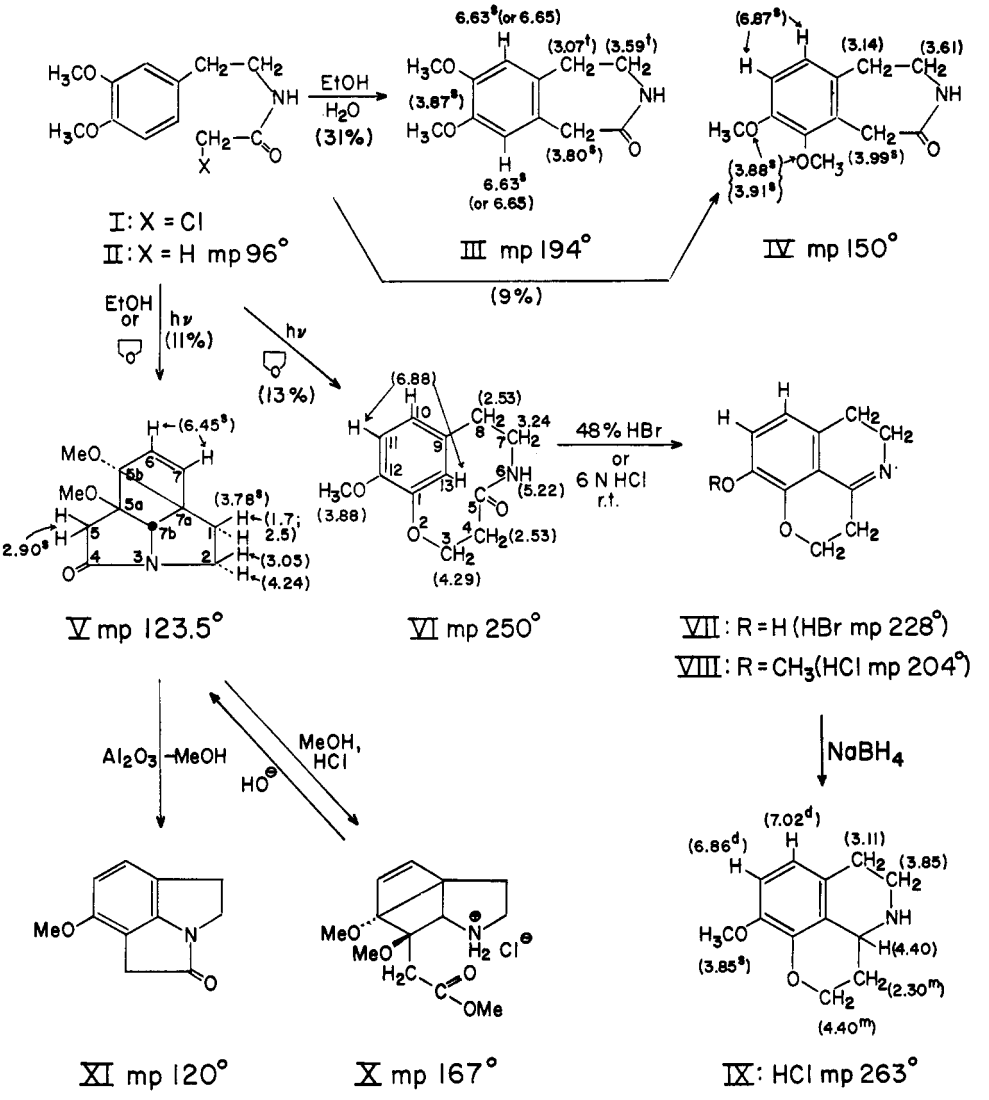
a few minutes, an example of an unusually facile *transannular Bischler–Napieralski reaction.*

mation of VI. Such a dualism of photolysis has recently been observed for α-sulfonyloxy ketones.[7]

Chart I. Photocyclizations of N-Chloroacetyl-3,4-dimethoxyphenethylamine. The figures in parentheses refer to nmr values (δ, $CDCl_3$; IX in D_2O).



