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The NIH Shift During the Hydroxylation of Aromatic Substrates by Fungi

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Summary The migration and retention of deuterium which occurs during aromatic hydroxylation of several substrates (NIH Shift) with a range of fungi is compared to the migration and retention obtained with liver microsomes and a chemical model system; enzymatic mechanisms are discussed in terms of arene oxide intermediates.

The fungus, *Aspergillus niger*, effects hydroxylation,[1] *O*-demethylation,[2] and *S*-oxidation[3] of aromatic compounds in a manner similar to that observed for the mono-oxygenases of hepatic microsomes. Since mono-oxygenases also appear to be involved in the fungal oxidations,[4] intramolecular migration of aromatic ring substituents (the NIH Shift) may occur. Such migrations have previously been observed with mono-oxygenases from plant, animal, and bacterial sources.[5,6] A migration of halogen during the metabolism of 2,4-dichlorophenoxyacetic acid by *A. niger*[1] is analogous to the formation of 3-chlorotyrosine from 4-chlorophenylalanine catalysed by mammalian or bacterial phenylalanine hydroxylase.[7] Migration and retention of tritium occurs during metabolism of *meta*-tritiophenylalanine to cyclopenin and cyclopenol in *Penicillium cyclopium*.[8]

The extent of intramolecular migrations during fungal metabolism has now been examined using several specifically deuteriated aromatic substrates and a range of fungi. The majority of the fungi in Table 1, with the exception of *A. niger*,[1] have not previously been reported to effect hydroxylation of aromatic substrates. A broad substrate specificity and a multiplicity of oxidative pathways was observed. The pathways included *para*- and *ortho*-aryl hydroxylation, *O*-demethylation, and benzylic hydroxylation. The yields and proportions of various products depended on the stage of growth of the micro-organism. *O*-Demethylation was favoured during early stages of growth, while *ortho*-hydroxylation became preponderant in the mature fungi.

The extent of deuterium migration and retention was determined after incubations using both normal and replacement shake culture techniques (Tables 1 and 2).

Table 1

Fungus	% D-retention of 4-hydroxyanisole produced by oxidation of 4-D-anisole	% D-retention of 2-hydroxyanisole produced by oxidation of 2-D-anisole
Aspergillus niger (NRRL337)	69	
Aspergillus niger (NRRL382)	71	
Sporotrichum sulphurescens		52
Cunninghamella elegans	71	57
Rhizopus arrhizus	72	53
Helicostylum piriforme		40
Mucor parasiticus	72	35
Rhizopus stolonifer		39
Culvularia falcata		40

For anisole, *para*-hydroxylation led to a 69—72% migration and retention of deuterium with the fungi of Table 1. *ortho*-Hydroxylation was accompanied by a migration and retention of 35—57%. These values are similar to those observed with anisole for hepatic microsomes[9,10] and certain chemical oxidants[11] (unpublished results). *ortho*- or *para*-Hydroxylation of deuteriated anisoles was not accompanied by a primary isotope effect in oxidations with either the micro-organisms or with hepatic microsomes.[10]

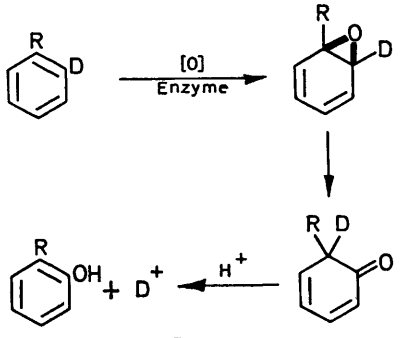
TABLE 2[a]

R Substrates (Ph–R)	Deuterium label position	Product	*A. Niger* (NRRL382)[b]	% Retention chemical model system	Hepatic microsomes[d]
1-OMe	2-D	2-OH			60
1-OMe	4-D	4-OH	71	45	60
1-OCH_2CO_2H	2,4-D	2-OH	4	23	
1-Me	2-D	2-OH	11		43
1-Me	4-D	4-OH	49	59	59
1-CH_2CO_2H	2-D	2-OH	7		
2-Naphthyloxyacetic acid	6-D	6-OH	13		

[a] Synthesis of labelled compounds, separation of products and methods used in determination of D-contents will be detailed in the full paper. [b] Results represent an average value of several experiments. [c] Photolysis of pyridine *N*-oxide.[11] [d] Determined with hepatic microsomes as described.[9,10]

A detailed comparison of migration and retentions of deuterium following aryl hydroxylation of a range of substrates was made using *A. niger*, a representative fungus, and the hepatic microsomal system. The values for retentions after *para*-hydroxylation of anisole and toluene were comparable for *A. niger*, microsomes and a chemical oxidant[11] thought to function *via* an "oxene" mechanism[11,12] (Table 2). These results are consistent with the formation of arene oxide intermediates which then rearrange *via* the NIH Shift to phenols.[13]

There are, however, several unexpectedly low retentions reported in Table 2 which do not appear to be compatible with the usual 2,3- or 3,4-arene oxide intermediates. A possible explanation for the very low retentions (4—11%) obtained during *ortho*-hydroxylation of phenoxyacetic acid, phenylacetic acid, and toluene may be that these compounds preferentially form 1,2-arene oxides involving migration of either D or R which on isomerization must lose deuterium (see Scheme). This hypothesis is under investigation with



SCHEME

alkylbenzenes in which the course of rearrangement of relevant arene oxides has been determined.[14] Such an explanation does not, however, hold in the case of 6-hydroxylation of 2-naphthyloxyacetic acid where a low retention of 13% occurred.

The intermediacy of an arene oxide isomerase, suggested to be present in certain plants and to effect the stereoselective isomerization of arene oxides to phenols,[15] appears to require further investigation. Such a hypothesis seems inconsistent with previous[16,17] and more recent data on the NIH Shift in plants[18] and animals[18] with a variety of substrates including deuteriated and tritiated phenylalanines, acetanilides, anisoles, and cinnamic acids. The results obtained for hydroxylation of 4-D-anisole (Table 1) and 3,5-dideuterioanisole with *A. niger* and other fungi are also inconsistent with the presence of an isomerase that selectively removes isotopic label from the position adjacent to that of hydroxylation. Thus, during *para*-hydroxylation of 3,5-dideuterioanisole, 36% deuterium is lost from the adjacent position while in *ortho*-hydroxylation only 15% is lost. Even those losses are, however, much greater than are observed with animal systems *in vivo* and *in vitro*.[18] The biological significance and possible practical importance of aryl hydroxylation by fungi is, as yet, a relatively unexplored area; of relevance is the recently reported formation of DOPA from tyrosine with a variety of fungi.[19] Several generalizations, however, can now be made: (i) the aryl oxidative enzymes of fungi appear to be similar to the mono-oxygenases of hepatic microsomes since both display the NIH Shift and have broad substrate specificities, (ii) *ortho*-hydroxylation to ring substituents is usually predominant in fungi while *para*-hydroxylation tends to predominate in hepatic systems, (iii) polar substrates are readily oxidized in fungi, but are not readily oxidized in hepatic systems, and (iv) there is no evidence for the presence of a stereoselective arene oxide isomerase in these microbial systems.

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