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INTERNATIONAL JOURNAL OF QUANTUM CHEMISTRY, VOL. XXV, 493–502 (1984)

Some Reactive Properties of Chlorooxirane, a Likely Carcinogenic Metabolite of Vinyl Chloride

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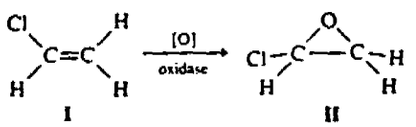
Abstract

We have carried out a computational study of the reactive properties of chlorooxirane, the metabolically produced epoxide of vinyl chloride that is believed to be a direct-acting carcinogenic form of this molecule. An *ab initio* SCF-MO procedure (GAUSSIAN 70) was used to compute the energy requirements for stretching the C—Cl and both C—O bonds (S_N1 reactivity) and to determine the course of the epoxide's possible S_N2 reactions with ammonia, taken as a model for nucleophilic sites on DNA. The epoxide was assumed to be protonated; both the oxygen- and chloro-protonated forms were considered. At each step along the various reaction pathways, the structure of the system was reoptimized. For the oxygen-protonated epoxide, the C_1—O bond has a significantly lower energy barrier to stretching than does the C_2—O. (The carbon bearing the chlorine is designated C_1.) However, both are very much higher than that of the C—Cl bond in the chloro-protonated form, confirming our earlier finding of the relative weakness of this bond. In the S_N2 processes involving ammonia, intermediate complexes are formed with both carbons of the oxygen-protonated epoxide, the C_2-complex being the more stable. However, the most stable ammonia complex occurs at C_1 of the chloro-protonated epoxide. Our calculated results, both the energies and also the geometry changes, allow us to propose two possible mechanisms for the formation of the 7-N-(2-oxoethyl) derivative of guanine that has been observed to be the major *in vivo* DNA alkylation product of vinyl chloride and has been suggested as possibly being responsible for its carcinogenicity. One of these mechanisms is S_N1 and starts with the chloro-protonated epoxide; the other is S_N2 and involves the oxygen-protonated form.

1. Introduction

Numerous studies on animals, cell cultures, and bacteria have shown vinyl chloride (**I**) to be both mutagenic and carcinogenic in these systems [1–5]. In addition, an increased incidence of certain types of cancer has been observed among industrial workers who are exposed to vinyl chloride [6, 7].

Experimental evidence indicates that the first step in the mutagenic and carcinogenic action of vinyl chloride is its metabolic conversion to the epoxide, a process that is catalyzed by the mixed function oxidase system [2, 8-11]:



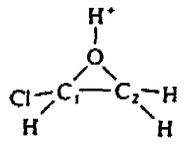
I **II**

This epoxide, chlorooxirane (**II**), is a strong mutagen [2, 10, 12], and is regarded as very likely to be a direct-acting carcinogen, an "ultimate" carcinogenic form of vinyl chloride [8, 10–13].

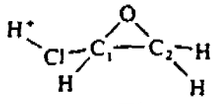
CCC 0020-7608/84/030493-10$04.00

Chlorooxirane is known to alkylate various nucleic acid bases, as well as DNA, RNA, and protein residues [2, 13–16], forming covalent bonds to nucleophilic sites on these systems, and it has also been found to alkylate DNA *in vivo* [17]. Such interactions could lead to miscoding [11, 16, 18], and might result in replicational and transcriptional errors [17a, 19], and the development of tumors.

We have earlier computed the structures and various properties for chlorooxirane and its oxygen- and chloro-protonated forms (structures **III** and **IV**) [20].* Protonation of the oxygen was found to weaken both C—O bonds,



III



IV

particularly the C_1—O.† (The chlorine-bearing carbon shall be designated C_1.) An even more sriking effect was the very marked weakening of the C—Cl bond that resulted from protonation of the chlorine. This suggests that rupture of this bond may conceivably play a significant role in the biological functioning of this molecule.

In order to understand better the alkylating properties of chlorooxirane, we have now made a detailed computational study of its behavior in both S_N1 and S_N2 reactions. To simulate the former processes, we simply stretched each of the key bonds in turn, and computed the energy requirements for doing so. For S_N2 reactions, the ammonia molecule was used as a model nucleophile. As shall be shown, one of the consequences of this investigation is that it permits us to suggest mechanisms for the formation of the primary *in vivo* DNA alkylation product that has been observed for vinyl chloride [17].

