

analogues such as inorganic phosphate or the phosphomonoester of ethylene glycol. A small reversible contraction of the unit cell (1.7% by volume) occurs in the presence of saturating concentrations of α -glycerophosphate, an inhibitor which combines structural features of both triosephosphates and appears to be slightly more tightly bound than either. When crystals are soaked in saturating concentrations of 2-phosphoglycollate (the potent inhibitor VI which resembles the hypothetical enediolate intermediate), a major change occurs in the X-ray diffraction pattern, including a reduction of approximately 5% in the volume of the unit cell. Contraction occurs without major disordering of the crystal structure and is fully reversed when the inhibitor is removed.²⁷

A change in unit cell dimensions may result from changes in tertiary structure or quaternary structure of the protein or merely from a rearrangement of molecules in the crystal lattice; further crystallographic studies will be required to decide between these alternatives. However, the magnitude of the change in crystal structure is positively correlated with the tightness of binding of inhibitors in solution and with the degree to which saturating concentrations of inhibitors protect the enzyme in solution against thermal inactivation.¹⁴ It therefore seems likely that the contraction of the unit cell represents a change in protein conformation.

These findings suggest that this enzyme may undergo a small change in structure as it passes from the ground state to the Michaelis complex, followed by additional larger change in structure as it passes to the transition state. This is understandable if the altered substrate in the transition state (and its analogs) possesses additional points of interaction with the enzyme, not present in the substrate or its analogs.

Possible Developments

During evolution, enzymes have presumably been

selected for structural complementarity to the altered substrate in the transition state of the reaction catalyzed. Exact structural techniques, in conjunction with appropriate analogs, may make it possible to define the approximate structure of this transition state. The strong forces of attraction should tend to stabilize analogs and protein binding residues in position, leading to sharp diffraction patterns. In conjunction with structural studies, equilibrium binding properties of analogs may also be helpful in elucidating the effects of pH, ionic strength, temperature, pressure, and allosteric effectors on catalytic activity.

Analogues of substrates which are intermediates in metabolic pathways would normally be expected to show affinity for two or more enzymes, including those responsible for substrate formation and breakdown. In contrast, analogs approaching the structure of the transition state for a particular reaction should often show a high and unique affinity for the enzyme or enzymes responsible for that reaction in a given organism. This may help to reduce undesirable side effects of antimetabolites, the design of which is based on these principles. The affinity and specificity of these analogs also recommend them for use as protective agents against heat inactivation and other forms of nonspecific destruction and as ligands for affinity chromatography. If analog affinity of this type can be combined with reactivity as a protein derivatizing agent, it may be possible to generate irreversible inhibitors with enhanced potency and selectivity.

It is a pleasure to acknowledge the work of Mr. R. M. Cohen, Dr. Ben Evans, and Mr. L. D. Byers, who have been responsible for the development of analogs in this laboratory. This work was supported by research grants (GM-12725 and GM-18325) and a career development award (AM-08560) from the National Institutes of Health.

Chemically Induced Dynamic Nuclear Polarization (CIDNP).

I. The Phenomenon, Examples, and Applications

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Students of nmr spectroscopy are taught that, when presented with a spectrum taken under normal high-

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resolution conditions, they may expect to observe absorption signals with relative areas proportional to the relative concentrations of the nuclei under inspection. In the past few years, spectra taken of certain reacting systems have transgressed this rule with signal enhancements of several orders of magnitude over the expected intensities. Figure 1A illustrates such posi-

