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PHOTOLYSIS OF PYRIDINE-N-OXIDE: AN OXYGEN ATOM TRANSFER MODEL FOR ENZYMATIC OXYGENATION, ARENE OXIDE FORMATION, AND THE NIH SHIFT

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The observations of i) intramolecular migration of aryl ring substituents during enzymatic aryl hydroxylation - the NIH Shift,[1] ii) a similar migration of deuterium[2] during nonenzymatic isomerization of 3,4-toluene-4-^{2}H oxide to 4-hydroxytoluene-3-^{2}H, and iii) 1,2-naphthalene oxide as the initial product from the microsomal metabolism of naphthalene[3] provide evidence for the formation of arene oxide intermediates during enzymatic "hydroxylation" of aromatic compounds. Elaboration of chemical oxidants which exhibit the NIH Shift and form arene oxides should permit further insight into the nature of the "active oxygen" involved in enzymatic oxidations.

Since peroxytrifluoroacetic acid does cause the NIH Shift[4], a number of similar but milder oxidants (i.e., oxygen atom transfer reagents[5] causing epoxidation, etc.) were studied with anisole-4-^{2}H. This substrate exhibits a high migration and retention (60%) of deuterium during microsomal hydroxylation[6], while oxidation with peroxytrifluoroacetic acid leads to a low retention (8%). A more satisfactory model than the peracid should result in a higher retention of deuterium with this substrate. Several chemical oxidants were found to be capable of producing the NIH Shift (Table I). Photolysis of aromatic-N-oxides produced generally high deuterium retentions.** In addition, reasonable yields of phenols obtain.

Substituent effects on the degree of deuterium retention during pyridine-N-oxide photolysis were similar to those observed with microsomes (Table II). Addition of acetamide to the photo-

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**Since substituted pteridines are cofactors for phenylalanine hydroxylase, two pteridine-8-N-oxides were also irradiated but no hydroxylation was observed.

lysis medium caused an increase in deuterium retention similar to that noted during the isomerization of 3,4-toluene-4-^{2}H oxide.[2] This result strongly suggests arene oxide intermediates.

The stability of benzene oxide and 1,2-naphthalene oxide was studied under the photolytic conditions (CH_2Cl_2 solvent, 25°, 20 min) before attempting to demonstrate arene oxide formation. When the concentration of pyridine-N-oxide was sufficient to absorb nearly all the light, 1,2-

Table I. Oxidants Which Cause Aryl Hydroxylation and Produce the NIH Shift[a]

Oxidant	% Deuterium Retention in 4-Hydroxyanisole
9-diazofluorene, O_2, hν[7]	16[b]
N_2O, hν[8]	25[c]
t-Bu-OOH, $Mo(CO)_6$[9]	58[d]
dimethylaniline-N-oxide, hν	20[e]
pyridazine-N-oxide, hν	34[e]
pyridine-N-oxide, hν	45[e]
pyrazine-N-oxide, hν	52[e]

[a]All irradiations (Nuclear Supplies, Inc. low pressure mercury lamp, model W-K^2, 2537 Å) were done in quartz cells for 10-20 min with N_2 bubbled through. [b]Anisole solvent with O_2 rather than N_2 bubbled through. The migration product observed here may result via a minor oxygen atom transfer pathway since radicals[7] do not cause the NIH Shift.[4] [c]Gas phase. [d]Anisole solvent in sealed tube at 60°. [e]CH_2Cl_2 solvent, phenol and epoxide formation has been reported [10,11,12] during photolysis of aromatic-N-oxides.

Table II. Comparison of Deuterium Retentions

Substrate-4-^{2}H	% Retention in 4-Hydroxylated Product	
	Microsomes	N-Oxide Photolysis[a]
anisole	60	45[b]
chlorobenzene	54	62
bromobenzene	40	49
acetanilide	30	28
toluene	54	59[c]

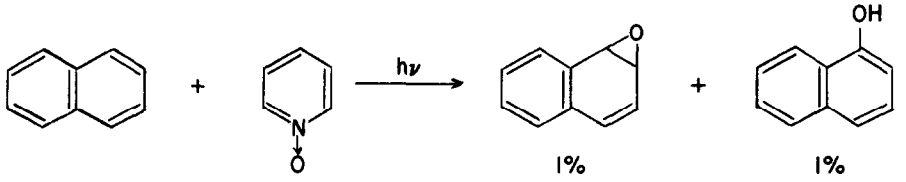
[a]Values for CH_2Cl_2 solutions ~25°. [b]Retention at -78° = 60%. [c]Substrate as solvent.

Table III. Range and Scope of Photolytic Oxidation

Substrate	Products	Ratio[a]
anisole[b]	2-hydroxyanisole	1
	4-hydroxyanisole	2
	phenol	1
tetralin	5-hydroxytetralin	3
	6-hydroxytetralin	3
	1-hydroxytetralin	2
naphthalene	naphthol[c]	1
	1,2-naphthalene oxide	1
cyclohexane	cyclohexanone	1
	cyclohexen-3-ol	1
	cyclohexene oxide	4
styrene	acetophenone	1
	styrene oxide	10
tetrahydrothiophene	sulfoxide	100
	sulfone	1

[a]In a typical experiment 4 mM substrate and 20 mM pyridine-N-oxide were irradiated 20 min with N_2 bubbled through the solution. The yields (1-4%) were kept low to prevent secondary reactions. Products were separated and identified by combined gas chromatography - mass spectrometry. [b]Dealkylation occurs only in aqueous solution. [c]About 95% 1-naphthol.

naphthalene oxide was quite stable, while benzene oxide completely isomerized to phenol. Without the pyridine-N-oxide, 1,2-naphthalene oxide photoisomerized to naphthol. Solutions of naphthalene (10 mg) and pyridine-N-oxide (100 mg) were irradiated (2537 Å) in CH_2Cl_2 (4 ml) for 20 min. Analysis of the reaction mixture (tlc) showed Gibbs positive[4] spots corresponding to 1,2-naphthalene oxide (0.14 mg) and naphthol (0.13 mg). Whether the naphthol (~95% 1-



naphthol) is produced in the photolysis via 1,2-naphthalene oxide by oxygen addition or directly by oxygen insertion is under investigation. The combined solutions from 10

irradiations were washed (aqueous $NaHCO_3$), dried (Na_2SO_4), reduced to a small volume (in vacuo, <20°), and subjected to countercurrent distribution.[4] Tubes showing the presence of 1,2-naphthalene oxide (uv spectrum) were pooled, subjected to a second countercurrent distribution, and concentrated to provide ~1 mg of 1,2-naphthalene oxide; the nmr spectrum of which confirmed the assigned structure. This constitutes the first example of chemical epoxidation of an aromatic double bond.

Pyridine-N-oxide photolysis as a mechanistic model for enzymatic oxidation is capable of many oxidation reactions typical of mixed function oxidases; i.e., aliphatic hydroxylations, dealkylations, S-oxidations, and epoxidation of olefins as well as aromatic hydroxylation (Table III). Further results on the nature and mechanisms of the oxygen atom transfer reactions studied here and their relationship to enzymatic oxygenations will be presented shortly.

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