Rearrangements on Acid-Catalyzed Dehydration of Regioisomeric Thiol Adducts Formed from K-Region Arene Oxides

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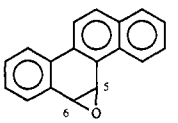
Abstract: K-Region 5,6-oxides of chrysene, benz[*a*]anthracene, and benzo[*c*]phenanthrene undergo nucleophilic trans attack by *tert*-butylthiolate anion in aqueous dioxane to give mixtures of regioisomeric thiol adducts. These adducts have been separated by HPLC, and the positions of thiol addition have been assigned by NMR and/or CD spectroscopy. Adducts characterized were as follows: from chrysene 5,6-oxide, 5-hydroxy-6-(*tert*-butylthio) (minor) and 5-(*tert*-butylthio)-6-hydroxy (major); from benz[*a*]anthracene 5,6-oxide, 5-(*tert*-butylthio)-6-hydroxy and 5-hydroxy-6-(*tert*-butylthio) (equal amounts); and from benzo[*c*]phenanthrene 5,6-oxide, 5-(*tert*-butylthio)-6-hydroxy (minor) and 5-hydroxy-6-(*tert*-butylthio) (major). Reaction of either one of a pair of regioisomeric adducts with boron trifluoride in ether gave the same mixture of aryl alkyl thioethers, as a result of sulfur migration, presumably via a common episulfonium ion intermediate. Under these acid conditions, both chrysene adducts gave exclusively 6-(*tert*-butylthio)chrysene, both benz[*a*]anthracene adducts gave predominantly (87%) 5-(*tert*-butylthio)benz[*a*]anthracene, and both benzo[*c*]phenanthrene adducts gave predominantly (90%) 5-(*tert*-butylthio)benzo[*c*]-phenanthrene. We have observed for the first time that thiol adducts of K-region arene oxides also undergo a facile *base-catalyzed* elimination of water in the presence of sodium methoxide in THF. In contrast to the acid-catalyzed reactions, the reaction of each pure adduct under these basic conditions gave a single aryl alkyl thioether that resulted from dehydration without migration of the sulfur substituent. Because of the dissimilarity in mechanism between the acid and base reactions of the adducts, it is essential that a distinction be drawn between acid- and base-induced dehydrations of K-region thiol adducts, such that the structures of the original adducts can only be deduced from the aromatized products of the *base-catalyzed* dehydration. Reaction of several thiol adducts of benzo[*c*]phenanthrene 5,6-oxide in acid has been observed to yield benzo[*c*]phenanthrene. A proposed sulfenyl chloride intermediate from this reaction in hydrochloric acid has been trapped by its addition to cyclohexene. It is suggested that hydrocarbon formation occurs by attack of a nucleophile on the sulfur of a cationic intermediate.

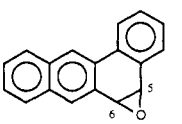
Several years ago we had noted that benzo-ring (non-K-region) arene oxides are more susceptible to attack by nucleophiles at the oxirane carbon that forms the more stable carbocation.[1-3] Thus, naphthalene 1,2-oxide is regioselectively attacked at the allylic oxirane carbon C_2 in preference to the benzylic oxirane carbon C_1. Acid-catalyzed dehydration of such trans thiol adducts was found to occur with migration of the thiol group; i.e., *trans*-1-hydroxy-2-(thioethyl)-1,2-dihydronaphthalene forms 1-(thioethyl)naphthalene. An episulfonium ion that regenerates the more stable carbocation at C_2 was suggested as an intermediate in the reaction, since oxidation to the sulfoxide prior to acid-catalyzed dehydration led to dehydration without migration of the sulfoxide residue.

Beland and Harvey[4] extended these studies to a series of K-region arene oxides using *tert*-butylthiolate as the nucleophile. In refluxing tetrahydrofuran (typically 2-5 h) containing *tert*-butylthiol and an excess of sodium ethoxide (added in a small amount of ethanol), adducts were presumed to form and subsequently to dehydrate to *tert*-butyl aryl thioethers, which were isolated as products. In the case of the nonsymmetric benz[*a*]-anthracene 5,6-oxide, benzo[*a*]pyrene 4,5-oxide, and chrysene 5,6-oxide, mixtures of positional isomers of the fully aromatized thioethers were obtained. The ratio of these positional isomers corresponded to the ratio of positional isomers of the adducts formed when the arene oxides were allowed to react with the thiolate anion in alkaline, 50% dioxane-water for 3 h at 70 °C. This latter ratio was determined from NMR spectra of the acetylated adduct mixtures since difficulty in separation of the adducts was encountered. In the case of benzo[*c*]phenanthrene 5,6-oxide, a single adduct isomer and corresponding fully aromatic thioether (both with sulfur at C_6) were reported. These results prompted the claim that dehydration of thiolate adducts is not accompanied by sulfur migration for these K-region derivatives.[4] As this conclusion was at variance with the earlier results on the acid-catalyzed dehydration of thiolate adducts of non-K-region arene oxides,[1] further investigation was warranted.

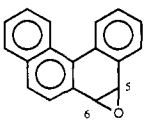
The regioisomeric *tert*-butylthiol adducts from chrysene, benz[*a*]anthracene, and benzo[*c*]phenanthrene 5,6-oxides (**1-3**, respectively) have now been separated chromatographically, and



chrysene 5,6-oxide (**1**)



benz[a]anthracene 5,6-oxide (**2**)



benzo[c]phenanthrene 5,6-oxide (**3**)

the acid- and base-catalyzed dehydrations of the individual isomers have been investigated. As expected, base-catalyzed dehydration proceeds without migration of sulfur. However, *under acid conditions*, sulfur migration occurs, such that the same mixture of aryl alkyl thioethers is obtained upon reaction of either positionally isomeric adduct from a given hydrocarbon.

