View Article Online / Journal Homepage / Table of Contents for this issue

Regioselective Dihydroarene Oxide Formation during *ortho*-Hydroxylation of Halogenobenzenes by Fungi

Barbara J. Auret, Suresh K. Balani, Derek R. Boyd,* and Ruth M. E. Greene
Department of Chemistry, The Queen's University of Belfast, Belfast, BT9 5AG, N. Ireland
Glenn A. Berchtold *
Department of Chemistry, The Massachusetts Institute of Technology, Cambridge, Mass 02135, U.S.A.

ortho-Hydroxylation of chloro- and bromo-benzene occurred in the presence of growing cultures of the fungi *Rhizopus arrhizus, Rhizopus stolonifer*, and *Cunninghamella elegans*. The absence of a primary kinetic isotope effect and the presence of the NIH shift are consistent with dihydroarene oxides being initial metabolites. N.m.r. analysis of the deuterium-labelled *o*-halogenophenol products suggests that enzyme-catalysed epoxidation occurs preferentially at the 2,3-bond.

Published on 01 January 1984. Downloaded by East Carolina University on 09/08/2013 11:08:24.

The mechanism of enzyme-catalysed hydroxylation of arenes has received considerable attention over the past 15 years.[1] The intermediacy of dihydroarene oxides in such hydroxylations has been inferred from (*a*) the metabolites which have been identified (phenols, dihydrodiols and premercapturic acids); (*b*) the migration of labelled hydrogen atoms (^2H, ^3H) from the site of hydroxylation to a neighbouring carbon atom, and the retention of a substantial proportion of the label (NIH shift); (*c*) the detection of dihydroarene oxide metabolites of several polycyclic aromatic hydrocarbons which were found to isomerize to phenols.

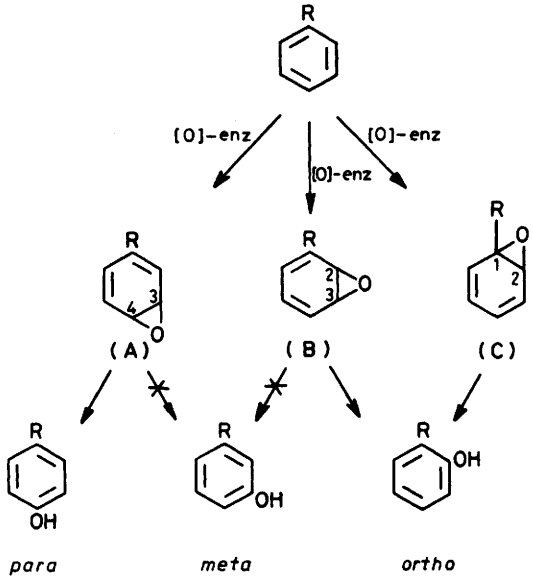
The problem of stereoselectivity occurring during the initial epoxidation step has been examined for polycyclic aromatic hydrocarbons. Thus, it is now clearly established that mono-oxygenase enzymes from mammalian liver catalyse the addition of an oxygen atom exclusively to one stereoheterotopic face of benz[*a*]anthracene[2] and benzo[*a*]pyrene.[3]

The enzyme-catalysed epoxidation of a monosubstituted arene may be regioselective, *i.e.* preferential formation of a dihydroarene 1,2- (C), 2,3- (B) or 3,4-oxide (A) (Scheme 1). Formation of a *para*-substituted phenol can only arise by isomerization of a 3,4-oxide (A) and thus this type of regioselectivity is readily confirmed.

In principle, it is possible for a dihydroarene 2,3- (or 3,4-) oxide to isomerize to a *meta*-phenol (Scheme 1), but in practice only an *ortho* (or a *para*) phenol appears to be formed.[4–6] *meta*-Substituted phenols are also generally only observed as very minor products both in animal[7] (*para*-isomer favoured) and fungal[8–11] (*ortho*-isomer favoured) metabolism. On the basis of studies with labelled substrates,[12] evidence has been obtained that where enzyme-catalysed *meta*-hydroxylation of mono-substituted arenes occurs, dihydroarene oxide intermediates are not generally involved. The question of regioselectivity occurring during epoxidation of monosubstituted arenes, as the initial step toward the formation of *meta*-substituted phenols, thus appears to be of less relevance.

Regioselectivity is, however, of considerable importance in the process of *ortho*-hydroxylation of monosubstituted arenes which can occur *via* either dihydroarene 1,2- (C) or dihydro-arene 2,3-oxide (B) intermediates (Scheme 1). Using a wide range of fungi both in these[8,9] and other[10,11] laboratories, the major metabolites of monosubstiuted aromatic rings have generally been *ortho*-phenols. Possible *ortho*-hydroxylation products from the metabolism of ^2H labelled monosubstituted arenes are shown in Scheme 2 (X = Cl or Br).

Both dihydroarene 1,2- (**1**)—(**3**) and 2,3-oxide (**4**)—(**6**) derivatives of monosubstituted arenes will isomerize to phenols (**7**)—(**11**). If a substrate is selected where substituent X can



Scheme 1.

