Di- and Trinitrophenanthrenes: Synthesis, Separation, and Reduction Property[1]

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Although nitrated polycyclic aromatic hydrocarbons (nitrated PAHs), specifically, dinitrated PAHs, have been indicated as potent mutagenic and carcinogenic environmental pollutants, only a few di- and trinitrophenanthrenes (DNPHs and TNPHs) have been reported up to the present time. Methods for the preparation of DNPHs and TNPHs by direct nitration of phenanthrene were established in this study. The reaction of phenanthrene with fuming nitric acid in acetic anhydride gave a complex mixture of DNPHs. Eleven DNPHs were isolated by using preparative HPLC with a total of 62.6% yield. Their chemical structures were determined using ^{1}H NMR and electron impact mass spectrometry (EI-MS). The isolated DNPHs and their yields were 2,10-DNPH (14.2%), 1,10-DNPH (8.0%), 3,10-DNPH (6.9%), 3,6-DNPH (6.6%), 2,9-DNPH (5.3%), 1,6-DNPH (5.1%), 3,5-DNPH (4.6%), 4,9-DNPH (3.2%), 4,10-DNPH (3.1%), 1,5-DNPH (2.8%), and 2,6-DNPH (2.8%). Similarly, nine TNPHs were obtained from the nitrated reaction mixture of phenanthrene with fuming nitric acid without solvent: 3,6,9-TNPH (9.2%), 2,6,9-TNPH (9.0%), 1,6,9-TNPH (8.2%), 1,5,10-TNPH (9.0%), 2,6,9-TNPH (9.0%), 1,6,9-TNPH (4.0%), 1,7,9-TNPH (3.4%), 2,5,10-TNPH (2.5%), 2,6,10-TNPH (2.4%), 3,5,10-TNPH (1.8%), and 2,7,9-TNPH (0.2%) in 46.5% of the total yield. The reduction properties of the DNPHs and TNPHs were examined using cyclic voltammetry. The LUMO energy levels of the DNPHs and TNPHs calculated by the AM1 method were correlated to the first reduction potentials ($E_{1/2}$). The coplanar or noncoplanar conformations of the nitro substituents to the phenanthrene ring system were also discussed.

Introduction

Mutagenic and carcinogenic nitrated polycyclic aromatic hydrocarbons (nitrated PAHs)[2] have become of enormous concern because of their existence in urban air (*1–7*). The toxicity of nitrated PAHs possessing a wide variety of polycyclic aromatic rings has been extensively studied (*8–14*). The direct-acting mutagenicity of nitrated PAHs has been proposed to result from their metabolic reduction to the ultimate mutagens, such as *N*-hydroxyarylamines (*15–17*). From the data of nitrated PAHs tested for mutagenicity, a quantitative structure–activity relationship (QSAR) has been derived. The main determinants of mutagenicity are the hydrophobicity and the energy of the lowest unoccupied molecular orbital (LUMO) (*18, 19*). Nitrated PAHs, which have nitro substituents coplanar or nearly coplanar to the aromatic ring, are classified to be a direct-acting mutagen, and decreased mutagenicity is generally observed when the nitro substituents are perpendicular to the aromatic ring (*20–22*). It is also known that nitrated PAHs with three or more fused rings are significantly more mutagenic than those with one or two condensed aromatic rings (*10, 18*). Actually, most of the studies on nitrated PAHs have been focused on dinitropyrenes, which are among the most potent bacterial mutagens known (*4, 23, 24*). The levels in the environment of di- and trinitropyrenes are below that of 1-nitropyrene, but their mutagenicity in the *Salmonella* test system is extremely higher than that of 1-nitropyrene (*10*).

In contrast to a great deal of studies on nitrated pyrenes, the chemical, toxicological, and biological studies on the nitrated phenanthrenes possessing three fused benzene rings are limited. The presence of mononitrophenanthrene (NDP) and DNPH in the extracts of coal combustion particles was proved by Hanson *et al.* without any characterizations of their chemical structure (*25*). Hirayama *et al.* has clarified the mutagenic potency of 2-NPH, 9-NPH, and 2,7-DNPH in the Ames *Salmonella typhimurium* assay (*26, 27*). The syntheses of three DNPHs (2,5-, 2,7-, and 4,5-DNPH) were reported, but they were from biphenyl derivatives (*28–31*). The formation of large quantities of polynitrated products by the nitration of phenanthrene was known in the 1950s (*32–34*), but their separation and structural determination have not been achieved until now. The difficulty in the separation of pure isomers from the reaction mixture of nitrated phenanthrenes may be the main barrier for the study of DNPHs and TNPHs. Since recent progress on the preparative HPLC technique is remarkable (*35*) and we have already succeeded in separating several nitroarenes using HPLC on a preparative scale (*12, 14*), it is now thought that the separation of the mixture of DNPHs and TNPHs by HPLC is promising. We now report the separation and the structure determination of DNPHs and TNPHs synthesized by the direct nitration of phenanthrene. The reduction property and the conformation of the nitro substituents, which are closely related to their biological properties, were also clarified.

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[1] The data in this paper are taken from a thesis submitted by K.F. for the Doctor of Philosophy in Pharmacology, University of Tokyo.
[2] Abbreviations: PAH, polycyclic aromatic hydrocarbon; NPH, mononitrophenanthrene; DNPH, dinitrophenanthrene; TNPH, trinitrophenanthrene; EI-MS, electron impact mass spectrometry; QSAR, quantitative structure–activity relationship; LUMO, lowest unoccupied molecular orbital; COSY, correlation spectroscopy; TMS, tetramethylsilane.

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Experimental Section

General. Proton (^{1}H) and homonuclear two-dimensional chemical shift correlation spectroscopy (^{1}H–^{1}H COSY) NMR spectra were recorded using a Varian VXR-400S instrument in deuterated chloroform with tetramethylsilane (TMS) as an internal standard (0 ppm). Chemical shifts (δ) are reported relative to TMS, and the coupling constants (J value) are given in hertz (Hz). Mass spectra were obtained with a JEOL DX-300 mass spectrometer. Melting points were determined with a Yanagimoto MP-500V and are uncorrected. Cyclic voltammetric measurements were done with a Bioanalytical Systems BAS-100B electrochemical analyzer, using platinum working and auxiliary electrodes. Preparative HPLC was conducted on a Shimadzu LC8A HPLC instrument with a silica gel column (5 μm, 20 mm i.d. × 300 mm length) obtained from Soken Chemical & Engineering Co. (Tokyo). The flow rate was 30 mL/min, and the eluent was monitored with a Shimadzu SPD-10A detector at 280 nm. Analytical HPLC was conducted on a Shimadzu LC6AV HPLC instrument with the same detector equipped with a Nucleosil 50-5 column (5 μm, 4.3 mm i.d. × 250 mm length). For the purification of the crude reaction mixture, silica gel 70–325 mesh from E. Merck (Darmstadt) was used as specified in the text.

