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The importance of keto-enol tautomerism in alloxan. A semi-empirical quantum mechanical study

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The various tautomers and rotamers of alloxan are examined in detail by the MNDO method. The keto form is predicted to be the most important in the gas phase, while in solution the monohydroxy forms may also contribute. Dihydroxy forms are contraindicated. The alloxan ring is found to be most susceptible to nucleophilic attack. In spite of the fact that the carbon C_2 carries a larger positive charge, the site of nucleophilic attack is C_5. This is explained on the basis of the molecular electrostatic potential (MEP) maps in various planes parallel to the alloxan ring. The larger contribution of the C_5O_{11} antibonding π orbital in the lowest unoccupied molecular orbital (LUMO) also explains why this carbon is most susceptible to nucleophilic attack. The large negative energies of the LUMOs and highest occupied molecular orbitals (HOMOs) of all the systems indicate that these systems are good electron acceptors and bad electron donors. The anions are predicted to be highly stable. Even in the anions, the ring remains electron deficient.

Tautomerism is a very important phenomenon affecting the activity of several biologically active compounds. One such compound is alloxan (see Figure 1), which was first synthesized by Wöhler[1] in 1838. Oxidation of uric acid yields alloxan. It has the unusual property of causing a type of diabetes in experimental animals. It has been suggested[2] that it might be a circulating hormone controlling insulin secretion and arising from the metabolism of pyrimidines and purines.

Since the molecule has complex behaviour and is extremely reactive, very few studies of its physicochemical properties are available. Theoretical studies on the compound are also limited[3-6], and are mainly restricted to HMO calculations on the keto form. Hall[3] carried out an INDO study of the various possible tautomers and rotamers. *Abinito* calculations[7] are limited to a few of the possible tautomers.

In the present work, the more sophisticated MNDO method [8] has been used to carry out a detailed examination of the various possible tautomers and rotamers of alloxan with a view to understanding its structure and reactivity.

Method of Calculation

The MNDO method[8] has been used for all the calculations. The geometries of all structures were completely optimized with respect to the energy, keeping the molecule planar.

Results and Discussion

Figure 1 gives the structures and heats of formation of the various systems studied in this work. It can be seen that the keto form, alloxan itself, is the most stable and is favoured over the tautomer of least energy, the 4-hydroxy form by 11.7 kJ/mol. This value is much lower than the results[3] obtained from INDO studies, which predicted the difference in stabilities to be 79 kJ/mol. Also, they predict that the next in order of energy, the 2-hydroxy compound, has an energy which is 112.5 kJ/mol above the keto form. The present results suggest that the 2-hydroxy compound is less stable than the keto form by only 23.2 kJ/mol. The INDO results are in any case unreliable as they were obtained without geometry optimization, keeping the ring geometry fixed at the experimental geometry of the keto form. Since enolization is accompanied by large changes in the ring geometry due to the reorganization of bonds, complete geometry optimization has been carried out in the present work. The two dihydroxy forms (2,4- and 4,6-) are predicted to be less stable than the keto form by 32.6 kJ/mol and 37.7 kJ/mol, respectively.

The rotamers of the same tautomer, displayed in Figure 1, are labelled A - D in order of increasing

