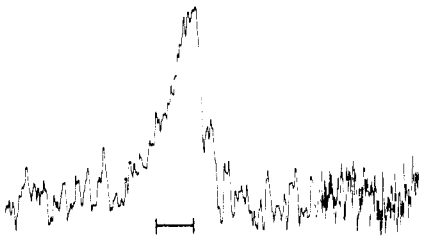

Figure 1. A typical measurement of T_2 for calcium-43 in aqueous solution; $T_2 = 0.85$ sec. Mark equals 1 sec.

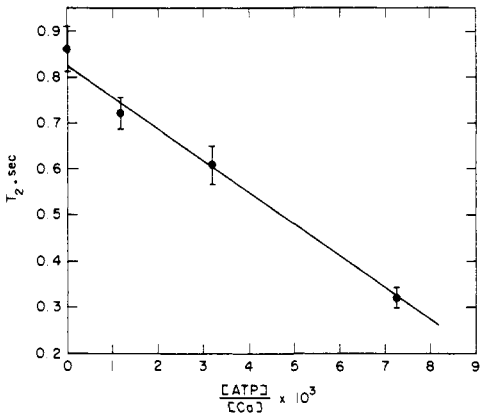

Figure 2. The transverse relaxation of calcium-43 as a function of ATP concentration at pH 6.4.

rapid for the rapid exchange approximation to obtain.[6] In this fast exchange limit the observed relaxation time is a weighted average of the relaxation times that characterize each site in the spectrum

$$\frac{1}{T_2} = \sum_{\text{sites}} P_i \left(\frac{1}{T_2}\right)_i \qquad (2)$$

where P_i is the probability that a calcium-43 ion is found at site i with a relaxation rate $(1/T_2)_i$.[7] Equation 2 may be simplified to the sum of two terms to permit estimation of an average $1/T_2$ for the ATP site.[8] At this pH $(1/T_2)_{atp}$ is 262 sec^{-1}. Since the exchange rate of calcium with ATP is about three orders of magnitude more rapid than $(1/T_2)_{atp}$, the fast-exchange approximation will obtain even if there is an increase in the product $(e^2qQ)^2\tau_c$ by about three orders of magnitude. Assuming that values of the field gradient do not vary drastically between one type of phosphate and another, the fast exchange approximation may be applied even if τ_c increases by three orders of magnitude, as it might if ATP were to bind to an enzyme for example. This suggests that enzyme- or protein-bound calcium should be observable at physiologically reasonable concentrations of protein because the exchange serves as a chemical amplifier in the same way that chemical exchange of chloride amplifies the chloride ion interaction with bovine plasma albumin.[9] As with other rapid exchange systems such as the halogen ion probe technique,[9,10] appropriate control experiments may serve to isolate the bound calcium contribution to the total relaxation time. This contribution then reflects the accessibility of the metal to the exchange site, the electric environment of the metal site, and the mobility of the site.

The binding of calcium to ATP is certainly a very well-known phenomenon; however, this experiment has shown that calcium nuclear magnetic resonance is potentially a powerful probe for investigating the calcium environment in systems where the calcium interactions may be important. Furthermore the exchange rate of calcium with potential binding sites is likely to be rapid enough so that the observation and characterization of bound calcium should be possible when the binding site is protein, enzyme, or even tissue such as muscle or nerve fiber.

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(6) M. Eigen and G. G. Hammes, *J. Am. Chem. Soc.*, **83**, 2786 (1961).
(7) T. J. Swift and R. E. Connick, *J. Chem. Phys.*, **37**, 307 (1962).
(8) Due to the ionization of ATP eq 2 will in general consist of more than two terms; however, the assumption that one species dominates permits reasonable estimation of what is an average $1/T_2$ for an ATP-bound calcium. Measurements as a function of pH should permit separation of these added terms.
(9) R. G. Bryant, *J. Am. Chem. Soc.*, **91**, 976 (1969).
(10) T. R. Stengle and J. D. Baldeschwieler, *Proc. Natl. Acad. Sci. U. S.*, **55**, 1020 (1966).

