Synthesis of the Active Diol Epoxide Metabolites of the Potent Carcinogenic Hydrocarbon 7,12-Dimethylbenz[a]anthrene

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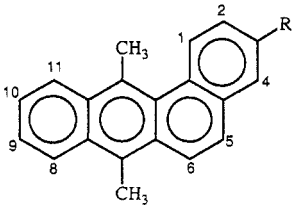
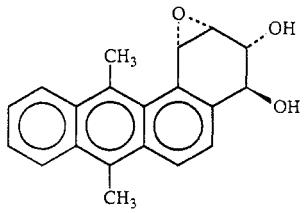
Efficient syntheses of the anti and syn diol epoxide derivatives of 7,12-dimethylbenz[a]anthracene (DMBA), *trans*-3,4-dihydroxy-*anti*-(and *syn*)-1,2-epoxy-1,2,3,4-tetrahydro-DMBA (**2a, 2b**), implicated as the active metabolites of this potent carcinogenic hydrocarbon, are described. The failure of previous attempted syntheses is ascribed to the exceptional facility of photooxidation of the synthetic precursor of **2a, 2b**, *trans*-3,4-dihydroxy-3,4-dihydro-DMBA (**3**), and **2a, 2b** coupled with the instability of the DMBA diol epoxide derivatives. It is shown that these complications can be surmounted by conducting all operations in controlled light, monitoring reactions by HPLC to optimize reaction time, and utilizing mild conditions for the conduct of reactions and product isolation.

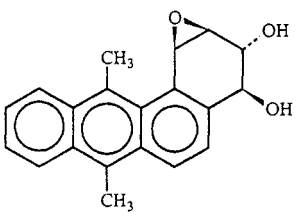
7,12-Dimethylbenz[a]anthracene (DMBA) (**1a**) is the most potent carcinogenic polycyclic aromatic hydrocarbon commonly employed in carcinogenesis research, and the DMBA induced rat mammary carcinoma is the standard laboratory animal model in the study of human breast cancer.[1] A diol epoxide metabolite of DMBA, *trans*-3,4-dihydroxy-*anti*-(or *syn*)-1,2-epoxy-1,2,3,4-tetrahydro-DMBA (**2a,b**), has been implicated as the principal active form of DMBA which binds covalently to DNA in vivo.[2]



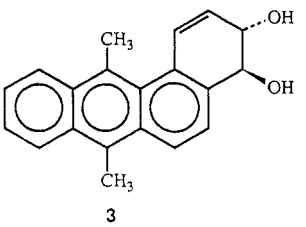
1a: R = H
b: R = OH
c: R = OMe



2a: Anti



2b: Syn



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Although synthesis of the 3,4-dihydrodiol of DMBA (**3**) was achieved in 1979,[3] attempts to convert it to the corresponding anti diol epoxide **2a** were unsuccessful. Direct epoxidation of **3** by peracids using procedures employed previously to prepare the diol epoxide derivatives of other carcinogenic hydrocarbons[4] afforded mainly tars. When peracid oxidation was conducted in the presence of DNA, there was isolated a small percentage of an adduct tentatively identified as arising from covalent binding of **2a** to DNA.[5] Since DMBA undergoes relatively facile oxidation with peracids to afford mixtures of meso-region oxidation products, it appeared likely that this was the predominant pathway of oxidation of **3**. Moreover, since DMBA is severely distorted from planarity due to steric interaction between the bay region methyl group and the hydrogen atom in the 1-position,[6] it seemed probable that its diol epoxide derivatives **2a,b** might be too unstable to isolate due to similar strain. However, our subsequent success in synthesizing the bay region diol epoxide of 3-methylcholanthrene,[7] which has a reactive meso region, and the bay region diol epoxide of 5-methylchrysene,[8] which has a bay methyl group, stimulated our renewed efforts to synthesize the diol epoxide derivatives of DMBA. We now report the first successful synthesis of the diastereomeric anti and syn diol epoxides of DMBA, **2a,b**.

Results and Discussion

Initial efforts were directed toward development of an improved synthesis of DMBA 3,4-dihydrodiol (**3**) in order to make larger quantities of this key intermediate available for studies of its conversion to the corresponding diol epoxides.

Synthesis of 3-Hydroxy-DMBA (1b). Synthesis of **1b**, the starting compound in the preparation of **3**, was accomplished initially by a modification of the method of Newman.[9] This approach is outlined in Scheme I. Condensation of the Grignard reagent of 6-methoxy-2-bromonaphthalene with phthalic anhydride gave the keto acid **4** which underwent reduction with zinc and alkali to yield **5**. Treatment of **5** with zinc chloride and acetic anhydride in acetic acid provided 3-methoxy-6-acetoxybenz[a]anthracene (**6**),[10] which was oxidized with sodium

(1) Welsch, C. W. *Cancer Res.* **1985**, *45*, 3415.
(2) Moschel, R. C.; Baird, W. M.; Dipple, A. *Biochem. Biophys. Res.* **1977**, *76*, 1092. Ivanovic, V.; Geacintov, N. E.; Jeffrey, A. M.; Fu, P. P.; Harvey, R. G. *Cancer Lett.* **1978**, *4*, 131. Malaveille, C.; Bartsch, H.; Tierney, B.; Grover, P. L.; Sims, P. *Biochem. Biophys. Res. Commun.* **1978**, *83*, 1468. Dipple, A.; Nebzydoske, J. A. *Chem.-Biol. Interact.* **1978**, *20*, 17. Chou, M. W.; Yang, S. K. *Proc. Natl. Acad. U.S.A.* **1978**, *75*, 5466. Bigger, C. A.; Sawicki, J. T.; Blake, D. M.; Raymond, L. G.; Dipple, A. *Cancer Res.* **1983**, *43*, 5647. Dipple, A.; Pigott, M.; Moschel, R. C.; Constantino, N. *Cancer Res.* **1983**, *43*, 4132.
(3) Sukumaran, K. B.; Harvey, R. G. *J. Am. Chem. Soc.* **1979**, *101*, 1353; *J. Org. Chem.* **1980**, *45*, 4407.
(4) Harvey, R. G. In *Polycyclic Hydrocarbons and Carcinogenesis*; Harvey, R. G., Ed.; ACS Symp. Series No. 283; American Chemical Society: Washington, DC, 1985; pp 35-62.
(5) Jeffrey, A. M.; Weinstein, I. B.; Harvey, R. G. *Proc. Am. Assoc. Cancer Res.* **1979**, *20*, 131.
(6) Iball, J. *Nature (London)* **1964**, *201*, 916. Glusker, J. In *Polycyclic Hydrocarbons and Carcinogenesis*; Harvey, R. G., Ed.; ACS Symp. Series No. 283; American Chemical Society: Washington, DC, 1985; pp 125-185.
(7) Jacobs, S.; Cortez, C.; Harvey, R. G. *Carcinogenesis* **1983**, *4*, 519.
(8) Pataki, J.; Lee, H.; Harvey, R. G. *Carcinogenesis* **1983**, *4*, 399. Harvey, R. G.; Pataki, J.; Lee, H. In *Polycyclic Aromatic Hydrocarbons*, IXth Internat. Symp. on Polynuclear Aromatic Hydrocarbons; Cooke, M., Dennis, A. J., Eds.; Battelle: Columbus, OH, 1986; pp 371-386. Harvey, R. G.; Pataki, J.; Lee, H. *J. Org. Chem.* **1986**, *51*, 1407.
(9) Newman, M. S.; Khanna, J. M.; Kanakarajan, K.; Kumar, S. *J. Org. Chem.* **1978**, *43*, 2553.