Results and Discussion

Assignment of Structure to the Isomeric Adducts. Upon reaction with excess sodium *tert*-butylthiolate in aqueous dioxane, each of the arene oxides **1-3** formed a pair of regioisomeric trans adducts. The positional isomers in each pair were readily separated by HPLC, and their NMR spectra were recorded (Table I). Assignment of structure to the adducts from chrysene 5,6-oxide (**1**) is straightforward in that C_5 forms part of a bay region.[5] Consequently, H_5 suffers edge deshielding and is further downfield than would otherwise be expected. Since hydroxyl substitution relative to thioether substitution causes a greater downfield shift

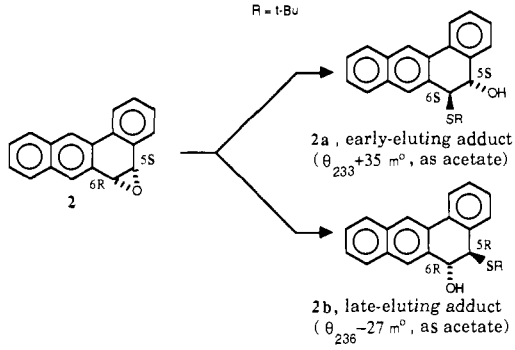
(1) Jeffrey, A. M.; Jerina, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 4427-4428.
(2) Jeffrey, A. M.; Yeh, H. J. C.; Jerina, D. M.; DeMarinis, R. M.; Foster, C. H.; Piccolo, D. E.; Berchtold, G. A. *J. Am. Chem. Soc.* **1974**, *96*, 6929-6937.
(3) Bruice, P. Y.; Bruice, T. C.; Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1976**, *98*, 2973-2981.
(4) Beland, F. A.; Harvey, R. G. *J. Am. Chem. Soc.* **1976**, *98*, 4963-4970.
(5) Bartle, K. D.; Jones, D. W. *Adv. Org. Chem.* **1972**, *8*, 317-423.

Table I. NMR Spectra (300 MHz, $CDCl_3$) of the Pairs of Trans Adducts Formed from Chrysene, Benz[*a*]anthracene, and Benzo[*c*]phenanthrene 5,6-Oxides on Reaction with *tert*-Butylthiolate (SR)

K-region oxide	elution time[a] (HPLC, min)	adduct structure	chem shift H_5	chem shift H_6	coupling[b] ($J_{5,6}$, Hz)	adduct ratio[c] (early:late)
chrysene 5,6-oxide (**1**)	5.2	**1a** 5-OH,6-SR	5.70	4.36	2.1	1:2
	5.9	**1b** 5-SR,6-OH	5.11	5.05	2.7	
benz[*a*]anthracene 5,6-oxide (**2**)	3.4	**2a** 5-OH,6-SR	4.80	4.29	6.8	1:1
	3.7	**2b** 5-SR,6-OH	4.21	4.94	6.1	
benzo[*c*]phenanthrene 5,6-oxide (**3**)	3.1	**3a** 5-SR,6-OH	4.10	4.71	7.2	1:3
	3.5	**3b** 5-OH,6-SR	4.78	4.18	6.1	

[a] HPLC on a Du Pont Zorbax SIL column (9.4 × 250 mm) eluted at 10 mL/min with 1.25% methanol and 7.5% ethyl acetate in hexane. The column had a void time of 1.3 min based on elution of toluene. Separations were base line. [b] The hydrogens on methine carbons bearing hydroxyl groups showed coupling to the OH prior to addition of deuteriomethanol. [c] Adducts formed upon reaction of the arene oxides with 14 mM *tert*-butylthiol at pH 12.2 in 3:7 (vol) dioxane–water, 40 °C, for 4 h.

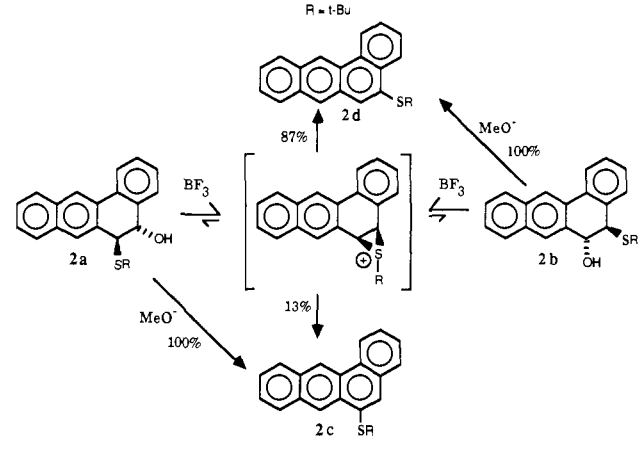
Scheme I



of the methine hydrogen, the major (late eluting) adduct **1b** results from attack by the thiolate anion at C_5 in the hindered bay region. Structures of the positional isomers formed from benz[*a*]anthracene and benzo[*c*]phenanthrene 5,6-oxides (**2** and **3**, respectively) were not apparent from their NMR spectra (**2a,b** and **3a,b**, respectively, in Table I). In order to assign the position of thiolate attack on the positional isomers **2a** and **2b**, optically active (+)-benz[*a*]anthracene 5(*S*),6(*R*)-oxide[6] was used in the reaction (Scheme I). Comparison of the CD spectra (in acetonitrile, normalized to 1 AU at λ_{max}) of these *tert*-butylthiolate adducts, as their acetate esters, with those previously reported for the trans adducts of glutathione anion[7] to this arene oxide established that the early-eluting *tert*-butylthiolate adduct **2a** resulted from attack at C_6 and the late-eluting adduct **2b** from attack at C_5 (Scheme I). Diaxial orientation of the K-region substituents was confirmed by NMR spectra in acetonitrile-d_3: acetate of early-eluting adduct **2a**, H_5 6.07 ppm, H_6 4.61 ppm with $J_{5,6}$ = 2.95 Hz; acetate of late-eluting adduct **2b**, H_5 4.47 ppm, H_6 6.23 ppm with $J_{5,6}$ = 2.7 Hz. Structures of the adducts (**3a,b**) formed by attack of *tert*-butylthiolate on benzo[*c*]phenanthrene 5,6-oxide (**3**) were established through use of **3** that was 50% deuterated[8] at C_5. For the major (late-eluting) adduct **3b**, the NMR signal for the methine hydrogen geminal to the hydroxyl substituent (4.78 ppm) integrated for less than one hydrogen, indicating partial deuteration. The signal for the hydrogen geminal to sulfur (4.18 ppm) appeared as an asymmetric triplet, due to partial deuterium substitution at the *adjacent* position, instead of the doublet ($J_{5,6}$ = 6.1 Hz) observed in the nondeuterated counterpart. In the minor (early-eluting) adduct **3a** the methine hydrogen geminal to sulfur (4.10 ppm) gave a doublet ($J_{5,6}$ = 7.1 Hz) that integrated for less than one hydrogen. Thus, the major adduct resulted from attack by the thiolate anion at C_6 (Table I). The ratio of minor to major adducts formed from the three arene oxides with *tert*-butylthiolate in dioxane–water is generally in agreement with that previously reported,[4] with the exception that the minor adduct (25%) from **3** had not been detected.

