tween the ions is 4.1 Å[12] (in the range of the iron–iron distance of **1**, 3.8–5.1 Å). This concentration, when multiplied by the bimolecular rate constant, gives a pseudo-first-order rate constant of about 4×10^9 sec^{-1}. This value is in very good agreement with the rate constant calculated from the near-ir transition (1.3×10^{10} sec^{-1}).

The electron-transfer transition is also observed in thin-film solid-state spectra of **1** (maximum at 1900 mμ). In addition, the relative absorptivities of the solid-state and acetonitrile solution spectra are the same. The band maximum and relative absorptivities probably indicate that very similar distances, geometries, and interactions are involved in the solid and in solution.

Conductivity measurements along the long axis of single crystals of **1** were performed in a nitrogen atmosphere using the two-probe technique.[13] The conductivity (σ) was determined, at a given temperature, from the slope of a plot of current (I) *vs.* voltage (V) or log I *vs.* log V when the current and voltage range was very large.

Compound **1** was observed to behave in an ohmic fashion ($\sigma = 2.3 \times 10^{-8}$ ohm^{-1} cm^{-1} at 298°) over a very large range of field strengths (10^{-2} to 8×10^3 V/cm). For most organic aromatic molecular solids both the dark and photoconductivity cease to be ohmic when field strengths exceed about 1000 V/cm.[14] The ohmic behavior of **1** at very high field strengths could be due to a high mobility (μ) of the carriers, a large number of carriers (n), or particularly good electrode behavior (injecting) compared to the other cases studied. Regardless of the cause, it has not been possible to observe the space-charge limited (SCL) region usually found for organic compounds.

If the conductivity is ionic and not electronic the compound should be slowly electrolyzed when current flows through the crystal. To test for ionic conduction a larger amount of charge was passed through the sample than would be allowed by Faraday's law without a drastic change in the conductivity. No conductivity change could be detected.

In addition to the measurements of current *vs.* voltage at room temperature, the conductivity as a function of temperature was determined and found to obey the following functional relationship.

$$\sigma(T) = \sigma_0 \exp[-E_a/kT]$$

From the slope of a plot of σ *vs.* $1/T$, the activation energy (E_a) for the conduction process was determined. The interpretation of the constant (σ_0) will depend upon the nature of this conduction process involved.[15] Values of σ, σ_0, and E_a are given in Table I for ferrocene, ferrocenium picrate, and biferrocene[Fe(II)Fe(III)] picrate.

The conductivity of the mixed valence compound **1** *is observed to be six orders of magnitude larger than either ferrocene or ferrocenium picrate.* The comparison of **1** and ferrocenium picrate is particularly important inasmuch as both picrates were prepared *via* the same method and subjected to identical isolation and purification procedures, and the conductivity measurements performed in the same manner. The increase in conductivity could be due to an increase in the number of charge carriers (n), an increase in the mobility (μ) of the carriers, or both ($\sigma = ne\mu$).

Table I. Conductivity of Ferrocene Compounds

Compound	σ(298°), ohm^{-1} cm^{-1}	E_a, eV	σ_0, ohm^{-1} cm^{-1}
Ferrocene[a]	1×10^{-13}	0.61	10^{-4}
Ferrocenium picrate	7.3×10^{-14}		
Biferrocene[Fe(II)Fe(III)] picrate	2.3×10^{-8}	0.43	0.4

[a] D. C. Hoesterey and G. M. Letson, *J. Chem. Phys.*, **41**, 675 (1964).

The activation energy (0.43 eV) observed for the electronic conduction in **1** is much larger than the calculated thermal activation energy (0.16 eV) for intramolecular electron transfer in **1**. This indicates that the rate-limiting process is not intramolecular electron transfer but must be related to the intermolecular carrier transfer mechanism. While we have been able to effect a very large (10^6) change in the conductivity with very minor structural modifications it would appear that in order to increase the conductivity even more, to obtain either a metal or a superconductor, will require structural or crystal changes[16] aimed at reducing intermolecular as well as intramolecular barriers. A number of mixed valence ferrocene compounds, including the analogous ferrocene polymer, are currently under investigation.

Acknowledgment. The authors acknowledge many helpful discussions on the experimental methods of solid state physics and chemistry with Drs. Roger Westgate and Jerome Perlstein. Generous support of this work by the Petroleum Research Fund, administered by the American Chemical Society, is acknowledged.

