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Quantum Chemical Studies of the Metabolism of a Series of Chlorinated Ethane Anesthetics

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SUMMARY

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Using a working molecular orbital program based on an iterative extended Hückel method, we have calculated the conformational and electronic properties of a series of nine chloroethane anesthetics. The object of this study was to determine the molecular factors which correlated with the observed specificity of the extent of dechlorination *in vitro* of these compounds, all believed to be substrates of the same liver microsomal enzyme system. The results of our energy conformation studies reveal that all these compounds have a common minimum energy conformer, characterized by adjacent chlorine atoms as far apart as possible. Chemically, our calculations point to a unique and striking correlation between the extent of dechlorination and the extent of electron deficiency in the most electron-deficient carbon valence atomic orbital in each compound. No such correlation was found with other calculated molecular properties such as C—Cl or C—H bond strengths, or the net charge on the carbon, hydrogen, or chlorine atoms in this series of compounds. Our calculation also show that the electron-deficient carbon atom orbital is used in bonding to a chlorine atom in each compound. Our results are most consistent with a mechanism of dechlorination in which the rate-determining step involves an anionic attack on the electron-deficient carbon atomic orbital. Other calculated molecular characteristics reinforce this conclusion, and allow the further inference that such an anionic attack is accompanied by a chlorine displacement, perhaps aided by a proton on a nearby acidic residue in the metabolizing enzyme.

INTRODUCTION

Until recently, almost all volatile anesthetics were considered to be inert substances eliminated from the body without alteration. This concept is no longer acceptable, and evidence is now accumulating to indicate that the most commonly used anesthetics do metabolize appreciably. Thus biodegradation of the volatile anesthetics has become an area of concern to both the anesthesiologist and the clinical pharmacologist. One of the most significant questions to be answered is the possible toxic effects produced by anesthetic metabolites on vital organs such as the liver and kidney. To help answer this question the metabolic pathways of a number of widely used anesthetics, such as halothane (1, 2) and methoxyflurane (3, 4), are being extensively

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studied. Progress in this field has recently been reviewed (5–7).

In addition to experimental studies, theoretical calculations which could link molecular properties to observed metabolic behavior would be useful in the design of future safe anesthetics. With this goal in mind we have attempted to correlate properties calculated by molecular orbital theory with the observed extent of dechlorination (8) of the entire series of chlorinated ethanes by an enzyme system located in rat hepatic microsomes. The main aim of these studies was to determine systematically the effect of the number and distribution of the chlorine atoms on the extent of metabolism, and also to gain some insight into possible metabolic pathways involving dehalogenation.

In the experimental work reported (8), the extent of enzymatic dechlorination was found to be highly substitution-dependent within this series of compounds. These results are summarized in Table 1. The extent of metabolism given in Table 1 was determined after a fixed period by assay *in vitro* of the percentage of ^{36}Cl enzymatically removed, compared to control systems without the NADPH needed for enzyme action. The enzyme system used in these studies was known to cleave carbon-halogen bonds (9, 10) in the presence of NADPH and oxygen, and was thought to be similar in function to a mixed-function oxidase system. The first two rows of Table 1, with one subunit constant as CH_3— or CH_2Cl—, clearly indicate that the rate of dechlorination within a methy subunit follows the order—$CHCl_2$ > —CCl_3 > —CH_2Cl. From column 3 we also note that the rate of dechlorination of the $CHCl_2$ group decreases as the number of chlorine atoms on the adjacent carbon increases.

TABLE 1

Enzymatic dechlorination of chloroethanes

The percentage of ^{36}Cl enzymatically removed was determined by assay *in vitro* (8).

Substituent	1. —CH_3	2. —CH_2Cl	3. —$CHCl_2$	4. —CCl_3
	%	%	%	%
CH_3—		<0.5	13.5	<0.5
CH_2Cl—	<0.5	<0.5	9.8	0.8
$CHCl_2$—	13.5	9.8	6.0	1.7
CCl_3—	<0.5	0.8	1.7	3.9[a]

[a] High control value.

