Migration and Retention of Deuterium on Aromatization of Toluene 1,2-Oxide and 2,3-Oxide to *o*-Cresol

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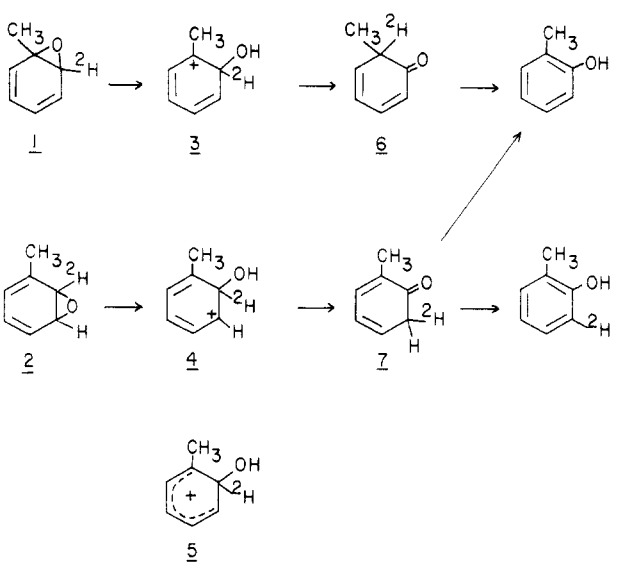
Received October 2, 1980

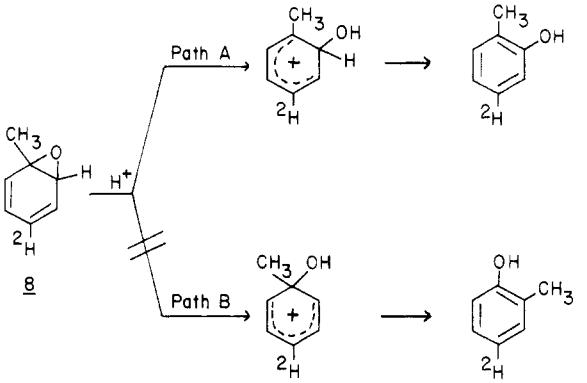
Hydroxylation of aromatic substrates by monooxygenase-catalyzed oxidation[1] to arene oxides that subsequently rearrange via the NIH shift pathway to phenolic derivatives is a recognized metabolic pathway in plants and animals.[2-4] Experimental evidence supporting the pathway commonly includes observation of substituent migration and retention consistent with the mechanism for aromatization of arene oxides via the NIH shift.[2-5] The hydroxylation of toluene in animals (or by liver microsomal preparations)[6-8] and fungi[9,10] affords *o*- and *p*-cresol. Although para hydroxylation is predominant in mammalian metabolism, ortho hydroxylation is more common in fungi. Ortho hydroxylylation could occur by initial formation of the 1,2- or 2,3-oxide since either isomer aromatizes exclusively to *o*-cresol.[3,11,12] Arguments have been presented to suggest the pathway involving the 2,3-oxide of toluene is more important in mammalian metabolism,[11,12] whereas the 1,2-oxide may be more important in fungal metabolism.[9] Thus, ortho hydroxylation of [2-^{2}H]toluene by rat liver microsomal preparations affords *o*-cresol with 43% migration and retention of deuterium[7] and by fungi affords *o*-cresol with 11% migration and retention by deuterium.[9]

Previous studies of the aromatization of toluene 1,2-oxide and other methyl-substituted benzene oxides[3,11,12] have indicated that toluene 1,2-oxide undergoes oxirane ring opening by acid catalysis or spontaneous rearrangement to *o*-cresol. These reactions proceed in the direction to afford the more stable cationic intermediate, i.e., that stabilized by the methyl substituent. Subsequent migration of hydrogen should occur to the adjacent carbon atom of higher positive charge density, i.e., the carbon atom bearing the methyl substituent. [2-^{2}H]Toluene 1,2-oxide (**1**) might, therefore, be expected to aromatize to *o*-cresol with complete loss of deuterium label to the reaction medium (Scheme I). In contrast, [2-^{2}H]toluene 2,3-oxide (**2**), which is also known to isomerize exclusively to *o*-cresol,[3,11,12] might be expected to show a substantial migration and retention (NIH shift) of deuterium (Scheme I). Notably, however, carbocations **3** and **4** are equally well represented by the common carbocation **5** from which both **1** and **2** would be expected to give the same degree of migration and retention of deuterium. The present study was undertaken to establish the degree of migration and

