crystal structures of polyether antibiotics. Although the structure of the 2:1 antibiotic–divalent cation complex is not yet known, one can speculate that the principal ligands to the metal ion are the oxygen atoms of the carbonyl and carboxyl group, as well as one of the ether oxygen atoms from the spiro ring system.

**Acknowledgment.** We wish to thank Dr. R. L. Hamill and Mr. E. A. Presti for supplying the antibiotic used in this investigation and Dr. D. E. Dorman for his help in the preparation of this manuscript. Appreciation is also due Mr. D. W. Smith for computer assistance and Mr. J. W. Paschal and Mr. J. P. Hettle for their help in obtaining nmr and mass spectra.

**Supplementary Material Available.** A listing of refined atomic coordinates will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-1932.

**Michael O. Chaney,* Paul V. Demarco**
**Noel D. Jones, John L. Occolowitz**
*The Lilly Research Laboratories, Eli Lilly and Company*
*Indianapolis, Indiana 46206*
*Received November 9, 1973*

## Rapid Relaxation of Spin Equilibrium in Ferric Myoglobin Hydroxide

*Sir:*

The hypothesis[1] that changes in the spin states of the cytochromes are intimately related to the mechanism of oxidative phosphorylation has attractive features. Electron transfer and phosphorylation could be coupled through the conformational changes induced in the

(1) D. F. Wilson, P. L. Dutton, M. Erecinska, J. G. Lindsay, and N. Sato, *Accounts Chem. Res.*, **5**, 234 (1972), and references therein.

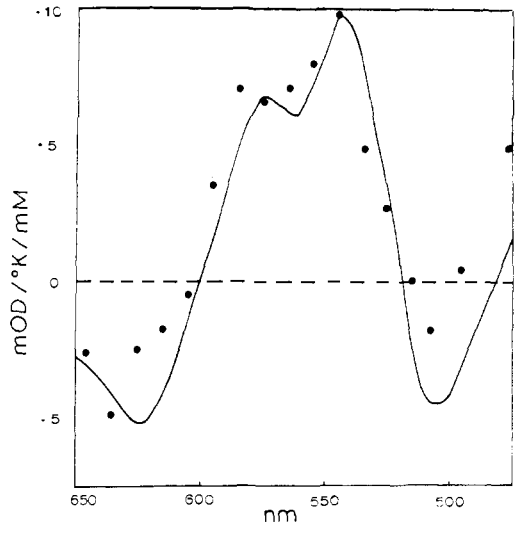


**Figure 1.** Temperature difference spectrum (—) and the amplitudes of optical density changes observed in relaxation experiments for ferric myoglobin hydroxide. The data have been normalized for a 1° change of a 1 m$M$ solution (mOD = $10^{-3}$ OD, m$M$ = $10^{-3}$ $M$).

protein by the movement of the iron atom into or out of the plane of the porphyrin ligand,[2-4] and energy could be conserved through a spin-state-dependent redox potential of the cytochrome. Some evidence implicating phosphate-dependent spin-state changes in mitochondria has recently been presented.[1] For these reasons it is imperative to examine the dynamics of spin-state changes in iron–porphyrin complexes and in hemoproteins. We report here that the interconversion of the high-spin and low-spin states of ferric myoglobin hydroxide has a lifetime of less than 5 μsec, indicating that such spin-state changes can be sufficiently rapid to participate in coupled electron transport.[5]

The existence of thermal equilibrium between the high-spin sextet and low-spin doublet electron configurations of ferric myoglobin hydroxide has been established from the temperature dependence of its electronic absorption spectrum,[6,7] of its magnetic susceptibility,[6] and of its electron spin resonance spectrum.[8] The reported temperature difference spectrum between 5 and 35° is reproduced in Figure 1.[6]

Temperature-jump relaxation experiments were performed using apparatus supplied by Messanlangen Studiengesellschaft. Both equine and sperm whale myoglobins supplied by Sigma Chemical Co. were used as received with similar results. Solutions of $2 \times 10^{-4}$ $M$ protein were prepared in 0.1 $M$ glycine buffer at pH 11.0. Optical density changes between 650 and 450 nm were recorded for temperature jumps of approximately 1° from 22°. The amplitude of the optical density change at 545 nm agreed within the experimental error of ±20% with the optical density change reported[6] from the static temperature difference spectra. The relaxation data are plotted as points in Figure 1. The agreement between the static temperature difference spectrum and the amplitudes of the relaxation effect indicates that the observed relaxation is the result of the change in spin state.

