N, 5.76. Found: C, 78.49; H, 8.56; N, 5.73.

Cone Conformer of 5,11,17,23-Tetracarbamoyl-25,26,27,28-tetrakis(octyloxy)calix[4]arene (III). A mixture of 0.206 g (0.211 mmol) of cone conformer of 5,11,17,23-tetracyano-25,26,27,28-tetrakis(octyloxy)calix[4]arene, 0.100 g (0.295 mmol) of $Bu_4N{\cdot}HSO_4$, 0.8 mL of 30% H_2O_2, 0.8 mL of 20% NaOH, and 1.0 mL of CH_2Cl_2 was stirred at rt for 48 h. The product mixture was then concentrated under reduced pressure and the organic residue dissolved in 1 mL of CH_2Cl_2 and subjected to identical hydrolysis conditions for an additional 16 h. Addition of 13 mL of 1 N HCl, followed by concentration under reduced pressure, washing of the solid product with 3 × 30 mL of water, and recrystallization from 30 mL of MeOH afforded 0.163 g of white solid which consisted of four components having R_f = 0.78 (w), 0.55 (s), 0.42 (w), and 0.33 (m) (silica, $CHCl_3/MeOH/H_2O$ (75:20:3), v/v). Purification of that component having R_f = 0.55 via preparative TLC and recrystallization (3 × from CH_3OH) afforded 0.111 g (51%) of **III**, having mp 302-4 °C dec: ^1H NMR (500 MHz, $CDCl_2CDCl_2$, 100 °C) 0.95 (t, 12 H, CH_3), 1.35 (m, 40 H, CH_2), 1.90 (m, 8 H, OCH_2CH_2), 4.49-4.52, 3.28-3.30 (2 d, J = 14.0 Hz, 8 H, endo, exo-CHAr); 3.99 (t, 8 H, CH_2O), 5.57 (s, 8 H, NH_2), 7.15 (s, 8 H, ArH). Anal. Calcd for $C_{64}H_{92}N_4O_8$: C, 73.52; H, 8.87; N, 5.36. Anal. Calcd for $C_{64}H_{92}N_4O_8{\cdot}2CH_3OH$: C, 71.45; H, 9.08; N, 5.50. Found: C, 71.60; H, 8.81; N, 5.54.

Registry No. I, 141344-71-0; II, 141344-72-1; III, 141434-03-9; 25,26,27,28-tetrakis(octyloxy)calix[4]arene, 141344-73-2; tetrahydroxycalix[4]arene, 74568-07-3; bromooctane, 111-83-1; 5,11,17,23-tetracarboxy-25,26,27,28-tetrakis(1-*n*-octyl)calix[4]arene, 141434-74-4; 5,11,17,23-tetrabromo-25,26,27,28-tetrakis(1-*n*-octyl)calix[4]arene, 141344-74-3; 5,11,17,23-tetracyano-25,26,27,28-tetrakis(octyloxy)calix[4]arene, 141344-75-4.

Supplementary Material Available: ^1H NMR (500-MHz) spectrum of **III** (1 page). Ordering information is given on any current masthead page.

Synthesis of Nitropolycyclic Aromatic Hydrocarbons with the Substituent at the Longest Axis[1]

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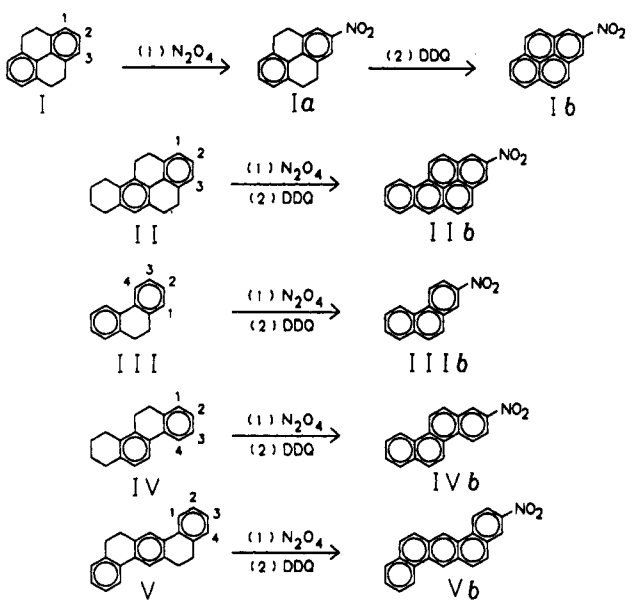
Introduction

