# A Proton Magnetic Resonance Study of the Interaction of Hexachlorophene with Amides and Polypeptides[1,2]

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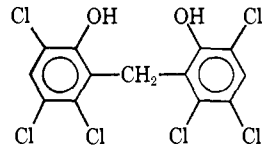
**Abstract:** The addition of simple amides, acetone, and dimethyl sulfoxide to a $CCl_4$ solution of hexachlorophene resulted in large low-field chemical shift and line broadening of the phenolic proton resonance peak. These findings are interpreted in terms of intermolecular hydrogen bond formation between the phenolic protons and the oxygen atoms of amides and related compounds. Structures of the various hydrogen bonds are discussed. Similar studies when extended to the interaction of hexachlorophene with polypeptides (poly-γ-benzyl-L-glutamate and poly-L-methionine) also confirmed the formation of similar hydrogen bonds.

While the binding of hexachlorophene [2,2′-methylenebis(3,4,6-trichlorophenol)] (I) and other chlorinated bisphenol germicides to proteins[3,4] may play an important role in determining their biological



I

activity, little is known about the mechanism of such interactions. The proton magnetic resonance spectrum[5] (pmr) of I in dimethyl sulfoxide solvent was reported previously but the phenolic protons were not detected because of line broadening.

Phenols usually form strong hydrogen bonds with compounds having proton-accepting properties resulting in line broadening and large low-field shifts of the phenolic proton resonance. In this paper we shall present a pmr study of the interaction of hexachlorophene with the simple amides, *N*,*N*-dimethylformamide (DMF), *N*-methylacetamide (NMA), and *N*-methylpyrrolidone (NMP), the related compounds, acetone and dimethyl sulfoxide (DMSO), and the polypeptides, poly-γ-benzyl-L-glutamate and poly-L-methionine. The results are discussed in terms of intermolecular hydrogen bond formation between the phenol and the amides or related compounds.

## Experimental Section

Hexachlorophene (USP grade) was a gift of the Givaudan Corp. and was recrystallized twice from 2-propanol–water before use. Poly-γ-benzyl-L-glutamate (mol wt = 200,000) was obtained from the International Chemical and Nuclear Co. and poly-L-methionine (mol wt = 45,000) from Miles Laboratories, Inc. All other chemicals employed in these investigations were of analytical reagent grade. Proton magnetic resonance spectra were recorded on Varian A-60 and Varian HA-100 nuclear magnetic resonance spectrometers. Chemical shifts are reported with reference to cyclohexane or tetramethylsilane (TMS) as an internal standard. A hexachlorophene concentration of 0.05 *M* was used throughout these investigations since this was the minimum concentration that yielded usable pmr measurements in the presence of proton-acceptor molecules.

## Results and Discussion

The pmr spectra of I in $CCl_4$ showed resonance peaks for the aromatic, methylene, and phenolic protons at 7.3, 4.4, and 5.5 ppm from internal TMS. The phenolic proton peak was considerably broader than that of the methylene and ring protons, suggesting that intra- and intermolecular hydrogen bonding occurred between phenolic protons in solution. Addition of simple amides or carbonyl compounds to solutions of I in $CCl_4$ caused additional line broadening of the phenolic proton peak accompanied by a shift to low field. The chemical shift and line width changes for the phenolic proton peak approached 150 and 20 Hz, respectively, depending on the concentration of the reactants. Changes in chemical shift of the phenolic protons for a 0.05 *M* solution of I in $CCl_4$ upon addition of various amides, acetone, and DMSO are shown in Figure 1. Formation of hydrogen bonds between the phenolic protons of I and the added proton-acceptor molecules changed the electronic environment around the phenolic proton[6-9] resulting in a shift of the pmr resonance toward low field. If one assumes that binding can occur only *via* the phenolic protons of I, two molecules of proton-acceptor solvent theoretically could bind to one molecule of I. However, the observed minima of the chemical shift concentration curves (Figure 1) appear to occur at a ratio of donor solvent to hexachlorophene that is somewhat greater than 2:1. This difference could be the result of the increasing influence of dielectric constant and pH at the higher proton-acceptor concentrations or the fact that the equilibrium constant for complex formation may not be infinitely high.

The two aromatic rings of I are not necessarily in the same plane[10] and a possibility of both intramolecu-

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(2) We thank Miss M. Rasmusson, Miss S. Lilley, and Mr. D. Schmedding for their assistance at various stages of the experiment.

