Biologically Active Dihydrodiol Metabolites of Polycyclic Aromatic Hydrocarbons Structurally Related to the Potent Carcinogenic Hydrocarbon 7,12-Dimethylbenz[*a*]anthracene

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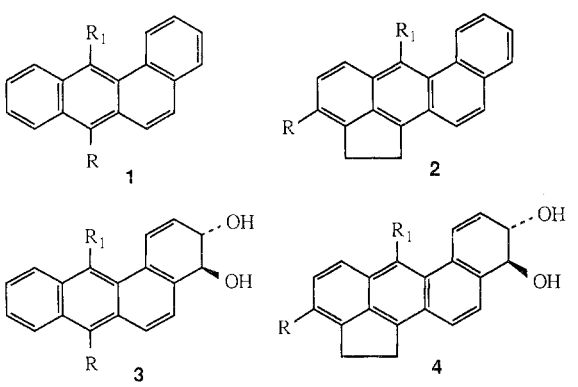
Syntheses of the *trans*-dihydrodiol derivatives implicated as the *proximate* carcinogenic metabolites of the polycyclic hydrocarbons cholanthrene, 6-methylcholanthrene, benz[*a*]anthracene, and 7- and 12-methylbenz[*a*]anthracene are described. These compounds are useful models for research to determine the molecular basis of the strong enhancement of carcinogenicity consequent upon methyl substitution in nonbenzo bay molecular sites and meso regions of polycyclic hydrocarbons. Synthesis of the bay region *anti*-diol epoxide derivative of cholanthrene, its putative *ultimate* carcinogenic metabolite, is also described. Tumorigenicity assays indicate that the 9,10-dihydrodiol derivatives of cholanthrene and its 3- and 6-methyl derivatives are all potent tumor initiators on mouse skin. The most active member of the series is the dihydrodiol derivative of 6-methylcholanthrene, which contains a bay region methyl group. The ability of the dihydrodiols **3a–c** and the *trans*-3,4-dihydrodiol of 7,12-dimethylbenz[*a*]anthracene (**3d**) to induce chromosomal aberrations in rat bone marrow cells was also examined. The observed order of activity was **3d > 3c > 3b > 3a**. These findings are consistent with the hypothesis that the diol epoxide metabolites of these dihydrodiols are the active carcinogenic forms of the parent hydrocarbons.

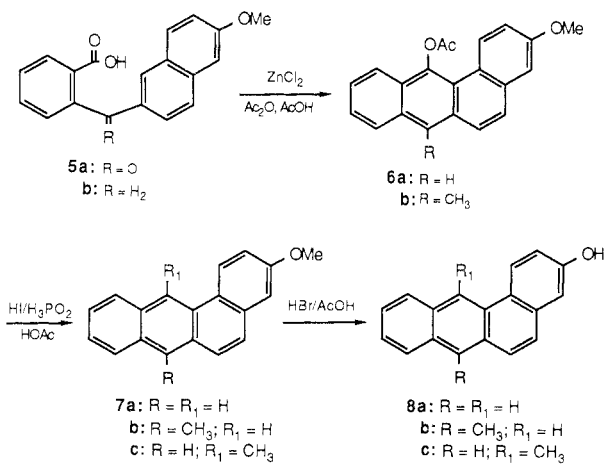
Methyl substitution in appropriate molecular regions, particularly in bay regions, of polycyclic aromatic hydrocarbons (PAHs) often dramatically influences their activities as carcinogens.[1-5] While benz[*a*]anthracene (**1a**) is inactive as a complete carcinogen in animal tests, 7,12-dimethylbenz[*a*]anthracene (**1d**) is the most potent carcinogenic hydrocarbon employed in carcinogenesis research.[1,2] 7-Methyl- and 12-methylbenz[*a*]anthracene (**1b**, **1c**) and the related hydrocarbons cholanthrene (**2a**) and 3-methylcholanthrene (**2b**) exhibit intermediate carcinogenic potency.[1,4] These findings lead us to predict that 6-methylcholanthrene (**2c**),[6] which contains a bay region methyl group and is a close structural analogue of **1d**, should exhibit a high level of tumorigenic activity.



a: R = R_1 = H; **b**: R = CH_3; R = H; **c**: R = H; R_1 = CH_3; **d**: R = R_1 = CH_3

There is now substantial evidence that the mechanism of carcinogenesis of alternant PAHs such as **1d** involves metabolic activation by the P-450 microsomal enzymes via a sequence that involves formation of an arene oxide in the appropriate molecular site (the 3,4-bond in the case of **1d**), hydration to form a *trans*-dihydrodiol (e.g. **3** and **4**), and epoxidation of the olefinic bond to yield the bay region diol epoxide derivative.[7] The diol epoxide metabolites bind covalently to DNA, leading initially to mutation and ultimately to tumor induction.[8-10]

Scheme I



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⊥ University of Texas.

(1) Huggins, C. B.; Pataki, J.; Harvey, R. G. *Proc. Natl. Acad. U.S.A.* **1967**, *58*, 2253.
(2) Harvey, R. G. In *Safe Handling of Chemical Carcinogens, Mutagens, Teratogens and Highly Toxic Substances*; Walters, D. B., Ed.; Ann Arbor Science: Ann Arbor, MI, 1980; Vol. 2, p 439.
(3) Hecht, S. S.; Amin, S.; Melikian, A. A.; LaVoie, E. J.; Hoffmann, D. In *Polycyclic Hydrocarbons and Carcinogenesis*, ACS Monograph No. 283; Harvey, R. G., Ed.; American Chemical Society: Washington, D.C., 1985; p 85.
(4) Dipple, A.; Moschel, R. G.; Bigger, C. A. H. In *Chemical Carcinogens*, ACS Monograph No. 182; Searle, C. E., Ed.; American Chemical Society: Washington, D.C., 1984; Vol. 1, p 41.
(5) DiGiovanni, J.; Diamond, L.; Harvey, R. G.; Slaga, T. J. *Carcinogenesis (London)* **1983**, *4*, 403.
(6) Syntheses of cholanthrene and its 3-methyl and 6-methyl derivatives have recently been described. (a) Harvey, R. G.; Cortez, C.; Jacobs, S. *J. Org. Chem.* **1982**, *47*, 2120. (b) Jacobs, S.; Harvey, R. G. *Tetrahedron Lett.* **1981**, *22*, 1093. (c) Harvey, R. G.; Cortez, C. *J. Org. Chem.* **1987**, *52*, 283.
(7) (a) Harvey, R. G. *Acc. Chem. Res.* **1981**, *14*, 218. (b) Conney, A. H. *Cancer Res.* **1977**, *37*, 3356. (c) Harvey, R. G. *Polycyclic Hydrocarbons and Carcinogenesis*, ACS Monograph No. 283; American Chemical Society: Washington, D.C., 1985.

