ip on days 1-5 in accordance with the protocols described by the National Cancer Institute.[15] The mean survival time (MST) for each treatment group (eight mice/group) was calculated and the percent $T/C$ determined by using the following formula:

$$\% \ T/C = \frac{\text{MST treated}}{\text{MST control}} \times 100$$

NADH dehydrogenase activity was determined with cytochrome $c$ as the electron acceptor.[16] It was examined by following cytochrome $c$ reduction at 550 nm with an extinction coefficient for cytochrome $c$ (reduced minus oxidized) of 19600. The reaction mixture (1.0 mL) contained 0.05 M TRIS-HCl buffer (pH = 7.2), 50 $\mu$M cytochrome $c$, 100 $\mu$M NADH, and 1.0 unit/mL NADH dehydrogenase. Enzymatic activity has been expressed in units, where 1 unit of activity is the amount of NADH dehydrogenase capable of reducing 1 $\mu$M of cytochrome $c$ per min at pH 7.2 at 25 °C. NADH oxidation was measured at 340 nm with an extinction coefficient of 6.22 $mM^{-1}$ $cm^{-1}$. The 1-mL reaction mixture contained 0.05 M TRIS-HCl buffer (pH = 7.2), 100 $\mu$M tested compound, 100 $\mu$M NADH, and 1 unit/mL NADH dehydrogenase. NADH consumption was initiated by the addition of enzyme.

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**Registry No.** **3a**, 69895-67-6; **3b**, 131042-03-0; **3c**, 69895-69-8; **4a**, 131042-04-1; **4b**, 131042-05-2; **4c**, 131042-06-3; **4d**, 131042-07-4; **4d** (Boc deriv.), 131042-11-0; **5a**, 86991-03-9; **5b**, 131042-08-5; **6a**, 131042-09-6; **6b**, 131042-10-9; **7**, 96502-06-6; quinizarin, 81-64-1; 1,4-dihydroxy-5-methoxy-9,10-anthracenedione, 64831-67-0.

(15) Geran, R. J.; Greenberg, N. R.; MacDonald, H. M.; Schumacher, A. M.; Abolt, B. Y. *Cancer Chemother. Rep.* **1972**, *3* (Part 3), 1-103.
(16) Malher, H. R. *Method Enzymol.* **1955**, *2*, 688.

# Synthesis and Biological Activity of Bay-Region Metabolites of a Cyclopenta-Fused Polycyclic Aromatic Hydrocarbon: Benz[*j*]aceanthrylene

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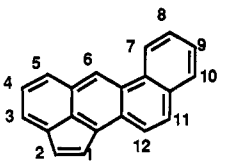
The possibility of bay-region activation of the cyclopenta PAH (polycyclic aromatic hydrocarbon with a peripherally fused cyclopenta ring) benz[*j*]aceanthrylene (**1**) was investigated by synthesis and bioassay of the bay-region metabolites *trans*-9,10-dihydroxy-9,10-dihydrobenz[*j*]aceanthrylene (**4**), *trans*-9,10-dihydroxy-*anti*-7,8-epoxy-7,8,9,10-tetrahydrobenz[*j*]aceanthrylene (**2**), and 9,10-dihydrobenz[*j*]aceanthrylene 9,10-oxide (**3**). The known 1,2-dihydrobenz[*j*]aceanthrylene-9,10-dione (**5**) was obtained by published methods; however, the direct route to target dihydrodiol **4**, dehydrogenation of the saturated five-membered ring of **5** followed by $NaBH_4$ reduction, gave a poor yield of **4** contaminated with tetrahydrogenated products. Acceptable yields of **4** were obtained by reduction of **5** to the corresponding tetrahydro diol, diacetylation of the diol, and dehydrogenation of the five-membered ring followed by base-catalyzed deacetylation to **4**. *anti*-Diol epoxide **2** was generated by *m*-chloroperoxybenzoic acid oxidation of **4**. Oxide **3** was synthesized by treatment of the monotosylate of **4** with NaOH in monoglyme. Diol epoxide **2** was an active mutagen in *Salmonella typhimurium* strain TA98 in the absence of metabolic activation, **3** showed marginal activity, while **3** and **4** were mutagenic with metabolic activation. These results coupled with previous studies support activation of benz[*j*]aceanthrylene via both **2** and cyclopenta ring epoxidation.