There is precedent for the participation of an aromatic methoxy substituent in the photocyclization of quercetin pentamethyl ether.[5] The $-OCH_2\cdot$ radical may be formed by abstraction of H· through Cl· generated in the photoinduced homolysis of the C–Cl bond which is known to depend on the nature of the solvent.[6] The results presented here point to two simultaneous primary reactions. The n,π^* activation of the carbonyl group of I may lead to elimination of the α substituent, Cl^-, as an anion, and a positive fragment which is capable of electrophilic aromatic substitution to give photoproducts III, IV, and possibly V. In nonpolar aprotic solvents a radical mechanism would favor for-

(5) A. C. Waiss, Jr., R. E. Lundin, A. Lee, and J. Corse, *J. Am. Chem. Soc.*, **89**, 6213 (1967).
(6) G. A. Russell, *ibid.*, **80**, 4987, 4997 (1958); *cf.* J. A. Barltrop and A. Thomson, *J. Chem. Soc., C*, 155 (1968).

(7) S. Iwasaki and K. Schaffner, *Helv. Chim. Acta*, **51**, 557 (1968).

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The Role of Arene Oxide–Oxepin Systems in the Metabolism of Aromatic Substrates. II. Synthesis of 3,4-Toluene-4-^{2}H Oxide and Subsequent "NIH Shift" to 4-Hydroxytoluene-3-^{2}H[1]

Sir:

Arene oxides have been postulated as intermediates in the oxidative metabolism of aromatic substrates.[2]

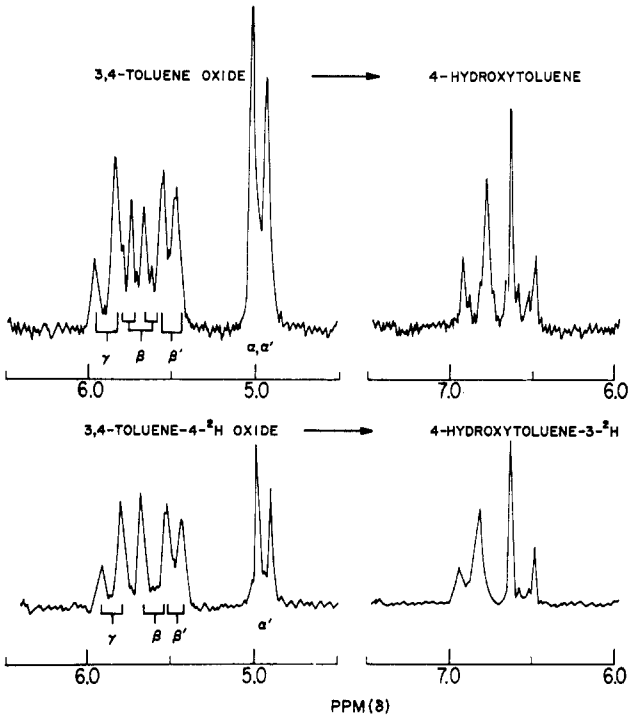
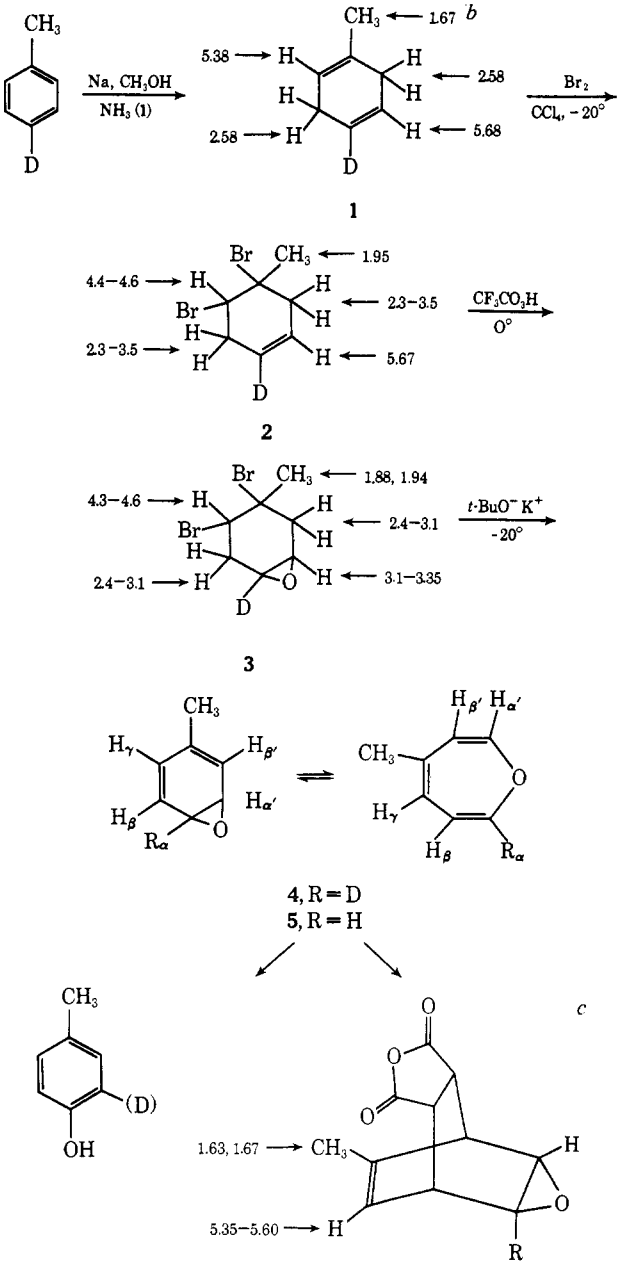