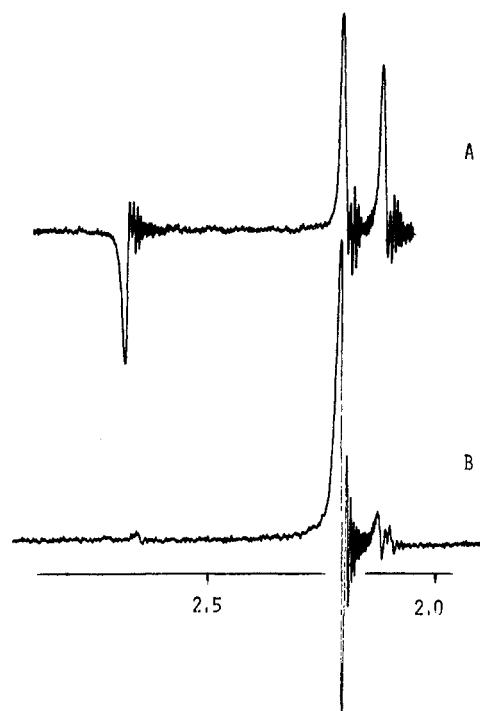
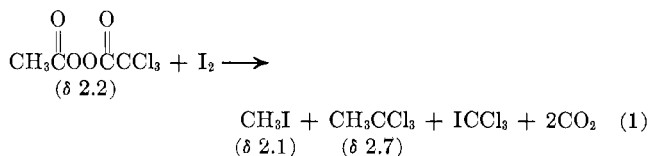
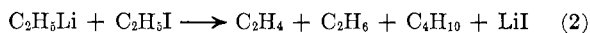


Figure 1. (A) Spectrum recorded 5 min after a solution of acetyltrichloroacetyl peroxide and iodine in carbon tetrachloride was warmed to 50°. (B) Spectrum taken at 0° immediately following scan A.

tive (δ 2.1) and negative (δ 2.7) enhancements taken during a peroxide decomposition reaction¹ (eq 1) at 50°.



It should be compared with Figure 1B, taken of the same solution at the same spectrum amplitude, after the reaction was stopped by lowering the temperature to 0°. Further, both enhanced absorption and emission signals can appear in a single multiplet, *e.g.*, in the case of the methyl and methylene protons of ethyl iodide during reaction with ethyllithium² (Figure 2).



It will be useful to distinguish between polarizations of the types shown in Figures 1 and 2, which actually represent extremes in a continuum of observable spectra. *Net polarization* (Figure 1) can be either enhanced absorption (A) or emission (E), while the *multiplet effect* polarization in Figure 2 can show either first emission and then enhanced absorption with increasing field (EA) or the reverse (AE).

From the first,^{3,4} these spectra have been assumed to arise solely from the products of radical reactions, an assumption which seems increasingly safe since the

(1) P. Livant, unpublished results.
 (2) H. R. Ward, R. G. Lawler, and R. A. Cooper, *J. Amer. Chem. Soc.*, **91**, 746 (1969).
 (3) J. Bargon, H. Fischer, and U. Johnsen, *Z. Naturforsch. A*, **22**, 1551 (1967).
 (4) H. R. Ward and R. G. Lawler, *J. Amer. Chem. Soc.*, **89**, 5518 (1967).

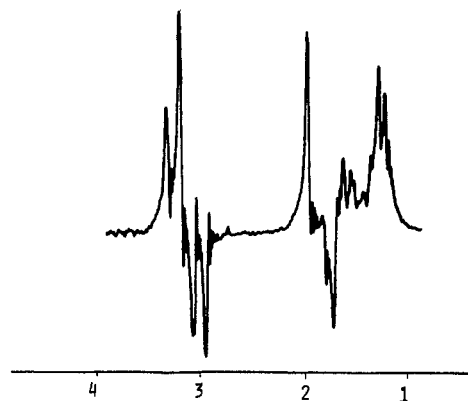


Figure 2. Spectrum recorded during the reaction of ethyllithium with ethyl iodide in benzene solution.

useful chemical systems continue to be ones which either are well known (eq 1) or can rationally be expected (eq 2) to involve radical intermediates. Furthermore, established ionic and concerted reactions do not show the effect.