Nitropolycyclic aromatic hyrocarbons (nitro-PAHs) are environmental contaminants and are present in the food chain.[2-5] Since many nitro-PAHs are metabolized to highly mutagenic and tumorigenic metabolites by mammalian and/or bacterial enzymes, there is concern regarding possible adverse human health effects from exposure to these compounds. We have been interested in structure–activity relationships as a means of utilizing the structural and electronic features of nitro-PAHs for the interpretation and/or prediction of their metabolism and their biological activities, including mutagenicity and tumorigenicity.[4-10] Recent studies by several groups have demonstrated that the geometric location and the orientation of the nitro substituent[6-11] and the first half-wave

Scheme I



reduction potential[8,10,12] are all important features that may affect the mutagenic potency of nitro-PAHs. As a continuation of our interest in the biological studies of nitro-PAHs, we need to prepare the nitro-PAHs with the nitro substituent situated at the longest axis of the molecule. Because carbons on the longest axis of a PAH are not the most reactive positions for electrophilic aromatic substitution reactions, direct nitration will not produce the nitro-PAHs with the nitro group in these positions.[13] As a consequence, alternative approaches are required for the synthesis of nitro-PAHs of this type. We report here a general synthetic method for the synthesis of ten nitro-PAHs with the nitro substituent located on the longest axis of the molecule. The nitro-PAH compounds synthesized include 2-nitro-4,5,9,10-tetrahydropyrene (Ia), 2-nitro-4,5,7,8,9,10,11,12-octahydrobenzo[*a*]pyrene (IIa), 2-nitro-9,10-dihydrophenanthrene (IIIa), 2-nitro-7,8,9,10,11,12-hexahydrochrysene (IVa), 3-nitro-5,6,12,13-tetrahydrobenz[*a*,*h*]anthracene (Va), 2-nitropyrene (Ib), 2-nitrobenzo[*a*]pyrene (IIb), 2-nitrophenanthrene (IIIb), 2-nitrochrysene (IVb), and 3-nitrodibenz[*a*,*h*]anthracene (Vb).

Results and Discussion

Synthesis of nitro-PAHs with the substituent situated on the longest axis of the molecules started with the partially saturated PAHs I–V, all of which contained a biphenyl moiety (Scheme I). This strategy is based on the prediction by molecular orbital calculations that nitration of biphenyl occurs at the 2- and 4-positions,[13] the reported nitration of 4,5,9,10-tetrahydropyrene (I) to 2-

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nitro-4,5,9,10-tetrahydropyrene (Ia),[14,15] and the following considerations. For compounds I and II, the positions equivalent to the 2-positions of a biphenyl molecule are the bridged positions. Thus, nitration of I and II should provide only one product, the desired nitro-PAH with the nitro group at the longest axis. For compounds III–V, the positions equivalent to the 2-positions of a biphenyl molecule are accessible for reaction. However, these positions are situated at a crowded bay region and thus should be less favored for nitration. Furthermore, the ratio of nitration to the 2- or 4-position of a biphenyl molecule varies, depending upon the reagents and conditions employed. Nitration of biphenyl by nitric acid in acetic anhydride regiospecifically favors the 2-position. With sodium nitrate in trifluoroacetic acid,[16,17] nitration of biphenyl provided 2- and 4-nitrobiphenyl in a nearly equal ratio. Nitration of biphenyl with N_2O_4 in methylene chloride[18–22] with the presence of an acid gave 2- and 4-nitrobiphenyl in a 3 to 2 ratio. However, we have found that nitration of biphenyl with N_2O_4 in methylene chloride in the absence of added acid provided 2- and 4-nitrobiphenyl in 35 and 61%, respectively. Similar high regioselectivity has also been found on the nitration of compounds I–V by this method. Thus, this method was selected for preferential nitration on the longest axis.

