proposed here for reorientation by jumps on a tetrahedral lattice is certainly a simplification but is acceptable in this experimental context because the activation energy is large; i.e., the time in transit is negligible in comparison to the residence time. Both the activation energy and dynamical rates determined here are directly comparable to those from dielectric relaxation.

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Quantum Chemical Studies of the Products of Decomposition of Anticancer (2-Haloethyl)nitrosoureas under Physiological Conditions

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Abstract: Ab initio (Hartree–Fock) calculations are performed on plausible intermediates leading to the observed products of decomposition of HENU's ((2-haloethyl)nitrosoureas) under physiological conditions. The entities investigated are diazohydroxides, diazonium ions, and diazoate salts. It is found that ethane- and 2-fluoroethanediazohydroxides are more prone to a soft electrophilic attack on the DNA bases than methane and 2-chloroethanediazohydroxides, and the same trend is found for their diazonium ions.

(2-Haloethyl)nitrosoureas (HENU's) have an established place in the clinical treatment of human malignancies, including Burkitt's lymphoma, Hodgkin's disease, and cerebral neoplasms.[1-3] It has been established that HENU's decompose spontaneously under physiological conditions, producing electrophiles, which attack sensitive cellular macromolecules, including DNA. Specific lesions have been identified, including base alkylation, cyclic nucleotide adduct formation, and interstrand cross-linking, the latter being a lethal event to the cell.[5,6]

Simple nitrosoureas, such as methylnitrosourea, are highly mutagenic and carcinogenic, but more complex analogues, such as HENU's, have much higher antitumor activity in relation to their mutagenicity.[7] It was found that the extent of the interstrand cross-linking correlates with activity against L-1210 murine leukemia and with the G + C content of the DNA.[8,9] The sequence of reactions leading to interstrand cross-link formation by HENU's begins with the generation of reactive intermediates responsible for the transfer of the 2-haloethyl group to nucleophilic sites on the DNA bases. The monoadduct thus formed can then react with a nucleophilic site on another base, eliminate the halogen, and generate a cross-link consisting of an ethylene bridge. It has been shown that nucleophilic sites alkylated by 2-haloethyl groups comprise the O^6 site on guanine as well as the N^7 site, while the interstrand cross-linking sites that have been identified to date involve a cytosine amine group.[6,10,11] The O^6-alkylation leads to interstrand cross-linking, while the alkylation at N^7 apparently does not lead directly to a cytotoxic event.

The ability to repair the alkylation at O^6, before interstrand cross-linking can occur, has been related to the selective toxic action of HENU's. The tumor cell lines were found to be deficient in the removal of O^6-methylguanine from their DNA and thus were found to belong to the phenotype termed Mer^-, while normal cells belong to the phenotype Mer^+, that is, show proficiency in removing O^6-methylguanine from DNA.[12]

Therefore, the determination of the site of attack by the products of decomposition of HENU's under physiological conditions, as well as their ability to form interstrand cross-links, becomes a crucial issue in respect to their antitumor activity and preferential cytotoxicity.

In order to establish the preferred site of attack, one also has to gather information about the nature of the intermediates in the decomposition, which result in the alkylating reactions. The possibility that a carbonium ion acts as the alkylating entity has been rejected.[13] The putative alkylating agents are now considered to be either the 2-haloethanediazohydroxides or the corresponding kinetically equivalent diazonium ions.[14,15] Kinetic experiments cannot distinguish between the latter species, and, therefore, it is of interest to study both species and to clarify their similarities

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Table I. Energies (au) and Optimized Geometrical Parameters (Å, deg) for Ethane-, 2-Fluoroethane-, and 2-Chloroethanediazohydroxides[a]