The working model originally proposed⁵⁻⁷ to explain these spectral enhancements depended on an electron-nuclear cross-relaxation (Overhauser effect)⁸ in the radical intermediates, and, in recognition of related double resonance effects, a name of chemically induced dynamic nuclear polarization (CIDNP)⁹ was chosen. The Overhauser model was never adequate as a quantitative explanation of the experimental spectra because it did not allow emission signals of enhancements greater than 128. Also it could not accommodate multiplet spectra of the type shown in Figure 2. In 1969 a superior theory was proposed by Kaptein and Oosterhoff¹⁰ and by Closs¹¹ which satisfactorily explains large enhancements and multiplet spectra and allows spectral simulation. Quantitative applications and extensions of this theory are presented in the following Account.¹² The qualitative aspects are given here, along with a simple classical explanation of the model, to allow comparison with the examples of CIDNP spectra.

Radical Pair Model

The current theory focuses on radical pairs, and especially on the effect of nuclear magnetic moments on their reaction rates. In the decomposition of a diamagnetic molecule AB, for example, if the nuclei in fragments A and B can influence the reactivity of the

(5) R. G. Lawler, *ibid.*, **89**, 5519 (1967).
 (6) J. Bargon and H. Fischer, *Z. Naturforsch. A*, **22**, 1556 (1967).
 (7) H. Fischer and J. Bargon, *Accounts Chem. Res.*, **2**, 110 (1969).
 (8) K. H. Hausser and D. Stehlik, *Advan. Magn. Resonance*, **3**, 79 (1968).
 (9) The CIDNP designation might be construed to imply, by its association with DNP, a cross-relaxation process which the current theories of spin selection do not employ. However, to avoid the confusion that a change in the title of the effect might cause at this time, a majority of the participants in a recent CIDNP symposium (held in Houston, Texas, Feb 24-25, 1970) agreed to retain CIDNP as a general phenomenological designation for nmr enhancement observed during chemical reaction.
 (10) R. Kaptein and L. J. Oosterhoff, *Chem. Phys. Lett.*, **4**, 195, 214 (1969).
 (11) (a) G. L. Closs, *J. Amer. Chem. Soc.*, **91**, 4552 (1969); (b) G. L. Closs and A. D. Trifunac, *ibid.*, **92**, 2184 (1970).
 (12) R. G. Lawler, *Accounts Chem. Res.*, **5**, 25 (1972).

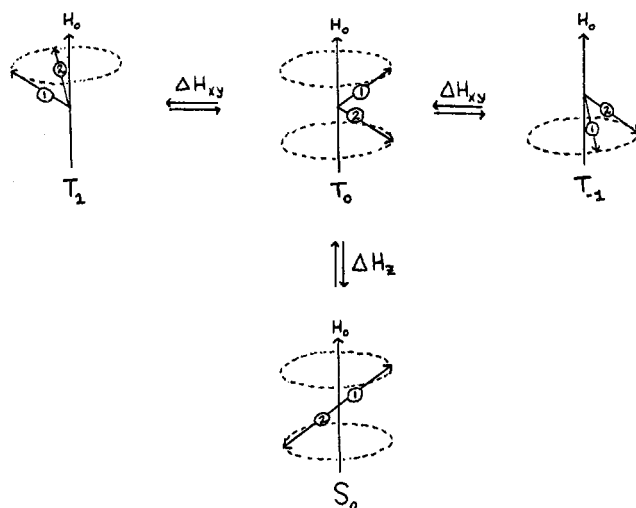
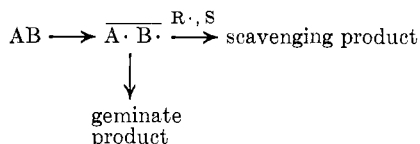


Figure 3. Schematic representation of the reaction of two weakly coupled electron spins in a magnetic field. The Z axis is the direction of the applied field, H_0 .

pair $\overline{A \cdot B \cdot}$, then certain nuclear states may favor product formation while radicals with other nuclear states will be scavenged by solvent or nonpartner radicals.



Even though the energy difference (Zeeman splitting) between nuclear states is small (0.006 cal at 60 MHz), when radicals have separated to distances where the energy of interaction between electrons (exchange interaction) is of a comparable magnitude, the nuclear magnetic moments can induce singlet-triplet mixing in the radical pairs. Since nearly all radical-radical reactions produce singlet-state products, the reaction rate is proportional to the singlet-state character of the pair and is therefore dependent on nuclear spin.