(12) E. M. Kosower, "Physical Organic Chemistry," John Wiley & Sons, Inc., New York, N. Y., 1968, p 344.
(13) L. Marton, Ed., "Methods of Experimental Physics," Vol. 6B, "Solid State Physics," Academic Press, New York, N. Y., 1959.
(14) D. R. Kearns in "Advances in Chemical Physics (VII). The Structure and Properties of Biological Systems," Interscience Publishers, New York, N. Y., 1964, p 282.
(15) F. Gutmann and L. E. Lyons, "Organic Semiconductors," John Wiley & Sons, Inc., New York, N. Y., 1967.
(16) The picrate salt was chosen as the first candidate for study inasmuch as it is known (R. C. Petterson, Ph.D. Thesis, University of California, Berkeley, Calif., 1966) that the picrate ions and ferrocenium ions stack in alternate columns. However, the crystal structure of biferrocene[Fe(II)Fe(III)] picrate has not been determined.
(17) A. P. Sloan Fellow.
(18) National Institutes of Health Predoctoral Fellow.

Dwaine O. Cowan,[17] Frank Kaufman[18]
Department of Chemistry, The Johns Hopkins University
Baltimore, Maryland 21218
Received November 12, 1969

Thallium-205 Nuclear Magnetic Resonance as a Probe for Studying Metal Ion Binding to Biological Macromolecules. Estimate of the Distance between the Monovalent and Divalent Activators of Pyruvate Kinase

Sir:

Recently, there has been considerable interest in applying nonproton nmr spectroscopy for studying molecular interactions of biochemical importance.[1] Thal-

(1) (a) T. R. Stengle and J. D. Baldeschwieler, *Proc. Nat. Acad. Sci. U. S.*, **55**,1020 (1966); *J. Amer. Chem. Soc.*, **89**, 3045 (1967); (b) T. L. James and J. H. Noggle, *Proc. Nat. Acad. Sci. U. S.*, **62**, 644 (1969), and references cited therein; (c) R. G. Bryant, *J. Amer. Chem. Soc.*, **91**, 1870 (1969).

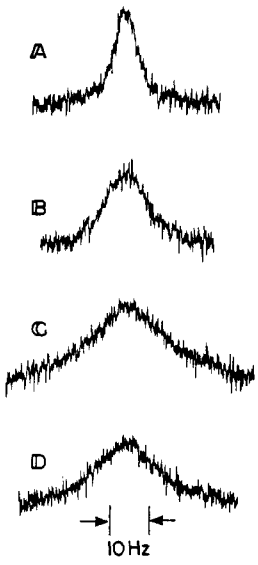

Figure 1. Thallium-205 nmr spectra: A, 0.10 M $TlNO_3$, 0.05 M tris(hydroxymethyl)aminomethane·HNO_3, pH 7.4; B, 4.4×10^{-5} M PK in A; C, 4.7×10^{-4} M $Mn(NO_3)_2$ in B; D, 5.0×10^{-3} M $Mg(NO_3)_2$ in C. The rf power was adjusted to produce signals of similar intensity.

lium-205 nmr seems to be a particularly suitable probe for studying binding of metal ions to biological macromolecules. The main relevant features of thallium-205 are its spin quantum number ($I = 1/2$), natural abundance (70.48%), and high relative sensitivity for nmr experiments (19.2% of that for protons).[2] This communication reports preliminary results of a ^{205}Tl linewidth study of metal ion binding to pyruvate kinase.

Rabbit muscle pyruvate kinase (hereafter referred to as PK) has long been known to exhibit an absolute requirement for a monovalent as well as a divalent cation.[3] Although the function of the divalent ion seems to be fairly well understood,[4] the mode of action of the monovalent cation has not yet been made clear.[5] It has recently been demonstrated[6] that PK is activated by Tl^+ to a degree one would expect on the basis of its ionic similarity to the cations of the alkali metal series. The present study, utilizing ^{205}Tl nmr, permitted the setting of narrow limits for some important parameters of this system.