(6) Armstrong, R. N.; Kedzierski, B.; Levin, W.; Jerina, D. M. *J. Biol. Chem.* **1981**, *256*, 4726–4733.
(7) Cobb, D.; Boehlert, C.; Lewis, D.; Armstrong, R. N. *Biochemistry* **1983**, *22*, 805–812.
(8) Sayer, J. M.; van Bladeren, P. J.; Yeh, H. J. C.; Jerina, D. M. *J. Org. Chem.* **1986**, *51*, 452–456.

Scheme II



Dehydration of Adducts Derived from *tert*-Butylthiol. In the earlier study,[4] no distinction was made between dehydration of adducts in *refluxing, basic THF* versus elimination of water under *acidic* conditions. The present study demonstrates that these are two mechanistically distinct dehydration processes that yield markedly different product distributions.

We have observed that dehydration of the adducts in refluxing THF does not proceed unless an excess of alkoxide over thiol is present. Thus, the reaction is presumably catalyzed by *alkoxide* but *not by thiolate* anions. The procedure used in the previous study[4] in which the oxides were refluxed for 3–5 h with 1.5 molar equiv of *tert*-butylthiol and 1.65 molar equiv of sodium ethoxide gave high yields of aryl *tert*-butyl thioethers. However, when the preformed adducts **1a,b** and **2a,b** were stored in sealed vials at 80 °C overnight in the same medium except that the *tert*-butylthiol was in excess over the alkoxide (such that the thiolate anion was the only base present), *the adducts remained intact.* When the adducts of **1–3** were individually treated with a several-fold molar excess of dry sodium methoxide in THF at room temperature, facile elimination of water occurred to form aryl *tert*-butyl thioethers *without migration* of the *tert*-butyl group. Presumably this base-catalyzed reaction occurs by proton abstraction from the sulfur-bearing carbon followed by (or simultaneous with) expulsion of hydroxide ion. According to such a mechanism, no migration of sulfur would be expected, and none was observed. There was a substantial difference in the rates at which the regioisomeric thiol adducts underwent base-catalyzed dehydration (see Experimental Section). Particularly notable is the rapid rate ($t_{1/2}$ < 5 min) of base-catalyzed dehydration of the benzo[*c*]phenanthrene adduct **3b** as compared with $t_{1/2}$ = 30 min to 2 h for the other adducts investigated.

All three pairs of adducts underwent rapid dehydration when treated with boron trifluoride in ether at room temperature. In contrast to the base-catalyzed reaction, dehydration under these acidic conditions led to migration of the *tert*-butylthiol residue, as illustrated for the benz[*a*]anthracene derivatives in Scheme II. The same product distribution was observed from either member of a pair of adducts (Schemes II and III). Thus, **1a**

Scheme III

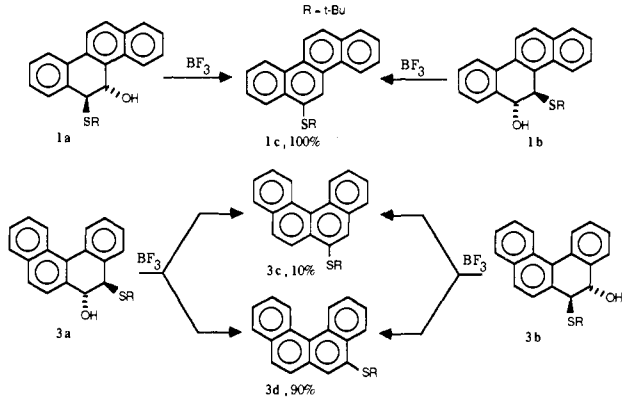


Table II. Comparison of $\Delta E_{deloc}/\beta$ for Carbocations Resulting from Opening of Arene Oxides with the Ratio of *tert*-Butylthiolate Adducts and with Acid-Catalyzed Dehydration Products

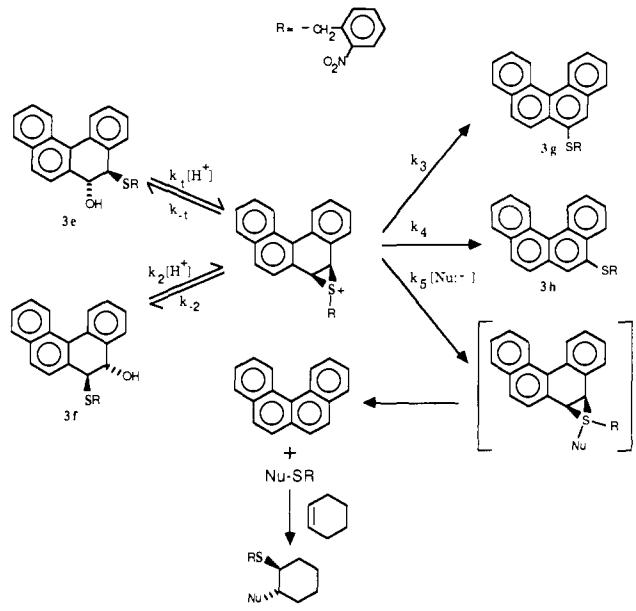
arene oxide	position of carbocation	$\Delta E_{deloc}/\beta$	corresponding thiol adducts	acid products
1	5	0.749	**1b** 66% (5-SR)	**1c** 100% (6-SR)
	6	0.586	**1a** 33% (6-SR)	
2	5	0.576	**2b** 50% (5-SR)	**2c** 13% (6-SR)
	6	0.576	**2a** 50% (6-SR)	**2d** 87% (5-SR)
3	5	0.606	**3a** 25% (5-SR)	**3c** 10% (6-SR)
	6	0.658	**3b** 75% (6-SR)	**3d** 90% (5-SR)