A qualitative idea of the requirements for this mixing may be obtained from Figure 3, which presents a schematic representation of the motion of two electron spin vectors (S_1 and S_2) which are weakly coupled to each other but strongly coupled to a magnetic field. The three magnetic substates of the triplet state, T , differ in the Z components of the net spin ($0, \pm 1$), while in the singlet state, S , all components of the two electron spins cancel.

All that is required for the mixing of S and T_0 is a rotation of one of the electron spin vectors relative to the other about the Z axis.¹³ The net magnetic field in the Z direction experienced by an electron is the sum of the applied field and the internal fields arising from nearby nuclear spins and electron orbital motion, so that the precessional frequencies of S_1 and S_2 about the Z axis are given in eq 3 and their differences in eq 4.

(13) In magnetic fields such as those typically employed for high-resolution nmr (which are much larger than the internal fields, *i.e.*, greater than a few hundred gauss), the hyperfine field components in the XY plane will be essentially zero, thus preventing mixing of the states $T_{\pm 1}$.

$$\omega_{S_1} = \beta \hbar^{-1} [g_1 H_0 + \sum a_1 m_1] \quad (3)$$

$$\omega_{S_2} = \beta \hbar^{-1} [g_2 H_0 + \sum a_2 m_2]$$

$$(\omega_{S_1} - \omega_{S_2}) = 2\delta = \beta \hbar^{-1} [(g_1 - g_2) H_0 + (\sum a_1 m_1 - \sum a_2 m_2)] \quad (4)$$

The first term in eq 4 arises from the Zeeman interaction in cases where g factors for the two electrons are different, and the second term is from precession in the "hyperfine field" arising from nuclei with hyperfine splittings, a , and magnetic quantum numbers, m . If the two unpaired electrons in the radical partners have the projections of their spins in the XY plane separated by an angle $\theta(0)$ at $t = 0$, after a time τ , the angle between the spins will be $\theta(\tau) = \theta(0) + 2\delta\tau$. Since $\theta = 0$ and 180° correspond to T_0 and S states, respectively, if $\delta \neq 0$, a pair which is born in the singlet state will develop some triplet character at later times and *vice versa*. Furthermore, the rate of mixing of states will depend on differences of both the g factors and hyperfine fields characterizing S_1 and S_2 . The former term represents a field dependence of the intersystem crossing rate; the latter term, a nuclear spin dependence. The reaction rate of a radical pair will be proportional to its singlet-state probability, ρ_{ss} , which, for times less than 10^{-8} sec, can be expressed at a time τ in terms of δ and of the probability of finding the pair in the S or the T_0 state at the moment of its formation ($\rho_{ss}(0)$ and $\rho_{tt}(0)$). For a pair formed in the singlet state (as in re-

$$\rho_{ss}(\tau) = \rho_{ss}(0) - [\rho_{ss}(0) - \rho_{tt}(0)]\delta^2\tau^2 \quad (5)$$

actions 1 and 2) eq 5 reduces to $\rho_{ss}(\tau) = 1 - \delta^2\tau^2$.

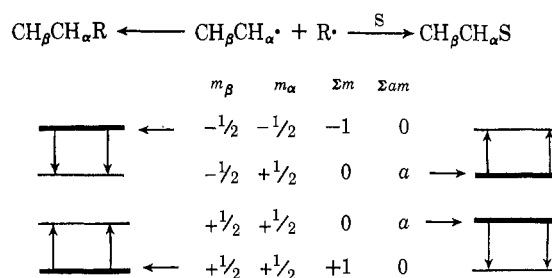
Typical values of Zeeman and hyperfine field differences for organic free radicals are a few gauss, which amounts to a frequency of dephasing of electron spins ("intersystem crossing" frequency) of 10^7 – 10^8 radians sec^{-1} . During the lifetime of a typical radical encounter pair in solution (10^{-9} – 10^{-10} sec) one would, therefore, expect a per cent or so of mixing of singlet and triplet states with $\delta \neq 0$ in the limit of very weak exchange coupling, and less mixing if the exchange interaction is larger than δ . The significance of this small admixture is evident if one realizes that 50% mixing of an unreactive triplet for some states ($\delta\tau > 1$) and no mixing for others ($\delta = 0$) would give rise to an nmr enhancement of approximately 10^5 , or about 10^2 larger than has so far been observed!