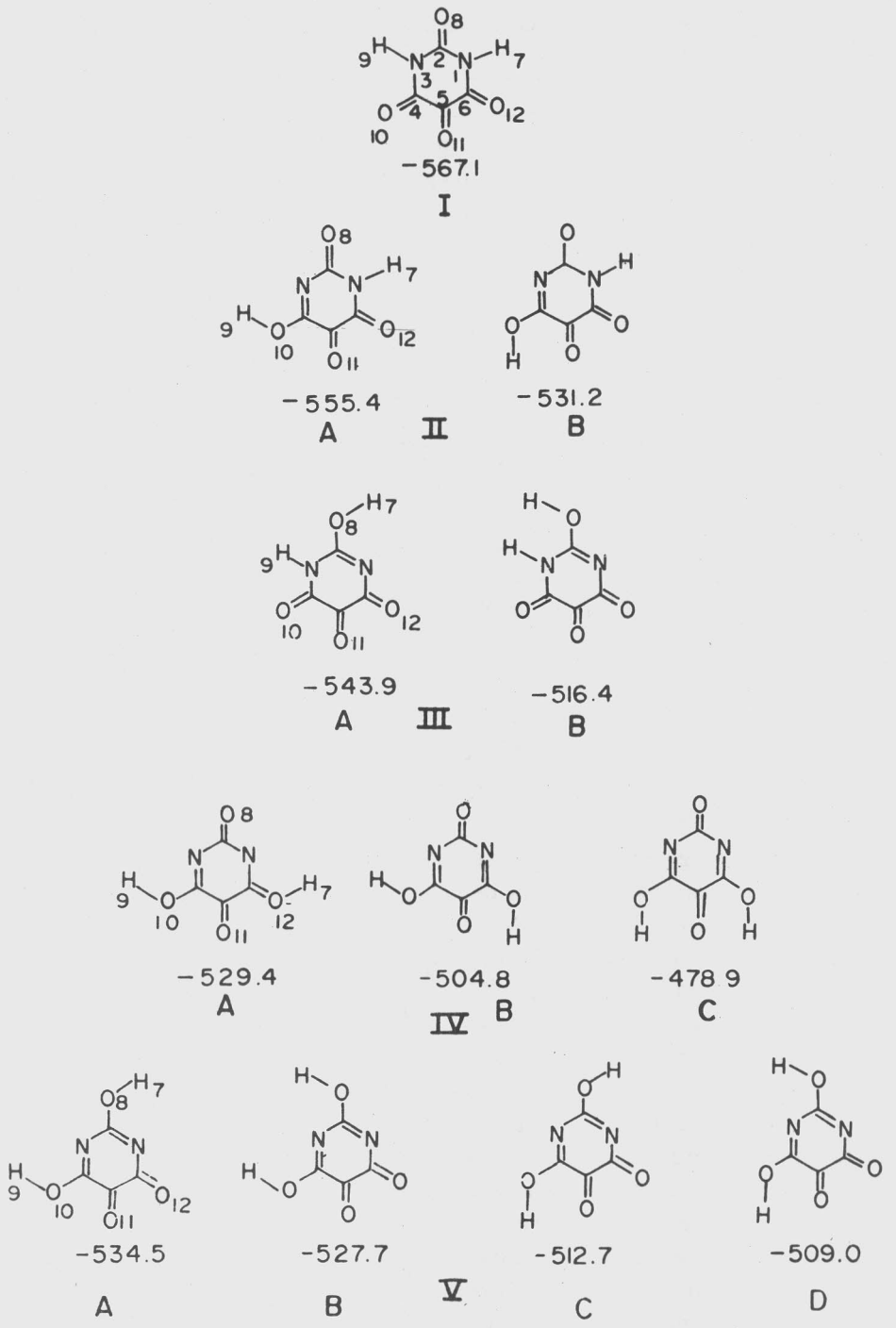

Figure 1 — Various tautomers and rotamers of alloxan and their heats of formation (kj/mol).

energy. In all cases, the A form, the rotamer of minimum energy, is the one in which the hydrogens are directed towards the nitrogens on which they were originally placed in the keto form. Hence, no rotation of the hydroxyl group occurs after enolization. The difference in energies between the various rotamers of the same tautomer is also observed to be quite high, i.e. 24.2 kJ/mol in the case of the 4-hydroxy compound, and 27.5 kJ/mol for the 2-hydroxy compound.

Thus it seems likely that the keto form is the dominant one in the gas phase, and the monohydroxy forms also make some contribution. The dihydroxy forms are not expected to play any part in the

Table I—Optimized bond distances and Mulliken overlap populations for alloxan

Bond[a]	Distance (Å)		Overlap population			
	Calc.	Expt.[b]	Calc.		Other work[c]	
			σ	π	σ	π
N_1C_2	1.410	1.388	0.840	0.127	1.730	0.378
N_1C_6	1.411	1.364	0.825	0.149	1.585	0.405
C_4C_5	1.528	1.521	0.817	0.020	1.757	0.240
C_2O_8	1.225	1.289	1.137	0.676	1.794	0.814
N_1H_7	1.008	1.010	0.900	-	1.643	-
C_4O_{10}	1.222	1.213	1.124	0.776	1.940	0.862
C_5O_{11}	1.218	1.186	1.099	0.917	1.648	0.934

*see Figure 1; [b]From ref 10 and 11; [c]From ref 3

tautomerization. The predominance of the keto form is confirmed by NMR studies[9] which do not show an enolic signal in alloxan solutions studied in DMSO solvent. Thus, in nonpolar solvents, too, the keto form is found to predominate.