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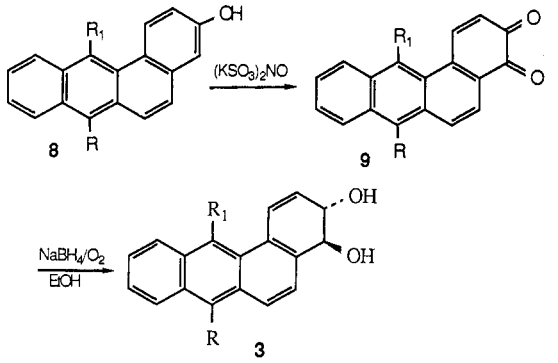
Scheme II



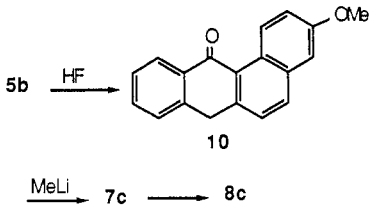
a: R = R_1 = H. **b**: R = CH_3; R_1 = H. **c**: R = H; R_1 = CH_3

As part of a program to elucidate the molecular basis of the strong enhancement of carcinogenic activity that often results from methyl substitution in bay and meso regions of polycyclic hydrocarbons,[5] we have undertaken to synthesize the bay region dihydrodiol and diol epoxide derivatives of a series of PAHs structurally related to 7,12-dimethylbenz[*a*]anthracene. We have recently reported convenient syntheses of the dihydrodiol and diol epoxide derivatives of 7,12-dimethylbenz[*a*]anthracene[11] and 3-methylcholanthrene.[12] We now report syntheses of the dihydrodiols **3a–c** and **4a,c**. Although syntheses of two of these compounds (**3a** and **3b**) were previously described,[11b,13–15] the synthetic approaches were relatively unsatisfactory, entailing relatively large numbers of steps, some of which gave low yields.

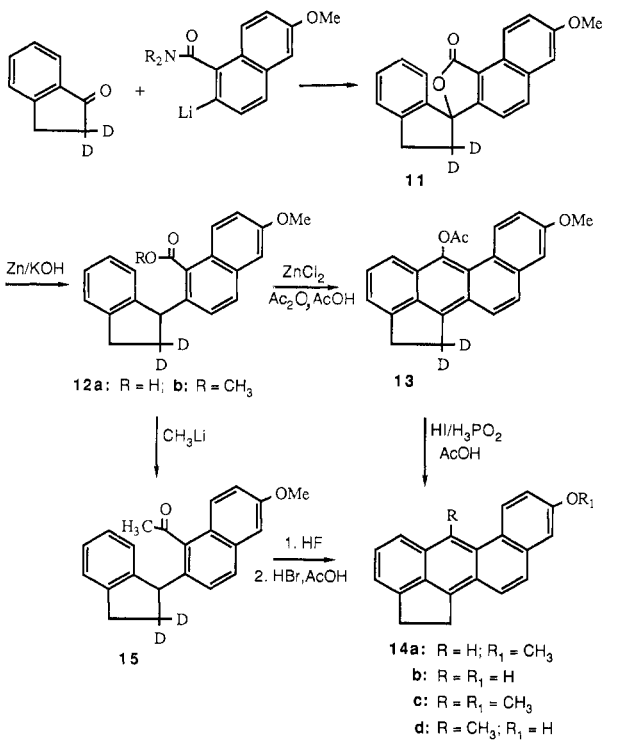
Synthesis of Benz[*a*]anthracene Dihydrodiols. The approach that proved generally applicable to the synthesis of the oxidized derivatives in the benz[*a*]anthracene series (**3a–c**) is outlined in Schemes I and II. The key intermediates in this sequence are the *β*-phenolic derivatives of the parent hydrocarbons (**8**), previously shown to be convenient starting compounds for the preparation of *trans*-dihydrodiols.[11,12,16]

The synthesis of 3-hydroxybenz[*a*]anthracene (**8a**) is based upon 12-acetoxy-3-methoxybenz[*a*]anthracene (**6a**) (Scheme I), which is conveniently accessible via reaction of the Grignard reagent of 2-bromo-6-methoxynaphthalene with phthalic anhydride and reduction of the resulting keto

Scheme III



Scheme IV



acid **5a** with zinc and alkali to yield the carboxylic acid **5b**, followed by treatment of **5b** with zinc chloride and acetic anhydride in acetic acid.[11a] Reduction of **6a** with hydriodic acid and hypophosphorus acid in acetic acid[17,18] yielded 3-methoxybenz[*a*]anthracene (**7a**). Demethylation of **7a** with HBr in acetic acid provided the free phenol **8a** in good overall yield.

Conversion of 3-hydroxybenz[*a*]anthracene to *trans*-3,4-dihydroxy-3,4-dihydrobenz[*a*]anthracene was accomplished by the general procedure (Scheme II) developed in our prior studies.[11,16] Oxidation of **8a** with Fremy's salt [$(SO_3K)_2NO$] in a two-phase methylene chloride–water system furnished benz[*a*]anthracene-3,4-dione (**9a**). Reduction of this quinone with $NaBH_4$ in absolute ethanol with oxygen bubbling through the reaction mixture took place smoothly and stereospecifically to provide the dihydrodiol **3a**. Light was excluded to prevent potential photoxidation.[11a] The utility of O_2 for the reoxidation of catechol byproducts back to quinones has been previously demonstrated.[11a,13,16] The ^1H NMR spectrum and other physical properties of **3a** were in good agreement with those reported earlier.[11b,13,16]

(8) In the terminology introduced by Miller, the dihydrodiol and the related bay region diol epoxide are termed the *proximate* and the *ultimate* carcinogens, respectively, and the unmetabolized PAH is referred to as a precarcinogen: Miller, J. A.; Miller, E. C. In *Environmental Carcinogenesis*; Emmelot, P., Kriek, E., Eds.; Elsevier: Amsterdam, 1979; p 25.
(9) Levin, W.; Wood, A.; Chang, R.; Ryan, D.; Thomas, P.; Yagi, H.; Thakker, D.; Vyas, K.; Boyd, C.; Chu, S.-Y.; Conney, H.; Jerina, D. *Drug Metab. Rev.* **1982**, *13*, 555.
(10) Harvey, R. G. In *Molecular Mechanisms of Carcinogenic and Antitumor Activity*; Chagas, C., Pullman, B., Eds.; Pontifical Academy of Sciences, Vatican Press: Vatican City, 1987; pp 95.
(11) (a) Lee, H.; Harvey, R. G. *J. Org. Chem.* **1986**, *51*, 3502. (b) Sukumaran, K. B.; Harvey, R. G. *J. Org. Chem.* **1980**, *45*, 4407. (c) Sukumaran, K. B.; Harvey, R. G. *J. Am. Chem. Soc.* **1979**, *101*, 1353.
(12) Jacobs, S.; Cortez, C.; Harvey, R. G. *Carcinogenesis (London)* **1983**, *4*, 519.
(13) Platt, K. L.; Oesch, F. *J. Org. Chem.* **1983**, *48*, 265.
(14) Lehr, R. E.; Schaefer-Ridder, M.; Jerina, D. M. *J. Org. Chem.* **1977**, *42*, 736.
(15) Lee, H. M.; Harvey, R. G. *J. Org. Chem.* **1979**, *44*, 4948.
(16) Harvey, R. G. In *Polycyclic Hydrocarbons and Carcinogenesis*, ACS Monograph No. 283; Harvey, R. G., Ed.; American Chemical Society: Washington, D.C., 1985; p 35.
(17) Konieczny, M.; Harvey, R. G. *J. Org. Chem.* **1979**, *44*, 4813.
(18) Harvey, R. G.; Cortez, C.; Jacobs, S. *J. Org. Chem.* **1982**, *47*, 2120.
(19) The usual byproducts of reduction of *o*-quinones with $LiAlH_4$ are the tetrahydrodiols arising from attack on the olefinic bonds of the dihydrodiols and the catechols formed by incomplete reduction.[11b,20]
(20) Harvey, R. G.; Goh, S. H.; Cortez, C. *J. Am. Chem. Soc.* **1975**, *97*, 3468.

