(9) The mechanistic implications of the generation of the "natural" configuration at C-7 will be discussed in our full paper.

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Received March 24, 1975

Novel Rearrangements during Dehydration of Nucleophile Adducts of Arene Oxides. A Reappraisal of Premercapturic Acid Structures

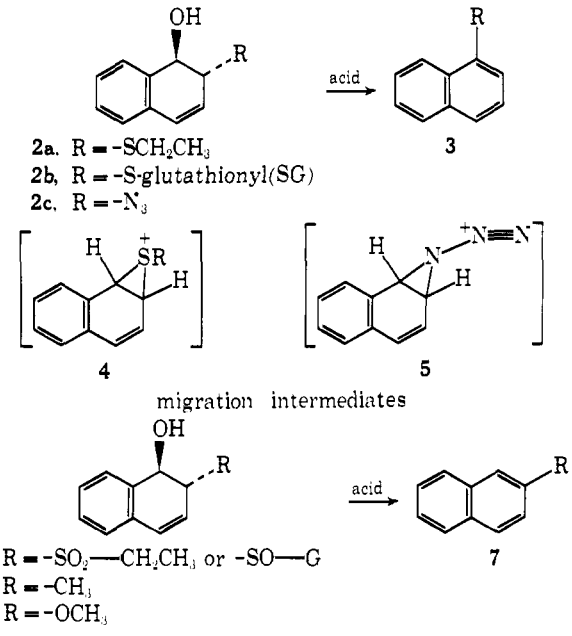
Sir:

Covalent binding of environmental agents to cellular entities such as protein and nucleic acid has been proposed as a prerequisite for the chemical induction of cancer.[1] For the polycyclic aromatic hydrocarbon class of carcinogens, metabolically formed arene oxides have emerged as the most viable candidates to account for this binding.[2] Structural information on the nature of arene oxide adducts, thus, acquires special significance. The present study establishes that the structures of arene oxide adducts must be determined prior to dehydration since unanticipated rearrangements can accompany aromatization.

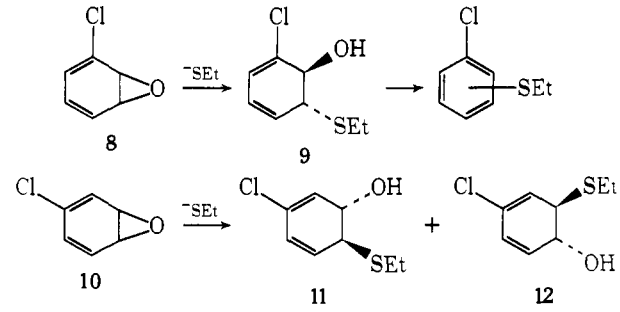
The most common binding reaction of arene oxides within the cell consists of opening of the oxirane ring by the thiol group of the tripeptide glutathione to form a premercapturic acid.[2,3] Reaction of arene oxides with glutathione occurs both spontaneously and enzymatically with glutathione-*S*-epoxide transferase.[4,5] For naphthalene 1,2-oxide (**1**), the adduct prepared chemically or enzymatically has been assigned the structure 1-*S*-glutathionyl-2-hydroxy-1,2-dihydronaphthalene based primarily on the observations[6,7] that dehydration results in a C-1 substituted naphthalene ring. The ^{1}H NMR spectrum of the chemically synthesized adduct[6] (D_2O at 220 MHz) *suggests that sulfur is attached at C-2 rather than C-1.* Partial coincidence of signals from the peptide residue with the critical signals from the dihydronaphthalene ring, however, prevent rigorous assignment of sulfur substitution at C-2 in the adduct. Dehydration of *trans*-1-hydroxy-2-thioethyl-1,2-dihydronaphthalene[8] (**2a,** Scheme I) was, therefore, examined as a simpler model. Treatment of **2a** with 5% trifluoroacetic acid in methanol at room temperature caused rapid dehydration to 1-thioethylnaphthalene[9] (**3a**), *via* migration of the thioethyl residue.

Since a cyclic sulfonium ion (**4,** Scheme I) is implicated as the intermediate during dehydration and rearrangement of **2a,** decrease in the electron density at sulfur should inhibit migration without preventing dehydration. Treatment of **2a** with H_2O_2 and tungstic acid[10] in water led to a 90:10 mixture of 2- and 1-naphthyl ethyl sulfone,[11] respectively. Application of this technique to inhibit migration during dehydration of **2b** was also successful. An aqueous solution of the peptide conjugate was treated with 15% H_2O_2 at pH 7 and 0° for 6 hr. After destruction of excess H_2O_2 with catalase, the oxidized conjugate (λ_{max} 269 nm) was made strongly acidic and heated at 100° for 5 min to effect dehydration. The product consists principally of **7a** based on a λ_{max} of 276 nm with minor peaks at 265, 282, and 321 nm, a spectrum typical of the 2-substituted naphthalenes employed for this study.[12] Dehydration without oxidation gave a λ_{max} of 298 nm.

Scheme I



Scheme II



The facility of migration for groups other than thioethers was then examined. *trans*-1-Hydroxy-2-methyl-1,2-dihydronaphthalene (**6b**) is known to dehydrate without migration of the methyl group to produce 2-methylnaphthalene[8] (**7b**). Addition of methoxide to **1** provided *trans*-1-hydroxy-2-methoxy-1,2-dihydronaphthalene (**6c**)[13] which decomposed (concentrated HCl in $CHCl_3$, 100° for 5 min) into 1-naphthol and 2-methoxynaphthalene (GLC–MS, 15% QF-1, 170°) in a ratio of 10:3, respectively. While other products were present, neither 2-naphthol nor 1-methoxynaphthalene (10% Carbowax 20 M, 150°) could be detected. Thus for **6c,** migration of the hydroxy group to C-2 or of the methoxy group to C-1 does not occur. In contrast, *trans*-1-hydroxy-2-azido-1,2-dihydronaphthalene[8] (**2c**) dehydrates ($t_{1/2} \sim 11$ min, 1 *N* HCl, 50°) to 1-azidonaphthalene (**2c**) with essentially complete migration of the azido group.[14] The facility with which intermediate **5** may be formed from a carbonium ion accounts for the facile migration. Evidently, only those substituents which readily stabilize positive charge (-SR, $-N_3$) undergo migration. The directed isomerization of **1** to form 1-naphthol,[15] the preferential ring opening of **1** by attack of nucleophiles at C-2, and the direction of migration during dehydration of these adducts all point to the greater stability of the carbonium ion at C-2.

Decomposition of the thiol adducts from 3- and 4-chlorobenzene oxides[16] (**8** and **10,** Scheme II) is also accompanied by migration of the thioether group. The adducts were prepared by allowing 0.2 m*M* solutions of the oxides in methanol to stand for 2 hr at 0° in the presence of 3 equiv each of ethanethiol and KOH. The reactions went to completion and were not accompanied by the production of phenols.

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.

References and Notes

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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-