Figure 1. The 60-MHz spectra ($\delta_{CCl_4}^{TMS}$) of the labeled and nonlabeled oxides and the cresol which forms on isomerization. The respective methyl groups appear at 1.86 and 2.21 ppm. Integration of the aromatic proton region suggested the 4-hydroxytoluene-3-²H contained 64% deuterium assuming the two broad downfield peaks contain 2.0 hydrogen atoms.

For example, 1,2-naphthalene oxide is the logical common intermediate on the metabolic routes to dihydroarenes, such as *trans*-1,2-dihydro-1,2-dihydroxynaphthalene, on the one hand, and N-acetyl-S-(1,2-dihydro-2-hydroxynaphthyl)-L-cysteine, a so-called premercapturic acid, on the other hand. Benzene oxide has been shown to undergo enzymatic hydration to (−)-*trans*-1,2-dihydro-1,2-dihydroxybenzene, enzymatic addition of glutathione to S-(1,2-dihydro-2-hydroxyphenyl)glutathione, and nonenzymatic rearrangement to phenol.[1] In order to qualify as intermediates, deuterated arene oxides must retain their label on rearrangement to phenolic metabolites; they must undergo the "NIH shift."[3] In this communication we wish to report such a deuterium shift for toluene oxide.

3,4-Toluene-4-²H oxide was synthesized from 4-bromotoluene (Chart I), which in the presence of deuterium was hydrogenolyzed to toluene-4-²H.[4] Birch reduction[5] of both deuterated and normal toluene led to diene **1** which was brominated in CCl_4 at −20°. The trisubstituted double bond was brominated exclusively to form **2** [bp 60–70° (1.0–0.5 mm)].[6] Peroxytrifluoroacetic acid in the presence of sodium carbonate oxidized **2** to the dibromo oxide **3** (mp 56–62°). That **3** consisted of an equal mixture of the *cis* and *trans* oxide, relative to the methyl group, was established by two equal methyl peaks at 1.88 and 1.94 ppm in $CDCl_3$ and at 1.57 and 1.69 ppm in benzene-²H_6. In addition, column chromatography (silica gel–benzene) partially separated the isomers. Although the heaviest ions in the mass spectrum of **3** appeared at nominal masses 190 and 192 ($C_7H_9DBrO^+$) due to loss of bromine, the

Chart I[a]



[a] The numbers are $\delta_{TMS}^{CDCl_3}$ in parts per million. [b] For comparison purposes, nonlabeled diene was simultaneously carried through the sequence from this point. [c] Spectrum run in DMSO-²H_6. All the remaining hydrogens appear in a multiplet at 3.15–3.55 ppm.