Chemicals. Phenanthrene was purchased from Wako Pure Chemical Industries, Ltd. (Osaka), and used without purification. Three mononitrophenanthrenes (1-NPH, 3-NPH, and 9-NPH) were synthesized according to the reported method (*32*) and purified by preparative HPLC eluted with *n*-hexane/CH_2Cl_2 (4:1). The isolated yields and the melting points are listed in Table 1. 2-NPH was a gift from Dr. Hirayama, Kyoto Pharmaceutical University.

Caution! *Because of the expected hazardous nature of the nitrated PAHs, all products were carefully handled.*

Synthesis of Dinitrated Phenanthrenes. A solution of fuming HNO_3 (sp. gravity = 1.52, 1 mL) and acetic anhydride (Ac_2O, 1 mL) was added dropwise to a solution of phenanthrene (1.00 g, 5.6 mmol) in Ac_2O (8 mL) with stirring at 0 °C. After an additional 20 min of stirring at room temperature, crushed ice was added and stirring was continued for an additional 1 h. The resulting mixture was neutralized with $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was then washed, dried, and evaporated. The residue was chromatographed on a silica gel column with elution by *n*-hexane/CH_2Cl_2 (1:1) to give the mixture of DNPHs (1.13 g), which was further separated into ten fractions by preparative HPLC eluting with *n*-hexane/benzene (1:1). Further purification was subsequently conducted by preparative HPLC using *n*-hexane/CH_2Cl_2 (1:1) for fractions 4 and 10 and *n*-hexane/CH_2Cl_2/EtOAc (7:2:1) for fraction 6. Fraction 2 was separated by preparative HPLC into two fractions (2a and 2b) using *n*-hexane/CH_2Cl_2 (1:1). All fractions were then subjected to electron impact mass spectrometry (EI-MS) and NMR analyses.

4,10-Dinitrophenanthrene (4,10-DNPH, fraction 1, 47.2 mg, 3.1% yield): light yellow powder; mp 172 °C; ^{1}H NMR δ 7.79 (m, 6- and 7-H), 7.82 (dd, J = 7.6 and 8.5, 2-H), 7.93 (d, J = 7.6, 3-H), 8.09 (m, 8-H), 8.17 (m, 5-H), 8.57 (s, 9-H), 8.66 (d, J = 8.5, 1-H); EI-MS m/z (rel intensity) 268 (38, M^+), 238 (24, $M^+ - NO$), 192 (100), 176 (37).

4,9-Dinitrophenanthrene (4,9-DNPH, fraction 2a, 47.5 mg, 3.2% yield): pale yellow crystals; mp 218 °C; ^{1}H NMR δ 7.74 (m, 6-H), 7.78 (dd, J = 7.7 and 8.3, 2-H), 7.84 (m, 7-H), 7.97 (d, J = 7.7, 1-H), 8.19 (d, J = 8.3, 3-H), 8.20 (d, J = 8.0, 5-H), 8.43 (s, 10-H), 8.44 (d, J = 8.7, 8-H); EI-MS m/z (rel intensity) 268 (47, M^+), 238 (44, $M^+ - NO$), 221 (72), 191 (100), 176 (47), 163 (43).

1,5-Dinitrophenanthrene (1,5-DNPH, fraction 2b, 42.9 mg, 2.8% yield): pale yellow needles; mp 139 °C; ^{1}H NMR δ 7.68 (dd, J = 7.8 and 8.6, 3-H), 7.76 (dd, J = 7.6 and 8.0, 7-H), 7.91 (d, J = 7.6, 8-H), 7.99 (d, J = 9.3, 9-H), 8.13 (d, J = 8.0, 6-H), 8.24 (d, J = 7.8, 2-H), 8.36 (d, J = 8.6, 4-H), 8.40 (d, J = 9.4, 10-H); EI-MS m/z (rel intensity) 268 (27, M^+), 192 (100), 176 (22).

3,10-Dinitrophenanthrene (3,10-DNPH, fraction 3, 103.9 mg, 6.9% yield): light yellow needles; mp 243 °C; ^{1}H NMR δ 7.86 (m, 7-H), 8.00 (m, 6-H), 8.15 (d, J = 8.0, 8-H), 8.55 (d, J = 9.3, 2-H), 8.76 (s, 9-H), 8.78 (d, J = 9.3, 1-H), 8.83 (d, J = 8.5, 5-H), 9.69 (s, 4-H); EI-MS m/z (rel intensity) 268 (100, M^+), 222 (14, $M^+ - NO_2$), 176 (81), 164 (34).

2,9-Dinitrophenanthrene (2,9-DNPH, fraction 4, 79.1 mg, 5.3% yield): pale yellow needles; mp 216 °C; ^{1}H NMR δ 7.90 (m, 7-H), 7.93 (m, 6-H), 8.51 (m, 8-H), 8.55 (s, 10-H), 8.60 (d, J = 9.2, 3-H), 8.83 (m, 5-H), 8.88 (d, J = 9.7, 4-H), 8.94 (s, 1-H); EI-MS m/z (rel intensity) 268 (80, M^+), 240 (23), 210 (29), 176 (100), 164 (60).

1,6-Dinitrophenanthrene (1,6-DNPH, fraction 5, 76.8 mg, 5.1% yield): colorless crystals; mp 248 °C; ^{1}H NMR δ 7.90 (dd, J = 7.8 and 8.7, 3-H), 8.07 (d, J = 9.3, 9-H), 8.13 (d, J = 8.8, 8-H), 8.33 (d, J = 7.8, 2-H), 8.52 (d, J = 8.8, 7-H), 8.56 (d, J = 9.3, 10-H), 9.08 (d, J = 8.7, 4-H), 9.65 (s, 5-H); EI-MS m/z (rel intensity) 268 (100, M^+), 240 (36), 210 (54), 192 (21), 176 (99), 164 (74).

