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SYNTHESES OF "UNSTABLE" DIOL EPOXIDE METABOLITES OF THE POTENT CARCINOGENS 7- AND 12-METHYLBENZ[*a*]ANTHRACENE

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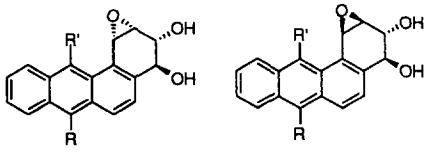
Abstract: Stereospecific syntheses of the *anti*- and *syn*-diol epoxides of 7- and 12-methylbenz[*a*]anthracene, suspected as active metabolites of the parent PAHs but previously thought to be too chemically reactive and unstable to isolate, are described and the pure compounds are shown to be moderately stable.

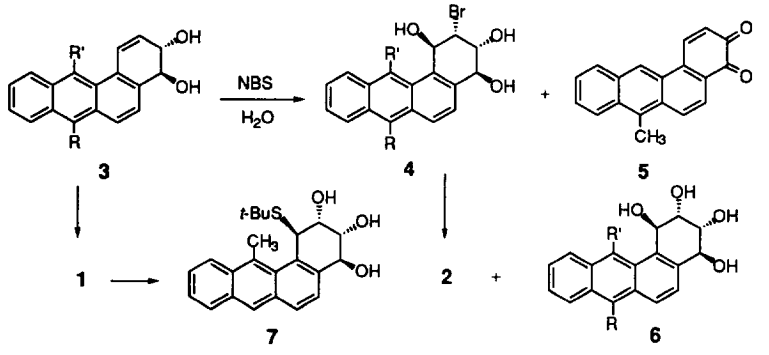
The 7-12-methyl-substituted derivatives of benz[*a*]anthracene (7-MBA, 12-MBA, and DMBA) are among the most potent known carcinogenic polycyclic aromatic hydrocarbons.[1] They are activated by microsomal enzymes to highly tumorigenic diol epoxide metabolites that bind covalently to DNA.[1,2] The DMBA *syn*-diol epoxide (**2c**) has been shown to bind selectively to deoxyadenosine residues in DNA,[3] and mutagenesis studies show that DMBA induces AT → TA transversion mutations in the 61st codon of the H-*ras* oncogene.[4,5] However, chemical and biological studies have been hampered by the unavailability of some diol epoxide isomers due to their apparent exceptional reactivity and instability. While syntheses of the *anti*- and *syn*-diol epoxides of DMBA[6,7] (**1c** and **2c**) and the *anti*-diol epoxide of 7-MBA[8] (**1a**) were reported, attempted syntheses of the *syn*-diol epoxide of 7-MBA (**2a**)[9] and the *anti*- and *syn*-diol epoxides of 12-MBA (**1b** and **2b**) have been frustrated by their apparent facility of decomposition.[10]



1 *anti* **2** *syn*

a: R = CH_3 ; R' = H; **b**: R = H; R' = CH_3; **c**: R = R' = CH_3

We now report efficient syntheses and complete characterization of the diol epoxides **1b**, **2a**, and **2b**. While the synthetic approach to these diol epoxides is similar to that devised earlier for the preparation of other diol epoxide isomers, its success with these reactive PAH diol epoxides was found to be highly dependent upon the purity of the dihydrodiol precursors and the diol epoxide products. The most significant modification of the purification procedures used earlier was flash chromatography of the dihydrodiols and diol epoxides through a short silica gel column deactivated with THF:hexane:Et_3N (15:15:1) and eluted with the same solvent.[7,11] Transformation of the dihydrodiol **3a** to **2a** was accomplished via reaction with *N*-bromosuccinimide[12] (1.1 equiv) in moist Me_2SO (5%) to form the bromotriol **4a**. On addition of NBS to the degassed solution of **3a**, it turned yellow immediately, and reaction was complete in 45 min.[13] The resulting yellow-orange mixture was poured into ice-water and worked up to provide pure **4a** (69%) and a lesser amount of quinone **5** (12%).[14]



a: R = CH_3 ; R' = H; **b**: R = H; R' = CH_3

Quinones were not previously detected as products in these types of reactions. Optimum yield of **4a** was obtained from reactions on 30-40 mg scale. Larger scale led to increase in the ratio of **5** and tarry products.