The heating risetime of the apparatus with a discharge capacitor of 20 pF is 3–5 μsec using the pH indicator Alizarin Yellow with the glycine buffer. Under identical operating conditions, the relaxation time observed with the myoblogin solutions was found to be equivalent to this heating risetime. This places a lower limit of $2 \times 10^5$ sec$^{-1}$ on the sum of the forward and reverse rate constants for the interconversion of the spin states. An upper limit on this sum of about $10^{10}$ sec$^{-1}$ can be inferred from the reported observation[8] of separate X-band epr signals at $g = 2$ and $g = 6$ for the two spin states.

These results are consistent with the recent observation[9] that relaxation between the singlet and quintet states of an iron(II) complex in solution occurs with rate constants of $10^7$ sec$^{-1}$. In both cases a spin change with $\Delta S = 2$ is involved, although in the iron(II) complex the accompanying stereochemical change involves expansion of the octahedral coordination sphere while in ferric myoglobin hydroxide the reorganization is presumably from six-coordinate to five-coordinate.

These rapid spin-state changes cause some doubt about slower spin-state interconversions reported for iron(III) hemin–pyridine systems.[2,10,11] A rate constant of $10^3$ sec$^{-1}$ has been reported from pmr line broadening data.[10] In another study, separate pmr resonances with relatively narrow lines were assigned to high-spin and low-spin forms of the complex,[11,12] implying a rate constant for interconversion of $<10^2$ sec$^{-1}$. The resonances ascribed to the high-spin isomer have been subsequently reassigned to a hemin–pyridine chloride complex.[13] This suggests that the dynamic process identified as a spin-state interconversion[10] is probably related to a substitution process on iron(III).[14]

The present results indicate that the rate of change of spin state in at least one iron(III) hemoprotein is sufficiently rapid to be consistent with a role in coupled electron transport. It is significant that relaxation effects observed in carboxymethylated cytochrome $c^{II}$ have been interpreted as a high-spin to low-spin conversion with a rate constant of $1.5 \times 10^4$ sec$^{-1}$ with a reverse rate constant of 25 sec$^{-1}$.[15] This suggests that formation of a six-coordinate low-spin complex requiring a protein conformation change to provide the sixth ligand may be slower than formation of a low-spin complex with a solvent molecule as the sixth

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(14) G. N. La Mar and F. A. Walker, *J. Amer. Chem. Soc.*, **94**, 8607 (1972); G. N. La Mar, *ibid.*, **95**, 1662 (1973); J. Hodgkinson and R. B. Jordan, *ibid.*, **95**, 763 (1973).
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ligand. Furthermore, it suggests that, for rapid turnover in electron transport, a low-spin complex with a weakly coordinated sixth ligand may be a prerequisite.[16]

**Acknowledgment** is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We thank Dr. C. T. Lin for a preliminary temperature-jump measurement.

(16) Parallel experiments on ferric myoglobin azide, which exists in spin equilibrium at pH 7,[6] give similar results, *i.e.*, the spin-equilibrium relaxation occurs within the heating risetime of 5 μsec. This relaxation process is considerably more rapid than the formation and dissociation of the azido ferrimyoglobin.[17]

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**James K. Beattie,* Robert John West**
*School of Chemistry, The University of Sydney*
*Sydney, New South Wales 2006, Australia*
*Received October 11, 1973*

## High-Pressure High-Resolution Nuclear Magnetic Resonance. Pressure Dependence of the Proton Chemical Shift of Chloroform in Aromatic Solvents

*Sir:*

Recent experiments on the high-pressure high-resolution nmr have shown that the technique provides a very useful means of studying the behavior of a molecule in the liquid phase at high pressure.[1]

In the previous paper,[1c] one of us described a convenient device in which a pressure-resisting glass cell was connected to a thermal expansion type pressure generator and a piezometer to realize the high-resolution nmr experiment up to near 1700 kg cm$^{-2}$. The device, however, required considerable skill and effort to be constructed for the correct functioning. To overcome this difficulty, we have developed a method of connecting the glass cell with standard high-pressure equipment, and, in the course of the nmr experiments utilizing the improved apparatus, we have found a notable pressure dependence of the proton chemical shift of chloroform in aromatic solvents.