Robert G. Bryant
Department of Chemistry, Stanford University
Stanford, California 94305
Received November 12, 1968

Diastereomeric Solute–Solute Interactions of Enantiomers in Achiral Solvents. Nonequivalence of the Nuclear Magnetic Resonance Spectra of Racemic and Optically Active Dihydroquinine

Sir:

It is generally accepted that the nmr spectra of a racemate and of either one of its enantiomers are identical when measured under the same conditions in an achiral solvent.[1] For instance, it is now a matter of routine to establish the structural identity of an optically active natural product and its corresponding synthetic racemate by comparing their nmr spectra. We report that our data refute this method as a principle. The nmr spectra of optically active dihydroquinine and of racemic dihydroquinine[2] are significantly different when taken at the same

(1) In chiral solvents[1a] or upon addition of chiral additives in achiral solvents,[1b] two enantiomers reside in diastereomeric environments and, hence, can in principle be distinguished by their nmr spectra. Recently these phenomena were applied to the determination of optical purity[1c] and to the assignment of absolute configuration by nmr spectroscopy.[1d] (a) K. Mislow and M. Raban in "Topics of Setreochemistry," Vol. 1, N. L. Allinger and E. L. Eliel, Ed., Interscience Publishers, New York, N. Y., 1967, p 22 ff, and references therein; (b) J. C. Jochims, G. Taigel, and A. Seeliger, *Tetrahedron Lett.*, 1901 (1967); F. A. L. Anet, L. M. Sweeting, T. A. Whitney, and D. J. Cram, *ibid.*, 2617 (1968); W. H. Pirkle and S. D. Beare, *J. Amer. Chem. Soc.*, **90**, 120 (1968); (c) M. Raban and K. Mislow in "Topics in Stereochemistry," Vol. 2, N. L. Allinger and E. L. Eliel, Ed., Interscience Publishers, New York, N. Y., 1967, p 216, and references therein; (d) W. H. Pirkle and S. D. Beare, *J. Amer. Chem. Soc.*, **89**, 5485 (1967).

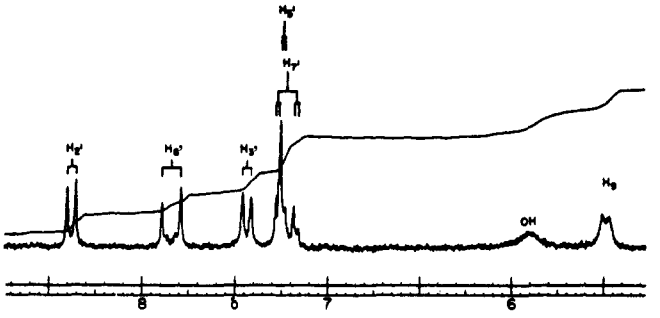


Figure 1. Portion of the 100-MHz spectrum of natural (−)-dihydroquinine, *ca.* 0.36 *M* $CDCl_3$ solution.

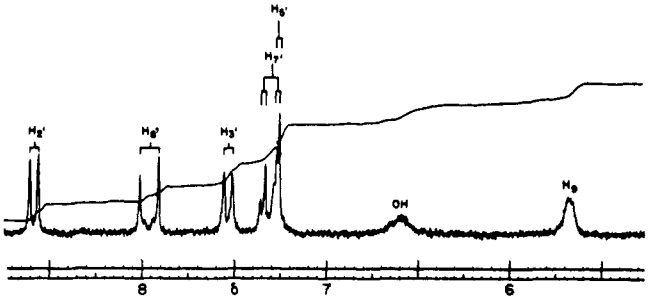
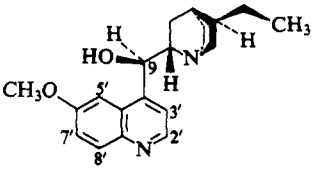


Figure 2. Portion of the 100-MHz spectrum of racemic dihydroquinine, *ca.* 0.35 *M* $CDCl_3$ solution.

concentration in deuteriochloroform. Furthermore, if the ratio of (−)- and (+)-dihydroquinines[2] in a mixture differs from 1:1, two sets of peaks for some protons are observed, and the peak areas are proportional to the relative amount of each enantiomer.