dehydrated without migration and **1b** with complete migration, both **2a** and **2b** gave the same 87:13 mixture of 5-(*tert*-butylthio)- to 6-(*tert*-butylthio)benz[*a*]anthracene, and both **3a** and **3b** gave the same 90:10 mixture of 5-(*tert*-butylthio)- to 6-(*tert*-butylthio)benzo[*c*]phenanthrene. The results are highly suggestive that each pair of adducts dehydrates exclusively via a common episulfonium ion intermediate (Scheme II). Furthermore, evidence was obtained that indicated that some degree of scrambling (**a** ⇌ **b**) occurred between individual pairs of adducts on treatment with boron trifluoride in ether. Analysis by HPLC during the course of boron trifluoride treatment of each of the pure adducts from benz[*a*]anthracene 5,6-oxide indicated the transient formation of an HPLC peak with a retention time identical with that of the other member of each pair. The rapid rates of these dehydrations and the very small amounts of the apparent minor adduct made characterization of the component responsible for these peaks impractical. A serendipitous experiment, however, proved quite informative. Samples of pure **2a** and of pure **2b** were allowed to stand at room temperature in $CDCl_3$ for 4 days. Analysis of both samples by HPLC showed the presence of three major peaks. The components in each sample were separated and characterized as the mixed benz[*a*]anthracene *tert*-butyl thioethers (80:20 mixture of the 5- and 6-derivatives, respectively), adduct **2a** and adduct **2b** in their order of elution. Adducts **2a** and **2b** were present in a 1:1 ratio from either adduct based on their absorbance at 280 nm. Presumed traces of DCl in the $CDCl_3$ had caused some dehydration as well as equilibration between **2a** and **2b**.

Effect of Structure on Regioselectivity. Attempts have been made to correlate the regioselectivity of attack of *tert*-butylthiolate on these arene oxides with reactivity indices.[4,8] The present results suggest that such an approach may not be justified in its simplest form. Since the extent of positive charge development at carbon in the transition state may be quite small for these presumably S_N2-type reactions, factors other than the ability to stabilize a positive charge at carbon may be of crucial importance in determining the direction of attack. Ease of formation of the two possible carbocations from each of the arene oxides, as predicted by the Dewar PMO method,[9] is compared with the ratio of adducts formed by *tert*-butylthiolate in Table II. While it is true that

(9) Dewar, M. J. S. In *The Molecular Orbital Theory of Organic Chemistry*; McGraw-Hill: New York, 1969; pp 214–217, 304–306.

Scheme IV



the major adduct corresponds to attack of thiolate at the more stable carbocation, we have previously noted[10] that methoxide ion in methanol prefers to attack **1** at C_6. More surprising is the observation that the principal (87%) dehydration product formed upon treatment of **2a** and **2b** with boron trifluoride arises from the carbocation at the 6-position, although both the 5- and 6-carbocations are predicted to form with equal facility, leading to a *predicted* 1:1 ratio of the two aryl alkyl thioethers. This result is not a consequence of equilibration between the two fully aromatic thioethers once formed, since samples of pure 5- or 6-(*tert*-butylthio)benz[*a*]anthracene were unchanged after storage in ether in the presence of excess boron trifluoride for 1–2 h. Thus, ease of carbocation formation also appears not to be the sole determinant of transition-state stability in reactions of the hypothetical episulfonium ion intermediate.

Hydrocarbon Formation from Thiolate Adducts of Benzo[*c*]phenanthrene 5,6-Oxide. A number of years ago Boyland and Sims[11a] made the novel observation that *S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)cysteine, a urinary metabolite of phenanthrene presumed to be formed from phenanthrene 9,10-oxide, rapidly liberated phenanthrene (28% yield) on treatment with aqueous HCl. In a like fashion, quinoline is produced from an acid-labile urinary metabolite.[11b] We have also obtained evidence for an analogous reaction in the case of the methyl esters of *N*-acetylcysteine adducts, as well as an ethanethiol adduct, derived from benzo[*c*]phenanthrene 5,6-oxide, upon treatment with 3 M HCl in 1:4 dioxane–water at 40 °C. Benzo[*c*]phenanthrene was also detected as a product of the reaction of **3b** with HCl in 1:1 dioxane–water, although only in trace amounts.

To facilitate investigation of this novel reaction, a chromophoric thiol, (*o*-nitrobenzyl)thiol, was selected in order to trace the fate of the thiol portion of the molecule, which is presumably eliminated from the aromatic system as an unstable sulfenic acid derivative that might be subject to appropriate trapping. Reaction of benzo[*c*]phenanthrene 5,6-oxide with (*o*-nitrobenzyl)thiolate anion at 37 °C in 1:1 dioxane–water gave a mixture of adducts in a ratio of 30:70. By analogy with other thiol adducts of benzo[*c*]-phenanthrene, the structure of the major adduct was assumed to be the 5-hydroxy-6-((*o*-nitrobenzyl)thio) derivative, **3f**, and the

(10) Balani, S. K.; van Bladeren, P. J.; Cassidy, E. S.; Boyd, D. R.; Jerina, D. M. *J. Org. Chem.* **1987**, *52*, 137–144.

(11) (a) Boyland, E.; Sims, P. *Biochem. J.* **1962**, *84*, 564–570. In a related metabolic process, 1,2-dihaloethanes are converted to ethylene. See: Schasteen, C. S.; Reed, D. J. *Toxicol. Appl. Pharmacol.* **1983**, *70*, 423–432. (b) See: Gorrod, J. W. In *Biological Oxidation of Nitrogen in Organic Molecules*; Bridges, J. W., Gorrod, J. W., Parke, D. V., Eds.; Taylor and Francis: London, 1972; p 41.