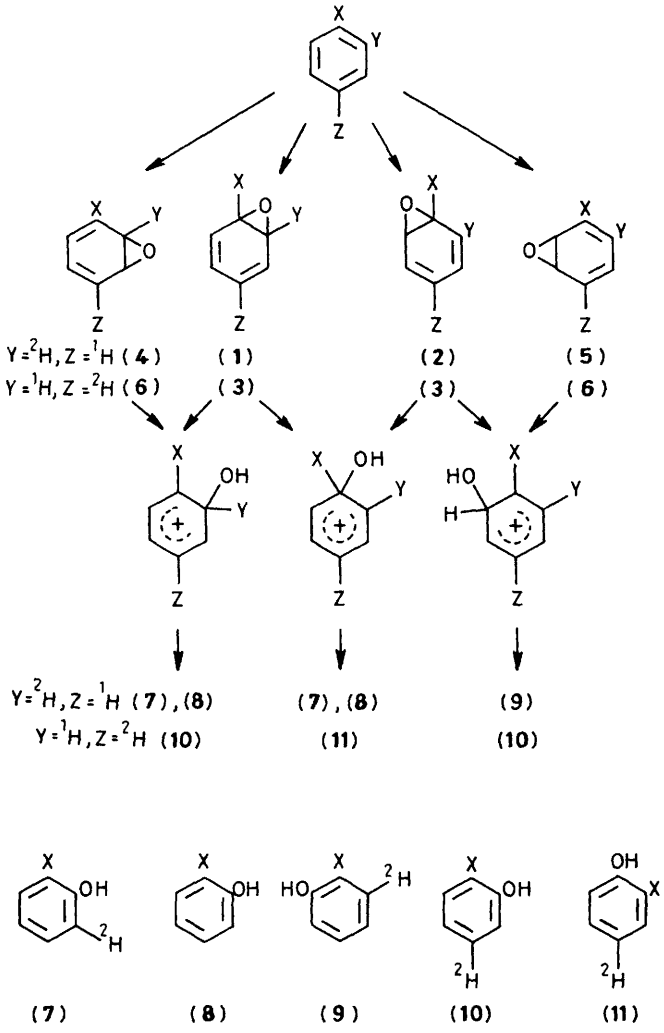
readily migrate then the phenol product (**11**) can only be formed *via* a dihydroarene 1,2-oxide.

The synthesis and aromatization of a series of arene 1,2-oxides[5,13–16] indicated that the only group (X) (from the range studied) to show a strong migratory aptitude was alkoxy-carbonyl ($-CO_2R$). Preliminary experiments with fungi revealed that substrates containing a methoxycarbonyl group were readily hydrolysed to the parent carboxylic acid, presumably owing to the presence of esterase enzymes. Using t-butyl benzoate as substrate, however, it was possible to observe aromatic hydroxylation without complete ester hydrolysis occurring. Thus, *para*-hydroxylation was observed using *Aspergillus foetidus*, *Rhizopus arrhizus*, and *Cunninghamella echinulata* (Table 1). Since *ortho*-hydroxylation of t-butyl benzoate could not be detected using the range of fungi indicated, no further investigations of arenes substituted with a alkoxycarbonyl group were carried out.

The possibility of using chlorobenzene and bromobenzene as substrates was examined since both chlorine and bromine atoms (X) are known to migrate readily. The metabolism of chlorobenzene[6,17–19] and bromobenzene[20] in mammals and mammalian liver systems has been extensively studied. The

View Article Online

Published on 01 January 1984. Downloaded by East Carolina University on 09/08/2013 11:08:24.



Scheme 2.

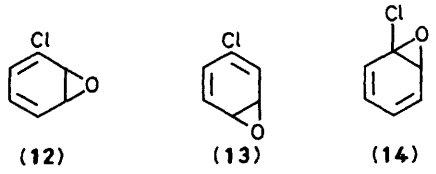
intermediacy of dihydroarene 3,4-oxides of chlorobenzene[6] (or bromobenzene[20]) has been established by using ^{14}C labelled chlorobenzene and chemically synthesised 1-chloro-3,4-dihydrobenzene 3,4-oxide (or by the isolation of *trans*-glutathione conjugates of bromobenzene). To date it has not proved possible to distinguish between the involvement of either dihydroarene 1,2-oxide or 2,3-oxide metabolites during mammalian metabolism of chlorobenzene or bromobenzene to form *ortho*-halogenophenols. The aromatic hydroxylation of chlorobenzene has previously been reported using the fungi *Aspergillus ochraceous* and *Rhizopus stolonifer*.[11] Unfortunately, the chlorobenzene substrate used in this microbial study was unlabelled and no information was available on the possible involvement of dihydroarene oxide intermediates.

The migration of a chlorine atom during enzyme-catalysed hydroxylation of aromatic rings has been observed in animal, plant, and microbial systems.[21–29] The strong migratory aptitude of a chlorine atom during isomerization of an α-chloro epoxide to an α-halogeno ketone is well established.[30–34] On the basis of this evidence it appears reasonable to assume that the enzyme-catalysed aromatic hydroxylations accompanied by a chlorine atom migration[21–29] have occurred *via* dihydroarene oxides bearing a chlorine atom on the oxirane ring. Thus, the formation of dihydroarene oxide intermediates of type (**1**) or (**2**) (where X = Cl or Br) would be expected to isomerize to form *ortho*-halogenophenols with concomitant halogen migration.

