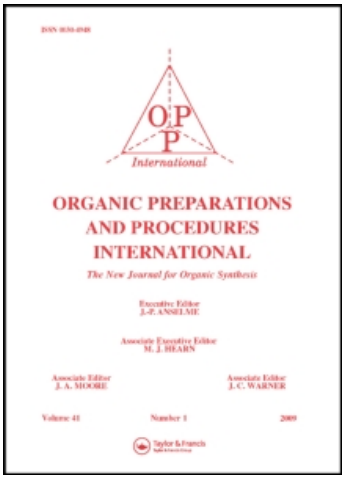

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SYNTHESIS OF 6-SUBSTITUTED 3-DEUTEROBENZO[A]PYRENE

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OPPI BRIEFS

SYNTHESIS OF 6-SUBSTITUTED 3-DEUTEROBENZO[A]PYRENE

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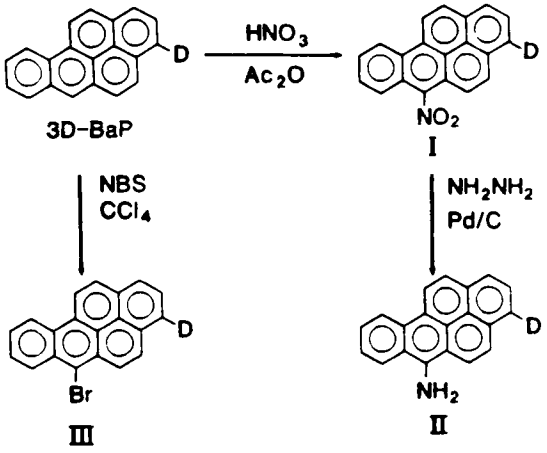
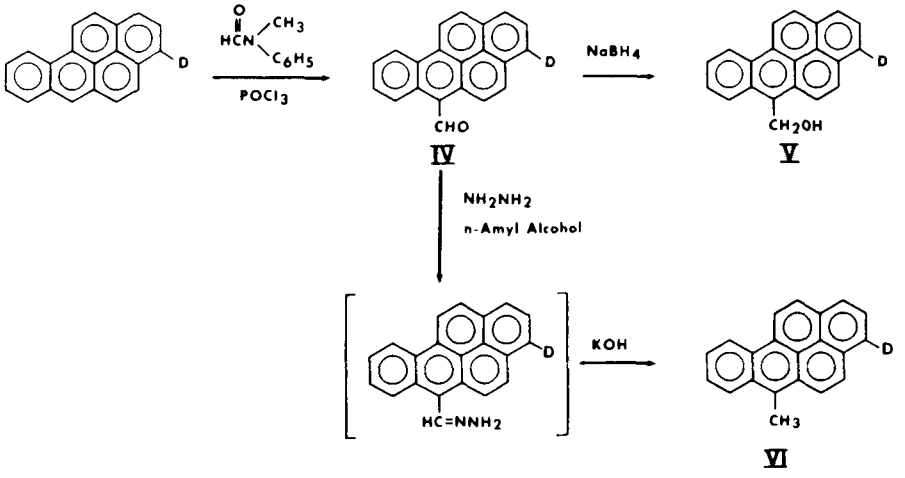
We report the synthesis of the 3-deuterobenzo[a]pyrene (3-D-BaP) from which 6-nitro-3-D-BaP (I), 6-amino-3-D-BaP (II), 6-bromo-3-D-BaP (III), 6-formyl-3-D-BaP (IV), 6-hydroxymethyl-3-D-BaP (V) and 6-methyl-3-D-BaP (VI) were obtained. For quantitation of the metabolic studies of 6-nitro-BaP, [G-^{3}H]-6-nitro-BaP (VII) and [7-^{14}C]-6-nitro-BaP were also prepared.