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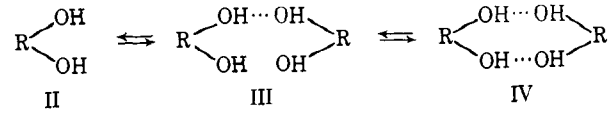
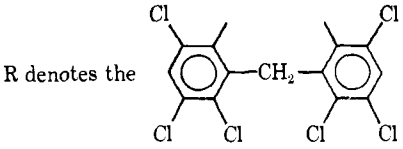
lar hydrogen bonding (II) as well as intermolecular hydrogen bonding (III, IV) between the phenolic protons of hexachlorophene exists. Because of the rapid exchange of protons among the various hydrogen bonded species, however, separate resonance peaks are not observable at room temperature.

Alteration of the concentration of I within its limited solubility range in $CCl_4$ had very little effect on the chemical shift of the phenolic proton. A 0.05 *M* solu-



II III IV



R denotes the portion of hexachlorophene

tion of hexachlorophene showed only a small low-field shift of about 0.1 ppm of the phenolic proton resonance with changes in temperature from 33 to −10°.

These results suggest that the large chemical shifts observed in the presence of simple amides and carbonyl compounds are not the result of inter- or intramolecular interactions between hexachlorophene molecules but are caused by interaction of I with the proton-acceptor molecules. Hydrogen bond formation probably occurs between the phenolic protons and the lone pair of electrons of the carbonyl oxygens in the proton-acceptor molecules. Previous studies[11-13] on the protonation of simple amides by strong acids have shown that hydrogen bond formation occurs with the carbonyl oxygen rather than on the amide nitrogen atom. Furthermore, DMSO and acetone which have no amide groups also form hydrogen bonds with I (Figure 1), indicating that interaction in this case probably occurs at the oxygen atom.

The equilibrium in the systems under investigation could be represented as shown in Scheme I.

The low-field shift (Figure 1) obtained at the lowest concentrations of proton-acceptor molecules is probably due to formation of the intermolecular hydrogen bonded configuration V and VI. Depending on the acidity of the phenol, species VI could rearrange to yield a hydrogen bonded associated ion-pair complex VII. As the concentration of the acceptor molecule increases, the chemical shift also increases until a minimum is reached at some molar ratio of reactants and the chemical shift then reverses direction. Simple phenols do not show such a minimum in their chemical shift curves.[7] The change in the direction of the chemical shift of phenolic protons of I can be compared with the previous extensive studies on the protonation of amides in strongly acid media.[14] The upswing in the chemical shift at high concentrations of donor solvents probably results from a dissociation[11,12,14] of the hydrogen bonded associated ion-pair complex VII to form the protonated species IX. Dissociation ap-

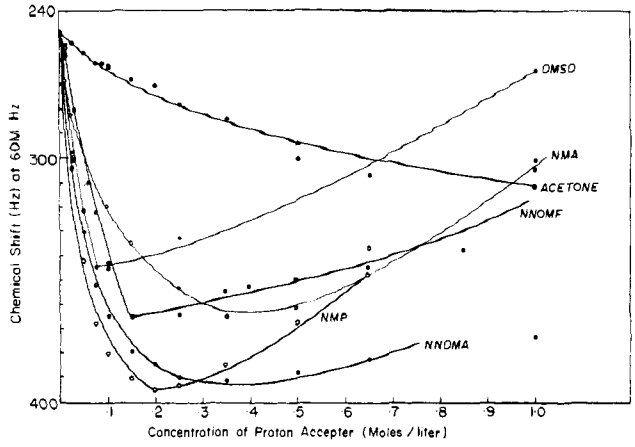
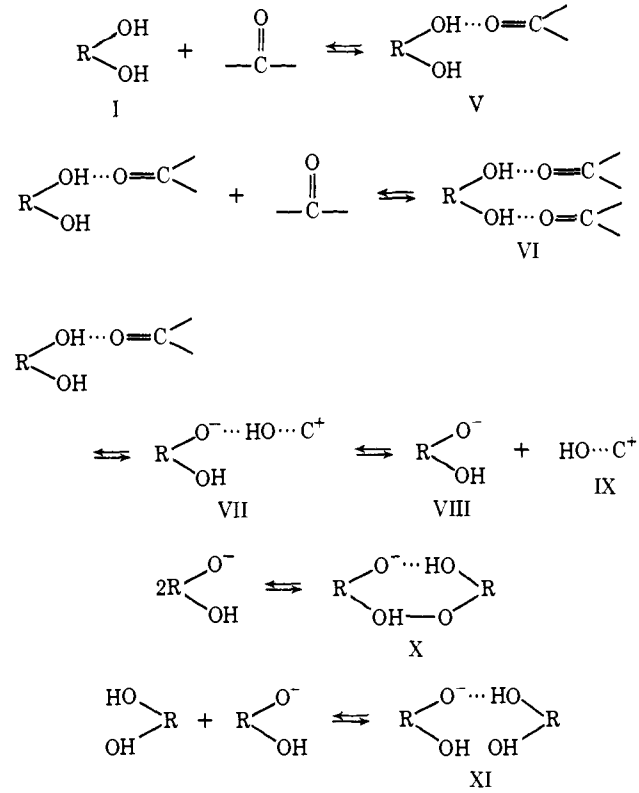