The nitrations proceeded at room temperature by adding N_2O_4 in methylene chloride to the PAHs in methylene chloride in a 1:1 molar ratio with stirring. These reactions were complete within 1 h, except for III (Scheme I). Compounds I and II gave one product in near quantitative yield. Compound III required 18 h for completion of the reaction. However, with excess N_2O_4 (3 mol), the reaction was complete within 4.5 h. The desired product IIIa was produced in 95% yield, while 4-nitro-9,10-dihydrophenanthrene was formed in less than 1%. Nitration of IV gave two products, IVa in 75% yield and another mononitro isomer, identified as 5- or 6-nitro-7,8,9,10,11,12-hexahydrochrysene (IVa′). Compound V gave the desired product Va in 70% yield. Upon separation of the nitration products by chromatography, two minor products were identified, 7-nitro-12,13-dihydrodibenz[*a*,*h*]anthracene (Va′) and a product tentatively assigned as 3,10-dinitro-5,6,12,13-tetrahydrodibenz[*a*,*h*]anthracene (Va″). Compound V was the only case where dinitration occurred. These data indicate that nitration of III–V with N_2O_4 results in major substitution at the position corresponding to the 4-position with only minor substitution at the position corresponding to the 2-position of the biphenyl moiety in each case. This reduced level of substitution at the position corresponding to the 2-position of biphenyl for III–V is most reasonably due to steric hindrance. Thus, our results described above have demonstrated that nitration of compounds I–V with N_2O_4 in methylene chloride is highly regioselective, affording nitro-PAHs with the nitro substituent at the longest axis in high yield.

Aromatization of Ia–Va was accomplished in good yield by refluxing with DDQ in benzene or dioxane (Scheme I). The yields were virtually quantitative except for IVa. Compound IVa upon refluxing 6 days in dioxane with 12 equiv of DDQ gave a 75% yield of IVb.

The structures of the synthesized nitro-PAHs were confirmed by analysis of their mass and high-resolution proton nuclear magnetic resonance (NMR) spectral data. In compounds Ia–Va and Ib–Vb, the chemical shifts of the protons ortho to the nitro group are downfield as compared with those of the corresponding parent PAHs. The magnitude and direction of the ortho proton chemical shift changes associated with a nitro group being substituted at the longest axis are consistent with the nitro π-bond being conjugated with the aromatic π-system.[17,23,24] The nitro group deactivation via mesomeric and σ electronic effects as predicted by the chemical shift data could explain the control over dinitration except for V. The relative ease of dinitration might be expected due to the distance between the two terminal rings.

Experimental Section

Materials. 9,10-Dihydrophenanthrene and DDQ were purchased from Aldrich Chemical Co. (Milwaukee, WI). Compounds I, II, and V were prepared by catalytic hydrogenation of pyrene, 7,8,9,10-tetrahydrobenzo[*a*]pyrene, and dibenz[*a*,*h*]anthracene, respectively, with Pd/C in ethyl acetate at mild conditions following published procedures,[25] with the exception that a Parr apparatus was employed for hydrogenation. Compound IV was prepared in an overall yield of 90% by reduction of 4-oxo-1,2,3,4-tetrahydrochrysene to 1,2,3,4-tetrahydrochrysene followed by hydrogenation with Pd/C in ethyl acetate at 30 psig. Dinitrogen tetraoxide was purchased from the Matheson Division of Searle Medical Products (East Rutherford, NJ). Proton NMR spectral data were obtained on a Bruker AM-500 instrument in acetone-d_6 and the chemical shifts are reported in ppm downfield from TMS. The mass spectral data were obtained on a Finnigan 4023 mass spectrometer. Electron ionization at 70 V was used to ionize the sample. The source temperature was set at 270 °C.