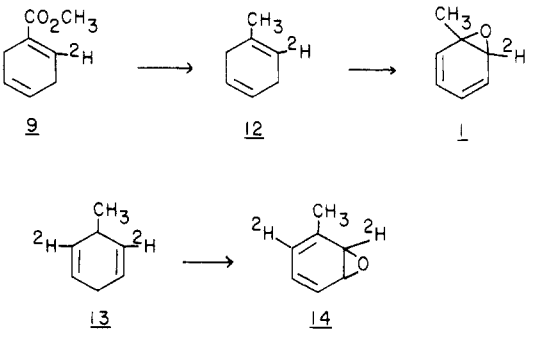
Scheme I



Scheme II



Scheme III



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0022-3263/81/1946-1948$01.25/0

Table I. Migration and Retention of Deuterium on Rearrangement of [2-^{2}H]Toluene 1,2-Oxide and [2,6-$^2H_2$]Toluene 2,3-Oxide to *o*-Cresol

arene oxide	pH (time)	% migration and retention
[2-^{2}H]toluene 1,2-oxide[a]	1.1 (2 min)	10[c]
	4.0 (2 days)	30
	7.0 (2 days)	31
	10.0 (2 days)	28
[2,6-$^2H_2$]toluene 2,3-oxide[b]	2 (30 min)	9[d]
	8.5 (1 day)	30

[a] Reactions were run in 2 parts tetrahydrofuran and 1 part water with the aqueous portion at pH 1.1 (HCl), 4.0 (biphthalate buffer), 7.0 (phosphate buffer), or 10.0 (carbonate-borate buffer). [b] The arene oxide (2–3 mg) was added to 40 mL of water at pH 2 ($HClO_4$), 7.4 (phosphate buffer), or 8.5 (bicarbonate buffer). [c] Analyzed by integration of the 250-MHz ^{1}H NMR spectrum (acetone-d_6) [6.71 (H_4), 6.80 (H_6), 6.99 (H_5), and 7.0 (H_3) ppm] for *o*-cresol. [d] The isolated *o*-cresol was ortho methylated (1 N NaOH, dimethyl sulfate, 10 min, 90 °C) and analyzed by GC–MS (6 ft, column of 10% SP1000 on Chromosorb W at 72 °C).

retention which occurs when [2-^{2}H]toluene 1,2-oxide (1) and 2,3-oxide (2) isomerize to *o*-cresol.

In order to do this, it was first necessary to establish that toluene 1,2-oxide isomerizes to *o*-cresol without migration of the methyl group. This was achieved through study of the isomerization of [4-^{2}H]toluene 1,2-oxide (8) obtained from [4-^{2}H]toluene[9] by a slight modification of literature procedures[11,13] for the preparation of the unlabeled arene oxide. If isomerization of the arene oxide to *o*-cresol occurs without methyl migration (Scheme II, path a), the deuterium will be meta to the phenolic hydroxyl group, whereas methyl migration (Scheme II, path b) results in deuterium para to the phenolic hydroxyl group. Since bromination of the resultant *o*-cresol to afford 4,6-dibromo-2-methylphenol failed to cause any detectable loss of deuterium, it was concluded that toluene 1,2-oxide isomerizes without methyl migration as expected.