Based partly on the results of this study and other existing studies, some possible metabolic pathways involving dehalogenation of volatile anesthetics are tentatively proposed. One scheme involves the initial radical or anionic displacement of a hydrogen atom by a hydroxyl group. A second involves the insertion of an active oxygen atom, possibly bound to an enzyme such as a peroxidase, between the carbon and hydrogen atoms of a C—H bond. In both these mechanisms the initial reaction would lead to formation of an active HO—C—Cl group, which would rapidly lose HCl to form an aldehyde. A third scheme involves the homolytic (radical) cleavage of either a C—H or C—Cl bond, with the aid of a radical form of the enzyme created by its interaction with both NADP and a peroxide radical. The radical substrate fragment thus formed is then depicted as adding another peroxide radical to form a hydroperoxide, which would rapidly decompose. A fourth mechanism involves the replacement of chlorine atom by a hydroxyl group. All four mechanisms could lead to oxidative products such as alcohols, aldehydes, and acids, which are suspected metabolites. All schemes admittedly would require a great deal more experimental verification, such as isolation of metabolites and further kinetic studies.

In this paper we report quantum chemical studies of the series of chlorinated ethanes listed in Table 1. The aim of this study was to determine both conformational and electronic properties which would correlate with and explain the observed specificity of this series of compounds as substrates of the dechlorinating enzyme system used in the experimental study *in vitro*. Using a working molecular orbital program called iterative extended Hückel theory, we have calculated the energy of each molecule as a function of rotation about the C—C bond. For each conformer we have also obtained the distribution of electrons in the molecule, from which we have calculated bond densities, net atomic

charges, number of electrons in each valence atomic orbital, nature of the bonding, and number and nature of nonbonding electrons. These calculations have led to identification of some of the molecular factors responsible for the observed specificity, and have pointed to a plausible mechanism involving dechlorination consistent with the observed behavior. Our results indicate which of the four proposed mechanisms seems most reasonable on a molecular level.

METHOD OF CALCULATION

To obtain a fundamental description of a molecule in terms of its energy and electron distribution, we solve in some approximation the molecular Schroedinger equation,

$$\mathcal{H}\psi_i = E_i\psi_i$$

In this equation $\mathcal{H}$ is the Hamiltonian operator and represents the kinetic and potential energy of interaction of all the nuclei and electrons in the molecule. E_i is the minimum electronic energy, corresponding to the optimum spatial distribution of all electrons given by the wave function for a specified molecular geometry. The semiempirical method used to solve this equation is embodied in a working computer program based on the iterative extended Hückel theory method. This method, in a form similar to the one we use, has been described elsewhere (11, 12) and we shall not give details here. It is characterized by the use of a semiempirical, independent electron, molecular Hamiltonian which allows evaluation of 1-electron energy matrix elements from input values of atomic ionization potentials. All valence atomic orbitals and electrons are allowed to interact in the chosen nuclear geometry. A single Slater type orbital function (2*s*, 2*p*, etc.) is used to describe each orbital, and all overlaps between atomic orbitals are calculated. In addition, we use a charge iteration scheme developed by Zerner and Gouterman (11) to solve the Schroedinger equation successively until input and output values of net atomic charges agree to a stipulated consistency. The self-consistent charge aspect of the model permits inclusion of some electron interactions in this essentially independent electron model. It leads to a better description of the electron distributions in the molecule than results from the simpler, noniterative extended Hückel theory method. This is evidenced, for example, by the calculation of much more reasonable dipole moments. The iterative extended Hückel theory method has now been used extensively by ourselves (13–18) and others (19–21) to study a variety of molecules of biological interest. We have also analyzed its strengths and weaknesses with respect to the most accurate calculations *ab initio* (13). Taking into account its limitations, the iterative extended Hückel theory method has been a useful tool in understanding a large number of physical and chemical properties of molecules in a consistent framework.

The iterative extended Hückel theory method cannot be used to calculate accurate total molecular energies. However, molecular energies thus calculated can be used with some confidence to determine the relative stability and minimum energy conformer of a series of different conformations of the same molecule (13, 22, 23). This is the use we have made of the calculated energies in the present study.

From these calculations we have also obtained the spatial distribution of all the valence electrons in the molecule, expressed as a set of molecular orbitals which are linear combinations of the original valence atomic orbitals that have interacted in molecule formation. Each molecular orbital can describe the spatial distribution of 2 electrons with opposite spins, which can be occupied by 1 unpaired or 2 spin-paired electrons. The specification of the occupancy of each orbital describes a total electron configuration of the molecule.