Dinitrogen Tetraoxide (N_2O_4) Nitrating Reagent. 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 methylene chloride until the methylene chloride was saturated with N_2O_4. The stoppered flask was weighed to determine the grams of N_2O_4 per milliliter of methylene chloride (0.3 mg per mL).

General Procedure for Nitration Reactions with N_2O_4. In general, nitration of I–V was accomplished as follows: To 100 mg (0.35–0.55 mmol) of the substrate (I–V) in 50 mL of methylene chloride was added slowly a 10% molar excess of the N_2O_4 solution, with the only exception that a two-fold excess of N_2O_4 was used for the nitration of compound III. The reaction mixture was monitored for the disappearance of the starting material by silica gel TLC eluting with methylene chloride/hexane (1:1). After nitration was completed, the excess N_2O_4 was removed by bubbling argon through the reaction mixture. A 0.5-mL sample was removed and the solvent was removed under a stream of argon. The resulting material was dissolved in acetone-d_6 and the proton NMR spectrum was obtained to assess the extent of reaction. The reaction mixture was then extracted with ethyl acetate. Upon removal of the solvent, the products were purified by passing the crude reaction mixture through silica with hexane/methylene chloride (1:1).

2-Nitro-4,5,9,10-tetrahydropyrene (Ia). The nitration of I was completed within 1 h, giving compound Ia in a 98% yield.

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The product was further purified by column chromatography: mp 110-111 °C (lit.[26] mp 110-111 °C); mass spectrum, m/z 251 (M^+); NMR 2.90-3.02 (m, 8, CH_2), 7.16 (dd, 2, $H_{6,8}$, $J_{6,7}$ = 7.7 Hz), 7.25 (d, 1, H_7), and 7.97 ppm (s, 2, $H_{1,3}$).

2-Nitro-4,5,7,8,9,10,11,12-octahydrobenzo[*a*]pyrene (IIa). The nitration of II was completed in 20 min, giving the product IIa in a 98% yield: mp 152-153 °C; mass spectrum, m/z 305 (M^+); NMR 1.69-1.90 (m, 4, $H_{8,9}$), 2.63-3.10 (m, 8, $H_{4,5,7,10,11,12}$), 6.88 (s, 1, H_6), and 7.95 ppm (s, 2, $H_{1,3}$).

2-Nitro-9,10-dihydrophenanthrene (IIIa). The nitration of III was accomplished by reaction of a two-fold excess of N_2O_4 to 0.55 mmol (100 mg) of III in 50 mL of methylene chloride for 4.5 h. Determined by NMR spectral analysis, the crude reaction mixture contained 95% IIIa, 2.5% III, and 2.5 of an isomer of IIIa. Upon chromatography of the crude product followed by recrystallization from ethyl ether, pure IIIa was obtained as pink prisms: mp 80-82 °C (lit.[27] mp 81-82 °C); mass spectrum, m/z 225 (M^+); NMR 2.89-3.03 (m, 4, $H_{9,10}$), 7.32-7.40 (m, 3, H_{6-8}), 7.92 (dd, 1, H_5, $J_{5,6}$ = 6.8 Hz), 8.05 (dd, 1, H_4, $J_{3,4}$ = 8.1 Hz), 8.14 (s, 1, H_1), and 8.15 ppm (d, 1, H_3).

2-Nitro-7,8,9,10,11,12-hexahydrochrysene (IVa). The nitration of IV was complete within 1 h. Purification of IVa (75% yield) was accomplished by column chromatography: mp 123-124 °C; mass spectrum, m/z 279 (M^+); NMR 1.73-1.87 (m, 4, $H_{8,9}$), 2.71-2.99 (m, 8, $H_{7,10-12}$), 7.08 (d, 1, H_6, $J_{5,6}$ = 8.6 Hz), 7.69 (d, 1 H_5), 7.98 (d, 1, H_4, $J_{3,4}$ = 9.4 Hz), 8.11 (d, 1, H_1), and 8.12 ppm (d, 1, H_3).