## Introduction

Polycyclic aromatic hydrocarbons with peripherally fused cyclopenta rings (cyclopenta PAH) are environmental contaminants[1,2] and potential carcinogens.[3-7] Biological activity has been observed for a number of cyclopenta PAH that also contain a bay region.[6,7] Of these compounds, benz[*j*]aceanthrylene (**1**) is a potent mutagen,[8,9] cell-transforming agent,[6] and tumor initiator[7] in rodents. The activity of **1** is dependent upon microsomal metabolism and for this reason activation is expected to proceed via arene oxide formation. Although epoxidation of the cyclopenta ring is known to be an activation pathway[2] for biologically active cyclopenta PAH, including **1**, molecular orbital correlations[10] and metabolism studies[6,8] on **1** also indicate that bay-region diol epoxide **2** and 9,10-oxide **3** are potentially active metabolites, suggesting multiple activation pathways. The importance of the cy-

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(1) Grimmer, G. In *Air Pollution and Cancer in Man*; Mohr, Y., Schmel, D., Tomatis, L., Eds.; International Agency for Research on Cancer: Lyon, France, 1977, p 93.
(2) Gold, A.; Sangaiah, R.; Nesnow, S. In *Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure-Activity Relationships*; Yang, S. K., Silverman, B. D., Eds.; CRC Press: Boca Raton, FL, 1988; Vol. 1, pp 178-207.
(3) Gold, A.; Nesnow, S.; Moore, M.; Garland, H.; Curtis, G.; Howard, B.; Graham, D.; Eisenstadt, E. *Cancer Res.* **1980**, *40*, 4482.
(4) Wood, A. W.; Levin, W.; Chang, R. L.; Huang, M.-T.; Ryan, D. F.; Thomas, P. E.; Lehr, R. E.; Kumar, S.; Koreeda, M.; Akagi, H.; Ittah, Y.; Dansette, P.; Yagi, H.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1980**, *40*, 642.
(5) Cavalieri, E.; Rogan, E.; Munhall, A. *Carcinogenesis* **1981**, *2*, 277.
(6) Mohapatra, N.; MacNair, P.; Bryant, B. J.; Ellis, S.; Rudo, K.; Sangaiah, R.; Gold, A.; Nesnow, S., *Mutat. Res.* **1987**, *188*, 323.
(7) Nesnow, S.; Gold, A.; Sangaiah, R.; Triplett, L. L.; Slaga, T. J. *Cancer Lett.* **1984**, *22*, 263.
(8) Nesnow, S.; Leavitt, S.; Easterling, R.; Watts, R.; Toney, S. H.; Claxton, L.; Sangaiah, R.; Toney, G. E.; Wiley, J.; Fraher, P.; Gold, A. *Cancer Res.* **1984**, *44*, 4493.
(9) Sangaiah, R.; Gold, A.; Toney, G. E.; Toney, S. H.; Easterling, R.; Claxton, L. D.; Nesnow, S. *Mutat. Res.* **1983**, *119*, 259.
(10) Silverman, B. D.; Lowe, J. P. *Cancer Biochem. Biophys.* **1984**, *7*, 203.

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clopenta epoxide as an ultimate active metabolite has been confirmed by synthesis and bioassay.[11] However, our continuing investigation of alternative pathways of bioactivation of this novel PAH required the synthesis of the possible ultimate active metabolites **2** and **3** and the metabolic precursor of **2**, *trans*-9,10-dihydro diol **4**.

## Results and Discussion

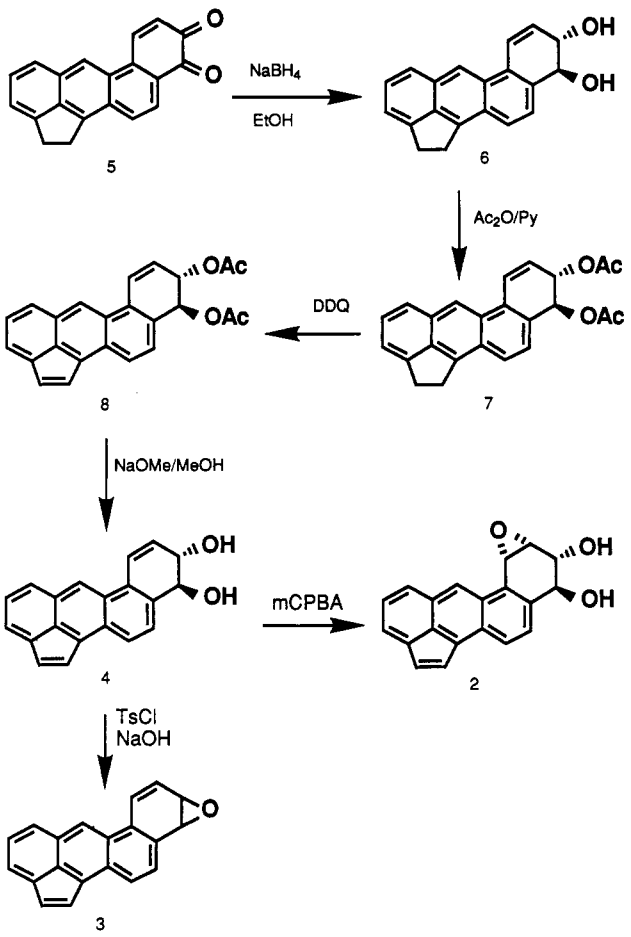
The general approach to the synthesis of diol epoxide **2** and diol **4** was similar to that described for the synthesis of the structurally analogous cholanthrene[12] and 3-methylcholanthrene[13] derivatives. However, the presence of the unsaturated cyclopenta ring in **2** and **4** necessitated a modification of the published procedures[12,13] which should prove useful for the synthesis of other putative cyclopenta PAH metabolites. 9,10-Oxide **3** was obtained from *trans*-9,10-dihydro diol **4**.