I

The differences are best illustrated in the chemical shifts of the protons $H_{2'}$, $H_{3'}$, $H_{8'}$, and H_9. Thus, the 100-MHz spectrum[3] of a 0.36 *M* solution of (−)-dihydroquinine (I) in deuteriochloroform (Figure 1) shows $H_{2'}$ at δ 8.38 (d, J = 4.5 Hz), $H_{3'}$ at 7.44 (d, J = 4.5 Hz), $H_{8'}$ at 7.85 (J = 10 Hz), and H_9 at 5.48, whereas the spectrum of racemic dihydroquinine (Figure 2) exhibits $H_{2'}$ at δ 8.58 (d, J = 4.5 Hz), $H_{3'}$ at 7.54 (d, J = 4.5 Hz), $H_{8'}$ at 7.95 (J = 10 Hz), and H_9 at 5.68.

In the spectrum of a mixture of (−)- and *rac*-dihydroquinines in a ratio of 1:1 (Figure 3) the above mentioned protons give rise to two sets of peaks with areas in a ratio of 3:1 and with the following chemical shifts: $H_{2'}$ at δ 8.46 (d, J = 4.5 Hz) and 8.58 (d, J = 4.5 Hz), $H_{3'}$ at 7.47 (d, J = 4.5 Hz) and 7.55 (d, J = 4.5 Hz), $H_{8'}$ at 7.88 (J = 10 Hz) and 7.96 (J = 10 Hz), and H_9 at 5.57 and 5.77.

(2) P. Rabe and A. Schulzte, *Chem. Ber.*, **66**, 120 (1933).
(3) Nmr spectra were recorded on a Varian HA-100 spectrometer using tetramethylsilane as an internal reference.

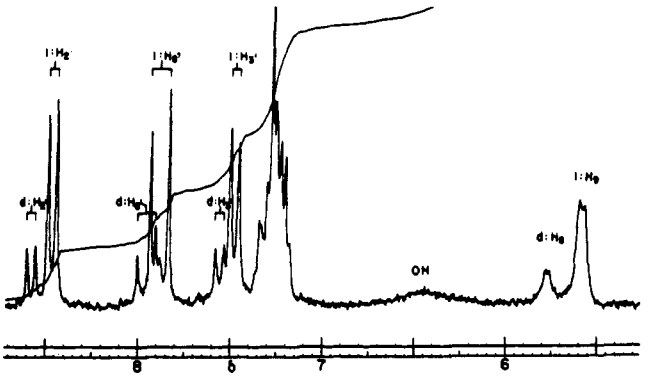


Figure 3. Portion of the 100-MHz spectrum of an artificial 1:1 mixture of racemic and natural (−)-dihydroquinine, *ca.* 0.27 *M* $CDCl_3$ solution.

Concentration[4] studies revealed that the nmr spectra of the pure enantiomers, the racemate, and various mixtures[5] thereof on high dilution tend to become identical. The chemical shifts of the aromatic protons are most susceptible to change in concentration, whereas those of protons H_9 are mainly dependent upon the relative concentration of the enantiomers.

The spectral differences are greatly reduced when deuterated methanol is used as a solvent. The nmr spectra of the acetates of optically active and racemic dihydroquinine, recorded in deuteriochloroform solution, show significantly smaller differences than those observed with dihydroquinine.

These facts can be rationalized by consideration of solute–solute interactions of the enantiomers. In solutions of the pure enantiomers, the racemate, and mixtures thereof, the molecules of each individual enantiomer reside in environments which are by intrasolution comparison identical, enantiomeric, and diastereomeric, respectively. By intersolution comparison the environments experienced by the molecules of individual enantiomers are diastereomeric when solutions of different, nonreciprocal compositions are considered.

The above-illustrated effects were also observed in the spectra of other compounds in the same class and will be found for other types of compounds. One practical application of these effects would be the determination of optical purity.

(4) Solutions ranging from 0.54 to 0.01 *M* in deuteriochloroform.
(5) (−)-Dihydroquinone:(+)-dihydroquinone: 87.5:12.5, 75:25, 62.5:37.5.

T. Williams, R. G. Pitcher, P. Bommer
J. Gutzwiller, M. Uskoković
Chemical Research Department
Hoffmann-La Roche Inc., Nutley, New Jersey 07110
Received January 15, 1969

Kinetics and Mechanism of the Reaction of the Thianthrene Cation Radical with Water

Sir:

The reactions of organic cation radicals are not at all clearly defined. For the most part these radical ions are