Synthesis of the Biologically Reactive Bay-Region Diol Epoxide of the Mutagenic Environmental Contaminant 1-Nitrobenzo[a]pyrene

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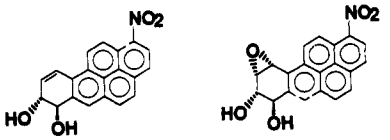
Received April 16, 1993

Introduction

Nitropolycyclic aromatic hydrocarbons (nitro-PAHs) are genotoxic environment contaminants, which require metabolic activation in order to exert their biological activities, including mutagenicity and tumorigenicity.[1] Among the nitro-PAHs studied so far for carcinogenic properties, 1-, 3-, and 6-nitrobenzo[a]pyrenes (nitro-BaP) are the nitro-PAHs derived from a potent carcinogenic PAH, such as benzo[a]pyrene (BaP). Both 1- and 3-nitro-BaP are environmental contaminants and are potent mutagens in the *Salmonella typhimurium* reversion assay with and without exogenous S9 activation.[2-9] However, while 3-nitro-BaP is also a potent mutagen in the Chinese Hamster Ovary mammalian mutagenicity assay, 1-nitro-BaP exhibits very weak activity.[6,8] It was also found that hepatic microsomal metabolism of 1-nitro-BaP generates *trans*-7,8-dihydroxy-7,8-dihydro-1-nitrobenzo[a]pyrene (1) as the predominant metabolite and 1-nitro-BaP

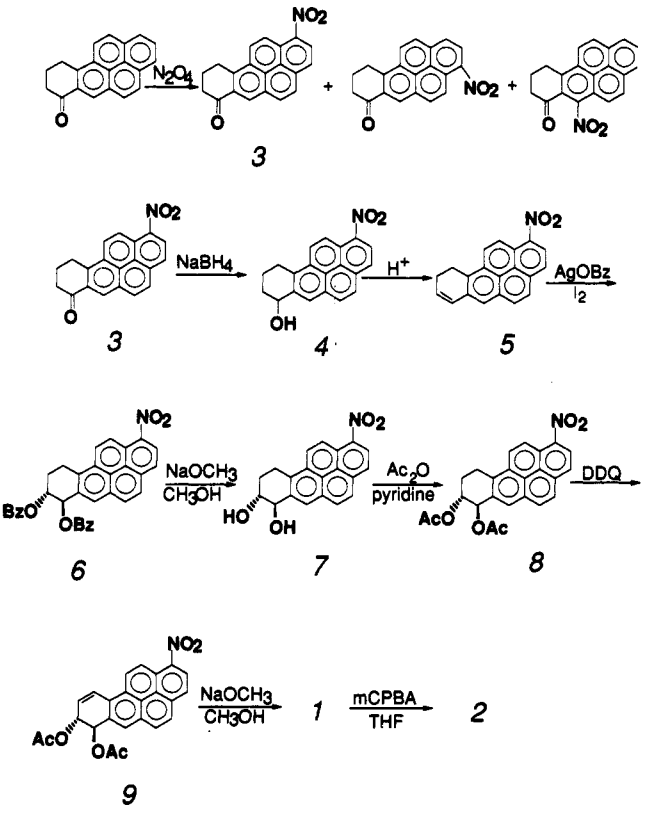


1 2

7,8,9,10-tetrahydrotetraol as a minor metabolite.[10,11] 1-Nitro-BaP *trans*-7,8-dihydrodiol is a potent mutagen in *S. typhimurium* TA98 with and without S9 activation.[10,11] 1-Nitro-BaP 7,8,9,10-tetrahydrotetraol was also formed from further metabolism of 1-nitro-BaP *trans*-7,8-dihydrodiol.[11] This tetrahydrotetraol, with a *trans–cis–trans* configuration,[10] is presumably formed from nonenzymatic hydrolysis of the diol epoxide metabolite, 1-nitro-BaP *trans*-7,8-diol *anti*-9,10-epoxide. These results suggest that *trans*-7,8-dihydroxy-7,8-dihydro-1-nitrobenzo[a]pyrene and 9,10-epoxy-*trans*-7,8-dihydroxy-7,8-dihydro-1-nitrobenzo[a]pyrene (2), respectively, are the proximate and ultimate mutagenic metabolites of 1-nitro-BaP.[10,11] In this paper, we report a convenient route for the synthesis of 1 and 2 which will permit the study of their biological activity, including DNA adduct formation and tumorigenicity. This represents the first time that a *bay*-region diol epoxide of a nitro-PAH has been prepared by total organic synthesis.