Synthesis of the *trans*-3,4-dihydroxy-3,4-dihydro-7-methylbenz[*a*]anthracene (**3b**) was accomplished by modification of the foregoing approach. The intermediate 12-acetoxy-3-methoxy-7-methylbenz[*a*]anthracene (**6b**) was prepared from the keto acid **5a** by reaction with methyllithium, reduction of the resulting lactone with zinc and alkali, and $ZnCl_2$-catalyzed cyclization in acetic anhydride–acetic acid as previously described.[17] Reduction of **6b** with HI–H_3PO_2 in acetic acid took place smoothly to furnish 3-methoxy-7-methylbenz[*a*]anthracene (**7b**). Demethylation of the latter with HBr in acetic acid gave the free phenol **8b**. Oxidation of **8b** with Fremy's salt furnished 7-methylbenz[*a*]anthracene-3,4-dione (**9b**). Reduction of **9b** with $NaBH_4$ in ethanol in the presence of O_2 took place smoothly and stereospecifically to afford the *trans*-3,4-dihydrodiol **3b**. The excellent yield of **3b** obtained by the $NaBH_4/O_2$ reduction procedure (96%) contrasts with the poor yield (15%) obtained previously from reduction with $LiAlH_4$.[15] The melting point of the pure **3b** was notably higher (222–224 °C) than previously reported (188–189 °C),[15] probably indicative of the absence of traces of secondary products produced by $LiAlH_4$ reduction.[18] The 500-MHz NMR spectrum of **3b** was in good agreement with the assigned structure.

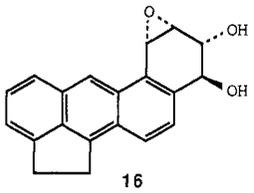
The synthesis of the *trans*-3,4-dihydroxy-3,4-dihydro-12-methylbenz[*a*]anthracene (**3c**) is based on the carboxylic acid **5b** (Scheme III). Cyclodehydration of **5b** in liquid HF furnished the ketone intermediate **10**, which underwent reaction with methyllithium to yield 3-methoxy-12-methylbenz[*a*]anthracene (**7c**). Demethylation of **7c** furnished the free phenol **8c**, which was converted to **3c** by the method in Scheme II.

Synthesis of Cholanthrene Dihydrodiols. The β-phenolic compounds (**14b,d**) were synthesized by the method in Scheme IV.[18] Condensation of 2,2-dideuterioindan-1-one with *N,N*-diethyl-2-lithio-6-methoxy-1-naphthamide in ether at −78 °C yielded an adduct, which on treatment with *p*-toluenesulfonic acid in refluxing benzene gave the lactone **11**. The dideuterio analogue of the indanone was employed to minimize competing enolization of the carbonyl function.[21] Reduction of **11** with zinc and alkali took place smoothly to furnish the free acid **12a**, which on treatment with $ZnCl_2$ in acetic acid–acetic anhydride yielded the cyclized product 9-methoxy-6-acetoxycholanthrene-d_2 (**13**). The 6-acetoxy group of **13** was efficiently removed by reduction with hydriodic acid in the presence of hypophosphorous acid in refluxing acetic acid.[17,18] The deuterium isotope was also lost in this step by HI-catalyzed exchange. 9-Methoxycholanthrene (**14a**) was converted to the free phenol 9-hydroxycholanthrene (**14b**) by treatment with HBr in acetic acid. Good yields were obtained in all steps except the first, which furnished **11** in only moderate yield (41%). It is likely that this could be improved by more careful study of experimental conditions.[22]

Conversion of **14b** to *trans*-9,10-dihydroxy-9,10-dihydrocholanthrene (**4a**) was carried out by the method in Scheme II. Oxidation of **14b** with Fremy's salt under the usual conditions furnished cholanthrene-9,10-dione, which on reduction with $NaBH_4$ in the presence of O_2 provided **4a**.

Synthesis of *trans*-9,10-dihydroxy-9,10-dihydro-6-methylcholanthrene (**4c**) was accomplished by modification of the method employed for the preparation of **4a** (Scheme IV). Esterification of the carboxylic acid **12a** with methyl iodide and KOH in hexamethylphosphoramide gave the methyl ester **12b**. This was transformed to the corresponding methyl ketone **15** by treatment with methyllithium in hexamethylphosphoramide and cyclized in liquid HF to yield 9-methoxy-6-methylcholanthrene-d_2 (**14c**-d_2). The latter on heating with HBr in refluxing acetic acid underwent demethylation and exchange of the deuterium atoms for hydrogen to yield 9-hydroxy-6-methylcholanthrene (**14d**). Oxidation of **14d** with Fremy's salt gave 6-methylcholanthrene-9,10-dione. This quinone underwent reduction with $NaBH_4/O_2$ to furnish the corresponding *trans*-9,10-dihydrodiol **4c**.

The bay region diol epoxide derivative of the cholanthrene *trans*-9,10-dihydroxy-*anti*-7,8-epoxy-7,8,9,10-tetrahydrocholanthrene (**16**) was synthesized stereospecifically by reaction of the dihydrodiol **4a** with *m*-chloroperbenzoic acid. Syntheses of the corresponding diol epoxide derivatives of benz[*a*]anthracene[11b,14] and 7-methylbenz[*a*]anthracene[15,23] from **3a** and **3b** have been described previously. The analogous *anti*-diol epoxide derivatives of 12-methylbenz[*a*]anthracene and 6-methylcholanthrene, predicted to be the ultimate active metabolites of these potent carcinogens, are anticipated to be chemically reactive and unstable by analogy with the related diol epoxide derivative of 7,12-dimethylbenz[*a*]-anthracene,[11a] which also contains a methyl group in the sterically crowded bay region. Their syntheses were not attempted.