**Synthesis of *trans*-9,10-Dihydroxy-9,10-dihydrobenz[*j*]aceanthrylene (4).** 1,2-Dihydrobenz[*j*]aceanthrylene-9,10-dione (**5**), the key intermediate in the synthetic routes to 2–4, was prepared by a published procedure involving condensation of indanone-2,2-$d_2$ with regioselectively lithiated *N*,*N*-dimethyl-6-methoxynaphthamide.[12] Attempts to obtain the target 9,10-dihydrobenz[*j*]aceanthrylene-9,10-diol (**4**) by the straightforward approach of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) oxidation of **5** to the 1,2-dehydroquinone followed by $NaBH_4$ reduction gave **4** in low yield (20%) contaminated by an equal amount of a mixture of 1,2,9,10- and 7,8,9,10-tetrahydro products from which **4** could be separated only by tedious high-performance liquid chromatography. No reaction conditions could be established to improve the yield of **4** and to decrease the extent of overreduction. Since the electron-withdrawing effect of the cyclopenta ring could explain the surprisingly facile overreduction of the benz[*j*]aceanthrylene-9,10-dione, Scheme I was adopted in which the dihydroquinone **5** was reduced with $NaBH_4$ to diol **6** and protected by acetylation prior to dehydrogenating the cyclopenta ring.

A number of variations have been reported[12-14] for the reduction procedure, and we found the yield and purity of **6** to be extremely sensitive to reaction conditions. Yields of ~50% without significant formation of the 7,8,9,10-tetrahydro contaminant could be consistently obtained if $O_2$ were bubbled through the reaction, and progress was monitored (16–24 h) by comparison of the catechol and diol bands at 250 and 266 nm, respectively. The $A_{250nm}/A_{266nm}$ ratio for optimal yield was 0.75.

After protecting the diol functionality of **6** by acetylation to **7**,[12] dehydrogenation of the cyclopenta ring to yield diacetate **8** was accomplished with DDQ. The acetyl groups were removed with sodium methoxide in THF/MeOH to give **4** in 38% overall yield from **5**. Structural assignment of **4** is based on the $^1H$ NMR and UV–vis spectra, which were identical with the spectra reported[8] for the metabolically generated dihydro diol. In the $^1H$ NMR spectrum, four resonances appear in the vinyl region. The chemical shift of doublets at 7.19 and 7.76 ppm and coupling constant ($J_{1,2}$ = 5 Hz) are typical of cyclopenta ring protons.[15] Since a downfield shift is expected for H1

**Scheme I**




in the pseudo bay region,[15] the signal at 7.76 ppm can be assigned to H1, and that at 7.19 ppm to H2. The remaining vinyl resonances at 6.33 (H8) and 7.50 (H7) ppm are coupled ($J_{7,8}$ = 10.5 Hz) and display a pattern typical of vinylic protons of dihydro diols situated distal to a bay region, with the signal of the proton within the bay region (H7) shifted to extremely low field.[12-14,16] Additional small splitting of both resonances indicates the expected coupling to carbinyl H9. Although coupling with the hydroxyl protons causes the two carbinyl resonances (H9, H10) to appear as broadened doublets, the higher field signal can be assigned to H9 by analogy to the spectrum of diacetate **8** in which the coupling of H9 with H8, H10 and homoallylic coupling with H7 are resolved. The difference in coupling between vicinal carbinyl protons (H9, H10) of diol **4** ($J_{9,10}$ = 12.0 Hz) and diacetate **8** ($J_{9,10}$ = 6.7 Hz) parallels the behavior reported for carbinyl proton coupling in the *trans*-9,10-dihydro diols and diacetates of cholanthrene[12] and 3-methylcholanthrene[13] and the *trans*-3,4-dihydro diol and diacetate of benz[*a*]anthracene.[16] Hence, the decrease in $J_{9,10}$ observed for the diacetate of dihydrobenz[*j*]aceanthrylenediol suggests that diacetate **8** is in a predominantly trans diaxial conformation and diol **4** is in a predominantly trans diequatorial conformation, in accord with conformational preferences established for the other benzanthracene congeners.