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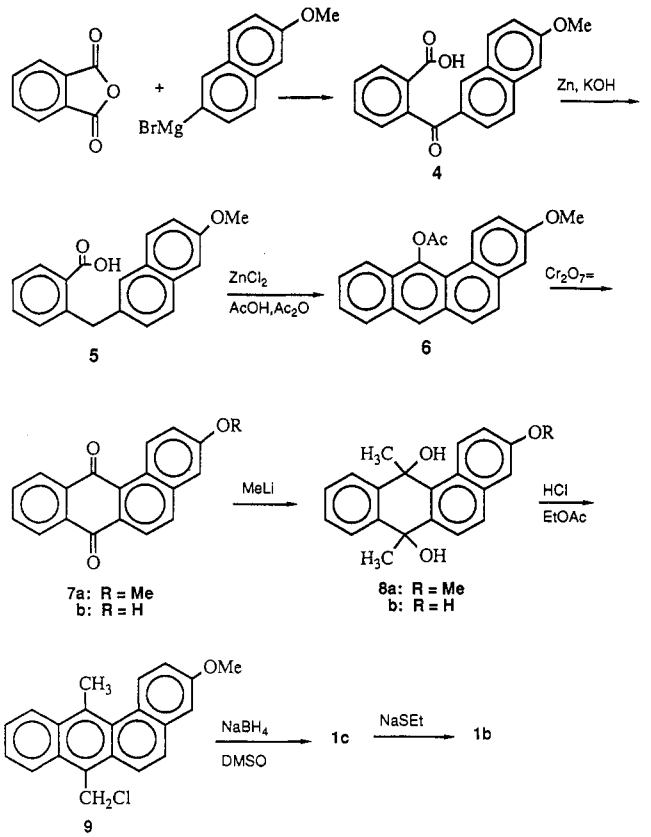
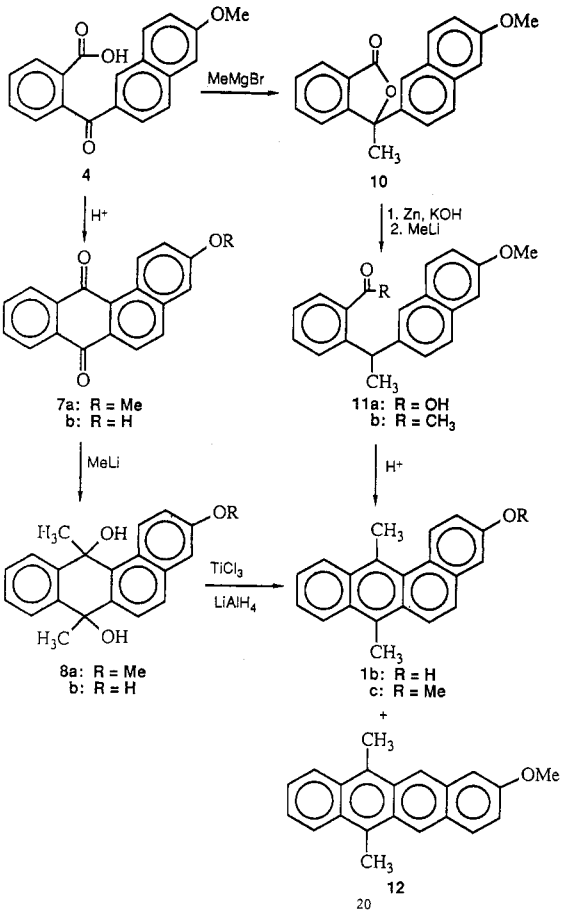
Scheme I



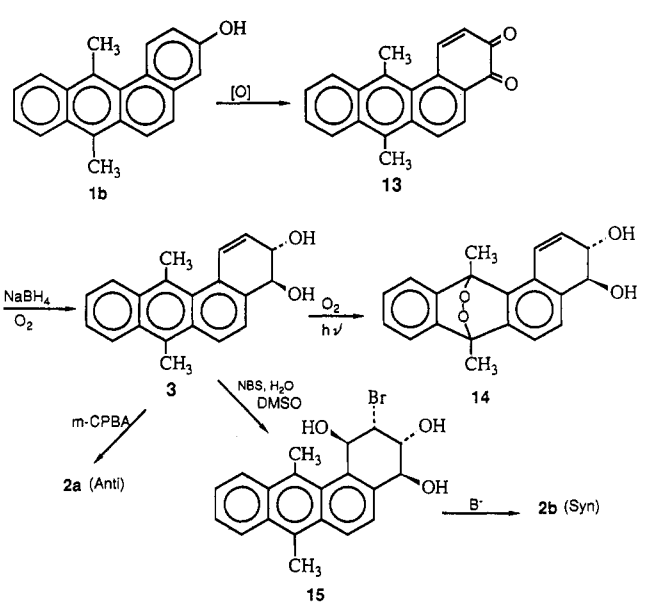
dichromate in acetic acid to furnish the quinone **7**. Addition of methyllithium to **7** followed by treatment of the resulting dimethyl adduct **8a** with dry HCl gas in ethyl acetate afforded smoothly 7-(chloromethyl)-3-methoxy-DMBA (**9**). Reduction of the latter with $NaBH_4$ gave 3-methoxy-DMBA (**1c**) which underwent demethylation with NaH and ethanethiol in DMF to yield the free phenol **1b**. Although this synthetic sequence entails a considerable number of steps, the reactions are relatively straightforward and the yields in the individual steps are generally high.