Nmr spectra were recorded at ambient temperature (24 ± 1°) on a Varian DA-60 spectrometer operating at 24.3 MHz in the high-resolution mode. The samples, 2.5 ml in volume, were contained in 15-mm o.d. spinning sample tubes. PK was prepared from frozen rabbit muscle according to the procedure of Tietz and Ochoa[7] with a final $(NH_4)_2SO_4$ fractionation. The precipitate from this was dialyzed twice at 4° against a solution of 0.10 M $TlNO_3$ in 0.05 M tris(hydroxymethyl)-aminomethane, pH 7.4. The line widths, obtained under slow-sweep conditions with the rf power level well below saturation, were reproducible to within 10%. Typical tracings are shown in Figure 1. The ^{205}Tl line width, Δ, of 0.10 M $TlNO_3$ in the buffer solution is 7 Hz. Gasser and Richards have reported a line width of 50 Hz for a nonspinning sample.[8] From their T_1 value (obtained by progressive saturation) and assuming $T_1 = T_2$, a "natural" line width of 5.5 Hz is calculated.

The ^{205}Tl line is broadened by 9.5 Hz (Δ = 16.5 Hz) by adding PK to a total concentration of 4.38×10^{-5} M. Only after examining the effects of changing the temperature could a detailed interpretation of this broadening be given. Meanwhile, we offer a tentative rationalization; however, the analytical implications of the observed phenomenon are obvious irrespective of its origin. Dipolar interaction of bound ^{205}Tl with nuclei (protons and nitrogen-14) at the binding site is ruled out since a calculation shows that the correlation time for it has to be several orders of magnitude longer (of the order of 10^{-4} sec) than what might be anticipated for this system. Thallium-205, however, experiences enormously large chemical shifts when associated, *e.g.*, to anions (see ref 8 and references cited therein). Depending upon the relation between the mean residence time, τ_M, of Tl^+ on the enzyme and the magnitude of its shift, $\Delta\omega_M$ (in rad/sec), relative to the ion in solution, the broadening may assume two limiting forms:[9] (a) $1/$

$$\frac{1}{T_{2p}} = \frac{P_M}{\tau_M}\left[\frac{1/T_{2M}^2 + 1/(T_{2M}\tau_M) + \Delta\omega_M^2}{(1/T_{2M} + 1/\tau_m)^2 + \Delta\omega_M^2}\right]$$

$T_{2p} = P_M/\tau_M$, when $\tau_M \gg 1/\Delta\omega_M$, or (b) $1/T_{2p} = P_M\tau_M \cdot \Delta\omega_M^2$, when $\tau_M \ll 1/\Delta\omega_M$, where P_M is the ratio $[Tl^+]_{bound}/[Tl^+]_{total}$.[10] We attempted to measure the chemical shift of the Tl^+–PK solution relative to the sample without enzyme. Nonspinning samples were used for this experiment and the shifts were referred to the resonance of a 1 M CH_3COOTl solution contained in a small tube placed in the solution. Within the experimental uncertainty of ±15 Hz no shift was observed.[11] Thus, limits could be set for the mean residence time of bound $^{205}Tl^+$ and its chemical shift. Case a gives the lower limits for τ_M and $\Delta\omega_M$, which are 6×10^{-6} sec and 109 ppm, respectively, and case b gives an upper limit of 6×10^{-5} sec for τ_M using 350 ppm as the upper limit of $\Delta\omega_M$.

A more pronounced broadening (Δ = 30.5 Hz) is observed upon addition of Mn^{2+} (total concentration of 4.7×10^{-4} M in Mn^{2+}) to the solution containing Tl^+ and PK. No broadening was observed in solutions containing $TlNO_3$ and $Mn(NO_3)_2$ in the absence of enzyme. The effect of Mn^{2+} was followed by stepwise addition of microliter aliquots of a 0.47 M $Mn(NO_3)_2$ solution in the buffer. The line width *vs.* $[Mn^{2+}]$ plot appears as a titration curve leveling at about 40 Hz with the saturation occurring at a total Mn^{2+} concentration of *ca.* 5×10^{-4} M, which is about where recent esr studies indicate that approximately four Mn^{2+} ions are tightly