**Synthesis of *trans*-9,10-Dihydroxy-*anti*-7,8-epoxy-7,8,9,10-tetrahydrobenz[*j*]aceanthrylene (2).** Since the vinylic C7–C8 bond of **4** is more highly localized than the cyclopenta ring double bond,[2] epoxidation of **4** by per-

(11) Bartczak, A. W.; Sangaiah, R.; Ball, L. M.; Warren, S. H.; Gold, A. *Mutagenesis* **1987**, *2*, 101.
(12) Harvey, R. G.; Cortez, C.; Sugiyama, T.; Ito, Y.; Sawyer, T. W.; DiGiovanni, J. *J. Med. Chem.* **1988**, *31*, 154.
(13) Jacobs, S. A.; Cortez, C.; Harvey, R. G. *Carcinogenesis* **1983**, *4*, 519.
(14) Lee, H.; Harvey, R. G. *J. Org. Chem.* **1986**, *51*, 3502.
(15) Sangaiah, R.; Gold, A.; Toney, G. E. *J. Org. Chem.* **1983**, *48*, 1632.
(16) Lehr, R. E.; Schaefer-Ridder, M.; Jerina, D. M. *J. Org. Chem.* **1977**, *42*, 736.

Table I. Mutagenicity[a] of Bay-Region Metabolites of Benz[j]aceanthrylene (1) toward *Salmonella typhimurium* TA98

| dose, | benz[j]aceanthrylene (1) | | 9,10-oxide (3) | | 9,10-dihydro diol (4) | | 9,10-diol 7,8-oxide (2) | |
|---|---|---|---|---|---|---|---|---|
| μg/plate | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| 0.5 | 35 (8) | 76 (16) | 28 (5) | 58 (15) | 36 (18) | 67 (33) | 37 (7) | 51 (13) |
| 1.0 | 39 (8) | 103 (28) | 26 (3) | 71 (4) | 40 (29) | 100 (35) | 53 (10) | 86 (4) |
| 5.0 | 38 (6) | 429 (65) | 34 (3) | 176 (14) | 43 (23) | 335 (89) | 186 (12) | 283 (16) |
| 10.0 | 32 (11) | 694 (111) | 39 (12) | 272 (22) | 45 (26) | 469 (98) | 172 (20) | 287 (65) |
| 20.0 | 38 (11) | 915 (148) | 46 (9) | 350 (47) | 75 (45) | 543 (111) | 93 (6) | 148 (49) |
| rev/nmol | 0.1 ± 0.1 | 12.9 (2) | 0.2 (0.02) | 5.6 (1.5) | 0.7 (0.4) | 9.4 (3) | 10.0 (1.1) | 12.9 (5.9) |

[a] Quantities in parentheses are standard deviations.

oxyacid was expected to favor formation of the bay-region diol epoxide 2 over epoxidation of the cyclopenta ring, as indicated in Scheme I.

Additionally, the trans diequatorial geometry of 4 was expected to lead to anti geometry in the diol epoxide on the basis of the well-documented stereoselectivity of peroxyacid epoxidation of trans diequatorial dihydro diols distal to a bay region.[12,13,17] In accord with prediction, treatment of 4 with *m*-chloroperoxybenzoic acid yielded a single epoxide having an $^1$H NMR spectrum consistant with the anti bay-region dihydro diol structure. Doublets at 7.22 and 7.77 ppm ($J_{1,2}$ = 5 Hz) indicate that the cyclopenta ring double bond remains intact in the epoxidation. Additional evidence is provided by the UV–vis spectrum, which is identical with that of the aceanthrylene chromophore.[18-20] Support for the anti isomeric structure of 2 is provided by the coupling constants $J_{9,10}$ (=8.3 Hz) and $J_{7,8}$ (=4.2 Hz), which are similar to those reported for the corresponding protons of the saturated angular benzo ring of the anti bay-region diol epoxides of cholanthrene,[12] 3-methylcholanthrene,[13] and benz[a]anthracene[16] and the lack of resolved coupling between H8 and H9.

