Further Studies on Polycyclic Arene Sulfides. Preparation and Mutagenic Activity of 9,10,10a,11a-Tetrahydrotriphenyleno[1,2-*b*]-thiirene, 1a,2,3,10b-Tetrahydro-5*H*-thiereno[3,4]benzo[1,2-*b*]fluorene and 1a,2,3,11b-Tetrahydroacenaphtho[1',2':6,7]naphth[1,2-*b*]thiirene

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The title compounds **6**, **8** and **10**, which are dihydroarene sulfides of the environmental pollutants triphenylene, benzo[*b*]fluorene and benzo[*k*]fluoranthene, have been synthesized from the corresponding epoxides and *N*,*N*-dimethylthioformamide. The mutagenicity of the episulfides has been investigated using *Salmonella typhimurium* strains TA98 and TA100. While compounds **6** and **10** were mutagenic, the tetrahydrobenzo[*b*]fluorene episulfide **8** was inactive.

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The involvement of arene oxides in metabolic activation of carcinogenic polycyclic compounds [1] and the exceptionally high mutagenic potencies of the corresponding arene imines [2] also arose considerable interest in the analogous episulfides.

Unsubstituted polycyclic arene sulfides have not yet been prepared owing to their instability [3], but the synthesis of three dihydroarene sulfides (**1-3**) [4] and of a pair of diastereomeric diol sulfides (**4**) [5] has lately proven feasible. As one of these episulfides, 7,8,8a,9a-tetrahydrobenzo[10,11]chryseno[3,4-*b*]thiirene (**2**) was shown to exhibit considerable mutagenicity in *S. typhimurium* cell cultures (*vide infra*), we found it imperative to extend these studies by preparing some further representative dihydroarene sulfides and determining their mutagenic potencies. In this paper we report the preparation of the title compounds, which are episulfide derivatives of biologically active alternant and non-alternant polycyclic aromatic environmental pollutants [6-8].

9,10,10a,11a-Tetrahydrotriphenyleno[1,2-*b*]thiirene (**6**) was obtained in 41% yield from the corresponding epoxide **5** [9] by treatment at room temperature with 2.1 equivalents of *N*,*N*-dimethylthioformamide, followed by a catalytic amount of trifluoroacetic acid. The structure of the product was determined by its elemental analysis and ^{1}H nmr spectrum. The episulfide protons in the nmr appear as expected at 3.85 and 4.70 ppm (*cf.*, [4]). The base peak in the EI mass spectrum is that of the sulfur-free ion radical $C_{18}H_{14}^{+\cdot}$.

In the same manner 1a,2,3,10b-tetrahydro-5*H*-thiereno[3,4]benzo[1,2-*b*]fluorene (**8**) was prepared in 43% yield from the corresponding oxirane **7**. The latter was obtained in 64% yield by *m*-chloroperbenzoic acid oxidation of 8,9-dihydrobenzo[*b*]fluorene [10]. The thiirane protons of **8** appeared at 3.73 and 4.11 ppm.

The precursor of thiirane **10**, 1a,2,3,11b-tetrahydroacenaphtho[1'2':6,7]naphth[1,2-*b*]oxirene (**9**) was prepared likewise from 8,9-dihydrobenzo[*k*]fluoranthene (**8**) and *m*-chloroperbenzoic acid (47% yield). Its conversion into the expected episulfide however was accomplished (in 55% yield) only when the trifluoracetic acid (that was used in the synthesis of **6** and **8**) was replaced by boron trifluoride etherate.

The new dihydroarene sulfides **6**, **8** and **10**, as well as compounds **1-3** reported in our previous paper [4], were subjected to mutagenicity tests using reversion of the histidine-dependent *S. typhimurium* strains TA98 and TA100 to histidine prototrophy as the experimental model. The results shown in Figure 1 and in Table 1 indicate that compounds **2**, **6** and **10** are mutagenic while **8** is inactive. **1** and **3** showed unambiguous, but very weak activities, which were just above the limit of detection. Since the active episulfides are thermally unstable and release sulfur in solution, we confirmed that the observed activities are indeed those of the thiiranes and not those of their desulfurization products. We have shown, *e.g.*, that 1,2-dihydrotriphenylene, which is formed by desulfurization of **6**, is inactive under our testing conditions (despite the fact that it is metabolized to an active diol epoxide in the presence of cytochrome P450 [6]).