	ethane		2-fluoroethane		2-chloroethane	
	E	*Z*	*E*	*Z*	*E*	*Z*
E	−262.7578	−262.7585	−361.5782	−361.5804	−721.6310	−721.6337
α	120	180	131	180	180	180
N1-N2	1.208	1.205	1.209	1.208	1.212	1.205
N2-O	1.401	1.443	1.393	1.431	1.377	1.432
O-H1	0.952	0.951	0.952	0.952	0.962	0.952
C1-N1	1.470	1.476	1.464	1.467	1.477	1.480
C1-C2	1.527	1.522	1.515	1.512	1.523	1.523
C-H	1.082	1.082	1.080	1.080	1.077	1.077
C-X			1.412	1.413	1.855	1.857
N1-N2-O	111.1	112.8	111.1	112.7	113.2	112.6
N2-O-H1	106.4	106.7	106.7	107.2	110.2	107.3
N2-N1-C1	115.1	123.5	115.3	123.5	116.9	123.6
N1-C1-C2	108.7	107.9	107.6	106.4	104.6	104.0
C1-C2-H	110.5	110.6	111.4	111.5	111.7	111.8
C1-C2-X			108.3	108.3	110.5	109.1

[a] X = H, F, and Cl.

and differences. An observation in favor of 2-halo diazohydroxides as candidates for the critical intermediate species is the observed in vivo antileukemic activity of (*E*)-2-chloro diazohydroxide.[15,17]

The theoretical approach of this work uses ab initio calculations to elucidate the structure of a number of appropriate diazohydroxides and the corresponding diazonium ions. Information is obtained about the geometry of the most stable conformations of *E* and *Z* isomers of the 2-haloethanediazohydroxides and the corresponding diazonium ions. Net atomic charges are calculated via Mulliken population analysis, and LUMO's are discussed in terms of atomic orbital participation. These results permit an interpretation of the observed base site selectivity via the utilization of the hard and soft acids and bases theory.[16]

Experiments using specific ^{15}N and ^{18}O labeling have confirmed the intermediacy of a 1,2,3-oxadiazoline during the decomposition of HENU's under physiological conditions plausibly arising from the (*Z*)-2-chloroethanediazohydroxide.[17] As such, it is of interest to elucidate the structure and properties of this intermediate by theoretical means.[17]

The entities investigated are the following.

1. Diazohydroxides: ethanediazohydroxide, 2-fluoroethanediazohydroxide, and 2-chloroethanediazohydroxides (*E* and *Z* isomers); structures and properties of the (*E*)- and (*Z*)-methanediazohydroxides have been reported by us elsewhere.[15b]
2. Diazonium ions: methanediazonium ion, ethanediazonium ion, 2-fluoroethanediazonium ion, and 2-chloroethanediazonium ion.
3. Diazoate ions and their affinities to cations such as Li^+, Na^+, and NH_4^+.
4. 1,2,3-Oxadiazoline.

Methods and Results

The calculations are performed within the Hartree-Fock approximation with the 3-21G, 6-31G, and 6-31G* basis sets, as implemented by the GAUSSIAN 80 and the GAUSSIAN 82 computer programs. The geometry optimization is performed by the gradient method (Berny optimization).[20]

Table II. Energies (au) and Optimized Geometrical Parameters (Å, deg) for the Methane-, Ethane-, and 2-Fluoroethanediazonium Ions

	methane	ethane	2-fluoroethane
E	−148.2160	−187.2624	−286.0912
N1-N2	1.073	1.073	1.073
C1-N1	1.510	1.550	1.551
C1-C2		1.528	1.536
C-H	1.078	1.080	1.078
C2-F			1.341
C1-N1-N2	180.0	180.0	180.0
H-C1-N1	105.0	104.5	103.1
H-C2-C1		110.0	110.8
F-C2-C1			107.6

Table III. Binding Energies of H^+, Li^+, Na^+, and NH_4^+ to Ethane- and 2-Chloroethanediazoates (kcal/mol)

	H^+	Li^+	Na^+	NH_4^+
(*E*)-ethane	392	211	174	151
(*Z*)-ethane	382	193	153	138
(*E*)-2-chloroethane	380	197	165	112
(*Z*)-2-chloroethane	372	177	145	123

Figure 1 shows the most stable conformations of the *E* and *Z* isomers of the diazohydroxides investigated. These were obtained by selecting a number of initial geometries and allowing them to relax. The rotation around the C-C bond is relatively free since this is a single bond. The N2-N1-C1-C2 dihedral angle is designated α, and the initial geometries will feature α = 0°, α = 60°, α = 120°, and α = 180°. α and the other parameters of the molecule are then allowed to relax, and the optimized values are presented in Table I. The α = 0° conformation of the *Z* isomers leads to considerable crowding. However, the 1,2,3-oxadiazoline is plausibly obtained by nucleophilic attack of O at C2, with the elimination of the halogen, and, as such, structures that feature a small C2-O distance are of interest. Therefore, α is given the fixed value of 30°, and the other parameters of the molecule are optimized. All of the above-mentioned calculations are performed with the 6-31G basis sets.