**Synthesis of 9,10-Epoxy-9,10-dihydrobenz[j]aceanthrylene (3).** Arene oxide 3 was obtained according to Scheme I by dehydration of diol 4 through treatment of the monotosylate, formed in situ, with base.

An initial procedure, involving heating the tosylate in an aprotic medium in the presence of NaH,[21] was unsuccessful, presumably because the desired product 3 was unstable under the reaction conditions. However in a modified procedure, the cyclization was accomplished by treatment of the monotosylate with powdered sodium hydroxide in monoglyme.[22] The assigned structure 3 is supported by the similarity of its UV–vis spectrum to that of diol 4 and by the $^1$H NMR spectrum. Although the chemical shifts of H9 and H10 are similar for 3 and 4, coupling constant $J_{9,10}$ decreases from 12.0 Hz in 4 to 4.0 Hz in 3, indicating a cis geometry for these protons as required by the assigned structure. Further coupling of H9 to H8 ($J_{8,9}$ = 4.0 Hz) and H7 ($J_{7,9}$ = 2.5 Hz) results in the appearance of the H9 resonance as a triplet of doublets. As expected, the chemical shift of H8 changes significantly upon epoxidation, moving 0.5 ppm downfield in 3 with respect to the H8 signal of 4.

**Biological Activity.** Table I presents the results of Ames mutagenicity assays of 1–4 in *S. typhimurium* strain TA98 with and without metabolic activation by Aroclor 1254 induced rat liver S9. Of the putative ultimate mutagens 2 and 3, only the bay-region diol epoxide 2 showed a level of mutagenicity in the absence of S9 requisite for a role in the activation of 1. However, the fact that the diol epoxide is less mutagenic than the metabolically activated parent PAH 1 (in contrast to (+)-*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, diol epoxide I) suggests that 2 is not the most mutagenic metabolite of 1. Coupled with the report that cyclopenta ring rather than bay-region metabolism predominates in rat liver microsomes,[8,9] the results of Table I make it unlikely that the bay region diol epoxide is a major contributor to the mutagenicity of 1 in the Ames assay. Comparison of the direct-acting mutagenicity of 2 with that reported[11] for the cyclopenta epoxide (benz[j]aceanthrylene 1,2-oxide; 200 rev/nmol) confirms the prediction based on the perturbational molecular orbital (PMO) delocalization energies of the carbonium ions derived from oxirane ring opening that the bay-region diol epoxide will be less active than the cyclopenta ring oxide.[10]

Metabolism and transformation studies in C3H 10T1/2 cells suggest that activation in this system, in contrast to the Ames assay, proceeds via the bay-region metabolite 2, since 4 is a major metabolite, while the 1,2-dihydro diol, indicative of initial cyclopenta ring epoxidation, is minor.[6] Although an explanation for the change in metabolic regioselectivity is not readily apparent, the level of mutagenic activity of 2 reported here supports the involvement of the bay region in the transformation assays. Some possible metabolic activation of 9,10-dihydro diol 4 through the cyclopenta ring as well as the bay region cannot be ruled out. Studies in progress on the metabolism of 4 should shed light on this question.

Because of its lability, 9,10-oxide 3 could be assayed only as a ~1:1 mixture with the 10-phenol. Since the phenol displayed no direct-acting mutagenicity (data not shown), 3 is responsible for the activity observed in the absence of S9. Even if allowance is made for sample purity, the marginal level of activity rules out the importance of 3 as an active metabolite. In the presence of S9, the level of activity observed for 3 is expected via enzymatic or nonenzymatic hydration to 4.

The bioassay results clearly demonstrate that both the cyclopenta epoxide and bay-region diol epoxide 2 can contribute to the biological activity of benz[j]aceanthrylene and have relative potencies in accord with predictions of PMO theory. The importance of each pathway appears to depend on the metabolizing system; nevertheless, the demonstration of major alternative activation pathways via different molecular sites represents an unusual situation thus far observed only for methylated benz[a]anthracenes and chrysenes, as well as additional confirmation of the utility of the relatively simple PMO technique in predicting activity.

## Experimental Section

$^1$H NMR spectra were obtained at 400 MHz on a Varian XL-400 spectrometer. Mass spectra were obtained by direct insertion probe on a VG 70-250SEQ mass spectrometer in the EI mode at

(17) Lehr, R. E.; Schaefer-Ridder, M.; Jerina, D. M. *Tetrahedron Lett.* **1977**, 539.
(18) Sangaiah, R.; Gold, A. *Org. Prep. Proc. Int.* **1985**, *17*, 53.
(19) Plummer, B. F.; Al-Saigh, Z. Y.; Arfan, M. *J. Org. Chem.* **1984**, *49*, 2069.
(20) Becker, H. D.; Hansen, L.; Anderson, K. *J. Org. Chem.* **1985**, *50*, 277.
(21) McCaustland, D. J.; Engel, J. F. *Tetrahedron Lett.* **1975**, 2549.
(22) Holland, S.; Epstein, R. *Synthesis* **1977**, 706.