Synthesis of [2-^{2}H]toluene 1,2-oxide (1) required [2-^{2}H]-1-(carbomethoxy)cyclohexa-1,4-diene (9) as starting material (Scheme III).[14,15] This was obtained with 99% deuterium incorporated at C-2 through Diels–Alder reaction between [3-^{2}H]propiolic acid[16] and butadiene. Through a sequence of steps, the carbomethoxy group was reduced to a methyl group, and the resultant methylcyclohexadiene (12) converted to arene oxide 1 as described for the unlabeled compound.[13] In order to synthesize an appropriately deuterated toluene 2,3-oxide, advantage was taken of the commercial availability of [2,4-$^2H_2$]-3-methylcyclohexa-1,4-diene (13) which was brominated, epoxidized, and dehydrohalogenated essentially as described[11] (Scheme III). On the basis of the starting material, the resultant arene oxide (14) had 90% deuterium incorporated at C-2 and at C-6.

Migration and retention of deuterium during aromatization of [2-^{2}H]toluene 1,2-oxide (1) and [2,6-^{2}H]toluene 2,3-oxide (14) to *o*-cresol was examined under conditions of spontaneous and acid-catalyzed rearrangement (Table I). The magnitude of the NIH shift of deuterium for both arene oxides was identical, but different in the two pH regions. Migration and retention of deuterium was 10% for the acid-catalyzed pathway and 30% for the spontaneous rearrangement. Thus, both arene oxides appear to isomerize by a common mechanism which presumably involves carbocation 5 (Scheme I). The difference in the magnitude of the NIH shift for the acid-catalyzed and spontaneous reactions may reflect differences in the isotope effect for the enolization of 7 as a function of pH, the extent to which 6 is formed, and/or differences in the rate of direct loss of deuterium from 5 to form unlabeled *o*-cresol.

Our present results indicate that either or both toluene 1,2- and 2,3-oxides could account for the magnitude of the NIH shift observed upon liver microsomal ortho hydroxylation of toluene. The much lower value for the ortho hydroxylation of toluene by fungi is less readily understood. Although the micro environment of the catalytic side of the fungal cytochrome P-450 could have a very low pH, a direct (non arene oxide) or radical-mediated hydroxylation may also be possible.

Experimental Section

Boiling points are uncorrected. ^{1}H NMR spectra were obtained at 60 or 250 MHz with Hitachi Perkin-Elmer R-24B and Bruker WP 250 FT spectrometers, respectively. Deuteriochloroform solvent was used unless otherwise stated. Chemical shift values (δ) are reported in parts per million downfield from Me_4Si. Mass spectra were determined with Varian MAT 44 and LKB 9000 mass spectrometers. Infrared spectra were obtained from a Perkin-Elmer Model 567 grating spectrophotometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN.

[2-^{2}H]-1-(Carbomethoxy)cyclohexa-1,4-diene (9). The diene was prepared as described previously.[14] The monopotassium salt of acetylenedicarboxylic acid was exchanged with ^2H_2O prior to decarboxylation to increase deuterium incorporation in the [3-^{2}H]propiolic acid[16] used in the Diels–Alder reaction. The ^{1}H NMR spectrum (250 MHz in acetone-d_6) of diene 9 indicated 99% deuterium incorporation at C-2; bp 49 °C (0.7 mm) [lit.[17] bp 94–96 °C (20 mm)].

[2-^{2}H]-1-(Hydroxymethyl)cyclohexa-1,4-diene (10). A suspension of $LiAlH_4$ (4.0 g, 0.11 mol) in 300 mL of anhydrous ether was added slowly to a stirring solution of diene 9 (23.6 g, 0.17 mol) in 250 mL of ether at room temperature. The mixture was stirred for 1 h, and the excess hydride was destroyed by addition of 5 mL of ethyl acetate. Water was added cautiously to precipitate aluminum salts. The ether solution was filtered, dried ($MgSO_4$), concentrated, and distilled to afford 16.1 g (87%) of diene 10, bp 81 °C (2.7 mm).

