(15) J. P. Hummel, D. Gust, and K. Mislow, *J. Am. Chem. Soc.*, **96**, 3679 (1974).
(16) (a) J. D. Andose and K. Mislow, *J. Am. Chem. Soc.*, **96**, 2168 (1974); (b) M. R. Kates, J. D. Andose, P. Finocchiaro, D. Gust, and K. Mislow, *Ibid.*, **97**, 1772 (1975).
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(18) J. Brocas, R. Willem, D. Fastenakel, and J. Buschen, *Bull. Soc. Chim. Belg.*, **84**, 483 (1975).
(19) H. S. Gutowsky and C. H. Holm, *J. Chem. Phys.*, **25**, 1228 (1956).
(20) H. Lankamp, W. Th. Nauta, and C. MacLean, *Tetrahedron Lett.*, 249 (1968).
(21) A clear-cut choice could be made by introducing a prochiral group[15] or by operating in a chiral environment.
(22) The computer program used was adapted from one developed by M. Saunders (see M. Saunders in "Magnetic Resonances in Biological Systems", A. Ehrenberg, B. C. Malmström, and T. Vänngard, Ed., Pergamon Press, New York, N.Y., 1967, p 85). We are grateful to Professor Saunders for providing us with a copy of the program, and to Drs. J. D. Andose and V. Librando for the modifications.
(23) H. Shanan-Atidi and K. H. Bar-Eli, *J. Phys. Chem.*, **74**, 961 (1970).
(24) Within the limitations of the present evidence, steric arguments must be resorted to in order to differentiate between modes $\mathbf{M}_{11}$ and $\mathbf{M}_{17}$ as threshold mechanisms. The group generated by these two modes ($\mathbf{G}_{4a}$ and $\mathbf{G}_{4b}$, respectively; see Table VI) contains elements in the same conjugacy classes of $\mathbf{G}_{64}$ and thus the same number of isomers and NMR signals will always be expected under the action of each of these two modes. Since there is only one mode equivalent flip mechanism for each, there is no possibility of differentiating between them on the basis of diastereomeric mode equivalent pathways.
(25) The first example of residual diastereotopism in a molecular propeller was reported by J. C. Martin and co-workers,[26] who found that the *o*-methyl and *o*-methoxy signals in dimesityl(2,4,6-trimethoxyphenyl)methane coalesce at −20 and 145 °C, respectively. The residual diastereotopism of the *o*-methoxy groups at temperatures above −20° and below 145° may be viewed[4,6] as the ghost of the residual stereoisomerism in dimesityl(3-methyl-2,4,6-trimethoxyphenyl)methane, an as yet unreported compound which will predictably[8] exist in two diastereomeric forms.
(26) M. J. Sabacky, S. M. Johnson, J. C. Martin, and I. C. Paul, *J. Am. Chem. Soc.*, **91**, 7542 (1969).
(27) D. Hellwinkel, M. Melan, W. Egan, and C. R. Degel, *Chem. Ber.*, **108**, 2219 (1975).
(28) J. F. Blount and K. Mislow, *Tetrahedron Lett.*, 909 (1975).
(29) P. Finocchiaro, D. Gust, and K. Mislow, *J. Am. Chem. Soc.*, **96**, 2176 (1974).
(30) M. G. Hutchings, J. d. Andose, and K. Mislow, *J. Am. Chem. Soc.*, **97**, 4562 (1975).
(31) See footnote 21 of part I.
(32) A. L. Van Geet, *Anal. Chem.*, **42**, 679 (1970); **40**, 2227 (1968).
(33) The transmission coefficient was assumed to be unity.[34]
(34) See G. Binsch, *Top. Stereochem.*, **3**, 97 (1968).

Reactions of the K-Region Oxides of Carcinogenic and Related Polycyclic Hydrocarbons with Nucleophiles: Stereochemistry and Regioselectivity

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Abstract: Reactions of the "K-region" oxides of a series of carcinogenic and related polycyclic hydrocarbons with the model nucleophile *tert*-butyl mercaptide afford the products of trans-stereospecific addition to the oxide ring in aqueous dioxane or addition–dehydration to *tert*-butylthioarenes in THF. Product structures accord with regioselective attack at the most electrophilic center(s) in agreement with MO theoretical prediction. Addition to the K-region oxide of the potent carcinogen 7,12-dimethylbenz[*a*]anthracene takes place selectively at the 6 position with rearrangement during dehydration to furnish 7,12-dimethyl-5-*tert*-butylthiobenz[*a*]anthracene. Structural assignments are aided by 270-MHz high-resolution NMR spectroscopy utilizing benzylic coupling constants, lanthanide-induced shifts, and nuclear Overhauser enhancement measurements in the 1:1 adducts and an observed strong downfield shift of the aryl protons adjacent to the thio ether group in the alkylthioarenes.

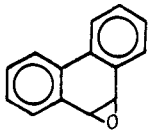
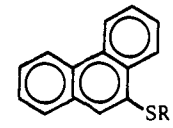
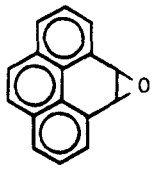
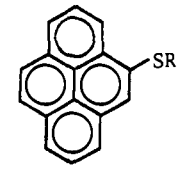
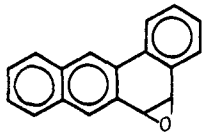
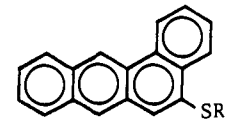
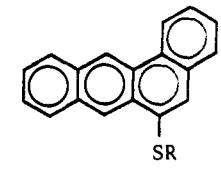
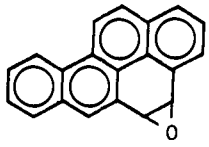
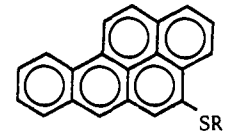
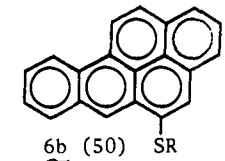
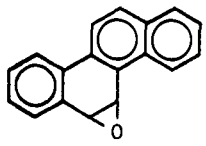
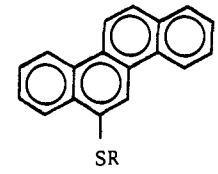
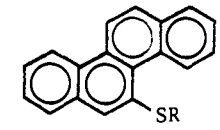
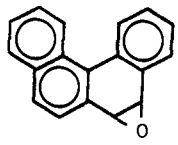
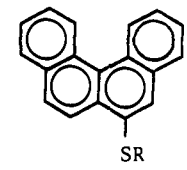
The hypothesis by Miller and Miller[1] that carcinogens or their metabolically activated derivatives act as electrophiles which initiate tumor formation through covalent interaction with nucleic acids or proteins is supported by an expanding body of evidence.[2] In the case of the carcinogenic hydrocarbons, the nature of the active intermediate is not established. However, the K-region oxides[3] have been shown to bind covalently to nucleic acids and proteins in vivo,[4-6] to be more active than the parent hydrocarbons in the transformation of cells in culture[7] and more highly mutagenic than either the hydrocarbons[8] or the non-K-region oxides.[9] On the other hand, diol oxide derivatives of benzo[*a*]pyrene (BaP) and benz[*a*]anthracene (BA) recently have been suggested[10,11] to be the principal metabolites of these hydrocarbons bound to DNA in vivo.