70 eV. IR spectra were recorded on a Beckmann 4250 spectrometer and UV–vis spectra on a Perkin-Elmer 124 double-beam spectrometer. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. HPLC was performed with an ISCO Model 2360 gradient programmer and 2300 pump connected to a Du Pont Zorbax C-8 9.4 × 250 mm column equilibrated at 45% methanol in water. Eluate was monitored with a Du Pont 842 spectrometer at 254 nm. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

***trans*-9,10-Diacetoxy-1,2,9,10-tetrahydrobenz[*j*]aceanthrylene (7).** $NaBH_4$ (2.5 g) was added to a stirred suspension of quinone **5** (250 mg, synthesized according to published procedures[12]) in ethanol (250 mL) and the reaction protected from light with aluminum foil. $O_2$ was bubbled through the reaction which was monitored by UV–vis at 250 and 266 nm. When the ratio of bands 250 nm/266 nm reached 0.75, the reaction was terminated by evaporation of the ethanol at room temperature under reduced pressure with a dry ice condenser. Distilled $H_2O$ (50 mL) was added and the fine yellow solid that precipitated was filtered and dried to yield 280 mg (95%) of the disodium salt of crude tetrahydro diol **6**.

The salt of crude **6** was stirred at room temperature with acetic anhydride (50 mL) and pyridine (10 mL) for 6 h. The reaction mixture was then poured into ice water (500 mL), the resulting precipitate filtered and purified on a short column of Florisil eluted with benzene. Diacetate **7** was collected as a yellow band, 123 mg (38% from **5**), with UV–vis and $^1H$ NMR spectra in agreement with published data.

***trans*-9,10-Diacetoxy-9,10-dihydrobenz[*j*]aceanthrylene (8).** A solution of the diacetate **7** (186 mg, 0.5 mmol) and DDQ (114 mg, 0.5 mmol) in benzene (100 mL) was refluxed under nitrogen for 2 h. The filtrate of the cooled solution was concentrated to 50 mL and passed through a short column of Florisil (benzene eluant), and the bright red fraction collected to yield diacetate **8**: 138 mg (75%); mp 161–162 °C (hexane); $^1H$ NMR (400 MHz, acetone-$d_6$) 2.05 ( 3 H, OAc), 2.17 (s, 3 H, OAc) 5.68 (br dd, 1 H, $J_{9,10}$ = 6.67 Hz, $J_{9,8}$ = 4.16 Hz, H9), 6.33 (d, 1 H, $J_{10,9}$ = 6.67 Hz, H10), 6.37 (dd, 1 H, $J_{8,7}$ = 10.0 Hz, $J_{8,9}$ = 4.16 Hz, H8), 7.22 (d, 1 H, $J_{2,1}$ = 5.33 Hz, H2), 7.64 (d, 1 H, $J_{7,8}$ = 10.0 Hz, H7), 7.68 (dd, 1 H, $J_{4,5}$ = 8.58 Hz, $J_{4,3}$ = 8.50, H4), 7.75 (d, 1 H, $J_{1,2}$ = 5.33 Hz, H1), 7.87 (br d, 1 H, $J_{3,4}$ = 8.50 Hz, H3), 7.93 (d, 1 H, $J_{11,12}$ = 8.50 Hz, H11), 8.15 (d, 1 H, $J_{1,12}$ = 8.50 Hz, H12), 8.42 (br d, 1 H, $J_{5,4}$ = 8.58 Hz, H5), 9.12 (s, 1 H, H6) ppm; UV (methanol) $\lambda_{max}$ ($\epsilon \times 10^{-4}$) 435 (0.49), 414 (0.60), 395 (0.51), 372 (0.66), 360 (sh, 0.51), 315 (sh, 0.57), 256 (9.26) nm; accurate mass $M^+$ 370.1199, calcd for $C_{24}H_{18}O_4$, 370.1204; mass spectrum, *m/z* (relative intensity), 370 (5, $M^+$), 310 (30, M – HOAc), 268 (100, M – OAc – Ac), 252 (48, M – 2 OAc), 239 (53, M – OAc – CHOAc). Anal. Calcd for $C_{24}H_{18}O_4$: C, 77.84; H, 4.86. Found: C, 78.22; H, 4.88.