2,10-Dinitrophenanthrene (2,10-DNPH, fraction 6, 213.5 mg, 14.2% yield): pale yellow powder; mp 207–209 °C; ^{1}H NMR δ 7.87 (m, 7-H), 7.97 (m, 6-H), 8.14 (d, J = 7.8, 8-H), 8.57 (d, J = 9.2, 3-H), 8.73 (s, 9-H), 8.77 (d, J = 8.7, 5-H), 8.92 (d, J = 9.2, 4-H), 9.56 (s, 1-H); EI-MS m/z (rel intensity) 268 (100, M^+), 222 (11, $M^+ - NO_2$), 210 (34), 176 (85), 164 (50).

2,6-Dinitrophenanthrene (2,6-DNPH, fraction 7, 41.8 mg, 2.8% yield): light yellow needles; mp 264–266 °C; ^{1}H NMR δ 8.00 (d, J = 8.8, 9-H), 8.10 (d, J = 8.8, 10-H), 8.13 (d, J = 8.7, 8-H), 8.54 (d, J = 8.7, 7-H), 8.57 (d, J = 9.1, 3-H), 8.90 (s, 1-H), 8.93 (d, J = 9.1, 4-H), 9.65 (s, 5-H); EI-MS m/z (rel intensity) 268 (100, M^+), 222 ($M^+ - NO_2$), 176 (65).

3,5-Dinitrophenanthrene (3,5-DNPH, fraction 8, 68.9 mg, 4.6% yield): light tan crystals; mp 159 °C; ^{1}H NMR δ 7.76 (dd, J = 7.7 and 8.0, 7-H), 7.91 (d, J = 7.7, 8-H), 7.94 (d, J = 9.0, 10-H), 7.99 (d, J = 9.0, 9-H), 8.08 (d, J = 8.8, 1-H), 8.14 (d, J = 8.0, 6-H), 8.46 (d, J = 8.8, 2-H), 9.10 (s, 4-H); EI-MS m/z (rel intensity) 268 (75, M^+), 222 (25, $M^+ - NO_2$), 192 (100), 176 (52), 163 (37).

3,6-Dinitrophenanthrene (3,6-DNPH, fraction 9, 99.4 mg, 6.6% yield): light yellow needles; mp 284 °C; ^{1}H NMR δ 8.05 (s, 9- and 10-H), 8.13 (d, J = 8.8, 1- and 8-H), 8.53 (d, J = 8.8, 2- and 7-H), 9.67 (s, 4- and 5-H); EI-MS m/z (rel intensity) 268 (100, M^+), 238 (13, $M^+ - NO$), 176 (54).

1,10-Dinitrophenanthrene (1,10-DNPH, fraction 10, 120.0 mg, 8.0% yield): light tan crystals; mp 260 °C; ^{1}H NMR δ 7.85 (m, 7-H), 7.90 (dd, J = 7.8 and 8.7, 3-H), 7.96 (m, 6-H), 8.13 (d, J = 7.9, 8-H), 8.32 (d, J = 7.8, 2-H), 8.66 (s, 9-H), 8.74 (d, J = 8.5, 5-H), 9.04 (d, J = 8.7, 4-H); EI-MS m/z (rel intensity) 268 (18, M^+), 222 (100, $M^+ - NO_2$), 164 (90).

Synthesis of Trinitrated Phenanthrenes. To 1.00 g (5.6 mmol) of phenanthrene was added dropwise 3 mL of fuming HNO_3, with stirring at room temperature. Stirring was continued for an additional 10 min. The reaction mixture was treated with ice water, neutralized with $NaHCO_3$, and then extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed and dried over Na_2SO_4. After removal of the solvent by evaporation, the residue was dissolved in CH_2Cl_2 and column chromatographed over silica gel eluted with *n*-hexane/CH_2Cl_2 (2:1) to obtain the mixture of TNPHs (1.35 g). Separation of the mixture of TNPHs was conducted by preparative HPLC using *n*-hexane/CH_2Cl_2 (1:1) to give six fractions (fractions 1–6). Fractions 2 and 4 were each rechromatographed by HPLC, eluting with *n*-hexane/CH_2Cl_2/EtOAc (60:35:5) into two fractions (fraction 2a and 2b and fraction 4a and 4b), respectively. Fraction 6 was separated into four fractions by preparative HPLC using *n*-hexane/CH_2Cl_2/EtOAc (70:25:5). Fraction 3 was a mixture of TNPHs, but no pure isomers were obtained by further HPLC separation.

1,5,9-Trinitrophenanthrene (1,5,9-TNPH, fraction 1, 69.9 mg, 4.0% yield): light tan crystals; mp 164–165 °C; ^{1}H NMR δ 7.87 (dd, J = 7.8 and 8.6, 7-H), 7.95 (dd, J = 7.7 and 8.5, 3-H), 8.08 (d, J = 7.7, 4-H), 8.41 (d, J = 8.6, 6-H), 8.45 (d, J = 7.8, 8-H), 8.59 (d, J = 8.5, 2-H), 9.17 (s, 10-H); EI-MS m/z (rel

intensity) 313 (26, M^+), 266 (14), 237 (100), 207 (19), 191 (36), 175 (13), 174 (21), 163 (41).

3,5,10-Trinitrophenanthrene (3,5,10-TNPH, fraction 2a, 30.9 mg, 1.8% yield): light yellow powder; mp 223–225 °C; ^{1}H NMR δ 7.93 (dd, J = 7.7 and 8.8, 7-H), 8.06 (d, J = 7.7, 8-H), 8.32 (d, J = 9.4, 1-H), 8.54 (d, J = 9.4, 2-H), 8.66 (s, 9-H), 8.67 (d, J = 8.8, 6-H), 8.99 (s, 4-H); EI-MS m/z (rel intensity) 313 (34, M^+), 283 (23, $M^+ - NO$), 266 (22), 237 (100), 191 (26), 175 (41), 174 (41), 163 (44).