In order to shorten this synthetic route, several modifications were also examined (Scheme II). One approach entailed addition of methylmagnesium bromide to the keto acid **4** to provide the lactone **10**. Reduction of **10** with zinc and alkali followed by addition of methyllithium afforded the methyl ketone **11b**. However, cyclization of **11b** with HF, polyphosphoric acid, or methanesulfonic acid took place nonregiospecifically to yield 3-methoxy-DMBA (**1c**) and 2-methoxy-6,11-tetracene (**12**) in approximately equimolar ratio.[11] A second, more efficient, alternative synthetic route to 3-hydroxy-DMBA (**1b**) involved cyclization of the keto acid **4** in concentrated sulfuric acid to yield the demethylated quinone **7b**. Addition of methyllithium to this quinone furnished the dimethyl adduct **8b**. Deoxygenation of this intermediate with the $TiCl_3$–$LiAlH_4$ by the method of Walborsky[13] gave 3-hydroxy-DMBA (**1b**) in good yield. This synthetic route to **1b** offers the significant advantages of fewer steps and elimination of the unpleasant demethylation step with NaSEt. Its only

Scheme II



Scheme III



drawback is the relatively low yield (50%) of **7b** in the initial cyclization of the keto acid.

More efficient cyclization of the keto acid **4** was accomplished by substitution of methanesulfonic acid for concentrated sulfuric acid. Cyclization took place smoothy and regiospecifically to afford only the benz[a]anthracene quinone derivative **7a** which retained the methyl group (80%). Addition of methyllithium to this quinone followed by deoxygenation of the resulting dimethyl adduct **8a** with $TiCl_3$–$LiAlH_4$ furnished 3-methoxy-DMBA (**1c**). Demethylation of **1c** with NaSEt in DMF took place quantita-

(10) Smith, D. C. *J. Chem. Soc.* **1962**, 673.
(11) In the previous report[12] of acid-catalyzed cyclization of **11b** the tetracene derivative **12** was not identified.
(12) Newman, M. S.; Khanna, J. M.; Khanna, V. K.; Kanakarajan, K. *J. Org. Chem.* **1979**, *44*, 4994.
(13) Walborsky, H. M.; Wust, H. H. *J. Am. Chem. Soc.* **1982**, *104*, 5807.

tively to yield **1b**. This synthetic sequence provides the best overall yield of 3-hydroxy-DMBA (**1b**). It is shorter than alternative methods[3,9,12] and is readily adaptable to preparation on any scale.

Synthesis of DMBA 3,4-Dihydrodiol (3). Conversion of 3-hydroxy-DMBA to **3** was carried out by a modification of our general procedure for the conversion of phenols to dihydrodiols (Scheme III).[4] Oxidation of **1b** with Fremy's salt in a two-phase methylene chloride–water system in the presence of Adogen 464 gave a dark purple solid identified as DMBA-3,4-dione (**13**).[14] Although reduction of **13** with $LiAlH_4$ was previously reported to afford the trans dihydrodiol, the yield of **3** was low and poorly reproducible.[3] Reduction of **13** with $NaBH_4$ in absolute ethanol in the dark, or under yellow light with oxygen bubbling through the reaction mixture, took place smoothly and stereospecifically to provide **3**. The utility of O_2 for the reoxidation of catechol byproducts back to quinones in reactions of this type has previously been demonstrated.[4,7,15] The yield of **3** obtained by reduction with $NaBH_4$ under these conditions was much higher (90–94%) and more reproducible than that previously obtained[3] via reduction with $LiAlH_4$. Moreover, precautions for the exclusion of traces of moisture were not required for the $NaBH_4$ method, and **3** was obtained essentially free of secondary products. The melting point of pure **3** was somewhat higher (193–194 °C) than previously reported (182–184 °C),[3] indicative of the higher purity of **3** obtained by this method.

The 500-MHz high resolution NMR spectrum of **3** was fully consistent with the DMBA 3,4-dihydrodiol structure. The H_1 and H_2 vinylic protons each appeared as a doublet of doublets ($J_{1,2}$ = 10.2 Hz, $J_{1,3}$ = 1.8 Hz, $J_{2,3}$ = 2.0 Hz) at δ 6.97 and 6.10, respectively. The lower field peak is assigned to H_1 since it is a bay region proton anticipated to exhibit "edge-deshielding".[16] The H_3 and H_4 carbinol proton signals appeared as doublets at δ 4.51 and 4.59, respectively. The large value of the coupling constant ($J_{3,4}$ = 11.3 Hz) is consistent with the existence of this molecule predominantly in the diequatorial conformation.[17]

The detailed ^1H NMR assignments were supported by two-dimensional chemical shift correlation and NOE difference spectroscopy. Rigorous assignment of the H_3 and H_4 carbinol protons and the H_6, H_8, and H_{11} aromatic protons was not possible by decoupling techniques due to the proximity of these protons resonances. The high resolution COSY spectrum of **3** (Figure 1) clearly indicates that the proton at δ 4.51 is coupled with the protons at δ 5.30, 6.10, and 6.97, the proton at δ 4.59 is coupled with the proton at δ 5.70, and the protons at δ 4.59 and 4.51 are coupled with each other. This clearly demonstrates connectivities between H_1/H_3, H_2/H_3, H_1/H_2, H_3/3-OH, H_4/4-OH. Also, the proton at δ 4.51 is coupled with the proton at δ 6.97, indicating not only ^3J coupling but also long range coupling, $^4J_{1,3}$. The COSY experiment via long range coupling (not shown) revealed ^5J epi coupling between H_1 and H_5. This coupling is expected because in olefinic and aromatic systems ^5J couplings are generally larger than ^4J couplings, with the exception of aromatic meta couplings.[18] Expansion of the 7.3–8.4 ppm region