The structures of the hydrocarbon nucleic acid and protein conjugates have not been established. Hydrolysis of the nucleic acid conjugates of the K-oxides of BaP and 7,12-dimethylbenz[*a*]anthracene (DMBA) gave products in which the hydrocarbon was bound principally to guanosine.[5,6,12] Recent experiments have revealed these products to be further separable by HPLC into several, presumably isomeric, components.[13] Because of the low extent of binding, the quantities of these bound adducts isolable are quite small, i.e., submilligram, adding to the difficulty of ultimate structural elucidation.

In order to gain some insight into the nature of these reactions, particularly their regio- and stereoselectivity, and to obtain NMR and other data on compounds of authentic structure, we undertook to investigate the reactions of a series of K-region oxides, including several derivatives of carcinogenic hydrocarbons, with the model nucleophile *tert*-butyl mercaptide anion. The choice of this reagent was dictated by the known effectiveness of sulfur nucleophiles in the cleavage of epoxide rings[16] and the anticipated relative simplicity of the NMR spectra. In addition, *tert*-butyl mercaptan provides a convenient model for cysteine and glutathione, the former the principal site of reaction of arene oxides on proteins,[14] the latter one of the principal means of detoxification of arene oxides in vivo.

No systematic study of the reactions of the K-region arene oxides of carcinogenic hydrocarbons with nucleophiles has been reported. Swaisland et al.[15] investigated the relative reactivity of various K-region oxides with 4-(*p*-nitrobenzyl)pyridine, but did not determine product structure. Reactions of the benzene and naphthalene (non-K-region) oxides with several nucleo-

Table 1. Reactions of Polycyclic Arene Oxides with *tert*-Butyl Mercaptan in THF[a]

Oxide	Reaction Time (hr)	Temp.	Products[b,c] (ratio)	Yield (%)
 3	2 21	reflux RT	 3a	91 90
 4	38	RT	 4a	45
 5	3	reflux	 5a (50)  5b (50)	86
 6	4	reflux	 6a (50)  6b (50)	90
 7	3.75	reflux	 7a (25)  7b (75)	59
 8	5	reflux	 8a	70

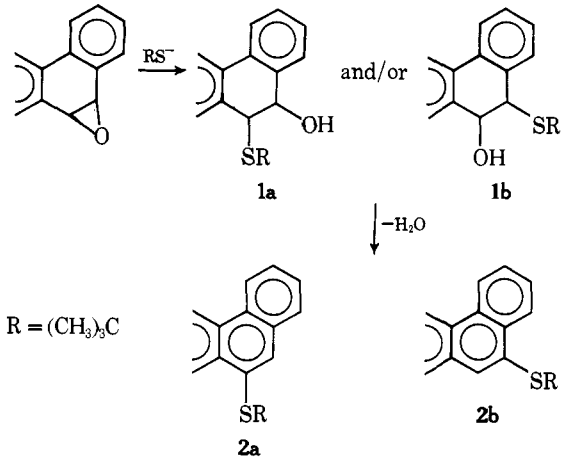
[a] See Experimental Section. [b] Ratios of isomers were determined by GLC analysis. [c] R = $C(CH_3)_3$.

philes were investigated by Jeffrey et al.,[16] who demonstrated cis and trans 1,6-addition to the former and trans 1,2-addition to the latter.[17] Most recently solvolysis of several K-oxides was shown by Keller and Heidelberger[18] to be neither regiospecific nor stereospecific, affording mixtures of isomeric phenols along with both cis and trans diols. Also Battistini et al.[19] have

demonstrated hydrolysis of aryloxiranes to afford both cis and trans diols, the proportion of the former being related to the degree of carbocation character in the transition state.

Results

The course of addition of sodium *tert*-butyl mercaptide to polycyclic arene oxides proved highly dependent upon the solvent. Experiments conducted in aqueous dioxane furnished the products of addition (**1**) in good yield, while those carried out in dry tetrahydrofuran led principally to the aromatic products (**2**) arising from subsequent dehydration (Table I). Both types of adduct are of biological interest, since analogous derivatives of 7,12-dimethyl-BA were found as products of the interaction of the 5,6-oxide with poly(G).[6]



The fully aromatic products permitted most convenient determination of isomer structure(s). They were stable crystalline solids separable by preparative gas chromatography and distinguished through analysis of their 270-MHz NMR spectra. In particular, there was noted a strong downfield shift of the aryl proton adjacent to the *tert*-butylthio group in comparison with the unsubstituted polycyclic hydrocarbon. Detailed analysis of the NMR spectra of the parent hydrocarbons and assignment of all proton resonances was reported earlier by Bartle et al.[20] and others (Table II). The chemical shift of the 4- and 7-protons of BA reported by Bartle et al.[20] were δ 7.73 (d, J = 7.5 Hz) and 8.14 (s), respectively. The *tert*-butylthio derivative here designated as the 5-isomer exhibited peaks assigned to these two protons at δ 8.78 (d, J = 6 Hz) and 8.10 (s), respectively, while the analogous protons of the 6-isomer appeared at δ 7.86 (d, J = 7.5 Hz) and 9.28 (s), respectively. Although the remaining protons exhibited minor differences in chemical shift from those of the parent hydrocarbon, the shifts were considerably less dramatic than those of the protons adjacent to the substituent and were in accord with expectation. On the basis of similar spectral analysis all the isomeric structures in Table I were assigned. The essential NMR spectral data are summarized in Table II. In general, the peri protons adjacent to the sulfur atom exhibited downfield shifts of 0.95–1.14 ppm (av $\Delta\delta$ = 1.04), while the K-region ortho protons were displaced 0.33–0.60 ppm (av $\Delta\delta$ = 0.46). Details of the individual 270-MHz NMR spectra may be found in the Experimental Section.