However, aqueous solutions of alloxan exhibit[10] a pK_a, value of 7.2 suggesting a labile hydrogen. Since the second ionization is not observed, the dihydroxy forms are not stabilized in solution, also.

Table I gives the optimized geometry of the keto form of alloxan and the experimental geometry determined by X-ray crystallography[11,12]. The MNDO method is found to predict slightly larger CN distances and a smaller C_2O_8 distance (see Figure 1). The optimized geometries of the rotamers of the two monohydroxy forms are given in Table II. This indicates that enolization at the 4-position results in a large decrease in the N_3C_4 bond distance since this bond now acquires a double bond character. There is also a large increase in the $N_3C_4C_5$ bond angle. The two rotamers (A and B, see Figure 1) have similar structures. Similarly, in the case of the 2-hydroxy tautomer, the N_1C_2 bond distance decreases, as this becomes a double bond. The ring bond angle at C_2 also increases by 6° (see Table II).

Charge distributions

Table III gives the partial charge distributions on the various atoms for the keto form of alloxan, as well as the rotamers of the monohydroxy tautomers. There is a large variation in the dipole moments. Also, the dipole moment of the 2-hydroxy tautomer is greater than that for the 4-hydroxy tautomer. Secondly, the

Table II—Optimized geometries of rotamers of the 4-hydroxy and 2-hydroxy tautomers

Bond parameter[a]	4-hydroxy		2-hydroxy	
W	N_1		O_8	
X	O_{10}		N_3	
Y	C_6		C_2	
Z	C_4		C_2	
	A	B	A	B
N_1C_2	1.427	1.427	1.308	1.312
C_2N_3	1.417	1.405	1.398	1.409
C_3C_4	1.307	1.422	1.427	1.422
C_4C_5	1.522	1.307	1.536	1.532
N_1C_6	1.409	1.527	1.408	1.418
C_5C_6	1.530	1.529	1.536	1.533
WH_7	1.007	1.008	0.951	0.950
C_2O_8	1.219	1.218	1.340	1.314
C_4O_{10}	1.336	1.343	1.219	1.220
H_9X	0.953	0.950	1.007	1.005
C_5O_{11}	1.215	1.218	1.216	1.217
C_6O_{12}	1.222	1.222	1.220	1.219
$N_1C_2N_3$	116.8	118.1	122.3	123.5
$C_2N_3C_4$	122.3	121.3	123.2	123.2
$N_3C_4C_5$	124.8	124.3	115.9	115.9
$C_2N_1C_6$	126.3	125.9	124.5	121.7
YWH_7	117.0	117.3	112.9	115.5
$N_3C_2O_8$	122.3	121.7	115.6	119.7
$N_3C_4O_{10}$	118.6	113.5	117.6	118.1
ZXH_9	115.2	114.7	118.8	119.5
$C_4C_5O_{11}$	123.7	122.7	119.6	120.6
$N_1C_6O_{12}$	119.9	120.4	119.5	118.2

[a]see Figure 1. Bond distances are in Angstroms and bond angles in degrees.