The diazonium ions are subjected to geometry optimization using the 6-31G* set. The only exception is the 2-chloroethanediazonium ion **4d** for which the geometry of the 2-fluoroethanediazonium ion **4c** is used, setting the C-Cl bond length at 1.85° Å. These results are presented in Table II and Figure 2.

The diazoate ions are investigated with the 3-21G basis set, and the results are shown in Figure 3. Since the diazoates are negatively charged, it is improbable that they would act as electrophiles. However, it has been shown experimentally and theoretically that Li^+ ions catalyze the decomposition of nitrosoureas.[21,27] Accordingly, it is possible that, in the presence of suitable counterions, the entities initially formed are the salts of diazohydroxides. Therefore, values of the affinities of diazoates for cations are obtained and presented in Table III.

Tables IV and VI show the net atomic charges on the atoms of the compounds, and Tables V and VII show the LUMO composition.

Figure 4 shows the geometrical parameters of the 1,2,3-oxadiazoline as calculated with the 3-21G basis set. The net atomic charges and the LUMO composition are shown in Table VIII.

Discussion

Inspection of Table I reveals that the *Z* isomers of the diazohydroxides are slightly more stable than the *E* isomers. The most

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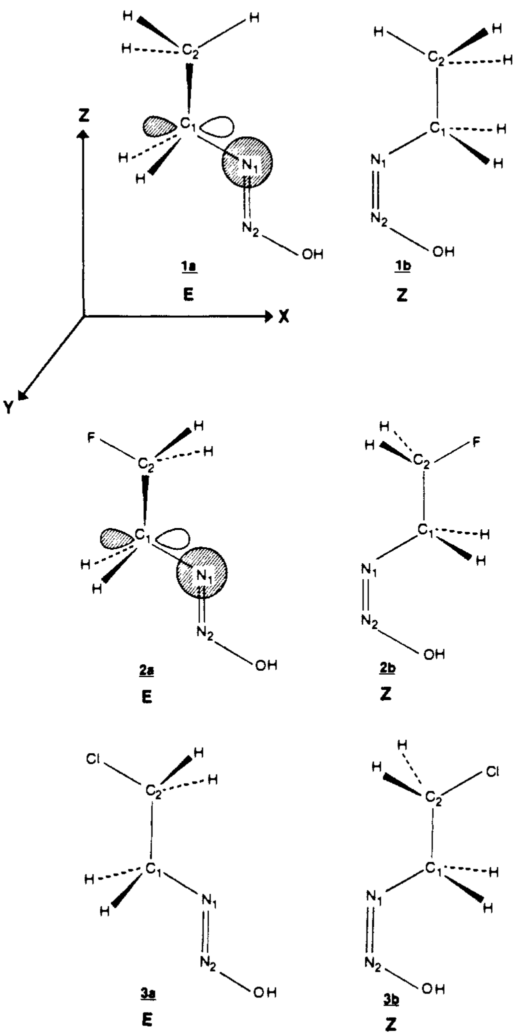

Figure 1. Geometry-optimized conformations of the *E* and *Z* isomers of alkanediazohydroxides: (1) ethane, (2) 2-fluoroethane, and (3) 2-chloroethane.