Initial attempts to convert **4a** to **2a** with *t*-BuOK in THF-*t*-BuOH under conditions previously found effective for the synthesis of DMBA *syn*-diol epoxide (**2c**) failed.[8] However, **4a** was efficiently transformed to **2a** by treatment of its solution in THF with preactivated Amberlite®-IRA 400 under argon in subdued light.[15] It was found subsequently that the *t*-BuOK method could be employed, if the crude product was worked up cold and rapidly purified by flash chromatography through a deactivated silica gel column as described above. The *syn*-diol epoxide **2b** purified by this means was readily characterized by NMR, UV, MS, etc.[16] and shown to be free of tetraols formed by hydrolysis. Moreover, pure **2b** was found to be approximately as stable as **2c**, surviving for weeks on storage in the cold. It is suspected that trace impurities removable by flash chromatography, such as tetraols or peroxides, catalyze decomposition of this and other diol epoxides.

The *syn*-diol epoxide of 12-MBA (**2b**) was synthesized from the pure 3,4-dihydrodiol of 12-methylbenz[*a*]anthracene (**3b**)[8] via the corresponding bromotriol (**4b**) by an analogous procedure.[17] Compounds **4b** and **2b** were obtained in 69% and 64% yields, respectively, from reactions conducted on 30 mg scale. Attempted preparation of **4b** on larger scale (100 mg of **3b**) gave a 50% decrease in yield.

The *anti*-diol epoxide of 12-MBA (**1b**) was synthesized from **3b** by reaction with *m*-chloroperbenzoic acid by the procedure described for the synthesis of the DMBA *anti*-diol epoxide (**1c**) modified by purification of **3b** and **1b** by flash chromatography as described above.[6] The diol epoxides **1b** and **2b** were obtained pure, free of tetraols, and sufficiently stable to characterize by NMR, MS, etc.[17,18] The structure of **1b** was further confirmed by its reaction with *t*-BuSH to form adduct **7**.[19] Like **2a**, **1b** and **2b** are moderately stable and can be stored for weeks in the cold without significant decomposition.

These diol epoxide metabolites, previously unavailable or considered too unstable for most studies, are now readily available for DNA binding studies and a wide range of biological investigations.

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References

1. (a) *Review:* Harvey, R. G. *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity,* Cambridge University: Cambridge, England, 1991, Chaps. 3 and 4. (b) Huggins, C. B.; Pataki, J.; Harvey, R. G. *Proc. Natl. Acad. Sci. USA* **1967**, *58*, 2253. (c) Harvey, R. G.; Dunne, F. B. *Nature (London)* **1978**, *273*, 566.

2. Harvey, R. G.; Geacintov, N. E. *Acc. Chem. Res.* **1988**, *21*, 66.

3. Cheng, S. C.; Prakash, A. S.; Pigott, M. A.; Hilton, B. D.; Lee, H.; Harvey, R. G.; Dipple, A. *Carcinogenesis* **1988**, *9*, 1721.

4. Bizub, D.; Wood, A. W.; Skalka, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6048. Dandekar, S.; Sukumar, S.; Zarbl, H.; Young, L.; Cardiff, R. D. *Mol. Cell Biol.* **1986**, 4104.

5. Chen, J. X.; Pao, A.; Zheng, Y.; Ye, X.; Kiselyov, A. S.; Morris, R.; Slaga, T. J.; Harvey, R. G.; and Tang, M.-S. *Biochemistry* **1996**, *35*, 9594.

6. Lee, H.; Harvey, R. G. *J. Org. Chem.* **1986**, *51*, 3502.

7. Koreeda, M.; Gopalaswamy, R. *J. Am. Chem. Soc.* **1995**, *117*, 10595-96.

8. (a) Lee, H.; Harvey, R. G. *J. Org. Chem.* **1979**, *44*, 4948. (b) Harvey, R. G.; Cortez, C.; Sugiyama, T.; Ito, Y.; Sawyer, T. W. *J. Medic. Chem.* **1988**, *31*, 154.

