the direct coupling to the two protons in the $^{13}CH_2$ group.

The spectra, as shown in Figure 2, can be semiquantitatively reproduced using the LAOCOON computer program with reduced values for the carbon–proton coupling constants.[7] These calculations are approximate because they do not include the asymmetry introduced by the decoupling field or any relaxation effects.

We have performed additional experiments which show that it is not necessary for the protons to be equivalent to observe the additional splittings in a CWSD experiment, but the unusual effects shown in the figures are diminished as the difference between the chemical shifts of the vicinal protons increases or the vicinal proton–proton coupling constant decreases. For identical reduced J_{CH}, the "extra" middle peak in 1,1,2,2-tetrachloroethane is less than 20% that observed in fumaric acid because J_{HH} is 16 Hz in the latter but only 4 Hz in the former. A more detailed explanation of the conditions required for virtual coupling is given by Musher and Corey.[4] Our main point is to stress that caution must be exercised in interpreting CWSD experiments when there is strong coupling between two or more protons with similar chemical shifts on different carbon atoms in a molecule.

(7) Quantum Chemistry Program Exchange, Program No. 111, Indiana University, Bloomington, Ind.

Richard A. Newmark,* James R. Hill
Central Research Laboratories, 3M Company
St. Paul, Minnesota 55101
Received April 17, 1973

Pulsed Spin Decoupling in Nuclear Magnetic Resonance

Sir:

Time sharing versions of nmr, for example, time sharing pulsed double resonance,[1] are becoming increasingly popular these days. Concurrently, a number of myths concerning their theoretical interpretation have begun to pervade the literature. We should like to present a very brief preview of some of the problems which may be encountered in applying naively the concepts of coherent averaging theory[2] to time sharing or multiple-pulse experiments.

Examples of common misconceptions can be found in some recent communications on pulsed versions of spin decoupling and spin locking.[3] Basically, they take a common form; the rf field $H_1(t)$, at frequency ω, is applied in a series of pulses, of duration t_w and repetition period t_c, to permit facile simultaneous observation of the signal during the intense irradiation. For the case where $t_c \rightarrow 0$, the problem is then analyzed in terms of an "average perturbation" which is described as a continuous irradiation of intensity $\bar{H}_1$ at frequency ω, where $\bar{H}_1 = (t_w/t_c)H_1$. This corresponds to Fourier analyzing the rf pulse train and discarding the side bands (since, for $t_c \rightarrow 0$, they lie outside the spectral region) and retaining the resonant center band of intensity $\bar{H}_1$. This approach is successful on some occasions, but we would like to mention that in general it is simply wrong! Coherent averaging theory does *not* apply to the rf excitation; it is the complete interactions of rf and spins which must be transformed and averaged.

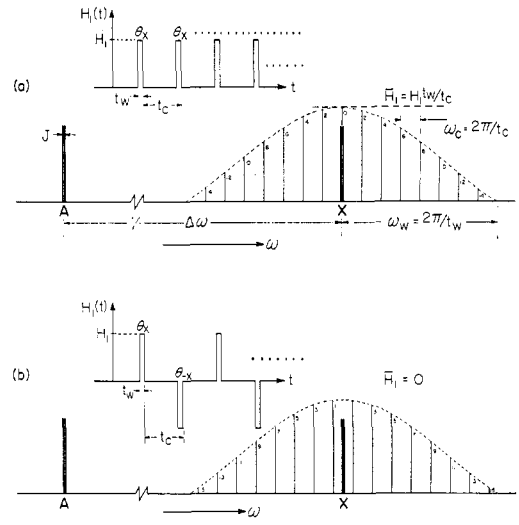


Figure 1. Pulsed version of spin decoupling in AX spectrum. The rf field is applied at the X frequency and modulated by the pulse trains (corresponding to (a) constant phase and (b) phase alternated rf) of duty factor t_w/t_c (here = 1/8). Only the A peak is sampled and $\gamma H_1 \ll \Delta\omega$. Shown schematically are also the AX spectrum and the side bands (absolute amplitudes) in the first $(\sin \omega t)/\omega t$ lobe from the Fourier decomposition of the pulse trains. The case of interest is that of $t_w, t_c \rightarrow 0$. In (a), even though $\bar{H}_1 \neq 0$ and $\gamma\bar{H}_1 \gg J$, there is essentially no spin decoupling (here $^7/_8$) for $\theta = 2n\pi$. In (b), even though $\bar{H}_1 = 0$, there can be complete spin decoupling for $\theta \sim (2n+1)\pi$ [here $\theta = (2n + 1 + [0.06/(2n+1)])\pi$].