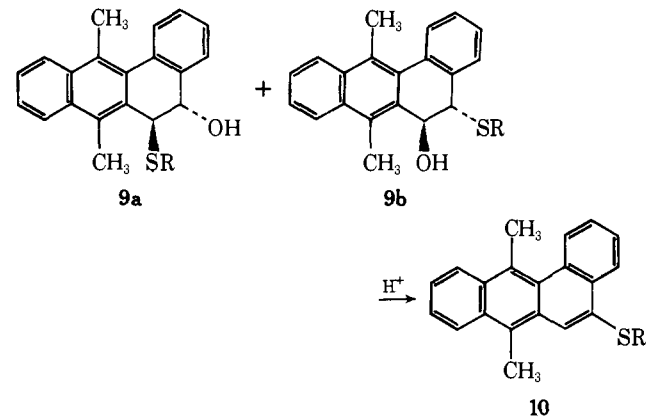
The results summarized in Table I indicate no regioselectivity of attack on either BA 5,6-oxide (**5**) or BaP 4,5-oxide (**6**), while reactions of both chrysene 5,6-oxide (**7**) and benzo[*c*]phenanthrene 5,6-oxide (**8**) exhibit strong preference for formation of a single isomer. Although no product could be isolated from reaction of the 5,6-oxide of DMBA in THF, an 86% yield of a 1:1 conjugate was obtained from reaction in aqueous dioxane. The latter (**9a,b**) was a mixture of two isomers in a 4:1 ratio differing in the position of attachment of the

Table II. Chemical Shifts of Aromatic Protons Adjacent to the *tert*-Butylthio Group of *t*-BuSAr[a]

Compd	Proton Position	Proton Type	δ, ppm	$\Delta\delta$,[b] ppm
3a	8	Peri	8.83	0.97[c]
	10	Ortho	8.03	0.33
4a	3	Peri	9.05	0.95[c]
	5	Ortho	8.37	0.37
5a	4	Peri	8.78	1.05[d]
	6	Ortho	8.27	0.60
5b	7	Peri	9.28	1.14[d]
	5	Ortho	7.95	0.45
6a	3	Peri	9.09	1.10[e]
	5	Ortho	8.42	0.51
6b	6	Peri	9.48	1.06[e]
	4	Ortho	8.34	0.50
7a	7	Peri	8.93	1.03[c]
	5	Ortho	9.09	0.43
7b	6	Ortho	8.39	0.46[c]
8a	7	Peri	8.87	1.12[f]
	5	Ortho	8.30	0.48

[a] Measured at 270 MHz in $CDCl_3$. [b] Values represent differences from those of the corresponding protons of the parent hydrocarbons reported in the reference cited. [c] C. W. Haigh and R. B. Mallion, *Mol. Phys.*, 18, 737 (1970). [d] K. D. Bartle, D. W. Jones, and R. S. Matthews, *Spectrochim. Acta, Part A*, **25**, 1603 (1969). [e] C. W. Haigh and R. B. Mallion, *J. Mol. Spectrosc.*, **29**, 478 (1969). [f] R. H. Martin, N. Defray, N. P. Figeys, M. Flammang-Barbieux, J. P. Cosyn, M. Gelbcke, and J. J. Schurter, *Tetrahedron*, **25**, 4985 (1965).

tert-butylthio group. However, dehydration with *p*-toluenesulfonic acid in refluxing benzene afforded exclusively 5-*tert*-butylthio-DMBA (**10**).[21] This assignment is based on analysis of the aromatic region of the NMR spectrum in



comparison with those of DMBA and the authentic 5- and 6-methyl analogues[22] of this hydrocarbon.[20] The most distinctive feature of these spectra was the downfield shift of the H_4 protons of 5,7,12-trimethyl-BA (δ 7.97, $J_{3,4}$ = 9 Hz) and **10** (δ 8.79, $J_{3,4}$ = 9 Hz) relative to those of DMBA (δ 7.59) and 6,7,12-trimethyl-BA (part of a multiplet with H_2 and H_3 at δ 7.58–7.67). The strong induced shift of H_4 of **10** is clearly indicative of substitution of the *tert*-butylthio group in the adjacent 5 position. Since only a single isomeric product, **10**, is formed from the mixture of **9a,b**, it is evident that rearrangement accompanies aromatization of one of the isomers of **9a,b**. Evidence that initial attack of the mercaptide anion on the 5,6-oxide of DMBA occurs principally at the 6 position with subsequent rearrangement to the 5-position will be presented.

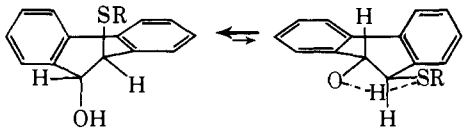
The addition products from phenanthrene 9,10-oxide and pyrene 4,5-oxide (**11** and **12**)[23] were obtained in high yield (95 and 75%, respectively) from reaction in aqueous dioxane. The stereochemistry of the products is assigned as trans on the basis

Table III. NMR Coupling Data for the Benzylic Protons of the *tert*-Butyl Mercaptan Addition Products[a]

Compd	Chemical shifts, δ		J
	*CH*OH	*CH*SR	
9a	4.77	4.57	3.6
9a acetate	6.06	4.77	3
9b	5.13	4.03	3.4
11	4.67	4.05	8
11 acetate	5.97	4.17	3.5
11 trimethylsilate	4.87	4.03	3
12	5.13	4.48	8
12 acetate	6.42	4.65	3
15	4.52	3.93	8
15 acetate	5.93	4.26	2.5
16a acetate	6.83	4.30	3
16b acetate	6.17	5.03	3
17a,b	4.93	4.33	8
17a,b acetate	6.33, 6.42	4.62, 4.70	3.2–3.5
21a,b	4.53, 4.68	3.96, 4.05	8
21a,b acetate	5.97, 6.15	4.28, 4.43	3

[a] Measured at 60 Hz in $CDCl_3$ or CCl_4.

of NMR and chemical evidence. The coupling constants for the benzylic protons of **11** and **12** were $J = 8$ Hz in nonpolar solvents (CCl_4, $CDCl_3$), which decreased to $J = 3$ Hz in ionizing solvents (Me_2SO-d_6, acetone-d_6). The latter value is consistent with that expected for the diequatorial benzylic protons in a 9,10-dihydrophenanthrene ring system bearing the substituent groups in the preferred diaxial conformation (**13**).[24] The somewhat larger value of J in the nonpolar media



13 (diaxial) **13** (diequatorial)

is indicative of a partial shift of the dynamic equilibrium due to stabilization of the trans-diequatorial conformer by internal hydrogen bonding between hydroxyl and sulfur. The trimethylsilyl and acetate derivatives of **11** exhibited smaller couplings ($J_{9,10} = 3.5$–4 Hz) consistent with the expectation that these larger groups are capable of locking the conformation into the favored trans-diaxial structure.[24] If the stereochemistry were cis, the introduction of bulky groups would not be expected to alter appreciably the benzylic hydrogen coupling constants.

Reaction of the nonsymmetrical K-oxides of benzo[*c*]phenanthrene, chrysene, benz[*a*]anthracene, and benzo[*a*]pyrene (**5–8**) with *tert*-butyl mercaptide in aqueous dioxane also afforded the corresponding addition products. The ratios of the isomers (determined by NMR analysis of the acetylated products) corresponded with the ratios of *tert*-butylthioarenes obtained after dehydration (Table I), confirming the absence of rearrangement during the latter step. *trans*-5-Acetoxy-6-*tert*-butylthio-5,6-dihydrobenzo[*c*]phenanthrene (**15**) was obtained as a crystalline solid (mp 136.5–137.5 °C); the NMR spectrum exhibited $J_{5,6} = 2.5$ Hz, consistent with the trans-diaxial assignment. The unacetylated precursor of **15** had $J_{5,6} = 8$ Hz (CCl_4) in agreement with an internally hydrogen-bonded diequatorial conformation. Attempts to separate the isomeric addition products of **5–7** by HPLC and other means have thus far not been successful. However, the coupling constants of the acetate derivatives of the mixed isomers (Table III) were close to those of the corresponding phenanthrene and pyrene derivatives (**11** and **12**) and consistent with the trans assignment.

