trans-2-Chloro-6-thioethylcyclohexa-2,4-dienol (**9**) was the sole product from **8**.[17] Acid-catalyzed dehydration of **9** (TFA in $CHCl_3$, 100°, 1 min) produced 2-, 3-, and 4-chlorophenyl ether sulfide in a ratio of 61:5:34.[18] Migration of the thioethyl group occurs to both canonical forms of the carbonium ion in which the positive charge is adjacent to sulfur. Pyrolysis (injection port of the GLC at 500°) of the acetate of **9** led to dehydration with little migration; 2-, 3-, and 4-chlorophenyl ethyl sulfide and 2-chlorophenyl acetate were formed in the ratio of 9:75:12:4, respectively. Instability of the adducts[19] from **10** caused considerable difficulty. Storage as a neat oil at −70° for 1 week resulted in decomposition to equal amounts of 4-chlorophenol and 4-chlorophenyl ethyl sulfide. Acetylation of the mixture of adducts followed by HPLC[20] produced equal amounts of 4-chlorophenyl ethyl sulfide (from **11**) and the acetate of **12**.[21] On storage at room temperature in CCl_4, the acetate of **12** slowly converts to 4-chlorophenyl acetate and 4-chlorophenyl ethyl sulfide. In contrast, GLC (injection port at 175°) produces 2-, 3-, and 4-chlorophenyl ethyl sulfide in the ratio 5:4:91 by migration of the thioethyl group. Thus, thiol adducts of the chlorobenzene oxides aromatize to produce alkyl aryl sulfides in which the sulfur substituent is preponderantly at the 2- and 4-positions relative to chlorine. Since most of the epoxide opening had occurred at the 3-position, the aromatic products must result from extensive sulfur migration. Formation of **11** from **10** cannot proceed *via* a carbonium ion or tight ion pair and must result from an SN2 reaction since **10** isomerizes only to 4-chlorophenol under a variety of conditions.[16] Thus, carbonium ion (or ion pair) trapping as well as direct nucleophilic opening are feasible mechanisms[8,22] for the reactions of arene oxides with nucleophiles.

The high concentration of glutathione in mammalian liver[23] and the poor K_m's of glutathione-*S*-epoxide transferases[24] for epoxides and arene oxides suggest that a substantial portion of the premercapturic acids which form *in vivo* are the result of purely chemical reaction. The present study establishes that the structure of the premercapturic acid formed from naphthalene[6,7] and probably those of chlorobenzene[25] have been missassigned. Structures of many other premercapturic acids[3] are now in question. The observations of distal attack[8,26] and rearrangement on aromatization suggest that the products formed on interaction of arene oxides with DNA will prove to be difficult in their structural elucidation.

Acknowledgment. We are most grateful to Dr. H. J. C. Yeh of this laboratory for the decoupled 220-MHz spectra and to Drs. H. Selander and R. Moriarty for helpful discussions.