From the calculated electron distribution, utilizing a Mulliken population analysis (24) of filled molecular orbitals, the net charge on each atom, the electron density in each valence atomic orbital, and the electron density between adjacent bonding atoms in the molecule were calculated. Finally, a description of the bonding and of the nature and number of nonbonding electrons was obtained from a detailed analysis of the nature of each filled molecular orbital.

RESULTS

Conformational Characteristics of Series of Chlorinated Ethanes

Using the iterative extended Hückel theory program, we have calculated the energy of each molecule as a function of rotation about the C—C bond. The results of these calculations yield energy conformation behavior of the isolated molecules. This behavior could be related to their conformation as substrates at the site of action of a given enzyme in several possible ways. It is possible that the same minimum energy conformer obtains in the biophase as in the isolated molecule. It is also possible that another conformer, which is an excited one in the isolated molecule, becomes the preferred one at the site of action. Third, it is possible that the molecule acts as a substrate in an excited but energetically accessible conformation, i.e., requiring $\leqq 6$ kcal/mole. Given these alternatives, it is still of considerable interest to delineate the energy conformation behavior of the isolated molecule, just as it is to determine its conformation in a crystal by X-ray analysis and in solution by various techniques. The combination of findings of these kinds can only shed light on the conformational properties of the molecule as a substrate. From the theoretical calculations one obtains very important information about the flexibility of the molecule, i.e., a mapping of the energy conformation behavior which permits identification of the minimum energy and low-lying conformer that would be biologically accessible, on the one hand, and could obtain in solution, on the other.

In this particular investigation the aim of our conformational studies was simply to determine whether any obvious differences exist in the energy conformation behavior within the series of compounds which could immediately account for the observed differences in extent of dechlorination by the enzyme system used in the experimental study. We find that for each molecule a staggered conformation, with the chlorine atoms as far apart as possible, is the lowest energy conformer.

For each of the six molecules which have a symmetrical CH_3 or CCl_3 group, there is only one staggered form and one eclipsed form; that is, rotation by 60 degrees about the C—C bond defines all the unique conformations. Experimentally, it has been inferred that the three molecules with a CCl_3 group have a staggered ground state conformation (25), in agreement with our results. For the three molecules with a CH_3 group, it also appears from dipole moment measurements in the gas phase (26) that the staggered form has the lower energy, in agreement with our calculations. For the remaining three molecules in the series (which can have two staggered forms, called *trans* and *gauche*), we calculate that the *trans* form, with maximum separation of adjacent chlorine atoms, is the more stable of the two, and that the eclipsed forms have a still higher energy. Again, from dipole moment measurements in the gas phase (25, 27), the *trans* form has been established as the lowest energy form for each molecule.

Based on these comparisons with experiment, we conclude that our calculations have correctly selected the *trans* and staggered forms, respectively, as the correct minimum energy conformers for these two types of compounds.

Recently, using a completely empirical method of calculating the charge distribution and energy of a group of atoms as a function of their geometry, the dipole moments of all the chloroethanes have been calculated (28). For the three compounds which can have a *trans* and a *gauche* form, the energy barrier to internal rotation was also determined (28). The results are in qualitative agreement with ours and further substantiate the conclusions we draw based on conformation. In this instance it is gratifying to obtain agreement with a more empirical method.

Since all the molecules have similar ground state conformations and similar energy-ordered conformers, with all conformers within the biologically accessible energy range, it is clear from conformational considerations alone that all these molecules could be substrates of the same enzyme system. It then would appear that it is their electronic rather than conformational properties which are primarily responsible for the

observed specificity to the dechlorinating enzyme system used in the experimental study. The chemical properties presented and discussed in subsequent sections are those of the minimum energy conformer. However, these properties were determined for all conformers and found to be quite insensitive to rotations about the C—C bond. Thus the entire subsequent discussion is applicable regardless of the molecular conformer which is the actual substrate at the site of action.

Bond Densities

The C—C, C—Cl, and C—H bond densities, calculated from filled molecular orbitals for all 10 molecules, are given in Table 2A–C. Table 2A shows that C—C bond strength increases as the number of chlorine atoms on either carbon increases. From each column of Table 2B we see that the C—Cl bond strength increases in the order CH_2Cl < $CHCl_2$ < CCl_3. Additionally, from each row it may be noted that the C—Cl bond strength is relatively insensitive to the nature of the adjacent carbon group. From Table 2C, the C—H bond density is in the order $CHCl_2$ > CH_2Cl > CH_3, but is more affected by the nature of the neighboring carbon atom group.