The same procedure was used to prepare unlabeled 10: IR (neat) 3350, 1650 cm^{-1}; ^{1}H NMR (60 MHz) δ 1.70 (br s, 1 H), 2.67 (s, 4 H), 3.97 (s, 2 H), 5.66 (s, 3 H). Anal. ($C_7H_{10}O$) C, H.

[2-^{2}H]-1-(Bromomethyl)cyclohexa-1,4-diene (11). To a stirring solution of triphenylphosphine (41.0 g, 0.156 mol) in 110 mL of CH_3CN at 0 °C was added bromine (24.0 g, 0.15 mol) in a dropwise fashion. The cooling bath was removed, and a solution of diene 10 (16.1 g, 0.145 mol) in 20 mL of CH_3CN was added in portions with continued stirring. After the addition was complete, the mixture was heated at 60–70 °C for 30 min, at which time all of the precipitate had dissolved. The solution was cooled, concentrated, and distilled in vacuo to afford 15.5 g (58%) of diene 11, bp 41 °C (1.2 mm).

The same procedure was used to prepare unlabeled diene 11: IR (neat) 1645 cm^{-1}; ^{1}H NMR (60 MHz) δ 2.73 (s, 4 H), 3.93 (s, 2 H), 5.70 (s, 2 H), 5.87 (br s, 1 H). Anal. (C_7H_9Br) C, H.

[2-^{2}H]-1-Methylcyclohexa-1,4-diene (12). To a suspension of $LiAlH_4$ (1.63 g, 43 mmol) in 50 mL of anhydrous ether stirred at room temperature was added diene 11 (15 g, 86 mmol) in a dropwise fashion. After addition was complete, the mixture was stirred for 4 h. Ether saturated with water was added to destroy excess hydride, and the precipitated salts were removed by filtration. The ether layer was dried and distilled through a Vigreux

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column to remove solvent and to afford 4.5 g (56%) of diene **12**, bp 115 °C.

[2,4-$^2H_2$]-3-Methylcyclohexa-1,4-diene (13). Diene **13** was purchased from Isomet Crop., NJ. The route of synthesis consisted of Birch reduction of [2,6-$^2H_2$]benzoic acid followed by a sequence of steps analogous to those above to convert the carboxyl group into a methyl group (cf. ref, 11). The resultant methylcyclohexadiene (**13**) had 90% deuterium incorporated at C-2 and at C-4.

[4-^{2}H]-1-Methylcyclohexa-1,4-diene (15). Diene **15** (80% ^2H) was prepared by Birch reduction of [4-^{2}H]toluene[9] (80% ^2H) as described previously.[18]

Deuterated Toluene Oxides (1, 8, 14). Toluene 1,2-oxides **1** and **8** were prepared from dienes **12** and **15**, respectively, as described previously for the preparation of toluene 1,2-oxide[13] except that dehydrobromination of the dibromo epoxide was effected with 1,5-diazabicyclo[4,3.0]non-5-ene (DBN) in ether at room temperature. Toluene 2,3-oxide **14** was prepared from diene **13** as described for the unlabeled compound.[11] Oxide **8** had 80% ^2H at C-4 (^1H NMR), oxide **1** had 99% ^2H at C-2, and oxide **14** had 90% ^2H at C-2 and C-6.

Aromatization of 8. *o*-Cresol from **8** was brominated to afford 4,6-dibromo-2-methylphenol,[19] and the deuterium content at C-5 was determined from the 60-MHz ^1H NMR spectrum [δ 7.32 (H_3), 7.49 (H_5)]. The deuterium content at C-5 of the dibromo compound from aromatization at different pH's was 78% (pH 1.1), 79% (pH 4.0), 79% (pH 7.0), and 80% (pH 10.0).

Acknowledgment. We are grateful to the National Institutes of Health, Grant 1 RO1 GM 26388, and the North Atlantic Treaty Organization, Grant 1807, for financial support. We are also pleased to acknowledge the assistance of Dr. H. Fales and Mr. W. Comstock of the NIH in obtaining mass spectra.

