and 2-chlorophenol are produced, respectively.[16] The substantial percentage of 3-chlorophenol (>20%) produced in vivo,[12] and by the perfused liver,[17] appears to result from an enzyme which catalyzes the insertion of oxygen directly into the carbon-hydrogen bond without intervention of an arene oxide.[18]

Prior induction of animals with 3-methylcholanthrene greatly enhances the extent of ortho hydroxylation of halobenzenes.[17,19] To determine the effect of induction on the profile of metabolites, 13 male Sprague-Dawley rats (200 g) were given two intraperitoneal injections of chlorobenzene (1.1 g/kg in cotton seed oil) 1 day apart, and urine was collected for 2 days. In addition to the known 4-chlorodihydrodiol[13] (1%), the unknown 3-chloro isomer, *trans*-1,2-dihydroxy-1,2-dihydro-3-chlorobenzene (0.5%), was isolated from the urine of the induced animals.[20] Separate incubations of **1** and **2** with microsomal and solubilized epoxide hydrase[16] resulted in the formation of the 4- and 3-chlorodihydrodiols, respectively, by the direct trans addition of water.[21] Formation of 2-chlorophenol as the major phenolic isomer[17] and the identification of the 3-chlorodihydrodiol strongly implicates **2** as a primary metabolite of chlorobenzene when animals are induced by 3-methylcholanthrene.

Results of trapping experiments in which [^{14}C]chlorobenzene was incubated with liver microsomes from control and induced rats in presence of carrier chlorobenzene oxides are shown in Table I. After incubation, the residual oxides were extracted into ethyl acetate. In separate experiments, the recoveries of **1** and **2** to this point were determined to be 47 and 29%, respectively, by titration of the extracts with the highly colored methyl triazolinedione[9] used to stabilize the oxides as Diels–Alder adducts. The comparable recoveries for the two arene oxides from the incubation medium suggests that their rates of isomerization are similar. Remarkably, the reisolated **2** was free of radioactivity while as much as 15% of the total metabolism was trapped as [^{14}C]-**1**.

The above trapping experiments are of profound importance in explaining the hepatotoxicity of halobenzenes as mediated by halobenzene oxides which covalently bind to tissue constituents.[1a,22] Prior induction of animals with 3-methylcholanthrene has been noted to cause an increase in the rate of metabolism,[17,19] yet unexpectedly results in markedly decreased toxicity. While induction by 3-methylcholanthrene favors metabolic pathways originating from 3- rather than 4-halobenzene oxides (e.g., **2** rather than **1**), the basis for the protection against necrosis has remained unclear. Studies of the toxicity of **1** and **2** toward liver cells in suspension suggest these compounds have comparable activity.[23] Failure to trap significant amounts of **2** in the present study (Table I) is indicative that little of the **2** formed in vivo enters the cytosol from the endoplasmic reticulum, and thus never has the opportunity to inactivate critical macromolecules in the cell through covalent interaction.[24]

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(9) The NMR spectra of **1** and **2** were measured in CS_2: **1** (220 MHz), H_1 5.37, H_2 5.39, H_3 5.57, H_5 6.11, and H_6 5.52; **2** (100 MHz), $H_{1,2}$ 4.17 and $H_{4,5,6}$ 6.24. The chemical shifts of the α-hydrogens (H_1 and H_2) established that **1** exists mainly as the oxepin isomer while **2** is mainly in the oxide form when compared to benzene oxide.[5] Both **1** and **2** were further characterized as Diels–Alder adducts with 4-methyl-1,2,4-triazoline-3,5-dione (prepared by the method of R. C. Cookson, S. S. Gupte, I. D. Stevens, and C. T. Watts, *Org. Synth.*, **51**, 121 (1971)): adduct from **1**, mp 164–166° from acetone, and adduct from **2**, mp 136–137° from methanol. Both adducts gave acceptable NMR and mass spectra as well as elemental analyses.
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(20) Preliminary isolation of a dihydrodiol fraction was as in ref 13. Separation of the isomeric 3- and 4-chlorodihydrodiols was achieved by multiple development on alumina TLC plates on which the 3-chloro isomer migrated faster. The isomers also separate on high pressure liquid chromatography.[17] The NMR spectra of the diols were measured $CDCl_3$ at 220 MHz. The spectrum of the trans 3-chloro isomer was not first order and showed H_1 4.54 H_2 4.42, H_4 6.13, and $H_{5,6}$ 5.93 with $J_{1,2}$ = 9.5 Hz, while the spectrum of the trans 4-chloro isomer showed H_1 4.47, H_2 4.53, H_3 6.00, H_5 5.91, and H_6 6.05 with $J_{1,2}$ = 10.8, $J_{1,5}$ = 1.6, $J_{1,6}$ = 2.4, $J_{2,3}$ = 2.6, $J_{3,5}$ = 2.9, $J_{3,6}$ = 0.4, and $J_{5,6}$ = 10 Hz. For comparison, the cis 3-chloro isomer, a microbial product from chlorobenzene which was kindly provided by Dr. D. T. Gibson at the University of Texas, showed H_1 4.52, H_2 4.19, H_4 6.11, and $H_{5,6}$ 5.87 with $J_{1,2}$ = 6.6 Hz.
(21) Both **1** and **2** were poor substrates for epoxide hydrase. Adequately sensitive assays to determine kinetic parameters for the hydrations were not found. Large scale incubations provided sufficient product to establish structure by NMR. Much higher turnover numbers might be realized with fresh liver microsomes due to the fragile nature of "benzene oxide hydrase" on freezing microsomes.
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Photochemistry in the Electronic Ground State. III. Isotope Selective Decomposition of Methylene Chloride by Pulsed Carbon Dioxide Laser