The isomeric adducts from reaction of DMBA 5,6-oxide

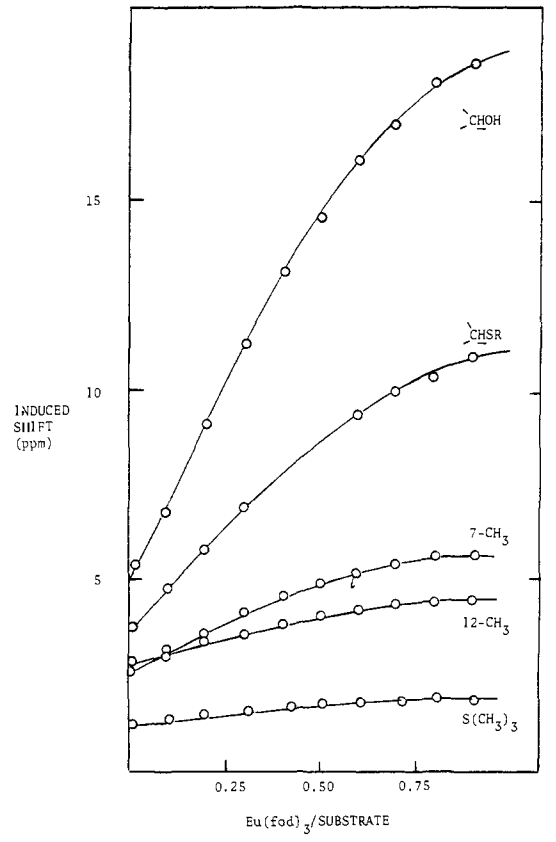
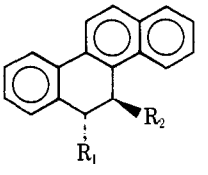
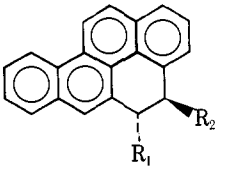


Figure 1. Lanthanide-induced shifts of the benzylic, methyl, and *tert*-butyl protons of **9b**.



16a: $R_1 = t$-BuS; R_2 = OAc
b: R_1 = OAc; $R_2 = t$-BuS



17a: $R_1 = t$-BuS; R_2 = OH
b: R_1 = OH; $R_2 = t$-BuS

with *tert*-butyl mercaptide were separated by HPLC on Silica (10 μm) eluted with CH_2Cl_2–hexane. The major and minor isomers were assigned the structures *trans*-5-hydroxy-6-*tert*-butylthio- and 6-hydroxy-5-*tert*-butylthio-5,6-dihydro-DMBA (**9a** and **9b**), respectively, on the basis of NMR spectral data. Thus, the couplings between the benzylic protons (J = 3.6 and 3.4 Hz, respectively) were in the expected range for the trans relationship. Lanthanide-induced shift measurements[26] with Eu(fod)$_3$ exhibited strong shifts of the benzylic and methyl protons of both isomers (Figure 1). Most revealing, however, was the much larger induced shift of the 7-methyl protons of the minor isomer (Figure 2), indicating the hydroxyl group associated with the rare earth ion to be in the adjacent 6 position and fixing this isomer as **9b**. Further support was provided by observation of significant nuclear Overhauser enhancement (NOE) effects between the 7-methyl and 6-benzylic protons of **9a** and **9b**, which were in agreement with the respective assignments. Thus, irradiation of the 7-methyl protons of **9a** gave a substantial NOE (~12%) of the doublet (δ 4.57) associated with the benzylic protons on the carbon bearing the sulfur group and no effect on the lower field benzylic doublet (δ 4.77). Conversely, irradiation of the 7-methyl protons of **9b** gave an NOE effect (~11%) on the doublet (δ 5.13) associated with the benzylic protons on the carbon atom bearing the hydroxyl group and no effect on the higher field

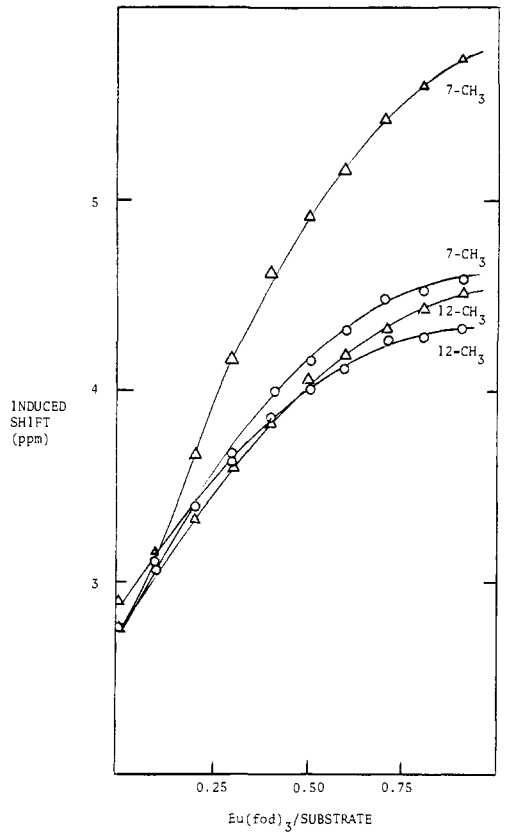
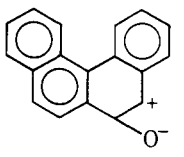


Figure 2. Comparison of the induced shifts of the methyl protons of **9a** (○) and **9b** (△).

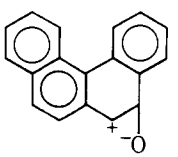
benzylic doublet (δ 4.03). Therefore, the major isomer is established to be **9a,** and it is this isomer which rearranges during dehydration to afford **10.** A similar rearrangement of *trans*-1-hydroxyethylthio-1,2-dihydronaphthalene was recently reported.[27]

Discussion

The present results are most simply interpreted as involving direct SN2 displacement on the arene oxides by the potent sulfur nucleophile. The observed regioselectivities are consistent with kinetic control over product structure, with the attacking nucleophile combining with the most electrophilic carbon atom. The latter is predictable by molecular orbital theory. The predominant isomer is in all cases that for which the calculated Dewar reactivity number[28] (N_t) of the corresponding carbocation in the ionized form of the oxide ring is minimum (Table IV).[29] To illustrate, for benzo[*c*]phenanthrene the two structures to consider are **18a** and **18b,** which



18a



18b

bear the positive charge at the 5 and the 6 positions, respectively. Since the latter exhibits a lower reactivity number (N_t = 1.79) than the former (N_t = 1.86), reaction is predicted to occur preferentially at the 6 position, which accords with experimental finding.