Results and Discussion

The preparation of 1 and 2 started with nitration of 9,10-dihydrobenzo[a]pyren-7(8*H*)-one by N_2O_4 in methylene chloride. The desired product, 1-nitro-9,10-dihydrobenzo[a]pyren-7(8*H*)-one (3), was separated from the other mononitro isomers by column chromatography over silica gel. The nitrating agent, N_2O_4, was found to be better than fuming nitric acid in a strong acid[12-16] or sodium



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nitrate in acetic anhydride and trifluoroacetic acid,[17,18] both of which generated dinitrated byproducts that caused difficulty in purification. Conversion of **3** to **1** and **2** was then accomplished *via* modification of the general synthetic route for the synthesis of BaP *trans*-7,8-dihydrodiol and BaP *trans*-7,8-diol *anti*-9,10-epoxide.[19,20] Reduction of **3** with sodium borohydride generated 7-hydroxy-7,8,9,-10-tetrahydro-1-nitrobenzo[*a*]pyrene (**4**) in 96% yield. Upon acid-catalyzed dehydration, **4** was converted to 9,-10-dihydro-1-nitrobenzo[*a*]pyrene (**5**). Conversion of **5** into 7,8-dihydroxy-7,8,9,10-tetrahydro-1-nitrobenzo[*a*]pyrene *trans*-7,8-dibenzoate (**6**) was accomplished by carrying out the Prevost reaction with silver benzoate and iodine. Attempts to convert **6** into 1-nitro-BaP *trans*-7,8-dibenzoate by dehydrogenation with 2,3-dichloro-5,6-dicyano-1,6-benzoquinone (DDQ) in refluxing benzene or dioxane failed. This contrasts the ease of dehydrogenation of the analog, BaP 7,8,9,10-tetrahydro-*trans*-7,8-dibenzoate, to the BaP *trans*-7,8-dibenzoate by DDQ under similar conditions.[19,20] The failure is apparently due to steric hindrance caused by the bulky benzoate groups and the electron-withdrawing effect of the nitro group on hydride abstraction from **6** by DDQ.[21] As an alternative, **6** was converted to *trans*-7,8-dihydroxy-7,8,9,10-tetrahydro-1-nitrobenzo[*a*]pyrene (**7**), followed by acetylation to form the 7,8-dihydro-7,8,9,10-tetrahydro-1-nitrobenzo[*a*]pyrene *trans*-7,8-diacetate (**8**). Dehydrogenation of **8** with DDQ in refluxing dioxane afforded the desired compound, 7,8-dihydroxy-7,8-dihydro-1-nitrobenzo[*a*]pyrene *trans*-7,8-diacetate (**9**), in 60% yield. Upon methanolysis with sodium methoxide in methanol and THF, 7,8-dihydroxy-7,8-dihydro-1-nitrobenzo[*a*]pyrene (**1**) was obtained in 92% yield. Synthesis of the reactive *trans*-9,10-epoxy-7,8-dihydroxy-7,8-dihydro-1-nitrobenzo[*a*]pyrene (**2**) was attempted with several different conditions. The highest yield was obtained using a 30-fold excess of *m*-chloroperbenzoic acid at ice–water temperature under N_2 for 3 h. The reaction mixture was partitioned between ethyl acetate and dilute NaOH, and the organic layer was washed with water (all being conducted as quick as possible at ice–water temperature) to afford **2** in 80% yield.

The nitro orientation of nitro-PAHs has been found to be a critical structural feature in determining the biological activities of these compounds.[1g,22,23] Analysis of UV–vis absorption and high-resolution proton NMR spectral data indicates that both **1** and **2** have their nitro groups coplanar or nearly coplanar to the aromatic moiety.[18] We have also demonstrated that **1** is a potent mutagen when tested in *S. typhimurium* TA98 in the absence of S9 activation system and that the mutagenicity decreased substantially in the nitroreductase-deficient strain TA98NR.[11] These results indicate the involvement of the nitro functional group on the metabolic activation.[11] Preliminary data have shown that diol epoxide **2** binds to cellular DNA *via* its epoxy ring to form an N^2-deoxyguanosine adduct (unpublished data). Thus, it is likely that **1** can be metabolically activated by both the nitroreduction and ring-oxidation pathways.