Table III—Partial charges on the various atoms in alloxan and its monohydroxy tautomers

Atom*	Alloxan	4-hydroxy		2-hydroxy	
		A	B	A	B
N_1	-0.407	-0.433	-0.431	-0.457	-0.357
C_2	0.493	0.451	0.434	0.434	0.407
N_3	-0.407	-0.355	-0.264	-0.394	-0.429
C_4	0.330	0.231	0.198	0.325	0.326
C_5	0.210	0.231	0.207	0.181	0.184
C_6	0.330	0.325	0.330	0.312	0.283
H_7	0.299	0.217	0.219	0.247	0.218
O_8	-0.331	-0.275	-0.266	-0.228	-0.202
H_9	0.229	0.239	0.224	0.220	0.204
O_{10}	-0.260	-0.211	-0.196	-0.259	-0.259
O_{11}	-0.154	-0.153	-0.190	-0.165	-0.162
O_{12}	-0.260	-0.267	-0.266	-0.222	-0.213
m	1.24	1.69	2.65	4.41	5.79

*see Figure 1

less stable rotamers in the gas phase have the larger dipole moment. This suggests that, in aqueous solution, too, the less stable rotamers may get greater stabilization, and the 2-hydroxy tautomer may gain more stability as compared to the 4-hydroxy tautomer.

In all the systems, the nitrogen atoms carry large negative charges while the carbon atoms are positively charged. The total charge on the ring is, respectively, 0.549, 0.450, 0.474, 0.401 and 0.414, for the keto form and the A and B rotamers of the 4- and 2-hydroxy tautomers. Hence, the ring carbon atoms of the keto form are most susceptible to nucleophilic attack. In all the systems, at first sight, the site of nucleophilic attack appears to be C_2, which carries the largest positive charge. However, this carbon is flanked by three highly electronegative atoms — two nitrogens and an oxygen, all of which carry large negative charges. C_5, on the other hand, is between two positively charged atoms. It is, therefore, expected that a nucleophile would experience difficulty in attacking C_2, but can easily attack C_5. Furthermore, enolization at the 4-position increases the positive charge on C_5, and the negative charges on the nitrogens, and decreases the positive charge on C_2 (see Table III). This increases the possibility of a nucleophilic attack at C_5.

To check this point, molecular electrostatic potential (MEP) maps were drawn for alloxan. In the molecular plane, two minima are observed close to C_4 and C_6 (potential = - 239.9 kJ/mol) and another near C_2 (-183.1 kJ/mol, see Figure 2). However, in

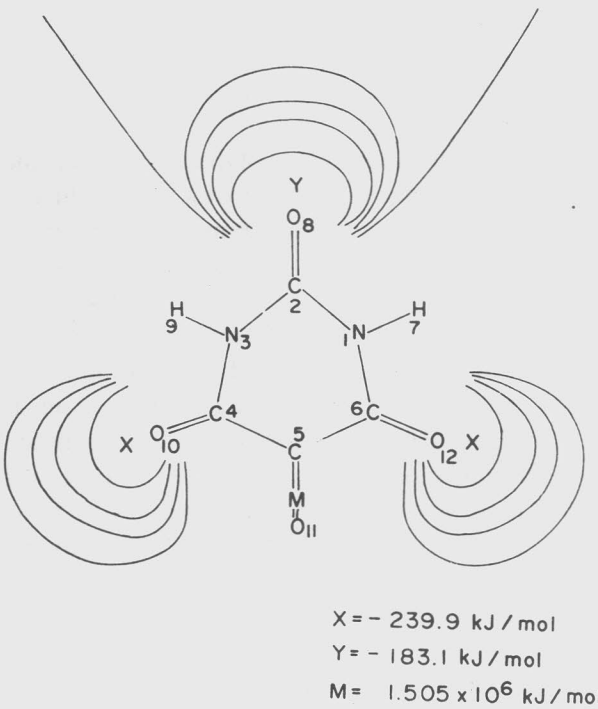

Figure 2 — Molecular electrostatic potential (MEP) maps for alloxan in the molecular plane (X=primary minimum, Y=secondary minimum, M=maximum)