Although the relationship with N_t appears generally valid, other factors undoubtedly contribute to determination of product structure. The steric factor, as might be anticipated, is one of these. Thus, chrysene 5,6-oxide, for which the values

Table IV. Product Structures vs. MO Theoretical Prediction[a]

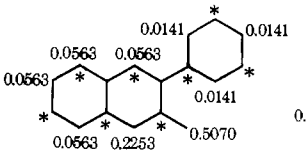
Product isomer	Reactivity no. N_t	Isomer (%)
5a	1.66	50
5b	1.66	50
6a	1.55	50
6b	1.55	50
7a	1.90	25
7b	1.67	75
8a	1.79	100
8b	1.86	0

[a] Reactivity numbers are calculated[28] for the two ionized forms of the parent arene oxide.

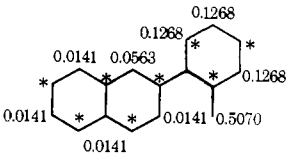
of N_t (Table IV) differ by 0.23 affords a 75:25 ratio of the predicted isomer, whereas benzo[*c*]phenanthrene 5,6-oxide, for which the difference is only 0.13, provides a 100:0 ratio. This may be partially a consequence of the somewhat greater steric crowding at the 5 than at the 6 position of the chrysene oxide. More important, perhaps, is the distortion from planarity introduced by the steric interference between the hydrogen atoms at the 4 and 5 positions, which tends to force the oxirane proton out of the plane of the polycyclic ring system[35] into the pseudoaxial orientation, hampering approach of the nucleophile from this direction.

Also consistent with SN2 mechanism is the observed trans stereospecificity of ring opening and the stability of the K-region arene oxides in anhydrous THF in the absence of mercaptide ion. Formation of phenols, the usual products of acid-catalyzed or uncatalyzed ring-opening of arene oxides[18,30] under anhydrous conditions, was not observed,[31] indicating an SN1 process to be not seriously competitive. The trans stereospecific character of these reactions contrasts with the previous observations of a relatively large proportion of cis ring opening during hydrolysis of K-region oxides[18] and other aryloxiranes.[19] It is probable that at neutral or low pH similar SN1 pathways may govern the reactions of mercaptans and other nucleophiles with the K -region oxides.[32] Investigation of the effect of pH on the course of these reactions was, however, outside the scope of the present study.

Rearrangement during dehydration was detected only in the product from DMBA 5,6-oxide. Preferential attack at the 6 position of this oxide is consistent with prediction of MO theory. The positive charge densities in the transition states of the two isomeric ions may be approximated from the eigenvalues of the nonbonding MO of the two zwitterions.[34] For BA 5,6-oxide (**5**) the charge densities at positions 5 and 6 in the two zwitterions are equal (**19a** and **19b**). Alkyl substitution

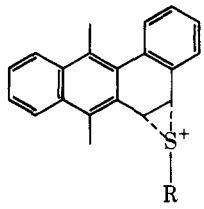


19a



19b

on position 7 in **19a** (which bears a large positive charge) would lead to stronger inductive stabilization of the transition state leading to that ion than would substitution on the same carbon atom in **19b,** since the latter is at a node bearing no charge.[18] Favored attack at C-6 is also in accord with the x-ray crystallographic data on DMBA 5,6-oxide,[35] which indicates polarization of the oxide ring and a considerable positive charge associated with position 7. Migration of the sulfur group probably proceeds via the cyclic sulfonium intermediate[36] **20.** The driving force for this migration is provided by the steric interference between the bulky 6-*tert*-butylthio group and the 7-methyl group and by the greater stability of the carbonium ion at C-6.



20

While the products of the foregoing reactions were found to accord with predictions of MO theory, there is evidently no correlation between regioselectivity and carcinogenicity. BA and chrysene are inactive or borderline in the latter respect, while BaP is among the most potent of known carcinogens.[37]

The observed nonregiospecificity of the reactions of the K-region oxides of BA and BaP with *tert*-butylthiol casts some doubt on previous reports of reactions of these and other K-region oxides with cysteine and glutathione to furnish only one isomeric product. Thus, reactions of cysteine with the 5,6-oxides of BA and dibenz[*a*,*h*]anthracene were reported to each afford a single isomeric product assumed to be the products of attack at the 5 position; the stereochemistry of addition was not established.[38] In the light of our results with BA 5,6-oxide and molecular orbital theoretical prediction, the two possible positional isomers would be expected to be formed in equal ratio in this case. Experiments are in progress on the reactions between cysteine and the series of K-region oxides to assign the structures of the isomers formed and to determine whether similar factors govern product structure. Since cysteine is a major site of binding of carcinogenic hydrocarbons to proteins,[14] the structures of the hydrocarbon-cysteine conjugates take on additional significance. Similar reservations are justified concerning the regioselectivity of formation of the glutathione conjugates. Only a single isomeric glutathione conjugate is reported formed chemically or enzymatically from the K-region oxides of phenanthrene, BA, 7-methyl-BA, 7-hydroxymethyl-BA, DMBA, BaP, dibenz[*a*,*h*]anthracene, and 3-methylcholanthrene (cf. review by Sims and Grover[3]). It is, of course, conceivable that reactions catalyzed by the enzyme glutathione *S*-epoxide transferase may exhibit a site specificity different from that of the chemical processes. In any case, reinvestigation to confirm or correct the original assignments utilizing the powerful techniques of separation (HPLC) and structural analysis (NMR) not available to Boyland, Sims, and their associates who conducted the pioneering studies in this field is in progress.

The NMR spectroscopic methods developed herein to solve the problems of structural assignment are potentially applicable to the structures of the arene oxide conjugates formed in vivo and in vitro. Although the quantities of material available from these sources is generally submilligram, this is not a serious limitation with the availability of Fourier transform techniques. Analysis of the structures of the products of interaction of DMBA 5,6-oxide and nucleic acids (following degradation to the nucleoside level) utilizing high resolution NMR spectroscopy and Fourier transform will be reported shortly.[39]

Experimental Section

Materials and Methods. Proton NMR spectra were obtained on Varian T-60 and Bruker 270-MHz spectrometers; chemical shifts are reported relative to Me_4Si in $CDCl_3$, or CCl_4 unless otherwise specified. Integration was consistent with all assignments. The NOE measurements were made on dilute, deoxygenated samples in CCl_4 as an average of repeated integrations of the signal in question with the external oscillator on and off resonance. Gas chromatographic analyses were performed on a Varian Aerograph Series 2400 chromatograph employing a 6 ft × 0.25 in. 10% DEGS 60-80 mesh Chromosorb W column at 210 °C or a 6 ft × 0.25 in. 3% OV-101 60-80 mesh Chromosorb W column at 215 °C. Detector and injector temperatures were maintained ~30° above column temperature. The K-region oxides were synthesized by the general method previously described.[40] *tert*-Butyl mercaptan was employed as supplied by Aldrich Co. Tetrahydrofuran (THF) was purified by distillation from $LiAlH_4$. Benzene and dioxane were dried over molecular sieves, type 4A. Analyses for all new compounds were correct to ±00.3%. High-pressure liquid chromatography separations were performed on a ½ × 24 in. column of silica (LiChrosorb, 10 μm, supplied by Varian) with CH_2Cl_2–hexane as solvent or by reverse phase on a ½ × 24 in. column of Permaphase ODS (Dupont) with methanol-water. A Variscan ultraviolet detector was employed.