2. Methods

The oxygen-protonated epoxide was used for investigating the S_N1 reactivities of the C—O bonds. To simulate S_N1 ring opening, each bond separately was stretched in several incremental steps, the entire structure being reoptimized at each step. Essentially the same procedure was used to study the S_N1 reactivity of the C—Cl bond, but involving now the chloro-protonated form of the epoxide.

* The extent to which chlorooxirane is protonated *in vivo* is uncertain. However, there are known to exist subcellular regions with high proton activities [21]. It is of particular interest that the nuclear membrane contains certain redox enzymes [22], among which are the cytochrome P-450 systems that activate some carcinogens, including vinyl chloride [10, 11, 23]. These redox processes are believed to generate high local proton activities [21c, 24]. It seems significant that the eynzymatic processes involved in DNA replication are also localized in the nuclear membrane. In addition, there is evidence specifically for chlorooxirane which suggests that its biological activity involves a protonated form [25].

† Bond weakening was inferred from increases in bondlengths and decreases in force constants.

S_N2 processes were examined by allowing the ammonia to approach either C_1 or C_2; at each different C—N distance, all structural parameters were again reoptimized. This was done for both the oxygen- and chloro-protonated species.

The computational method used for this work was the GAUSSIAN 70 *ab initio* self-consistent-field molecular orbital procedure, at the STO-3G level [26]. This has been shown to be an effective technique for structure calculations [27].

3. Results and Discussion

A. S_N1 Processes

(a) C—O bonds: The energies of opening the epoxide ring by simply stretching either C—O bond from its equilibrium distance of 1.50 Å, without any approaching nucleophile, were investigated for the oxygen-protonated form of chlorooxirane. The relative energies of the reoptimized structures at each different C—O distance are given in Table I. Significantly more energy is required to stretch the C_2—O bond by the arbitrary amount of 0.7 Å than the C_1—O: 44 vs. 32 kcal/mol. This is consistent with our earlier findings concerning the relative strengths of these two bonds [20].

TABLE I. Calculated relative energies for bond-stretching processes.

Oxygen-Protonated Chlorooxirane:							
C_1-O Bond:							
Bond Length (A):	1.50	1.52	1.60	1.75	1.90	2.20	
Relative Energy: (kcal/mole)	0.0	0.1	2.9	13.0	23.0	32.4	
C_2-O Bond:							
Bond Length (A):	1.50	1.52	1.60	1.75	1.90	2.20	
Relative Energy: (kcal/mole)	0.0	0.3	3.6	15.7	28.6	44.0	
Chlorine-Protonated Chlorooxirane:							
C_1-Cl Bond:							
Bond Length (A):	2.07	2.23	2.50	2.75	3.00	3.25	3.50
Relative Energy: (kcal/mole)	0.0	0.3	1.5	2.1	2.7	3.5	4.0

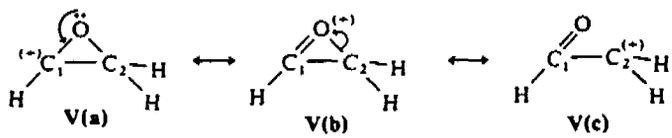
As one C—O distance is increased, the other carbon is observed to move toward a more tetrahedral configuration; for instance, its H—C—C angles change from approximately 120° to roughly 112°. The C—C and the other C—O bond lengths also change toward their normal single bond values. One notable feature is a significant shortening of the C_1—Cl bond, from 1.77 to 1.71 Å, that accompanies the C_1—O stretch.

An examination of the calculated atomic charges (population analysis [28]) in these systems shows a pattern very similar to what was observed in a previous

study of protonated ethylene oxide [29]. As one of the C—O bonds is stretched, there is a general movement of electronic charge from that carbon and its attached atoms (whether H or Cl) to the remainder of the molecule. Most of this apparent charge shift (the total amount of which is about 0.25 e.u.) goes to the oxygen and the proton. Thus the bond openings can be regarded as having a $C^{+\delta} \cdots O^{-\delta}$ character.

(b) C—Cl bond: Table I also lists the energies involved in stretching the C_1—Cl bond from its equilibrium value in the chloro-protonated epoxide, 2.07 Å, to 3.50 Å. Only 4 kcal/mol are required, much less than for either C—O bond. This is again in agreement with our earlier work [20], and demonstrates the weakness of the C—Cl bond in chlorooxirane.