(1) Paper I: D. Jerina, J. Daly, B. Witkop, P. Zaltzman-Nirenberg, and S. Udenfriend, *Arch. Biochem. Biophys.*, **128**, 176 (1969).

(2) E. Boyland, "Proceedings of the First International Pharmacology Meeting," Vol. 6, The Macmillan Co., New York, N. Y., 1962, p 65; E. Boyland and J. Booth, *Ann. Rev. Pharmacol.*, **2**, 129 (1962); H. Taniuchii and O. Hayaishi, *J. Biol. Chem.*, **238**, 283 (1963).

(3) G. Guroff, J. W. Daly, D. M. Jerina, J. Renson, B. Witkop, and S. Udenfriend, *Science*, **158**, 1524 (1967).

(4) When the mass spectrum of toluene was determined at low electron voltage (~11 eV), the normally large M − 1 peak decreased to a negligible intensity compared to the molecular ions at M = 92. By this technique the deuteriotoluene synthesized was calculated to consist of 83% monodeuterio species (4-²H) along with 5% dideuterio and 11% normal toluene.

(5) W. Hückel, B. Graf, and D. Munkner, *Ann.*, **614**, 42 (1958).

(6) F. A. Haak and J. P. Wibaut, *Rec. Trav. Chim.*, **69**, 1382 (1950), suggest that 1-methyl-4,5-dibromocyclohexene-1 is the principal product formed on bromination of 1-methylcyclohexadiene-1,4. The nmr spectrum (2.0 vinyl hydrogens) of the product formed under our conditions clearly indicates the structure as 4-methyl-4,5-dibromocyclohexene-1.

structure was confirmed by elemental analysis and molecular weight (271; osmometry in $CHCl_3$).

Oxide **3** was dehydrohalogenated to 3,4-toluene-4-^{2}H oxide (**4**) with potassium *t*-butoxide in THF or ether at −20° for 15 min. Water and ether were added. The ether layer was separated and dried over $MgSO_4$. All operations were conducted at 0° in glassware which had previously been washed with 10% NaOH to prevent acid-catalyzed isomerization to cresol. In order to obtain an nmr spectrum (Figure 1), the ether solution was concentrated to a small volume *in vacuo* at 0° after addition of an equal volume of CCl_4. The uv spectrum of the bright yellow oxide–oxepin mixture [$\lambda_{max}^{85\%\ methanol}$ 267 mμ (ε 2200); $\lambda_{max}^{cyclohexane}$ 273 mμ with tailing to longer λ (ε 1910)][7] resembles benzene oxide, suggestive of the presence of both valence isomers in solution. 3,4-Toluene oxide is much more unstable than benzene oxide. It could not be distilled and had a half-life (CCl_4 solution) of ~24 hr at room temperature in *base-treated* glassware. The structure of oxide **4** was further confirmed by formation of the Diels–Alder adduct with maleic anhydride in ether at 0° (mp 141–142°; 50% yield based on **3**; *m/e* 207). By comparison of the mass spectra of the Diels–Alder adducts of **4** and **5** (calcd *m/e* 206.0579; found 206.0577), the isotopically labeled oxide had neither lost nor randomized the deuterium initally present.

The isomerization of oxide **4** was studied under a variety of conditions. According to tlc, glpc, and the nmr spectra (Figure 1), **4** isomerizes almost exclusively to *p*-cresol. Such a strong orienting influence of a methyl substituent is noteworthy. During isomerization deuterium migrates from the 4 position of oxide **4** to the 3 position of the product (4-hydroxytoluene), as is clearly shown by the nmr of this material (Figure 1). The deuterium retention values in Table I can be compared with values of 56 or 68%

Table I[a]

Isomerization method	% deuterium[b] retention
0.1 *N* HCl	37
Room temperature in CCl_4	58
Liver microsomes[c] at pH 8.0	70
10% aqueous acetamide	75[d]