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NMR Spectral Analysis. The 500-MHz ^1H NMR spectra of all of the dihydrodiol derivatives **3a–c** and **4a,c** and the *anti*-diol epoxide **16** were entirely consistent with their structural assignments. The large values of the coupling constants between the carbinol protons of **3b** and **3c** ($J_{3,4}$ = 11.0 and 11.3 Hz, respectively) support existence of both these dihydrodiols predominantly in the diequatorial conformation.[24,25] The coupling constants of the carbinol protons of **4a** and **4c** ($J_{9,10}$ = 11.3 and 11.4 Hz, respectively) were closely similar to these values, indicating that these dihydrodiols are also predominantly diequatorial. The carbinol protons of the diol epoxide **16** exhibited a lower value of $J_{9,10}$ (8.0 Hz), indicating a slight excess of the diequatorial conformer (56%) in solution.[26] These observations are consistent with previous findings for other nonsterically hindered *trans*-dihydrodiol derivatives of polycyclic aromatic hydrocarbons.[24]

The syntheses of the dihydrodiol derivatives of benz[*a*]anthracene (**3a–c**) and cholanthrene (**4a,c**) outlined above provide convenient access to these compounds. Although syntheses of two of these dihydrodiols (**3a** and **3b**) were reported previously,[11b,13–15] the new synthetic

(21) Jacobs, S. A.; Harvey, R. G. *J. Chem. Soc., Chem. Commun.* **1981**, 1215.
(22) Analogous reaction of *N,N*-dimethyl-2-lithio-6-methoxy-1-naphthamide with 2,2-dideuterio-4-methyl-1-indanone furnished the corresponding lactone in 84% yield.[9]
(23) Lee, H.; Harvey, R. G. *Tetrahedron Lett.* **1981**, *22*, 1657.
(24) Zacharias, D.; Glusker, J. P.; Fu, P. P.; Harvey, R. G. *J. Am. Chem. Soc.* **1979**, *101*, 4043.
(25) Harvey, R. G. In *The Conformational Analysis of Cyclohexenes, Cyclohexadienes, and Related Hydroaromatics*; Rabideau, P. W., Ed.; VCH: New York, in press.
(26) The percentage is calculated as a simple arithmetical average of the pure diequatorial and diaxial conformations by assuming a normal half-chair structure.

Table I. Tumor-Initiating Activity of Cholanthrene Derivatives[a]

compound	dose, nmol	papillomas per mouse	% of mice with papillomas
cholanthrene	200	6.90	93
cholanthrene-9,10-diol	200	3.32	80
3-methylcholanthrene[b]	100	7.13	97
3-methylcholanthrene-9,10-diol[b]	100	7.70	100
6-methylcholanthrene	50	14.67	100
6-methylcholanthrene-9,10-diol	50	16.50	100

[a] Thirty female SENCAR mice were used for each experimental group. All mice were initiated with the various compounds (doses shown) in 0.2 mL of acetone. Two weeks after initiation, mice recieved twice-weekly applications of TPA (3.4 nmol). Data are given at 18 weeks of promotion. Animals receiving 0.2 mL of acetone at initiation followed by TPA promotion had 0.03 papillomas per mouse. [b] Data taken for DiGiovanni, J.; Diamond, L.; Prichett, W. P.; Fisher, E. P.; Harvey, R. G. *Cancer* Lett. (*Shannon, Irel.*) **1985**, *28*, 223.

approaches described herein represent significant improvements, entailing fewer steps, providing higher yields, and allowing preparation on relatively larger scale than heretofore possible.

Biological Activity. Tumorigenicity studies indicate the 9,10-dihydrodiols of cholanthrene, 3-methylcholanthrene, and 6-methylcholanthrene (**4a–c**) are all potent tumor initiators on the skins of female SENCAR mice following promotion by 12-*O*-tetradecanoylphorbol 13-acetate (TPA; Table I). While the level of activity of **4a** was slightly lower than that of the parent hydrocarbon, the two methyl-substituted dihydrodiols **4b** and **4c** exhibited activity essentially equivalent to that of the parent hydrocarbons at the dosage tested. The most active members of the series were the bay region methyl-substituted compounds 6-methylcholanthrene (**2c**) and the corresponding dihydrodiol **4c**. These findings are consistent with the hypothesis that bay region diol epoxide metabolites (or their further oxidized metabolites)[27] are the ultimate active carcinogenic forms of these hydrocarbons and provide an additional example of the enhancement of carcinogenic activity by a bay region methyl group.[3,5]

Tumorigenicity data has been previously reported on the 3,4-dihydrodiols of benz[a]anthracene (**3a**)[28,29] and its 7-methyl derivative **3b**,[30] which indicate that these dihydrodiols are more potent tumor initiators on mouse skin than either the parent hydrocarbons or the other isomeric dihydrodiols.

The ability of the dihydrodiols **3a–c** to induce chromosomal aberrations in rat bone marrow cells was also examined. In a prior study, Sugiyama reported that the capacity of benz[a]anthracene (**1a**) and its meso region mono and dimethyl derivatives (**1b–d**) to induce chromosomal damage paralleled their abilities to induce sarcomas in the rat by intramuscular injection.[31] In similar tests, the *trans*-3,4-dihydrodiol derivative of 7,12-dimethylbenz[a]anthracene exhibited the highest level of activity (Table II). The dihydrodiols **3b** and **3c** induced intermediate levels of aberrations and the related dihydrodiol derivative of benz[a]anthracene exhibited much lower activity.[32] These findings parallel the findings from tumorigenicity tests and are also consistent with the hypothesis that bay region diol epoxides are the in vivo carcinogenic metabolites of the parent hydrocarbons.

Table II. Chromosomal Aberrations Induced in Rat Bone Marrow Cells by the Dihydrodiol Derivatives of Methyl-Substituted Benz[a]anthracenes **3a–d**[a]

dihydrodiol	dose, mg/kg	no. of aberrations per cell	incidence of aberrant cells,[b] %
3a	50	0.02 ± 0.01	2.2 ± 1.0
3b	50	0.07 ± 0.03	6.6 ± 2.6
3c	25	0.17 ± 0.05	12.8 ± 3.4
3d	25	0.31 ± 0.07	22.2 ± 3.9
3d	50	1.44 ± 0.31	53.0 ± 4.8

[a] Experiments were conducted with noninbred Long–Evans male rats, 28–35 days of age, weighing 50–80 g. [b] Aberrations include chromosomal breaks and exchanges, but do not include chromosomal gaps.

Experimental Section

Materials and Methods. 2-[(6-Methoxy-2-naphthyl)-methyl]benzoic acid (**5b**) was synthesized via the reaction of phthalic anhydride with the 2-bromomagnesium salt of 6-methoxynaphthalene followed by reduction with zinc and alkali.[11a] 12-Acetoxy-3-methoxybenz[a]anthracene (**6a**) was prepared from **5b** by reaction with $ZnCl_2$ in acetic anhydride and acetic acid by the procedure described.[11a,33] 12-Acetoxy-3-methoxy-7-methylbenz[a]anthracene (**6b**) was prepared by the method of Lee and Harvey.[15] 2,2-Dideuterio-1-indanone was synthesized from 1-indanone by K_2CO_3-catalyzed exchange in CH_3OD as described.[6c] *N,N*-Diethyl-2-lithio-6-methoxy-1-naphthamide was synthesized by metalation of the naphthamide derivative with *sec*-butyllithium by the method reported.[12] *m*-Chloroperbenzoic acid (Aldrich) was purified by washing with pH 7.5 phosphate buffer and drying under reduced pressure. Fremy's salt [$(KSO_3)_2NO$] was freshly prepared according to the literature method.[34] *N,N,N',N'*-Tetramethylethylenediamine (TMEDA) was dried over $LiAlH_4$ and redistilled. Tetrahydrofuran (THF) was freshly distilled from benzophenone ketyl. Ether was dried over sodium. Magnesium turnings used in the preparation of Grignard reagents were obtained from the Reade Manufacturing Co., Lakehurst NJ.

