PROPERTIES OF PYRIDINE N-OXIDE IN EXCITED STATES.
A MODEL STUDY OF MICROSOMAL HYDROXYLATIONS

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A semiempirical PPP method which involves σ bond polarization was employed for computations of the ground and excited state properties of pyridine N-oxide. The possibility of N—O bond fission is discussed in terms of the gradient changes of the π electronic energy upon excitation. On energy grounds the N—O bond fission appears to be more facile in the T_1 state than in the S_0 state, and more facile in the S_0 state than in the S_1 state. The π–π complex of the pyridine N-oxide molecule with a C—C double bond was also computed. The calculation suggests that the N—O bond fission is more feasible in both the S_1 and T_1 states than in the S_0 state of the complex or in the S_0 state of pyridine N-oxide alone. The results are discussed with regard to the "oxene" mechanism of microsomal hydroxylation.

Isolation of naphthalene 1,2-oxide[1] as an intermediate in the microsomal biotransformation of naphthalene to 1-naphthol and the discovery of the so called NIH shift[2] (for details see reviews[3,4]) has necessitated new requirements for model chemical systems of the microsomal hydroxylation (oxidation) of aliphatic and aromatic compounds. The systems known previously (the systems of Fenton[5], Udenfriend[6], Hamilton[7] and others[8]) mimic the action of monooxygenases or mixed function oxygenases. They oxidize the substrates labelled by deuterium or tritium as the liver microsomes[9,10], however, they do not give products with the same retention of the heavy hydrogen atom shifted to the adjacent position. Besides the peroxytrifluoroacetic acid, photoactivated N-oxides of various heterocycles[11,12], in particular pyridine N-oxide[13], proved the most convenient model systems for the migration and retention of the heavy hydrogen atom. Photochemical reactivity of heterocyclic N-oxides has been the subject of numerous studies[14–16] and of a recent review[17]. The authors of the NIH shift suggested that atomic oxygen in a triplet state is the effective agent in oxidations of aliphatic substrates and atomic singlet oxygen in oxidations of olefinic and aromatic substrates. In analogy with isoelectronic carbene and nitrene they call the atomic oxygen oxene and therefore speak about the oxenoid mechanism of microsomal oxidations.

This paper is devoted to the theoretical study of properties of pyridine N-oxide in excited states. We presumed that the results of such a study might facilitate the formulation of the mechanism of microsomal oxidations of foreign compounds. Pyridine N-oxide is a suitable model for a theoretical treatment. Since its lower excited states are $\pi - \pi^*$ states, the π electronic approximation can be employed.

EXPERIMENTAL

A π electronic LCI–SCF method is used throughout and involves implicit changes in the σ electronic structure due to bond polarization[18]. The gradient of the π electronic energy in excited states was computed by making use of LCI wave functions. The LCI basis set covered all singly excited states. A more detailed description of the gradient computation and the respective formulas will be published later. Calculations were performed on IBM 7040 and IBM 360/40 computers with programs in FORTRAN and PL/I.

RESULTS AND DISCUSSION

Our first goal was to ascertain to what extent the semiempirical method accounts for observed physicochemical properties of pyridine N-oxide. Fig. 1 presents the singlet-singlet spectrum measured in hexane (full line) and water (dashed line) with the locations and intensities of calculated electronic transitions. It is seen that the theory
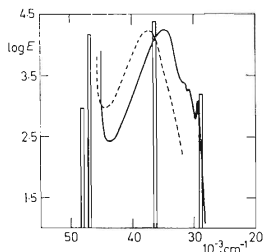

Fig. 1

Singlet-Singlet Spectrum of Pyridine N-Oxide

TABLE I

Change in the π Electronic Energy for the Unit Stretch of the N—O Bond (A) and the Stabilization Energy of the Formation of the Complex of Pyridine N-Oxide with the C—C Double Bond (B) in eV/Å.

State	A (pyridine N-oxide)	A (complex of pyridine N-oxide with C=C)	B
S_0	8·721	8·223	2·184
S_1	9·355	7·104	4·414
S_2	6·860	7·404	3·920
T_1	6·053	3·151	3·123
T_2	10·152	7·300	4·526