The more rigorous extension of this classical picture presented in the following Account¹² shows that both terms in eq 4 must be included to account for the observed effects of spin selective reactions, since dephasing induced by g -factor differences may be canceled or reinforced by the hyperfine fields arising from nuclei with opposite spins.

The effect that this cancelation and reinforcement has on the nuclear spin state populations in the S and T_0 radical pairs is most easily demonstrated with two simplified model systems. Consider a radical pair,

formed in the S state, in which the electron of radical **1** precesses more slowly in the external field (H_0) than does that of radical **2** (*i.e.*, $g_1 < g_2$). After some time (which depends on the magnitude of δ), the more rapid precession of the electron of radical **2** will lead to the T_0 state. If, however, radical **1** contains a proton of $m_1 = -1/2$ coupled to the unpaired electron with a negative hyperfine coupling constant, the hyperfine field will supplement the external field and will lead to a relatively higher precessional frequency for the electron in **1**, a lower value of δ , and consequently a longer time required to reach the triplet state. If $m_1 = +1/2$, the opposition of hyperfine and external fields leads to a lower precessional frequency for **1**, a larger δ , and a more rapid intersystem crossing. Clearly, in the case of $m_1 = -1/2$, the radical pair with higher singlet character will have a greater opportunity to lead to product, and the product will have an overpopulation of the $-1/2$ nuclear state and will show net nmr emission (E). The T_0 pairs, enriched in the $+1/2$ state, will have a longer life (since reaction is improbable) and a higher probability of dissociation or scavenging. The scavenging product must, of course, show enhanced absorption (A). If scavenging occurs in times much less than the nuclear T_1 in the radical and if T_1 for the protons in the products are equivalent, the magnitudes of emission and enhanced absorption must be equal (see, for example, Figure 1), a necessity because only spin selection has occurred and no nuclear spin transitions are involved in the polarization process.¹⁴ The same reasoning applied to a pair formed in the T state leads to the expectation of geminate encounter product enriched in the $+1/2$ state (A) and scavenging product with the complementary $-1/2$ state (E), since $m_1 = +1/2$ will more effectively induce singlet character than the $-1/2$ state. *The polarization is precisely opposite to that resulting from spin selection in the S pair.*

If the radical pair has $\Delta g = 0$, as with paired alkyl radicals, hyperfine fields are the only interactions available for the singlet-triplet mixing. Those pairs which contain radicals whose hyperfine fields are most likely to differ from the hyperfine fields averaged over all states will be the pairs most likely to undergo intersystem crossing. These will contain radicals with the largest absolute value of the hyperfine field and will result in a symmetric distribution of spin-state populations on either side of zero hyperfine field (for first-order spectra). For a two-spin system with one proton α ($a < 0$) and one β ($a > 0$) to the unpaired electron, the highest hyperfine fields result from the inner nuclear levels ($\Sigma m = 0$). If the two protons are coupled with a positive nuclear spin-spin coupling constant, the geminate product will show a multiplet effect of phase EA and the scavenging product phase AE. If the pair were to be formed in the T state, the large $|\Sigma am|$ states would induce mixing with the S state and would



lead to geminate products with an AE phase, again *opposite* to polarization in the S pair.

Pairs formed by diffusive encounters of free radicals would seem at first sight to lead to no polarization, since, with equal population of S and T_0 , no spin selection occurs ($\rho_{ss}(0) = \rho_{tt}(0)$; see eq 5). However, if during the lifetime of the diffusive encounter pair some reaction occurs from S states (as it must, since diffusive encounters of radicals certainly give reaction), the remaining pairs will acquire triplet character, and the same type of polarization is expected as from a triplet geminate pair.