Nitration of 3-D-BaP by fuming nitric acid in acetic anhydride at 0° yielded I with a trace of the 1- and 3-nitro-BaP and dinitro-BaP.[1] Reduction of I with hydrazine and palladium on carbon afforded II. Compound III was synthesized directly by bromination of 3-D-BaP with N-bromosuccinimide (NBS) in carbon tetrachloride (Scheme 1).



Scheme 1

Vilsmeier-Haack reaction[2] of 3-D-BaP provided IV (Scheme 2). Reduction of IV with sodium borohydride in methanol gave V in high yield. Compound VI was synthesized by a Wolff-Kishner reduction. The structure and the



Scheme 2

deuterium content of each compound (I-VI) was determined by the analysis of the 500 MHz proton NMR spectra. The assigned 500 MHz proton NMR spectra of 6-NO_2-BaP and compound I are shown in Fig. 2.

Nitration of [G-^{3}H]-BaP and [7-^{14}C]-BaP under similar conditions as described for compound I gave the radioactive VII and [7-^{14}C]-6-nitro-BaP, respectively. The availability of compound VII enabled quantification of rat liver microsomal metabolism of 6-nitro-BaP.[3] Because compound I was prepared, the major metabolite of 6-nitro-BaP, 3-hydroxy-6-nitro-BaP, was found to be formed *via* rearrangement of an unstable 2,3-epoxide intermediate.[4]

EXPERIMENTAL SECTION

$NaBD_4$ (98% atom) and $NaBH_4$ were purchased from ICN Corp., Irvine, CA. DDQ, NBS, phosphorus oxychloride, and N-methylformanilide were purchased from Aldrich Chemical Co., Milwaukee, WI. The ketone, 1,6,10b,11,12,12b-hexahydro-BaP-3(2H)-one, was synthesized according to the method of Cook *et.al.*[5] [7-^{14}C]-BaP (8.56 mCi/mm) was purchased from Pathfinder Laboratories Inc., St. Louis, MO. ^{1}H NMR spectra were recorded with a Bruker

WM-500 spectrometer. Acetone-D_6 was employed as the solvent and chemical shifts were reported downfield from the internal standard tetramethylsilane. Mass spectra were obtained on a Finnigan 4023 GC/MS/data system at 70eV with a solid probe. The radioactivity was determined on a Tracor Analytic Mark III liquid scintillation spectrometer system.