***trans*-9,10-Dihydroxy-9,10-dihydrobenz[*j*]aceanthrylene (4).** A solution of diacetate **8** (100 mg, 0.27 mmol) and $NaOCH_3$ (50 mg, 0.93 mmol) in dry THF (10 mL) and methanol (50 mL) was stirred under reflux for 15 min. The reaction mixture was concentrated to ~20 mL and diluted with water (100 mL), and the precipitated solid was filtered. The solid was triturated with ether to give red crystals: 77 mg (100%); mp 214 °C; $^1H$ NMR (400 MHz, acetone-$d_6$) 4.56 (br d, 1 H, $J_{9,10}$ = 12.0 Hz, H9), 4.95 (br d, 1 H, $J_{10,9}$ = 12.0 Hz, H10), 6.33 (dd, 1 H, $J_{8,7}$ = 10.5 Hz, $J_{8,9}$ = 2.0 Hz, H8), 7.19 (d, 1 H, $J_{2,1}$ = 5.0 Hz, H2), 7.50 (dd, 1 H, $J_{7,8}$ = 10.5 Hz, $J_{7,9}$ = 1.83 Hz, H7), 7.65 (dd, 1 H, $J_{4,3}$ = 8.7 Hz, $J_{4,5}$ = 8.3 Hz, H4), 7.75 (d, 1 H, $J_{1,2}$ = 5.0 Hz, H1), 7.89 (d, 1 H, $J_{11,12}$ = 8.07 Hz, H11 or 12), 8.03 (d, 1 H, $J_{3,4}$ = 8.7 Hz, H3), 8.12 (d, 1 H, $J_{11,12}$ = 8.07 Hz, H11 or 12), 8.38 (d, 1 H, $J_{5,4}$ = 8.3 Hz, H5), 8.98 (s, 1 H, H6) ppm; UV (methanol) $\lambda_{max}$ ($\epsilon \times 10^{-4}$) 438 (0.60), 415 (0.73), 395 (0.67), 373 (0.94), 355 (0.74), 317 (sh, 1.14), 276 (sh, 5.40), 250 (8.85) nm; HPLC retention time (gradient program, percent methanol in water at 1 mL/min: 45 → 60% in 5 min, 5 min at 60%, 60 → 100% in 10 min) 16.6 min; accurate mass $M^+$ 286.0994, calcd for $C_{20}H_{14}O_2$ 286.0993; mass spectrum, *m/z* (relative intensity) 286 (72, $M^+$), 269 (39, M – OH), 268 (95, M – $H_2O$), 240 (99, M – $H_2O$ – CO), 239 (110, M – $H_2O$ – CHO), 226 (35, M – 2 $CH_2O$). Anal. Calcd for $C_{20}H_{14}O_2$: C, 83.92; H, 4.90. Found: C, 83.50; H, 4.86.

***trans*-9,10-Dihydroxy-*anti*-7,8-epoxy-7,8,9,10-tetrahydrobenz[*j*]aceanthrylene (2).** A solution of dihydro diol **4** (57 mg, 0.2 mmol) and *m*-chloroperoxybenzoic acid (172 mg, 1.0 mmol) in freshly distilled THF (25 mL) was stirred under argon at room temperature for 1.5 h. The reaction mixture was treated with ice-cold water (100 mL) and the product extracted into ether (2 × 50 mL). The combined ether layers were washed with cold NaOH (10%, 2 × 50 mL) and ice water (1 × 100 mL), dried ($K_2CO_3$), and evaporated. Evaporation of solvent gave a quantitative yield of the orange-red bay-region diol epoxide **2**: 60 mg, mp 200–202 °C (softening at 187 °C); $^1H$ NMR (400 MHz, acetone-$d_6$) 3.89 (d, 1 H, $J_{8,7}$ = 4.16 Hz, H8), 4.05 (br d, 1 H, $J_{9,10}$ = 8.33 Hz, H9), 4.75 (br d, 1 H, $J_{10,9}$ = 8.33 Hz, H10), 5.15 (d, 1 H, $J_{7,8}$ = 4.16 Hz, H7), 7.22 (d, 1 H, $J_{2,1}$ = 5.0 Hz, H1), 7.68 (dd, 1 H, $J_{4,3}$ = 9.17 Hz, $J_{4,5}$ = 8.96 Hz, H4), 7.77 (d, 1 H, $J_{1,2}$ = 5.0 Hz, H1), 7.92 (d, 1 H, $J_{11,12}$ = 8.33 Hz, H11), 8.08 (d, 1 H, $J_{3,4}$ = 9.17 Hz, H3), 8.16 (d, 1 H, $J_{12,11}$ = 8.33 Hz, H12), 8.46 (d, 1 H, $J_{5,4}$ = 8.96 Hz, H5), 9.25 (s, 1 H, H6) ppm; UV (methanol) $\lambda_{max}$ ($\epsilon \times 10^{-4}$) 425 (0.36), 404 (0.45), 367 (0.63), 353 (0.41), 259 (4.77), 244 (4.83) nm; accurate mass, $M^+$ 302.0929, calcd for $C_{20}H_{14}O_3$ 302.0942; mass spectrum, *m/z* (relative intensity 302 (65, $M^+$), 284 (33, M – $H_2O$), 256 (100, M – $H_2O$ – CO), 255 (80, M – $H_2O$ – HCO).