2,5,10-Trinitrophenanthrene (2,5,10-TNPH, fraction 2b, 44.4 mg, 2.5% yield): light tan powder; mp 292–293 °C; ^{1}H NMR δ 7.59 (dd, J = 7.7 and 8.3, 7-H), 8.08 (d, J = 7.7, 8-H), 8.27 (d, J = 8.8, 4-H), 8.57 (s, 9-H), 8.59 (d, J = 8.8, 3-H), 8.63 (d, J = 8.3, 6-H), 9.17 (s, 1-H); EI-MS m/z (rel intensity) 313 (100, M^+), 267 (57, $M^+ - NO_2$), 237 (60), 221 (24), 207 (25), 191 (57), 175 (63), 174 (68).

1,6,9-Trinitrophenanthrene (1,6,9-TNPH, fraction 4a, 144.0 mg, 8.2% yield): pale yellow needles; mp 256 °C; ^{1}H NMR δ 8.11 (dd, J = 7.9 and 8.7, 3-H), 8.53 (d, J = 7.9, 2-H), 8.66 (d, J = 9.2, 7-H), 8.70 (d, J = 9.2, 8-H), 9.16 (d, J = 8.7, 4-H), 9.37 (s, 10-H), 9.73 (s, 5-H); EI-MS m/z (rel intensity) 313 (100, M^+), 236 (10), 221 (24), 209 (20), 191 (12), 175 (37), 174 (42), 163 (73).

1,5,10-Trinitrophenanthrene (1,5,10-TNPH, fraction 4b, 102.2 mg, 5.8% yield): colorless crystals; mp 229 °C; ^{1}H NMR δ 7.85 (dd, J = 7.8 and 8.6, 3-H), 7.92 (dd, J = 7.7 and 8.0, 7-H), 8.10 (d, J = 7.7, 8-H), 8.31 (d, J = 8.0, 6-H), 8.37 (d, J = 7.8, 4-H), 8.41 (d, J = 8.6, 2-H), 8.67 (s, 9-H); EI-MS m/z (rel intensity) 313 (15, M^+), 267 (100, $M^+ - NO_2$), 237 (29), 221 (33), 209 (18), 191 (24), 163 (74).

1,7,9-Trinitrophenanthrene (1,7,9-TNPH, fraction 5, 60.5 mg, 3.4% yield): light tan crystals; mp 213–214 °C; ^{1}H NMR δ 8.08 (d, J = 8.6, 3-H), 8.53 (d, J = 7.8, 2-H), 8.68 (d, J = 9.2, 6-H), 8.99 (d, J = 9.2, 5-H), 9.11 (d, J = 8.6, 4-H), 9.34 (s, 10-H), 9.48 (s, 8-H); EI-MS m/z (rel intensity) 313 (100, M^+), 267 (14, $M^+ - NO_2$), 221 (39), 209 (20), 191 (8), 175 (26), 174 (26), 163 (58).

2,6,9-Trinitrophenanthrene (2,6,9-TNPH, fraction 6a, 157.4 mg, 9.0% yield): pale yellow powder; mp 288–290 °C; ^{1}H NMR δ 8.69 (d, J = 9.3, 7-H), 8.75 (d, J = 9.1, 3-H), 8.81 (d, J = 9.2, 8-H), 8.83 (s, 10-H), 9.01 (d, J = 9.1, 4-H), 9.05 (s, 1-H), 9.72 (s, 5-H); EI-MS m/z (rel intensity) 313 (100, M^+), 237 (8), 221 (31), 209 (13), 191 (14), 175 (57), 163 (45).

3,6,9-Trinitrophenanthrene (3,6,9-TNPH, fraction 6b, 161.4 mg, 9.2% yield): white powder; mp 285–287 °C; ^{1}H NMR δ 8.33 (d, J = 8.7, 1-H), 8.65 (d, J = 8.7, 2-H), 8.67 (d, J = 9.2, 7-H), 8.74 (s, 10-H), 8.76 (d, J = 9.2, 8-H), 9.71 (s, 4-H), 9.72 (s, 5-H); EI-MS m/z (rel intensity) 313 (100, M^+), 267 (7, $M^+ - NO_2$), 221 (30), 191 (16), 175 (51), 163 (43).

2,6,10-Trinitrophenanthrene (2,6,10-TNPH, fraction 6c, 43.0 mg, 2.4% yield): white powder; mp 261 °C; ^{1}H NMR δ 8.33 (d, J = 8.8, 8-H), 8.66 (d, J = 8.8, 7-H), 8.72 (d, J = 9.2, 3-H), 8.74 (s, 9-H), 9.05 (d, J = 9.2, 4-H), 9.54 (s, 1-H), 9.68 (s, 5-H); EI-MS m/z (rel intensity) 313 (100, M^+), 255 (9), 221 (32), 191 (10), 175 (38), 163 (35).

2,7,9-Trinitrophenanthrene (2,7,9-TNPH, fraction 6d, 3.1 mg, 0.18% yield): white powder; mp 258–260 °C; ^{1}H NMR δ 8.69 (d, J = 9.6, 6-H), 8.73 (d, J = 9.4, 3-H), 8.83 (s, 10-H), 8.96 (d, J = 9.4, 4-H), 9.01 (d, J = 9.6, 5-H), 9.05 (s, 1-H), 9.57 (s, 8-H); EI-MS m/z (rel intensity) 313 (100, M^+), 267 (25), 255 (22), 237 (9), 221 (40), 209 (31), 175 (41), 163 (58).

Electrochemical Reduction by Cyclic Voltammetry. In dimethylformamide, tetraethylammonium perchlorate was used as the supporting electrolyte at a 0.1 M concentration. The reference electrode was an Ag/Ag^+ electrode in acetonitrile with 0.1 M tetrabutylammonium perchlorate. After transfer of the solution containing the test chemical to the cell, it was purged of oxygen by bubbling with N_2 for 15 min. The cyclic voltammograms were recorded at a scan rate of 100 mV/s while maintaining the test solution under a steady stream of N_2.

Electronic Descriptors. The electronic descriptor, LUMO energy levels, of nitrated phenanthrenes was calculated by "PASOCON MOPAC/386 ver. 6.03", which is based on the MOPAC (ver. 6.0, Quantum Chemistry Program Exchange No. 455), of Toray System Center by using the AM1 method. The initial geometries were constructed from standard bond lengths and angles. The geometries were then completely optimized using algorithms in the MOPAC program.