Reactions of the K-Region Oxides with *tert*-Butyl Mercaptan in THF. (1) General Procedure. All glassware was flame dried and purged with N_2. To a solution of the oxide (1.0 mmol) in 30 ml of freshly distilled THF was added a solution of sodium ethoxide (1.65 mmol) in 0.6 ml of ethanol followed by *tert*-butyl mercaptan (1.5 mmol). The resulting solution was stirred under N_2 at the temperature and for the time specified in Table I. Water was then added, and the product was extracted with ether, washed three times with water, dried over $MgSO_4$, and evaporated to dryness in vacuo. NMR analysis was routinely carried out before further purification to determine the extent of dehydration and as a check on possible decomposition or rearrangement during subsequent chromatography.

(2) Dehydration Procedure. To a solution of the addition product in 30 ml of dry benzene was added *p*-toluenesulfonic acid (10 mg), and the resulting solution was maintained at reflux under N_2 for 3 h and worked up in the usual manner.[40]

(3) Phenanthrene 9,10-Oxide. NMR analysis of the crude product revealed the presence of the 1:1 conjugate **11** (20%) and **3a** (80%). Dehydration of the mixture with *p*-toluenesulfonic acid afforded a product which was chromatographed on Florisil (5 g). Elution with hexane gave pure **3a:** mp 91.5–92.0 °C (from MeOH); NMR δ 1.33 (s, 9, CH_3), 7.5–8.0 (m, 5, $H_{1,2,3,6,7}$), 8.03 (s, 1, H_{10}), and 8.5–9.0 (m, 3, $H_{4,5,8}$).

(4) Pyrene 4,5-Oxide. The crude product shown by NMR analysis to have been converted completely to **4a** was chromatographed on Florisil and recrystallized from hexane to afford pure **4a:** mp 108.5–109.0 °C; NMR δ 1.37 (s, 9, CH_3), 7.9–8.2 (m, 7, $H_{1,2,6-10}$), 8.37 (s, 1, H_5), and 9.05 (d of d, $J_{1,3}$ = 2 Hz, $J_{2,3}$ = 6 Hz, H_3).

(5) BA 5,6-Oxide. NMR and GLC analysis of the crude product revealed only the two dehydrated addition products in a 1:1 ratio. Following preliminary cleanup by passage through a column of Florisil eluted with benzene, the isomers were separated by GLC on a 10% DEGS column at 210°. The first isomer to emerge was **5b,** isolated as white needles: mp 157.5–158.0 °C; NMR δ 1.35 (s, 9, CH_3), 7.47 (m, 2, $H_{9,10}$), 7.54 (t, 1, H_3), 7.60 (t, 1, H_2), 7.86 (d, 1, H_4), 7.95 (s, 1, H_5), 8.02 (m, 2, $H_{8,11}$), 8.70 (d, 1, H_1), 9.06 (s, 1, H_{12}), and 9.28 (s, 7, H_7), $J_{1,2} = J_{2,3} = J_{3,4}$ = 7.5 Hz. Isomer **5a** was isolated as white needles, mp 138–139 °C; NMR δ 1.33 (s, 9, CH_3), 7.47 (t, 2, $H_{9,10}$), 7.58 (t, 2, $H_{2,3}$), 7.93 (d of d, 1, H_8), 8.01 (d of d, 1, H_{11}), 8.10 (s, 1, H_7), 8.27 (s, 1, H_6), 8.59 (d, 1, H_1), 8.78 (d, 1, H_4), 9.05 (s, 1, H_{12}): $J_{1,2} = J_{2,3} = J_{3,4} = J_{8,9} = J_{9,10} = J_{10,11}$ = 6 Hz; $J_{8,10} = J_{9,11}$ = 2 Hz.

(6) BaP 4,5-Oxide. The dehydrated addition products **6a,b** were shown by NMR and GLC analysis to be the sole products and to be formed in a 1:1 ratio. Following preliminary cleanup by chromatography on Florisil eluted with benzene–hexane (1:1), the individual isomers were isolated by preparative GLC on a 10% OV 101 column at 200°. Isomer **6b,** which eluted first, was obtained as pale yellow needles: mp 146.5–147 °C; NMR δ 1.43 (s, 9, CH_3), 7.76–7.89 (m, 2, $H_{8,9}$), 7.93 (t, 1, H_2), 8.11 (d, 1, H_3), 8.27 (d, 1, H_1), 8.32 (d, 1, H_{12}), 8.34 (s, 1, H_4), 8.40 (d, 1, H_7), 9.06 (d, 1, H_{10}), 9.09 (d, 1, H_{11}), and 9.48 (s, 1, H_6); $J_{1,2} = J_{2,3} = J_{7,8} = J_{9,10}$ = 8.5 Hz, and $J_{11,12}$ = 9.0 Hz. Isomer **6a** was also a pale yellow solid: mp 169–170 °C; NMR δ 1.43 (s, 9, CH_3), 7.74–7.89 (m, 2, $H_{8,9}$), 8.04 (t, 1, H_2), 8.27 (d, 1, H_1), 8.30 (d, 1, H_7), 8.34 (d, 1, H_{12}), 8.42 (s, 1, H_6), 8.52 (s, 1, H_5), 9.03 (d, 1, H_{11}), 9.09 (d, 2, $H_{10,3}$); $J_{1,2} = J_{9,10}$ = 8.5 Hz; $J_{7,8} = J_{11,12}$ = 9 Hz.

(7) Chrysene 5,6-Oxide. NMR and GLC analysis of the crude product indicated a 75:25 ratio of the two dehydrated products **7a,b.** The pure individual isomers were isolated by GLC on a 10% OV-101 column at 200° following preliminary chromatography on Florisil with hexane. The major isomer **7b** eluted first and was isolated as white needles: mp 152–153 °C; NMR δ 1.00 (s, 9, CH_3), 7.58–7.76 (m, 4,

$H_{4,5,9,10}$), 7.90–8.00 (br d, 3, $H_{8,3,9}$), 8.39 (s, 1, H_2), 8.69 (d, 1, H_7), 8.71 (d, 1, H_6), and 10.44 (d, 1, H_{12}); $J_{5,6} = J_{7,8} = J_{11,12} = 9$ Hz. The minor isomer **7a** was obtained as white needles: mp 181.5–183.0 °C; NMR δ 1.30 (s, 9, CH_3), 7.69 (m, 4, $H_{4,5,10,11}$), 7.99 (d, 1, H_9), 8.03 (d, 1, H_8), 8.71 (d, 1, H_7), 8.79 (d, 2, $H_{6,12}$), 8.93 (d, 1, H_3), and 9.09 (s, 1, H_1); $J_{3,4} = 8$ Hz; $J_{5,6} = J_{9,10} = J_{11,12} = 8.5$ Hz; $J_{7,8} = 9$ Hz.