TABLE 2
Calculated bond densities of chloroethanes

A. C—C bonds

Group	—CH_3	—CH_2Cl	—$CHCl_2$	—CCl_3
CH_3—	0.330	0.355	0.377	0.397
CH_2Cl—	0.355	0.373	0.395	0.402
$CHCl_2$—	0.377	0.395	0.408	0.421
CCl_3—	0.388	0.402	0.421	0.430

B. C—Cl bonds

Group	CH_3	CH_2Cl	$CHCl_2$	CCl_3
CH_2Cl	0.292	0.294	0.294	0.295
$CHCl_2$	0.314	0.316	0.319	0.313
CCl_3	0.335	0.335	0.332	0.331

C. C—H bonds

Group	—CH_3	—CH_2Cl	—$CHCl_2$	—CCl_3
CH_3—	0.420	0.416	0.411	0.407
CH_2Cl—	0.424	0.420	0.414	0.412
$CHCl_2$—	0.427	0.422	0.416	0.412

TABLE 3
Net atomic charges in chloroethanes

A. H^+ charge

Group	—CH_3	—CH_2Cl	—$CHCl_2$	—CCl_3
CH_3	0.04	0.05	0.06	0.07
CH_2Cl	0.08	0.09	0.10	0.11
$CHCl_2$	0.11	0.12	0.14	0.14

B. Cl^- charge

Group	—CH_3	—CH_2Cl	—$CHCl_2$	—CCl_3
CH_2Cl^-	0.20	0.18	0.17	0.17
$CHCl_2$	0.15	0.13	0.13	0.12
CCl_3	0.11	0.10	0.09	0.09

C. C charge

Group	—CH_3	—CH_2Cl	—$CHCl_2$	—CCl_3
CH_3—	−0.11	−0.10	−0.08	−0.09
CH_2Cl—	−0.01	0.01	0.02	0.03
$CHCl_2$	0.09	0.11	0.12	0.14
$CHCl_3$	0.18	0.20	0.20	0.23

Net Atomic Charges

We have calculated the net charges on the carbon, hydrogen, and chlorine atoms in the 10 molecules studied. The results are given in Table 3. As might be expected, the charge on the hydrogen atom, given in Table 3A, becomes more positive as the number of chlorine atoms on its carbon atom, or on the adjacent carbon atom, increases. As seen from each column of Table 3B, the net negative charge on the chlorine atom decreases with the number of chlorine atoms on the same carbon. Finally, in Table 3C we see that the carbon atom becomes more positive as the number of chlorine atoms on it or its neighbor increases. The most positive carbon is in CCl_3CCl_3, with a net charge of +0.25, while in ethane the carbon atoms are somewhat negative, with a charge of −0.12 electron unit, indicating the reverse polarities of the C←H vs. C→Cl bonds.

The small fractional charges on all the atoms listed in Table 3 are typical of the

TABLE 4

Electron distribution in carbon valence atomic orbitals

Values indicate number of electrons in orbital.

Bond	C^α				C^β			
	$2s$	$2p_x$	$2p_y$	$2p_z$	$2s$	$2p_x$	$2p_y$	$2p_z$
$C^\alpha H_3$—$C^\beta H_3$	1.04	0.96	1.05	1.05	1.05	0.76	1.05	1.05
CH_3—CH_2Cl	1.04	1.06	1.06	0.94	1.10	0.88	1.03	0.99
CH_3—$CHCl_2$	1.03	1.07	1.07	0.92	1.14	0.76	0.99	1.01
CH_3—CCl_3	1.00	0.93	1.08	1.08	1.15	1.03	0.82	0.82
CH_2Cl—CH_2Cl	1.09	0.97	0.89	1.04	1.09	0.97	0.89	1.04
CH_2Cl—$CHCl_2$	1.07	0.96	0.90	1.05	1.12	1.00	0.77	1.00
CH_2Cl—CCl_3	1.06	0.94	0.91	1.06	1.14	1.00	0.83	0.82
$CHCl_2$—$CHCl_2$	1.11	0.98	0.78	1.00	1.11	0.98	0.78	1.01
$CHCl_2$—C_3	1.10	0.97	0.79	1.01	1.12	0.99	0.83	0.83
CCl_3—CCl_3	1.11	0.98	0.84	0.84	1.11	0.98	0.84	0.84

extent of charge build-up on atoms in molecules, which rarely exceeds 0.25 electron unit. Thus there are in fact no integral charges associated with these atoms, as is usually depicted in chemical pictures of functional groups in molecules.