These qualitative ideas can be used to generate spectral predictions based on four parameters: the sign of the hyperfine coupling constants, the sign of Δg (both of which are usually available or estimatable from esr data), the sign of the nuclear spin-spin coupling constant, and the multiplicity of the radical pair at birth. The effects of these quantities on polarization are summarized in Table I for pair products. In each case the

Table I
Polarization Character of Geminate Products

Entry	a_A	a_B	Birth	g	J_{AB}	H_A	H_B
HA· + ·BH → HABH							
1	-	-	S	A > B	+	A, EA	E, EA
2	-	+	S	A > B	+	A, AE	A, AE
3	-	-	T	A > B	+	E, AE	A, AE
4	-	-	S	B > A	+	E, EA	A, EA
5	-	-	S	A > B	-	A, AE	E, AE
R· + CH_BCH_A· → RCH_ACH_B							
6	-	+	S	R > C	+	E, EA	A, EA
7	+	-	S	R > C	+	A, EA	E, EA
8	-	+	T	R > C	+	A, AE	E, AE
9	-	+	S	C > R	+	A, EA	E, EA
10	-	+	S	R > C	-	E, AE	A, AE

scavenging product will show complementary polarization. If $\Delta g = 0$, the net polarization will vanish leaving only the multiplet effect. If Δg is large the multiplet may be obscured by net polarization. In common with most generalizations, this table should be applied with caution and is no substitute for the complete simulation of the polarized spectrum.¹⁵

Examples

It is not appropriate to attempt to present here a complete review of CIDNP investigations, but a brief mention of the important applications is proper and

(14) This balance holds only for reactions conducted at high fields (>1000 G) and in pairs formed by doublet species. In low field, T_1 -S mixing may involve nuclear spin transitions. Triplet-triplet encounters apparently give a similar effect, even at high field.

(15) Because of breakdown in approximations used in deriving the table, its predictions should not be applied for multiplet effects when $\Delta g > 0.005$.

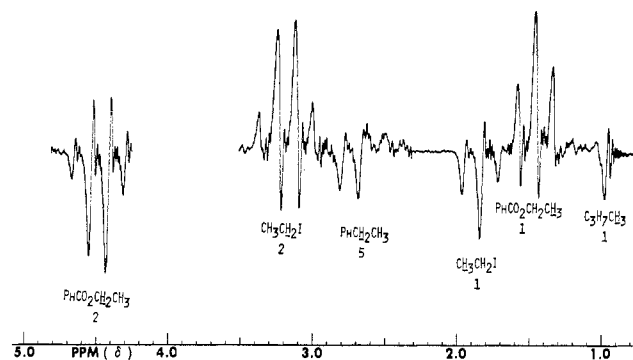
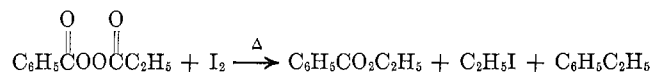


Figure 4. Spectrum recorded during the decomposition of a solution of propionyl benzoyl peroxide and iodine in *o*-dichlorobenzene at 100°. The numbers below the formulas indicate the relative spectrum amplitudes for the underlined protons.

useful in the evaluation of CIDNP as a mechanistic technique.

Acyl Peroxides. The thermal decomposition of propionyl benzoyl peroxide¹⁶ in the presence of iodine is taken as an example of singlet geminate encounters because it provides simple spectra from cage and scavenging products (Figure 4) and because it offers results of mechanistic interest. Benzoyloxy radicals



decarboxylate slowly¹⁷ while propionyloxy decarboxylation is rapid¹⁸ (compared to diffusion); thus the predominant geminate pair should contain a benzoyloxy and an ethyl radical, the former of higher *g* factor than the latter. Combination during the encounter should be aided by $-1/2$ nuclei on the α and $+1/2$ nuclei on the β positions of the ethyl radical, since both will lead to a higher precessional frequency for the ethyl electron and oppose the mixing by Δg (see Table I, line 6). The methylenes of the ethyl benzoate then show emission (δ 4.5) and the methyls (δ 1.5) enhanced absorption. Of the ethyl radicals which avoid coupling, most are trapped by iodine to give ethyl iodide with the opposite polarization (CH_2 , δ 3.2; CH_3 , δ 1.8), and the remainder dimerize to butane, from which emission can be seen from the methyl protons (δ 0.9).