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(11) While the degree of oxidation at the time of spontaneous dehydration is unknown, most of the sample must have been oxidized at least to the sulfoxide. Product analysis was by GLC–MS (5% OV-17, 250°).
(12) The uv spectra of 1- vs. 2-substituted naphthalenes are very characteristic in that 2-substitution causes a complex spectrum, while 1-substitution results in a smooth curve with the λ_{max} at longer wavelength. The general shape of the absorption spectrum and the position of the λ_{max} are relatively independent of the oxidation state of the sulfur. Typical spectra are reported in ref 6. The labels on the traces in Figure 4 of this reference should be reversed.
(13) Storage of 0.1 m*M* solution of 1 in 5% $NaOCH_3$ for 15 hr at room temperature results in complete conversion into two methoxide adducts according to ^1H NMR. Preparative TLC (R_f 0.3, $CHCl_3$) allowed isolation of **6c** (80%) while the minor isomer decomposed to naphthol. The NMR spectrum of **6c** (H_1 4.08, H_2 4.90, H_3 6.03, H_4 6.48, $-OCH_3$ 3.52, aromatic 7.0–7.6 with $J_{1,2}$ = 10.0, $J_{2,3}$ = 2.5, $J_{2,4}$ = 2.0, $J_{3,4}$ = 10.0 Hz at 100 MHz in $CDCl_3$) establishes that trans opening of **1** had occurred *via* attack of methoxide at C-2 (cf. ref 8): λ_{max} 262 nm (H_2O); M^+ 176 (34%), M − 58 (100%).
(14) After dehydration the product was extracted into hexane and its uv spectrum recorded (λ_{max} 299 (major) and 326 with shoulders at 290, 310, and 318 nm). Presence of naphthol in the extract was excluded by TLC. The spectrum of the dehydration product was virtually identical with that of 1-azidonaphthalene (K. Suga and S. Watanabe, *Isr. J. Chem.*, **6**, 521 (1968)) and markedly different from that of the 2-isomer prepared in the same manner; λ_{max} 274, 282 (major), 292, 316, and 332 nm in hexane. For further examples of azidonium ions as intermediates see A. Streitwieser and S. Pulver, *J. Am. Chem. Soc.*, **86**, 1587 (1964).
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(17) Synthesis of **8**[16] is accompanied by minor (10%) production of **10**. Preparative TLC of **9** (R_f 0.35, $CHCl_3$) removes the adducts from **10** as they rapidly decompose on dry silica. The structure of **9** was assigned from its ^1H NMR spectrum (H_1 4.25, H_3 6.20, H_4 5.93, H_5 5.84, H_6 3.60 with $J_{1,6}$ = 2.0, $J_{3,4}$ = 6.0, $J_{4,5}$ = 9.0, $J_{5,6}$ = 5.0 Hz at 220 MHz in acetone; λ_{max} 264 nm, ethanol) which is consistent with related benzene oxide adducts.[8]
(18) Chlorophenyl ethyl sulfides were separated by GLC (10% Bentone 34 and 5% Carbowax 20 M on Chromasorb W, 150°). We thank R. Kruppa of Applied Science Laboratories for suggesting this phase. 2- and 3-Chlorophenyl acetate separate on 15% SE-30 at 130°.
(19) The 220-MHz ^1H NMR spectrum (acetone) of the crude reaction product established the presence of two nearly identical compounds in equal amounts. Double resonance indicated one isomer had signals at δ 3.52 and 4.39 for the sulfur- and oxygen-bearing carbons with J = 1.8 Hz; other isomer δ 3.64 and 4.27 with J = 2.7 Hz. Since reaction of benzene oxide with sulfur nucleophiles is known to proceed by direct trans 1,2-opening,[6] the isomers were tentatively assigned as **11** and **12**.
(20) Separation with a Chromatronix 3500 high pressure liquid chromatograph (HPLC) equipped with a 2.1 mm × 25 cm Du Pont Zorbax-Sil column; 0.025% ethanol in hexane at 1.2 ml/min as mobile phase. Attempted chromatography on silica gel TLC plates resulted in extensive aromatization.
(21) The structure of the acetate of **12** was assigned from its 220 MHz spectrum in CCl_4: H_1 5.32, H_2 5.84, H_3 5.91, H_5 5.84, H_6 3.52 with $J_{1,2}$ = 5.6, $J_{1,6}$ = 2.0, $J_{1(3\text{ or }5)}$ = 0.8, $J_{2,3}$ = 9.8, $J_{3,5}$ = 1.8, $J_{5,6}$ = 6.0, $J_{6,(2\text{ or }3)}$ = 0.8 Hz (cf. ref 8).
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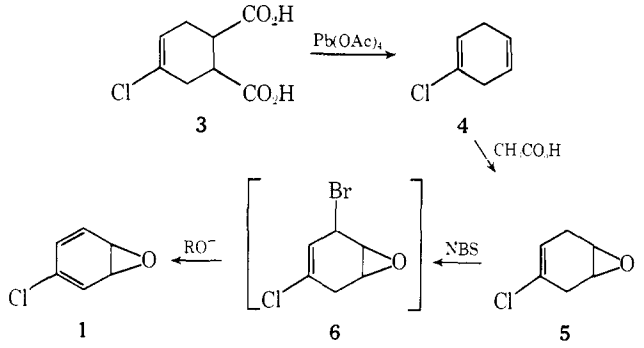
Received January 20, 1975

Synthesis of 3- and 4-Chlorobenzene Oxides. Unexpected Trapping Results during Metabolism of [^{14}C]Chlorobenzene by Hepatic Microsomes

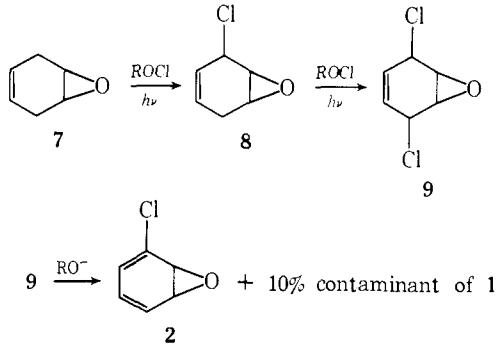
Sir:

Direct evidence has been presented for the metabolic formation of arene oxides from polycyclic aromatic hydrocarbons ranging in size from naphthalene to benzo[*a*]pyrene.[1a] For hydrocarbons larger than naphthalene, only the relatively stable K-region arene oxides have been detected.[1b] Direct demonstration of the metabolic formation of an arene oxide from benzene and its derivatives has been im-

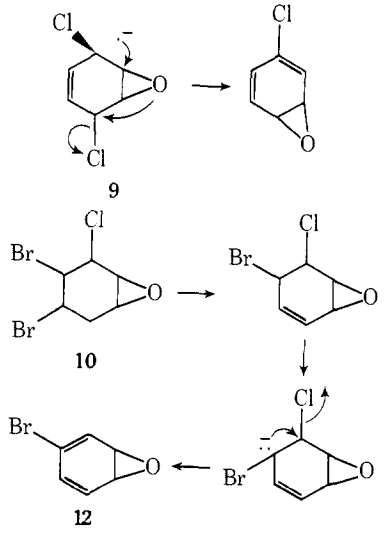
Scheme I



Scheme II



Scheme III



possible since the available benzene oxides isomerize too rapidly to phenols to survive the conditions of incubation.[2,3] Both 4- and 3-chlorobenzene oxides (**1**, **2**) have now been synthesized in order to determine if direct evidence could be obtained for the formation of these arene oxides from [^{14}C]chlorobenzene in the presence of liver microsomes. A preliminary study had established that benzene oxides with electron-withdrawing substituents would be more stable toward isomerization to phenols.[4]

Synthetic routes to the desired chlorobenzene oxides (Schemes I and II) were patterned after procedures devised by Vogel and coworkers for the synthesis of benzene oxide.[5] Availability of chloroprene[6] provided a convenient starting material for the synthesis of **1**. Acid **3**[7] was decarboxylated with lead tetraacetate in dimethyl sulfoxide–pyridine to provide 1-chlorocyclohexa-1,4-diene (**4**).[8] The diene was selectively epoxidized with peroxyacetic acid at the more reactive, unsubstituted double bond. NBS bromination of **5** (boiling CCl_4, 2 hr) produced an unstable, viscous oil (presumably **6**) which was treated directly with dry NaOMe in boiling ether for 1 hr to obtain the desired oxide **1** after distillation (50–60% based on **5**, bp 20° (0.1 mm)).[9]

Table I. Trapping of Chlorobenzene Oxides Formed during Metabolism of [^{14}C]Chlorobenzene by Liver Microsomes[d]

Induction	Total dpm in trapped oxides minus zero time[a]		nmol products/incubation	
	3-Chloro	4-Chloro	As 4-chloro oxide	As total phenols
None[b]	Nil	86,000	5.9	55.1
Phenobarbital	Nil	54,000	3.2	29.6
3-Methylcholanthrene	Nil	79,000[c]	5.4	36.4

[a] Trapped counts were greater than ten times this blank. [b] The higher metabolism with noninduced microsomes is due to storage of the induced preparations. [c] After five recrystallizations from methanol, 97% of the original specific activity remained. [d] Microsomes were prepared from the livers of normal and induced male Sprague-Dawley rats (175–200 g) as described.[17] Incubation mixtures were reconstituted to 14–16 mg of microsomal protein in 1.0 ml (0.1 *M* phosphate, pH 8.5) and contained an NADPH regenerating system.[17] After a 1-min preincubation at 37°, 1760 nmol of [^{14}C]chlorobenzene (16 nCi/nmol) was added and incubation continued 3.5 min. Addition of 5 mg of **1** and **2** was followed by an additional 1.5-min incubation. Products were extracted into ethyl acetate, **1** and **2** were trapped with methyl triazolinedione, and phenols extracted into aqueous NaOH. The adducts were separated by high pressure liquid chromatography on a 0.25-m Du Pont Zorbax-Sil column eluted with 0.5% ethanol in hexane as mobile phase (retention of adduct of **2** relative to adduct of **1** = 0.83).