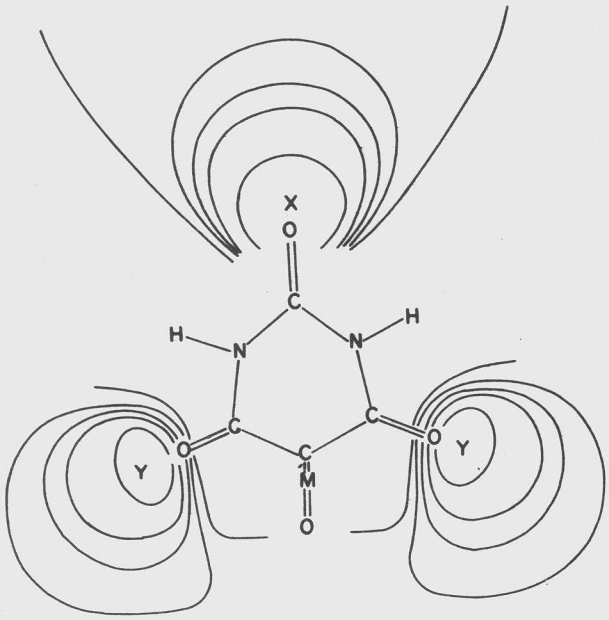

Figure 3 — Molecular electrostatic potential (MEP) maps for alloxan 1.0 Å above the molecular plane (X=primary minimum, Y=secondary minimum, M=maximum)

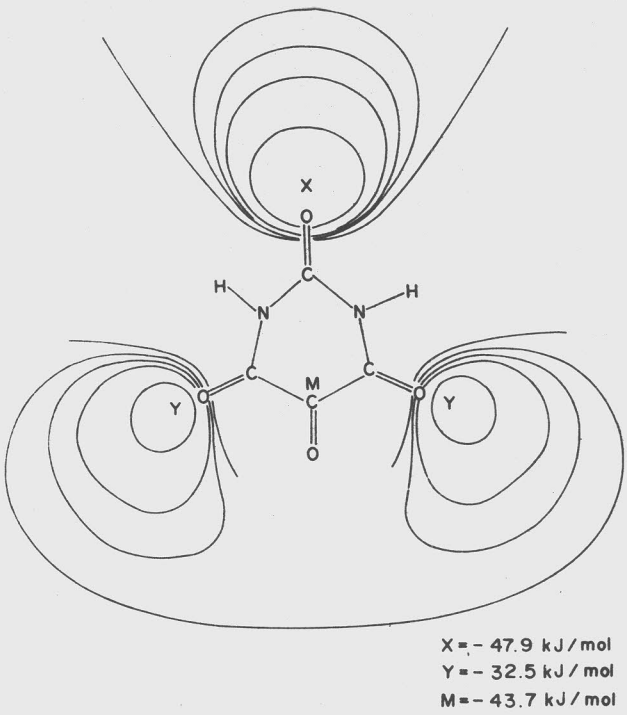

Figure 4 — Molecular electrostatic potential (MEP) maps for alloxan 1.6 Å above the molecular plane (X=primary minimum, Y=secondary minimum, M=maximum)

the plane parallel to the plane of the alloxan ring and situated at a distance of 1 Å, at which the influence of the π electron density is a maximum, the potential minimum is at the 2- keto side (-127.7 kJ/mol, see Figure 3).

In the plane 1.6 Å above the molecular plane, the potential minimum is again at the 2-position (-47.9 kJ/mol, see Figure 4). This plane has been chosen as this is roughly half the distance of separation between atoms having 'close contact' in crystals of molecular polarization or charge-transfer complexes of these molecules[13]. Hence, the 2-keto group is the likely site for electrophilic attack. Since all the MEP minima are close to the carbons C_2,C_4 and C_6, the only remaining carbon, i.e. C_5, must be the site for nucleophilic attack. In fact, in all the cases, the potential maximum is found close to the C_5C_{11} moiety (see Figures 2-4).

This is confirmed by experimental observations[14], which indicate that α-amino acids attack at the 5-position to produce alloxantin. Reduction of alloxan with hydrogen sulphide also yields alloxantin. Also, alloxan is normally obtained as the monohydrate with hydration at the 5-position. The formation of this monohydrate, which is difficult to dehydrate, is similar to that in chloral hydrate and is due to the nucleophilic attack by water at the 5-position. Hence, although C_2 carries a larger positive charge, C_5 is more prone to nucleophilic attack.