The methylenes (δ 2.6) of the minor amount of ethylbenzene formed show a polarization pattern that is clearly a combination of E and EA, from which the mode of formation can be deduced. Phenyl and ethyl radicals differ so slightly in *g* factors ($\Delta g = 0.0002$) that the net emission must have arisen in benzoyloxy-ethyl pairs, in which the benzoyloxy radical decarboxylated before reaction. The phenyl-ethyl pair thus formed (still as the singlet pair) can superimpose an EA phase on the E already present in the ethyl radical. Alternative routes of formation, diffusive encounter of phenyl and ethyl radicals or a concerted three-bond cleavage of the peroxide, would give easily distinguishable polarization patterns (AE and EA, respectively,

(16) R. A. Cooper, unpublished results.

(17) D. F. DeTar, *J. Amer. Chem. Soc.*, **89**, 4058 (1967).

(18) R. C. P. Cubbon, *Progr. React. Kinetics*, **5**, 29 (1970).

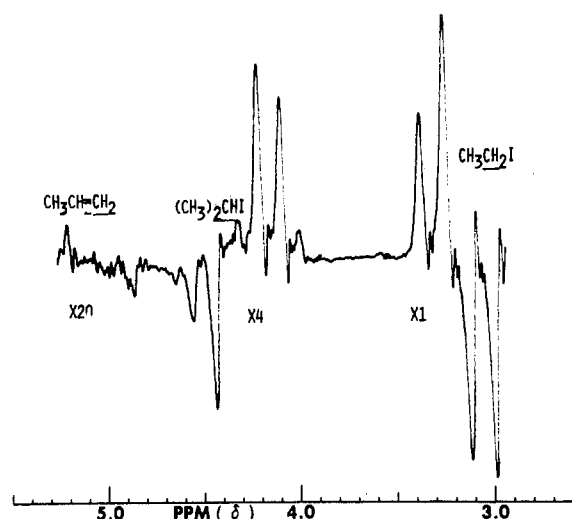
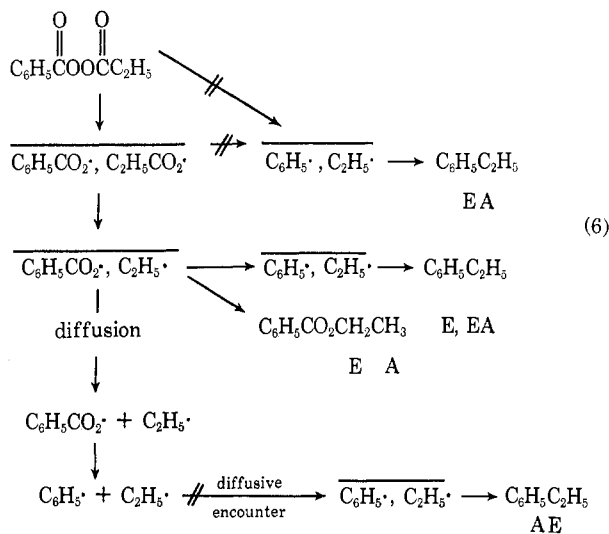
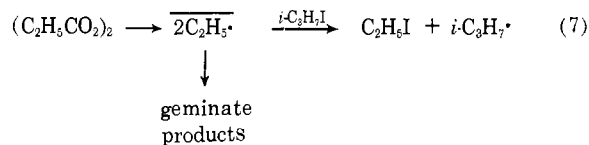


Figure 5. Spectrum recorded during the decomposition of a solution of propionyl peroxide and isopropyl iodide in *o*-dichlorobenzene at 100°.

with no net emission; reaction 6). The choice between the concerted and stepwise decarboxylation could not readily have been made without CIDNP spectra, since the reaction occurs entirely within the geminate pair and is not susceptible to scavenging experiments.