Sir:

When a mixture of two compounds in the gas phase is irradiated with monochromatic infrared (ir) light at a fre-

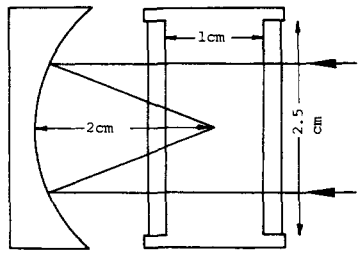

Figure 1. Scheme of the irradiation cell equipped with two NaCl windows and a focusing mirror.

quency which is absorbed by one of the compounds, vibrational excitation is selectively induced in the compound absorbing the ir light. However, because of collisional energy transfer, the vibrational energy is redistributed, and thermal equilibrium is restored. The number of molecules excited by a laser pulse depends on the light intensity and the absorption coefficient. Very intense light pulses may lead to multiple photon absorption. Under conditions of slow relaxations, near saturation conditions are attained, and cascade excitation takes place. Collisions between like molecules usually lead to degradation of the excitation energy but, under near saturation conditions, collisions between excited molecules become probable, resulting in the generation of molecules possessing very high vibrational energy, according to eq 1.

$$A^+ + A^+ \rightarrow A^{2+} + A^0 \qquad (1)$$

A^0 is a vibrational unexcited molecule, A^+ is an excited molecule, and A^{2+} is a doubly excited molecule.

Whenever the vibrational excitation exceeds the activation energy of a certain reaction, a chemical reaction may take place. This reaction may have an activation energy higher than the energy per Einstein in the laser light. Collisions between unlike molecules will always lead to degradation of the excitation energy. If the pulse duration is shorter than the collisional relaxation time, a compound can selectively undergo chemical reactions in the gas phase. Such reactions may occur in the presence of other components in the same mixture, which have comparable energies of activation but which do not absorb the laser light.

In a previous experiment,[1] we have shown that *trans*-2-butene can be selectively decomposed by intense ir laser pulses in the presence of *cis*-2-butene which is practically transparent to the applied laser light.

We report here on an observation where deuterated methylene chloride (CD_2Cl_2) is selectively decomposed by 10-MW ir pulses of 943 cm^{-1} and 10^{-7} sec duration, in the presence of methylene chloride (CH_2Cl_2). The absorption coefficient of CD_2Cl_2 measured at low power is ϵ^{943} ($Torr^{-1}$ cm^{-1}) 1.6×10^{-3} and is related to the CD_2 rocking vibration. CH_2Cl_2 is practically transparent at 943 cm^{-1} (the CH_2 rocking vibration of CH_2Cl_2 is at 1266 cm^{-1}).

In a typical experiment at a gas pressure of 2 Torr, 3600 pulses at a rate of 2 PPS were applied. Reaction of CD_2Cl_2 was obtained only with focused ir light. The reaction cell is shown in Figure 1. Under the same conditions, CH_2Cl_2 was not reactive. When a mixture of CD_2Cl_2 and CH_2Cl_2 with a molar ratio of 1:1.2 (0.83) was irradiated under the same conditions, 20% of the total methylene chloride was decomposed (as determined gas chromatographically[2]).

However, the ratio of CD_2Cl_2 to CH_2Cl_2 changed to 0.74.[3] According to this result the ratio of overall rates of decomposition of CD_2Cl_2 and CH_2Cl_2 is $V_D/V_H = 1.2$. The thermal decomposition was reported in the literature.[4-8] A kinetic study was carried out, and the reaction was found to be second order[4,5] with respect to the methylene chloride.