[a] The isomerization products obtained in the three aqueous systems were isolated by extraction into ether and concentration to a small volume for glpc. Deuterium retentions were measured on an LKB 9000 mass spectrometer–gas chromatograph at 70 eV using a column (6 ft × 1/8 in. o.d.) of Bentone 34–tricresyl phosphate–80–100 mesh Gas Chrom CL (1:1:1) operated at 125°. [b] Values are corrected to represent amount of initial 4-^{2}H retained in the product *p*-cresol. [c] This is a protein-catalyzed nonenzymatic rearrangement which occurs both with active and boiled microsomes. [d] Mass spectrometric deuterium analysis for hydroxytoluene and toluene is difficult because of the large M − 1 peaks which in the 3,5-dinitrobenzoates of the three *p*-cresols become negligible. In this way it was determined that 4-hydroxytoluene 3,5-dinitrobenzoate obtained by rearrangement of the oxide in acetamide solution retained 85% deuterium.

when toluene-4-^{2}H is hydroxylated either with rabbit liver microsomes or with peroxytrifluoroacetic acid, respectively.[8] Migration and retention of deuterium during rearrangement of the oxide **4** is similar to that obtained during enzymatic hydroxylation of toluene-4-^{2}H. *Arene oxides are thus possible metabolic intermediates in the formation of phenols since they undergo spontaneous or catalyzed "NIH shifts" to an extent comparable to enzymatic hydroxylation.*

Due to the great instability of 3,4-toluene oxide, it has not been possible to demonstrate its role as a substrate for the enzymes which add water or glutathione to benzene oxide.[1] Although at present no direct evidence is available for the existence of a monocyclic arene oxide in a biological system, our preliminary observations suggest that 1,2-naphthalene oxide is an intermediate in the metabolism of naphthalene.[9]

(7) The oxide stock solution (CCl_4) was washed with base to remove cresol immediately prior to running the spectra. Extinction coefficients were estimated from the *p*-cresol which spontaneously formed in the cell after 24 hr.

(8) D. M. Jerina, J. W. Daly, and B. Witkop, *Arch. Biochem. Biophys.*, in press.

(9) D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg, and S. Udenfriend, *J. Am. Chem. Soc.*, **90**, 6525 (1968).

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The Role of Arene Oxide–Oxepin Systems in the Metabolism of Aromatic Substrates. III. Formation of 1,2-Naphthalene Oxide from Naphthalene by Liver Microsomes[1]

Sir:

The possibility that arene oxides function as intermediates during the oxidative metabolism of aromatic substrates to phenols, catechols, dihydrodiols, and premercapturic acids has been investigated in three stages. First, benzene oxide was shown to serve as an *in vitro* precursor for phenol, catechol, (−)-*trans*-1,2-dihydro-1,2-dihydroxybenzene, and S-(1,2-dihydro-2-hydroxyphenyl)glutathione.[2] Then the nonenzymatic rearrangement of 3,4-toluene-4-^{2}H oxide to 4-hydroxytoluene was demonstrated to occur with up to 85% deuterium retention,[1] making the isomerization of arene oxides to phenols compatible with the "NIH shift."[3,4] In this communication *we describe the first isolation and identification of an arene oxide from a biological system.*

Attempts were made to demonstrate the *in vitro* formation of benzene oxide in an earlier study,[2] but the results were inconclusive, since benzene is a poor substrate for the aromatic hydroxylating system in liver microsomes. Naphthalene (I), on the other hand, is readily metabolized by rat liver microsomes to 1-naphthol (III) and *trans*-1,2-dihydro-1,2-dihydroxynaphthalene (IV). If the liver supernatant fraction and glutathione are included in the incubation, the principal product becomes a glutathione conjugate which has been assigned the structure S-(1,2-dihydro-

(1) Paper II: D. M. Jerina, J. W. Daly, and B. Witkop, *J. Am. Chem. Soc.*, **90**, 6523 (1968).

(2) D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg, and S. Udenfriend, *Arch. Biochem. Biophys.*, **128**, 176 (1968).

(3) G. Guroff, J. W. Daly, D. M. Jerina, J. Renson, S. Udenfriend, and B. Witkop, *Science*, **157**, 1524 (1967).

(4) J. W. Daly, G. Guroff, D. M. Jerina, S. Udenfriend, and B. Witkop, Advances in Chemistry Series, American Chemical Society, Washington, D. C., in press.