(2) See, *e.g.*, NMR Table, 5th ed, Varian Associates, Palo Alto, Calif., 1965.
(3) P. D. Boyer, H. A. Lardy, and P. H. Phillips, *J. Biol. Chem.*, **146**, 673 (1942).
(4) A. S. Mildvan and M. Cohn, *ibid.*, **240**, 238 (1965); **241**, 1178 (1966).
(5) For a review see H. J. Evans and G. J. Sorger, *Ann. Rev. Plant Physiol.*, **17**, 47 (1966).
(6) F. J. Kayne, Abstracts of the 6th FEBS Meeting, Madrid, April 1969, p 153.
(7) A. Tietz and S. Ochoa, *Arch. Biochem. Biophys.*, **78**, 477 (1958).
(8) R. P. H. Gasser and R. E. Richards, *Mol. Phys.*, **2**, 357 (1959).
(9) T. J. Swift and R. E. Connick, *J. Chem. Phys.*, **37**, 307 (1962). For easy reference we give eq 9 of that paper.
(10) In the conditions of the experiment there are probably four thallous ions bound to each protein molecule (F. J. Kayne, to be published).
(11) Under favorable conditions, however, *i.e.*, average shifts of the order of magnitude of (or larger than) the apparent line width, such measurements and comparison with model systems may allow mapping of the Tl^+ binding site.

bound to the enzyme.[12] The amount of bound Mn^{2+} is reduced by back-titrating with the diamagnetic Mg^{2+} resulting in a narrowing of ^{205}Tl line. The line width was reduced from 30.5 to 25 Hz in the presence of 5×10^{-3} *M* $Mg(NO_3)_2$ and 4.7×10^{-4} *M* $Mn(NO_3)_2$. Apparently, bound Mg^{2+} has no effect on the line width of bound $^{205}Tl^+$. The width was further reduced to 20.5 Hz by back-titrating the bound Tl^+ itself with K^+ at a final concentration of 0.5 *M* in KNO_3. The control experiments show that the observed phenomena result from the binding of Tl^+ to the enzyme and the interaction of bound $^{205}Tl^+$ with the bound Mn^{2+} ions. The fact that no additional broadening is produced by the diamagnetic Mg^{2+} strongly suggests that the factor that changes in the presence of Mn^{2+} is the relaxation rate, $1/T_{2M}$, of bound $^{205}Tl^+$ due to dipolar interactions with the unpaired electronic spin of Mn^{2+}. An additional broadening (upon adding Mn^{2+} to the Tl–PK solution) of the observed magnitude could only occur if the system were originally in the limit of fast exchange [case b], but now, owing to the increase in $1/T_{2M}$, the condition $1/T_{2M} \gg \Delta\omega_M$ prevails. Thus, the upper limit for τ_M is set now at 1.7×10^{-5} sec, which is also the upper limit for T_{2M}. The correlation time for the dipolar interaction is determined predominantly by the shortest among the rotational correlation time, the electron spin relaxation time, and the mean lifetime of the complex. Recent proton relaxation studies[12] indicate that the magnitude of the relevant correlation time is approximately 10^{-8} sec. We have used the well-known relation[13]

$$1/T_{2M} = (1/15)S(S + 1)\gamma_I^2 g^2 \beta^2 r^{-6} f(\tau_c),$$

where $f(\tau_c) = \tau_c[4 + 3/(1 + \omega_I^2\tau_c^2) + 13/(1 + \omega_s^2\tau_c^2)]$, with $T_{2M} = 1.7 \times 10^{-5}$ sec and correlation times of 10^{-9}, 10^{-8}, and 5×10^{-8} sec and obtained values of 4.23, 5.85, and 7.40 Å, respectively, for the distance (r) between the interacting nuclear (^{205}Tl) and electronic (of Mn^{2+}) spins. (The calculated distance is relatively insensitive to the various assumptions since it is dependent on the sixth root of $f(\tau_c)T_{2M}$.) These tentative figures representing the upper limit indicate that the monovalent and divalent binding sites are in very close proximity and thus the monovalent as well as the divalent[4] metal ion activators could directly participate in the catalysis of the pyruvate kinase reaction as suggested recently by Suelter.[14] For example, the distance between the phosphate and carboxylate coordination sites of phosphoenol pyruvate has recently been estimated at approximately 6 Å.[15] These observations seem to contradict the generally held concept[5] that monovalent cation activation is due mainly to conformational changes stabilized by the otherwise passive monovalent ions. Although this role is not ruled out by the present study, the site of action would then have to be very near the active site itself.

The effects of temperature and added substrates on the ^{205}Tl line broadening due to Mn^{2+} are currently under investigation and the results will be published in due course.