Table 1.

	Chlorophenol metabolites[a]		Hydroxy t-butyl benzoate metabolites[a]	
	ortho	*meta*/*para*	*ortho*	*meta*/*para*
1. *Aspergillus foetidus*	+	+	−	++
2. *Aspergillus ochraceous*	−	−	−	−
3. *Rhizopus stolonifer*	+++	+	−	−
4. *Rhizopus arrhizus*	+++	+	−	++
5. *Cunninghamella elegans*	++	+	−	(+)[b]
6. *Cunninghamella echinulata*	+	+	−	+
7. *Mortierella isabellina*	+	−	−	−
8. *Helminthosporium sp.*	−	−	−	−

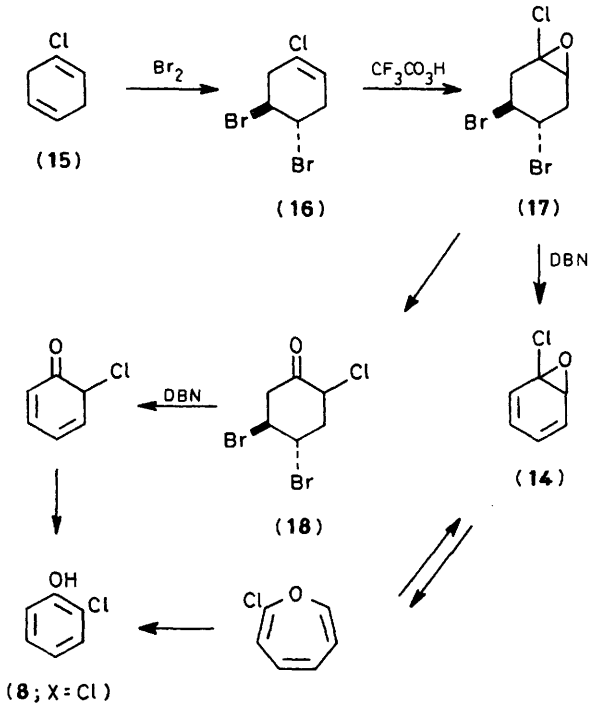
[a] Relative proportions based upon g.l.c.–m.s. analysis. [b] Impurity peak superimposed.



While the dihydroarene 2,3- (**12**) and 3,4-oxides (**13**) of chlorobenzene have previously been synthesised,[6] the dihydroarene 1,2-oxide (**14**) has not to date been synthesised chemically.

As part of the present investigation a synthesis of (**14**) based upon the previous route to the dihydroarene oxide (**13**),[6] was undertaken (Scheme 3).

The diene (**15**) was prepared by a literature method[6] and was readily converted into the dibromo adduct (**16**). The dibromo



Scheme 3.

View Article Online

Published on 01 January 1984. Downloaded by East Carolina University on 09/08/2013 11:08:24.

Table 2.

Expt.	Substrate (%D)	Fungus	Deuterium content of *o*-halogenophenol[a] (%D)
(a)	Chlorobenzene (0) –perdeuteriochlorobenzene (100)[b]	*R. arrhizus*	51[b]
(b)	Chlorobenzene (0) –perdeuteriochlorobenzene (100)[b]	*R. stolonifer*	50[b]
(c)	Bromobenzene (0) –perdeuteriobromobenzene (100)[c]	*R. arrhizus*	50[c]
(d)	Bromobenzene (0) –perdeuteriobromobenzene (100)[c]	*R. stolonifer*	50[c]
(e)	(*o*-^{2}H)-Chlorobenzene (96)	*R. arrhizus*	67 (39)[d]
(f)	(*o*-^{2}H)-Chlorobenzene (96)	*R. stolonifer*	73 (52)[d]
(g)	(*o*-^{2}H)-Bromobenzene (97)	*R. arrhizus*	69 (44)[d]
(h)	(*o*-^{2}H)-Bromobenzene (97)	*R. stolonifer*	64 (33)[d]
(i)	(*p*-^{2}H)-Chlorobenzene (94)	*R. arrhizus*	>95[e]
(j)	(*p*-^{2}H)-Chlorobenzene (94)	*R. stolonifer*	>95[e]
(k)	(*p*-^{2}H)-Chlorobenzene (94)	*C. elegans*	>95[e]
(l)	(*p*-^{2}H)-Bromobenzene (96)	*R. arrhizus*	>95[e]
(m)	(*p*-^{2}H)-Bromobenzene (96)	*R. stolonifer*	>95[e]
(n)	(*p*-^{2}H)-Bromobenzene (96)	*C. elegans*	>95[e]

[a] Mass spectral analysis (±3%). [b] Used as a mixture of chlorobenzene–perdeuteriochlorobenzene (1:1). [c] Used as a mixture of bromobenzene–perdeuteriobromobenzene (1:1). [d] NIH shift values, corrected for incomplete labelling of substrate. [e] Approximately (±5%) based upon n.m.r. analysis.