9. The *anti* diol epoxide isomer has the epoxide function on the face opposite the benzylic hydroxyl group, and the *syn* isomer has these groups on the same face.

10. All diol epoxides are susceptible to decomposition on heating or exposure to mild acids. Diol epoxides and dihydrodiols that contain a reactive meso region, such as the 7- and 12-MBAs and DMBA derivatives, are also susceptible to formation of 7,12-epidioxides on exposure to air and light.[6] Therefore, it is essential that all reactions be conducted in an inert atmosphere in subdued light.

11. Purification of **3a** was most efficiently accomplished by formaion of the diacetate or dibenzoate ester, chromatography on Florisil®, reconversion to the dihydrodiol by reaction with NaOMe in refluxing MeOH, and flash chromatography as described. The ^{1}H NMR spectrum of **3a** purified by this method matched that reported previously[6] and indicated the absence of impurities; TLC on silica gel and HPLC on a Zorbax Sil column eluted with MeOH:H_2O (25:1) further confirmed its essential purity.

12. NBS was freshly recrystallized from water and dried prior to use.

13. Reaction was monitored by HPLC on a Zorbax ODS column eluted with MeOH:H_2O (20:1) and TLC on silica gel eluted with Et_2O:EtOAc (5:1).

14. The reaction mixture was poured into 50 mL of ice-water and extracted with 5 x 25 mL of Et_2O-THF (4:1). The combined extracts were washed with 3x25 mL of ice-cold brine, passed through a short plug of Na_2SO_4, concentrated at 5-10 °C (bath temperature) to give a yellow oily residue. Treatment of this with 3 x 2 mL of cold Et_2O gave a white precipitate of bromotriol **4a** (69%) which was sufficiently pure to be used directly in the next step. Concentration of the Et_2O extract gave quinone **5**, mp 200-202 °C, lit.[8b] 202-203 °C (12%). Further purification of **4a** was achieved by passing its solution through a short plug of silica gel, eluted with EtOAc:THF, 6:1, however, the overall yield was decreased by 20-25%. Prior to its use in the next step, **4a** was dried for 24 h under high vacuum at room temperature. Data for **4a**: mp 85-86 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.02 (s, 3, CH_3), 4.32 (d, 1, H_3, J = 6.5 Hz), 4.50 (d, 1, H_4, J = 6.5 Hz), 4.63 (dd, 1, H_2, J = 4.5 Hz, J = 2.5 Hz), 5.53 (d, 1, H_1, J = 4.5 Hz), 7.53-7.56 (m, 2, $H_{9,10}$), 7.61 (d, 1, H_5, J = 9.0 Hz), 8.13 (d, 1, H_6, J

= 9.0 Hz), 8.29 (d, 1, H_8, J = 9.0 Hz), 8.40 (d, 1, H_{11}, J = 9.0 Hz), 8.79 (s, 1, H_{12}); UV (MeOH) λ_{max} 202 (ε = 31 300), 259 (63 400) nm. Anal. calcd for $C_{19}H_{17}BrO_3$: C, 61.14; H, 4.59; Br, 21.41. Found: C, 61.26; H, 4.67; Br, 21.30.

15. This procedure is similar to one employed in other syntheses e.g. Bushman, D. R.; Grossman, S. J.; Jerina, D. M.; Lehr, R. E. *J. Org. Chem.* **1989**, *54*, 3533. Amberlite®-IRA 400 (5 g) was activated by washing with 5x50 mL of 30% KOH followed by 10x50 mL of distilled water until neutral pH, 2 x 25 mL of THF, 2 x 25 mL of dry THF, 3x25 mL of Et_2O, and dried under high vacuum at 30 °C. Elimination of traces of moisture in the diol epoxide product is essential.

16. Data for **2a**: mp 177-178 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.02 (s, 3, CH_3), 4.62 (d, 1, H_3, J = 6.5 Hz), 4.73 (d, 1, H_4, J = 6.5 Hz), 5.21 (dd, 1, H_2, J = 4.5 Hz, J = 2.5 Hz), 5.63 (d, 1, H_1, J = 4.5 Hz), 7.53-7.56 (m, 2, $H_{9,10}$), 7.63 (d, 1, H_5, J = 9.0 Hz), 8.16 (d, 1, H_6, J = 9.0 Hz), 8.30 (d, 1, H_8, J = 9.0 Hz), 8.39 (d, 1, H_{11}, J = 9.0 Hz), 8.85 (s, 1, H_{12}); UV (MeOH) λ_{max} 204 (ε = 14 100), 263 (116 800) nm; HRMS: Calcd for $C_{19}H_{16}O_3$: 292.1099. Found: 292.1092.