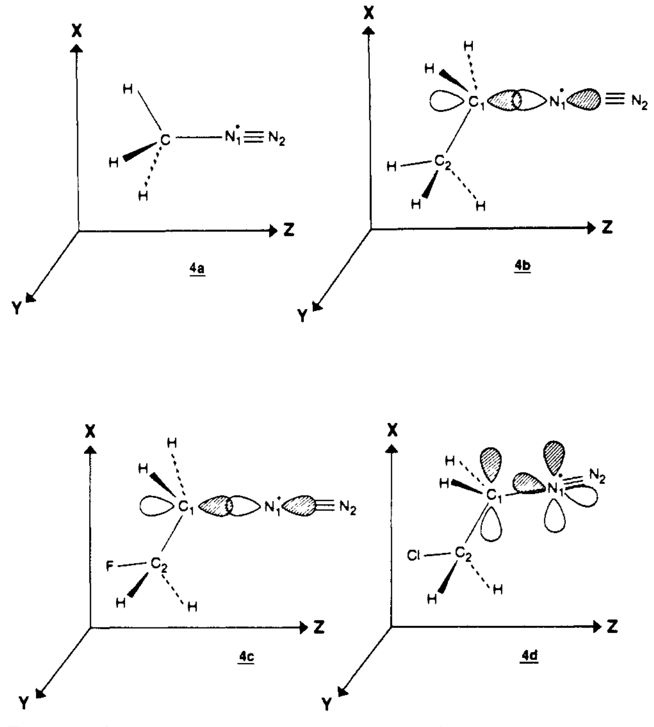

Figure 2. Geometry-optimized conformations of alkanediazonium ions: (**4a**) methane, (**4b**) ethane, (**4c**) 2-fluoroethane, and (**4d**) 2-chloroethane. They are calculated with the 6-31G* basis set.

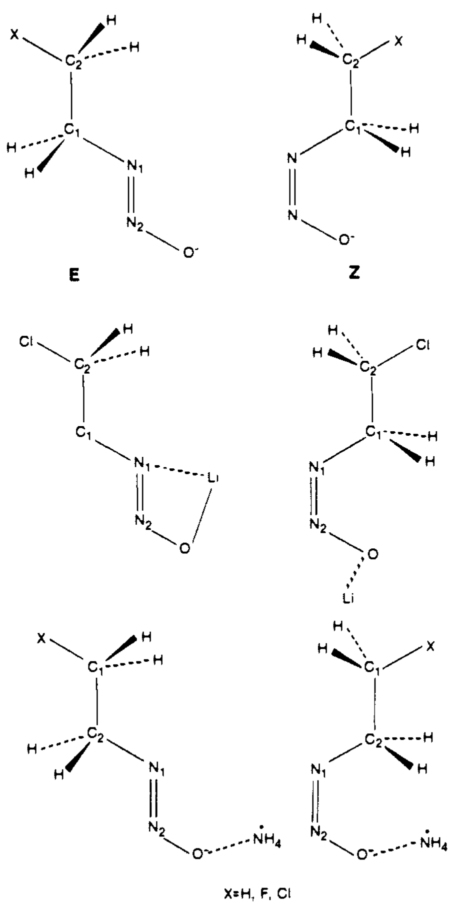

Figure 3. Geometry-optimized conformations of (*E*)- and (*Z*)-alkanediazoate anions and the corresponding lithium and ammonium salts calculated with the 3-21G basis set.

Table IV. Net Atomic Charges and Group Charges for the Diazohydroxides

	ethane		2-fluoroethane		2-chloroethane	
	E	*Z*	*E*	*Z*	*E*	*Z*
N1	−0.183	−0.237	−0.209	−0.262	−0.336	−0.249
N2	+0.053	+0.138	+0.072	+0.155	+0.189	+0.153
O	−0.573	−0.652	−0.566	−0.644	−0.569	−0.644
C1	−0.176	−0.172	−0.216	−0.214	−0.131	−0.150
C2	−0.449	−0.442	+0.148	+0.154	−0.448	−0.441
F			−0.460	−0.464		
Cl					−0.057	−0.065
H2						
C1	+0.210	+0.254	+0.219	+0.258	+0.335	+0.334
H3						
H4						
C2	+0.068	+0.060				
H5						
H5						
H4						
C2						
H5			+0.053	+0.053		
F						
H4						
C2						
H5					−0.037	
Cl						

stable conformation of the *Z* isomers for all the compounds considered are those with $\alpha = 180°$. As mentioned above, the formation of the 1,2,3-oxadiazoline would require proximity of the O and C2 atoms. This would be best accomplished for $\alpha = 0°$, but the energy of such a system would be very high, owing to considerable crowding. When $\alpha = 30°$ is maintained and the other parameters of the systems are optimized, the O–C2 distance takes the value of 2.7 Å and the energy a value of −361.5720 au for the 2-fluoroethanediazohydroxide. For the chloro compounds,