Acknowledgments. We wish to express our thanks to Drs. Mildred Cohn, John S. Leigh, Jr., and George H. Reed for helpful discussions. This work was supported in part by Grant Nos. GM-12202 and GM-12446 from the U. S. Public Health Service.

(12) J. Reuben and M. Cohn, to be published. *Cf.* also G. L. Cottam and R. L. Ward, *Arch. Biochem. Biophys.*, **132**, 308 (1969).

(13) I. Solomon, *Phys. Rev.*, **99**, 559 (1955); A. Abragam, "The Principles of Nuclear Magnetism," Oxford University Press, London, 1961, Chapter 8; see also R. E. Connick and D. Fiat, *J. Chem. Phys.*, **44**, 4103 (1966).

(14) C. H. Suelter, Abstracts, 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, BIOL. 056.

(15) A. S. Mildvan, J. R. Hunsley, and C. H. Suelter in "Johnson Foundation Symposium on Probes for Macromolecular Structure and Function," B. Chance, R. Yonetani, and M. Cohn, Ed., in press.

(16) On leave from the Weizmann Institute of Science, Rehovoth, Israel. This work was done during the tenure of a Career Investigator Fellowship of the American Heart Association.

F. J. Kayne, Jacques Reuben[16]
Johnson Research Foundation
University of Pennsylvania, School of Medicine
Philadelphia, Pennsylvania 19104
Received October 23, 1969

Alkaloid Studies. LXII.[1] X-Ray Crystallographic Structure Determination of Dichotine Hydrobromide

Sir:

In 1965 the isolation from *Vallesia dichotoma* Ruiz *et* Pav of 28 alkaloids containing a remarkable variety of indole structural types was reported.[2] All but six of them were identified by physical and analytical methods and by chemical correlation with known compounds. Of the remaining alkaloids, four were available in sufficient amount for further investigation. Two of them, (+)-vallesiachotamine[3] and (−)-vallesamidine,[4] were subsequently shown by us to have biogenetically very intriguing structures and thus provided additional stimulus to elucidate the constitution of the last two alkaloids (alkaloids **26** and **25** in ref 2, now named dichotine and 11-methoxydichotine), which exhibit an unusually high degree of oxygenation.

(+)-Dichotine ($C_{22}H_{26}N_2O_6$, I, R = H) and (+)-11-methoxydichotine ($C_{23}H_{28}N_2O_7$, I, R = OCH_3) differ from each other by a methoxyl group on the benzene ring. Using extensive chemical and spectroscopic data, the two alkaloids were found[5] to encompass partial formulation II, which contains several structural features hitherto unencountered in the field of indole alkaloids. Since the supply of these rare alkaloids was virtually exhausted, the complete structure was determined by X-ray diffraction analysis of dichotine hydrobromide.

Dichotine hydrobromide was crystallized from absolute ethanol to give orthorhombic hexahedrons. The space group is $P2_12_12_1$ with unit cell dimensions of a = 14.017 ± 0.005, b = 17.241 ± 0.005, c = 9.913 ± 0.005 Å; V = 2395.6 Å^3. The density (measured by flotation) and microanalysis indicate four molecules of alkaloid and four molecules of ethanol per unit cell (calculated density: 1.500 ± 0.001 g/cm^3; found: 1.498 ± 0.005 g/cm^3. *Anal.* Calcd for $C_{24}H_{33}N_2O_7Br$: C, 53.23; H, 6.15; N, 5.17; Br, 14.76. Found: C, 52.95; H, 6.24; N, 5.44; Br, 15.07.)

A total of 21,816 diffraction intensities was collected by a Hilger–Watts computer-controlled Y290 diffractometer, using monochromatic Cu Kα radiation. Averaging according to Friedel's law gave 2773 unique

(1) For paper LXI see B. V. Milborrow and C. Djerassi, *J. Chem. Soc., C*, 417 (1969).

(2) A. Walser and C. Djerassi, *Helv. Chim. Acta*, **48**, 391 (1965).

(3) H. J. Monteiro, A. Walser, L. J. Durham, and C. Djerassi, *J. Am. Chem. Soc.*, **88**, 1792 (1966).

(4) S. H. Brown, C. Djerassi, and P. G. Simpson, *ibid.*, 90, 2445 (1968).

(5) N. C. Ling and C. Djerassi, paper in preparation.