Figure 1. Changes in the phenolic proton chemical shift of 0.05 *M* hexachlorophene in $CCl_4$ by the addition of donor solvent; cyclohexane as internal standard.

**Scheme I**



parently occurs only in solvents with higher dielectric constants and the absence of minima in acetone (Figure 1) is probably due to the relatively low dielectric constants[15] as well as the low basicity[16] in this solvent. The presence of a minimum for hexachlorophene is also related to its relatively low p$K$[17] since only strong acids have been reported to exhibit such a minimum.[11-14] Dissociation of the hydrogen bonded associated ion-pair complex VII also could result in the formation of intermolecular hydrogen bonded species X and XI by hexachlorophene and its anion. A rapid exchange of phenolic protons between species I and XI produces the single broad OH peak. Chemical shift changes for the aromatic and methylene proton of I

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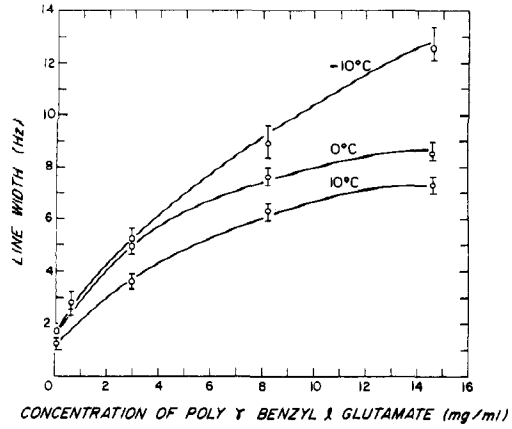


Figure 2. Line width changes of phenolic protons of hexachlorophene (0.05 $M$ in $CDCl_3$) by the addition of poly-γ-benzyl-L-glutamate.

and for the proton-acceptor molecules were very small in all experiments.

Although high concentrations of the peptides, poly-γ-benzyl-L-glutamate or poly-L-methionine, in $CDCl_3$ produce viscous solutions, good high-resolution pmr spectra could be obtained when relatively low concentrations of these polypeptides were employed. Addition of increasing amounts of poly-γ-benzyl-L-glutamate (1–14 mg/ml, monomer concentration of 0.0046–0.064 $M$) to a 0.05 $M$ solution of hexachlorophene, thus, produced a small but significant low-field chemical shift of the phenolic proton. The total chemical shift change was about 0.1 ppm depending upon the concentration of polypeptide and the temperature of the solution (Table I). Line width of the phenolic proton peak also broadened considerably in the presence of the polypeptide (Figure 2). However, neither a chemical shift or significant line broadening was observed for the methylene or aromatic protons of hexachlorophene at all peptide concentrations employed. No minimum was observed in either the chemical shift–concentration or line width–concentration relationships. Therefore, it was not possible to estimate the molar ratio of I bound to the poly-γ-benzyl-L-glutamate on a basis of pmr measurements, especially in view of a significant peak broadening due to viscosity alone at polypeptide concentrations greater than 14 mg/ml.

**Table I.** Changes in the Chemical Shift of the Phenolic Protons of Hexachlorophene (0.05 $M$ in $CDCl_3$) by the Addition of Poly-γ-benzyl-L-glutamate[a]