3-Nitro-5,6,12,13-tetrahydrodibenz[*a*,*h*]anthracene (Va). The nitration of V was complete within 1 h. Compound Va (70% yield) was separated from Va′ and Va″ by preparative TLC on silica gel eluting with methylene chloride/hexane (1:1): mp 137-139 °C; mass spectrum, m/z 327 (M^+); NMR 2.86-3.10 (m, 8, $H_{5,6,12,13}$), 7.23-7.38 (m, 3, $H_{9,10,11}$, $J_{8,9}$ = 7.6 Hz), 7.81 (s, 1, H_7), 7.87 (s, 1, H_{14}), 7.89 (d, 1, H_8), 8.11 (d, 1 H_1, $J_{1,2}$ = 8.3 Hz), 8.16 (s, 1 H_4), and 8.21 ppm (d, 1, H_2).

General Procedure for Dehydrogenation with DDQ. Compounds Ia-Va were aromatized by DDQ in benzene or dioxane with refluxing temperature under an argon atmosphere. The resulting reaction mixture was filtered through Celite, and the filtrate was chromatographed on neutral alumina eluting with benzene. The yellow band that eluted from the column was stripped of solvent and recrystallized from methylene chloride/hexane (1:1).

2-Nitropyrene (Ib). A solution of 50 mg (0.2 mmol) of Ia and 180 mg of DDQ (0.8 mmol) in 50 mL of benzene was refluxed for 48 h. After workup and chromatography, the residue was recrystallized, yielding pure Ib (48 mg, 98%): mp 201-203 °C (lit.[26] mp 201-202.5 °C); mass spectrum, m/z 247 (M^+); NMR 8.23 (d, 1, H_7), 8.37 (d, 2, $H_{4,10}$), 8.41 (d, 2, $H_{5,9}$), 8.44 (d, 2, $H_{6,8}$), and 9.12 ppm (s, 2, $H_{1,3}$).

2-Nitrobenzo[*a*]pyrene (IIb). A solution of 50 mg (0.16 mmol) of IIa and 446 mg of DDQ (1.97 mmol) in 50 mL of benzene was refluxed for 15 h. Upon chromatography and recrystallization, compound IIb was obtained (46 mg, 98%): mp 142-144 °C; mass spectrum, m/z 297 (M^+); NMR 7.92 (dd, 1, H_8), 7.97 (dd, 1, H_9), 8.22 (d, 1, H_4), 8.29 (d, 1, H_5), 8.45 (d, 1, H_7), 8.69 (d, 1, H_{12}), 8.83 (s, 1, H_6), 8.96 (s, 1, H_3), 9.20 (s, 1, H_1), 9.24 (d, 1, H_{11}), and 9.27 ppm (s, 1, H_{10}). Anal. Calcd for $C_{20}H_{11}NO_2$: C, 80.80; H, 3.73; N, 4.71. Found: C, 80.72; H, 3.79; N, 4.78.

2-Nitrophenanthrene (IIIb). A solution of 50 mg (0.22 mmol) of IIIa and 101 mg (0.44 mmol) of DDQ in 50 mL of dioxane was refluxed 48 h. After chromatography and recrystallization, pure compound IIIb was obtained (47 mg, 96%): mp 100-101 °C (lit.[28] mp 100-101 °C); mass spectrum, m/z 223 (M^+); NMR 7.75-7.83 (m, 2, $H_{6,7}$), 7.99-8.09 (m, 3, H_{8-10}), 8.42 (d, 1, H_5, $J_{5,6}$ = 7.7 Hz), 8.89 (s, 1, H_1), 8.91 (d, 1, H_3, $J_{3,4}$ = 9.2 Hz), and 9.04 ppm (d, 1 H_4).