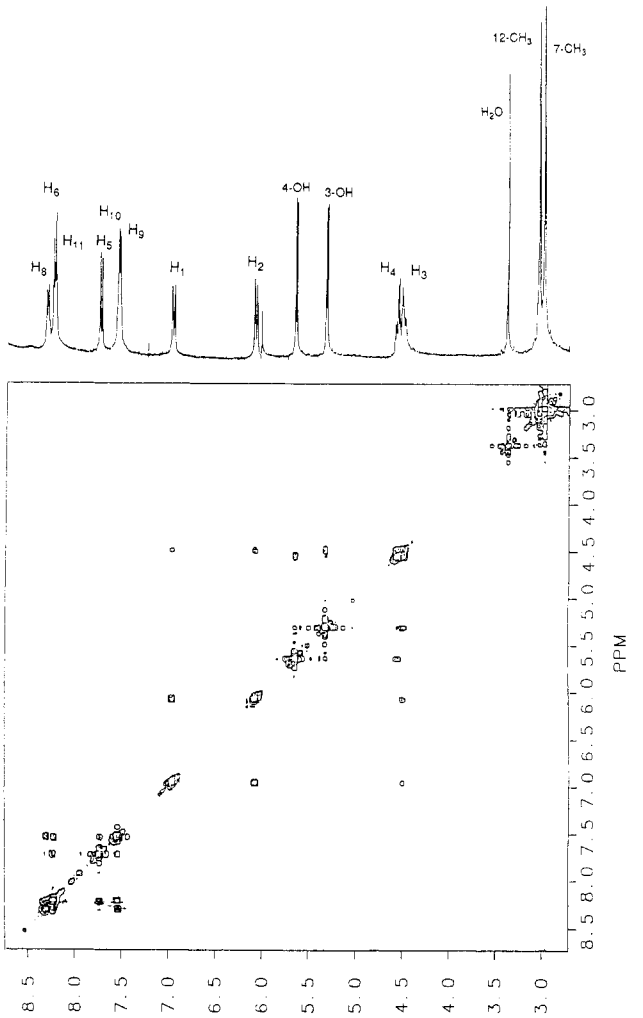


Figure 1. Contour plot of a high resolution COSY experiment with **3** at 400 MHz. The normal 400-MHz ^1H NMR spectrum of **3** is shown above.

of the high resolution COSY spectrum unambiguously indicated connectivity between H_5 and H_6 and coupling between the protons at δ 7.53 and 8.31 and the protons at δ 7.56 and 8.21. However, there was no clear information to identify the proton at δ 8.21 or 8.31 as H_8 or H_{11}. This ambiguity was readily clarified by NOE difference experiments in which the methyl resonances at δ 2.99 and 3.04 were irradiated. Thus, irradiation of the methyl singlet at 2.99 showed NOE effects on the signals at δ 8.24 and 8.31, identifying these two signals as H_6 and H_8, respectively, and the irradiated signal as the 7-CH_3 peak. Similarly, irradiation at δ 3.04 resulted in a major NOE effect at δ 6.97 and 8.21, identifying these two signals as H_1 and H_{11}, respectively, and the irradiated signal as the 12-CH_3 resonance. By these means all of ^1H peaks of **3** have been unequivocally assigned.

Compound **3** in solution underwent facile photooxidation in the air on exposure to sunlight or fluorescent light to yield the stable transannular peroxide derivative **14**.[19] The structural assignment of **14** is supported by its mass spectrum, m/e 322.1207 (M^+, calcd 322.1200) and its high resolution 500 MHz NMR spectrum which exhibited methyl singlets at δ 2.02 and 2.20 shifted upfield from those of **3** (δ 3.02 and 3.10). The sensitivity of **3** to photooxidation is markedly greater than that of the structurally analogous dihydrodiols of benz[*a*]anthracene and 3-

(14) Conversion of **3** was complete under these conditions; similar reaction in the absence of the phase-transfer agent Adogen 464 was previously reported to lead to recovery of unreacted **3** even with large excess of Fremy's salt.[12]

(15) Platt, K. L.; Oesch, F. *J. Org. Chem.* **1983**, *48*, 265.
(16) Bartle, K. D.; Jones, D. W. *Adv. Org. Chem.* **1972**, *8*, 317.
(17) Zacharias, D.; Glusker, J. P.; Fu, P. P.; Harvey, R. G. *J. Am. Chem. Soc.* **1979**, *101*, 4043.
(18) Barfield, M.; Chakrabarti, B. *Chem. Rev.* **1969**, *69*, 757.

(19) Photooxidation was essentially complete within 30 min.

methylcholanthrene and partially accounts for the previous lack of success in converting **3** to its diol epoxide derivatives.

Synthesis of the Anti and Syn Diol Epoxide Derivatives of DMBA (2a and 2b). The syn diol epoxide isomer **2b** was synthesized from **3** via the bromohydrin intermediate **15**. In view of the likely sensitivity of the intermediates and products to photooxidation and thermal decomposition, all reactions and workup procedures were conducted in subdued light, avoiding heating. Direct treatment of **3** with *N*-bromosuccinimide (NBS) in moist dimethyl sulfoxide[4,20] resulted in substantial reoxidation of **3** back to the quinone **13**, as evidenced by HPLC, the dark violet color of the solution, and the moderate yield of **15** (55–65%). Treatment of **15** with *t*-BuOK in THF while monitoring the extent of reaction by HPLC on a DuPont Zorbax Sil column gave the syn diol epoxide **2b** in good yield. The 500-MHz high resolution NMR spectrum of **2b** was fully consistent with its assigned structure. The NMR signals of the H_1 and H_2 protons appeared at δ 4.33 (doublet, $J_{1,2}$ = 4.3) and δ 3.78 (multiplet), respectively, at considerably higher field than those of the corresponding protons of **3** and consistent with the oxiranyl assignment. The H_3 and H_4 carbinol protons of **2b** exhibited characteristic resonances at δ 3.78 and 4.60; the value of the coupling constant ($J_{3,4}$ = 8.1 Hz) was intermediate between 2.0 Hz expected for pure diaxial and 12.7 Hz for pure diequatorial, indicating a slight predominance of the diequatorial (57%)[21] over the diaxial conformer in solution.[17] The ultraviolet spectrum of **2b** contains an absorption maximum at 269.2 nm which is shifted 5 nm to shorter wavelength than that of **3** (273.1 nm), which is consistent with loss of the olefinic bond.

Compound **2b** was further characterized by its reaction with *t*-BuSK to afford the product of trans addition to the epoxide ring **16**. The *tert*-butyl group was utilized in order to lock the conformation of the adduct into the structure with the bulky *tert*-butyl group in the diaxial orientation. The integrated 500-MHz NMR spectrum of **16** was consistent with its assigned structure, confirming the syn isomeric assignment of its diol epoxide precursor **2b**.

Synthesis of the anti diastereomeric diol epoxide **2a** was complicated by its thermal instability which exceeded that of the syn isomer. Oxidation of the DMBA 3,4-dihydrodiol (**3**) with *m*-chloroperbenzoic acid was carried out at room temperature and monitored by HPLC. Formation of the epoxide, which had a longer retention time than **3**, was complete in 30 min. The workup was conducted at 0 °C, and the ether extract was dried over Na_2SO_4 (decomposition occured with $MgSO_4$). The isolated anti diol epoxide **2a** was stable in ether solution but deteriorated on evaporation of the solvent under vacuum, even at 0 °C using a dry ice condenser. The presence of the diol epoxide in solution was demonstrated by its UV spectrum which closely ressembled that of **2b** (Figure 2) and by trapping with *tert*-butyl mercaptan to form the product of transstereospecific addition to the epoxide ring **17**. The 500-MHz NMR spectrum of **17** was consistent with the assigned structure and characteristically different from that of the adduct **16** formed by the syn diol epoxide.