olefin (**16**) was purified by flash distillation, characterized by n.m.r. analysis, and used directly. Peroxytrifluoroacetic acid in the presence of disodium hydrogen phosphate was used as oxidant in the synthesis of the epoxide (**17**). The latter product was insufficiently stable to allow final purification by chromatography or distillation. Confirmation of the structure of compounds (**16**) and (**17**) relied upon spectroscopic analysis (i.r. and n.m.r.). The dihydroarene oxide (**14**) could not be detected on treatment of (**17**) with base; but since *o*-chlorophenol (**8**; X = Cl) was the major product, this provides evidence to support the conclusion that the dihydroarene oxide (**14**) had been formed initially but had rapidly and spontaneously isomerized to (**8**; X = Cl). Although i.r. spectroscopy confirmed that the α-chloro epoxide (**17**) was free from the chloro ketone (**18**) before the final dehydrobromination step, the rearrangement of (**17**) to (**18**) during the course of the reaction cannot be totally excluded. Since α-chloro epoxides are relatively unstable compounds it was expected that chlorodihydrobenzene 1,2-oxide (**14**) would be extremely unstable.

A range of fungi was initially examined for their ability to produce *o*-chlorophenol (**8**; X = Cl) as a major metabolite of chlorobenzene. The selection of fungi was made from those previously found to be capable of aromatic hydroxylation or other oxidation reactions,[8–11] and the results are summarized in Table 1.

The maximum recovered yields were found for the fungi *Rhizopus arrhizus*, *Rhizopus stolonifer*, and *Cunninghamella elegans* and the results obtained (Table 2) are confined to these three species.

In order to exclude the possibility of aromatic hydroxylation occurring by a direct C–H insertion mechanism (as found in aliphatic hydroxylation[2,7] and *meta*-hydroxylation of monosubstituted arenes[12]), equivalent quantities of chlorobenzene and perdeuteriochlorobenzene (or bromobenzene and perdeuteriobromobenzene) were added as substrates to cultures of *R. arrhizus* and *R. stolonifer* (Table 2). In experiments (**a**—**d**) the proportion of perdeuteriated phenol metabolite (50—51% $^2H_4$) was almost identical to the proportion of perdeuteriated halogenobenzene substrate (50% $^2H_5$). The absence of a primary kinetic isotope effect in experiments (**a**—**d**) cannot provide unequivocal evidence against a direct insertion mechanism. Nevertheless, based upon previous reports of primary kinetic isotope effects observed in experiments of this type,[1,2] this negative evidence appears to favour an arene oxide pathway.

The strongest positive evidence of a dihydroarene oxide intermediate being formed during enzyme-catalysed hydroxylation of a monocyclic arene is the presence of the NIH shift.[1] Values of the NIH shift observed during the enzyme-catalysed *ortho*-hydroxylation of [2-^{2}H]-labelled toluene (11—43%[4,9]), and [2-^{2}H]-labelled anisole (35—67%[7–9]) are generally comparable with values obtained during *para*-hydroxylation. The magnitude of the NIH shift values observed during fungal *ortho*-hydroxylation of [2-^{2}H]-labelled chlorobenzene (experiments **e** and **f**, 39—52%) and [2-^{2}H]-labelled bromobenzene (experiments **g** and **h**, 33—43%) are comparable with the value obtained from *para*-hydroxylation of [4-^{2}H]-chlorobenzene (54%) using mammalian liver enzymes.[7] Although the NIH shift values recorded in experiments (**e**—**h**) were determined by g.l.c.–m.s. analysis, the 250 MHz n.m.r. spectra of the purified *o*-halogenophenol metabolites confirmed these results and established that a substantial proportion of the ^{2}H atoms had migrated from C-2 to C-3. The present observations of (i) the lack of a primary kinetic isotope effect and (ii) the presence of the NIH shift provide compelling evidence in favour of arene oxide intermediate formation during the *ortho*-hydroxylation of chlorobenzene and bromobenzene by fungi.

The formation of *o*-halogenophenol [(**11**) Scheme 2)], if observed, may be taken as clear evidence of halogen migration during the enzyme-catalysed *ortho*-hydroxylation. This metabolic pathway could be most readily explained by an arene 1,2-oxide intermediate (**3**) and thus the use of [4-^{2}H]-labelled chlorobenzene or bromobenzene substrates could provide evidence of regioselective formation of an arene 1,2-oxide.

The *o*-halogenophenol products obtained in experiments (**i**—**n**) were readily purified by preparative t.l.c. on silica gel. The aromatic protons in these phenols were clearly resolved by 250 MHz n.m.r. spectroscopy (Figure). The proton assignments previously reported[35,36] were confirmed by extensive proton decoupling. The n.m.r. spectra were virtually identical for both *o*-chloro- and *o*-bromophenols and thus only the spectrum of compound (**8**; X = Cl) is shown (Figure).