The NMR spectra were obtained on a Varian EM 360 and/or the University of Chicago 500-MHz NMR spectrometer in $CDCl_3$ unless stated otherwise with tetramethylsilane as an internal standard. Integration was consistent with all molecular structural assignments. The ultraviolet spectra were obtained on a Perkin-Elmer Lamda 5 spectrophotometer. Melting points are uncorrected. All new compounds gave satisfactory microanalyses for C and H within ±0.3% and/or mass spectra consistent with the assigned structures.

3-Methoxybenz[*a*]anthracene (7a). A solution of 57% HI (55 g) and 50% hypophosphorous acid (30 g) and acetic acid (200 mL) was brought to reflux and added to a suspension of **6a** (21.6 g, 68 mmol) in acetic acid (300 mL) at 100 °C. The reaction mixture was heated at the same temperature for 90 s, whereupon it decolorized and then was poured into ice water. The precipitate was collected by filtration, washed with water, dried, dissolved in benzene, and passed through a short column of Florisil. Elution with benzene–hexane gave **7a** (16.7 g, 95%): mp 160–161 °C (benzene–hexane) (lit.[35] mp 159–161 °C); the NMR spectrum

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(28) Slaga, T. J.; Gleason, G. L.; DiGiovanni, J.; Berry, D. L.; Juchau, M. R.; Fu, P. P.; Sukumaran, K. B.; Harvey, R. G. In *Polynuclear Aromatic Hydrocarbons*; Jones, P. W., Leber, P., Eds.; Ann Arbor Science: Ann Arbor, MI, 1979; p 753.
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(31) Sugiyama, T. *Gann* **1973**, *64*, 637.
(32) Ito, Y.; Ueda, N.; Maeda, S.; Murao, S.; Sugiyama, T.; Lee, H.; Harvey, R. G., submitted for publication in *J. Natl. Cancer Instit.*
(33) Smith, D. C. *J. Chem. Soc.* **1962**, 673.
(34) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.

agreed closely with that reported.[35]

3-Hydroxybenz[*a*]anthracene (8a). To a solution of **7a** (2.58 g, 10 mmol) in refluxing acetic acid (180 mL) was added HBr (60 mL) dropwise over 30 min. The mixture was heated at reflux for 3.5 h, cooled, and poured into ice water. The precipitate was filtered, washed with water, dried, and chromatographed on a short column of Florisil to yield **8a** (1.98 g, 81%): mp 208–209 °C (lit.[35] mp 209–210 °C); NMR (500 MHz in acetone-d_6–D_2O) δ 7.23 (1, d of d, H_2, $J_{2,4}$ = 1.6 Hz, $J_{1,2}$ = 8.7 Hz), 7.26 (1, d, H_4), 7.49 (2, m, $H_{9,10}$), 7.53 (1, d, H_5, $J_{5,6}$ = 9.0 Hz), 7.76 (1, d, H_6, $J_{5,6}$ = 9.1 Hz), 8.01 (1, d, $H_{8 \text{ or } 11}$, J = 7.8 Hz), 8.11 (1, d, $H_{8 \text{ or } 11}$, J = 8.0 Hz), 8.37 (1, s, H_7), 8.75 (1, d, H_1), 8.96 (1, s, OH), 9.15 (1, s, H_{12}); UV max (EtOH) 212 (33 720), 244.5 (40 320), 288 (72 000) nm.

Benz[*a*]anthracene-3,4-dione (9a). To a solution of **8a** (2 g, 8.2 mmol) in benzene (200 mL) and CH_2Cl_2 (100 mL) was added Adogen 464 (~1 mL). The solution was stirred under Argon, and Fremy's salt (5 g) in $^1/_6$ M KH_2PO_4 (300 mL) was added, and stirring was continued for 1 h. The product was extracted with CH_2Cl_2, washed with H_2O, evaporated to dryness, and triturated with acetone to afford **9a** (1.3 g, 62%), a dark purple solid, mp 213–214 °C. Anal. ($C_{18}H_{10}O_2$) C, H.

***trans*-3,4-Dihydroxy-3,4-dihydrobenz[*a*]anthracene (3a).** To a partial solution of the quinone **9a** (1.72 g, 6.7 mmol) in 1.5 L of ethanol was added $NaBH_4$ (18 g). Oxygen was bubbled through the solution, which was stirred for 24 h in the dark and worked up conventionally. The crude dihydrodiol (1.74 g) was purified by acetylation with acetic anhydride (90 mL) in pyridine (10 mL) at room temperature for 4 h. The product was chromatographed on Florisil and eluted with benzene to afford **3a** diacetate (1.7 g, 74%), mp 148–149 °C (benzene–hexane). Deacetylation of **3a** diacetate (1.0 g) with $NaOCH_3$ (0.8 g) in methanol (100 mL) and THF (50 mL) at reflux (10 min) gave pure **3a** (700 mg, 96%): mp 215–216 °C (lit.[14] mp 215–217 °C); NMR spectrum matched that reported.[11b,14]

3-Methoxy-7-methylbenz[*a*]anthracene (7b). Reduction of **6b** (4 g, 12.2 mmol) with HI and H_3PO_2 in acetic acid by the procedure employed for the analogous reduction of **6a** afforded **7b** (3.0 g, 91%), mp 165–167 °C (lit.[15] mp 165–167 °C).

3-Hydroxy-7-methylbenz[*a*]anthracene (8b). Demethylation of **7b** (3.0 g, 1.1 mmol) with HBr in AcOH by the procedure employed for the preparation of **8a** gave **8b** (2.6 g, 93%): mp 208–210 °C (lit.[15] mp 210–212 °C); UV max (EtOH) 211 (36 130), 245 (34 950) 292 (61 220) nm.

7-Methylbenz[*a*]anthracene-3,4-dione (9b). Oxidation of **8b** (500 mg, 1.9 mmol) with Fremy's salt by the procedure employed for preparation of **9a** yielded **9b** (460 mg, 87%), mp 202–203 °C (lit.[15] mp 197–198 °C).

***trans*-3,4-Dihydroxy-3,4-dihydro-7-methylbenz[*a*]anthracene (3b).** Reduction of **9b** (206 mg, 0.76 mmol) with $NaBH_4$ in the presence of O_2 by the procedure employed for **3a** gave the crude dihydrodiol, which was triturated with ether (without acetylation) to provide pure **3b** (200 mg, 96%): mp 222–224 °C (lit.[15] 188–189 °C); NMR (500 MHz in Me_2SO-d_6–D_2O) δ 3.05 (3, s, CH_3), 4.39 (1, d, H_3), 4.75 (1, d, H_4), 5.28 (1, d, OH), 5.62 (1, d, OH), 6.21 (1, d of d, H_2), 7.43 (1, d of d, H_1), 7.49 (2, m, $H_{9,10}$), 7.79 (1, d, H_5), 8.10 (1, d, H_6), 8.28 (2, d of d, $H_{8,11}$), 8.78 (1, s, H_{12}), $J_{1,2}$ = 10.0, $J_{1,3}$ = 2.0, $J_{2,3}$ = 2.4, $J_{3,4}$ = 11.0, $J_{5,6}$ = 9.0 Hz; UV max (EtOH) 214 (31 630), 264 (170 500), 372 (7800), 392 (11 870), 414.5 (11 200) nm.