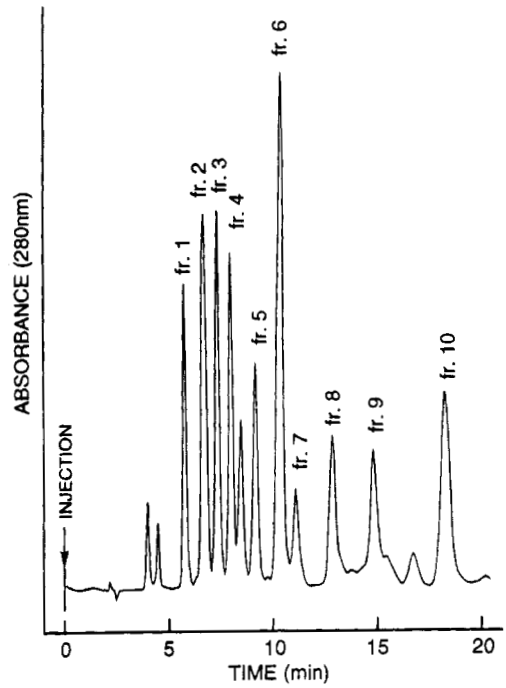

Figure 1. Chromatogram obtained by HPLC separation of the dinitrated phenanthrenes mixture. HPLC conditions are described in the Experimental Section.

Results and Discussion

To obtain a DNPH mixture, nitration of phenanthrene with fuming nitric acid was carried out in Ac_2O at 0 °C. From the reaction mixture, a crude fraction of DNPHs was obtained by silica gel column chromatography eluted by n-hexane/CH_2Cl_2 (1:1) in a yield of 75.0%. The isomeric mixture was separated by using preparative HPLC eluted by n-hexane/benzene (1:1), and 10 fractions were obtained which were determined as DNPHs by NMR and MS spectrometries. The elution profile and the collected fraction numbers corresponding to the peaks are shown in Figure 1. Among the separated fractions, fractions 1, 3, 5, 7, 8, and 9 consisted of the pure respective isomers of DNPHs, but the other fractions (2, 4, 6, and 10) were mixtures of DNPHs. These fractions were each rechromatographed by preparative HPLC as described in the Experimental Section, and fractions 2a, 2b, 4, 6, and 10 were obtained as pure isomers. Finally, 11 DNPHs, shown in Figure 2, were obtained as pure isomers. The total yield of DNPHs was 62.6%.

Nitration of phenanthrene with fuming HNO_3 without solvent yielded a mixture of TNPHs. Their separation was also performed by preparative HPLC. The elution profile is shown in Figure 3. Nine TNPHs, shown in Figure 4, were obtained in 46.5% total yield as pure isomers.

The structures of the DNPHs and TNPHs were determined as follows. In the case of the DNPHs, 25 isomers are theoretically possible. Among them, 1,2-DNPH, 1,3-DNPH, 1,4-DNPH, 2,3-DNPH, 2,4-DNPH, 3,4-DNPH, and 9,10-DNPH, in which two nitro groups are on the same benzo ring, and 4,5-DNPH, in which two nitro groups are in the bay region, were ruled out since their

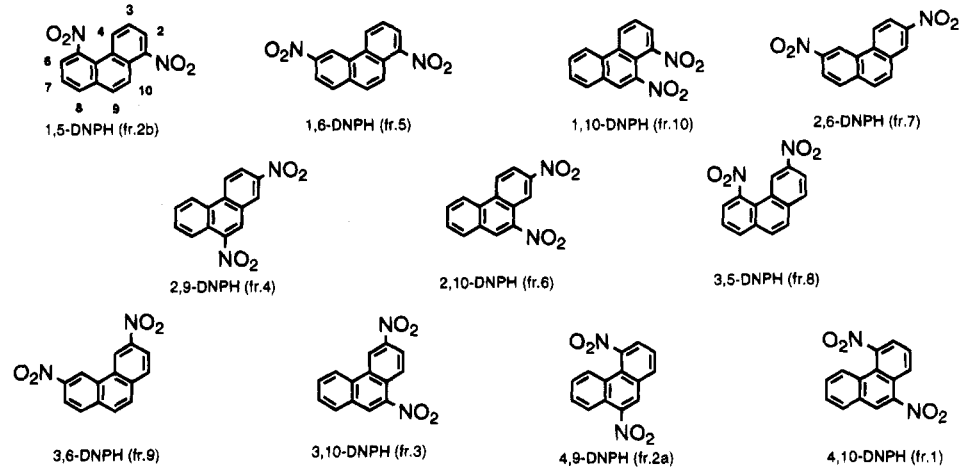

Figure 2. Structures of dinitrophenanthrenes.

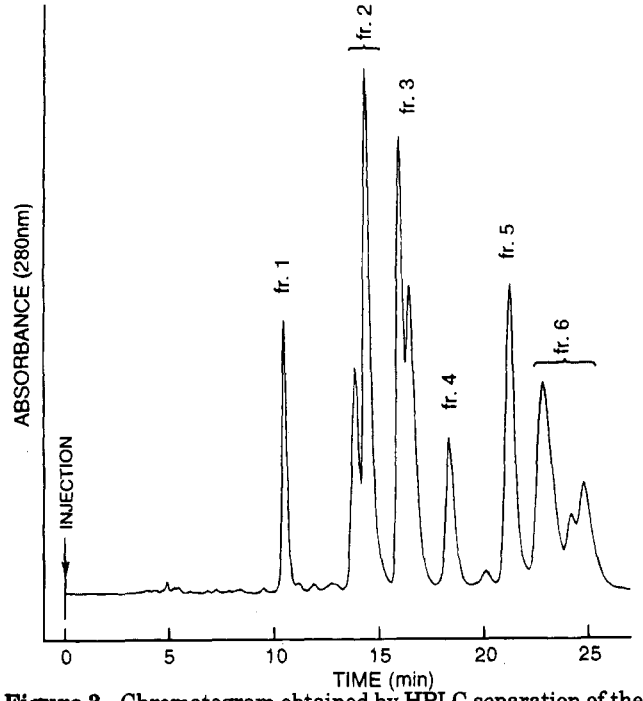

Figure 3. Chromatogram obtained by HPLC separation of the trinitrated phenanthrenes mixture. HPLC conditions are described in the Experimental Section.