Nature of Bonding and Electron Distribution in Valence Atomic Orbitals

Bonding of carbon atoms. The net electron density in each of the carbon valence atomic orbitals, obtained from a Mulliken population analysis of the molecular orbitals, is given for all molecules in Table 4. Because of molecule formation there are nonintegral values of electrons in each atomic orbital. The most striking feature in this table is that for each molecule there is one carbon orbital with substantial electron deficiency. Furthermore, an inspection of the filled molecular orbitals of each molecule reveals that in every molecule this electron-deficient carbon orbital is involved exclusively in bonding to a chlorine atom.

We have further characterized the way in which the carbon valence electrons and orbitals are used in bonding, as illustrated in Table 5 for carbon atom bonding in $CHCl_2$—$CHCl_2$. The carbon bond to hydrogen (obtained from summing contributions found mainly in molecular orbitals 5, 6, and 7) has 80% p character and 0.93 carbon valence electron. The C—C bond (from molecular orbitals 1–5 and 7) has 66% p character and 1.24 carbon valence electrons. The C—Cl bond (from contributions in molecular orbitals 1–5 and 8–11) has 60% p character and 1.07 valence electrons from the carbon atom. The carbon atom does not then form three equivalent sp^3 bonds, each with 1 electron, with its three neighbors. Instead the percentage of p character and the number of electrons it uses depend on the atom to which it bonds. While the actual hybridization and number of carbon electrons in each bond vary from molecule to molecule, the effect of hybridization on the "electronegativity" of the carbon orbitals illustrated for 1,1,2,2-tetrachloroethane is typical. The percentage of p character in the bond is in the order C—H > C—C >

TABLE 5

Carbon bonding in $CHCl_2$—$CHCl_2$

Bond	Molecular orbitals[a]	Hybridization[b]	Total[b]	p[c]
				%
C—H	5, 6, 7	0.20s–0.73p	0.93	80
C—C	1–5, 7	0.45s–0.79p	1.24	66
C—Cl	1–5, 8–11	0.45s–0.62p	1.07	60

[a] Molecular orbitals which make contribution to the specified bond.

[b] Number of s and p electrons in each bond.

[c] p character of carbon bond orbital.

C—Cl, and the number of carbon electrons in the bond in the order C—C > C—Cl > C>H.

Bonding of chlorine atoms. It is of particular interest in the interpretation of our results to describe the nature of the chlorine atom to which the electron-deficient carbon atom orbital is bound. As we have just indicated, the carbon is bound to the chlorine atom with an *sp* orbital of approximately 60% *p* character. This carbon orbital is shown in Fig. 1 as an (sp_r) orbital along the C—Cl bond direction (r).

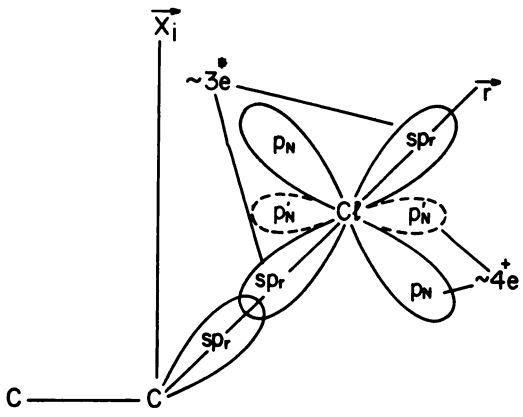

FIG. 1. *Prototype carbon-chlorine bond in chlorinated ethanes involving an electron-deficient carbon atom p orbital*

The four valence atomic orbitals of the chlorine atom participate in both bonding and nonbonding molecular orbitals. Thus the electron density in each chlorine orbital can be divided into a bonding and a nonbonding fraction. These electron densities are given in Table 6 for each molecule in the series studied. For all molecules, most of the chlorine 3*s* atomic orbital is involved in bonding. Also given in Table 6 is the projection of the C—Cl bond direction (r) on the x, y, and z coordinate axes. For all molecules the *p* orbital that has the largest projection along the internuclear line also contains the most bonding electrons. Conversely, the two *p* orbitals with only small projections along the internuclear line have mainly nonbonding character. The number of *s*- and *p*-bonding electrons, as well as the character of the main *p*-orbital contributor, is given in the column labeled "Bonding." In the last column is given the total number of nonbonding electrons found mainly in two *p* orbitals perpendicular to the bond direction.