Figure 1

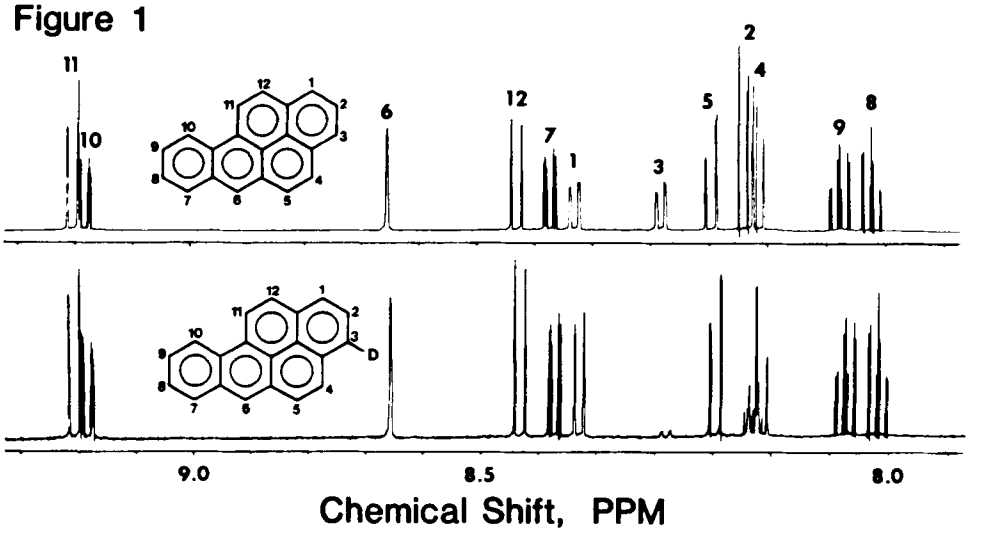
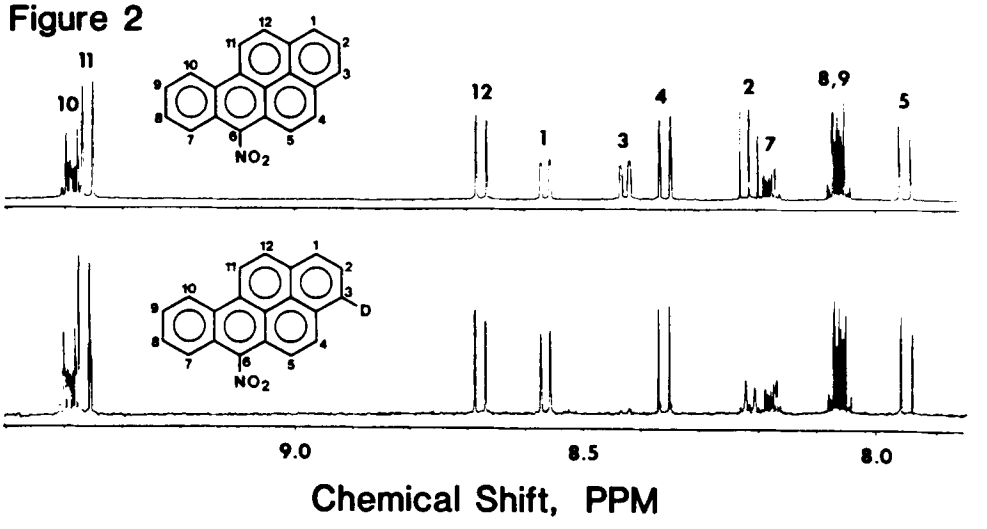


Figure 2



3-Deuterobenzo[a]pyrene (3-D-BaP).- This compound was synthesized as previously described,[5] except a larger scale reaction was carried out, and the product (75% yield) was analyzed by high resolution ^1H NMR spectroscopy; mp 178-179°, lit.[5] 178-179°; mass spectrum: m/e 253 (M^+); NMR (Fig. 1): 7.83

(dd, 1, H_9), 7.89 (dd, 1, H_8), 8.03 (d, 1, H_4), 8.04 (d, 1, H_2), 8.11 (d, 1, $J_{4,5}$ = 9.2 Hz, H_5), 8.34 (d, 1, $J_{1,2}$ = 7.7 Hz, H_1), 8.39 (d, 1, $J_{7,8}$ = 8.1 Hz, H_7), 8.45 (d, 1, H_{12}), 8.67 (s, 1, H_6), 9.20 (d, 1, $J_{9,10}$ = 8.2 Hz, H_{10}) and 9.22 ppm (d, 1, $J_{11,12}$ = 9.1 Hz, H_{11}). NMR spectral analysis indicated that the product contained 92 atom % deuterium.

3-Deutero-6-nitrobenzo[a]pyrene (I).- Sodium nitrate (170 mg, 2 mmol) in 50 ml of trifluoroacetic acid was added to a benzene (50 ml) solution of 3-D-BaP (506 mg, 2 mmol) and stirred 6 hrs under nitrogen at 0^0. The reaction mixture was partitioned between benzene and water. The organic layer was separated, dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was chromatographed on Florisil and eluted with benzene. Recrystallization from benzene gave I (360 mg, 60%), mp. 251-253^0; mass spectrum: m/e 300 (M^+); NMR: 7.93 (d, 1, H_5), 8.06-8.02 (m, 2, $H_{8,9}$), 8.16 (m, 1, H_7), 8.19 (d, 1, H_2), 8.34 (d, 1, $J_{4,5}$ = 7.2 Hz, H_4), 8.55 (d, 1, $J_{1,2}$ = 8.1 Hz, H_1), 8.65 (d, 1, H_{12}), 9.35 (d, 1, $J_{11,12}$ = 9.2 Hz, H_{11}) and 9.37 ppm (m, 1, H_{10}).