(8) Benzo[*c*]phenanthrene 5,6-Oxide. NMR and GLC analysis indicated formation of a single dehydrated product, **8a**. Purification by passage through a column of Florisil, elution with hexane, and recrystallization from hexane gave pure **8a** as white clusters: mp 103–104 °C; NMR δ 1.33 (s, 9, CH_3), 7.66 (m, 4, $H_{2,3,10,11}$), 7.93 (d, 1, H_8), 8.02 (m, 2, $H_{4,9}$), 8.30 (s, 1, H_5), 8.87 (d, 1, H_7), and 9.04 (d, 2, $H_{1,12}$); $J_{1,2} = J_{7,8} = J_{11,12} = 9$ Hz.

Reactions of the K-Region Oxides with *tert*-Butyl Mercaptan in Aqueous Dioxane. (1) General Procedure. To a solution of the oxide (1 mmol) in 40 ml of 50% aqueous dioxane was added NaOH (2 mmol) and *tert*-butyl mercaptan (1.5 mmol). The resulting solution was stirred at 65–70 °C under N_2 for 3 h and worked up as the reactions in THF.

(2) Acetylation Procedure. A solution of pyridine (1 ml) in acetic anhydride (9 ml) was heated at reflux for 10 min, cooled to room temperature, and added to the addition product. The resulting solution was stirred at ambient temperature under N_2 overnight and worked up in a conventional manner.

(3) Silylation Procedure. To a solution of the addition product (1 mmol) in 20 ml of pyridine was added chlorotrimethylsilane (5 mmol). The resulting solution was stirred under N_2 for 1.5 h at reflux and worked up in the usual manner.

(4) Phenanthrene 9,10-Oxide. The crude product was chromatographed on Florisil, eluted with ether, and recrystallized from hexane to afford pure **11** (95%): mp 71–76 °C dec; NMR δ 1.40 (s, 9, CH_3), 2.77 (br s, 1, OH), 4.05 (d, 1, H_9), 4.67 (d, 1, H_{10}), and 7.2–7.8 (m, 8, aromatic); $J_{9,10} = 8.0$ Hz. Silylation followed by chromatography on Florisil eluted with benzene gave the trimethylsilyl derivative of **11** as a clear oil (91% yield): NMR δ 0.0 (s, 9, CH_3), 1.43 (s, 9, CH_3), 4.03 (d, 1, H_9), 4.87 (d, 1, H_{10}), 7.1–7.9 (m, 8, aromatic); $J_{9,10} = 3$ Hz. Acetylation followed by chromatography on activity IV neutral alumina and recrystallization from benzene–methanol gave **11** acetate as white needles: mp 136–137 °C; NMR δ 0.00 (s, 9, CH_3), 1.83 (s, 3, OAc), 4.17 (d, 1, H_9), 5.97 (d, 1, H_{10}), 7.1–7.8 (m, 8, aromatic); $J_{9,10} = 3.5$ Hz.

(5) Pyrene 4,5-Oxide. The initial product was purified on Florisil eluted with benzene and recrystallized from hexane–benzene to furnish **12** (75%): mp 130.5–131.0 °C; NMR δ 1.45 (s, 9, CH_3), 2.52 (br s, 1, OH), 4.48 (d, 1, H_5), 5.13 (d, 1, H_4), and 7.2–8.0 (m, 8, aromatic); $J_{4,5} = 8$ Hz. The acetate of **12** was recrystallized from benzene–hexane: mp 153.5–155 °C; NMR δ 1.52 (s, 9, CH_3), 1.85 (s, 3, OAc), 4.65 (d, 1, H_4), 6.42 (d, 1, H_5), and 7.5–8.0 (m, 8, aromatic); $J_{4,5} = 3.0$ Hz.

(6) BaP 4,5-Oxide. NMR analysis indicated virtually quantitative formation of the expected isomeric adducts **17a,b** in 1:1 ratio. Since considerable decomposition was observed during attempted chromatography on Florisil, the crude product was acetylated directly and the resulting acetates purified by passage through a column of activity IV neutral alumina eluted with 2% dioxane in hexane. Attempts to separate the mixture of acetates of **17a,b** (1:1 by NMR) by HPLC on silica gel or reverse phase on Permaphase ODS were unsuccessful. However, dehydration of **17a,b** afforded **6a,b** in the same ratio as obtained from reaction in THF.

(7) Chrysene 5,6-Oxide. The initial products were acetylated and purified by chromatography on Florisil eluted with hexane. The ratio of **16a,b** was identical with the ratio of the dehydrated derivatives **7a,b** from reaction in THF. NMR analysis of the mixture gave **16a:** δ 1.46 (s, 9, CH_3), 1.86 (s, 3, OAc), 4.30 (d, 1, H_2), 6.83 (d, 1, H_1), and 7.2–8.1 (m, 10, aromatic); $J_{1,2} = 3.0$ Hz; **16b:** δ 1.60 (s, 9, CH_3), 1.80 (s, 3, OAc), 5.03 (d, 1, H_1), 6.17 (d, 1, H_2), and 7.2–8.1 (m, 10, aromatic); $J_{1,2} = 3.0$ Hz.

(8) Benzo[*c*]phenanthrene 5,6-Oxide. The addition product was purified by chromatography on activity IV neutral alumina eluted with 2% dioxane in hexane. NMR analysis of 5-hydroxy-6-*tert*-butylthio-5,6-dihydrobenzo[*a*]phenanthrene showed δ 1.43 (s, 9, CH_3), 2.88 (br peak, 1, OH), 3.93 (d, 1, H_6), 4.52 (d, 1, H_5), and 7.2–8.6 (m, 10, aromatic); $J_{5,6} = 8$ Hz. Acetylation followed by chromatography on Florisil, elution with 2% dioxane in hexane, and recrystallization from hexane gave the acetate of **15:** mp 136.5–137.5 °C; NMR δ 1.46 (s, 9, CH_3), 1.80 (s, 3, OAc), 4.26 (d, 1, H_6), 5.93 (d, 1, H_5), and 7.2–8.6 (m, 10, aromatic); $J_{5,6} = 2.5$ Hz.

(9) DMBA 5,6-Oxide. NMR analysis of the addition product (86% yield) indicated the presence of the two isomers **9a,b** in a 4:1 ratio. Following chromatography on Florisil and elution with benzene, the individual isomers were isolated by HPLC on 10-μm silica gel with CH_2Cl_2–hexane (70:30) as the liquid phase. The major isomer **9a** eluted first: NMR δ 1.36 (s, 9, CH_3), 2.10 (br peak, 1, OH), 2.72 (s, 3, 7-CH_3), 2.82 (s, 3, 12-CH_3), 4.57 (d, 1, H_6), 4.77 (d, 1, H_5), and 7.2–8.2 (m, 8, aromatic); $J_{5,6} = 3.6$ Hz. The minor isomer **9b** had NMR δ 1.33 (s, 9, CH_3), 1.87 (br peak, 1, OH), 2.72 (s, 3, 7-CH_3), 2.85 (s, 3, 12-CH_3), 4.03 (d, 1, H_5), 5.13 (d, 1, H_6), and 7.1–8.2 (m, 8, aromatic); $J_{5,6} = 3.4$ Hz.