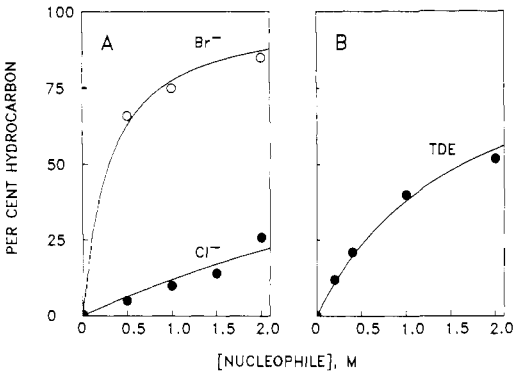


Figure 1. Effect of nucleophiles on product distribution upon reaction of **3f** in acidic solutions at 37 °C. Panel A shows the effect of added lithium chloride (solid symbols) or lithium bromide (open symbols) in the presence of 1 M perchloric acid, at a constant ionic strength (maintained with lithium perchlorate) of 3 M. Panel B shows the effect of replacing bis(2-hydroxyethyl) ether in the reaction medium with bis(2-hydroxyethyl) sulfide (thiodiethanol (TDE)) in the presence of 3 M perchloric acid, at a constant total concentration of (ether + sulfide); the sulfide, but not the ether, is nucleophilic.

minor adduct to be its isomer, **3e** (Scheme IV).

Solvent composition exerts a substantial effect on the product distribution obtained upon reaction of **3f** with 3 M HCl. Changing the solvent composition from 1:5 dioxane–water to 1:1 dioxane–water resulted in an increase in the yield of benzo[*c*]phenanthrene relative to fully aromatic thioethers **3g** and **3h** from 6% to 37%, accompanied by an increase in the half-life of the reaction from 73 to 185 s at 37 °C. Consequently, subsequent experiments were carried out in 1:1 dioxane–water to optimize hydrocarbon yield.

To determine the fate of the sulfur-containing moiety, reaction of **3f** with 3 M HCl was carried out in the presence of cyclohexene, to yield a mixture containing benzo[*c*]phenanthrene, *trans*-1-chloro-2-((*o*-nitrobenzyl)thio)cyclohexane, and thioethers **3g** and **3h** in a molar ratio of ca. 1.0:1.2:0.26:1.6. Chromatographic (255 nm) quantitation of these products was calibrated by NMR spectroscopy of a product mixture (see Experimental Section). This result indicates that the sulfur-containing reaction product formed concurrently with the hydrocarbon was quantitatively trapped by reaction with cyclohexene and suggests that the species trapped was a sulfenyl chloride,[12] since sulfenyl chlorides are known to react via trans addition to alkenes.

A possible mechanism for hydrocarbon formation is shown in Scheme IV. Support for a mechanism involving nucleophilic attack on sulfur is provided by the following observations: (i) Bromide ion, a relatively "soft" nucleophile, is much more effective than chloride ion in bringing about the formation of hydrocarbon, relative to rearrangement. Figure 1A shows the effects of increasing chloride or bromide ion concentration at constant ionic strength (maintained with lithium perchlorate) on the relative yield of benzo[*c*]phenanthrene from **3f** in the presence of 1 M perchloric acid. If it is assumed that replacement of chloride with bromide does not affect the rate of formation of thioethers **3g** and **3h**, the data are consistent with a ca. 25-fold increase in the rate of hydrocarbon formation in the presence of bromide relative to chloride. A similar enhancement in reactivity (35-fold) of bromide relative to chloride was observed by Kice and Large[13] for nucleophilic attack on the divalent sulfur atom of phenyl benzenethiolsulfinate (PhSS(O)Ph). Because of apparent large specific salt effects, including a pronounced inhibition by chloride, on the observed rates of reaction upon replacement of perchlorate with halide ions, it was not possible to assign a kinetic order to the dependence of the reaction rate on chloride or bromide ions. (ii) Addition of a neutral nucleophile, bis(2-hydroxyethyl) sulfide, to reaction mixtures of **3f** in the presence of 3 M perchloric acid also results in the formation of benzo[*c*]phenanthrene (Figure 1B).

(12) Kharasch, N.; Buess, C. M. *J. Am. Chem. Soc.* **1949**, *71*, 2724–2728. Havlik, A. J.; Kharasch, N. *Ibid.* **1956**, *78*, 1207–1210.
(13) Kice, J. L.; Large, G. B. *J. Am. Chem. Soc.* **1968**, *90*, 4069–4076.

Table III. Time Course of Adduct Isomerization and Product Composition in the Acid-Catalyzed Rearrangement of **3e** and **3f**[a]

time, s	approx extent of reaction,[b] %	% **3e** or **3f** in remaining adducts[c]	% of B[*c*]Ph in product mixture[d]
	Starting with Adduct **3e**		
0		98	
20	10	96	
60	30	90	27
240	76	67	40
480	94	41	34
	Starting with Adduct **3f**		
0		98	
15	8	95	22
60	22	92	30
180	50	87	34
360	70	83	35

[a] At 37 °C in 1:1 dioxane–water, [HCl] = 3 M. [b] Calculated from pseudo-first-order rate constants of 5.8×10^{-3} and 4×10^{-3} s^{-1} for **3e** and **3f**, respectively, measured spectrophotometrically (280 nm) under the same reaction conditions. [c] Determined by reverse-phase HPLC with detection at 318 nm; for chromatographic conditions, see text. [d] Determined by reverse-phase HPLC with detection at 255 nm. Correction was made for a small difference in the extinction coefficients of the hydrocarbon and thioethers based on standardization of the method by NMR; see text for details.

Formation of the hydrocarbon under these conditions was shown not to be a result of any reaction involving possible trace contamination of mercaptoethanol in the sulfide (see Experimental Section). To eliminate medium effects of the sulfide (which replaces a significant portion of the dioxane solvent at the concentrations used), these experiments were done at a constant total concentration (2 M) of the sulfide plus its oxygen analogue, bis(2-hydroxyethyl) ether. Under these conditions a small rate increase is observed when the analogous ether is replaced by the sulfide. The magnitude of this rate increase is consistent with a mechanism in which rate and products are determined by a common step: for example, the observed increase (from 0 to 52% at 2 M sulfide) in the mole percent of benzo[*c*]phenanthrene found should correspond to a 2-fold rate increase, if the sulfide does not affect the rate of aryl alkyl thioether formation and if the product-determining process is rate-determining. The observed rate increase was 1.9-fold.