[7-^{14}C]-6-Nitrobenzo[a]pyrene.- A solution of [7-^{14}C]-BaP (8.5 mg, specific activity 8.56 mCi/mmol), acetic acid (10 ml), acetic anhydride (10 ml) and fuming nitric acid (34 mmol) was stirred at 4^0 under nitrogen overnight. After conventional workup, crude [7-^{14}C]-6-nitro-BaP was obtained. Upon purification by HPLC (Zorbax SIL 6.2 X 250 mm) eluted with 3% THF in hexane at 2 ml/min, pure [7-^{14}C]6-nitro-BaP was obtained (2.2 mg, specific activity 10.8 mCi/mmol).

[G-^{3}H]-6-nitro-BaP.- A solution of red fuming nitric acid (d = 1.6, 1.7 ml) in ice-cold water (2.8 ml) was added rapidly with stirring to a solution of [G-^{3}H]-BaP (Amersham) (699 mg, 2.77 mmoles, 333 mCi) in doxane (50 ml). After stirring for 30 min, the yellow-orange precipitate was collected and washed with 10 ml of dioxane. This crude product was crystallized twice

from hot benzene to yield 270 mg of pure [G-^{3}H]-6-nitrobenzo(a)pyrene. The solvent was evaporated from the mother liquors, and the residue was chromatographed on a column of silica gel 60 (2.5 x 30 cm) eluting with hexane, gradually changing to hexane:benzene, 3:1. Pure fractions were combined and the recovered solid was crystallized from hot benzene to yield 150 mg of [G-^{3}H]-6-nitrobenzo(a)pyrene, with a specific activity of 93.2 mCi/mmole.

3-Deutero-6-aminobenzo[a]pyrene (II).- Compound I (150 mg, 0.5 mmol) in 95% ethanol (30 ml), 5% Pd/C (5 mg) and 99% hydrazine (0.5 ml) were heated at reflux for 1.5 hrs. The cooled reaction mixture was poured onto anhydrous Na_2SO_4 and filtered through Celite. The filtrate was concentrated to ca. 5 ml under reduced pressure. The concentrated solution was chromatographed over neutral alumina by eluting with benzene affording II, 116 mg (86%), mp. 236-238° (benzene); mass spectrum: m/e 268 (M^+); NMR: 6.33 (bs, 2, NH_2), 7.76-7.80 (m, 2, $H_{4,8}$), 7.84 (d, 1, $J_{1,2}$ = 7.3 Hz, H_2), 7.85 (dd, 1, $J_{9,10}$ = 9.0 Hz, H_9), 8.00 (d, 1, $J_{11,12}$ = 9.0 Hz, H_{12}), 8.05 (d, 1, H_1), 8.29 (d, 1, $J_{4,5}$ = 9.5 Hz, H_5), 8.63 (d, 1, $J_{7,8}$ = 8.2 Hz, H_7), 8.96 (d, 1, H_{11}) and 9.12 ppm (d, 1, H_{10}).

3-Deutero-6-bromobenzo[a]pyrene (III).- A mixture of 3-D-BaP (200 mg, 0.75 mmol) and NBS (135 mg, 0.75 mmol) in CCl_4 (15 ml) was heated at reflux for 2 hrs. The precipitate (succinimide) was removed by filtration. Chromatography of the filtrate on Florisil with benzene as the eluant afforded III (220 mg), mp. 223-224° (benzene); mass spectrum: m/e 331 (M^+); NMR: 7.93-8.00 (m, 2, $H_{8,9}$), 8.08 (d, 1, H_4), 8.18 (d, 1, H_4), 8.39 (d, 1, $J_{1,2}$ = 7.7 Hz, H_1), 8.48 (d, 1, H_{12}), 8.54 (d, 1, $J_{4,5}$ = 9.5 Hz, H_5), 8.85 (m, 1, H_7), 9.21 (d, 1, $J_{11,12}$ = 9.5 Hz, H_{11}) and 9.23 (m, 1, H_{10}).