Experimental Section

Materials. 9,10-Dihydrobenzo[*a*]pyren-7(8*H*)-one, silver benzoate, and DDQ were purchased from Aldrich Chemical Co. (Milwaukee, WI). Dinitrogen tetraoxide was purchased from the Matheson Division of Searle Medical Products (E. Rutherford, NJ). All the mass spectra were performed with a solid probe by electron impact at 70 eV. UV–vis absorption spectra were obtained in methanol. Proton NMR spectra were obtained in acetone-d_6 or $CDCl_3$, and the chemical shifts are reported in ppm downfield from TMS.

1-Nitro-9,10-dihydrobenzo[*a*]pyren-7(8*H*)-one (3). SAFETY NOTE: Dinitrogen tetraoxide (N_2O_4) should only be used in a well vented hood, and flammable solvents should be avoided. Dinitrogen tetraoxide (N_2O_4) was passed into a preweighed stoppered Erlenmeyer flask containing 100 mL of CH_2Cl_2 until saturated. The stoppered flask was weighed to determine the grams of N_2O_4 per milliliter of CH_2Cl_2 (0.3 mg per mL). To 540 mg (2.0 mmol) of 9,10-dihydrobenzo[*a*]pyren-7(8*H*)-one in 100 mL of CH_2Cl_2 was added slowly a 10% molar excess of the N_2O_4 solution. The reaction mixture was monitored for the disappearance of the starting material by silica gel TLC eluting with CH_2Cl_2/hexane (v/v, 1/1). After nitration was complete, the excess N_2O_4 was removed by bubbling argon through the reaction mixture, and the resulting residue was purified by column chromatography on silica gel. Elution with hexane–ethyl acetate (v/v, 5/1) afforded **3** (258 mg, 41% yield): mp 241–242 °C; MS (relative intensity), *m/z* 315 (M^+, 76), 287 ($[M-CO]^+$, 100), 269 ($[M-NO_2]^+$, 12); UV–vis spectrum, λ_{max} (log ϵ_{max}) 388 (4.03), 267 (4.58), 202 (4.33) nm; NMR ($CDCl_3$) δ 2.46 (m, 2H, H_9), 2.93 (t, 2H, $J_{8,9}$ = 6.3 Hz, H_8), 3.72 (t, 2H, $J_{9,10}$ = 5.6 Hz, H_{10}), 8.03 (d, 1H, $J_{4,5}$ = 9.0 Hz, H_5), 8.15 (d, 1H, $J_{2,3}$ = 8.7 Hz, H_3), 8.23 (d, 1H, $J_{4,5}$ = 9.0 Hz, H_4), 8.56 (d, 1H, $J_{11,12}$ = 9.6 Hz, H_{12}), 8.70 (d, 1H, $J_{2,3}$ = 8.7 Hz, H_2), 8.97 (s, 1H, H_6), 8.98 (d, 1H, $J_{11,12}$ = 9.6 Hz, H_{11}). Anal. Calcd for $C_{20}H_{13}NO_3$: C, 76.18; H, 4.16; H, 4.44. Found: C, 76.42; H, 4.05; N, 4.39.

Further elution with the same solvent gave a mixture of presumably the 3- and 6-mononitro isomers (284 mg, 45% yield).

7-Hydroxy-7,8,9,10-tetrahydro-1-nitrobenzo[*a*]pyrene (4). Compound **3** (180 mg) in THF (40 mL) was reduced with excess sodium borohydride (159 mg) in methanol (45 mL) at ambient temperature for 40 min. The reaction product was poured into ice–water and neutralized with 1 N HCl, affording the alcohol **4** (173 mg, 96% yield): mp 183–184 °C; MS *m/z* 317 (M^+); UV–vis spectrum, λ_{max} (log ϵ_{max}) 398 (4.65), 290 (4.18), 239 (4.70) nm; NMR (acetone-d_6) δ 1.85–2.28 (m, 4H, $H_{8,9}$), 3.6–3.8 (m, 2H, H_{10}), 5.08–5.30 (m, 1H, H_7), and 7.26–8.77 (m, 7H, aromatic). Anal. Calcd for $C_{20}H_{15}NO_3$: C, 75.69; H, 4.77; N, 4.41. Found: C, 75.87; H, 4.84; N, 4.47.