2-Nitrochrysene (IVb). A solution of 20 mg (0.07 mmol) of IVa and 97 mg of DDQ (0.43 mmol) in 50 mL of dioxane was refluxed for 6 days. The resulting reaction mixture was chromatographed, and further purification by recrystallization provided pure compound IVb in a 75% yield: mp 138-139 °C; mass spectrum, m/z 273 (M^+); NMR 7.76 (dd, 1, H_8, $J_{7,8}$ = 8.6 Hz), 7.82 (dd, 1, H_9, $J_{9,10}$ = 8.6 Hz), 8.14 (d, 1, H_7), 8.22 (d, 1, H_6, $J_{5,6}$ = 9.1 Hz), 8.39 (d, 1, H_{12}, $J_{11,12}$ = 9.3 Hz), 8.48 (d, 1, H_4, $J_{3,4}$ = 9.1 Hz), 8.93 (d, 1, H_5), 9.00 (dd, 1, H_{10}), 9.03 (s, 1, H_1), 9.09 (d, 1, H_{11}), and 9.17 ppm (d, 1, H_4). Anal. Calcd for $C_{18}H_{12}NO_2$: C, 79.11; H, 4.06; N, 5.13. Found: C, 79.02; H, 4.17; N, 5.19.

3-Nitrodibenz[*a*,*h*]anthracene (Vb). A solution of 30 mg (0.09 mmol) of Va and 83 mg of DDQ (0.36 mmol) in 50 mL of dioxane was refluxed for 15 h. The reaction mixture was chromatographed and recrystallization of the product yielded Vb (28 mg, 98%): mp 151-153 °C; mass spectrum, m/z 323 (M^+); NMR 7.74 (dd, 1, H_{10}, $J_{10,11}$ = 7.7 Hz), 7.79 (dd, 1, H_9, $J_{8,9}$ = 8.6 Hz), 7.93 (d, 1, H_{12}, $J_{12,13}$ = 9.5 Hz), 8.04 (d, 1, H_{11}), 8.10 (d, 1, H_6, $J_{5,6}$ = 8.6 Hz), 8.14 (d, 1, H_{13}), 8.30 (d, 1, H_5), 8.52 (d, 1, H_2, $J_{1,2}$ = 8.6 Hz), 8.92 (s, 1, H_4), 9.07 (d, 1, H_8), 9.27 (d, 1, H_1), 9.52 (s, 1, H_7), and 9.55 ppm (s, 1, H_{14}). Anal. Calcd for $C_{22}H_{13}NO_2$: C, 81.72; H, 4.05; N, 4.33. Found: C, 81.62; H, 4.12; N, 4.32.

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Enzymatic Halohydration of Glycals

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Haloperoxidase-catalyzed halogenation reactions are important biological processes. They are, for example, involved in the biosynthesis of the hormone thyroxine and in many biological defense systems.[1] They catalyze the following general reaction (eq 1):

$$AH + X^- + H_2O_2 + H^+ \xrightarrow{\text{haloperoxidase}} AX + 2H_2O \quad (1)$$

X = Cl, Br, or I

Chloroperoxidase (EC 1.11.1.10) can utilize chloride, bromide, and iodide ions as donors for the enzymatic halogenation reactions.[2-3] The enzyme also possesses the catalase activity for the disproportionation of hydrogen peroxide.[4] The chloroperoxidase-catalyzed halogenation seems to involve a hypohalous acid (HOX) as halogenating reagent, but the mechanism is not well understood. A mechanism involving an enzyme-bound electrophilic halogenating species was proposed for chloroperoxidase;[5] another mechanism involving free hypohalous acid as the halogenating species was proposed for myeloperoxidase.[6] Both reactions seem to proceed through a reactive "iron-oxo" intermediate. Previous studies indicate that chloroperoxidase showed poor stereoselectivity in reactions with a series of substrates.[7]

Chloroperoxidase catalyzes the oxidative formation of the carbon-halogen bond in a large number of substrates,[8]

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