The mutagenicities of several episulfides have been compared with those of the respective epoxides. For example, the specific mutagenicity of epoxide **9** was 2,400 and 16,000 revertants per nmole for TA98 and TA100 respectively, while the mutagenicity of **10** was only 200 and 700 revertants per nmole for these strains. In all investigated cases the episulfides (**1**, **2**, **3**, **8**, **10**) were found less active than the corresponding epoxides, and even the epoxide analog of the inactive episulfide **8** revealed mutagenic activity of

3,500 and 24,000 revertants per nmole for TA98 and TA100, respectively.

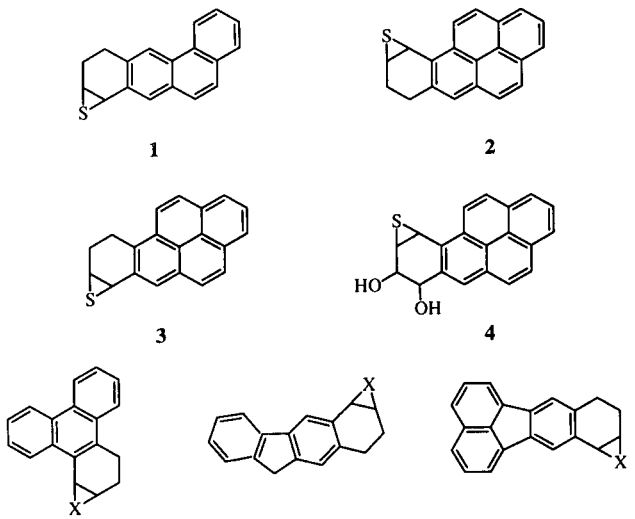

Table 1

Summary of the Mutagenicity Results

Compound	Mutagenic activity (revertants per nmol) [a] TA98	TA100
1	~0.3	~0.4
2	15	100
3	~0.4	~0.2
6	800	4000
8	<1	<1
10	200	700
positive control [b]	52000	64000

[a] Calculated from the initial, approximately linear part of the dose-response curves. For each condition, 2-4 independent experiments were carried out. Interexperimental variations of the results were within a factor of 2, except for **1** and **3**, which consistently led to small increases in the number of revertants in all experiments, but their effects were too small for accurate quantification; **8** did negative results, the limit of detection is given; [b] Benzo[*a*]pyrene 4,5-oxide.

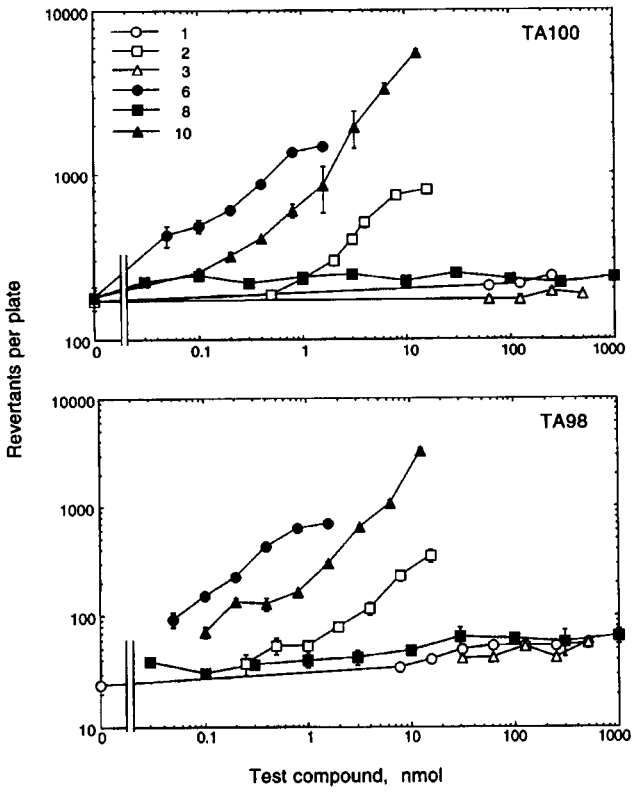

Figure 1. Dose-response curves of the mutagenicity of episulfides to *S. typhimurium* strains TA100 (upper panel) and TA98 (lower panel). Values are means and SE of 3 incubations. Where no bar is shown, SE falls within the symbol. In preceding experiments, **1** and **2** had also been tested at lower dose levels.

EXPERIMENTAL

9,10,10a,11a-Tetrahydrotriphenyleno[1,2-*b*]thiirene (**6**).

