

range of initial conditions, for some of which (Figure 3) the dynamics are considerably more complicated than suggested by a reaction path calculation.

The results reported in this paper represent only the first step in dynamic investigations of organic reactions by trajectory techniques on quantum mechanical potential surfaces. It is clear, nevertheless, that such investigations are now feasible and that they can lead to conclusions that are not evident from an analysis of the reaction path. Moreover, they can serve as an efficient way of isolating the portions of the potential surface of importance for the reaction *prior* to an examination by static methods. In future calculations, it would be helpful to utilize more reliable potential surfaces than the one employed here. One way of doing so, without a great increase in computing time, could be to employ a simple semiempirical functional form, such as that of the CNDO type, with parameters specifically chosen to fit accurate *a priori* calculations for the system of interest.

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π -Chlorobenzene

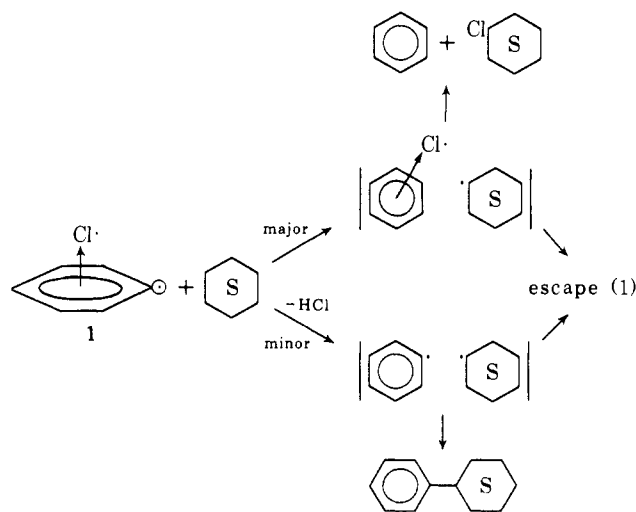
Sir:

We wish to present evidence that irradiation (2537 Å) of chlorobenzene¹ in solution yields the high-energy isomer **1**, which we call π -chlorobenzene, and to comment on the nature and reactivity of this novel biradical.

An early clue to its existence was the finding that chlorocyclohexane (~53%) accompanies benzene (~90%) and HCl (~35%) as the major photolysis products of chlorobenzene in dilute (0.025 M) cyclohexane solution. Formation of chlorocyclohexane by combination of free cyclohexyl radicals and chlorine

atoms is out of the question,² for a chlorine atom cannot survive more than a few collisions in the solvent cyclohexane before abstracting hydrogen.³ One might propose that the phenyl radical of a geminate phenyl radical-chlorine atom pair attacks the solvent, producing a cyclohexyl radical-chlorine pair which collapses to chlorocyclohexane. This possibility is clearly ruled out by the fact that *phenyl radicals attack cyclohexane at least 10,000 times slower than do chlorine atoms*.⁴

Due to π complexation, however, chlorine atoms in benzene solvent show selectivities so enhanced^{3,5} that they resemble phenyl radical selectivities⁶ (e.g., for tertiary *vs.* primary hydrogens). Moreover, the observed chlorine selectivities undershoot the true values for complexed atoms by an unknown amount since the observed quantities represent a weighted average of complexed and free chlorine atom selectivities.⁷ The product composition from photolysis of chlorobenzene in cyclohexane is thus understandable in terms of eq 1. Indeed, the very small amount of phenylcyclohexane formed (~1%) argues for considerably greater reactivity at the carbon radical center of **1** than at chlorine.



Solvent exerts a dramatic influence over the efficiency of chlorobenzene photolysis at 2537 Å: *the quantum yield for destruction (Φ_D) is 0.38 in cyclohexane, but only 0.012 in the inert Freon 113*.⁸ This contrast is readily explained in terms of the π intermediate, which

(2) Furthermore, chlorocyclohexane is still formed in abundance when a radical scavenger such as O₂ is present. The possibility that this product results from HCl addition to cyclohexene is eliminated by the finding that triethylamine fails to prevent its formation.

(3) G. A. Russell, A. Ito, and D. G. Hendry, *J. Amer. Chem. Soc.*, **85**, 2976 (1963).

(4) Compare ref 3 with G. A. Russell and R. F. Bridger, *J. Amer. Chem. Soc.*, **85**, 3765 (1963).