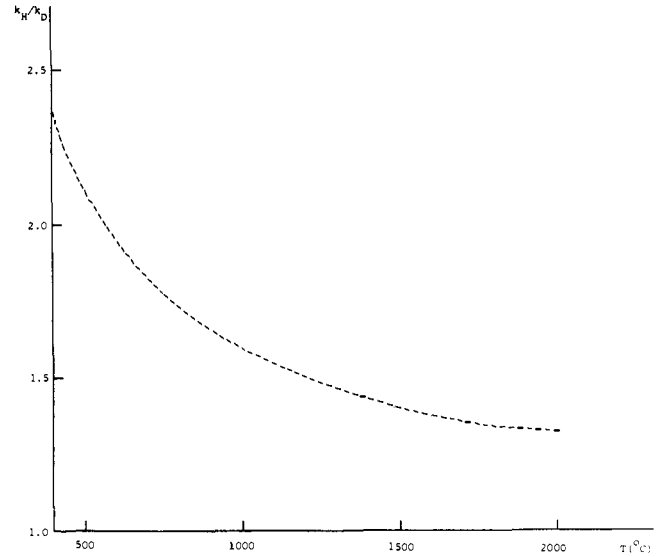

Figure 2. Plot of the isotope effect (k_H/k_D) vs. the temperature (T).

The main products were identified as carbon and hydrochloric acid. The Arrhenius energy of activation was 65 kcal/mol.

It is assumed that our reaction may be compared with a thermal decomposition. In order to show the selectivity of our reaction, we must consider the isotope effect. A theoretical study[9] based on bond stretching constants indicated that the isotope effect of the rate of decomposition of both compounds is temperature dependent. The temperature dependence is shown in Figure 2. According to Figure 2, the isotope effect decreases with the rise in the temperature.

Since the system in our experiment is not under thermal equilibrium, it is difficult to treat it in terms of temperature and reaction rate constants. For simplicity let us assume that the CH_2Cl_2 and the CD_2Cl_2 are at different vibrational temperatures. The vibrational temperature is measured by the corresponding rate of decomposition of the compound. The duration of the reaction initiated by each pulse must be shorter than the relaxation time since we obtain a selective reaction. The relaxation time is estimated to be 10 sec Torr by comparison with the experimental data for methyl chloride.[10] In order to use the kinetic data for the thermal decomposition, we have to estimate the change in the partial pressure ΔP occurring after each pulse. It is difficult to estimate ΔP because the reaction takes place in an unknown volume around the focal spot. For each estimate of ΔP the vibrational temperatures of CH_2Cl_2 and CD_2Cl_2 are defined. (In order to calculate the vibrational temperature, we use the integrated form of rate equation from ref 5 and the temperature dependence of the isotope effect from ref 9.)

Considering now an analogous experiment where both compounds are decomposed separately each at a different temperature, the set of possible temperatures corresponding simultaneously to each compound are shown in Figure 3.

In a test experiment, a mixture of the same composition at a pressure of 41 Torr was irradiated with a 50-W CW 943 cm^{-1} beam (as was indicated in a previous publication,[11] under these conditions it is impossible to get high conversion of material at lower pressure, and the reaction has all the features of a thermal reaction). The overall decomposition was 9%, and the molar isotope ratio changed from 0.83 to 0.90. Assuming complete thermal equilibrium and very fast diffusion into the reaction zone in the cell, the isotope effect is $k_H/k_D = 2.3$. This value corresponds to 700 K.

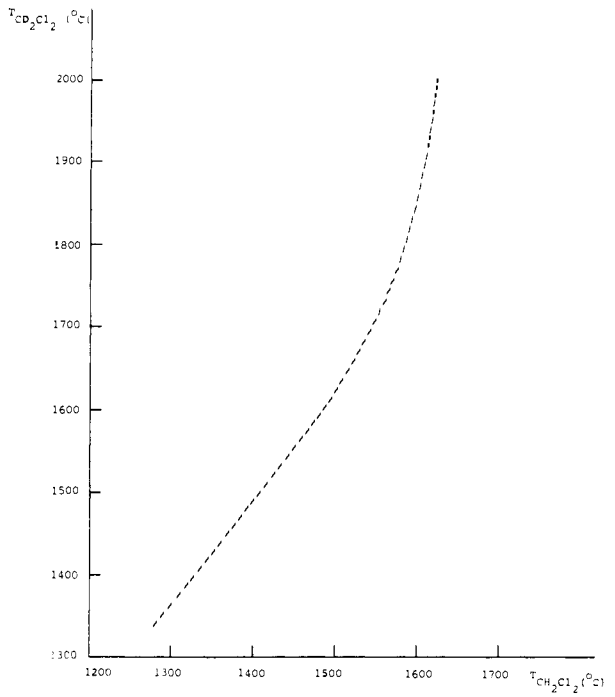

Figure 3. Plot of vibrational temperatures of CD_2Cl_2 ($T_{CD_2Cl_2}$) vs. various predicted vibrational temperatures of CH_2Cl_2 ($T_{CH_2Cl_2}$).