3-Methoxy-12-methylbenz[*a*]anthracene (7c). A solution of **5b** (10 g, 34 mmol) in liquid HF was stirred at ambient temperature overnight in a hood, evaporated to dryness, and worked up conventionally to afford 3-methoxy-12-oxo-7,12-dihydrobenz[*a*]anthracene (**10**) in equilibrium with 12-hydroxy-3-methoxybenz[*a*]anthracene.

To a solution of the mixture containing **10** (3.0 g) in benzene (300 mL) under argon was added methyllithium (30 mL of 1.3 M solution in ether), and the solution was stirred for 24 h. The usual workup followed by chromatography of the crude product over Florisil and recrystallization from benzene–hexane afforded **7c** (2.0 g, 68% based on **5b**): mp 114–115 °C; NMR (500 MHz) δ 3.33 (3, s, CH_3), 3.96 (3, s, OCH_3), 7.18 (1, d of d, H_2), 7.23 (1, d, H_4), 7.45 (1, d, H_5), 7.50 (1, t, $H_{9 \text{ or } 10}$), 7.58 (1, t, $H_{9 \text{ or } 10}$), 7.63 (1, d, H_6), 7.99 (1, d, H_8), 8.15 (1, s, H_7), 8.29 (1, d, H_{11}), 8.50 (1, d, H_1), $J_{1,2}$ = 9.0, $J_{2,4}$ = 2.8, $J_{5,6}$ = 8.9, $J_{8,9}$ = 8.2, $J_{10,11}$ = 8.8 Hz. Anal. ($C_{20}H_{16}O$) C, H.

3-Hydroxy-12-methylbenz[*a*]anthracene (8c). Demethylation of **7c** (1.9 g, 6.9 mmol) with HBr in HOAc by the procedure employed for preparation of **8a** gave **8c** (1.5 g, 84%): mp 180–181 °C (benzene–acetone); NMR (500 MHz in acetone-d_6–D_2O) δ 3.33 (3, s, CH_3), 7.18 (1, d of d, H_2), 7.29 (1, d, H_4), 7.47 (1, d, H_5), 7.54 (1, t, $H_{9 \text{ or } 10}$), 7.60 (1, t, $H_{9 \text{ or } 10}$), 7.66 (1, d, H_6), 8.03 (1, d, H_8), 8.23 (1, s, H_7), 8.33 (1, d, H_{11}), 8.51 (1, d, H_1), $J_{1,2}$ = 8.9, $J_{2,4}$ = 2.6, $J_{5,6}$ = 8.9, $J_{8,9}$ = 8.2, $J_{10,11}$ = 8.8 Hz; UV max (EtOH) 211 (28 370), 246 (25 200), 291 (81 280), 349 (7600) nm. Anal. ($C_{19}H_{14}O$) C, H.

12-Methylbenz[*a*]anthracene-3,4-dione (9c). Oxidation of **8c** (1.5 g, 5 mmol) with Fremy's salt by the usual procedure furnished **9c** (1.0 g, 73%): mp 185–186 °C; mass spectrum, *m/e* 272.305 (M^+), 244 (M^+ – CO), 215, 189.

***trans*-3,4-Dihydroxy-3,4-dihydro-12-methylbenz[*a*]anthracene (3c).** Reduction of **9c** (400 mg, 1.5 mmol) with $NaBH_4$ in the presence of oxygen by the procedure employed for the preparation of **3b** gave **3c** (255 mg, 62%): mp 220–221 °C; NMR (500 MHz in Me_2SO-d_6–D_2O) δ 3.33 (3, s, CH_3), 4.44 (1, d, H_3), 4.55 (1, d, H_4), 6.09 (1, d, of d, H_2), 7.07 (1, d, H_1), 7.48 (1, t, $H_{9 \text{ or } 10}$), 7.54 (1, t, $H_{9 \text{ or } 10}$), 7.67 (1, d, H_5), 7.92 (1, d, H_6), 8.00 (1, d, H_8), 8.21 (1, d, H_{11}), 8.34 (1, s, H_7), $J_{1,2}$ = 10.2, $J_{2,3}$ = 1.8, $J_{3,4}$ = 11.3, $J_{5,6}$ = 8.6, $J_{8,9}$ = 8.3, $J_{10,11}$ = 8.8 Hz; UV max (EtOH) 213 (27 870), 267 (138 200), 398 (9860), 422 (8930) nm. Anal. ($C_{19}H_{16}O_2$) C, H.

Synthesis of the Carboxylic Acid 12a. A solution of *N,N*-diethyl-2-lithio-6-methoxy-1-naphthamide in diethyl ether (150 mL) was prepared by metalation of *N,N*-diethyl-6-methoxy-1-naphthamide (6.0 g, 23.5 mmol) with *sec*-butyllithium and TMEDA by the procedure reported. To this solution at –78 °C under argon was added a solution of 2,2-dideuterio-1-indanone (6.29 g, 47 mmol) in anhydrous THF (50 mL), and the resulting solution was stirred at ambient temperature overnight. The usual workup gave the crude product, which was dissolved in benzene along with 10% by weight of *p*-toluenesulfonic acid. The solution was heated at reflux overnight and then passed through a column of silica gel to afford the lactone **11** (3.0 g, 41%), which was used directly in the next step.

A solution of the lactone **11** (2.3 g, 9.2 mmol) in pyridine (20 mL) was added to a mixture of 2.3 g of zinc dust (activated by treatment with 1.5 g of $CuSO_4 \cdot 5H_2O$ in 15 mL of H_2O) in a solution of 10% KOH (200 mL). The mixture was stirred at reflux overnight, cooled, and filtered. The filtrate was worked up to afford the free acid **12a** (2.0 g, 87%): NMR δ 3.1 (2, s, CH_2), 3.9 (3, s, OCH_3), 4.8 (1, s, methine), 7.25 (9, m, Ar), 9.2 (1, br s, OH); mass spectrum, *m/e* 320.138 (M^+), 302 ($M^+ - H_2O$), 275 ($M^+ - CO_2H$).

6-Acetoxy-9-methoxycholanthrene-*d*$_2$ (13). To a solution of **12a** (1.7 g, 5.3 mmol) in acetic anhydride (25 mL) and glacial acetic acid (25 mL) was added $ZnCl_2$ (170 mg), and the mixture was heated at reflux for 2 h. The product was precipitated by the addition of ice water, removed by filtration, dried, taken up in benzene, and chromatographed on a column of Florisil. Elution with benzene gave **13** (1.0 g, 56%): mp 238–239 °C (benzene–hexane); NMR δ 2.60 (3, s, OAc), 3.54 (2, s, CH_2), 3.95 (3, s, CH_3), 7.2–7.6 (6, m, Ar), 7.70 (1, d, H_{11}, $J_{10,11}$ = 9.0 Hz), 9.11 (1, d, H_1, $J_{1,2}$ = 9.2 Hz); mass spectrum, *m/e* 344.139 (M^+), 302, 301 ($M^+ - CH_3CO$), 259, 228.