The lesser stability of the anti than the syn diastereomeric bay region diol epoxide of DMBA is unexpected, since it is contrary to previous findings with analogous derivatives of other polycyclic aromatic hydrocarbons for which the anti isomers are generally the most stable.[4] This difference may result from a change in conformation from a half-chair to a half-boat form, but direct analysis by NMR methods is prevented by the instability of **2a**. The exceptional reactivity of the diol epoxide metabolites of DMBA may be an important factor in the high carcinogenic potency of DMBA.

Biological Investigations. The synthetic DMBA anti and syn diol epoxides (**2a**, **2b**) have proven sufficiently stable to conduct a variety of biological investigations. In experiments conducted in collaboration with Dr. Alan Jeffrey, the major adducts produced by either microsomal or *m*-chloroperbenzoic acid oxidation of the DMBA 3,4-dihydrodiol in the presence of DNA, or by direct reaction of **2a** (but not **2b**) with DNA were identical.[22] Moreover, the same major DNA-bound adduct was formed by the metabolism of DMBA by cultured human bronchial, colonic, and esophageal explants and primary hamster embryo cells. This adduct was shown by its fluorescence, circular dichroism, and mass spectra to be a deoxyguanosine derivative similar to the 2-aminodeoxyguanosine adduct earlier to be formed by metabolic activation of benzo[a]pyrene.[23] On the other hand, studies conducted in collaboration with Dr. Anthony Dipple on the adducts formed by metabolic activation of DMBA in mouse embryo cells reveal a somewhat different pattern.[24] The most notable difference was the formation of major levels of deoxyadenosine adducts arising from both **2a** and **2b** in addition to the deoxyguanosine products. The tentative isomer assignments made earlier on the basis of chromatographic retention times[25] were confirmed with authentic **2a** and **2b**. These finding support the hypothesis that **2a** and/or **2b** are the principal active carcinogenic metabolites of DMBA in mammalian cells.

Experimental Section

Materials and Methods. 12-Acetoxy-3-methoxybenz[a]anthracene (**6**) was synthesized from **5** in 87% yield by the method described;[10] mp 166–167 °C (lit.[10] mp 166–167 °C). 3-Methoxybenz[a]anthracene-7,12-dione (**7**), 3-methoxy-7,12-dihydro-7,12-dihydroxy-DMBA (**8a**), and 3-methoxy-7-(chloromethyl)-12-methylbenz[a]anthracene (**9**) were prepared from **6** by the method of Newman.[9] *m*-Chloroperbenzoic acid (Aldrich) was purified by washing with pH 7.5 phosphate buffer and drying under reduced pressure. *N*-Bromosuccinimide (NBS) was crystallized from water prior to use. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was recrystallized from benzene. Fremy's salt [$(KSO_3)_2NO$] was freshly prepared according to the literature method.[26] *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. $TiCl_3$–$LiAlH_4$ (2:1) was obtained from the Aldrich Co. 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 spectrometer, the University of Chicago 500-MHz NMR spectrometer, or on a Varian XL-400 instrument (in the case of **3**) in $CDCl_3$, Me_2SO-d_6, or acetone-d_6 as appropriate, with tetramethylsilane as an internal standard. The COSY experiments on **3** were

(20) Harvey, R. G.; Fu, P. P. In *Polycyclic Hydrocarbons and Cancer*; Gelboin, H. V., Ts'o, P. O. P., Eds.; Academic Press: New York, 1978; Vol. 1, pp 133–165.

(21) The percentage is calculated as a simple arithmetical average assuming a normal half-chair structure.

(22) Jeffrey, A. M.; Grzeskowiak, K.; Harvey, R. G.; Lee, H.; Autrup, H.; Harris, C., manuscript in preparation.

(23) Harvey, R. G. *Acc. Chem. Res.* **1981**, *14*, 218; *Am. Sci.* **1982**, *70*, 386.

(24) Dipple, A.; Lee, H.; Harvey, R. G., manuscript in preparation.

(25) Dipple, A.; Sawicki, J. T.; Moschel, R. C.; Bigger, A. H. In *Extrahepatic Drug Metabolism and Chemical Carcinogenesis*; Rydstrom, J., Montelius, J., Bengtsson, M., Eds.; Elsevier Science Publishers: Amsterdam, 1983; pp 439–448.

(26) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.

performed on the Varian XL-400 at 20 °C with 20 mg of **3** dissolved in 0.5 mL of Me_2SO-d_6. The proton spectra for 2D NMR were obtained through a decoupler coil with a pulse sequence of RD–60°–t_1–90°–t_2. The ^1H shift-correlated (COSY) two-dimensional spectra of **3** were collected into a 1K × 1K data matrix. The spectral width was 2408 Hz. Each point transient was sampled 45 times, with 0.5-s pulse delay. For COSY via the long range technique, the same pulse sequence was optimized for the observation of epi coupling (^5J of H_1 and H_5) with mixing pulse of D_3 = 0.2 s; in this case 128 increments and 36 repetitions with 1K × 1K data matrix were employed. Integration was consistent with all molecular structural assignments. Melting points are uncorrected. All new compounds gave satisfactory microanalyses for C, H within ±0.3% and/or mass spectra consistent with the assigned structures. The ultraviolet spectra were obtained on a Perkin-Elmer Lambda 5 spectophotometer.

2-(6-Methoxy-2-naphthoyl)benzoic Acid (4). A portion (15 mL) of a solution of 2-bromo-6-methoxynaphthalene (50 g, 210 mmol) in freshly distilled THF (150 mL) was added to a flame-dried flask containing Mg turnings (7.2 g) and a few crystals of iodine under N_2. The mixture was heated to reflux and the remaining bromo compound was added dropwise at a rate to maintain reflux. After addition was complete, the dark solution was heated at reflux for 30 min then added to a hot solution of phthalic anhydride (32.6 g, 220 mmol) in 60 mL of THF. The resulting mixture was heated at reflux for 2 h, cooled, treated with dilute HCl, washed with water 6× to remove any unreacted phthalic anhydride, and worked up conventionally to yield 45 g (69%) of **4**. Recrystallization from benzene gave pure **4**, mp 175–176 °C (lit.[12] mp 169–170 °C) as white flakes.

2-[(6-Methoxy-2-naphthyl)methyl]benzoic Acid (5). A mixture of **4** (40 g, 131 mmol), aqueous NaOH (150 g in 2 L of H_2O), and Zn dust (500 g activated by shaking with a solution of 17 g of $CuSO_4$, then washed with water) was maintained at reflux overnight, then cooled, filtered, and acidified with HCl to precipitate **5** (37 g, 98%). Crystallization from benzene afforded pure **5** as a white solid, mp 164–165 °C (lit.[10] mp 161–164 °C).