(5) (a) G. A. Russell, *J. Amer. Chem. Soc.*, **79**, 2977 (1957); **80**, 4987, 4997 (1958); C. Walling and M. F. Mayahi, *ibid.*, **81**, 1485 (1959); (b) E. S. Huyser, *Advan. Free-Radical Chem.*, **1**, 77 (1965); (c) R. E. Bühler and M. Ebert, *Nature (London)*, **214**, 1220 (1967); R. E. Bühler, *Helv. Chim. Acta*, **51**, 1558 (1968).

(6) R. F. Bridger and G. A. Russell, *J. Amer. Chem. Soc.*, **85**, 3754 (1963).

(7) Russell fit his data successfully (but not uniquely) using the assumption that complexed chlorine is *completely* selective, i.e., incapable of attacking primary hydrogen. The existence of both 1:1 and 2:1 (at least) complexes of aromatic rings with chlorine atoms considerably complicates analysis of the problem.

(8) Since very low Φ_D values are also obtained with small amounts of cyclohexane present, one can be certain that the value in pure Freon 113 is not low because of reconstitution of chlorobenzene from radicals turned loose into the medium.

(1) Earlier studies of chlorobenzene photolysis include the following: (a) T. Ichimura and Y. Mori, *J. Chem. Phys.*, **58**, 288 (1973); (b) G. E. Robinson and J. M. Vernon, *J. Chem. Soc. C*, 3363 (1971); (c) J. Wojtczak and W. Augustyniak, *Poznan. Tow. Przyj. Nauk, Pr. Kom. Mat.-Przyr., Pr. Chem.*, **12**, 61 (1971) (*Chem. Abstr.*, **75**, 43054m (1971)); J. Wojtczak and M. Elbanowski, *ibid.*, **12**, 87 (1971) (*Chem. Abstr.*, **74**, 43037h (1971)); (d) I. Loeff, H. Lutz, and L. Lindquist, *Isr. J. Chem.*, **8**, 141 (1970) (*Chem. Abstr.*, **73**, 93476e (1970)); (e) J. T. Pinhey and R. D. G. Rigby, *Tetrahedron Lett.*, 1267 (1969); (f) G. E. Robinson and J. M. Vernon, *J. Chem. Soc. D*, 977 (1969); (g) J. A. Barltrop, N. J. Bunce, and A. Thomson, *J. Chem. Soc. C*, 1142 (1967); (h) B. Brocklehurst, W. A. Gibbons, F. T. Lang, G. Porter, and M. I. Savadatti, *Trans. Faraday Soc.*, **62**, 1793 (1966); (i) A. Basinski and E. Latowska, *Rocz. Chem.*, **40**, 1747 (1966); (j) G. Porter and B. Ward, *Proc. Roy. Soc., Ser. A*, **287**, 457 (1965); (k) V. G. Vinogradova, B. N. Shelimov, N. V. Fok, and V. V. Voerdoskii, *Dokl. Akad. Nauk SSSR*, **154**, 188 (1964) (*Chem. Abstr.*, **60**, 14045f (1964)); (l) Y. S. Kim and Y. J. Park, *Ta Han Hua Hsueh Hui-Chih*, **6**, 148 (1962) (*Chem. Abstr.*, **60**, 2476b (1964)). See also J. A. Barltrop and D. Bradbury, *J. Amer. Chem. Soc.*, **95**, 5085 (1973).

is highly reactive yet capable of reverting quantitatively, or virtually so, to chlorobenzene.

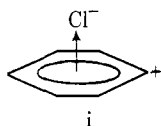
As increasingly concentrated solutions of chlorobenzene in cyclohexane are irradiated, the product composition changes strikingly: chlorocyclohexane almost vanishes while benzene yields remain high (<3% vs. ~86% at 1.0 M).⁹ The benzene:chlorocyclohexane ratio is, in fact, a (steep) linear function of chlorobenzene concentration (slope = 36 M⁻¹). Apparently, a 1:1 complex of π -chlorobenzene with chlorobenzene is formed (the " π dimer"), analogous to the 2:1 complexes of aromatic molecules with a chlorine atom.^{5,10} The reasonable surmise¹¹ that the chlorine is sandwiched between the rings suggests an explanation for the failure of this complex to yield appreciable chlorocyclohexane. Hydrogen abstraction by carbon occurs as in eq 1, but now the chlorine atom is sufficiently shielded from the cyclohexyl radical that escape from the cage completely overshadows coupling.