The description of the chlorine atom bonding which emerges from Table 6 is shown schematically in Fig. 1. Two hybrid *sp* orbitals directed along the internuclear line (r), i.e., two σ (sp_r) orbitals, are shown, each somewhat involved in bonding. The number of electrons in these two orbitals, given

TABLE 6

Bonding and nonbonding chlorine valence electrons in chloroethanes

Molecule	3*s*		3p_x			3p_y			3p_z			Total bonding electrons	Total nonbonding electrons
	B[a]	NB	B	NB	$r \cdot x$[b]	B	NB	$r \cdot y$[b]	B	NB	$r \cdot z$[b]		
CH_3CHCl_2	1.13	0.70	0.98	0.57	1.45	0.42	1.42	0.84	0.26	1.66	0.59	sp_x[c] 1.13–1.86	yz[d] 4.25
$CH_2Cl—CHCl_2$	1.33	0.49	0.27	1.65	0.58	0.89	0.66	1.45	0.42	1.42	0.84	sp_y 1.53–1.58	xz 4.22
$CHCl_2—CHCl_2$	1.36	0.47	0.21	1.66	0.58	0.88	0.65	1.45	0.44	1.40	0.84	sp_y 1.36–1.47	xz 4.18
$CCl_3—CHCl_2$	1.64	0.18	0.10	1.63	0.58	0.89	0.65	1.45	0.40	1.44	0.84	sp_y 1.64–1.39	xz 4.00
$CH_2Cl—CCl_3$	1.58	0.24	0.35	1.57	0.58	0.30	1.67	0	1.11	0.27	1.7	sp_z 1.58–1.76	xy 3.75
$CH_3—CCl_3$	1.82	0	0.97	0.56	1.45	0.53	1.29	0.84	0.29	1.63	0.60	sp_x 1.82–1.79	yz 3.48
$CH_2Cl—CH_2Cl$	1.22	0.62	0.40	1.53	0.58	0.97	0.59	1.45	0.50	1.35	0.84	sp_y 1.22–1.87	xz 4.09
$CH_3—CH_2Cl$	1.85	0	1.03	0.54	1.45	0.48	1.37	0.84	0.32	1.61	0.58	sp_x 1.85–1.83	yz 3.52
$CCl_3—CCl_3$	1.22	0.60	0.15	1.76	0.57	1.00	0.53	1.45	0.41	1.41	0.84	sp_y 1.22–1.56	zx 4.30

[a] B = number of bonding electrons; NB = number of nonbonding electrons.

[b] Projection of the interatomic C—Cl distance of r = 17.7 nm on each coordinate axis.

[c] Main atomic orbital components of bonding electrons.

[d] Main atomic orbital components of nonbonding electrons; $yz = p_y p_z$, etc.

in Table 6 under the column "Bonding," varies from 2.91 to 3.68. Also depicted are two essentially nonbonding orbitals, p_n and p_n', mutually perpendicular to the internuclear line, i.e., π- and π'-orbitals with a small amount of s character. The number of nonbonding electrons in these orbitals, given in the last column of Table 6, varies from 4.30 to 3.48 in the series of molecules studied.

SIGNIFICANCE OF RESULTS TO METABOLIC BEHAVIOR

Having obtained a detailed description of the halogenated ethanes from our molecular orbital calculations, we now attempt to establish the link between their calculated molecular properties and their metabolic behavior.

In Table 7 the compounds are listed in decreasing order of extent of enzymatic dechlorination (given in column 1), except for the dubious value for hexachloroethane obtained because of high control values (8). Also given in Table 7 is a summary of the calculated molecular factors which might be relevant to their enzymatic dechlorination. Bond strengths, listed in columns 2–4, are taken from Table 2; atomic charges (columns 5–7), from Table 3; electron densities (f_+) of the most electron-deficient carbon atom orbital (column 8), from Table 4; and the number of nonbonding electrons on the chlorine atom bound to the electron-deficient carbon orbital, (column 9), from Table 6.