The presence of the ^{2}H-labelled *o*-halogenophenol metabolite (**11**; X = Cl or Br) would be indicated by a reduction in intensity of the 4-H signal at (δ 6.86) relative to the 3-H, 5-H, and 6-H signal intensities which should remain constant. The n.m.r. spectrum of the *o*-chlorophenol metabolite of [4-^{2}H]-chlorobenzene produced by enzymes present in *R. arrhizus* (experiment **i**) is indicated in the Figure. The signal centred at δ 7.18 due to proton 5-H had been significantly reduced in intensity (>90% reduction) and the coupling between 5-H and the other protons had been largely eliminated. These observations are consistent with the presence of a high proportion of *o*-chlorophenol (**10**) (X = Cl). A closer examination of the weak proton signals centred at δ 7.18 suggest that they may be accounted for by non-deuteriated *o*-chlorophenol (**8**; X = Cl) formed from the small proportion (*ca.* 6%) of unlabelled substrate) and by a smaller contribution from the product of chlorine migration (**11**; X = Cl). Virtually identical results were obtained in experiments (**i**—**n**) (Table 2) using both chlorobenzene and bromobenzene as substrates and the fungi *R. arrhizus*, *R. stolonifer*, and *C. elegans*.

In view of the very small proportion (<5%) of *o*-halogenophenol (**11**; X = Cl or Br) which appeared to be present from n.m.r. analysis of the metabolites formed in experiments

View Article Online

Published on 01 January 1984. Downloaded by East Carolina University on 09/08/2013 11:08:24.

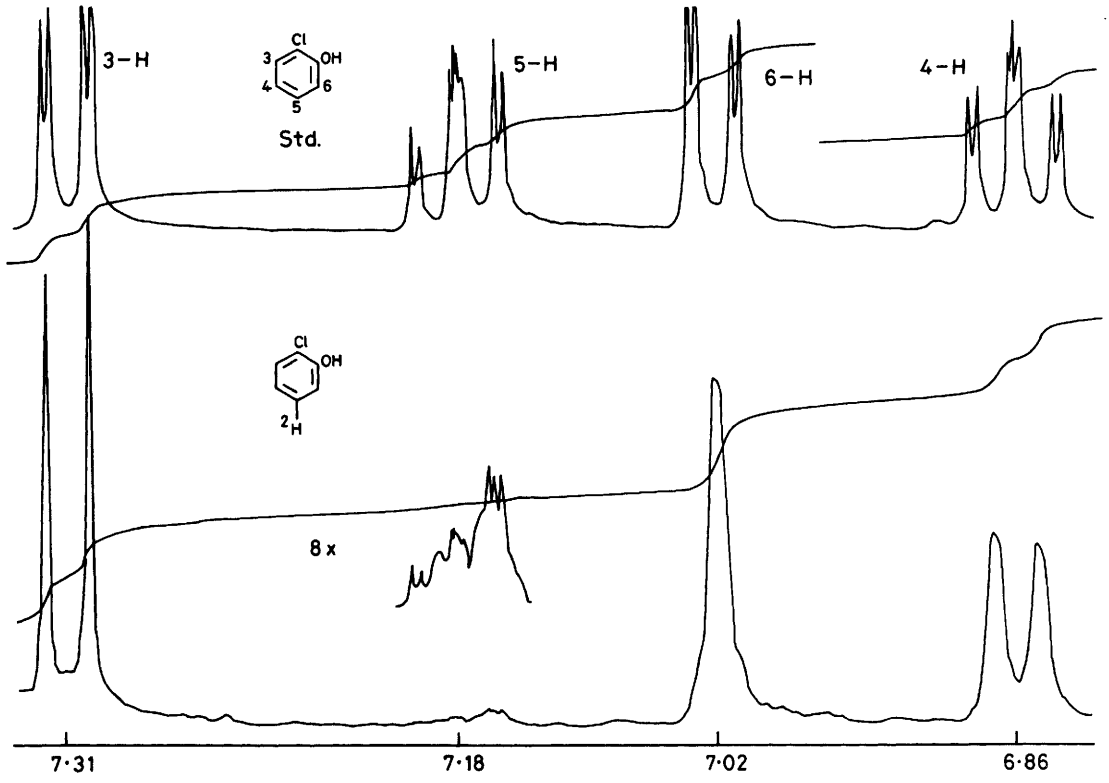


Figure. Aromatic region of the n.m.r. spectra [250 MHz, CCl_4–$(CD_3)_2CO$] of a standard *o*-chlorophenol sample (upper) and of the *o*-chlorophenol metabolite of [4-^{2}H]-chlorobenzene produced by *R. arrhizus* (lower)

(**i**—**n**), it was considered important to establish the degree of random labelling present in the substrate. The 4-^{2}H-labelled samples of chlorobenzene and bromobenzene were formed by decomposition of the appropriate Grignard reagents with deuterium oxide (99% ^2H_2O). These compounds have previously been prepared by this method[37] which has been shown to give high deuterium incorporations with no detectable scrambling of label. The location of the ^{2}H atom at C-4 was established by producing *p*-chlorophenol using Fenton's reagent which is known to effect aromatic hydroxylation with almost total loss of deuterium (*ca.* 97% loss).[37] A similar hydroxylation on chlorobenzene labelled with ^{2}H at C-2 gave *o*-chlorophenol containing 52% of the original ^{2}H atoms. These experiments confirm that the deuterium label was located exclusively at the specified position.

