Arene Oxides as Intermediates in the Metabolism of Aromatic Substrates: Alkyl and Oxygen Migrations during Isomerization of Alkylated Arene Oxides

(oxidation mechanisms/microsomal hydroxylations/multiple rearrangements/carcinogenesis)

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ABSTRACT Phenolic products obtained on isomerization of the three arene oxides of toluene, the nine arene oxides of *ortho*-, *meta*-, and *para*-xylene, the arene oxide of mesitylene, and 2-methyl and 1,2-dimethylnaphthalene-1,2-oxides are compared to the phenolic products obtained by hepatic metabolism of the parent aromatic hydrocarbons. The results are compatible with the intermediacy of certain of these arene oxides in the metabolic pathway from hydrocarbons to phenols. Migrations of methyl groups as well as a remarkable apparent migration of oxygen are observed. The isomerization of 1,4-dimethylbenzene oxide to 2,4-dimethylphenol is analogous to the methyl migration observed in the enzymatic conversion of 4-methylphenylalanine to 3-methyltyrosine with phenylalanine hydroxylase. There are multiple pathways of isomerization of alkylarene oxides to phenols and they are determined by environmental and structural factors. The proportion and nature of products vary greatly with pH, which suggests the presence of different mechanisms for acid-catalyzed or spontaneous isomerizations of arene oxides.

In 1968, naphthalene-1,2-oxide was demonstrated to be the initial product of naphthalene metabolism by hepatic microsomes and the precursor of all other metabolites of naphthalene (1). This was the first definite proof of the theory that arene oxides are labile key intermediates in the metabolism of aromatic substrates. This observation has stimulated considerable interest in both the chemistry and biochemistry of arene oxides; they have been suggested as intermediates in biosynthesis (2), as causal agents in tissue necrosis (3), and as chemical carcinogens more potent than the parent polycyclic hydrocarbon (4). An arene oxide has now been demonstrated as a metabolite of the carcinogenic hydrocarbon, dibenzanthracene (5). Because of their broad biological significance, we have now investigated more-labile monocyclic arene oxides. In benzene derivatives, the double bonds do not have as great a degree of individual reactivity as in the bi- and polycyclic hydrocarbons, and the great lability of the corresponding arene oxides militated against their isolation from a biological system. However, there is indirect evidence that points to arene oxides as intermediate metabolites of benzenoid substrates. Benzene oxide itself serves as a good substrate for liver enzymes and is, thereby, converted to all the secondary metabolites of benzene (6). In addition, enzymatic hydroxylation of the aromatic nucleus proceeds with retention of the ring substituent at the point where the hydroxyl group enters, and this 1,2-migration and retention of substituents, known as the *NIH Shift* (7, 8), occurs during isomeri-

zation of arene oxide intermediates; i.e., 4-methyl-1- ^{3}H -benzene oxide isomerizes to 4-methyl-2- ^{3}H phenol (9), and 1- ^{3}H naphthalene-1,2-oxide isomerizes to 2- ^{3}H -1-naphthol (10).

The objectives of the present study were 3-fold: (i) to determine the factors that control the stability of, and the pathways for isomerization of, arene oxides, (ii) to determine whether the phenolic products formed by metabolism of the parent alkylbenzenes are compatible with arene oxide intermediates, and (iii) to demonstrate directly an arene oxide intermediate in the metabolism of an alkylbenzene.

RESULTS AND DISCUSSION

The various arene oxides were synthesized by analogy to the published preparation of benzene (11) and naphthalene oxide (12). The respective methyl-substituted cyclohexadienes were prepared by Birch reduction of methylbenzenes or benzoic acids, followed by conversion of the carboxyl group to methyl. Methylated dialins for the substituted naphthalene oxides were obtained from 1-tetralone. Details of the synthetic routes will be reported in a subsequent paper. Metabolic studies were conducted *in vitro* with microsomal preparations from rat liver (13). Phenolic products were identified and analyzed by thin-layer chromatography and/or gas chromatography. Parent aromatic hydrocarbons, phenolic metabolites, arene oxides, and their acid-catalyzed rearrangement products are listed in Table 1.

Toluene

Microsomal hydroxylation of toluene leads to 4-hydroxy- and 2-hydroxytoluene. Toluene oxide 2 rearranges exclusively to 4-hydroxytoluene, while the isomeric oxides 3 and 4 rearrange to 2-hydroxytoluene on treatment with acid. The selectivity of oxirane ring opening must be the result of the additional stabilization of the cationoid intermediate by the contribution of a tertiary allylic carbonium ion, as shown for arene oxide 3. All three of the oxides could be intermediates for the observed metabolites of toluene;

TABLE 1. Phenolic metabolites from aromatic hydrocarbons and isomerization products from arene oxides

<i>In Vitro</i> Metabolism		Acid-Catalyzed Isomerization		<i>In Vitro</i> Metabolism		Acid-Catalyzed Isomerization	
Hydrocarbon →	Phenolic Metabolites	Arene Oxide →	Isomerization Products	Hydrocarbon →	Phenolic Metabolites	Arene Oxide →	Isomerization Products
→	1	^a →		→ +	10	^b → +

→ +	2	^b →		→ +	11	^b →
		3	^b →			12	^b → +
		4	^a →			13	→ unavailable
→	5	^{a, c} → +		→	14	^b →
		6	^a →		→	15	^a →
		7	^b → +		see text	16	^b →
		8	^b → +		see text	17	^b →

→	9	^b →

^a Refs. 11 and 12 for preparation and rearrangement.^b Prepared in this study and rearranged in pentane with trifluoroacetic acid.^c Rearranged in nonaqueous medium; Schubart, R., thesis, Köln 1967.

i.e., the 4-hydroxytoluene would arise from 2, and the 2-hydroxytoluene from 3 and/or 4. Of these arene oxides, only 4 is sufficiently stable to be studied under physiological conditions, and evidence obtained so far from metabolic studies suggests that formation of such substituted arene oxides is not a favored pathway.