Table V. LUMO Energies and Atom Contributions for Diazohydroxides

		ethane			2-fluoroethane			2-chloroethane	
		E	*Z*		*E*	*Z*		*E*	*Z*
energy		0.1410	0.1337		0.1209	0.1168		0.1102	0.1167
N_1	$2p_y$	-0.414	-0.414		-0.409	0.412		0.395	0.414
		-0.586	-0.552		-0.581	0.551		0.539	0.557
	2s	-0.103			-0.077				
N_2	$2p_y$	0.452	0.447		0.456	-0.453		-0.462	-0.455
		0.605	0.583		0.608	-0.588		-0.602	-0.592
O	$2p_y$	-0.150	-0.155		-0.155	0.159		0.160	0.158
		-0.181	-0.172		-0.187	0.178		0.200	0.177
C_1	$2p_x$	0.125		$2p_x$	0.158		$2p_y$	0.315	0.282
C	$2p_x$	-0.183		$2p_x$	-0.192		$2p_y$	-0.154	-0.187

Table VI. Net Atomic Charges (eu) for the Diazonium Ions

	methane	ethane	2-fluoroethane	2-chloroethane
N1	+0.195	+0.186	+0.169	+0.184
N2	+0.139	+0.120	+0.123	+0.121
C1	-0.320	-0.169	-0.200	-0.153
C2		-0.540	+0.117	-0.437
F			-0.339	
Cl				+0.054
H1	+0.329	+0.324	+0.338	+0.336
H2	+0.329	+0.324	+0.338	+0.336
H3	+0.329			
H4		+0.273	+0.227	+0.280
H5		+0.243	+0.227	+0.280
H6		+0.238		
CH_3	+0.667			
CH_2		+0.479	+0.476	+0.519
CH_2X		+0.214	+0.232	+0.177

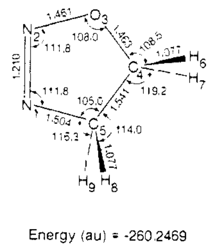

Figure 4. Optimized geometrical parameters of 1,2,3-oxadiazoline calculated with the 3-21G basis set.

these values become 2.8 Å and −712.6225 au. These distances are reasonable for a transition state, and the energies are within a few kilocalories per mole of those values of the most stable conformation, 5.3 and 7 kcal/mol, respectively. Thus, the calculations predict the formation of the 1,2;3-oxadiazoline intermediate to be geometrically and energetically feasible.

The *E* isomers show a different pattern for ethyl and 2-fluoroethyl species on the one hand and the 2-chloroethyl species on the other. In the former cases the conformations featuring $\alpha = 120°$ and $\alpha = 131°$, respectively, are more stable than the planar structures with $\alpha = 0°$ or 180°. The latter, shows $\alpha = 180°$ characterizing the most stable conformation. It should be pointed out however that α values of 120° and 131° do not imply chirality of the ethane- and 2-fluoroethanediazohydroxides because reference is made to rotation about a single bond, around which the CH_2X group rotates with relatively small energy barriers. For example, the $\alpha = 0°$ conformer is higher than the $\alpha = 120°$ conformer by only 2 kcal/mol in the case of the ethyl species. A similar situation exists for $\alpha = 180°$, and therefore the question of chirality does not arise.

The differences in preferred geometries are, however, significant from the point of view of the LUMO compositions. While the $\alpha = 0°$ conformations, as well as the methanediazohydroxide LUMO, feature a bonding contribution between N1 and C1 (implying that the addition of another pair of electrons would strengthen that bond rather than weakening it), the nonpolar conformations show an antibonding σ^* orbital between the 2s of N1 and the P_x of C1. The addition of another pair of electrons would then facilitate the breakage of this bond and consequently facilitate the alkylation of guanine by HOMO–LUMO interaction. When the frontier orbital theory is applied, such a mechanism will favor the attack at O^6 since O^6 is a softer nucleophilic site than N^7. Indeed, the net atomic charge of O^6 is −0.456 eu, while N^7 has a charge of −0.562 eu.[22]