It is clear from these results that selectivity was attained only when ir pulses with sufficient intensity and having durations shorter than the overall relaxation time were applied. By increasing either the pressure or the light intensity of the ir pulsed laser, experiment breakdown occurs. Under these conditions, most of the methylene chloride was decomposed after 100 pulses; the isotope ratio, however, remained constant.

As in our previous study,[1] the energy of activation (65 kcal/mol) was much higher than the energy per Einstein of the laser light. The detailed mechanism of energy accumulation was not studied. However, the reaction could not occur by single photon absorption. Multiphoton absorption, cascade excitation, or combination of the two mechanisms may be responsible for the observed reaction.

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The Stereochemically Correct Catalytic Site on Cyclodextrin Resulting in a Better Enzyme Model

Sir:

Cyclodextrin reactions show roughly the same rate enhancement with respect to hydroxide ion reactions as do chymotrypsin reactions.[1,2] There is one proviso, however. The rate constant for the chymotrypsin reaction was determined at pH 7.9, its maximum, whereas the rate constant for the cyclodextrin reaction was determined at pH 13, its maximum. If one can reduce the pH at which cyclodextrin operates, for example by introduction of an imidazole group into the molecule, its rate enhancement might truly parallel the reaction with chymotrypsin.[3]

The first such attempt by Cramer and Mackensen at introducing an imidazole group as a catalytic site shows only a slight rate enhancement over a combination of cyclodextrin and imidazole.[4] In their compounds, however, substitution occurs preferentially at carbon atom 6 of the glucose unit in the cyclodextrin. Namely the catalytic site (imidazole group) is attached to a *primary* alcohol group on the essentially closed face of the toroidal cyclodextrin molecule where the substrate could not be catalyzed in the cavity. The specific stereochemical relationship between the cyclodextrin and their substances in the cleavage of phenyl esters in alkaline solution is due to a nucleophilic reaction of an alkoxide ion derived from the *secondary* hydroxyl group at carbon atom 2 and 3, of the glucose unit in a cyclodextrin.[2]

Another attempt by Breslow and Overman, who prepared cyclodextrin with a metal ion coordinated nucleophile, showed that the reaction occurred at pH 5, the same general magnitude as Cramer's compounds.[5]

We wish to report here the preparation of an α-cyclodextrinhistamine compound (IV) in which the histamine group is attached to a *secondary* alcohol group on the more open face of the toroidal α-cyclodextrin. We wish also to demonstrate that this compound will accelerate the hydrolysis of *p*-nitrophenyl acetate by attack of the imidazole group on a substrate molecule bound in the cavity because of the appropriate position of the catalytic functionality (imidazole group) on the cyclodextrin.

The α-cyclodextrinhistamine compound (IV) was synthesized through the *p*-tosyl ester (I) and then the iodide (II) of α-cyclodextrin (α-CD). α-CD was tosylated with 10 equiv of *p*-toluenesulfonyl chloride in buffer solution of pH 11 at 25° for 1 hr, followed by ion-exchange chromatography using Amberlite MB-3, giving α-CD monotosylate (I), whose structure was confirmed by uv absorption (λ_{max} 263 nm, log ϵ 2.78, referred to ethyl tosylate, λ_{max} 262, log ϵ 2.75) together with proton, NMR spectra in D_2O which showed an absorption at δ 7.48 (ppm) due to the benzene ring hydrogens and 4.76 assigned to C_1H of the glucose ring. The relative areas of these peaks were 2:3, demonstrating monosubstitution on α-CD. Iodination of tosyl α-CD with NaI was carried out in water at 80° for 1 hr, then in situ free histamine was added and the mixture was kept at 80° for 48 hr. The product was purified by chromatography using Amberlite IR-120 and Sephadex G-15 columns, and gave IV in overall yield ca. 10%. The presence of an imidazole ring in IV was ascertained by the Pauly test. When histamine was replaced with ammonium hydroxide in the above treatment, the reaction was carried out in an autoclave (130° for 48 hr), followed by chromatography on Amberlite and Sephadex G-15, which then gave amino-α-CD (III). The intermediate iodo-α-CD (II) was also purified on Amberlite and Sephadex and was identified.

The products (I–IV) were found to be pure and free from cross-contamination as judged by paper chromatography