1,4-Cyclohexadiene 1,2-oxide (**7**) was employed in the synthesis of **2**. Irradiation (254 nm through quartz, 3 hr, 50°) of a solution of 0.1 mol of **7** and 0.4 mol of *tert*-butyl hypochlorite[10] followed by distillation and crystallization of the fraction which distilled at 49–50° (0.1 mm) from hexane (mp 73–74°) provided 3,6-dichlorocyclohex-4-ene 1,2-epoxide (**9**) (1.2 g, 7%) of unknown stereochemistry. The dichloro epoxide (**9**) was dehydohalogenated with *t*-BuOK (boiling ether, 3 hr) to provide **2** in 55% yield after distillation (30° (0.1 mm)).[9] The sample of **2** obtained by this route contained a 10% contaminant identified as **1**. Alternately, **2** was prepared by addition of bromine to the double bond in **8** followed by dehydrohalogenation of the bromination product (**10**). The sample of **2** was again contaminated (10%), this time by the bromo analog (**12**) of **1**. Possible mechanisms to account for these unexpected rearrangements are shown in Scheme III. An oxygen walk reaction[11] would allow reversible interconversion between **1** and **2** and would explain the contamination of **2** by **1**. This hypothesis is considered unlikely since pure **1** was readily isolated. Related syntheses of substituted benzene oxides[1b] have proceeded without detectable rearrangement to isomeric arene oxides.

A careful analysis of the in vivo metabolism of chlorobenzene has shown that the three isomeric chlorophenols, 4-chlorocatechol, and *S*-(4-chlorophenyl)-*N*-acetylcysteine are major metabolites.[12] Presumably, the phenols arise by isomerization of **1** and **2**, the catechol by enzymatic hydration of **1** to produce *trans*-1,2-dihydroxy-1,2-dihydro-4-chlorobenzene[13] and subsequent dehydrogenation,[14] and the sulfur conjugate by initial attack of glutathione on **1**. In the above metabolism study,[12] **1** was postulated as the sole initial metabolite of chlorobenzene. Several major and minor metabolites were speculated[12] to arise via homoallylic addition[15] of nucleophiles to **1**.

In order to clarify the origin of the isomeric chlorophenols, isomerization of **1** and **2** was examined over the range of pH 1–12 and in the presence of rat liver microsomes at pH 8. The presence of the chlorine has a marked influence on the isomerization of **1** and **2** in that only (>99.5%) 4-

and 2-chlorophenol are produced, respectively.[16] The substantial percentage of 3-chlorophenol (>20%) produced in vivo,[12] and by the perfused liver,[17] appears to result from an enzyme which catalyzes the insertion of oxygen directly into the carbon-hydrogen bond without intervention of an arene oxide.[18]

Prior induction of animals with 3-methylcholanthrene greatly enhances the extent of ortho hydroxylation of halobenzenes.[17,19] To determine the effect of induction on the profile of metabolites, 13 male Sprague-Dawley rats (200 g) were given two intraperitoneal injections of chlorobenzene (1.1 g/kg in cotton seed oil) 1 day apart, and urine was collected for 2 days. In addition to the known 4-chlorodihydrodiol[13] (1%), the unknown 3-chloro isomer, *trans*-1,2-dihydroxy-1,2-dihydro-3-chlorobenzene (0.5%), was isolated from the urine of the induced animals.[20] Separate incubations of **1** and **2** with microsomal and solubilized epoxide hydrase[16] resulted in the formation of the 4- and 3-chlorodihydrodiols, respectively, by the direct trans addition of water.[21] Formation of 2-chlorophenol as the major phenolic isomer[17] and the identification of the 3-chlorodihydrodiol strongly implicates **2** as a primary metabolite of chlorobenzene when animals are induced by 3-methylcholanthrene.

Results of trapping experiments in which [^{14}C]chlorobenzene was incubated with liver microsomes from control and induced rats in presence of carrier chlorobenzene oxides are shown in Table I. After incubation, the residual oxides were extracted into ethyl acetate. In separate experiments, the recoveries of **1** and **2** to this point were determined to be 47 and 29%, respectively, by titration of the extracts with the highly colored methyl triazolinedione[9] used to stabilize the oxides as Diels–Alder adducts. The comparable recoveries for the two arene oxides from the incubation medium suggests that their rates of isomerization are similar. Remarkably, the reisolated **2** was free of radioactivity while as much as 15% of the total metabolism was trapped as [^{14}C]-**1**.