17. Reaction times were 1 h each for the synthesis of **4b** and its conversion to **2b** by the *t*-BuOK method. Data for **4b**: mp 110-11 °C; ^{1}H NMR (500 MHz, DMSO-d_6+D_2O) δ 3.32 (s, 3, CH_3), 4.35 (d, 1, H_3, J = 6.0 Hz), 4.52 (d, 1, H_4, J = 6.0 Hz), 4.57 (dd, 1, H_2, J = 4.5 Hz, J = 2.5 Hz), 5.73 (d, 1, H_1, J = 4.5 Hz), 7.50-7.54 (m, 2, $H_{9,10}$), 7.59 (d, 1, H_5, J = 9.0 Hz), 7.96 (d, 1, H_6, J = 9.0 Hz), 8.07 (d, 1, $H_{8 \text{ or } 11}$, J = 9.0 Hz), 8.32 (d, 1, $H_{8 \text{ or } 11}$, J = 9.0 Hz), 8.35 (s, 1, H_7); UV (MeOH) λ_{max} 204 (ε = 30 600), 260 (112 100) nm; HRMS: Calcd for $C_{19}H_{17}BrO_3$ (^{79}Br): 372.0362. Found: 372.0355. Data for **2b**: mp 156-157 °C; ^{1}H NMR (500 MHz, DMSO-d_6+D_2O) δ 3.28 (s, 3, CH_3), 3.76 (m, 1, $H_{8 \text{ or } 11}$), 3.84 (m, 1, $H_{8 \text{ or } 11}$), 4.34 (d, 1, H_1, J = 5.0 Hz), 4.67 (d, 1, H_4, J = 9.0 Hz), 7.44-7.50 (m, 2, $H_{9,10}$), 7.76 (d, 1, H_5, J = 8.5 Hz), 7.96-8.07 (m, 2, $H_{8,11}$), 8.33 (s, 1, H_7), 8.36 (d, 1, H_6); UV (EtOH) λ_{max} 222 (ε = 11 400), 262 (114 400) nm.

18. Data for **1b**: mp 133-135 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.28 (s, 3, CH_3), 3.69 (m, 1, H_2), 3.75 (m, 1, H_3), 4.61 (m, 1, H_1), 4.74 (m, 1, H_4), 7.47-7.50 (m, 2, $H_{9,10}$), 7.86 (d, 1, H_5, J = 9.0 Hz), 8.24-8.28 (m, 4, $H_{6,7,8,11}$); UV (Et.OH) λ_{max} 225 (ε = 10 500), 263 (115 600) nm.

19. HPLC on Chiralcel OB eluted with *i*-PrOH:hexanes (30:70) indicated reaction to be complete in 35 min. Data for **7**: mp 158-159 °C; ^{1}H NMR (500 MHz, DMSO-d_6+D_2O) δ 1.12 (s, 9, CH_3), 3.28 (s, 3, CH_3), 4.26 (d, 1, H_3, J = 2.5 Hz), 4.34 (m, 1, H_2), 4.67 (d, 1, H_4, J = 3.5 Hz), 5.13 (d, 1, H_1, J = 3.5 Hz), 7.52-7.58 (m, 2, $H_{9,10}$), 7.63 (d, 1, H_5, J = 9.0 Hz), 8.09 (d, 1, H_6, J = 9.0 Hz), 8.27 (d, 1, H_8, J = 9.0 Hz), 8.34 (d, 1, H_{11}, J = 9.0 Hz), 8.65 (s, 1, H_7); UV (THF) λ_{max} 262 nm. Anal. calcd for $C_{23}H_{26}SO_3$: C, 72.22; H, 6.85; S, 8.38. Found: C, 72.42; H, 6.79; S, 8.29.

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