Table VII. LUMO Atomic Contributions for the Diazonium Ions

	methane		ethane		2-fluoro-ethane		2-chloro-ethane	
N1	p_y	-0.432	p_y	-0.260				
		-0.600		-0.353				
			p_x	-0.348	p_x	0.419	p_x	-0.346
				-0.477		0.590		-0.539
			p_z	0.082	p_z	0.080	p_z	0.095
								0.074
N2	p_y	0.476	p_y	0.273				
		0.638		0.370				
			p_x	0.382	p_x	-0.473	p_x	0.450
				0.511		-0.628		0.580
							p_z	0.100
								0.141
C1	p_y	-0.135						
			p_x	-0.110	p_x	0.132		
			p_z	0.118	p_z	0.322	p_x	0.147
C2			p_x	-0.114	p_x	0.131	p_x	-0.140
								-0.116
			2s	0.301	2s	-0.325	2s	-0.446
							p_z	0.017
								0.139
F					2s	0.142		
Li							3s	0.136

Table VIII. SCF 3-21G Completely Optimized Calculations for 1,2,3-Oxadiaxoline[a]

atom		contribution	net atomic charges atom and group
E		+0.142 54	N1 -0.236
N1	$2p_y$	+0.907	N2 +0.139
N2	$2p_y$	-0.993	C3 -0.483
C3	$2p_y$	+0.258	C4 -0.141
C4	$2p_y$	+0.100	C5 -0.323
C5	$2p_y$	+0.215	H6 +0.245
H6	1s	-0.145	H7 + 0.245
H7	1s	+0.145	H8 +0.277
H8	1s	-0.379	H9 +0.277
H9	1s	+0.379	C4–H6–H7 +0.344
			C5–H8–H9 +0.231

[a] LUMO energy is in atomic units.

The HOMO participation of the p_y orbital of O^6 is 0.362 au while the p_y of N^7 participates with only 0.189 au, as found by STO-3G calculations. Our calculations for cytosine using the 3-21G basis set and geometrical parameters determined from X-ray diffraction show a participation to the HOMO at 0.504 au for N^3. Kollman's calculations show a charge of −0.892 eu, which indicates that both hard and soft electrophilic attack could

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[22] Singh, U. C.; Kollman, P. A. *J. Comput. Chem.* **1984**, *5*, 129.

occur at N^3.[22] These results are in agreement with the experimental results of Singer, in that the ethyl species show a larger O^6/N^7 ratio for alkylation than the methyl species.[23,24]

The alkylation of nucleic acid bases by (2-chloroethyl)-nitrosoureas seems to occur by a different mechanism under certain conditions. The experimental results on BCNU of one of us and coauthors show the possibility of a direct nucleophilic attack at the carbon adjacent to chlorine in aprotic polar media.[25] Thus, although the calculations pertain initially to the gas phase, the possibility of the reactions of electrophiles generated in hydrophobic cellular regions adds to the significance of the present results.

There is no antibonding in LUMO between N1 and C1. Indeed, Table V shows a strong antibonding participation to the LUMO at C1 and C2.

The net atomic charges on the diazohydroxides show the following patterns: the planar compounds (all the *Z* isomers and (*E*)-2-chloroethanediazohydroxide) feature a higher negative charge on N1 than the *E* nonplanar entities and a larger positive charge on N2. The C–H charge separation is overestimated by this method while the C–Cl charge separation is underestimated (which is also observed in smaller molecules, such as CH_3CO, so the charges to be discussed and compared are the group charges CH2 and CH2X where X = H, F, and Cl. It is seen that the methylene adjacent to chlorine features a higher positive charge in 2-chloro diazohydroxide. This fact, coupled with the lack of antibonding participation to the LUMO by the Cl–N1 bond, seems to indicate for the chloro compound more of a hard nucleophile mechanism of attack at C1. It is possible that a soft nucleophile attack occurs at C2, in agreement with the experimental results on BCNU, since a charge-directed attack at this group would be less probable.[25]