Experiments (**i**—**n**) indicate that *o*-halogenophenol of structure (**10**; X = Cl or Br) is the major metabolite (*ca.* 90%), and that structure (**11**; X = Cl or Br), the product of halogen migration, if present, is only a very minor component (< 5%). Based upon the previous observations of an extensive degree of halogen migration occurring during enzyme-catalysed aromatic hydroxylation,[21–29] the present results suggest that regioselectivity occurs with a marked preference for epoxidation of the 2,3-bond during *ortho*-hydroxylation of monosubstituted arenes in fungi. There is at present no evidence to justify extrapolation of this conclusion to the *ortho*-hydroxylation of halogenobenzenes by hepatic enzyme systems from mammals.

Experimental

^{1}H N.m.r. spectra were obtained from a Brüker WH250 instrument using tetramethylsilane as internal reference and the solvent specified.

Mass spectra were recorded at 70 eV on an AE1-MS902 instrument (updated by V.G. Instruments). G.l.c.–mass spec. analyses were conducted using a Pye-Unicam 204 gas chromatograph in association with an OV-1701 capillary column operating over the temperature range 50—150 °C (12 °C/min) and VG 16F mass spectrometer.

Preparative t.l.c. was carried out using glass (20 × 20 cm) plates coated with Kieselgel $PG_{254,366}$ (Merck).

Microbiological Procedures.—The particular strains of fungi used in the present studies were obtained from either the Northern Regional Research Laboratory, Agricultural Research Service, U.S., Department of Agriculture, Petoria, Illinois (NRRL) or the American Type Culture Collection, Washington, D.C. (ATCC).

1. *Aspergillus foetidus* NRRL 337; 2. *Aspergillus ochraceous* ATCC 1008; 3. *Rhizopus stolonifer* NRRL 1477; 4. *Rhizopus arrhizus* ATCC 11145; 5. *Cunninghamella elegans* ATCC 9245; 6. *Cunninghamella echinulata* ATCC 9244; 7. *Mortierella isabellina* NRRL 1757; 8. *Helminthosporium sp.* NRRL 4671.

All fungi were grown by a shake culture technique (30 °C for 48 h using either Czapek Dox medium (fungi 1—3, 6—8) or a medium containing peptone (10 g), glucose (10 g), cornsteep liquor (10 ml) in water (1 000 ml). All experiments involved addition of substrate (0.034 g) to each of the flasks (× 100) containing the growing cultures in liquid medium (100 ml). The microbial transformation occurred over a period of 48 h, before the acidified (pH 4) medium and mycelium were extracted continuously with dichloromethane (7 days). Preliminary purification of the extract involved passage through a short column of silica gel. Final purification by preparative t.l.c. using benzene–ethylacetate (95:5) as eluant gave the *o*-chloro- or *o*-bromo-phenol metabolites (R_F 0.57). The *o*-halogenophenols were identified by spectral and chromatographic comparison with authentic samples. The final isolated yields of purified *o*-

View Article Online

Published on 01 January 1984. Downloaded by East Carolina University on 09/08/2013 11:08:24.

halogenophenols were found to be low (<1%), but the quantities available were adequate for both mass spectral and n.m.r. analysis.

Perdeuteriochlorobenzene (99% $^2H_5$) and perdeuteriobromobenzene (99% $^2H_5$) were purchased from Aldrich Chemical Co. (2-^{2}H)-chlorobenzene (96% $^2H_1$), (4-^{2}H)-chlorobenzene (94% $^2H_1$), (2-^{2}H)-bromobenzene (97% $^2H_1$), (4-^{2}H)-bromobenzene (96% $^2H_1$) were each produced by formation of a Grignard reagent from the appropriate dihalogenobenzene (*o*-chlorobromobenzene, *p*-chlorobromobenzene, *o*-dibromobenzene and *p*-dibromobenzene) and decomposition by the addition of an excess of deuterium oxide; yields 50—66%. t-Butyl benzoate was prepared in 84% yield from benzoyl chloride and anhydrous t-butyl alcohol in dry pyridine, b.p. 56—58 °C/0.5 mmHg (lit.,[38] 58 °C/0.5 mmHg).

The location of the ^{2}H label was determined by n.m.r. analysis and by hydroxylating the halogenobenzenes using Fenton's reagent.[37] In a typical example, the (2-^{2}H)-chlorobenzene (96% $^2H_1$) upon treatment (2 h, ambient temperature) with Fenton's reagent [ferrous sulphate, EDTA solution, phosphate buffer (pH 6.8), and hydrogen peroxide (30%)] gave a mixture of chlorophenols which was purified by passage through a short column of silica gel (eluting with diethyl ether). G.l.c.–mass spec. analysis indicated that the proportion of ^{2}H-present in the *o*-chlorophenol product was 52%.