9-Methoxycholanthrene (14a). Reduction of **13** (1.0 g, 2.9 mmol) with HI and H_3PO_2 in acetic acid by the procedure employed for the reduction of **6a** gave **14a** (790 mg, 96%): mp 163–164 °C; NMR δ 3.53 (2, d, CH_2), 3.70 (2, d, CH_2), 3.94 (3, s, CH_3), 7.2–7.8 (7, m, Ar), 8.68 (1, d, H_7), 8.79 (1, s, H_6); mass spectrum, *m/e* 284.120 (M^+).

9-Hydroxycholanthrene (14b). Demethylation of **14a** (800 mg, 2.8 mmol) with HBr in AcOH by the procedure used for the preparation of **8a** yielded **14b** (670 mg, 88%): mp 228–229 °C; NMR (500 MHz in acetone-d_6) δ 3.54 (2, m, CH_2), 3.74 (2, m, CH_2), 7.2–7.8 (7, m, Ar), 8.77 (1, d, H_7), 8.94 (1, s, H_6); mass spectrum, *m/e* 270.104 (M^+), 255, 239; UV max (EtOH) 220 (35 730), 243 (38 630), 284 (58 370), 293 (72 400), 305 (53 830), 347 (11 360) nm.

Cholanthrene-9,10-dione. Oxidation of **14b** (350 mg, 1.3 mmol) with Fremy's salt by the procedure utilized for the prep-

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aration of **9a** furnished cholanthrene-9,10-dione (250 mg, 68%): mp > 280 °C; NMR (Me_2SO-d_6) δ 3.53 (2, br s, CH_2), 3.75 (2, br s, CH_2), 6.56 (1, d, H_8), 7.2–8.7 (6, m, H_7 and Ar), 9.02 (1, s, H_6); mass spectrum, *m/e* 284.316 (M^+), 286, 256 ($M^+ - CO$), 239, 226.

***trans*-9,10-Dihydroxy-9,10-dihydrocholanthrene (4a).** Reduction of cholanthrene-9,10-dione (200 mg, 0.70 mmol) with $NaBH_4$ in the presence of O_2 by the procedure employed for **3a** gave **4a** diacetate (130 mg, 50%): mp 193.5–194.5 °C; NMR (500 MHz) δ 2.05 (3, s, OAc), 2.13 (3, s, OAc), 3.55 (2, m, CH_2), 3.75 (2, m, CH_2), 5.64 (1, t, H_9), 6.27 (1, d of d, H_8), 6.33 (1, d, H_{10}), 7.23 (1, d, H_3), 7.41 (1, d, H_{11}), 7.44 (1, m, H_4), 7.64 (1, d, H_7), 7.72 (1, d, H_5), 7.93 (1, d, H_{12}), 8.48 (1, s, H_6) ($J_{7,8}$ = 10.0, $J_{9,10}$ = 5.7, $J_{11,12}$ = 8.7 Hz); mass spectrum, *m/e* 372.38 (M^+), 312 ($M^+ - CH_3CO_2H$), 270 (312 - CH_2CO), 239.

Deacetylation of **4a** diacetate (80 mg) with $NaOCH_3$ (120 mg) in methanol (60 mL) and THF (15 mL) at reflux for 10 min gave pure **4a** (60 mg, 96%): mp 260 ° dec; NMR (500 MHz in Me_2SO-d_6) δ 3.51 (2, m, CH_2), 3.72 (2, m, CH_2), 4.36 (1, m, H_9), 4.74 (1, m, H_{10}), 5.23 (1, d, OH), 5.57 (1, d, OH), 6.18 (1, d of d, H_8); UV max (EtOH) 217 (27 660), 266.5 (155 550), 399.5 (11 170), 422 (9650) nm; mass spectrum, *m/e* 288.342 ($M^+ - H_2O$), 239.

***trans*-9,10-Dihydroxy-*anti*-7,8-epoxy-7,8,9,10-tetrahydrocholanthrene (16).** A solution of the dihydrodiol **4a** (50 mg, 0.17 mmol) and *m*-chloroperbenzoic acid (292 mg, 1.7 mmol) in freshly distilled THF (30 mL) was stirred at room temperature under argon for 1 h. The reaction was quenched with water, and the product was extracted into ether and washed three times with 10% NaOH and twice with H_2O. The solution was dried over $MgSO_4$, filtered, and evaporated, avoiding heating because of the well-known thermal instability of bay region diol epoxides. The product was triturated with ether to yield **16** (40 mg, 77%): mp 196–197 °C; NMR (500 MHz in Me_2SO-d_6) δ 3.81 (1, br s, H_8), 3.89 (1, d, H_9), 4.49 (1, d, H_{10}), 5.09 (1, br s, H_7), 7.2–8.8 (7, m, Ar), $J_{9,10}$ = 8.0 Hz; mass spectrum, *m/e* 304.110 (M^+) 286 ($M^+ - H_2O$), 258 ($M^+ - 2H_2O$), 229, 215, 202; UV max (EtOH) 201 (31 180), 227 (23 240), 263 (129 370), 385 (8820) nm.

Conversion of the Carboxylic Acid 12a to the Methyl Ketone 15. To a solution of KOH (400 mg) in H_2O (2 mL) was added a solution of **12a** (1.7 g, 5.3 mmol) in hexamethylphosphoramide (15 mL) and CH_3I (4 mL). The mixture was stirred overnight and worked up to afford the crude methyl ester **12b**. The ester was purified by chromatography on a short column of Florisil to yield pure **12b** (1.7 g, 96%): NMR δ 3.05 (2, q, CH_2), 3.86 (3, s, CH_3), 3.98 (3, s, CH_3), 4.49 (1, s, methine), 6.9–7.8 (9, m, Ar); mass spectrum, *m/e* 334.154 (M^+), 302 ($M^+ - CH_3OH$), 274 (302 - CO).

To a solution of **12b** (1.7 g, 5.1 mmol) in diethyl ether (150 mL) was added hexamethylphosphoramide (10 mL) and excess CH_3Li (30 mL of a 1.3 M solution). The solution was stirred at room temperature for 3 h and then worked up conventionally to provide the methyl ketone **15** (1.47 g, 91%) by crystallization from ether in two crops: mp 154–155 °C; NMR (500 MHz) δ 2.69 (3, s, CH_3), 3.05 (2, q, CH_2 J_{gem} = 15.8 Hz), 3.90 (3, s, OCH_3), 4.36 (1, s, methine), 6.9–7.7 (9, m, Ar); mass spectrum, *m/e* 318.416 (M^+).