formation is thought to be electronically and sterically unfavorable. The remaining 17 structures were considered as possible structures. To determine the isomeric structures of the DNPHs, NMR (^{1}H and ^{1}H–^{1}H COSY) spectroscopy was used as a decisive tool, because protons on the phenanthrene ring were characteristically shifted by the nitro substituent. In Table 1, the observed chemical shifts of the aromatic protons of phenanthrene and four mononitrophenanthrenes (1-NPH, 2-NPH, 3-NPH, and 9-NPH) are summarized. The incremental values (ppm from phenanthrene protons, +: downfield, −: upfield) are also listed in parentheses. Since we do not have an authentic sample of 4-NPH and since there is no reported NMR data on 4-NPH, the chemical shifts and the incremental values of the aromatic protons of 4-NPH were estimated from the data of 4,9-DNPH, 4,-10-DNPH, and 1,5-DNPH based on the assumption that the additivity of chemical shifts can be used to calculate the approximate shifts for DNPHs. The calculated incremental values and the chemical shifts for all protons of 4-NPH are also listed in Table 1 written in italics. By using these incremental values and the principle of additivity of chemical shifts, the chemical shifts of the aromatic protons of each DNPH were calculated, e.g., the calculated chemical shift, 8.16 ppm, for H_5 in 4,9-DNPH was obtained from the calculation of 8.69 + (−0.61) + 0.08 ([the observed δ value of H_5 in phenanthrene] + [the calculated incremental value for H_5 in 4-NPH] + [the calculated incremental value for H_5 in 9-NPH]). This value, 8.16, was close to the observed value, 8.20. All of the calculated chemical shifts are shown in Table 2 in

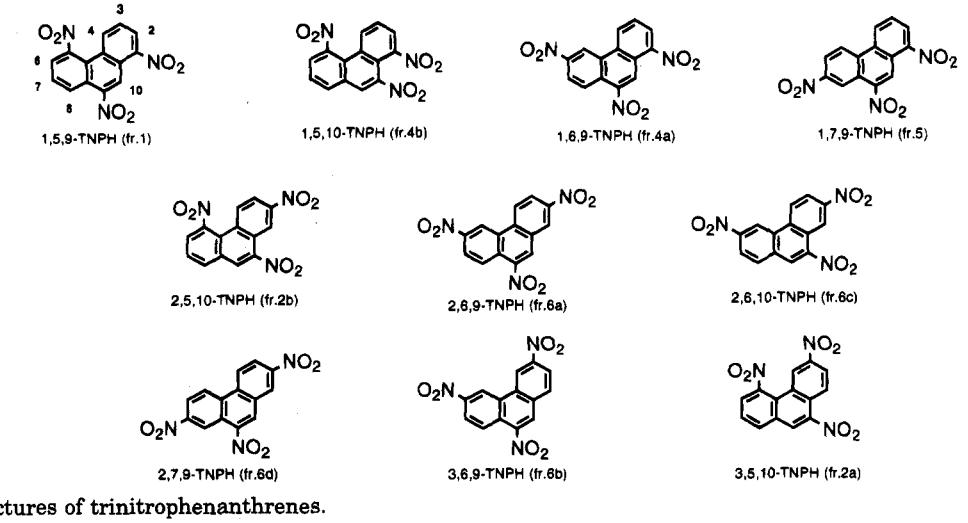

Figure 4. Structures of trinitrophenanthrenes.

Table 1. Proton NMR Spectral Data of Phenanthrene and Mononitrophenanthrenes

compound	yield (%)	mp (°C)	chemical shift (ppm)[a]									
			H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9	H_{10}
phenanthrene			7.89	7.60	7.65	8.69	8.69	7.65	7.60	7.89	7.74	7.74
1-NPH	5.9	131[b]		8.20 (+0.60)	7.73 (+0.08)	8.98 (+0.29)	8.70 (+0.01)	7.76 (+0.11)	7.70 (+0.10)	7.96 (+0.07)	7.97 (+0.23)	8.31 (+0.57)
2-NPH[c]		121[d]	8.79 (+0.90)		8.42 (+0.77)	8.78 (+0.09)	8.70 (+0.01)	7.74 (+0.09)	7.74 (+0.14)	7.96 (+0.07)	7.88 (+0.14)	7.85 (+0.11)
3-NPH	6.4	175[e]	8.03 (+0.14)	8.41 (+0.81)		9.63 (+0.94)	8.78 (+0.09)	7.77 (+0.12)	7.73 (+0.13)	7.98 (+0.09)	7.97 (+0.23)	7.83 (+0.09)
4-NPH[f]		–[g]	*7.91 (+0.02)*	*7.63 (+0.03)*	*8.03 (+0.38)*		*8.08 (−0.61)*	*7.62 (−0.03)*	*7.64 (+0.04)*	*7.81 (−0.08)*	*7.80 (+0.06)*	*7.73 (−0.01)*
9-NPH	14.9	116[h]	8.02 (+0.13)	7.78 (+0.18)	7.78 (+0.13)	8.71 (+0.02)	8.77 (+0.08)	7.78 (+0.13)	7.78 (+0.18)	8.51 (+0.62)		8.48 (+0.74)