1,2-Dimethylbenzene (*o*-xylene)

Rat liver microsomes convert *o*-xylene *only* to 2,3-dimethylphenol. This "hydroxylation" is accompanied by an NIH Shift of deuterium (14), suggestive of an intermediate arene oxide, such as 7 or 8. However, when the intermediate carbonium ions on the way from such arene oxides to the isomeric phenols are considered, the unique formation of 2,3-dimethylphenol would seem to exclude an intermediate arene oxide. This paradox was resolved by the synthesis of 7 and 8. As anticipated, under acid conditions 7 isomerized

to both 2,3- and 2,6-dimethylphenol, the 2,6-compound with concomitant migration of methyl, and 8 isomerized to 2,3- and 3,4-dimethylphenol. However, when 8 isomerized under the basic conditions of its generation, only 2,3-dimethylphenol, the observed metabolite, was formed. This remarkable selectivity demands that different mechanisms be operative under different isomerization conditions. On

the basis of kinetic data, benzene and naphthalene oxide

rearrange both by an acid-catalyzed isomerization and, more interestingly, by a slower spontaneous or uncatalyzed isomerization (15). Thus, both product distribution and kinetics corroborate the existence of multiple pathways for the isomerization of arene oxides.

1,4-Dimethylbenzene (*p*-xylene)

Metabolism produces exclusively 2,5-dimethylphenol. The xylene oxide **10** isomerizes in the presence of acid to a mixture consisting of equal amounts of 2,5-dimethylphenol and 2,4-dimethylphenol, the 2,4-compound with concomitant migration of methyl. The extent of methyl migration is a function of pH, and predominates under neutral and basic conditions. In this respect **10** provides an excellent model for the enzymic oxidation of 4-methylphenylalanine to a mixture of 4-hydroxy-3-methyl- and 3-hydroxy-4-methylphenylalanine (16). Since 2,4-dimethylphenol is not a metabolite of 1,4-dimethylbenzene, the arene oxide **10** cannot be

involved as a precursor. The other possible arene oxide **9**, however, isomerizes to the observed metabolite, 2,5-dimethylphenol. The oxide **9** was too unstable to attempt its isolation from a biological system. The more stable oxide, **10**, was not a substrate for the hepatic epoxide hydrazase, which converts arene oxides to "dihydrodiols" (17). The latter observation suggests that lack of "dihydrodiol" formation *in vivo* from an aromatic substrate could be a reflection of the substrate specificity of the epoxide hydrazase. Stability of the arene oxide is also a determining factor for dihydrodiol formation.

1,3-Dimethylbenzene (*m*-xylene)

2,4-Dimethyl- and 2,6-dimethylphenols, in a ratio of 10 to 1, are the metabolites of *m*-xylene. Under acidic conditions, **11** rearranges exclusively to 2,6-dimethylphenol, as anticipated from resonance considerations. The oxide **12** rearranges to 2,4-dimethyl- and 2,6-dimethylphenol, the latter *via* an apparent migration of oxygen. Rearrangement under acid conditions of **11** or **12** thus does not provide the correct proportions of the phenolic metabolites. However, **12** isomerizes under the basic conditions of its generation to 2,4-dimethylphenol and 2,6-dimethylphenol, in the correct ratio of about 10 to 1. The oxide **13** has not been synthesized, but would be predicted to isomerize preponderantly to 2,4-dimethylphenol. The mechanism of the novel "migration" of oxygen during rearrangement of **12** under acidic and neutral conditions requires further investigation.

1,3,5-Trimethylbenzene (mesitylene)

Metabolism produces 2,4,6-trimethylphenol, which is also produced by isomerization of **14**. Again, the oxide **14** is too unstable for biological studies.

Naphthalenes

The methyl-substituted naphthalene oxides are relatively stable compounds and, as predictable by resonance considerations, isomerize to 1-oxy- or 1-oxo- products, in analogy to the unsubstituted naphthalene oxide (**1**) (Table 1). Attack of oxygen at the 1,2-bond of the methyl- and dimethyl-naphthalenes could not be detected *in vitro*. Instead, naphthoic acids were the major products and naphthols and dihydrodiols were formed as minor products by oxygen attack at other ring positions.

SUMMARY

The findings reported here allow several conclusions: (i) the driving force of alkylated arene oxides to isomerize decreases with alkyl substitution of the oxirane ring, and increases with substitution in other ring positions. (ii) Acid-catalyzed and spontaneous isomerization of arene oxides are examples of two distinct mechanisms; each determines the nature and distribution of products. Prediction of isomerization products based on carbonium-ion stability alone is no longer feasible; (iii) of the 17 arene oxides listed in Table 1, more than half are intermediates compatible with the phenolic products obtained on microsomal hydroxylation of benzene, toluene, xylenes, and mesitylene. However, most of these potential arene-oxide intermediates are too unstable to be observed as such under physiological conditions.

Further studies on the formation and properties of substituted arene oxides will provide a sound basis for the prediction of the metabolic fate of aromatic substrates and for the involvement of arene oxides in long-range drug toxicity, allergic responses, tissue necrosis, and carcinogenesis.

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