Registry No. **1**, 76758-22-0; **8**, 76758-23-1; **9**, 76758-24-2; **10**, 76758-25-3; **11**, 76900-74-8; **12**, 76900-73-7; **13**, 76900-72-6; **14**, 76758-26-4; **15**, 76758-27-5.

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Organic Photochemistry with 6.7-eV Photons: *cis*- and *trans*-Bicyclo[6.1.0]nonanes

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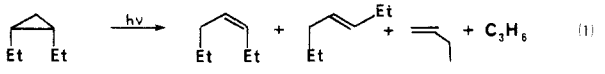
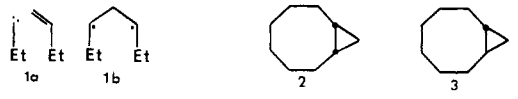
Received November 7, 1980

It had been reported previously[1] that photolysis of 1,2-dialkylcyclopropanes in solution with 185-nm radiation gave both one- and two-bond cleavage, typically as shown in eq 1. *cis*-1,2-Diethylcyclopropane (**1**) gave the products



(1)

shown in eq 1 which were visualized to arise via the intermediates **1a** and **1b**. The unanswered questions from



this study were (i) the failure to detect *trans*-1,2-diethylcyclopropane from the cis isomer by the reversal of the processes by which **1a** and **1b** were formed and (ii) the varying ratio of *cis*- to *trans*-3-heptene that was observed to depend on the starting material, which did not fit the idea of common intermediate(s) or agree with results by other workers[2] on the gas-phase photolysis of other 1,2-dialkylcyclopropanes.

A significant part of the problem in the study of 1,2-diethylcyclopropane was the meager yield of products from a one-bond cleavage and the formation of two small fragment molecules from a two-bond cleavage. In order to overcome these difficulties, an investigation of the photochemistry of the bicyclo[6.1.0]nonanes **2** and **3** was undertaken. The homologous relationship of these compounds to cyclooctene, the stereoisomerization of which is a successful reaction at 185 nm,[3] suggested that stereoisomerization may be observed in this instance as well.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer and/or a Beckman Acculab-6 spectrometer. The solvent was CCl_4 in all instances. NMR spectra were recorded on a Varian T60-1A spectrometer with CCl_4 as solvent and tetramethylsilane as internal reference. Mass spectra were recorded on a Du Pont 21-490B mass spectrometer.

Apparatus. The equipment for photolyses on both preparative (0.1–0.5 g) and quantitative scales has been described.[4] Irradiation at 214 nm was conducted in a cylindrical quartz cell of 50-mL volume the center of which was placed a Phillips Zn resonance lamp. The useful radiation was the Zn resonance line at 214 nm.

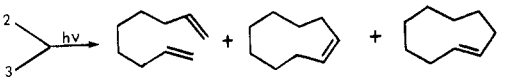
Materials. Compounds **2** and **3** were prepared by using methods described in the literature.[5] *trans*-Cyclononene was obtained from the photoisomerization of *cis*-cyclononene[6] on a preparative scale. All reactants were purified by GLC and their purities checked on two different columns.

Procedure. A solution of the reactant ($\sim 10^{-2}$ M) in pentane was placed in a cylindrical cell (volume 9.3 mL) with a Suprasil window and flushed with nitrogen. Photolyses were carried to conversions of <20%, and the initial, linear portion of the rate curve was extrapolated to zero time.

The products were analyzed on a Perkin-Elmer 3920B gas chromatograph fitted with a Carbowax column (14 ft × $^1/_8$ in.) or a gum rubber column of the same dimensions.

Results

Photolyses of both **2** and **3** gave rise to mixtures of 1,8-nonadiene (major product) and *cis*- and *trans*-cyclononenes (eq 2). There was no evidence for the formation



(2)

of **2** in the photolysis of **3** or of **3** from the photolysis of **2**. The limit of detection was 4% of the reactant that had disappeared.

The rates of formation of the products in the photolysis of either **2** or **3** are shown as a composite plot in Figure 1. The uncertainty in the determination which is principally in the analysis for the products is indicated by

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