gives a good account of the two observed electronic transitions. The molecular geometry in the S_0 state was computed from the density matrix given by the SCF wave function. The calculated N—O bond length of 1·284 Å reproduces the observed value[19] (1·28 Å). The dipole moment in the S_0 state is greatly overestimated (calc. 6·11 D, obsd.[20] 4·24 D). The direction of the dipole moment in the S_1 state is predicted to be the same as in the S_0 state, which conforms to experiment; the change in dipole moment upon the $S_0 \rightarrow S_1$ transition is however greatly overestimated. Fig. 2 comprises N—O bond orders, total charges and π electronic densities on the oxygen atom in the ground and two lowest excited states. The bond order is seen to increase upon the $S_0 \rightarrow S_1$ and $S_0 \rightarrow T_2$ transitions and to decrease upon the $S_0 \rightarrow T_1$ and $S_0 \rightarrow S_2$ transitions. Since pyridine N-oxide is stable in the S_0 state, the N—O bond fission is therefore to be expected to occur only in the T_1 and S_2 states. To support this conclusion we calculated the gradient of the π electronic energy. From the gradient we estimated changes in the π electronic energy which are due to the unit stretch of the N—O bond. These are entered in Table I. The changes are positive for all states considered, *i.e.* the π electronic energy increases on stretching the bond. However the total energy change also involves the contributions that are due to changes in the σ bond energy and electrostatic repulsion energy owing to translation of the oxygen atom. These contributions cannot be estimated rigorously within the framework of the π electronic theory alone. One can assume, however, that their value will not change appreciably on passing from the ground state to excited states. It thus appears that the changes in the gradient of the total energy should be accountable for by the mere changes in the gradient of the π electronic energy. The entries in Table I suggest that the considered component of the gradient falls down
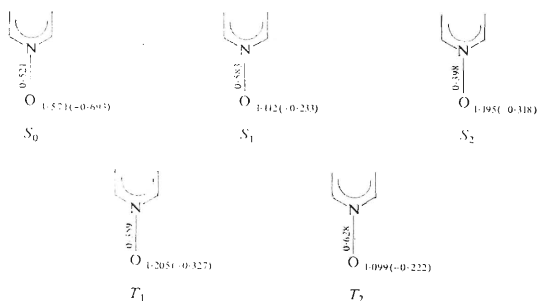

Fig. 2

Molecular Diagrams of the Ground and Lowest Excited States of Pyridine N-Oxide

only in the S_2 and T_1 states; this effect is more marked in the T_1 state. This is consistent with the conclusion based on the molecular diagrams that the loss of the oxygen can occur in the T_1 state but not in the S_1 state. This theoretical finding conforms to the enhanced loss of the oxygen observed in the sensizitization of the triplet state of pyridine N-oxide[21]. Similar observations were also reported for other heterocyclic N-oxides[22].

In order to have a deeper insight into the reaction studied, we also considered the formation of the complex between the pyridine N-oxide molecule and the substrate molecule followed by the fission of the N—O bond. The π electronic theory permits only study of a complex with a double bond. We selected the complex pyridine N-oxide-ethylene which is schematically drawn in Fig. 3. The results of calculations on the complex are given in the second column in Table I. The entries are changes in the π electronic energy which are due to the unit translation of the pyridine part of the complex from the epoxy part. The entries suggest that the dissociation of the N—O bond is more facile in all excited states than in the ground state. With the exception of the S_2 state, the values are lower than those for pyridine N-oxide. This implies that the formation of such a complex facilitates the N—O bond fission in both the lowest singlet and triplet states, the effect being particularly strong in the latter. The last column in Table I comprises stabilization energies of the complex formation, *i.e.* the changes in the π electronic energy that are due to the unit separation of ethylene from pyridine N-oxide. This change is positive in all instances because of the energy gain arising from the enlargement of the delocalized system upon complex formation. The tabulated data predict that the stabilization energy increases on going from the ground state to any excited state.

As stated above the dissociation of the N—O bond in pyridine N-oxide appears to be the most likely in the lowest triplet state on energy grounds. If a C—C double bond is involved in the oxidation, the process could be facilitated by the formation of a complex such as that drawn in Fig. 3. Of course, the formation of complexes of other types cannot be ruled out but the latter are not amenable to π electronic treatments. Finally, the reaction of atomic oxygen alone with the double bond should be taken into account. In this case it is desirable to decide whether the oxygen released in the dissociation of the N—O bond is in a singlet (excited) or triplet (ground) state. Since the process of the formation of pyridine and oxene proceeds in the triplet state, at least one of the products should be in a triplet state. Among all possible

FIG. 3

Complex of Pyridine N-Oxide with the C—C Double Bond
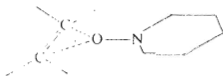
combinations the lowest energy one appears to be that of pyridine in the S_0 state and oxene in the T_1 state. Some authors prefer the addition of the singlet oxene to the C—C double bond[14]. The analogy with the addition of singlet and triplet carbene to olefins does not rule out participation of the triplet oxene in the formation of oxides or arene oxides in the photolysis of pyridine N-oxide or in microsomal oxidations. Recently, a photolysis of pyridine N-oxide was reported[23,24] in which the presence of cis-2-butene gives a mixture of cis- and trans-2,3-butene oxide; here the participation of the triplet oxygen in the oxidation appears to be very likely.

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