3-Methoxy-DMBA (1c). (a) From 9. To a solution of **9** (4.73 g, 14.7 mmol) in Me_2SO (250 mL) was added $NaBH_4$ (1.0 g), and the mixture was stirred at room temperature under N_2 for 2 h. After the usual workup, crystallization from benzene gave 2.72 g of **1c** as a white solid: mp 131–132 °C (lit.[9] mp 131–132 °C); NMR δ 3.1 (3, s, 7-CH_3), 3.3 (3, s, 12-CH_3), 3.9 (3, s, OCH_3), 7.02–8.4 (m, 9, Ar). Chromatography of the filtrate on a column of Florisil afforded another 1.1 g of **1c** (total yield 91%). **(b) From 7a.** To a solution of **7a** (180 mg, 0.6 mmol) in benzene (50 mL) and ether (50 mL) was added CH_3Li (40 mL of 1.3 M) and the solution was maintained at reflux for 16 h under N_2. Conventional workup furnished the adduct **8a** (190 mg, 99%) which was reduced directly with $TiCl_3$–$LiAlH_4$ (2:1) by the procedure described below for the preparation of **1b**. Similar workup afforded **1c** (142 mg, 83%): mp 131–132 °C; NMR (500 MHz) δ 3.03 (3, s, 7-CH_3), 3.29 (3, s, 12-CH_3), 3.95 (3, s, OCH_3), 7.13 (1, d of d, H_2, $J_{2,4}$ = 2.7 Hz, $J_{1,2}$ = 9.1 Hz), 7.47 (1, d, H_5, $J_{5,6}$ = 9.4 Hz), 7.54–7.58 (2, m, $H_{9,10}$), 7.99 (1, d, H_6, $J_{5,6}$ = 9.4 Hz), 8.29 (2, apparent t, $H_{8,11}$), 8.36 (1, d, H_1); UV max (THF) 226 nm (20 960), 243 (20 500), 292 (57 270), 297 (60 930), 367 (7 100).

3-Hydroxy-DMBA (1b). (a) From 1c. Treatment of **1c** with NaSEt by the procedure previously described[3,9] gave **1b**, mp 167–168 °C (lit.[12] mp 167–168 °C). **(b) From 7b.** To a solution of **7b** (149 mg, 0.52 mmol) in benzene (50 mL) and ether (50 mL) was added CH_3Li (40 mL of 1.3 M), and the solution was stirred at reflux for 16 h under N_2. Conventional workup provided the adduct **8b** (157 mg, 99%) which was reduced directly with $TiCl_3$–$LiAlH_4$ (2:1). The latter reagent (403 mg, 1.16 mmol) was placed in a flask under N_2 at 0 °C and THF was added cautiously. The adduct **8b** (157 mg) dissolved in THF (5 mL) was added, and the mixture was heated at reflux for 2 h. The mixture was cooled, 2 N HCl was added slowly, and the product was isolated by extraction with ether and purified by chromatography on Florisil. Elution with benzene yielded **1b** (110 mg, 75%), mp 167–168 °C (benzene–hexane) (lit.[12] mp 167–168 °C).

3-Methyl-3-(6-methoxy-2-naphthyl)phthalide (10). To a solution of **4** (6.12 g, 20 mmol) in anhydrous ether (100 mL) was added CH_3MgBr (2.86 M, 21 mL) dropwise. The solution was refluxed for 24 h and worked up in the usual manner to afford **10** (6.1 g, >99%) which was used directly in the next step: NMR δ 2.1 (3, s, CH_3), 3.9 (3, s, OCH_3), 7.0–7.9 (10, m, Ar).

***o*-[1-(6-Methoxy-2-naphthyl)ethyl]benzoic Acid (11a).** Reduction of **10** (28 g, 91 mmol) with Zn dust (123 g activated with 2 g of $CuSO_4$), KOH (1.4 L of 10%), and pyridine (110 mL) heated at reflux for 16 h by the procedure of Newman[12] gave **11a** (22.3 g, 81%) as a white solid: mp (THF/EtOAc) 212–213 °C (lit.[12] mp 207–208 °C); NMR δ 1.8 (3, d, CH_3), 3.9 (3, s, OCH_3), 5.5 (1, m, methine), 7.1–8.1 (10, m, Ar).

***o*-[1-(6-Methoxy-2-naphthyl)ethyl]acetophenone (11b).** To a solution of **11a** (10 g, 32 mmol) and TMEDA (10 g) in anhydrous ether (400 mL) was added CH_3Li (106 mL of 1.2 M) dropwise. The mixture was held at reflux for 24 h and worked up conventionally to afford **11b** (9.7 g, 97%) as a white solid: mp 113–114 °C (lit.[12] mp 106–107.5 °C); NMR δ 1.6 (3, d, CH_3), 2.3 (3, s, $COCH_3$), 3.9 (3, s, OCH_3), 4.9 (1, q, methine), 7.0–7.9 (10, m, Ar).

Cyclization of 11b. Attempts to synthesize 3-methoxy-DMBA (**1c**) via treatment of **11b** with acids gave mixtures of the two possible cyclization products **1c** and 6,11-dimethyl-2-methoxytetracene (**12**). In a typical run, a solution of **11b** (150 mg, 0.5 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a solution of methanesulfonic acid (10 mL) in CH_2Cl_2 (100 mL) and stirred at room temperature for 4 h. After the usual workup followed by chromatography on Florisil, there was obtained 83% of a mixture of **1c** and **12** in 1:1 ratio. Analogous reactions of **11b** with HF (18 h at ambient temperature) or polyphosphoric acid (2 h at 100 °C) afforded **1c** and **12** in similar ratio in 80% and 81% yields, respectively.[11] The NMR spectra of the products showed all the peaks found in the spectrum of **1c** plus the additional peaks assigned to **12**. An analytical sample of **12** was collected by HPLC (Zorbax Sil, 25 cm × 5.2 mm, 7% CH_2Cl_2/hexane, 5 mL/min); **12** which had a shorter retention time than **1c** was obtained as a red solid: mp 206–208 °C; NMR (500 MHz) δ 3.18 (3, s, CH_3), 3.19 (3, s, CH_3), 3.94 (3, s, OCH_3), 7.08 (1, d of d, H_3, $J_{1,3}$ = 2.3 Hz), 7.13 (1, d, H_1), 7.35–7.40 (2, m, $H_{8,9}$), 7.89 (1, d, H_4, $J_{3,4}$ = 9.2 Hz), 8.25–8.30 (2, m, $H_{7,10}$), 8.70 (1, s, $H_{5 \text{ or } 12}$), 8.79 (1, s, $H_{12 \text{ or } 5}$); UV max (THF) 288 nm (ε 137 670), 290 (137 920).