In 3 M HCl, both **3e** and **3f** give the same ratio of benzo[*c*]-phenanthrene to aryl alkyl thioether products, an observation that suggests but does not prove that these products arise from an intermediate that is common to both isomeric reactants. We suggest that this intermediate is the episulfonium ion, which either rearranges to the aryl alkyl thioethers via the 5- and 6-carbocations or undergoes nucleophilic attack to give a sulfurane, which forms the hydrocarbon upon extrusion of RSNu. Precedent for a sulfurane intermediate is provided by the observation (by NMR) of such an intermediate upon reaction of a cyclooctene-*S*-methylepisulfonium salt with chloride ion to yield cyclooctene as the final product.[14] In the present case, aromatization of the hydrocarbon should provide a strong driving force for elimination of the RSNu species. The time dependence of the product composition as well as the isomerization of the reactants **3e** and **3f** (Table III) indicates that, although there is some "scrambling" of the isomer composition of the adducts over the time required for complete reaction, this isomerization cannot be the cause of the identical product composition obtained from both adducts, especially at the earliest reaction times. The observed isomerization of the adducts presumably occurs by reversal of episulfonium ion formation, as previously discussed. On the basis of the mechanism shown in Scheme IV, the time course of reactant isomerization in 3 M HCl is consistent with a partitioning ratio, $(k_3 + k_4 + k_5[Cl^-])/(k_{-1} + k_{-2})$, of ca. 1.6; i.e., the hypothetical intermediate is converted to products 1.6 times more rapidly than it reverts to starting materials under the experimental conditions

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cited in the table. A *smaller* partitioning ratio (i.e., more rapid return of intermediate to reactants relative to rearrangement) would be required to account for the "scrambling" data if the hydrocarbon is formed by a *direct* reaction of the adducts (which are then removed from the possibility of isomerization) rather than via an intermediate, as suggested in Scheme IV.

Both thioether and azide adducts of arene oxides are known to undergo the facile acid-catalyzed migrations[1] described in the present study. Furthermore, the azide adduct of benzene oxide is known to exist at room temperature as an equilibrium mixture of three positional isomers resulting from rapid, consecutive [3,3]-sigmatropic rearrangements.[15] Although methanol adducts of arene oxides have not been reported to undergo migrations of either the OH or OCH_3 substituents on treatment with acid,[1,16] only a few oxygen adducts have been examined. We have, however, identified an instance where apparent migration of an oxygen substituent does occur. Storage of *trans*-6-bromo-5-acetoxy-5,6-dihydrochrysene[17] in acetic acid at 45 °C for 3 h results in complete decomposition to a mixture of 6-bromochrysene and 6-acetoxychrysene.[18] Since formation of 6-acetoxychrysene probably occurs via a rearrangement mediated by an acylium ion, it does not constitute a direct oxygen migration.

Experimental Section

Thiol Adducts of K-Region Arene Oxides. The *tert*-butylthiol adducts of chrysene, benz[*a*]anthracene, and benzo[*c*]phenanthrene 5,6-oxides (**1**–**3**) were prepared by reaction in 10–40% dioxane in water with a tenfold excess of sodium *tert*-butylthiolate for 2–4 h at 40 °C. After extraction of the adducts, the positional isomers from each hydrocarbon were separated by HPLC on a Du Pont Zorbax SIL column, 9.4 × 250 mm, eluted with 1.25% methanol and 7.5% ethyl acetate in hexane at a flow rate of 10 mL/min. Analytical HPLC of adduct mixtures was performed on a Du Pont Golden Series SIL column eluted with 0.8% methanol and 5% ethyl acetate in hexane at a flow rate of 2.5 mL/min. Peak areas were quantitated at or near a UV absorption peak for the adducts: 265 nm for **1a,b**, 257 nm for **2a,b**, and 315 nm for **3a,b**. NMR spectra of the adducts are reported in Table I. Mass spectra (EI) were measured for the benz[*a*]anthracene adducts (**2a,b**) and benzo[*c*]-phenanthrene adducts (**3a,b**) by using a direct-exposure probe and gave an M^+ peak at m/z 334 as well as peaks at m/z 245, corresponding to loss of $(CH_3)_3CS$, and at m/z 316, corresponding to loss of water. HRMS of the minor adduct **3a**, which had not previously been reported: calcd for $C_{22}H_{22}OS$, 334.1391; found, 334.1404. Optically active **2a** and **2b** from (+)-benz[*a*]anthracene 5(*S*),6(*R*)-oxide were acetylated with pyridine and acetic anhydride, and the CD spectra of the acetates were measured in acetonitrile (JASCO J500A spectropolarimeter). Observed ellipticities were normalized to 1.0 AU at λ_{max} for the acetates.

Adducts of benzo[*c*]phenanthrene with (*o*-nitrobenzyl)thiol were prepared by reaction of benzo[*c*]phenanthrene 5,6-oxide (0.07 mmol) with 0.6 mmol of (*o*-nitrobenzyl)thiol (K and K Laboratories) and 0.5 mmol of sodium hydroxide in ca. 20 mL of 1:1 dioxane–water at 37 °C for 4 h. The products were extracted with ethyl acetate and purified by chromatography on a Rainin Dynamax-60A silica column, 21.4 × 250 mm, equipped with a 50-mm guard column of the same material, eluted with 76:20:4 hexane–ethyl acetate–methanol at a flow rate of 25 mL/min. Material with a retention time (rt) of 9 min was collected and separated into two components by chromatography on a Du Pont phenyl column, 4.6 × 250 mm, eluted with 55% acetonitrile in water at a flow rate of 1.5 min; rt(minor), 13.7 min; rt(major), 15.1 min. HRMS ($C_{25}H_{19}NO_3S$): calcd, 413.1086; found, 413.1084 (minor isomer); 413.1053 (major isomer). NMR spectrum (300 MHz, $CDCl_3$) of the minor component: δ 4.11 (s, SCH_2), 4.17 (d, CHS), 4.84 (apparent t, CHOH), $J_{5,6}$ = 4.4 Hz. NMR spectrum of the major component: δ 3.97 (AB quartet, SCH_2), 4.26 (d, CHS), 4.85 (dd, CHOH), $J_{5,6}$ = 4.1 Hz. By analogy with the product distribution from *tert*-butylthiolate addition to **3**, the minor component is presumed to be the 5-((*o*-nitrobenzyl)thio) adduct, **3e**, and the major component is presumed to be the 6-((*o*-nitrobenzyl)thio) adduct, **3f**.