N.m.r. spectra of *o*-chlorophenol and *o*-bromophenol: *o*-chlorophenol δ_H [250 MHz; CCl_4–$(CD_3)_2CO$] 6.86 (1 H, ddd, *J* 8.1, 7.4, and 1.5 Hz, 4-H), 7.02 (1 H, dd, *J* 8.1 and 1.5 Hz, 6-H), 7.18 (1 H, ddd, *J* 8.1, and 7.4, and 1.5 Hz, 5-H), and 7.31 (1 H, dd, *J* 8.1 and 1.5 Hz, 3-H); *o*-bromophenol δ_H [250 MHz; CCl_4–$(CD_3)_2CO$] 6.79 (1 H, ddd, *J* 8.1, 7.4, and 1.5 Hz, 4-H), 7.01 (1 H, dd, *J* 8.1 and 1.5 Hz, 6-H), 7.21 (1 H, ddd, *J* 8.1, 7.4, and 1.5 Hz, 5-H), and 7.45 (1 H, dd, *J* 8.1 and 1.5 Hz, 3-H).

Attempted Synthesis of Chloro-1,2-dihydrobenzene 1,2-Oxide (**14**).—The chlorodiene[6] (**15**) (20 g, 0.175 mol) in dichloromethane solution was treated with bromine (28 g, 0.175 mol) at *ca.* 0 °C, and the product (**16**) was isolated by concentration and flash distillation. 4,5-Dibromo-1-chlorocyclohex-1-ene (**16**) was obtained in good yield (88%) after distillation, b.p. 65—88 °C/0.07 mmHg; δ (60 MHz, $CDCl_3$) 5.56—5.89 (1 H, m, 2-H), 4.3—4.6 (2 H, m, 4- and 5-H), 3.0—3.8 (2 H, m, 3- or 6-H), and 2.3—3.0 (2 H, m, 3- or 6-H).

The olefin (**16**), (10 g, 0.036 mol) was dissolved in dichloromethane (100 ml), cooled (0—5 °C) and then added dropwise to a vigorously stirred and cooled (*ca.* 0 °C) mixture of trifluoroacetic anhydride (50 ml, 0.176 mol), hydrogen peroxide (90%; 11 ml, 0.04 mol) and disodium hydrogen phosphate (150 g) in dichloromethane (400 ml). After the mixture had been stirred for 2 h the product epoxide (**17**) was isolated from the dried dichloromethane extract by concentration under reduced pressure at ambient temperature. The epoxide (**17**) was found to be an oil (9.0 g, 84%) which decomposed to (**18**) (i.r. 1 732 cm^{-1}) during attempted purification by distillation (<70 °C/0.07 mmHg). The epoxide (**17**) was identified from its i.r. spectrum (which gave no indication of carbonyl absorption found to be present in the decomposition products) and from its n.m.r. spectrum: δ (60 MHz, $CDCl_3$) 4.0—4.5 (2 H, m, 4- and 5-H), 3.4—3.7 (1 H, m, 2-H), 3.05—3.45 (2 H, m, 3- or 6-H), and 2.55—3.0 (2 H, m, 3- or 6-H).

Treatment of the epoxide (**17**) (5.0 g, 0.17 mol) in THF (40 ml) with diazabicyclo[4.3.0]non-5-ene (DBN) (4.2 ml, 0.031 mol) in THF (40 ml) at *ca.* 0 °C gave an immediate white precipitate of the hydrobromide salt of DBN. After the mixture had been stirred for 1—2 h and the temperature maintained at *ca.* 0 °C for a further 8 h, the dark red solution was concentrated under reduced pressure at ambient temperature. Extraction of the residue with dichloromethane, and drying and concentration of the extract gave a red-brown oil. Extraction of the latter product with pentane gave a product (1.1 g, 51%) which was indistinguishable from an authentic sample of *o*-chlorophenol (**8**; X = Cl).

Acknowledgements

We thank Dr. L. C. Waring (n.m.r. spectra), Mr. G. Jordan (mass spec.), Dr. D. B. Harper and Mr. R. Thompson (g.l.c.–mass spec.), and Mrs. F. Turley (microbiological assistance). The present results form part of a collaborative programme which has been assisted by receipt of a NATO travel grant (No. 1807). Financial support from the National Institutes of Health (Grant No. GM26388) to G. A. B. is gratefully acknowledged.