3-Hydroxybenz[*a*]anthracene-7,12-dione (7b). Compound **4** (300 mg, 0.95 mmol) was added to a solution of sulfuric acid (18 mL) and water (4.5 mL) at 100 °C, and the mixture was stirred at 100 °C for 1.5 h. The reaction mixture was diluted with ice water and extracted 4× with ether. The usual workup gave **7b** (149 mg, 50%) as an orange-red solid: mp >220 °C; NMR (500 MHz in acetone-d_6/D_2O) δ 7.33 (1, s, H_4), 7.40 (1, d, H_2, $J_{1,2}$ = 9.44 Hz), 7.84–7.90 (2, m, $H_{9,10}$), 8.09 (1, d, H_5, $J_{5,6}$ = 8.6 Hz), 8.17–8.24 (3, m, $H_{6,8,11}$), 9.53 (1, d, H_1).

3-Methoxybenz[*a*]anthracene-7,12-dione (7a). To methanesulfonic acid (5 mL) at 100 °C was added **4** (100 mg, 0.36 mmol), and the mixture was stirred at 100 °C for 1.5 h. The usual workup followed by chromatography on a column of Florisil eluted with CH_2Cl_2 afforded **7a** (85 mg, 79%) as an orange solid: mp 163–164 °C (lit.[9] mp 162–163 °C); NMR δ 3.9 (3, s, OCH_3), 7.0–8.4 (8, m, Ar), 9.6 (1, d, H_1).

7,12-Dimethylbenz[*a*]anthracene-3,4-dione (13). To a solution of **1b** (450 mg, 1.66 mmol) in benzene (200 mL) was added 10 drops of Adogen 464 followed by Fremy's salt (1.8 g, 6.64 mmol) in $^1/_6$ M KH_2PO_4 (50 mL) and water (50 mL). The heterogeneous solution was stirred vigorously for 30 min and then transferred to a separatory funnel. The aqueous layer was separated and washed with benzene 2×, and the combined organic layer and benzene washings were washed with water 3×, dried over $MgSO_4$, and evaporated to dryness. Trituration with MeOH gave **13** (400 mg, 83%) as a dark purple solid, mp 157–158 °C (lit.[12] mp 155.5–156.5 °C).

***trans*-3,4-Dihydroxy-3,4-dihydro-7,12-dimethylbenz[*a*]anthracene (3).** To a partial solution of **13** (200 mg, 0.7 mmol) in 300 mL of absolute ethanol was added granular $NaBH_4$ (700 mg). Immediately the solution turned from dark blue to yellow. Oxygen was bubbled through the solution and the flask was covered with aluminum foil to exclude light. The reaction was monitored by TLC on silica gel with CH_2Cl_2–EtOAc (1:1); HPLC was less satisfactory due to the low absorbance of the quinone in the UV at 254 nm. While the time for completion varied from 16–72 h, the best yields were obtained with the 72-h reaction time. The workup procedure was conducted in the dark or using yellow light to avoid photooxidation. The reaction mixture was diluted

with ether (400 mL) and the ether layer was extracted with water 2–3 times. The water wash was back-extracted with ether twice, the combined ether extracts were washed with water, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness, and the residue was triturated with ether to yield the dihydrodiol **3** (191 mg, 94%) as a bright yellow solid: mp 193–194 °C (lit.[3] mp 182–184 °C); NMR (500 MHz in THF-d_6/D_2O) δ 3.02 (3, s, 7-CH_3), 3.10 (3, s, 12-CH_3), 4.51 (1, d, H_3), 4.59 (1, d, H_4), 6.05 (1, d of d, H_2), 6.97 (1, d of d, H_1), 7.41–7.43 (2, m, $H_{9,10}$), 7.77 (1, d, H_5), 8.17 (1, d, H_6), 8.19–8.26 (2, m, $H_{8,11}$), $J_{1,2}$ = 10.1, $J_{1,3}$ = 1.8, $J_{2,3}$ = 2.0, $J_{3,4}$ = 11.3, $J_{5,6}$ = 9.0 Hz; NMR (400 MHz in Me_2SO-d_6) δ 2.99 (3, s, 7-CH_3), 3.02 (3, s, 12-CH_3), 4.51 (1, m, H_3), 4.59 (1, m, H_4), 5.30 (1, d, 4-OH), 5.70 (1, d, 3-OH), 6.10 (1, d, H_2), 6.97 (1, d, H_1), 7.53 (1, m, H_9), 7.56 (1, m, H_{10}), 7.72 (1, d, H_5), 8.21 (1, m, H_{11}), 8.24 (1, d, H_6), 8.31 (1, m, H_8); UV max (THF) 273.1 nm (ε 86 360).

***trans*-3,4-Dihydroxy-3,4-dihydro-7,12-dimethyl-7,12-epidioxybenz[*a*]anthracene (14).** Solutions of **3** in CH_2Cl_2, THF, or Me_2SO were converted essentially quantitatively on standing in the light for 30 min to the 7,12-peroxide **14** obtained as a white solid: mp 199–200 °C; NMR (500 MHz in Me_2SO-d_6) δ 2.02 (3, s, 7-CH_3), 2.20 (3, s, 12-CH_3), 4.17 (1, d of d, H_3), 4.31 (1, d of d, H_4, $J_{3,4}$ = 10.7 Hz), 5.15 (1, d, OH), 5.48 (1, d, OH), 6.03 (1, d of d, H_2, $J_{2,3}$ = 2.0 Hz), 6.95 (1, d of d, H_1, $J_{1,3}$ = 2.2 Hz), 7.28–7.32 (2, m, $H_{9,10}$), 7.34 (1, d, H_5), 7.42 (1, m, H_8), 7.48 (1, d, H_6, $J_{5,6}$ = 7.7 Hz), 7.50 (1, m, H_1); mass spectrum, *m/e* ($C_{20}H_{18}O_4$) parent peak obsd 322.1207, calcd 322.1200, (P – H_2O) 290.1306.

2α-Bromo-1β,3α,4β-trihydroxy-1,2,3,4-tetrahydro-7,12-dimethylbenz[*a*]anthracene (15). To a solution of the dihydrodiol **3** (30 mg, 0.115 mmol) in Me_2SO (10 mL) and water (0.2 mL) was added NBS (21 mg, 0.118 mmol). The solution turned dark blue. The reaction, which was monitored by HPLC [Zorbax Sil, 15 cm × 6.4 mm, THF/hexane (40/60), 3 mL/min], was complete in 30 min. The usual workup afforded crude **15** as a greenish solid (due to contamination with the quinone **7a**). Trituration with cold ether gave **15** (25 mg, 56%) as a white solid: mp 80–81 °C; NMR (500 MHz, Me_2SO-d_6/D_2O) δ 3.00 (3, s, 7-CH_3), 3.31 (3, s, 12-CH_3), 4.35 (1, d, H_3), 4.56 (1, d, H_4, $J_{3,4}$ = 6.7 Hz), 4.59 (1, d of d, H_2, $J_{2,3}$ = 2.5 Hz, $J_{1,2}$ = 4.2 Hz), 5.72 (1, d, H_1), 7.52–7.54 (2, m, $H_{9,10}$), 7.57 (1, d, H_5, $J_{5,6}$ = 9.3 Hz), 8.26 (1, d, H_6), 8.29 (1, m, $H_{8 \text{ or } 11}$), 8.33 (1, m, $H_{8 \text{ or } 11}$); UV max (*p*-dioxan) 273 nm (ε 43 050).