The lifetime of π -chlorobenzene is a central question which is best approached by considering first chlorobenzene's S₁ and T₁ states. Sensitization of biacetyl fluorescence by chlorobenzene in aerated solution¹³ revealed that the S₁ lifetime is very short: $\sim 8 \times 10^{-11}$ sec in Freon 113 and in cyclohexane. Similar experiments in degassed ampoules permitted measurement of sensitized biacetyl phosphorescence,¹³ and thus of the lifetime of T₁: 5×10^{-7} sec.¹⁴ Comparison of phosphorescence intensity with that using naphthalene (known triplet yield¹⁵) as sensitizer showed the triplet yield from chlorobenzene to be ~ 0.3 . The discovery that biacetyl (~ 0.01 M) fails to influence Φ_D for chlorobenzene revealed that the π species is (1) not formed *via* T₁ (which was completely quenched under these conditions) and (2) not quenched to (S₀) chlorobenzene by energy transfer to biacetyl even though that process

(9) The authors are greatly indebted to Professor R. G. Lawler of Brown University, who has searched to no avail for photo-CIDNP in this reaction. For intensity reasons the experiments have been carried out in concentrated solution.

(10) Steady-state kinetic analysis of this model "predicts" that the above function be linear, as found, if it be assumed that " π dimer" yields no chlorocyclohexane.

(11) The great difference in electron affinity between phenyl radical and chlorine atom (~ 50 kcal/mol¹²) argues for complexation by chlorobenzene at chlorine, not carbon. Moreover, no pronounced solvent effects on the reactivity of phenyl radicals have been discovered to our knowledge. The possibility that ionic resonance form i contributes



importantly to the observed electronic state(s) of π -chlorobenzene weakens the above argument, but there are reasons for considering i of minor significance (*e.g.*, much benzene but very little or no anisole is found when chlorobenzene is photolyzed in methanol; the π species resembles phenyl radical in relative rate of quenching by oxygen *vis-à-vis* cyclohexane; though much less reactive, a chlorine atom complexed with benzene behaves as if it is nearly as electrophilic as free chlorine^{5b}).

(12) D. K. Bohme and L. B. Young, *Can. J. Chem.*, **49**, 2918 (1971); B. L. Moiseiwitsch, *Advan. At. Mol. Phys.*, **1**, 61 (1965).

(13) J. T. Dubois and M. Cox, *J. Chem. Phys.*, **38**, 2536 (1963); J. T. Dubois and F. Wilkinson, *ibid.*, **38**, 2541 (1963); F. Wilkinson and J. T. Dubois, *ibid.*, **39**, 377 (1963); J. T. Dubois and R. L. Van Hemert, *ibid.*, **40**, 923 (1964).

(14) This value is identical with that obtained by pulsed laser photolysis of chlorobenzene (ref 1d).

(15) A. R. Horrocks and F. Wilkinson, *Proc. Roy. Soc., Ser. A*, **306**, 257 (1968); C. A. Parker and T. A. Joyce, *Trans. Faraday Soc.*, **62**, 2785 (1966).

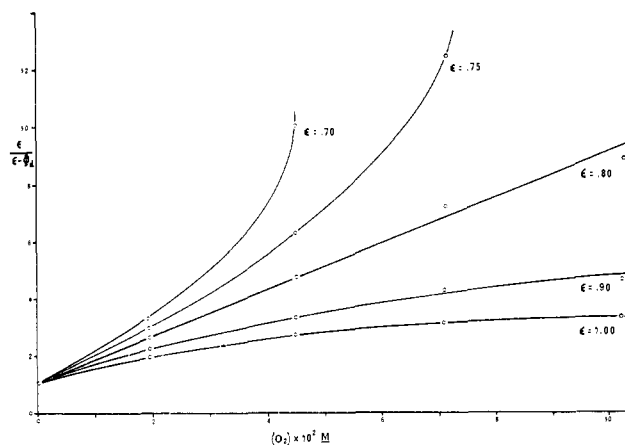


Figure 1. Oxygen quenching of π -chlorobenzene in Freon 113.

would be highly exothermic (whether singlet or triplet excitation be transferred). Hence the following simple Stern-Volmer expression (eq 2) is adequate¹⁶ for anal-

$$\epsilon/(\epsilon - \Phi_D) = 1 + k_q[Q]/k_r \quad (2)$$

ysis of the (reactive) quenching of π -chlorobenzene by Q, where ϵ represents the efficiency with which the π species is formed from chlorobenzene and k_q and k_r are the rate constants for quenching of π -chlorobenzene and its reversion to S₀,¹⁷ respectively. Figure 1 displays plots of $\epsilon/(\epsilon - \Phi_D)$ vs. [Q] for various values of ϵ , where Q = O₂ in Freon 113.¹⁸ We conclude that about 80% of the photoexcited chlorobenzene is transformed into the π species; from the slope of the $\epsilon = 0.8$ line (k_q/k_r) and the fact that $k_q \leq k_{diff}$, it follows that π -chlorobenzene is remarkably long-lived: $1/k_r \geq 7 \times 10^{-9}$ sec.¹⁹