The most obvious correlation that one might expect is between the strength of the C—Cl bond in a given group and its extent of dechlorination. No such correlation is obtained. As we have shown in Table 3 and again in column 2 of Table 7, the calculated C—Cl bond strength is in the order CCl_3 > $CHCl_2$ > CH_2Cl while the extent of dechlorination is in the order $CHCl_2$ > CCl_3 > CH_2Cl. Thus the group which has the weakest C—Cl bond dechlorinates least and the one with intermediate bond strength dechlorinates most. Clearly, then, the C—Cl bond strength is not a principal determining factor in the extent of dechlorination. From this result it can be inferred that the enzymatic breaking of the C—Cl bond alone, either homolytically (radical) or heterolytically (ionic), is not the initial rate-determining step in dechlorination. This conclusion is reinforced by the lack of correlation with the net charge on the carbon and chlorine atoms. As seen from column 5 of Table 7, the negative charge on the chlorine atom increases in the order CCl_3 < $CHCl_2$ < CH_2Cl. This is also the order of decreasing positive charge on the carbon atom and of C—Cl bond strength.

Turning now to the possibility that a

TABLE 7

Correlation of calculated molecular properties with percentage of dechlorination

Molecule	1. Dechlorination	Bond strength			Electron density				9. No. of nonbonding Cl electrons
		2. C—Cl	3. C—C	4. C—H	5. Cl	6. H	7. C	8. f_+[a]	
	%								
CH_3—$CHCl_2$	13.5	0.314	0.377	0.427	−0.15	+0.11	+0.09	0.76	4.25
CH_2Cl—$CHCl_2$	9.8	0.316	0.395	0.422	−0.14	+0.12	+0.11	0.77	4.22
$CHCl_2$—$CHCl_2$	6.0	0.315	0.408	0.416	−0.13	+0.13	+0.12	0.78	4.18
CCl_3—$CHCl_2$	1.7	0.313	0.420	0.412	−0.12	+0.14	+0.13	0.79	4.00
CH_2Cl—CCl_3	0.8	0.335	0.402		−0.10		+0.20	0.82	3.75
CH_3—CCl_3	<0.5	0.333	0.388		−0.11		+0.18	0.82	3.48
CH_3—CH_2Cl	<0.5	0.292	0.355	0.424	−0.20	+0.08	0.00	0.88	4.09
CH_2Cl—CH_2Cl	<0.5	0.294	0.373	0.420	−0.18	+0.09	+0.01	0.90	3.52
(CCl_3—CCl_3)[b]	(3.9)	0.331	0.431		−0.08		+0.24	0.84	4.30

[a] Fraction of electron in most electron-deficient carbon p orbital.

[b] Percentage of dechlorination value may be in error.

C—H bond cleavage is the initial step in dechlorination, one would expect that the weaker the C—H bond, the more the dechlorination. Yet the molecule with the strongest C—H bond, CH_3CHCl_2, dechlorinates most. Moreover, as seen in column 4 4 (Table 7), in the series of —$CHCL_2$-containing molecules, the weaker the C—H bond, the less the dechlorination. Finally, comparing the six compounds with either a $CHCl_2$ or CH_2Cl group in Table 7, there is no correlation between relative C—H bond strength and extent of dechlorination. The same lack of correlation is seen (from columns 6 and 7) with the variation in net charge on either the hydrogen or carbon atom in these six molecules. Hence radical or anionic cleavage of the C—H bond does not seem to be the rate-determining initial step in enzymatic dechlorination.

For the reaction in which an oxygen is inserted between the carbon and hydrogen atoms, one could also suppose that the C—H bond strength would be an important factor in the rate of this reaction. As we have just discussed, we obtain no such correlation.

In the postulated mechanism involving homolytic bond cleavages, it has been suggested that C—H bond cleavage occurs in the $CHCl_2$ group while C—Cl bond cleavage occurs in the CCl_3 group. If such homolytic bond cleavages were involved, since, as seen in Table 7, the C—H bond strength in the $CHCl_2$ group molecules is uniformly greater than the C—Cl bond strength in the CCl_3 group, our results would predict an order of reactivity $CCl_3 > CHCl_2$, which is the opposite of that observed.