***trans*-3,4-Dihydroxy-*syn*-1,2-epoxy-1,2,3,4-tetrahydro-7,12-dimethylbenz[*a*]anthracene (2b).** To a solution of the bromohydrin (14 mg, 0.036 mmol) in freshly distilled THF (5 mL) was added *t*-BuOK (7 mg, 0.063 mmol) in *t*-BuOH (1 mL). The reaction, which was monitored by HPLC under the same conditions used for **2b**, was complete in 30 min. The mixture was diluted with ether and the organic layer was washed with water 3× and dried over anhydrous Na_2SO_4 for 30 min at 0 °C. The solution was filtered through glass wool, and the solvent was evaporated at 0 °C using a dry ice condenser. Trituration of the product with cold ether afforded **2b** (9 mg, 90%) as a white solid: NMR (500 MHz, Me_2SO-d_6/D_2O) δ 3.01 (3, s, 7-CH_3), 3.28 (3, s, 12-CH_3), 3.78 (2, m, $H_{2,3}$), 4.33 (1, d, H_1, $J_{1,2}$ = 4.3), 4.60 (1, d, H_4, $J_{3,4}$ = 8.1 Hz), 7.54–7.57 (2, m, $H_{9,10}$), 7.69 (1, d, H_5, $J_{5,6}$ = 9.5 Hz), 8.31–8.34 (2, m, $H_{8,11}$), 8.35 (1, d, H_6); UV max (THF) 269.2 nm (ε 42 076).

1α-(*tert*-Butylthio)-2β,3α,4β-trihydroxy-1,2,3,4-tetrahydro-7,12-dimethylbenz[*a*]anthracene (16). To a solution of the bromotriol **15** (25 mg, 0.065 mmol) in dry THF (5 mL) was added *t*-BuOK (11.2 mg, 0.1 mmol) in 1 mL of *t*-BuOH, and the solution was stirred at room temperature under N_2. Conversion to **2b** was complete within 30 min (shown by HPLC). To this solution was added *t*-BuOK (22.4 mg, 0.2 mmol) in 1 mL of *t*-BuOH followed by *t*-BuSH (13.5 mg, 0.15 mmol). HPLC showed gradual disappearance of the epoxide peak and appearance of a new peak with longer retention time. The solution was stirred overnight at room temperature and worked up conventionally. Trituration of the product with cold ether gave **16** (7 mg, 28%) as a yellw solid: mp 180–181 °C; NMR (500 MHz, Me_2SO-d_6/D_2O) δ 1.19 (9, s, Me_3C), 3.01 (3, s, 7-CH_3), 3.33 (3, s, 12-CH_3), H_3 hidden in H_2O peak, 4.36 (1, d, H_2, $J_{2,3}$ = 3.8 Hz), 4.89 (1, d, H_4, $J_{3,4}$ = 9.4 Hz), 5.12 (1, apparent s, H_1), 7.52–7.54 (2, m, $H_{9,10}$), 7.71 (1, d, H_5, $J_{5,6}$ = 9.1 Hz), 8.19 (1, d, H_6), 8.28 (2, m, $H_{8,11}$).

***trans*-3,4-Dihydroxy-*anti*-1,2-epoxy-1,2,3,4-tetrahydro-DMBA (2a) and 1α-*tert*-Butyl-2β,3β,4α-trihydroxy-1,2,3,4-tetrahydro-DMBA (17).** A solution of the dihydrodiol **3** (30 mg, 0.103 mmol) and *m*-chloroperbenzoic acid (300 mg) in freshly distilled THF (30 mL) was stirred at ambient temperature under N_2. Conversion of **3** to the anti diol epoxide **2a** was complete in 30 min (by HPLC). The solution was diluted with cold ether and the ether layer was dried over anhydrous Na_2SO_4 for 30 min in an ice bath. The solution was decanted through a glass wool plugged funnel and washed in with cold dry THF. Attempts to isolate **2a** directly in several runs were unsuccessful due to its instability. An excess of *t*-BuOK (100 mg) was added to the combined solution at 0 °C to remove residual traces of water, then *tert*-butylmercaptan (0.10 mL) was added and the solution was allowed to stand at room temperature overnight. After the usual workup, the residue was triturated with cold ether to yield **17** (6 mg, 15%) as a yellow solid: mp 170–171 °C; NMR (500 MHz, Me_2SO-d_6/D_2O) δ 1.16 (9, s, Me_3C), 2.97 (3, s, 7-CH_3), 3.31 (3, s, 12-CH_3), 4.23 (1, d of d, H_3, $J_{2,3}$ = 2.5 Hz), 4.29 (1, m, H_2), 4.69 (1, d, H_4), 5.17 (1, d, H_1, $J_{1,2}$ = 3.0 Hz), 7.52 (2, m, $H_{9,10}$), 7.61 (1, d, H_5, $J_{5,6}$ = 9.2 Hz), 8.04 (1, d, H_6), 8.21 (1, m, $H_{8 \text{ or } 11}$), 8.26 (1, m, $H_{8 \text{ or } 11}$). Compound **2a** exhibited UV max (THF) 268.1 nm.

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Registry No. **1a**, 57-97-6; **1b**, 57266-83-8; **1c**, 66240-02-6; **2a**, 86941-58-4; **2b**, 86941-59-5; **3**, 68162-13-0; **4**, 71964-75-5; **5**, 94305-64-3; **6**, 66240-12-8; **7a**, 63216-11-5; **7b**, 60549-33-9; **8a**, 103500-28-3; **8b**, 103500-33-0; **9**, 66240-01-5; **10**, 71964-76-6; **11a**, 71964-77-7; **11b**, 71974-97-5; **12**, 103500-29-4; **13**, 70092-13-6; **14**, 103500-30-7; **15**, 103500-31-8; **16**, 103500-32-9; **17**, 103532-47-4; 2-bromo-6-methoxynaphthalene, 5111-65-9; phthalic anhydride, 85-44-9.