The closely related decomposition of propionyl peroxide in the presence of isopropyl iodide¹⁶ (an iodine atom source) confirms the rapid decarboxylation of the alkyl acyloxy radicals. The ethyl iodide shows a pure

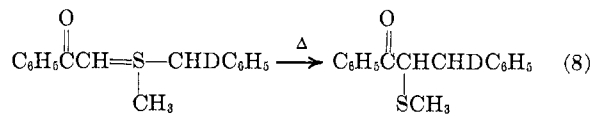


AE multiplet effect (Figure 5) with no suggestion of any propionyloxy-ethyl interaction, and the spectrum is explained by spin selection beginning with singlet pairs of ethyl radicals. No polarization is observed from the ethyl protons of ethyl propionate. Each iodine abstraction by an ethyl radical produces an isopropyl radical which is *not* formed as a partner in a radical pair and which should have the same macroscopic nu-

states have led to triplet radical pairs,²⁹ and the inter-system crossing apparently occurs in the product-forming step.

Molecular Rearrangements

The remaining group of reactions to which CIDNP has been of mechanistic assistance is composed of rearrangements of the Stevens type, illustrated by reactions of the sulfur ylide reported by Baldwin.³⁰



These reactions have been variously described as ionic, concerted, or radical (singly and in combination), based on the sensitivity of rate to solvent polarity and on retention of stereochemistry. In reaction 8, although the configuration at the benzyl carbon was 40% retained in the product, polarization from a singlet pair was nonetheless observed. A radical pair which can retain stereointegrity for the necessary 10^{-10} sec is a difficult concept, and it is probable that those pairs which lead to polarization also give racemized product. Retained product may form by an alternative, concerted path or, more likely, by radical pairs of shorter life span. Radical rearrangements must form intimate pairs (unlike azo compound or peroxide decomposition), and recombination of the majority of pairs in 10^{-12} – 10^{-13} sec is reasonable. Pairs which live longer, presumably by diffusive separation and reencounter, may contribute a minor part of the product but the majority of the polarization, since enhancement increases both with lifetime and with pair separation.

Summary

The exploitation of CIDNP is expected to proceed in the following directions. (a) Detection of radical

(29) Qualitatively, CIDNP spectra cannot distinguish between a geminate triplet and a diffusive encounter pair, so that a quantitative treatment or the addition of radical scavengers may be necessary to avoid misassignment.

(30) J. E. Baldwin, W. F. Erickson, R. E. Hackler, and R. M. Scott, *Chem. Commun.*, 576 (1970).

pairs: the indirect detection of radicals is more sensitive than direct esr observation (by an order of magnitude, at least) and less subject to misinterpretation, since polarization is observed in the product. Because of high enhancement factors, however, results still must be interpreted with caution. (b) Determination of spin multiplicity: for thermal reactions, the useful distinction is between geminate and diffusive encounter pairs; for photoreactions, the important differentiation is between singlet and triplet pairs. (c) Signal enhancements: the thousandfold amplification of spectra leads to a number of disparate applications, ranging from simple detection of products formed in trace amounts, through measurement of spectra of ^{13}C in natural abundance,³¹ to identification of unstable intermediates (necessarily of lifetime >0.1 sec). (d) Investigation of radical and radical-pair parameters: computer simulation of enhanced spectra can give signs and magnitudes of hyperfine coupling constants, relative magnitudes of g factors, and electron-exchange interactions, when, in each case, the remaining quantities necessary for the simulation are known. Scavenging experiments can give indications of the nuclear relaxation times in free radicals.³²

Recollection that the CIDNP phenomenon has been known for barely 4 years and the radical pair theory for less than 2 can only lead to the expectation that this listing is certain to be incomplete and that considerable and continuing effort will be expended to extend it.

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(31) E. Lippmaa, T. Pehk, A. L. Buchachenko, and S. V. Rykov, *Chem. Phys. Lett.*, **5**, 521 (1970).

(32) G. L. Closs and A. D. Trifunac, *J. Amer. Chem. Soc.*, **92**, 7227 (1970).