9,10-Dihydro-1-nitrobenzo[*a*]pyrene (5). Compound **4** (140 mg) underwent dehydration on refluxing benzene (50 mL) catalyzed by 20 mg of *p*-toluenesulfonic acid for 100 min. After extraction with ethyl acetate and removal of solvent under reduced pressure, **5** was obtained as a yellowish solid (135 mg, 98% yield): mp 218–219 °C; MS *m/z* 299 (M^+); UV–vis spectrum, λ_{max} (log ϵ_{max}) 390 (4.00), 265 (4.46), 203 (4.11) nm; NMR (acetone-d_6) δ 2.48 (m, 2H, H_9), 3.48 (t, 2H, H_{10}), 6.18 (dd, 1H, $J_{7,8}$ = 9.6 Hz, $J_{8,9}$ = 4.2 Hz, H_8), 6.87 (dd, 1H, $J_{7,8}$ = 9.6 Hz, $J_{7,9}$ = 1.8 Hz, H_7), and 7.80–8.45 (m, 7H, aromatic). Anal. Calcd for $C_{20}H_{13}NO_2$: C, 80.25; H, 4.38; N, 4.68. Found: C, 80.38; H, 4.25; N, 4.39.

7,8,9,10-Tetrahydro-1-nitrobenzo[*a*]pyrene *trans*-7,8-Dibenzoate (6). A solution of **5** (120 mg, 0.4 mmol) in 40 mL of benzene was added to a solution of silver benzoate (1.2 mmol) and iodine (0.6 mmol) in 20 mL of benzene that had been previously refluxed for 30 min. The resulting solution was heated at reflux for 18 h under nitrogen. The precipitate was removed by filtration through Celite and washed with 30 mL of benzene. The filtrate was partitioned between ethyl acetate and dilute aqueous NaOH. The organic fraction was washed with cold water

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and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the crude product was chromatographed on a silica gel column. Elution with hexane–ethyl acetate (v/v, 4/1) gave **6** as a yellowish solid (130 mg, 59% yield): mp 258–259 °C; MS *m/z* 541 (M^+); UV–vis spectrum, λ_{max} (log ϵ_{max}) 406 (4.04), 380 (4.05), 283 (4.11) 240 (4.56), 202 (4.49) nm; NMR (acetone-d_6) δ 2.60–2.80 (m, 2H, H_9), 3.60–3.82 (m, 2H, H_{10}), 6.10 (m, 1H, H_8), 7.24 (d, 1H, $J_{7,8}$ = 7.2 Hz, H_7), and 7.30–8.35 (m, 17H, aromatic). Anal. Calcd for $C_{34}H_{23}NO_6$: C, 75.40; H, 4.28; N, 2.59. Found: C, 75.51, H, 4.41; N, 2.68.

7,8-Dihydroxy-7,8,9,10-tetrahydro-1-nitrobenzo[*a*]pyrene (7). A solution of **6** (120 mg, 0.22 mmol) in 15 mL of THF and $NaOCH_3$ (27 mg, 0.5 mmol) in 10 mL of methanol was stirred at 65 °C for 30 min. The reaction mixture was partitioned between ethyl acetate and water. The organic fraction was collected and dried over $MgSO_4$, and solvent was removed under reduced pressure, affording **7** as yellow solid (66 mg, 90% yield): mp 204–205 °C; mass spectrum *m/z* 333 (M^+, 53), 315 ($[M - H_2O]^+$, 38), 298 ($[M - H_2O - OH]^+$, 17), 285 ($[M - H_2O - NO]^+$, 48), 269 ($[M - H_2O - NO_2]^+$, 35) and 213 (100); UV–vis spectrum, λ_{max} (log ϵ_{max}) 411 (3.85), 383 (3.81), 291 (3.90), 240 (4.44), 203 (4.30) nm; NMR (acetone-d_6) δ 2.60–2.80 (m, 2H, H_9), 3.60–3.82 (m, 2H, H_{10}), 6.10 (m, 1H, H_8), 7.24 (d, 1H, $J_{7,8}$ = 7.2 Hz, H_7), and 7.30–8.35 (m, 17H, aromatic). Anal. Calcd for $C_{20}H_{15}NO_4$: C, 72.06; H, 4.54; N, 4.20. Found: C, 72.20; H, 4.66; N, 4.38.