[a] Incremental shifts (ppm) from phenanthrene are listed in parentheses (+: downfield, −: upfield). [b] Lit. mp 133 °C (*32*). [c] Gift from *Dr.* Hirayama. [d] Lit. mp 119−120 °C (*32*). [e] Lit. mp 172−174 °C (*32*). [f] Chemical shifts and incremental shifts written in italics were calculated from the data of 4,9-DNPH, 4,10-DNPH, and 1,5-DNPH. A typical example for the calculation of the largest incremental value, −0.61 ppm, for H_5 in 4-NPH is as follows: (1) The incremental value, +0.08, for H_5 on the replacement of a hydrogen atom by the nitro substituent at the 9-position of phenanthrene was obtained by the subtraction of 8.69 from 8.77 ([the δ value of H_5 in 9-NPH] − [the δ value of H_5 in phenanthrene]). (2) The tentative incremental value, −0.49, for H_5 in 4,9-DNPH was obtained by the calculation of 8.20 minus 8.69 ([the δ value of H_5 in 4,9-DNPH] − [the δ value of H_5 in phenanthrene]). (3) Based on the assumption that this value, −0.49, was the sum of the shift values for H_5 by the nitro substitution on both the 4- and 9-positions, the calculated incremental value, −0.57, for H_5 by the nitro substitution at the 4-position of phenanthrene was obtained by the subtraction of +0.08 from −0.49 ([the tentative incremental value for H_5 in 4,9-DNPH] − [the incremental value for H_5 in 9-NPH]. (4) Two other values, −0.62 and −0.63, for H_5 were similarly obtained from the two sets of NMR data: [4,10-DNPH, 4-NPH, phenanthrene] and [1,5-DNPH, 5-NPH (synonym of 4-NPH), phenanthrene], respectively. (5) Finally, the average of three values [−0.61 = [(−0.57) + (−0.62) + (−0.63)]/3] was adopted as the incremental shift value for H_5 in 4-NPH. [g] The synthesis of 4-NPH from β-2-naphthoylpropionic acid was reported: lit. mp 82 °C (*32*). [h] Lit. mp 118 °C (*32*).

Table 2. Proton NMR Spectral Data of Dinitrophenanthrenes

compound	yield (%)	mp (°C)	chemical shift (ppm)[a]									
			H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9	H_{10}
1,5-DNPH	2.8	139		8.24 (8.24)	7.68 (7.70)	8.36 (8.37)		8.13 (8.14)	7.76 (7.73)	7.91 (7.98)	7.99 (7.96)	8.40 (8.37)
1,6-DNPH	5.1	248		8.33 (8.33)	7.90 (7.85)	9.08 (9.07)	9.65 (9.64)		8.52 (8.51)	8.13 (8.10)	8.07 (8.06)	8.56 (8.54)
1,10-DNPH	8.0	260		8.32 (8.38)	7.90 (7.86)	9.04 (9.06)	8.74 (8.72)	7.96 (7.89)	7.85 (7.88)	8.13 (8.09)	8.66 (8.71)	
2,6-DNPH	2.8	264−266	8.90 (8.88)		8.57 (8.54)	8.93 (8.87)	9.65 (9.64)		8.54 (8.55)	8.13 (8.10)	8.00 (7.97)	8.10 (8.08)
2,9-DNPH	5.3	216	8.94 (8.92)		8.60 (8.55)	8.88 (8.80)	8.83 (8.78)	7.93 (7.87)	7.90 (7.92)	8.51 (8.58)		8.55 (8.59)
2,10-DNPH	14.2	207−209	9.56 (9.41)		8.57 (8.55)	8.92 (8.86)	8.77 (8.72)	7.97 (7.87)	7.87 (7.92)	8.14 (8.09)	8.73 (8.62)	
3,5-DNPH	4.6	159	8.08 (7.95)	8.46 (8.45)		9.10 (9.02)		8.14 (8.15)	7.76 (7.76)	7.91 (8.00)	7.99 (7.96)	7.93 (7.89)
3,6-DNPH	6.6	284	8.13 (8.12)	8.53 (8.54)		9.67 (9.72)	9.67 (9.72)		8.53 (8.54)	8.13 (8.12)	8.05 (8.06)	8.05 (8.06)
3,10-DNPH	6.9	243	8.78 (8.65)	8.55 (8.59)		9.69 (9.71)	8.83 (8.80)	8.00 (7.90)	7.86 (7.91)	8.15 (8.11)	8.76 (8.71)	
4,9-DNPH	3.2	218	7.97 (8.04)	7.78 (7.81)	8.19 (8.16)		8.20 (8.16)	7.74 (7.75)	7.84 (7.82)	8.44 (8.43)		8.43 (8.47)
4,10-DNPH	3.1	172	8.66 (8.53)	7.82 (7.81)	7.93 (8.16)		8.13 (8.10)	7.79 (7.75)	7.79 (7.82)	8.09 (7.94)	8.57 (8.54)	

[a] Calculated chemical shifts using incremental values of mononitrophenanthrenes are listed in parentheses.

Table 3. Proton NMR Spectral Data of Trinitrophenanthrenes

compound	yield (%)	mp (°C)	chemical shift (ppm)[a] H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9	H_{10}
1,5,9-TNPH	4.0	164–165		8.59 (8.41)	7.95 (7.81)	8.08 (8.37)		8.41 (8.25)	7.87 (7.90)	8.45 (8.59)		9.17 (9.13)
1,5,10-TNPH	5.8	229		8.41 (8.41)	7.85 (7.81)	8.37 (8.43)		8.31 (8.25)	7.92 (7.90)	8.10 (8.10)	8.67 (8.68)	
1,6,9-TNPH	8.2	256		8.53 (8.49)	8.11 (7.94)	9.16 (9.05)	9.73 (9.68)		8.66 (8.67)	8.70 (8.70)		9.37 (9.26)
1,7,9-TNPH	3.4	213–214		8.53 (8.48)	8.08 (8.01)	9.11 (9.01)	8.99 (8.92)	8.68 (8.67)		9.48 (9.53)		9.34 (9.24)
2,5,10-TNPH	2.5	292–293	9.12 (9.41)		8.59 (8.55)	8.27 (8.31)		8.63 (8.33)	7.95 (7.92)	8.08 (8.11)	8.57 (8.67)	
2,6,9-TNPH	9.0	288–290	9.05 (9.06)		8.75 (8.68)	9.01 (8.94)	9.72 (9.72)		8.69 (8.69)	8.81 (8.71)		8.83 (8.80)
2,6,10-TNPH	2.4	261	9.54 (9.55)		8.72 (8.68)	9.05 (9.00)	9.68 (9.66)		8.66 (8.69)	8.33 (8.22)	8.74 (8.77)	
2,7,9-TNPH	0.2	258–260	9.05 (9.05)		8.69 (8.75)	9.01 (8.90)	8.96 (8.96)	8.73 (8.75)		9.57 (9.54)		8.83 (8.78)
3,5,10-TNPH	1.8	223–225	8.32 (8.58)	8.54 (8.62)		8.99 (9.08)		8.68 (8.26)	7.93 (7.93)	8.06 (8.12)	8.66 (8.68)	
3,6,9-TNPH	9.2	285–287	8.33 (8.23)	8.65 (8.70)		9.71 (9.70)	9.72 (9.76)		8.67 (8.70)	8.76 (8.72)		8.74 (8.78)

[a] Calculated chemical shifts using incremental values of mononitrophenanthrenes are listed in parentheses.