**9,10-Dihydrobenz[*j*]aceanthrylene 9,10-Oxide (3).** To a stirred suspension of powdered NaOH (40 mg, 0.1 mmol) in monoglyme (1 mL) under nitrogen was added a solution of dihydro diol **4** (28 mg, 0.1 mmol) and tosyl chloride (20 mg, 0.1 mmol) in monoglyme (1 mL). The reaction was stirred for 2 h at room temperature and then poured onto a neutral alumina activity grade IV column and eluted with benzene under positive nitrogen pressure. Epoxide **3** was collected as a red band. After removal of solvent under a stream of $N_2$, the red-orange residue was triturated with methanol to give pure **3**; 12 mg (46%). The UV–vis (methanol) was similar to that of diol **4**, except for a shift of the strongest band from 250 nm to 258 nm; $^1H$ NMR (400 MHz, acetone-$d_6$) 4.35 (td, 1 H, $J_{9,10}$ = $J_{8,9}$ = 4.0 Hz, $J_{7,9}$ = 2.5 Hz, H9), 4.79 (d, 1 H, $J_{10,9}$ = 4.0 Hz, H10), 6.84 (dd, 1 H, $J_{8,7}$ = 10.0 Hz, $J_{8,9}$ = 4.0 Hz, H8), 7.20 (d, 1 H, $J_{2,1}$ = 5.5 Hz, H2), 7.45 (br d, 1 H, $J_{7,8}$ = 10.0 Hz, H7), 7.71 (m, 1 H, H4), 7.75 (d, 1 H, $J_{1,2}$ = 5.5 Hz, H1), 7.90 (d, 1 H, $J_{11,12}$ = 9.6 Hz, H11), 8.00 (br d, 1 H, $J_{3,4}$ = 9.8 Hz, H3), 8.14 (d, 1 H, $J_{12,11}$ = 9.6 Hz, H12), 8.44 (d, 1 H, $J_{5,4}$ = 9.6 Hz, H5), 9.18 (s, 1 H, H6) ppm.

**Mutagenicity Assays.** Mutagenicity was determined in the Ames plate incorporation assay,[23] modified according to the method of Claxton.[24] The compounds were dissolved in DMSO. Each dose level was assayed in duplicate on two or three separate occasions. The values reported are means of $His^+$ revertants (rev)/plate. For exogenous metabolic activation, 0.8 mg of S9 protein from the livers of Aroclor 1254 treated male rats (Moltox, Inc., College Park, MD) was added per plate, along with NADPH-generating cofactors.[23] The solvent controls were 28 rev/plate without S9 and 53 rev/plate with S9. The positive controls were 2-nitrofluorene, 3 μg/plate, 377 rev/plate without S9, and 2-anthramine, 0.5 μg/plate, 701 rev/plate with S9. Specific mutagenicity, expressed as $His^+$ revertants/nmol, was calculated by least-squares linear regression from the linear portion of the dose–response curve, to 10 μg/plate for all compounds except **2** without S9 (to 5 μg/plate) and **3** without S9 (to 20 μg/plate).

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**Registry No.** 1, 202-33-5; *trans,anti*-**2**, 130933-91-4; **3**, 130933-92-5; *trans*-**4**, 93673-37-1; **5**, 111238-18-7; *trans*-**6**, 88262-31-1; *trans*-**6**-2Na, 130933-93-6; *trans*-**7**, 111238-20-1; *trans*-**8**, 130933-94-7.

(23) Ames, B. N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, *31*, 347.

(24) Claxton, L. D.; Kohan, M.; Austin, A. C.; Evans, C. "The Genetic Bioassay Branch Protocol for Bacterial Mutagenesis Including Safety and Quality Assurance Procedures"; HERL-0323; US EPA: Research Triangle Park, NC, 1982.