To a stirred solution of 0.25 g (1 mmole) of 9,10,10a,11a-tetrahydrotriphenyleno[1,2-*b*]oxirene (**5**) [9] in 40 ml of dichloromethane was added under nitrogen atmosphere a mixture of 0.19 g (2.1 mmoles) of *N,N*-dimethylthioformamide and 9 mg (0.08 mmole) of trifluoroacetic acid. After 30 minutes, when all the oxirane was consumed (tlc), 20 ml of water was added. Phase separation was followed by successive washing of the organic layer with 6 portions of 20 ml of ice cold water. The dichloromethane solution was dried on magnesium sulfate and the solvent was removed at room temperature under reduced pressure. The residue was chromatographed on silica gel pretreated with 17% water, using cyclohexane as eluent to give 110 mg (41%) of colorless **6**, mp 131-132°; ^{1}H nmr (deuteriochloroform): 400 MHz δ 2.37 (m, 1H, H10'), 2.68 (m, 1H, H10"), 2.86 (m, 1H, H9'), 3.39 (m, 1H, H9"), 3.85 (m, 1H, H10a), 4.70 (d, 1H, $J_{10a,11a}$ = 5.2 Hz, H11a), 7.60-8.72 (m, 8H, Ar*H*); ms: gc (70 eV) m/z (relative intensity) 230 [(M-S)$^{+\cdot}$, 100], 229 [(M-SH)$^+$, 48], 215 ($C_{17}H_{11}^+$, 38), 202 ($C_{16}H_{10}^{+\cdot}$, 13).

Anal. Calcd. for $C_{18}H_{14}S$: C, 82.44; H, 5.34; S, 12.21. Found: C, 82.02; H, 5.70; S, 11.79.

1a,2,3,10b-Tetrahydro-5*H*-oxireno[3,4]benzo[1,2-*b*]fluorene (**7**).

To a stirred solution of 0.6 g (2.5 mmoles) of 8,9-dihydrobenzo[*b*]fluorene [mp 151° [10]; ^{1}H nmr (deuteriochloroform): 300 MHz δ 2.39 (m, 2H, H8), 2.96 (t, 2H, $J_{8,9}$ = 8.0 Hz, H9), 3.90 (s, 2H, H11), 6.15 (m, 1H, H7), 6.69 (d, 1H, $J_{6,7}$ = 9.7 Hz, H6), 7.73-7.89 (m, 6H, Ar*H*)] in 100 ml of dry tetrahydrofuran was added 3.0 g of technical (90%) *m*-chloroperbenzoic acid. The oxidation was completed after 4 hours. A mixture of 100 ml of ether and 100 ml of 5% aqueous sodium hydroxide was added with strong agitation. After phase separation washing with three portions of 50 ml cold water and drying, the solvent was removed at room temperature under reduced pressure. The residue was recrystallized from a 1:5 mixture of ether and hexane to give 0.5 g (64%) of colorless **7**, mp 147°; ^{1}H nmr (deuteriochloroform): 300 MHz δ 1.81 (m, 1H, H2'), 2.43 (m, 1H, H2"), 2.62 (m, 1H, H3'), 2.87 (m, 1H, H3"), 3.76 (m, 1H, H1a), 3.86 (s, 2H, H5), 3.97 (d, 1H, $J_{1a,10b}$ = 4.2 Hz, H10b), 7.28-7.80 (m, 6H, Ar*H*); ms: gc (70 eV) m/z (relative intensity) 234 (M$^{+\cdot}$, 100), 192 ($C_{15}H_{12}^{+\cdot}$, 91), 191 ($C_{15}H_{11}^+$, 26), 178 ($C_{14}H_{10}^{+\cdot}$, 22), 165 ($C_{13}H_9^+$, 20).

Anal. Calcd. for $C_{17}H_{14}O$: C, 87.17; H, 5.98. Found: C, 86.89; H, 5.97.

1a,2,3,10b-Tetrahydro-5*H*-thiireno[3,4]benzo[1,2-*b*]fluorene (**8**).

A stirred solution of 500 mg (2.1 mmoles) of **9** and 0.4 ml of *N,N*-dimethylthioformamide in 100 ml of dichloromethane was treated at room temperature with 20 μl of trifluoroacetic acid for 30 minutes. The solvent was evaporated under reduced pressure. To the residue were added a few drops of tetrahydrofuran until a clear solution was formed. Dropwise addition of water afforded colorless crystals of the episulfide. The solid was washed 5 times with 50 ml of cold water to give 230 mg (43%) of **8**, mp 129-132°; ^{1}H nmr (deuteriochloroform): 300 MHz δ 2.12 (m, 1H, H2'), 2.45-2.58 (m, 2H, H2", H3'), 2.93 (m, 1H, H3"), 3.73 (m, 1H, H1a), 3.84 (s, 2H, H5), 4.11 (d, 1H, $J_{1a,10b}$ = 6.3 Hz, H10b), 7.23-7.81 (m, 6H, Ar*H*); ms: gc (70 eV) m/z (relative intensity) 218 [(M-S)$^{+\cdot}$, 100], 217 [(M-SH)$^+$, 34], 215 [(M-SH$_3$)$^+$, 22], 203 ($C_{16}H_{11}^+$, 29), 202 ($C_{16}H_{10}^{+\cdot}$, 28).