Table 4. First Reduction Potentials (E_{pc}), LUMO Energy Levels, and Dihedral Angles of Mono-, Di-, and Trinitrophenanthrenes

compound	E_{pc}	LUMO	dihedral angles (deg) 1(8)-	2(7)-	3(6)-	4(5)-	9(10)-
1-NPH	−1477	−1.345	16.9				
2-NPH	−1466	−1.300		2.5			
3-NPH	−1453	−1.367			0.3		
4-NPH		−1.065				85.4	
9-NPH	−1450	−1.413					11.1
1,5-DNPH	−1373	−1.644	39.2			85.8	
1,6-DNPH	−1357	−1.937	19.0		0.1		
1,10-DNPH	−1336	−1.767	63.0				64.5
2,6-DNPH	−1365	−1.836		1.9	0.9		
2,9-DNPH	−1311	−1.908		1.7			16.2
2,10-DNPH	−1368	−1.901		1.0			14.7
3,5-DNPH	−1363	−1.735			1.3	86.0	
3,6-DNPH	−1341	−1.955			0.5,0.5		
3,10-DNPH	−1242	−2.006			2.3		16.5
4,9-DNPH	−1277	−1.883				88.1	24.6
4,10-DNPH	−1297	−1.850				86.4	23.8
1,5,9-TNPH	−1063	−2.345	38.0			84.0	29.6
1,5,10-TNPH	−1150	−2.228	53.1			82.2	56.5
1,6,9-TNPH	−1058	−2.528	19.6		2.87		29.2
1,7,9-TNPH	−1058	−2.422	20.7	9.1			28.0
2,5,10-TNPH	−1050	−2.186		6.5		83.6	84.2
2,6,9-TNPH	−1116	−2.432		0.5	1.5		33.7
3,5,10-TNPH	−1139	−2.394			8.0	87.9	26.2
3,6,9-TNPH	−1042	−2.643			0.74,1.19		20.5

parentheses. By using these calculated values, we assigned all of the chemical structures of DNPs as shown in Figure 2. While the observed chemical shifts were widely dispersed from 7.68 to 9.69 ppm, the observed and calculated values were in good correlation and the differences were all less than 0.15 ppm. The observed $^1H-^1H$ coupling patterns and the $^1H-^1H$ correlations confirmed by COSY NMR spectra also supported these assignments.

The structures of TNPHs shown in Figure 4 were also confirmed by using NMR spectroscopy. The observed and calculated chemical shifts are listed in Table 3. Although the differences between the observed and calculated chemical shifts were somewhat larger that those of the DNPHs, the observed $^1H-^1H$ coupling patterns and the $^1H-^1H$ correlations confirmed by COSY NMR spectra were more useful since they were simpler than those of the DNPHs.

Both nitro reduction and ring oxidation are the main metabolic pathways of nitrated PAHs. Especially, nitro reduction is closely related to the metabolic activation of nitrated PAHs in bacterial systems (*15–17, 36*). Since the ease of the enzymatic reduction can be estimated by the ease of electrochemical reduction (*21, 37–39*), the reduction potentials of the NPHs, DNPHs, and TNPHs were measured by cyclic voltammetry. In Table 4, the first redox potentials ($E_{1/2}$) of NPHs, DNPHs, and TNPHs in dimethylformamide are summarized with the LUMO energy levels calculated by the AM1 method. The potentials (mV) of the DNPHs and TNPHs were in the range from −1242 to −1373 and from −1042 to −1150, respectively, while those of the NPHs were from −1450 to −1477. These results show that nitro substitution causes about a 100–300 mV positive shift of the reduction potentials. The calculated LUMO energy levels were in good correlation ($r^2 = 0.900$) with the observed reduction potentials among these nitrated phenanthrenes.

The orientation of the nitro substituent to the phenanthrene ring was also estimated from the results of chemical calculations by the AM1 method in complete optimization. The dihedral angles of the nitro substituents to the phenanthrene ring are shown in Table 4. As expected, nitro substituents on positions 4 and 5 were perpendicular due to the steric effect of the bay region aromatic proton, while those on positions 2, 3, 6, and 7 were nearly coplanar to the phenanthrene ring. The nitro groups on positions 1, 8, 9, and 10 were noncoplanar because of the steric hindrance of the aromatic proton on the peri position, and the calculated dihedral angles varied between 10° and 65°.

The orientation of the nitro group and the LUMO energy level generally predict the direct-acting bacterial mutagenicity of the nitrated PAHs (*20, 21*). Nitrated PAHs, which have a nitro function with perpendicular or nearly perpendicular orientation to the aromatic ring system, show little or no direct-acting mutagenicity in

either the *S. typhimurium* strain TA98 or TA100. The mutagenic potency of nitrated PAHs without a perpendicular nitro substituent is related to the energy of their LUMO level. The reported mutagenicities of three nitrated phenanthrenes (2-NPH, 9-NPH, and 2,7-DNPH) in *S. typhimurium* TA98 without the S9 mixture were 326.9, 176.0, and 3934.6 revertants/nmol, respectively (*26*). These potencies can be interpreted by the above structural and electronic features. Among the newly synthesized DNPHs and TNPHs, six DNPHs (1,6-DNPH, 2,6-DNPH, 2,9-DNPH, 2,10-DNPH, 3,6-DNPH, and 3,-10-DNPH) and four TNPHs (1,6,9-TNPH, 1,7,9-TNPH, 2,6,9-TNPH, and 3,6,9-TNPH), which have no nitro substituents perpendicular to the phenanthrene ring system and have relatively lower LUMO energy levels, are expected to be highly or moderately mutagenic.

Our approach to prepare DNPHs and TNPHs by the direct nitration of phenanthrene followed by HPLC separation afforded eleven DNPHs and nine TNPHs. The mutagenicity of these nitrated phenanthrenes is under study. Since it is quite possible that these nitrated phenanthrenes exist as environmental pollutants, further study to detect them in airborne particulates is also in progress.

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