| Temp, °C | Concn of poly-γ-benzyl-L-glutamate, mg/ml | Chemical shift change of phenolic proton, Hz |
|---|---|---|
| 33 | 0 | 0 |
| | 0.53 | 0.15 |
| | 1.29 | 0.65 |
| | 2.9 | 0.85 |
| | 4.6 | 2.10 |
| | 8.2 | 2.4 |
| | 14.6 | 4.05 |
| 20 | 0 | 0 |
| | 0.53 | 0.6 |
| | 1.29 | 0.9 |
| | 2.9 | 0.9 |
| | 4.6 | 2.7 |
| | 8.2 | 3.2 |
| | 14.6 | 5.0 |
| 10 | 0 | 0 |
| | 0.53 | 0.9 |
| | 1.29 | 1.0 |
| | 2.9 | 1.0 |
| | 4.6 | 3.1 |
| | 8.2 | 3.6 |
| | 14.6 | 6.2 |
| 0 | 0 | |
| | 0.53 | 1.0 |
| | 1.29 | 1.0 |
| | 2.9 | 1.5 |
| | 4.6 | 3.9 |
| | 8.2 | 4.6 |
| | 14.6 | 6.8 |
| −10 | 0 | |
| | 0.53 | 1.1 |
| | 1.29 | 1.7 |
| | 2.9 | 1.8 |
| | 4.1 | 5.0 |
| | 8.2 | 5.9 |
| | 14.6 | 8.1 |

[a] Mol wt = 200,000.

Similarities in the nature of the chemical shift changes elicited by poly-γ-benzyl-L-glutamate and the structurally similar amides tested suggest an analogous mechanism of interaction with hexachlorophene that involved formation of a hydrogen bond between the phenolic protons of hexachlorophene and the carbonyl groups of the polypeptide.

A rapid exchange of the phenolic protons between the bound and the unbound state always results in a single average peak. If we represent the concentration of hexachlorophene (H) as $C_1$, the concentration of polypeptide (P) as $C_2$, and the concentration of the complex as $\alpha$, we have the following equilibrium.

$$\underset{(C_1 - \alpha)}{\mathrm{H}} + \underset{(C_2 - \alpha)}{\mathrm{P}} \rightleftharpoons \underset{\alpha}{\mathrm{HP}} \qquad (1)$$

The equilibrium constant $K$ can be expressed as

$$K = \alpha/(C_1 - \alpha)(C_2 - \alpha) \qquad (2)$$

in terms of the concentration of H, P, and HP. If we assume that $\alpha \ll C_1$ or $C_2$, (2) can be reduced to

$$K = \alpha/C_1C_2 \qquad (3)$$

The observed chemical shift of the phenolic proton $\delta$ can also be written in terms of the chemical shifts corresponding to free $\delta_f$ and bound state $\delta_b$.

$$\delta = \frac{(C_1 - \alpha)}{C_1}\delta_f + \frac{\alpha}{C_1}\delta_b \qquad (4)$$

We can relate $\delta_b$ and $\delta$ to $\delta_f$ by

$$\delta = \Delta\delta + \delta_f \qquad (4a)$$

$$\delta_b = \Delta\delta_b + \delta_f \qquad (4b)$$

where $\Delta\delta$ and $\Delta\delta_b$ are the chemical shift changes at a particular concentration and in the bound state, respectively, with reference to $\delta_f$. Substituting (4a) and (4b) in eq 4, we obtain (5).

$$\Delta\delta = (\alpha/C_1)\Delta\delta_b \text{ or } K = \Delta\delta/C_2\Delta\delta_b \qquad (5)$$

The enthalpy of hydrogen bond formation, $\Delta H$, can be obtained from a consideration of the temperature-dependence nature of the chemical shift (Table I). The equilibrium constant $K$ is related to $\Delta H$ by (6).

$$\partial \ln K = \frac{-\Delta H^\circ}{R}\left(\partial\frac{(1)}{T}\right) \quad (6)$$

Equation 6 can be written in terms of chemical shifts at two temperatures as

$$\log\left(\frac{\Delta\delta^2}{\Delta\delta^1}\right) = \frac{-\Delta H^\circ}{2.303R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) + \log\frac{\Delta\delta^1{}_b}{\Delta\delta^2{}_b} \quad (7)$$

As expected for a hydrogen bonded system, a decrease in temperature produces larger chemical shifts (Table I). A plot between $1/T$ and log $\Delta\delta$ yielded a linear relationship (Figure 3) as predicted by eq 7. Solving this equation for $\Delta H^\circ$ at three different concentrations of polypeptides gave the following results.

| | | | |
|---|---|---|---|
| Concn of poly-γ-benzyl-L-glutamate (mg/ml) | 14.6 | 8.2 | 4.6 |
| $\Delta H^\circ$ (kcal/mol) | 2.56 | 3.24 | 3.18 |

The value of $\Delta H^\circ$ ranges from 2.5 to 3.25 kcal/mol which is in the range of enthalpy changes to be expected for the hydrogen bonded system.