Diazonium Ions. The geometry obtained through optimization for the diazonium ions is similar to that reported by Ford and Scribner with MNDO calculations.[26] It features a very short N–N bond. The LUMO composition is however not in accord with their conclusions but is in agreement with the experimental results, that is, that the ethyl compound has a higher O^6/N^7 alkylation ratio than the methyl, due to an enhanced soft electrophilic attack. Indeed, as in the case of diazohydroxides, in the methyl case the N1–C1 interaction in the LUMO is totally bonding, while in the ethyl and 2-fluoroethyl cases, there is a small interaction between the p_z of N1 and the p_z of O1 (see Figure 4). In the 2-chloroethyl case (see Figure 4), there is a slight π interaction but no σ^* interaction. There is however a σ^* interaction between C1 and C2. Therefore, in this respect the conclusions derived from the diazohydroxide LUMOs and the diazonium ion LUMOs are similar.

From Table VI it is apparent that the methyl group is strongly positive and as such can be charge-directed to attack the harder nucleophilic site N^7. In the other cases, the C1 methylene is the most positive charge in the chloro compound, which also features a lower positive charge at the C2 group. Again, there is the possibility that C1 in this case will be more charge-directed and C2 will act as a softer electrophile. In general, the positive charge is spread over the whole ion.

Diazoates. As shown in Figure 3, Li^+ and Na^+ become associated with the oxygen, replacing H1, while the NH_4^+ bonds to the oxygen via a hydrogen bond. This is why, for the former two cases, in the *E* isomers there is additional stability due to the proximity of the cation to the lone pair on N1. This effect can be termed as an "intramolecular Li^+ bond", similar to a hydrogen bond. This effect is absent in the *Z* isomers and in the NH_4^+ salts. Indeed, the Li^+ and Na^+ affinities are consistently smaller in the *Z* isomers than in the *E* isomers, which is not the case for NH_4^+. The affinities decrease from H^+ to NH_4^+, and this could be the reason that Li^+ addition to nitrosoureas catalyzes the decomposition while Na^+ and NH_4^+ ions do not.[27]

The diazoate ions, being negatively charged, cannot act as electrophiles. In water, the salts are dissociated, but in less polar environments (vide supra) they might exist in an undissociated state and attack the DNA bases. The LUMO analysis shows a strong participation by the s orbital of the cations and negligible contributions by other atoms.

Conclusions

It seems probable that the ethane- and 2-fluoroethanediazohydroxides will be somewhat more LUMO–HOMO interaction-directed in their attack on DNA bases than the methane- and 2-chloroethanediazohydroxides. The same holds true for the corresponding diazonium ions. The calculations show a high probability of formation of 1,2,3-oxadiazoline (implicated as an intermediate experimentally[17]) from the *Z* isomers, since the energy of the conformation favorable for the O attack at C2, with $\alpha = 30.0°$, is only a few kilocalories per mole higher than that of the most stable conformation, with $\alpha = 180°$.

The ultimate goal is an increased understanding of the chemical events induced by HENU's leading to cell death. Certainly, DNA alkylation events alone, which are subject to efficient cellular repair,[1,2] do not correlate with biological response. DNA alkylation is however a necessary prerequisite for DNA interstrand cross-linking,[5,6] which correlates well with anticancer efficacy.[8,9,12] The present results, focusing on certain essential chemical events, may contribute to that understanding.

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Registry No. 1a, 92219-52-8; **1b**, 92219-54-0; **2a**, 115204-23-4; **2b**, 115204-25-6; **3a**, 115204-24-5; **3b**, 115204-26-7; **4a**, 20404-06-2; **4b**, 84027-64-5; **4c**, 115204-27-8; **4d**, 115204-28-9; $H_3CCH_2N_2OLi$, 115204-29-0; $ClCH_2CH_2N_2OLi$, 115204-30-3; $H_3CCH_2N_2ONa$, 115204-31-4; $ClCH_2CH_2N_2ONa$, 115204-32-5; $H_3CH_2N_2O^{-+}NH_4$, 115204-33-6; $ClCH_2CH_2N_2O^{-+}NH_4$, 115204-34-7; 1,2,3-oxadiazoline, 13589-37-2.

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