A marked decrease in the C_1—O distance, from 1.37 to 1.28 Å, accompanies the stretching of the C_1—Cl bond, whereas the C_2—O bond lengthens from 1.46 to 1.50 Å. These changes can be explained by noting that the large increase in the C—Cl distance is nearly equivalent to removing HCl, which the calculated atomic charges show to come off as a neutral entity. The resulting positive charge on C_1 [structure **V(a)**] can be delocalized by contributions from resonance structures **V(b)** and **V(c)**, which should concomitantly shorten the C_1—O bond and lengthen the C_2—O:



The fact that the C_1—O bondlength is 1.28 Å, which is quite close to the standard $\geq C{=}O$ value of 1.21 Å [30], suggests that structures **V(b)** and **V(c)** make significant contributions.

B. S_N2 Processes

(a) Interactions of ammonia with oxygen-protonated chlorooxirane: In attacking either carbon atom, the ammonia molecule was given complete freedom regarding its line and angle of approach. These interactions led to the formation of two stable intermediate complexes, structures **VI** and **VII**.

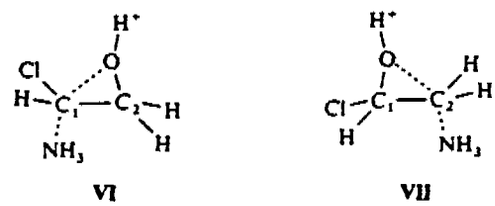


TABLE II. Calculated structures.[a]

Property	O-Protonated Chlorooxirane[b] (III)	Cl-Protonated Chlorooxirane[b] (IV)	Ammonia Complexes: O-Protonated at C_1 (VI)	Ammonia Complexes: Chlorooxirane at C_2 (VII)	Ammonia Complexes: Cl-Protonated Chlorooxirane (VIII)
Distance:					
C_1-O	1.50	1.36	2.34	1.40	1.42
C_2-O	1.50	1.46	1.42	2.32	1.44
C_1-C_2	1.50	1.48	1.56	1.55	1.49
C_1-Cl	1.77	2.07	1.80	1.84	3.52
C_1-H	1.10	1.10	1.10	1.10	1.09
C_2-H	1.10	1.10	1.10	1.09	1.09
O-H*	1.00	----	0.99	0.99	----
Cl-H*	----	1.34	----	----	1.32
C-N	----	----	1.55	1.55	1.54
Angle:[a]					
O-C_1-C_2	60	62	36	103	59
O-C_2-C_1	60	55	120	36	58
H-C_1-C_2	120	140	111	112	126
Cl-C_1-C_2	121	112	113	107	196
C_1-C_2-H_a[c]	120	118	108	111	120
C_1-C_2-H_b[c]	120	119	110	110	118
H-C_1-Cl	116	99	109	106	62
H_a-C_2-H_b	117	117	109	110	115
H*-O-C_1	114	---	124	106	---
H*-O-C_2	114	---	105	146	---
H*-Cl-C_1	---	105	---	---	164
N-C-C	---	---	112	111	118

[a] Distances are in Å; angles are in degrees.
[b] The structures given for the protonated chlorooxiranes are essentially the same as in ref. 20, with some small changes due to further optimization.
[c] H_a is *trans* to Cl.

Their optimized geometries are given in Table II. In both cases, the nitrogen is in the plane of the ring and forms a roughly tetrahedral N—C—C angle, although its initial approach was approximately perpendicular to the C—C bond.

The variation of the total energy during the course of each interaction is shown in Table III. In the formation of **VI**, a small energy barrier is observed during the early stages of the process, reaching its peak at a C_1—N distance of about 2.2 Å. This appears to reflect the presence of a hydrogen bond between the chlorine and the proton in the equilibrium form of the oxygen-protonated epoxide, structure **III**. This interpretation is supported by the fact that the Cl—H^+ distance in **III** is 2.74 Å, a reasonable value for this type of hydrogen bond [31].

TABLE III. Calculated interaction energies for reactions with ammonia.