The pmr spectrum of I could not be observed in the presence of poly-L-methionine concentrations greater than 3 mg/ml (monomer concentration 0.020 *M*) because the highly viscous polypeptide solution caused extreme broadening of all proton peaks. At lower polypeptide concentrations (less than 3 mg/ml), however, only the phenolic proton peak of I broadened with addition of poly-L-methionine. No significant chemical shift changes in the pmr spectrum of I resulted from addition of poly-L-methionine. Line width changes for a 0.05 *M* solution of I with the addition of small amounts of poly-L-methionine at two temperatures (30 and 40°) are given in Table II. The

Table II. Changes in the Chemical Shift and Line Width of the Phenolic Proton of Hexachlorophene (0.05 *M* in $CDCl_3$) by the Addition of Poly-L-methionine[a]

| Concn of poly-L-methionine, mg/ml | Chemical shift, Hz | | Line width, Hz | |
|---|---|---|---|---|
| | 33° | 40° | 33° | 40° |
| 0 | 0 | 0 | 1.0 | 1.0 |
| 0.08 | 0 | 0 | 1.2 | 1.1 |
| 0.40 | 0.1 | 0 | 1.4 | 1.2 |
| 1.30 | 0.3 | 0 | 1.3 | 1.9 |
| 1.68 | Too broad | 0 | 17.0 | 7.8 |
| 2.33 | Too broad | 0 | 17.0 | 9.8 |

[a] Mol wt = 45,000.

increase in viscosity of the solution mixture with a lowering of temperature prevented pmr measurements at temperatures lower than 30°.

The line width changes of the phenolic protons of I by the addition of poly-γ-benzyl-L-glutamate or poly-L-methionine can also be explained on the basis of a complex formation[18] between I and the polypeptide. The line width changes $\Delta\nu_{1/2}$ are related to the spin-spin lattice relaxation time $T_2$ as

$$1/T_2 = \Pi\Delta\nu_{1/2} \quad (8)$$

Apparently the $T_2$ of the phenolic protons is reduced on formation of the complex. A rapid exchange be-

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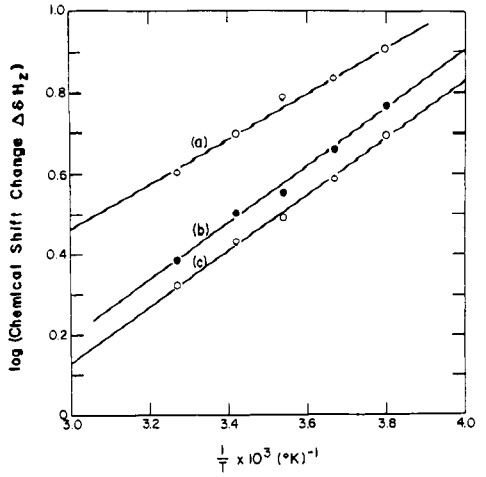


Figure 3. Plot between $1/T$ and chemical shift changes in the phenolic proton of hexachlorophene by the addition of (a) 14.6 mg/ml, (b) 8.2 mg/ml, and (c) 4.1 mg/ml of poly-γ-benzyl-L-glutamate (mol wt = 200,000) in $CHCl_3$ solution.

tween the free and the bound hexachlorophene always results in a single average peak on a pmr time scale. The relaxation time $T_2$ for the fast exchange process can be expressed as

$$(1/T_2)_{obsd} = \alpha(1/T_2)_{bound} + (1 - \alpha)(1/T_2)_{free} \quad (9)$$

The line width broadening of the phenolic proton was much more sensitive to the concentration of poly-L-methionine than that of poly-γ-benzyl-L-glutamate. This difference may be due to additional formation of hydrogen bonds between the phenolic protons of I and the lone-pair electrons of sulfur in poly-L-methionine.

Protonation of amide carbonyl oxygens by acid protons followed by dissociated ion-pair formation apparently occurs with simple amides in the presence of strong acids. In studies with a variety of amides in the presence of dichloroacetic and trichloroacetic acids, Klotz, *et al.*,[11,12] and Stewart and coworkers[14] showed by a variety of physical measurements (infrared, pmr, specific volume, and conductance) that the amides become protonated. An equilibrium exists between the amide, an ion pair consisting of the conjugate acid of the amide and the difluoroacetate or trifluoroacetate ion, and the dissociated ion pair. As the ratio of acid to amide increases, there is a complete dissociation of the ion pair. The evidence from present studies suggests that I is also capable of complexing with simple amides and that subsequent dissociation of the complex may occur.

