Tetrahedron Letters,Vol.23,No.7,pp 753-756,1982 0040-4039/82/070753-04$03.00/0
Printed in Great Britain

A MODEL FOR FAD-CONTAINING MONOOXYGENASE THE OXIDATION OF THIOANISOLE DERIVATIVES BY AN ISOALLOXAZINE HYDROPEROXIDE

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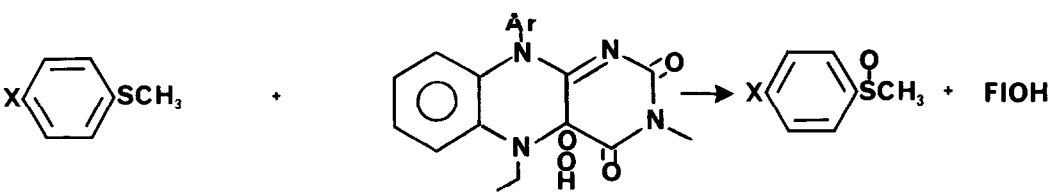
ABSTRACT A 4a-isoalloxazine hydroperoxide oxidizes thioanisole derivatives to the corresponding sulfoxides by a mechanism which involves nucleophilic attack of sulfur on electrophilic oxygen

Since the elegant work of Entsch, Ballou and Massey with p-hydroxybenzoate hydroxylase,[1] it has been generally accepted that oxidations by flavin monooxygenases proceed via a 4a-hydroperoxide FAD-containing monooxygenase, an enzyme in high concentration in the liver which oxidizes many different xenobiotic substances including a wide variety of sulfur-containing functionalities, is no exception to this hypothesis [2,3] What has been less clear is the mechanism (or mechanisms) by which flavin hydroperoxides effect oxidation. (Compare, for example, references 4-8.) Bruice and coworkers have reported that flavin 4a-hydroperoxide oxidizes thioxane to the S-oxide by a second order process which is considerably faster than the same oxidation effected by hydrogen peroxide or t-butyl hydroperoxide.[7] Speculation by Bruice[8] and a recent paper by Oae and coworkers on the oxidation of thioanisoles by cytochrome P-450[9] suggest that the mechanism with flavin may involve an initial electron transfer between the hydroperoxide and the sulfide to give a sulfur cation radical As a test of this mechanism versus the usual mechanism for oxidation of sulfides by peroxides (nucleophilic attack of sulfur on electrophilic oxygen)[10] we chose to investigate the rates of oxidation of a set of substituted thioanisoles, 1, by 4a-isoalloxazine hydroperoxide, 2



1 2 (FlOOH) 3

Ar = 2,6-dimethylphenyl

MATERIALS: Thioanisole was purchased from Aldrich Chemcial Co All other thiocompounds were synthesized by literature procedures Liquids were purified by fractional distillation under reduced pressure; solids were recrystallized to constant melting point. FlOOH, 2, was synthesized by the literature procedure.[11] The solvent for kinetic studies, t-butyl alcohol, was dried by refluxing over calcium hydride for two days and distilled with protection from moisture.

PRODUCT STUDY A solution of 0.020 g ($5 \cdot 10^{-5}$ moles) of FlOOH in 100 ml t-butyl alcohol was treated with 0.010 g ($6\ 5 \cdot 10^{-5}$ moles) of p-methoxythioanisole in 5 ml of t-butyl alcohol. The mixture was allowed to stand in the dark for several days. Removal of the solvent in vacuo followed by thick-layer chromatography (silica gel GF, ethyl acetate, eluant) gave 0.009 g (106%) of a pale yellow oil whose infrared spectrum was identical to that of authentic (p-methoxyphenyl)-methyl sulfoxide.[12] Tlc showed that 4a-hydroxyisoalloxazine, 3, was the major product from 2.