A lower limit for the π -binding energy in π -chlorobenzene can be established in the following way. Since an uncomplexed chlorine atom produced by dissociation should survive no more than a few collisions with the solvent cyclohexane, the probability of HCl formation must roughly equal that of return to π -chlorobenzene for a Cl·-C₆H₅· caged pair in that medium. More than three π species revert to S₀ for each which yields HCl, so $k_r \gtrsim k_{diss}$.²⁰ Hence the free energy of activation for dissociation, ΔG^\ddagger , $\gtrsim 6.5$ kcal/mol (25°); assuming no barrier for the back reaction, the same limit characterizes ΔG . The entropy change accompanying dissociation can be estimated from direct calculation on reasonable models for the π species or from entropy changes in closely related reactions such as the well-studied I₂-aromatics equi-

(16) These provisos are necessary: (1) chlorobenzene concentration be too low to permit significant complexation of the π species; (2) T₁ be assumed always to return to S₀, whether spontaneously or by quenching; (3) [Q] be low enough that S₁ is not quenched.

(17) Reversion *via* T₁ is possible as a minor pathway.

(18) Other quenchers, in order of decreasing reactivity toward the π species, are CS₂ (quenching rate nearly that for O₂), I₂, cyclohexane, and cyclohexane (<10⁻² that of O₂).

(19) Figure 1 is based on an experiment in which the initial chlorobenzene concentration was 0.025 M, insufficiently low to forestall formation of appreciable " π dimer." An experiment conducted at 0.0025 M produced similar values for ϵ and k_r , however, indicating that k_q/k_r for the " π dimer" is close to that for π -chlorobenzene.

(20) Indeed, our data do not require *any* dissociation of π -chlorobenzene. The tiny Φ_D in Freon 113 shows only slight temperature dependence, which argues against dissociation of π as the cause of net chlorobenzene destruction.

librium.²¹ Allowance of 2 kcal/mol for $T\Delta S$ (25°) is reasonable by either approach, so the heat of dissociation, ΔH , \gtrsim 8.5 kcal/mol. Since $D(\text{C}_6\text{H}_5\text{-Cl}) \simeq 93.8$ kcal/mol,²² π -chlorobenzene lies \gtrsim 85 kcal/mol above chlorobenzene (S_0). Transformations, energies, and lifetimes of $\text{C}_6\text{H}_5\text{Cl}$ species produced in chlorobenzene photolysis are summarized in Figure 2.

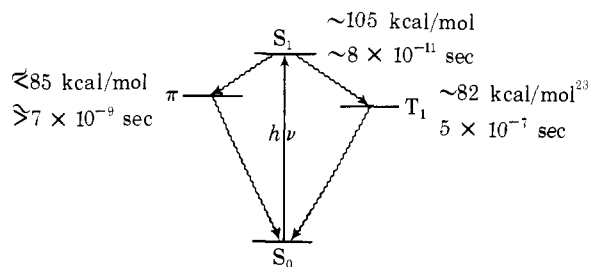
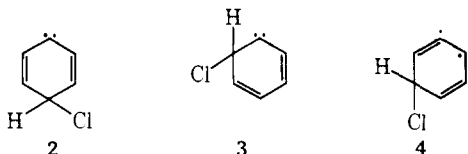


Figure 2. $\text{C}_6\text{H}_5\text{Cl}$ species formed by irradiation of chlorobenzene at 2537 Å in Freon 113.

The structure of π -chlorobenzene is not known in detail. Where the chlorine is located, whether or not it is capable of "ring whizzing," and whether the species is singlet or triplet²⁴ (if indeed only a single state of the biradical is involved) are all questions which remain to be answered.

There is clear evidence, however, against the possibility that the chlorine is σ bonded to the ring as in 2-4. For example, iodine quenching of 2-4 should



give major amounts of chloriodobenzenes, but photolysis of chlorobenzene in the presence of iodine yielded iodobenzene uncontaminated with any of the three chloriodobenzenes (<0.5% yield).