Klotz, *et al.*,[12,19] Hanlon,[20] and Glick, *et al.*,[21] have suggested that protonation and ion-pair formation is also the basis for the interaction between trifluoroacetic acid type solvents and polypeptides that result in helix to coil transitions. Stewart, *et al.*,[22] however, have interpreted the failure of the trifluoroacetic acid proton to undergo significant downfield shifts to the presence of poly-L-alanine or poly-L-leucine as an indication that the acid proton was not completely trans-

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ferred to the carbonyl group of these peptides. Instead, the pmr results suggest to these authors that the acid proton remains hydrogen bonded to the peptide carbonyl oxygen. As the concentration of acid increases, acid–polypeptide interaction and hydrogen binding without proton transfer increases. Intramolecular hydrogen bonding is, therefore, suppressed in favor of intermolecular hydrogen bonding between the acid and the polypeptide. This then is the driving force converting the polypeptide from the helical to the random coil form.

The slight low-field shift and the small degree of line broadening of the I phenolic proton peak in the presence of poly-γ-benzyl-L-glutamate and poly-L-methionine, respectively, encountered in the present studies also suggest that I hydrogen bonds to these polypeptides and that proton transfer does not occur. The pmr spectrum of poly-γ-benzyl-L-glutamate was not altered in the presence of I. This indicates that either the low solubility or the moderate acid strength of I could not elicit peptide confirmational changes of the type previously reported for trifluoroacetic acid–polypeptide systems.[21,23]

Hexachlorophene has now been shown to strongly hydrogen bond to polypeptides. The conclusions from the present investigations are useful in understanding the more complex interactions that occur between I and proteins.

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# Reactions of Sulfur Atoms. XIII. Experimental and Calculated Secondary Hydrogen–Deuterium Kinetic Isotope Effect for the S($^3$P) + Ethylene Reaction

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*Contribution from the Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.* *Received June 21, 1971*

**Abstract:** An experimental and theoretical study has been made of the secondary H/D kinetic isotope effect in the addition of S($^3$P) atoms to ethylene. The values of the experimental $k_D/k_H$ ratios were 1.14, 1.07, and 1.04 for the $C_2D_4$–$C_2H_4$, $CD_2CH_2$–$C_2H_4$, and *cis*-CHDCHD–$C_2H_4$ reactant pairs, respectively. Calculation of the isotope effect was carried out within the framework of transition-state theory for two basic models of the transition state, a symmetrical ring and an asymmetrical ring-distorted structure. Either model can be made compatible with the experimental results by an appropriate and reasonable choice for the structural parameters of the transition state. Calculation of the isotope effect arising from each normal vibrational mode clearly shows that, contrary to current notions, the most important single factor inducing a secondary H/D isotope effect in addition reactions involving olefinic double bonds is not the out-of-plane bending motions of the CH bonds, but the gain in the isotopically sensitive vibrational degrees of freedom on going from reactant to activated complex.

The addition of both $^1D_2$ excited and $^3P$ ground-state sulfur atoms to olefins has been extensively investigated.[1-6] The unique feature of the reaction is that not only singlet, but triplet atom addition as well, follows a stereospecific course. This has been attributed[7,8] to a correlation with an (n,σ*) excited state of the product thiirane, which retains CC bonding but is unstable with respect to CS ring opening. Thus the product thiirane would form essentially in its final nuclear configuration *via* a symmetric transition state in a suprafacial, spin- and symmetry-allowed concerted step. A conceptual alternative which is also supported by molecular orbital calculations[9] and kinetic considerations is an asymmetrical, ring-distorted (∠(CCS) ~ 100°) (n,σ*) triplet thiirane with a relatively large (23.6 kcal/mol) energy barrier for rotation around the CC bond. Formation of this intermediate does not conserve orbital symmetry; symmetry conservation, however, is not an *a priori* condition for reaction since, as discussed by Hoffmann and coworkers,[8] symmetry-allowed motions which are facile are initiated in the excited reactants and there is no need to reach the symmetry-allowed state of the product.

There is a small but clearly defined activation energy associated with the reaction.[5] The value of this is 1.5 kcal for the ethylene reaction and gradually decreases with increasing number of alkyl substituents on the doubly bonded carbons to a negative value of −1.5 kcal/mol in the tetramethylethylene reaction.[5] The negative activation energy is interpreted as being due to intersection of potential energy surfaces manifesting the nonadiabatic nature of the reaction. The frequency factor of the reaction is large, of the order

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