To make this more concrete, consider the simple examples of heteronuclear spin decoupling described in Figure 1. The approach described above would argue that for $t_c \rightarrow 0$ there should be spin decoupling of X in the constant-phase case (a), since $\bar{H}_1 \neq 0$ (if $\gamma\bar{H}_1 \gg J$), and no spin decoupling in the phase alternated case (b), since $\bar{H}_1 = 0$, *i.e.*, there is no center band! In fact, it is easy to verify that $\bar{H}_1 \neq 0$ *is neither a necessary nor a sufficient condition for spin decoupling;* this is made quite transparent by the following observations: (1) in Figure 1a, $\bar{H}_1 \neq 0$, but there will be essentially no spin decoupling for $\theta = 2n\pi$; and (2) in Figure 1b, $\bar{H}_1 = 0$, yet there can be full spin decoupling for $\theta \sim (2n + 1)\pi$.

Similar remarks apply to the case of homonuclear spin decoupling described in this journal recently by Jesson, *et al.*[3a] The claim that their problem reduces to one in which $\bar{H}_1$ is applied continuously (we replace their H_2, $\mathcal{H}'$ by H_1, $\bar{H}_1$) fails outright; consider the fact that *so long as* $\gamma H_1 \gg \Delta\omega(\Delta\omega = \delta_A - \delta_B)$ *there will be no spin decoupling no matter what the value of* H_1. At most a uniform collapse of the chemical shift can occur.[2] Thus it is not always possible to compensate for long dwell times by indiscriminately increasing H_1. Normally, examples such as 1 and 2 above will be not encountered in the homonuclear case, as $\gamma H_1 \ll \Delta\omega$ is adjusted experimentally, and since $t_c < \pi/\Delta\omega$ (to satisfy the Nyquist condition so that the whole spectrum is sampled) it follows that $\theta \ll \pi$. Details will be presented separately.

(1) S. L. Gordon and J. D. Baldeschwieler, *J. Chem. Phys.*, **41**, 571 (1964).
(2) J. S. Waugh, C. H. Wang, L. M. Huber, and R. L. Vold, *J. Chem. Phys.*, **48**, 662 (1968); U. Haeberlen and J. S. Waugh, *Phys. Rev.*, **175**, 453 (1968).
(3) (a) J. P. Jesson, P. Meakin, and G. Kneissel, *J. Amer. Chem. Soc.*, **95**, 618 (1973); (b) E. P. Jones and S. R. Hartmann, *Phys. Rev. B*, **6**, 757 (1972).

Acknowledgment. This work has been supported by the United States Atomic Energy Commission under Contract W-7405-eng-48.

A. Pines*
Department of Chemistry, University of California, Berkeley and Inorganic Materials Research Division Lawrence Berkeley Laboratory Berkeley, California 94720

J. D. Ellett, Jr.
Laboratory of Chemical Biodynamics Lawrence Berkeley Laboratory Berkeley, California 94720

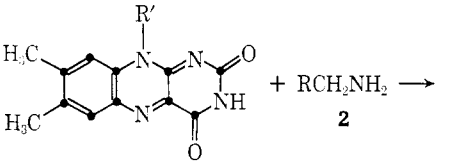
Received March 17, 1973

3-Bromoallylamine Induced Irreversible Inhibition of Monoamine Oxidase

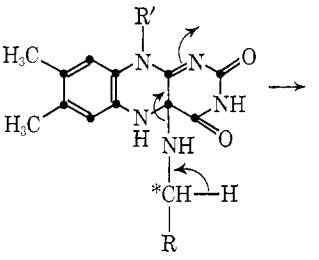
Sir:

Conventional methods for irreversibly inhibiting enzymes are based on the selectivity of binding of the labeling agent to the target enzyme.[1] In general, these agents are generated by attaching a reactive moiety, such as a bromoacetate group, to a molecule capable of binding to the receptor.[1] The lower the dissociation constant (K_s) for the labeling agent the higher the selectivity. Almost without exception affinity labeling agents of this kind cannot be used to produce selective labeling in crude systems or *in vivo*. The specificity of these inhibitors is simply not high enough and would be so only if biochemical methodology were such as to allow one to formulate the kind of molecule that would bind to a particular enzyme with $K_s \sim 10^{-8}$ or less. An alternative approach can be adopted, however, which promises unparalleled specificity of action. To develop these inhibitors one focuses on the selectivity implicity in the k_{cat} term rather than on the K_s term. Such a labeling agent should possess a chemically unreactive functional group which is rendered reactive at the active site of the enzyme in question. Then if the reactive moiety, once generated, were to engage in a reaction with an active-site residue, the enzyme would be irreversibly inhibited. This kind of inhibitor would require the enzyme to commit "suicide" by its specific mode of action. Since the grouping is normally masked, other biomolecules will not react with it and therein lies the basis of the specificity. The paradigm of this kind of inhibitor is to be found in studies on the inhibition of β-hydroxydecanoyl thioester dehydrase by 3-decynoyl-*N*-acetylcysteamine. In this case the enzyme first isomerizes the acetylene to an allene which then alkylates an active-site histidine.[2] In this paper the rational design of an inhibitor of this type is reported. Specifically, 3-bromoallylamine is demonstrated to be an irreversible inhibitor of mitochondrial monoamine oxidase.

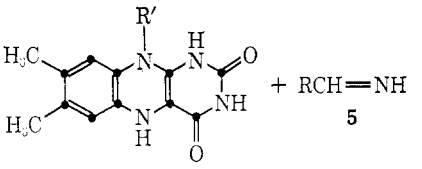
Rat liver monoamine oxidase, a flavine linked enzyme, catalyzes the oxidation of a host of aliphatic and aromatic amines to the corresponding imine, hydrolysis of which affords the aldehyde.[3] A plausible mechanistic hypothesis for this conversion has been forwarded by Hamilton.[4] In this mechanism a series of proton shifts are involved with the net oxidation of the amine and reduction of the flavine. Certainly a concerted mechanism, as drawn, need not be involved so that considerable carbanion may develop on the starred carbon **3**. Studies on D-amino acid oxidase,



1 **2**



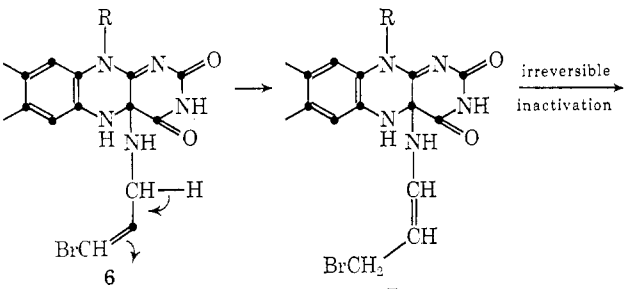
3



5

4

a similar flavine-linked enzyme, provide evidence for C–H bond cleavage in the oxidation of amino acids to α-keto acids.[5] Furthermore, D-β-chloroalanine can be nonoxidatively converted into pyruvic acid by the enzyme. This result demonstrates that the electron pair on the carbanion-like carbon can be subverted from the normal reaction course to an elimination route with the nonphysiological substrate. Given this precedent we would expect the mechanistically similar monoamine oxidase to behave as an isomerase in cases where the substrate contains a double bond β, γ to the amino group. Furthermore, we could expect it to be highly probable that 3-bromoallylamine would be an irreversible inhibitor of the enzyme since the enormously reactive intermediate **7** is generated at the active site and held there by covalent interaction with the flavine. Furthermore, the precursors of **7**, 3-bromoallylamine and **6**, are chemically unreactive as a consequence of



6 **7**

the bromine atom being attached to a vinyl group.

(1) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967.
(2) K. Bloch, *Accounts Chem. Res.*, **2**, 193 (1969).
(3) (a) R. Kapeller-Adler, "Amine Oxidases and Methods for their Study," Wiley-Interscience, New York, N. Y., 1970, Chapter 3; (b) S. R. Guha and C. R. Krishna Murti, *Biochem. Biophys. Res. Commun.*, **18**, 350 (1965).
(4) G. A. Hamilton, *Progr. Bioorg. Chem.*, **1**, 106 (1971).
(5) C. T. Walsh, A. Schonbrunn, and R. H. Abeles, *J. Biol. Chem.*, **246**, 6855 (1971).