The apparatus mainly consists of a stainless steel nozzle (NZ), a glass nozzle (NZG) fixed in (NZ) with an epoxy adhesive, and a glass cell having a long tail of flexible capillary, the end of which is fixed in (NZG) with the adhesive.[2] The (NZ) was mounted to the ordinary high-pressure system consisting of a 5000 kg cm$^{-2}$ Bourdon gauge[3] and a 3000 kg cm$^{-2}$ hand pump. The nmr measurements were carried out on a JEOL 3H-60 high-resolution spectrometer operating at 60 MHz, and the present sample cell, having i.d. $\simeq$ 1 mm, proved satisfactory to perform easily the high-resolution measurement to establish $\Delta\nu_{h/2} \simeq 1$ Hz for the chloroform resonance. The chemical shifts were determined by linear interpolation between known audio frequency side bands to obtain the accuracy of $\pm 0.1$ to $\pm 0.2$ Hz. The temperature of the sample, measured by the procedure described in the previous paper,[1c] was estimated to be maintained at 29–30° throughout the experiment.

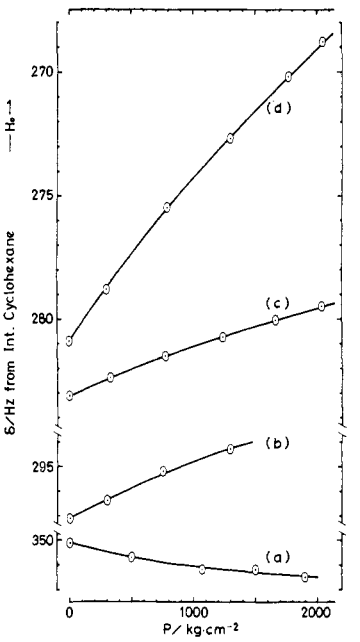


**Figure 1.** Pressure dependence of the chloroform proton chemical shift for chloroform (a) and chloroform in benzene (b), in mesitylene (c), and in 1,3,5-triisopropylbenzene (d).

Figure 1 summarizes the pressure dependences of the proton chemical shift of chloroform (a) and chloroform (20 mol %) in benzene (I), mesitylene (II), and 1,3,5-triisopropylbenzene (III) (b, c, and d, respectively) relative to cyclohexane (5 mol %) used as an internal reference. The pressure dependence was also measured for solutions of 10 mol % chloroform in II and 45 mol % chloroform in III. In both cases the result was similar to the observation (c) and (d), respectively. From this fact, it may possibly be said that the present data approximately represent the pressure shifts expected for very dilute solutions of chloroform in aromatics. Since the experiments use the internal referencing system, it is only possible to center the discussion about the difference of chloroform and cyclohexane in the degree of interaction with surrounding molecules. Chloroform differs from cyclohexane in polarity and also in shape and size of the molecule, which gives rise to the observed pressure dependences of the chemical shifts relative to the reference.

Little displacement to lower field (a) with increasing pressure is noted for the chloroform resonance in the absence of the aromatic solvent. This is interpreted in terms of the preferred reinforcement of the self-association of chloroform molecules, which promotes the polarization of the H–$CCl_3$ bond (decrease in $\sigma_E$)[4] and also intensifies the dispersion interaction between the molecules (decrease in $\sigma_w$).[4] In aromatic solvents, on the other hand, the resonance shifts to higher field (b, c, d) with increasing pressure. This might generally be explained by the picture that chloroform, in preference to the cyclohexane, gets closer with increasing pressure to the aromatic molecule to experience the increased diamagnetic anisotropy effect of the ring with a resultant increase in $\sigma_a$ which should overshadow the op-

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(3) Calibrated with Aminco dead weight tester.

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