Oxygen-Protonated Chlorooxirane:							
Reaction at C_1:							
C_1-N Distance (A):	3.00	2.50	2.20	2.00	1.80	1.55	
Interaction Energy: (kcal/mole)	−10.5	−10.3	−9.3	−26.6	−55.9	−72.7	
Reaction at C_2:							
C_2-N Distance (A):	3.00	2.50	2.20	2.00	1.80	1.55	
Interaction Energy: (kcal/mole)	−10.6	−12.0	−12.8	−24.5	−57.7	−86.9	
Chlorine-Protonated Chlorooxirane:							
Reaction at C_1 (approach along C_1-Cl line):							
C_1-N Distance (A):	3.50	3.00	2.50	2.20	2.00	1.80	1.54
Interaction Energy: (kcal/mole)	−9.8	−11.2	−14.9	−37.4	−54.6	−75.1	−95.9
Reaction at C_1 (approach along C_1-H line):							
C_1-N Distance (A):	3.50	3.00	2.50	2.20			
Interaction Energy: (kcal/mole)	−13.5	−23.8	−38.6	−4.8			

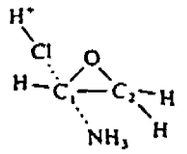
In addition, the calculated charge on the proton, which was varying quite gradually at large C—N separations, changes very markedly (from +0.33 to +0.25) as the C—N distance decreases from 2.2 to 2.0 Å.

Table III shows that the intermediate complex formed by S_N2 attack at C_2 is the more stable of the two possibilities, the computed interaction energies being −87 kcal/mol for **VII** and −73 kcal/mol for **VI**. This is in interesting contrast to the observation discussed above, that in the absence of a nucleophile it is the C_1—O bond that opens more readily.

The changes in geometry that accompany these two S_N2 processes are essentially similar (Table II). The C—O bond involving the carbon being attacked lengthens to approximately 2.3 Å, and there is inversion of the attached hydrogen atoms (or hydrogen and chlorine). The lengths of the C—O and C—C bonds involving the other carbon change in the direction of more typical single-bond values, and the orientation of the bonds around each carbon becomes more tetrahedral.

A notable feature of the reaction at C_2 is the concomitant lengthening of the C_1—Cl bond from 1.76 to 1.84 Å. Some possible implications of this will be discussed later.

(b) Interaction of ammonia with chloro-protonated chlorooxirane: In its interaction with the chloro-protonated epoxide, the favored position for the ammonia is essentially in line with the C_1—Cl bond (toward the carbon), and hence not in the plane of the ring (structure **VIII**). The optimized geometry of the intermediate complex that is formed is given in Table II. The major structural effects are the lengthening of the C_1—Cl bond, from 2.07 to 3.52 Å, and of the C_1—O bond, from 1.37 to 1.42 Å, plus an inversion of the hydrogen attached



VIII

to C_1. As seen from Table III, the product is more stable ($\Delta E = -96$ kcal/mol) than either of the complexes formed with the oxygen-protonated epoxide (**VI** and **VII**). On the other hand, we found no significant tendencies for complex formation between ammonia and C_2.

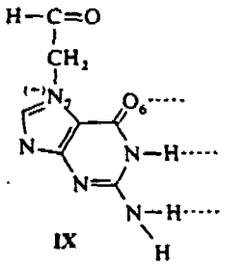
While examining various possible paths that the NH_3 could follow in attacking C_1, it was found that initially approach along the line of the C_1—H bond (toward the H) is favored over the C_1—Cl line. (See Table III.) This may reflect another instance of hydrogen bonding, this time involving (rather surprisingly) the ammonia nitrogen and the hydrogen on C_1. This route of attack is the more stable one until the C_1—N distance is approximately 2.5 Å; meanwhile the C_1—H bondlength increases from 1.1 to 1.35 Å, as the hydrogen interacts increasingly strongly with the ammonia. Eventually the approach along the C_1—Cl line does become the preferred one, although there is an energy barrier in going from the C_1—H line to the C_1—Cl, breaking the hydrogen bond in the process.

C. *Possible Mechanisms of Interaction with DNA*

The results that have been presented in this paper suggest several reactive pathways that chlorooxirane can follow. Two of these are of particular interest in the present context, because they represent possible routes to the formation of a DNA–chlorooxirane reaction product that may play a key role in the carcinogenic action of vinyl chloride.