Acetylation of **9a,b** gave a mixture of two isomeric acetates which were not separable by HPLC. NMR analysis of the mixture gave for **9a** acetate: δ 1.48 (s, 9, CH_3), 1.80 (s, 3, OAc), 2.75 (s, 3, 7-CH_3), 2.93 (s, 3, 12-CH_3), 4.77 (d, 1, H_6), 6.06 (d, 1, H_5), and 7.3–8.2 (m, 8, aromatic); $J_{5,6} = 3$ Hz; for **9b** acetate: δ 1.42 (s, 9, CH_3), 1.80 (s, 3, OAc), 2.66 (s, 3, 7-CH_3), 2.93 (s, 3, 12-CH_3), 4.23 (d, 1, H_5), 6.36 (d, 1, H_6), and 7.2–8.2 (m, 8, aromatic); $J_{5,6} = 3.0$ Hz.

Dehydration of the mixture of **9a,b** gave only **10:** NMR δ 1.33 (s, 9, CH_3), 3.02 (s, 3, 7-CH_3), 3.23 (s, 3, 12-CH_3), 7.05–7.68 (m, 4, $H_{2,3,9,10}$), 8.34 (d, 2, $H_{8,11}$), 8.39 (d, 1, H_1), 8.43 (s, 1, H_6), and 8.79 (d, 1, H_4); $J_{1,2} = J_{3,4} = J_{8,9} = J_{10,11} = 9$ Hz.

(10) BA 5,6-Oxide. NMR analysis indicated the expected adducts **21a,b** to be formed in a 1:1 ratio. The crude adduct was directly acetylated. Attempts to separate the mixture of acetates by HPLC were unsuccessful.

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Synthesis and Isolation of Optically Pure L- and D-2-^{2}H Amino Acids via Cobalt(III) Chelates

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Abstract: A nonenzymatic procedure for the facile preparation of enantiomorphically pure 2-^{2}H amino acids is described. The complexes $[Co(en)_2(L\text{-aa})]X_n$ (X^- = Cl^-, I^-, or NO_3^-), where aa = aspartate, glutamate, asparaginate, glutaminate, homoserinate, alaninate, leucinate, prolinate, or *S*-methylcysteinate, chelated through their five-membered glycinate rings, were synthesized and the diastereomers were separated by ion-exchange chromatography. At pH 9.6 and 37 °C each of the chelated amino acids exchanged deuterium at the 2-carbon with varying degrees of racemization, producing another set of diastereomers since configurational racemization did not occur under these conditions. Chromatography of the diastereomers produced in the deuteration of Λ-$[Co(en)_2(L\text{-Asp})]Cl$ separated the optically pure amino acid complexes Λ-$[Co(en)_2(L\text{-2-}^2H\text{-Asp})]Cl$ and Λ-$[Co(en)_2(D\text{-2-}^2H\text{-Asp})]Cl$. Sodium borohydride reduction of these isomers and of the complexes containing deuterated glutamate, asparaginate, and prolinate removed the amino acids from each complex with no loss of optical activity or deuterium at the 2-carbon. Sodium borohydride reduction of the *S*-methylcysteinate complex decomposed the amino acid, with alanine the major decomposition product. The complexes $[Co(en)_2(L\text{-Cys})]I$ (nitrogen-sulfur five-membered chelate ring), $[Co(en)_2(\beta Ala)]Cl_2$ (nitrogen-oxygen six-membered chelate ring), and [Co(EDDA)(L-Asp)] (anion under basic conditions) were synthesized and were found not to exchange deuterium at the amino acid 2-carbon under basic conditions.

Introduction

There exist a number of nonenzymatic methods for preparing deuterated amino acids. These include multistep organic procedures,[2] pyridoxal-metal ion catalyzed deuterium-exchange reactions,[3] and azlactonization by acetic anhydride[4] in deuterated solvents. Although of fairly general applicability, each of these procedures has limitations as to the type of amino acid that can be labeled and the ease of synthesis. Notably, all the methods produce racemic mixtures.

Enzymes which catalyze the racemization of amino acids have been used to isotopically label the 2-carbon during racemization.[5] Each enzyme, however, is specific for a particular amino acid, and, at present, very few have been identified or are available.

Reported here is an alternate nonenzymatic procedure for the facile preparation of enantiomorphically pure 2-^{2}H amino acids. The method is based on the metal ion catalyzed exchange of alkyl protons of chelated amino acids as first detailed by Williams and Busch[6] and the chromatographic separation of diastereomers.

Experimental Section

Analytical results are recorded in Table I. Yields of the diastereomers were of the order of 50%. The complex cysteinatobis(ethylenediamine)cobalt(III) iodide was prepared by the method of Kothari and Busch.[7] The syntheses and separations of the isomers of $[Co(en)_2(L\text{-aa})]X_n$, where aa = aspartate, glutamate, asparaginate, or glutaminate (X^- = Cl^-, I^-, or NO_3^-), have been reported previously.[8]

Synthesis and Separation of the Isomers of $[Co(en)_2(L\text{-aa})]X_2$ Where aa = Homoserinate, Alaninate, Leucinate, Prolinate, or *S*-Methylcysteinate (X^- = Cl^- or NO_3^-). The following procedure describes the synthesis and separation of the isomers of homoserinatobis(ethylenediamine)cobalt(III) chloride. The syntheses and separations of the isomers of the related complexes containing alanine, leucine, proline, or *S*-methylcysteine were identical with that of the homoserine complex except that the weight of amino acid used (0.01 mol) varied accordingly with its molecular weight.

A mixture of L-homoserine (L-Hse) (1.19 g, 0.01 mol) and NaOH (0.4 g, 0.01 mol) in 60 ml of water was heated to 40 °C. Solid *trans*-$[Co(en)_2Cl_2]Cl$[9] (2.86 g, 0.01 mol) was added to the warm solution, and the temperature was increased to 70 °C with stirring for 10 min. After cooling to room temperature, the orange solution was diluted to 500 ml with water and loaded on a Dowex 50W-X8 cation-exchange column (4 × 40 cm, 200–400 mesh, 1100 mequiv capacity, Na^+ form) at a rate of ½ ml/min. (A much longer column [4 × 150 cm] was employed for the separation of the isomers of $[Co(en)_2(L\text{-Ala})]^{2+}$.) Upon elution with 1 M NaCl (flow rate ½ ml/min) the complex separated cleanly into two orange bands. Circular dichroism spectra of fractions showed each band to consist of one isomer. The fractions were combined for each band, evaporated to near dryness in an air