In principle, π -chlorobenzene could be formed by intramolecular rearrangement, but our failure to detect it unambiguously in the gas phase (even at ~ 12 atm pressure of Freon 12) argues that its principal, if not exclusive, origin is C-Cl homolysis followed by cage recombination. Recombination must be nearly quantitative to explain the very low Φ_D found in Freon 113 solution.³

Preliminary experiments indicate that photogeneration of π isomers is a general phenomenon among more highly chlorinated benzenes and occurs with bromobenzene as well.

Acknowledgment. The authors are grateful to Professor C. L. Braun for valuable advice on the emission spectroscopy. They wish to thank the National Science Foundation, the donors of the Petroleum Research Fund, administered by the American Chemical Society,

(21) R. M. Keefer and L. J. Andrews, *J. Amer. Chem. Soc.*, **77**, 2164 (1955).

(22) This value is based on ΔH_1° (298°K) = 77.1 ± 3 kcal/mol for the phenyl radical (I. Szilagyi and T. Berces, *Int. J. Chem. Kinet.*, **2**, 199 (1970)).

(23) H. E. Zimmerman and G. E. Samuelson, *J. Amer. Chem. Soc.*, **91**, 5309 (1969); S. P. McGlynn, T. Azumi, and M. Kasha, *J. Chem. Phys.*, **40**, 507 (1964).

(24) Irradiation of chlorobenzene in a Fluorolube matrix at 77°K in an esr cavity failed to reveal a triplet species, but with a higher intensity source it may yet make an appearance.

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A Phosphodiesterase from *Enterobacter aerogenes*

Sir:

We wish to report the purification and preliminary characterization of a phosphodiesterase from *Enterobacter aerogenes*. This diesterase is the first to be isolated that is known to catalyze the hydrolysis of simple diesters of phosphoric acid, such as diethyl phosphate and trimethylene phosphate.¹ Recently the heats of enzyme-catalyzed hydrolysis of cyclic 3',5'-nucleotides and 2',3'-nucleotides^{2,3} were reported; since the large enthalpy of hydrolysis of cyclic AMP was unexpected, it was desirable to determine the heats of hydrolysis of simple aliphatic analogs for comparison. However, most diesters of phosphoric acid (except those containing five-membered rings) are exceedingly resistant to hydrolysis,⁴ so that determinations of heats of hydrolysis were out of the question until a suitable catalyst was discovered. The enzyme here described served for the determinations of the desired heats of hydrolysis,¹ and may have other uses.

Wolfenden and Spence⁵ observed that *Enterobacter aerogenes* can be grown on dimethyl phosphate as the only source of phosphorus. We have now shown that under these conditions (but not when the bacteria are grown with adequate inorganic phosphate), they produce large quantities (up to 3% of the total soluble protein) of a phosphodiesterase. The cells (American Type Culture Collection 13048) were grown at 37° on minimal medium⁵ without phosphate but with $1.0\text{--}1.4 \times 10^{-4}$ M sodium dimethyl phosphate in a rotary shaker at pH 7.5. The cells from 48 l. of culture medium were harvested and the cell paste washed with 0.01 M Tris buffer, pH 7.60. The cells were disrupted by a single pass through a French press⁶ (Aminco) at 16,000 psi; the subsequent purification procedure is outlined briefly in Table I.

The suspension from the French press was centrifuged at 34,800g, and the supernatant freed of nucleic acids with protamine sulfate. After centrifugation, the supernatant was heat-treated, cooled, and again centrifuged. The supernatant was then chromatographed on Whatman DE-52 cellulose. The esterase fractions were concentrated by an Amicon ultrafilter with a PM-10 membrane, and the protein was precipitated by bringing the concentration of ammonium sulfate to

(1) J. M. Sturtevant, J. A. Gerlt, and F. H. Westheimer, *J. Amer. Chem. Soc.*, **95**, 8168 (1973).

(2) P. Greengard, S. A. Rudolph, and J. M. Sturtevant, *J. Biol. Chem.*, **244**, 4798 (1969).

(3) S. A. Rudolph, E. M. Johnson, and P. Greengard, *ibid.*, **246**, 1271 (1971).

(4) (a) J. Kumamoto and F. H. Westheimer, *J. Amer. Chem. Soc.*, **77**, 2515 (1955); J. Kumamoto, J. R. Cox, and F. H. Westheimer, *ibid.*, **78**, 4858 (1956); (b) J. R. Cox and O. R. Ramsay, *Chem. Rev.*, **64**, 317 (1964).

(5) R. Wolfenden and G. Spence, *Biochim. Biophys. Acta*, **146**, 296 (1967).

(6) We wish to thank Professor Konrad Bloch for the use of this equipment.