7,8-Dihydroxy-7,8,9,10-tetrahydro-1-nitrobenzo[*a*]pyrene *trans*-7,8-Diacetate (8). Compound **7** (60 mg, 0.18 mmol) was acetylated by stirring with 10 mL of acetic anhydride and 1 mL of pyridine at ambient temperature overnight. After the reaction mixture was extracted with ethyl acetate and conventional workup, the residue was chromatographed over silica gel. Elution with hexane–ethyl acetate (v/v, 4/1) gave **8** as a light yellow solid in a 91% yield (68 mg, 0.16 mmol); mp 238–239 °C; mass spectrum *m/z* 417 (M^+, 18), 387 ($[M - NO]^+$, 5), 355 ($[M - CH_3CO_2H - H_2]^+$, 28), 325 ($[M - CH_3CO_2H - H_2 - NO]^+$, 4), 313 ($[M - CH_3CO_2H - H_2 - CH{=}C{=}O]^+$, 85), and 297 ($[M - 2CH_3CO_2H]^+$, 100); UV–vis spectrum, λ_{max} (log ϵ_{max}) 407 (4.03), 380 (4.00), 290 (4.07), 240 (4.62), 202 (4.27) nm; NMR ($CDCl_3$) δ 2.02 (s, 3H, OAc), 2.60–2.80 (m, 2H, H_9), 3.60–3.82 (m, 2H, H_{10}), 6.10 (m, 1H, H_8), 7.24 (d, 1H, $J_{7,8}$ = 7.2 Hz, H_7), and 7.30–8.35 (m, 17H, aromatic). Anal. Calcd for $C_{24}H_{19}NO_6$: C, 69.06; H, 4.59; N, 3.35. Found: C, 69.10; H, 4.61; N, 3.31.

7,8-Dihydroxy-7,8-dihydro-1-nitrobenzo[*a*]pyrene *trans*-7,8-Diacetate (9). A solution of **8** (60 mg) and an equimolar proportion of DDQ in 20 mL of dioxane was refluxed under nitrogen atmosphere. To monitor the reaction, a small portion (ca. 0.1 mL) of the reaction mixture was periodically removed and injected into a Zorbax ODS reversed-phase HPLC column (9.4 × 250 mm) for analysis. After 54 h, about 60% of **8** was consumed. Chromatography of the resulting residue on silica gel with hexane–ethyl acetate (v/v, 4/1) recovered **8**. Further elution provided **9** as a yellow solid (30 mg, 83% conversion yield): mp 237–238 °C; MS *m/z* 415 (M^+, 8), 355 ($[M - CH_3CO_2H]^+$, 25), 325 ($[M - CH_3CO_2H - NO]^+$, 26), and 313 ($[M - CH_3CO_2H - CH_2{=}C{=}O]^+$, 68); UV–vis spectrum, λ_{max} (log ϵ_{max}) 418 (4.21), 392 (4.35), 302 (4.27), 244 (4.63), 207 (4.53) nm; NMR ($CDCl_3$) δ 5.82 (dd, 1H, $J_{7,8}$ = 6.2 Hz, $J_{8,9}$ = 4.1 Hz, H_8), 6.28 (dd, 1H, H_9), 6.62 (d, 1H, $J_{7,8}$ = 7.5 Hz, H_7), 7.66 (dd, 1H, $J_{9,10}$ = 9.8 Hz, $J_{8,10}$ = 1.8 Hz, H_{10}), 8.31 (d, 1H, $J_{4,5}$ = 8.6 Hz, H_4), 8.44 (m, 2H, $H_{3,5}$), 8.71 (d, 1H, $J_{2,3}$ = 8.6 Hz, H_2), 8.70 (s, 1H, H_6), and 8.85 (AB, 2H, $H_{11,12}$). Anal. Calcd for $C_{24}H_{17}NO_6$: C, 69.39; H, 4.13; N, 3.37. Found: C, 69.38; H, 4.18; N, 3.32.