KINETIC STUDIES: A known amount of an approximately $2.5 \cdot 10^{-4}$ M solution of FlOOH in t-butyl alcohol was pipetted into the bottom of a Thunberg curvette. Into the top was pipetted a known amount of neat liquid sulfide or a solution of sulfide in t-butyl alcohol of known concentration. The concentrations of sulfide used were approximately 50-500 times that of FlOOH. The samples were thermally equilibrated at 30^{0} in a Cary 219 spectrophotometer for at least 0.5 hr and then mixed. The absorbance at 400 nm was measured continuously for the fast oxidations and at precise time intervals for the slow oxidations. The reactions were followed to at least three half lives and excellent pseudo first order kinetics were observed. Plots of first order rate constants vs concentration gave the second order rate constants reported in the table. All rate constants were determined by the least squares method.

A Hammett plot of the rates vs σ gave a slope (ρ) of -1.67 ($r = -0.994$) while a plot vs σ^{+} gave a slope (ρ^{+}) of -1.01 ($r = -0.979$). Thus the σ plot shows a better correlation coefficient than the σ^{+} plot. This result with a comparison of the ρ value of our reaction with those of similar reactions of thioanisoles strongly suggests that the mechanism of reaction of FlOOH with thioanisoles involves nucleophilic attack of sulfur, with little or no electron transfer. For example, using the σ values of Exner (see table) to recalculate data in the literature it is found that the oxidation of thioanisoles with hydrogen peroxide

gives ρ= -1.28 (aqueous ethanol)[13] while oxidation with singlet oxygen gives ρ = -1.63 (chloroform)[14] and ρ = -1.5 (methanol).[15,16] Since these reactions are believed to occur with little or no electron transfer, our reaction must involve the same mechanism. We anticipate that formation of a sulfur cation radical would entail an electron demand considerably greater than that observed. (See reference 15).

Table. Rates of Reaction of p-Substituted Thioanisoles with 2

p-Substituent in 1	Rate ($\ell M^{-1}sec^{-1}$)	σ^a	σ^{+a}
-CN	0.00307	0.70	0.70
-benzoyl	0.00781	0.46	0.46
-Cl	0.0198	0.24	0.11
-H	0.0377	0	0
$-CH_3$	0.0762	-0.14	-0.31
-NHAc	0.0815	-0.09	-0.6
$-OCH_3$	0.103	-0.28	-0.78
$-NH_2$	0.497	-0.57	-1.3

[a] O. Exner in N. B. Chapman and J. Shorter, "Correlation Analysis in Chemistry", Plenum Press N.Y., 1978, pp 439-540.

The oxidation of thioanisoles to sulfoxides by cytochrome P-450 (see above) and by dopamine β-hydroxylase[18] has been reported. Unfortunately, there are problems with these accounts which prevent a significant comparison with flavin hydroperoxide. The correlations made by Oae and coworkers with cytochrome P-450[9] are not meaningful for several reasons. First, it is unlikely that the slow step in oxidation by cytochrome P-450 is the oxidation of sulfur.[19] Since the rates measured were those of the reoxidation of NADPH, it is unlikely that the Hammett correlation of the data corresponds to the oxidation of sulfur. Also, the fact that one of the four substituted thioanisoles studied shows a V_{max} clearly out of line with the others precludes any meaningful linear free energy correlation of the data. In the work with dopamine β-hydroxylase the specificity of the enzyme precluded the study of a variety of substituents such that only thioanisole and the p-fluoro, chloro and bromo compounds gave meaningful results. Because of the small number of substituents and the small range of σ values for them (less than 0.3), the slope of the Hammett plot is clearly uncertain. It is curious that the ρ value of -3.6 (!) is claimed to be similar to that reported in Modena's work (-1.17).[13]

In conclusion, the results of this study strongly suggest that the oxidation of sulfides by FAD-containing monooxygenase occurs by nucleophilic attack of sulfur on the electrophilic oxygen of the flavin hydroperoxide with little or no electron transfer. Thus, this result rules out an electron transfer mechanism for the oxidation of sulfur as the reason for the rapid rate of oxidation of sulfides by flavin hydroperoxide relative to that by hydrogen peroxide and t-butyl hydroperoxide (see above).

ACKNOWLEDGMENTS The author wishes to acknowledge the gracious generosity of Professor Edward Leadbetter in the use of a Cary 219 spectrophotometer and helpful discussions with Professors Wassmundt and Rossi.

Footnotes

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(Received in USA 27 August 1981)