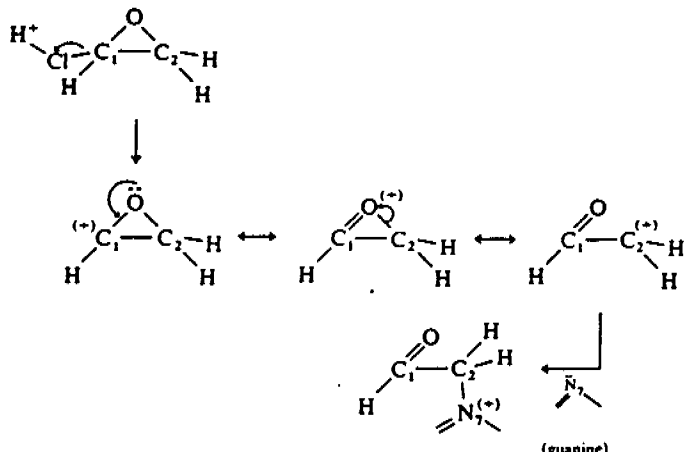
Recent studies have concluded [17], in support of earlier work [15], that the 7-N-(2-oxoethyl) derivative of guanine (structure **IX**) is the major *in vivo* DNA alkylation product of vinyl chloride. (There is some evidence suggesting that this aldehyde, **IX**, may be in equilibrium with a cyclic hemiacetal involving O-6 [17a].) It was proposed that the formation of **IX** may correspond to the "primary lesion" in DNA that might be responsible for the carcinogenicity of vinyl chloride [17b].

As pointed out earlier, the C—Cl bond in the chloro-protonated form of chlorooxirane is weakened to such an extent that only a small energy input is required for the removal



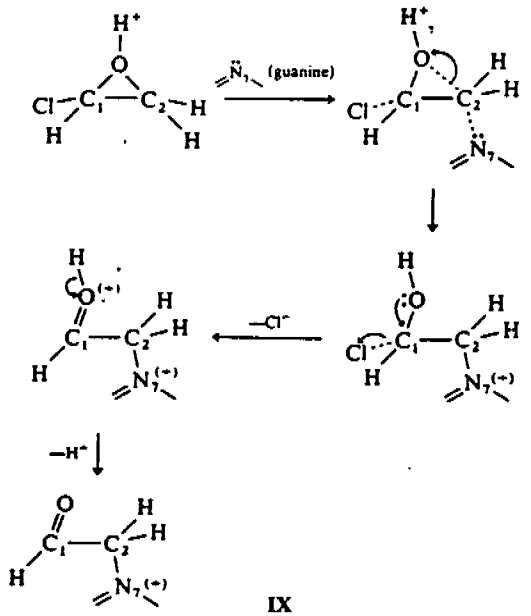
IX

of the chlorine. This leaves behind a charged species (V) having considerable aldehyde character. One can then readily envision the highly nucleophilic [32] N-7 position on guanine reacting with C_2 to produce **IX**. The sequence of steps would be:



IX

We can also account for the formation of **IX** by means of an S_N2 process involving oxygen-protonated chlorooxirane. Table II shows that the interaction of ammonia with C_2 that leads to the intermediate **VII** is accompanied by a rather surprising increase in the C_1—Cl bondlength, presumably indicating a significant weakening of this bond, as well as a shortening of the C_1—O distance, from 1.49 to 1.41 Å. This suggests the following mechanism, again involving N-7 of guanine:



IX

Thus, the results obtained in this work allow us to propose reasonable mechanisms, both S_N1 and S_N2, for the formation of the key alkylation product, **IX**. On purely thermodynamic grounds, the S_N2 process should be the favored one, since it starts with the more stable of the two possible protonated epoxides* and the nucleophile goes to the carbon, C_2, at which the more stable product is formed (Table III).

4. Summary

This study indicates several possible reactive pathways that protonated chlorooxirane can follow. It supports the suggestion [20] that the departure of the chlorine may play an important role in the biological mode of action of vinyl chloride. Of particular importance with regard to the carcinogenic activity of this molecule is the establishing of two possible routes, one S_N1 and the other S_N2, to the formation of what has been observed experimentally to be the primary *in vivo* DNA alkylation product of vinyl chloride, structure **IX** [17]. We are currently investigating mispairing possibilities that could result from this modification of guanine.

Acknowledgments

The authors greatly appreciate several very helpful discussions with Dr. G. Rickey Welch. They thank the U.S. Environmental Protection Agency for partial funding of this work under assistance agreement number CR808866-01-0 to Peter Politzer. The contents do not necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. They are also grateful for the financial support provided by the Research and Development program of the Louisiana Board of Regents and the University of New Orleans Computer Research Center.

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* Our calculated proton affinity for the oxygen in chlorooxirane is 216 kcal/mol, compared to 169 kcal/mol for the chlorine [20].

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Received January 25, 1983
Accepted for publication April 28, 1983