3-Deutero-6-formylbenzo[a]pyrene (IV).- A mixture of N-methylformanilide (0.28 ml) and phosphorus oxychloride (0.18 ml) was stirred at ambient

temperature under nitrogen until the formyl reagent formed as a solid cake. Compound I (253 mg, 1.0 mmol) and 2 ml of dimethylformamide were added and the mixture was heated at 70° for 6 hrs. The reaction products were partitioned between ethyl acetate and water. The organic layer was separated, dried over $MgSO_4$ and the solvent was removed under reduced pressure. Chromatography of the residue on Florisil with hexane as the eluant gave the recovered 3-D-BaP (36 mg). Elution with benzene-ethyl acetate (4:1) gave IV (172 mg), mp. 201-203° (acetone); mass spectrum: m/e 281 (M^+); NMR: 7.94- 7.98 (m, 2, $H_{8,9}$), 8.16 (d, 1, H_2), 8.32 (d, 1, H_4), 8.50 (d, 1, $J_{1,2}$ = 7.9 Hz, H_1), 8.65 (d, 1, $J_{11,12}$ = 9.2 Hz, H_{12}), 9.08 (d, 1, $J_{4,5}$ = 9.6 Hz, H_5), 9.30-9.35 (m, 3, $H_{7,10,11}$) and 11.69 ppm (s, 1, CHO).

3-Deutero-6-hydroxymethylbenzo[a]pyrene (V).- A solution of VI (56 mg, 0.2 mmol) in THF (15 ml) was stirred with $NaBH_4$ (38 mg, 1.0 mmol) in methanol (30 ml) for 1 hr at ambient temperature. The solution was concentrated to ca. 5 ml under reduced pressure, chromatographed on Florisil, and elution with benzene yielded 50 mg of V; mp. 270-271° (benzene); mass spectrum: m/e 283 (M^+); NMR: 5.77 (s, 2, CH_2), 7.86-7.90 (m, 2, $H_{8,9}$), 8.04 (d, 1, H_2), 8.09 (d, 1, H_4), 8.29 (d, 1, $J_{1,2}$ = 7.9 Hz, H_1), 8.43 (d, 1, H_{12}), 8.59 (d, 1, $J_{4,5}$ = 9.6 Hz, H_5), 8.86 (m, 1, H_7), 9.24 (d, 1, $J_{11,12}$ = 9.6 Hz, H_1) and 9.26 ppm (m, 1, H_{10}).

3-Deutero-6-methylbenzo[a]pyrene (VI).- A solution of VI (113 mg, 0.4 mmol), n-amyl alcohol (30 ml) and 99% hydrazine (0.8 ml) was heated at reflux for 24 hrs. After cooling, KOH pellets (220 mg) were added and the reaction mixture was refluxed for 24 hrs. The reaction mixture was partitioned between ethyl ether and water. The organic layer was separated, dried and the solvent removed under reduced pressure. The resulting solid was chromatographed on Florisil and elution with hexane yielded IV (98 mg), mp. 217-219° (benzene); mass spectrum: m/e 267 (M^+); NMR: 3.26 (s, 3, CH_3),

7.86-7.90 (m, 2, $H_{8,9}$), 8.00 (d, 1, H_2), 8.04 (d, 1, H_4), 8.29 (d, 1, $J_{1,2}$ = 9.0 Hz, H_1), 8.35 (d, 1, H_{12}), 8.43 (d, 1, $J_{4,5}$ = 9.0 Hz, H_5), 8.65 (m, 1, H_7), 9.19 (d, 1, $J_{11,12}$ = 9.0 Hz, H_{11}) and 9.23 ppm (m, 1, H_{10}).

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