In contrast to the lack of correlation of bond strengths and net atomic charges with extent and specificity of dechlorination, there is a striking correlation of the observed behavior with the degree of electron deficiency in the most electron-deficient carbon valence orbital. From Table 5 it is seen that (a) for all four molecules with a —$CHCl_2$ group, the carbon atom of that group has the most electron-deficient orbital; (b) for the remaining three molecules with a CCl_3 group, that group always has the most electron-deficient orbital; and (c) the CH_2Cl group has the carbon atom with the least electron-deficient orbital, except for the carbon in a methyl group.

Thus the electron deficiency of the carbon atom valence orbital decreases in the order —$CHCl_2$ > —CCl_3 > —CH_2Cl > —CH_3, and this order is preserved independent of the nature of the adjacent group. This is exactly the same order as the decrease in extent of dechlorination of a specific 1-carbon unit, as shown in Table 1. Neither variation is monotonic with the number of chlorine atoms, and both vary in the same way. Hence the correlation is all the more striking.

Not only do we obtain an explanation for group sensitivity to dechlorination on this basis, but, as seen in Table 7, we find that the degree of dechlorination generally decreases with the degree of electron deficiency of the most electron-deficient carbon orbital of each molecule. Despite the similarity of the extent of electron deficiency for two components, 1,1,1,2-tetra- and 1,1,1-trichloroethane, the trend is definite and indicates rather large sensitivity of the percentage of dechlorination to the degree of electron deficiency. The hexachloroethane is the only true exception to this trend. Remarkably, it is the one for which the experimental results are dubious. It is heartening indeed that the theoretical calculation reinforces the possibility of an experimental error. Thus the correlation appears to be real.

The correlation of the extent of dechlorination with the degree of electron deficiency of the most electron-deficient carbon orbital in the molecule strongly indicates that the initial rate-determining step is a nucleophilic attack at that carbon atom orbital. Further support for this hypothesis is obtained from the fact that, for each molecule, the lowest-lying empty molecular orbital has an appreciable contribution from the electron-deficient atomic orbital. This lowest-lying empty orbital would be the natural initial receptor of additional electrons and, by so doing, would alleviate the most electron-deficient orbital.

Other calculated molecular properties indicate that the initial nucleophilic attack is part of an SN_2 displacement reaction in-

volving cleavages of the C—Cl bond rather than either C—H cleavage or insertion. One strong indication of the chlorine displacement is that the electron-deficient p orbital, which we have postulated as the site of nucleophilic attack, is involved exclusively in bonding to a chlorine atom in each molecule. Thus, as an anion such as OH^- approaches and begins to mix with the electron-deficient carbon orbital, weakening of the C—Cl bond would automatically result. Moreover, from the last column of Table 7, there appears to be good correlation between the number of nonbonding electrons of the particular chlorine atom bonding to the electron-deficient carbon orbital and the extent of dechlorination. This correlation is particularly good for the four molecules which dechlorinate appreciably by the same reaction pathway. The smaller number of nonbonding electrons might help to explain the decreasing extent of dechlorination of this series. It is possible that the breaking of the C—Cl bond is helped by a well-placed proton on an amino acid residue in the enzyme. Such a proton could attract the chlorine atom away by interacting with its nonbonding electrons. This mechanism is consistent with the observed pH dependence (8) of the extent of dechlorination of 1,1,2-trichloroethane, which peaks at a pH of 8.4. This indicates that OH^- ion is needed to initiate the reaction but that in a more basic environment the leaving of the Cl^- ion would be hindered.

In order to test this mechanism further we have begun studies depicting a nucleophilic attack on the carbon atom by an OH^- ion and the leaving of a Cl^- ion, using the iterative extended Hückel theory program to characterize the reacting system along chosen points in the reaction pathway. Preliminary results thus far indicate that the initial bonding overlap interaction of the approaching OH^- ion is indeed with the electron-deficient carbon orbital. Furthermore, the C—Cl bond strength is rapidly and preferentially weakened. Thus our theoretical investigations consistently point to a chlorine displacement reaction initiated by an anionic attack as a key step in dechlorination of this series of chlorinated ethanes.

Upon completion of this manuscript, a recent study *in vivo* of the metabolism of 1,1,1,2-tetrachloroethane in the mouse was brought to our attention (29). In this careful study the conclusion tentatively reached was that the "initial metabolic reaction is a hydrolytic fission of a carbon-chlorine bond with the formation of trichlorethanol." a precursor of trichloracetic acid. These results and conclusions are an excellent experimental corroboration of the dechlorinating mechanism which our calculated results most clearly implicate.

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