9-Methoxy-6-methylcholanthrene (14c). A solution of **15** (1.1 g, 3.5 mmol) in liquid HF (sufficient to dissolve) was stirred overnight in a hood. The HF was evaporated in a stream of N_2, and the product was worked up conventionally to provide crude 1,1-dideuterio-**14c**, which was purified by chromatography on a column of Florisil. There was obtained 1,1-dideuterio-**14c** (950 mg, 91%): mp 168–169 °C (benzene–hexane); NMR (500 MHz) δ 3.27 (3, s, CH_3), 3.52 (2, s, CH_2), 3.96 (3, s, OCH_3), 7.17 (1, d of d, H_8), 7.24 (1, d, H_{10}), 7.29 (1, d, H_3), 7.46 (1, d, H_{11}), 7.54 (1, apparent t, H_4), 7.68 (1, d, H_{12}), 7.92 (1, d, H_5), 8.58 (1, d, H_7), $J_{3,4}$ = 6.6, $J_{7,8}$ = 9.1, $J_{8,10}$ = 2.7, $J_{11,12}$ = 8.9 Hz; mass spectrum, *m/e* 300.148 (M^+), 285 ($M^+ - CH_3$).

9-Hydroxy-6-methylcholanthrene (14d). Demethylation of **14c** (900 mg, 3 mmol) with HBr in AcOH by the usual procedure afforded the free phenol **14d** (600 mg, 70%): mp 225–226 °C; NMR (500 MHz in acetone-d_6) δ 3.24 (3, s, CH_3), 3.51 (2, m, CH_2), 3.67 (2, m, CH_2), 7.28 (1, m, H_8), 7.48 (1, d, H_{11}), 7.54 (1, m, H_4), 7.68 (1, d, H_{12}), 7.94 (1, d, H_5), 7.58 (1, d, H_7), 8.73 (1, s, OH); mass spectrum, *m/e* 284.359 (M^+), 269 ($M^+ - CH_3$); UV max (EtOH) 219 (28 900), 243 (27 000), 286 (55 660), 296 (65 100), 353 (10 355) nm.

6-Methylcholanthrene-9,10-dione. Oxidation of **14d** (230 mg, 0.81 mmol) with Fremy's salt by the procedure employed for the preparation of **9a** gave the title quinone (150 mg, 62%): mp > 250 °C; NMR (500 MHz in Me_2SO-d_6) δ 3.08 (3, s, CH_3), 3.47 (2, m, CH_2), 3.64 (2, m, CH_2), 6.33 (1, d, H_8), 7.34 (1, m, H_3)*, 7.55 (1, m, H_4), 7.81 (1, d, H_{11})*, 7.88 (1, d, H_{12})*, 8.03 (1, d, H_5)*, 8.36 (1, d, H_7), asterisk indicates assignment tentative, $J_{7,8}$ = 10.7 Hz; mass spectrum, *m/e* 298.34 (M^+), 300 ($M^+ + H_2$), 284.

***trans*-9,10-Dihydroxy-9,10-dihydro-6-methylcholanthrene (4c).** Reduction of 6-methylcholanthrene-9,10-dione (100 mg, 0.33 mmol) with excess $NaBH_4$ in the presence of O_2 by the usual procedure gave **4c** isolated as the diacetate (50 mg, 40%): mp 190–191 °C; mass spectrum, *m/e* 386.152 (M^+), 326 ($M^+ - CH_3CO_2H$), 284 (326 - CH_2CO), 270, 252, 239. Deacetylation of **4c** diacetate (50 mg, 0.13 mmol) with $NaOCH_3$ (70 mg) in methanol (40 mL) and THF (10 mL) at reflux for 10 min gave pure **4c** (35 mg, 90%): mp 218–220 °C; NMR (500 MHz in Me_2SO-d_6) δ 3.03 (3, s, CH_3), 3.74 (2, m, CH_2), 3.66 (2, m), 4.42 (1, m, H_9), 4.53 (1, m, H_{10}), 5.21 (1, d, OH), 5.55 (1, d, OH), 5.21 (1, d, OH), 6.06 (1, d of d, H_8), 7.11 (1, d of d, H_7), 7.23 (1, d, H_3)*, 7.47 (1, m, H_4), 7.69 (1, d, H_{11})*, 7.82 (1, d, H_5)*, 7.89 (1, d, H_{12})*, asterisk indicates tentative assignment, $J_{7,8}$ = 10.2, $J_{9,10}$ = 11.3 Hz; UV max (EtOH) 216 (27 400), 272.5 (120 100), 415 (11 800); mass spectrum, *m/e* 302.374 (M^+), 284 ($M^+ - H_2O$), 269 (284 - CH_3), 239.

Chromosome Experiments. Noninbred Long–Evans rats, 28–35 days of age, weighing 50–80 g were used. Each experimental group consisted of three to six rats. They were given NMF (Oriental Ferment Co., Tokyo) and water ad libitum. The BA derivatives were given in lipid emulsion as a single pulse dose of 25, 50, or 100 mg/kg body weight into the caudal vein. Colchine, 0.3 mg/rat ip, was injected 1 h before they were killed. Chromosome specimens were prepared from the femoral bone marrow by the conventional method 6 and 18 h after injection of the BA derivaties, stained in 2% Giemsa solution (pH 6.8) for 15 min, and then analyzed microscopically. The chromosomes in 100 well-spread metaphse cells in each animal (at least 300 metaphases per experimental group) were analyzed. Chromosomal aberrations (CA) were divided into gaps, breaks, and exchanges. Gaps were defined as complete discontinuity of one or both chromatids not exceeding the width of a chromatid, and breaks were defined as discontinuity greater than the width of a chromatid, irrespective of whether the distal fragment was dislocated. Multiple CA (above 10) were defined as uncountable CA. Cells were classified on the basis of the induced chromosome damage and placed only in a single category. The incidence of aberrant cells was defined by the precentage of cells with breaks, exchanges, and multiple CA. The severity of damage was expressed by the rate of aberrations per cell, and in this case multiple CA were counted as 10 aberrations.

Acknowledgment. This research was supported by grants from the American Cancer Society (BC-132) and the National Cancer Institute (CA 36097 and CA 14599).

Registry No. **3a**, 60967-89-7; **3a** diacetate, 60967-85-3; **3b**, 64521-14-8; **3c**, 80752-42-7; **4a**, 88262-31-1; **4a** diacetate, 111238-20-1; **4c**, 111238-24-5; **4c** diacetate, 111238-23-4; **5** (R = CH_3CH), 111238-07-4; **5a**, 71964-75-5; **5a** lactone, 71964-76-6; **5b**, 94305-64-3; **6a**, 66240-12-8; **6b**, 71988-98-2; **7a**, 69847-25-2; **7b**, 71988-99-3; **7c**, 111238-08-5; **8a**, 4834-35-9; **8b**, 71989-00-9; **8c**, 16053-79-5; **9a**, 74877-25-1; **9b**, 71989-02-1; **9c**, 111238-10-9; **10**, 111238-09-6; **11**, 111238-12-1; **12a**, 111238-13-2; **12b**, 111238-14-3; **13**, 111266-93-4; **14a**, 111238-15-4; **14b**, 111238-16-5; **14c**, 111238-19-8; **14d**, 111238-21-2; **15**, 111238-17-6; **16**, 111238-25-6; 2-bromo-6-methoxynaphthalene, 5111-65-9; 2-lithio-*N*,*N*-diethyl-6-methoxy-1-naphthamide, 111238-11-0; 2,2-dideuterio, 10036-02-9; cholanthrene-9,10-dione, 111238-18-7; 6-methylcholanthrene-9,10-dione, 111238-22-3.