Characterization and Quantitation of Aryl Alkyl Thioethers. Individual adducts were treated with an excess of dry sodium methoxide in THF at room temperature, and the course of reaction was followed by HPLC on the Zorbax SIL column as described. Approximate half-lives for the base-catalyzed dehydrations were as follows: **1a**, 30 min; **1b**, 90 min; **2a**, 40 min; **2b**, 120 min; **3a**, 90 min; **3b**, <5 min. The resultant aryl alkyl thioethers (single product from each adduct isomer) were characterized by their retention times on HPLC on a Du Pont Golden Series ODS column eluted with 15% water in acetonitrile at a flow rate of 2.5 mL/min: chrysene derivatives **1c** (6-SR), 7.37 min, and **1d** (5-SR), 5.79 min; benz[*a*]anthracene derivatives **2c** (6-SR), 6.92 min, and **2d** (5-SR), 7.55 min. The isomeric aryl alkyl thioethers derived from benzo[*c*]-phenanthrene were poorly resolved under the above conditions, but could easily be separated on a Du Pont Golden Series SIL column eluted with hexane at a flow rate of 3 mL/min: **3c** (6-SR), 5.04 min; **3d** (5-SR), 5.34 min. NMR spectra (300 MHz, $CDCl_3$) of the aryl alkyl thioethers agreed with those reported.[4] Thioether **3d**, which had not been previously reported, gave signals at δ 9.01 (m, H_4, peri to sulfur) and 8.21 (s, H_6, ortho to sulfur). HRMS ($C_{22}H_{20}S$): calcd, 316.1286; found, 316.1277.

Acid-catalyzed dehydration of the individual isomeric *tert*-butylthiolate adducts **1a,b**, **2a,b**, and **3a,b** was effected by treatment of each adduct with 1 drop of boron trifluoride etherate in ca. 1 mL of ether at room temperature. The reaction was complete within 10 min. The resultant mixtures of aryl alkyl thioethers were quantitated by HPLC using the conditions described. Product ratios were determined from peak areas integrated at 254 nm (products from **2a,b**) or 270 and 340 nm (products from **3a,b**). These ratios were confirmed by integration of the NMR spectra (300 MHz, $CDCl_3$) of the mixed aryl alkyl thioethers. In the case of **1a,b**, only a single product was detected.

Reactions of (*o*-Nitrobenzyl)thiol Adducts of Benzo[*c*]phenanthrene 5,6-Oxide in Acid: Investigation of Hydrocarbon Formation. For product identification, adduct **3f** was allowed to react with 3 M HCl in 20 mL of 1:1 dioxane–water in the presence of 100 μL of cyclohexene at 37 °C for 20 min. Products were extracted into ethyl acetate and separated by HPLC on a Rainin Microsorb silica column, 10 × 250 mm, eluted with 2.5% ethyl acetate in hexane at a flow rate of 8 mL/min: rt 2.4, 4.6, 6.0, and 6.4 min. The earliest eluting component had been identified as benzo[*c*]phenanthrene on the basis of its ultraviolet spectrum and mass spectrum (CI/CH_4, using a direct-exposure probe), m/z 229 (MH^+), 257 ($MC_2H_5^+$). The compound that was eluted at 4.6 min gave a base peak in the mass spectrum (CI/NH_3, using a direct-exposure probe) at m/z 303, corresponding to $M + NH_4^+$. This compound was identified as *trans*-1-chloro-2-((*o*-nitrobenzyl)thio)cyclohexane on the basis of its NMR spectrum, which exhibited complex multiplets centered at δ 2.8 (CHS) and 4.0 (CHCl), with $J_{1,2}$ = 6.85 Hz, as determined by decoupling. The benzylic *S*-methylene group appears as an AB quartet at 4.21 ppm: J_{gem} = 16.8 Hz. The aromatic proton ortho to the nitro group appears at δ 7.96 in the cyclohexene adduct and at δ 7.98 in the parent thiol, whereas the remaining aromatic signals are located at δ 7.27–7.60 and 7.37–7.60 in the cyclohexene trapping product and the thiol, respectively. The two compounds that were eluted at 6.0 and 6.4 min were identified as aryl alkyl thioethers **3g** (minor) and **3h** (major), respectively. HRMS of the mixed isomers: calcd for $C_{25}H_{17}NO_2S$, 395.0930; found, 395.0997. These isomers are easily distinguished from each other on the basis of the multiplicity of the NMR signal at δ ca. 8.6 (hydrogen peri to the sulfur substituent). In the minor (early) thioether **3g**, sulfur substitution is at C_6 and the peri hydrogen exhibits a doublet (J = 8.8 Hz), centered at δ 8.56, corresponding to H_7. In the major (late) thioether **3h**, sulfur substitution is at C_5, and the peri hydrogen (H_4) exhibits a multiplet centered at δ 8.65.

To calibrate the quantitation of reaction products by HPLC the four purified compounds were mixed, the HPLC profile of the mixture was determined (Perkin-Elmer HS-3 silica column, 4.6 × 10 mm, eluted at 2.0 mL/min with 2.5% ethyl acetate in hexane; detection at 255 nm), and the NMR spectrum of the mixture was measured. The relative areas of the signals at δ 4.21 (SCH_2 of the cyclohexene trapping product), δ 4.48 (SCH_2 of **3g**), and δ 4.54 (SCH_2 of **3h**) were determined. Benzo[*c*]phenanthrene was quantitated by difference between the total area of the bay-region proton signals at δ 9–9.2 (which represent a mixture of benzo[*c*]phenanthrene and the two aryl alkyl thioethers **3g** and **3h**) and the area of the two methylene signals at δ 4.45–4.6 (**3g** and **3h** only). Relative extinction coefficients on HPLC were as follows: benzo[*c*]-phenanthrene (rt 0.9 min), 0.94; 1-chloro-2-((*o*-nitrobenzyl)thio)cyclohexane (2.15 min), 0.2; **3g** (3.2 min), 1.0; **3h** (3.5 min), 1.0.