The diabetogenic activity of alloxan also appears to be partly due to the highly reactive carbonyl group at the 5-position, since substances similar to alloxan in structure, but lacking the 5-keto group, e.g. barbituric acid, violuric acid and ninhydrin are found to be nondiabetogenic[15,16]. Enolization also appears to play a role in the diabetogenic activity of alloxan as substitution at both nitrogens, which blocks the possibility of tautomerism, results in the loss of diabetogenic activity[17].

Reduction of alloxan with stannous chloride also occurs at the 5- position yielding dialuric acid suggesting the involvement of the monohydroxy tautomers and not the quinoid (4,6 dihydroxy) forms, as these would have resulted in the formation of isodialuric acid.

Since the MEP minimum is close to the negatively charged O_8, and the two nitrogens flanking it also carry large negative charges, there is a possibility of chelation at these positions by metal ions like Zn^{2+},

Table IV—Energies and nature of the two highest occupied and two lowest unoccupied molecular orbitals of the various systems

System[a]	35	36	37	38
Alloxan (**1**)	-11.862	-11.437*	-1.671	-0.026
4-hydroxy (**2**) A	-11.658*	-11.622	-1.921	-0.334
B	-11.655	-11.640*	-2.085	-0.432
2-hydroxy (**3**) A	-11.112	-10.782*	-1.533	-0.187
B	-11.098	-10.773*	-1.563	-0.235
4,6-dihydroxy (**4**) A	-11.517	-10.816*	-2.043	-0.305
B	-11.546	-10.780*	-2.197	-0.318
C	-11.574	-10.748*	-2.369	-0.345
2,4-dihydroxy (**5**) A	-11.043	-10.636*	-2.011	-0.137
B	-10.968	-10.558*	-2.026	-0.134
C	-11.070	-10.680*	-2.152	-0.343
D	-11.003	-10.612*	-2.156	-0.331

[a]see Figure 1. Orbital energies are in electron Volts.
The values marked with an asterisk are in-plane orbitals. The rest are out-of-plane orbitals.

Co^{2+}, Ni^{2+} and Cu^{2+}. This was demonstrated to be true by Lange and Foye[18]

Table IV contains the energies of the two highest occupied and two lowest unoccupied molecular orbitals of the various systems. For alloxan, the energy of the highest occupied molecular orbital (HOMO), MO # 36, is very low compared to other pyrimidines, which points to the fact that alloxan is not a good electron donor. In contrast to most other pyrimidines in which the HOMO is a π orbital, the HOMO of alloxan is a nonbonding orbital, consisting of the nonbonding MOs of O_{10} (21.4%) , O_{11} (22.0%) and O_{12} (see Figure 1). The penultimate occupied orbital, MO # 35, is basically a nitrogen nonbonding orbital (each nitrogen contributes 24.5%). Because of close proximity of the two nitrogens and O_8, this oxygen interacts with the nitrogen nonbonding orbitals and the involvement of O_8 in this orbital is 34.6%.

The energies of the two lowest unoccupied orbitals (LUMOs) are negative, showing that alloxan is a good electron acceptor. ESR studies in a water-methanol solution[19] confirm this. The LUMO, MO # 37, is basically the C_5O_{11} antibonding π^* orbital (C_5 = 29.6%, O_{11} = 22.8%). The next orbital in order of every, MO # 38, is also a π^* orbital comprising the C_4O_{10} and C_6O_{12} π bonds. Since the LUMO involves C_5 and O_{11}, this is the most reactive portion of the molecule towards nucleophilic attack.

For the other tautomers, too, the HOMO is usually an in-plane nonbonding orbital, the next lower level being an out-of-plane MO and lying about 0.5 eV below this level. However, both rotamers of the 4-hydroxy tautomer are exceptions as MOs # 35 and 36 are almost degenerate (see Table IV). In addition, in rotamer A (see Figure 1) there is a reversal in energy levels, the C_2O_8 π bonding orbital becoming the HOMO. Thus, this rotamer differs from the others in having a $\pi\pi^*$ transition as the longest wavelength transition, as opposed to the usual $n\pi^*$ transition in the other systems. The LUMOs in all cases, except all the rotamers of the 2,4 - dihydroxy tautomer, are the C_5O_{11} antibonding π^* orbitals. The ionization potentials, calculated on the basis of Koopmans' theorem, usually decrease on enolization. However, both rotamers of the 4-hydroxy tautomer have the highest ionization potentials. The electron accepting properties usually increase on enolization. Here the exception is the 2-hydroxy tautomer, both rotamers of which have higher LUMO energies as compared to alloxan.