Anal. Calcd. for $C_{17}H_{14}S$: C, 81.60; H, 5.60; S, 12.80. Found: C, 81.71; H, 5.84; S, 12.51.

1a,2,3,11b-Tetrahydroacenaphtho[1',2':6,7]naphth[1,2-*b*]oxirene (**9**).

A solution of 1.2 g (4.7 mmoles) of 8,9-dihydrobenzo[*k*]-fluoranthene [8] and 3.6 g of technical (90%) *m*-chloroperbenzoic acid in 150 ml of tetrahydrofuran was stirred at room temperature for 5 hours. To the mixture was added 200 ml of ether and 100 ml of 5% aqueous sodium hydroxide. The aqueous layer was extracted with another portion of ether and the combined organic solutions washed twice with 50 ml cold water. Evaporation of the solvent under reduced pressure at room temperature afforded 0.6 g (47%) of **9** as colorless crystals, mp 126°; ^{1}H nmr (deuteriochloroform): 300 MHz δ 1.85 (m, 1H, H2'), 2.47 (m, 1H, H2"), 2.69 (m, 1H, H3'), 2.90 (m, 1H, H3'), 3.80 (m, 1H, H1a), 3.99 (d, 1H, $J_{1a,11b}$ = 6.3 Hz, H11b), 7.62-7.93 (m, 8H, Ar*H*).

Anal. Calcd. for $C_{20}H_{14}O$: C, 88.23; H, 5.14. Found: C, 88.59; H, 5.31.

1a,2,3,11b-Tetrahydroacenaphtho[1',2':6,7]naphth[1,2-*b*]thiirene (**10**).

To a stirred solution of 0.43 g (1.5 mmoles) of the foregoing oxirene in 20 ml of dichloromethane, was added a mixture of 0.28 ml (3.1 mmoles) of *N,N*-dimethylthioformamide and 9.8 mg (6.9 x 10^{-2} mmole) of boron trifluoride etherate. After 15 minutes, when the reaction was completed (tlc), the solvent was removed under reduced pressure at room temperature. The residue was dissolved in a minimum volume of tetrahydrofuran and solid **10** was precipitated by dropwise addition of cold water. There was obtained 270 mg (55%) of the episulfide as pale yellow crystals, mp 127-130°; ^{1}H nmr (deuteriochloroform): 300 MHz δ 2.15 (m, 1H, H2'), 2.38 (m, 1H, 2"), 2.64 (m, 1H, H3'), 3.39 (m, 1H, H3"), 3.75 (m, 1H, H1a), 4.23 (d, 1H, $J_{1a,11b}$ = 6.6 Hz, H11b), 7.56-7.93 (m, 8H, Ar*H*).

Anal. Calcd. for $C_{20}H_{14}S$: C, 83.87; H, 4.93; S, 11.20. Found: C, 83.60; H, 5.02; S, 11.01.

Mutagenicity Tests.

Mutagenicity on *S. typhimurium* was determined using methods similar to those described by Maron and Ames [11]. Strain TA100 [11] has lost its ability to synthesize histidine due to a substitution mutation in a gene encoding an enzyme involved in the synthesis of histidine, and the reversion of this strain to histidine prototrophy usually is also produced by a substitution mutation. Strain TA98 is a (-1 bp) frameshift mutant and generally is reverted again by frameshift mutations. Bacteria were grown and resuspended at the 5-fold of the usual cell density as described previously [12]. The bacterial suspension (100 μl) and the test compound (in 10 μl dimethyl sulfoxide) were added sequentially to a glass tube containing 500 μl of 37° warm water. After incubation for 20 minutes at 37°, 2.0 ml of 45° warm soft agar [12] was added, and the mixture was poured onto a Petri dish containing 24 ml of minimal agar [12]. After incubation for 2 days in the dark, the colonies (*his*$^+$ revertants) were counted. The initial slope of the dose-response curve was used as a measure of the mutagenic activity [13].

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