***trans*-7,8-Dihydroxy-7,8-dihydro-1-nitrobenzo[*a*]pyrene (1).** A solution of **9** (25 mg, 0.06 mmol) in THF (10 mL) and $NaOCH_3$ (27 mg, 0.5 mmol) in methanol (10 mL) was stirred at 65 °C for 30 min. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure, affording **1** as a light yellow solid (18 mg, 92% yield): mp 261–262 °C; MS *m/z* 331 (M^+, 56), 313 ($[M - H_2O]^+$ 48), 285 ($[M - NO_2]^+$, 100), and 267 ($[M - H_2O - NO_2]^+$, 82); UV–vis spectrum, λ_{max} (log ϵ_{max}) 427 (4.06), 392 (4.15), 308 (4.09), 244 (4.50), 203 (4.49) nm: NMR (acetone-d_6 with trace of D_2O) δ 4.67 (dt, 1H, $J_{7,8}$ = 11.2 Hz, $J_{8,9}$ = $J_{8,10}$ = 2.2 Hz, H_8), 5.16 (d, 1H, $J_{7,8}$ = 11.2 Hz, H_7), 6.44 (dd, 1H, $J_{9,10}$ = 10.3 Hz, H_9), 7.65 (dd, 1H, H_{10}), 8.32 (d, 1H, $J_{4,5}$ = 8.6 Hz, H_4), 8.44 (m, 2H, $H_{3,5}$), 8.71 (d, 1H, $J_{2,3}$ = 8.6 Hz, H_2), 8.74 (s, 1H, H_6), and 8.85 (AB, 2H, $H_{11,12}$). Anal. Calcd for $C_{20}H_{13}NO_4$: C, 72.50; H, 3.96; N, 4.23. Found: C, 72.57; H, 3.99; N, 4.19.

9,10-Epoxy-*trans*-7,8-dihydroxy-7,8-dihydro-1-nitrobenzo[*a*]pyrene (2). A solution of **1** (10 mg, 0.03 mmol) in 10 mL of HPLC-grade THF and a 30-fold excess *m*-chloroperbenzoic acid (160 mg) was stirred at ice-bath temperature under N_2 for 3 h, and cold ethyl acetate (15 mL) was added to the reaction mixture. The mixture was extracted three times with cold dilute NaOH solution (3 × 5 mL). The organic layer was washed three times with cold water and dried over $MgSO_4$, and the solvent was removed under reduced pressure. Analysis of the resulting product **2** (8 mg, 80% yield) by a Zorbax ODS reversed-phase HPLC column (9.4 × 250 mm) indicated that this sample was 98% pure: mp 178–179.5 °C; MS *m/z* 347 (M^+); UV–vis spectrum, λ_{max} (log ϵ_{max}) 400 (3.90), 284 (4.08), 240 (4.58), 208 (4.42), 206 (4.44), nm; NMR (acetone-d_6) δ 3.88 (dd, 1H, $J_{8,9}$ = 1 Hz, $J_{9,10}$ = 4.5 Hz, H_8), 3.96 (d, 1H, $J_{7,8}$ = 9.0 Hz, H_8), 4.76 (d, 1H, $J_{7,8}$ = 9.0 Hz, H_7), 5.22 (apparent d, 1H, $J_{9,10}$ = 4.5 Hz, H_{10}), 8.32 (d, 1H, $J_{4,5}$ = 8.6 Hz, H_4), 8.44 (m, 2H, $H_{3,5}$), 8.71 (d, 1H, $J_{2,3}$ = 8.6 Hz, H_2), 8.74 (s, 1H, H_6), and 8.85 (AB, 2H, $H_{11,12}$). Anal. Calcd for $C_{20}H_{13}NO_5$: C, 69.16; H, 3.77; N, 4.03. Found: C, 69.39; H, 3.89; N, 4.00.