The above trapping experiments are of profound importance in explaining the hepatotoxicity of halobenzenes as mediated by halobenzene oxides which covalently bind to tissue constituents.[1a,22] Prior induction of animals with 3-methylcholanthrene has been noted to cause an increase in the rate of metabolism,[17,19] yet unexpectedly results in markedly decreased toxicity. While induction by 3-methylcholanthrene favors metabolic pathways originating from 3- rather than 4-halobenzene oxides (e.g., **2** rather than **1**), the basis for the protection against necrosis has remained unclear. Studies of the toxicity of **1** and **2** toward liver cells in suspension suggest these compounds have comparable activity.[23] Failure to trap significant amounts of **2** in the present study (Table I) is indicative that little of the **2** formed in vivo enters the cytosol from the endoplasmic reticulum, and thus never has the opportunity to inactivate critical macromolecules in the cell through covalent interaction.[24]

Acknowledgment. We thank Dr. H. J. C. Yeh at NIH for obtaining the 220-MHz ^{1}H NMR spectra and Dr. J. W. Daly for helpful discussions.

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(8) Diene **4** gave an acceptable mass spectrum and elemental analysis; NMR (60 MHz, $CDCl_3$), H_2 5.75, $H_{3,3,6,6}$ 2.83, and $H_{4,5}$ 5.60.
(9) The NMR spectra of **1** and **2** were measured in CS_2: **1** (220 MHz), H_1 5.37, H_2 5.39, H_3 5.57, H_5 6.11, and H_6 5.52; **2** (100 MHz), $H_{1,2}$ 4.17 and $H_{4,5,6}$ 6.24. The chemical shifts of the α-hydrogens (H_1 and H_2) established that **1** exists mainly as the oxepin isomer while **2** is mainly in the oxide form when compared to benzene oxide.[5] Both **1** and **2** were further characterized as Diels–Alder adducts with 4-methyl-1,2,4-triazoline-3,5-dione (prepared by the method of R. C. Cookson, S. S. Gupte, I. D. Stevens, and C. T. Watts, *Org. Synth.*, **51**, 121 (1971)): adduct from **1**, mp 164–166° from acetone, and adduct from **2**, mp 136–137° from methanol. Both adducts gave acceptable NMR and mass spectra as well as elemental analyses.
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(20) Preliminary isolation of a dihydrodiol fraction was as in ref 13. Separation of the isomeric 3- and 4-chlorodihydrodiols was achieved by multiple development on alumina TLC plates on which the 3-chloro isomer migrated faster. The isomers also separate on high pressure liquid chromatography.[17] The NMR spectra of the diols were measured $CDCl_3$ at 220 MHz. The spectrum of the trans 3-chloro isomer was not first order and showed H_1 4.54 H_2 4.42, H_4 6.13, and $H_{5,6}$ 5.93 with $J_{1,2}$ = 9.5 Hz, while the spectrum of the trans 4-chloro isomer showed H_1 4.47, H_2 4.53, H_3 6.00, H_5 5.91, and H_6 6.05 with $J_{1,2}$ = 10.8, $J_{1,5}$ = 1.6, $J_{1,6}$ = 2.4, $J_{2,3}$ = 2.6, $J_{3,5}$ = 2.9, $J_{3,6}$ = 0.4, and $J_{5,6}$ = 10 Hz. For comparison, the cis 3-chloro isomer, a microbial product from chlorobenzene which was kindly provided by Dr. D. T. Gibson at the University of Texas, showed H_1 4.52, H_2 4.19, H_4 6.11, and $H_{5,6}$ 5.87 with $J_{1,2}$ = 6.6 Hz.
(21) Both 1 and **2** were poor substrates for epoxide hydrase. Adequately sensitive assays to determine kinetic parameters for the hydrations were not found. Large scale incubations provided sufficient product to establish structure by NMR. Much higher turnover numbers might be realized with fresh liver microsomes due to the fragile nature of "benzene oxide hydrase" on freezing microsomes.
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(24) Formation of 2-chlorophenol from chlorobenzene by liver microsomes is linear[17] for time periods greatly in excess of that used in the trapping experiments. For this reason, complete binding of all **2** which is formed to microsomal components cannot explain the failure to trap **2** as a Diels–Alder adduct if **2** is indeed the precursor of 2-chlorophenol.

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Photochemistry in the Electronic Ground State. III. Isotope Selective Decomposition of Methylene Chloride by Pulsed Carbon Dioxide Laser

Sir:

When a mixture of two compounds in the gas phase is irradiated with monochromatic infrared (ir) light at a fre-