Rate and Product Studies. Reactions were carried out in 1:1 dioxane–water solutions, prepared with dioxane distilled from sodium. Rates of reaction were measured by following the increase in absorbance at 280

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(16) Acid decomposition of methanol adducts from the 5,6-oxides of chrysene (Weems, H. B.; Fu, P. P.; Yang, S. K. *Carcinogenesis* **1986**, *7*, 1221–1230) and 12-methylbenz[*a*]anthracene (Yang, S. K.; Mushtaq, M.; Weems, H. B.; Miller, D. W.; Fu, P. P. *Biochem. J.* **1987**, *245*, 191–204) has been reported.

(17) van Bladeren, P. J.; Jerina, D. M. *Tetrahedron Lett.* **1983**, *24*, 4903–4906.

(18) Results from the present study. 6-Acetoxychrysene was identified by comparison (UV, NMR) with acetylated 6-hydroxychrysene obtained from Cambridge Chemical Co., Milwaukee, WI.

nm. Reactions for kinetics were initiated by addition of 1-2 μL of **3e** or **3f** in acetonitrile per mL of the dioxane–water reaction mixture (1.0- or 3.0-mL total volume) which had been allowed to equilibrate to 37 °C. For product analyses, reaction mixtures were diluted with 1 volume of water and extracted with 1:1 hexane–ethyl acetate. After drying and evaporation of the organic solvents, the residue was dissolved in 2.5% ethyl acetate in hexane and analyzed by HPLC on the Perkin-Elmer HS-3 silica column as described above. The kinetics of these reactions generally exhibited an apparent lag phase during the first half-life; pseudo-first-order rate constants were determined from the later portion of the reaction where log $(A_\infty - A_t)$ vs time was linear. The cause of this apparent induction period is unclear. Neither the induction period, the final rate, nor the product distribution was affected by the presence of oxygen, as shown by a pair of parallel experiments in 3 M HCl in which one reaction mixture was degassed by purging with argon and a second was treated with pure oxygen prior to addition of adduct **3f**.

In an experiment to determine the effect of 2,2′-thiodiethanol [bis(2-hydroxyethyl) sulfide] on product distribution, 4 M solutions of 2,2′-thiodiethanol and bis(2-hydroxyethyl) ether in dioxane were prepared volumetrically and equal volumes of 6 M aqueous perchloric acid were mixed with appropriate mixtures of the above organic solutions such that the final concentration of 2,2′-thiodiethanol plus bis(2-hydroxyethyl) ether was held constant at 2 M. This was done to minimize medium effects that might result from the large amounts of thiodiethanol used. Because of the possibility that trace contamination of the thiodiethanol with free mercaptoethanol could influence product ratios, samples of reaction mixtures were neutralized, tested with Ellman's reagent,[19] and found to contain <0.05 mM free thiol. A reaction mixture containing 26 mM mercaptoethanol gave no benzo[*c*]phenanthrene product. Reverse-phase HPLC (Du Pont Zorbax phenyl column) of this mixture indicated the formation of the aromatic dehydration products as well as a broad peak that was slightly less polar than the initial adducts. This material (not seen in reaction mixtures containing 2,2′-thiodiethanol alone) had a UV spectrum similar to that of the initial adducts and was stable under the reaction conditions. Although this peak was not further characterized, we speculate that it corresponds to one or two isomeric bisthiol adducts formed by nucleophilic attack of the added mercaptoethanol.

The time course of adduct isomerization in the presence of 3 M hydrochloric acid was monitored by removal of 2-mL aliquots from a 10-mL reaction mixture and quenching with 5 mL of a solution containing 6 mmol of sodium hydroxide and 1 mmol each of sodium dihydrogen phosphate and disodium hydrogen phosphate. Products were extracted with three (ca. 1 mL) portions of ethyl acetate. After solvent evaporation, the residues were dissolved in 0.2 mL of methanol and portions were analyzed by HPLC on a Du Pont Zorbax phenyl column, 4.2 × 250 mm, eluted with 60% acetonitrile in water at 1.5 mL/min for 5 min, followed by a linear gradient to 100% acetonitrile in 15 min: rt 8.1 (**3e**), 8.6 (**3f**), 11.2 (benzo[*c*]phenanthrene), and 16.2 min (**3g** plus **3h**). The ratio of adducts **3e** and **3f** was determined at 318 nm, and the ratio of products was determined at 255 nm.

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Biosynthesis of NCS Chrom A, the Chromophore of the Antitumor Antibiotic Neocarzinostatin

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Abstract: Biosynthetic studies on neocarzinostatin chromophore A (NCS Chrom A) were carried out on the basis of the incorporation of singly and doubly ^{13}C labeled acetate precursors as well as radiolabeled [*methyl*-^{3}H]methionine, [^{14}C]sodium bicarbonate, and [^{14}C]sodium acetate by cultures of *Streptomyces carzinostaticus* (ATCC #15944 F-42). The results suggest that the *N*-methyl of the fucosamine and the *O*-methyl of the naphthoic acid moieties are derived from methionine via *S*-adenosylmethionine and the cyclic carbonate carbonyl carbon from carbonate. The acetate incorporation results show that the C_{12} naphthoic acid ring is derived from a hexaketide. The intriguing C_{14} cyclic carbonate/bicyclo[7.3.0]dodecadienediyne ring system, on the other hand, appears to be derived from a minimum of eight head to tail coupled acetate units which is discussed in terms of the oleate–crepenynate biosynthetic pathway for polyacetylenes. The related C_{15} enediyne ring skeleton in the esperamicin/calicheamicin class of antitumor antibiotics may be similarly derived. These incorporation experiments provide independent support for the unprecedented structure of NCS Chrom A.

Neocarzinostatin (NCS) is a member of a family of macromolecular antitumor antibiotics obtained from culture filtrates of *Streptomyces*.[1] The drug causes DNA strand breakage in vivo and in vitro in a reaction greatly stimulated by a sulfhydryl compound.[2] All biological activity resides in a methanol-extractable nonprotein chromophore that is tightly and specifically bound to an apoprotein (M_r = 11 000).[3] In previous reports[4] we have elaborated on the structure of the major component NCS Chrom A and its relationship to two active minor components B and C. We proposed that NCS Chrom A consists of a cyclic

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