Table I also contains the Mulliken overlap populations of the various bonds in alloxan. The structure of the keto form suggests that there should be six σ bonds and no π bonds in the ring. However, the calculated total σ bond order for the ring is only 4.964 and there is a π bond order of 0.592, making the total 5.556. Thus there is a weakening of the σ bonds in the alloxan ring. The π bond order arises from the

conjugation of the nitrogen lone pairs with the carbonyl π systems, particularly the C_2O_8 π bond. The weakest ring bonds are the bonds involving C_5, i.e. C_5C_4 and C_5C_6. This is because the π bond order is least for this bond as the C_5O_{11} carbonyl bond is furthest away from the two nitrogen atoms and there is thus less conjugation with the nitrogen lone pairs. Because of this lack of conjugation with the two nitrogens, this carbonyl bond is the strongest with a total bond order > 2. Although the σ bond order for the other carbonyl bonds is larger, the π bond orders in those cases are much smaller than unity and hence their smaller total bond order.

Charged species

As the above discussion indicates, the alloxans are good oxidizing agents but poor reducing agents. They readily undergo ionization to form anions.

The anion formed by ionization at the 1-position has a heat of formation of -769.0 kJ/mol, while its 2-hydroxy tautomer has a heat of formation of -764.7 kJ/mol. The 4-hydroxy tautomer is much less stable, the stable rotamer having a heat of formation of -705.0 kJ/mol. The charge on the ring in the keto form is -0.298 only, whereas in the 2-hydroxy tautomer, the ring remains positively charged (+0.038). Also, in this tautomer, the σ bond order reduces to 4.533 and the π bond order is 1.156, making the total similar to that in alloxan. Even in the dianion, the overall ring charge is only -0.067.

Since O_8 carries the largest negative charge, protonation should occur preferably at this oxygen. This is found to be so, this protonated compound being about 29 kJ/mol more stable than the next in order of stability, protonated at O_{10}.

Conclusions

The semi-empirical MNDO calculations corresponding to the gas phase indicate the keto form in the lactim-lactam pair to be the most important, followed by the 4-hydroxy and 2-hydroxy tautomers. The dihydroxy forms are unlikely to exist. The anion is formed readily and the keto form of the anion is more stable than the 2-hydroxy tautomer by only 4.3 kJ/mol. Thus both tautomers are likely to coexist. Protonation also occurs at this position with retention of the keto form. In no case are the quinoid forms indicated, refuting the usually drawn picture of alloxan. The greater stability of the triketo form is further borne out by NQR experiments on alloxan. H_2O[20] and X-ray diffraction studies[21].

Besides tautomerism, the important factor affecting the biological activity of alloxan is the highly reactive 5-keto group, which is most susceptible to nucleophilic attack.

The properties of alloxan have been satisfactorily explained on the basis of semiempirical MO studies. In particular, the greater reactivity of C_5 towards nucleophilic attack has been explained on the basis of the molecular electrostatic potential maps and the nature of the lowest unoccupied molecular orbitals.

The present calculations give a better insight to the structure and reactivity of alloxan than previous semi-empirical MO studies[3]. According to the INDO studies, the differences in energies between the various tautomers is upto 200 kJ/mol, which is unreasonable. The σ and π overlap populations calculated by the INDO method are also unreasonable, e.g. the overlap populations of carbonyl bonds are predicted to be 2.8 (see Table I). On analysing the σ and π contribution, it is found that the σ population is grossly overestimated by INDO calculations. Part of the reason that the INDO calculations gave unsatisfactory results seems to be the fact that the geometries were not optimized with respect to the energy. Since large chanrges in geometries are expected on tautomerization, fixing geometry at the experimental geometry for the triketo form leads to an overestimation of energy differences from the keto form.

Besides geometry optimizations, we have also investigated the natures of the frontier MOs and the MEP maps to understand the biological activity.

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