References

1 D. R. Boyd and D. M. Jerina in 'The Chemistry of Heterocyclic Compounds' Small Ring Heterocycles, Part III, ed. A. Hassner, Wiley, New York, 1984, 24, 197—282.
2 P. J. van Bladeren, R. N. Armstrong, D. Cobb, D. R. Thakker, D. E. Ryan, P. E. Thomas, N. D. Sharma, D. R. Boyd, W. Levin, and D. M. Jerina, *Biochem. Biophys. Res. Commun.*, 1982, **106**, 602.
3 W. Levin, M. K. Buening, A. W. Wood, R. L. Chang, B. Kedzierski, D. R. Thakker, D. R. Boyd, G. S. Gadaginamath, R. N. Armstrong, H. Yagi, J. M. Karle, T. J. Slaga, D. M. Jerina, and A. H. Conney, *J. Biol. Chem.*, 1980, **255**, 9067.
4 D. M. Jerina, N. Kaubisch, and J. W. Daly, *Proc. Natl. Acad. Sci. U.S.A.*, 1971, **68**, 2545.
5 H. S. I. Chao, G. A. Berchtold, D. R. Boyd, J. N. Dynak, J. E. Tomaszewski, H. Yagi, and D. M. Jerina, *J. Org. Chem.*, 1981, **46**, 1948.
6 H. G. Selander, D. M. Jerina, D. E. Piccolo, and G. A. Berchtold, *J. Am. Chem. Soc.*, 1975, **97**, 4428.
7 J. W. Daly, D. M. Jerina, and B. Witkop, *Arch. Biochem. Biophys.*, 1968, **128**, 517.
8 B. J. Auret, D. R. Boyd, P. M. Robinson, C. G. Watson, J. W. Daly, and D. M. Jerina, *J. Chem. Soc., Chem. Commun.*, 1971, 1585.
9 D. R. Boyd, R. M. Campbell, H. C. Craig, C. G. Watson, J. W. Daly, and D. M. Jerina, *J. Chem. Soc., Perkin Trans. 1*, 1976, 2438.
10 J. P. Ferris and M. J. Fasco, F. L. Stylianopoulou, D. M. Jerina, J. W. Daly, and A. M. Jeffrey, *Arch. Biochem. Biophys.*, 1973, **156**, 97.
11 R. V. Smith and J. P. Rosazza, *Arch. Biochem. Biophys.*, 1974, **161**, 551.
12 J. E. Tomaszewski, D. M. Jerina, and J. W. Daly, *Biochemistry*, 1975, **14**, 2024.
13 D. R. Boyd and G. A. Berchtold, *J. Am. Chem. Soc.*, 1979, **101**, 2470.
14 H. S. I. Chao and G. A. Berchtold, *J. Am. Chem. Soc.*, 1981, **103**, 898.
15 H. S. I. Chao and G. A. Berchtold, *J. Org. Chem.*, 1981, **46**, 813, 1191.
16 J. E. van Epp Jr., D. R. Boyd, and G. A. Berchtold, *J. Org. Chem.*, 1981, **46**, 1817.
17 H. G. Selander, D. M. Jerina, and J. W. Daly, *Arch. Biochem. Biophys.*, 1975, **168**, 309.
18 A. M. Jeffrey and D. M. Jerina, *J. Am. Chem. Soc.*, 1975, **97**, 4427.
19 J. R. Lindsay Smith, B. A. J. Shaw, and D. M. Foulkes, *Xenobiotica*, 1972, **2**, 215.
20 T. J. Monks, L. R. Pohl, J. R. Gillette, M. Houg, R. J. Highet, J. A. Ferretti, and J. A. Hinson, *Chem. Biol. Interact.*, 1982, **41**, 203.
21 E. W. Thomas, B. C. Loughman, and R. G. Powell *Nature* (*London*), 1964, **204**, 884.
22 J. K. Faulkner and D. Woodcock, *J. Chem. Soc.*, 1965, 1187.
23 G. Guroff, K. Kondo and J. W. Daly, *Biochem. Biophys. Res. Commun.*, 1966, **25**, 622.
24 D. M. Foulkes, *Nature* (*London*), 1969, **221**, 582.
25 D. M. Foulkes, *J. Pharmac. Exp. Ther.*, 1970, **172**, 115.
26 J. W. Daly, *Biochem. Pharmacol.*, 1970, **19**, 2979.
27 S. Safe, D. Jones and O. Hutzinger, *J. Chem. Soc., Perkin Trans. 1*, 1976, 357.
28 K. Ballschmiter, C. Unglert, and P. Heinzmann, *Angew. Chem., Int. Ed. Engl.*, 1977, 645.
29 M. T. M. Tulp, G. Sandström, J. C. de Graaff, and O. Hutzinger, *Chemosphere*, 1977, **6**, 109.
30 M. Mousseron, F. Winternitz, and R. Jacquier, *C.R. Acad. Sci.*, 1946, **223**, 1014.

RSC Article Online

31 R. N. McDonald and P. A. Schwab, *J. Am. Chem. Soc.*, 1963, **85**, 4004.
32 R. N. McDonald and T. E. Tabor, *J. Am. Chem. Soc.*, 1967, **89**, 6573.
33 R. N. McDonald and T. E. Tabor, *J. Org. Chem.*, 1968, **33**, 2934.
34 G. Bonse, T. Urban, D. Reichert, and D. Henschler, *Biochem. Pharmacol.*, 1975, **24**, 1829.
35 J. L. Roark and W. B. Smith, *J. Phys. Chem.*, 1969, **73**, 1043.
36 I. Morishima, K. Ishihara, K. Tomishima, T. Inubushi, and T. Yonezawa, *J. Am. Chem. Soc.*, 1975, **97**, 2749.
37 L. Castle, J. R. Lindsay Smith, and G. V. Buxton, *J. Mol. Catal.*, 1980, **7**, 235.
38 J. F. Norris and G. W. Rigby, *J. Am. Chem. Soc.*, 1932, **54**, 2098.

Received 9th April 1984; *Paper* 4/578

Published on 01 January 1984. Downloaded by East Carolina University on 09/08/2013 11:08:24.