Journal of Chromatography, 642 (1993) 107–116
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2689

Relationships of structures of nitro-polycyclic aromatic hydrocarbons with high-performance liquid chromatography retention order

Peter P. Fu*

National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079 (USA)

Yongming Zhang

Department of Animal Science, Shanghai, Agricultural College, Shanghai (China)

Yuh-Lin Mao

Department of Chemistry, Soochow University, Shihlin, Taipei (Taiwan)

Linda S. Von Tungeln

National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079 (USA)

Youngmee Kim and Hyewook Jung

National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079 (USA) and Department of Chemistry, Yonsei University, Seoul (South Korea)

Moo-Jin Jun

Department of Chemistry, Yonsei University, Seoul (South Korea)

ABSTRACT

Forty six structurally related nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) and their corresponding parent PAHs were employed to study the relationships between structure and HPLC retention time. Using reversed-phase HPLC, larger molecules had longer retention times, while saturation of the aromatic rings shortened the retention time. Isomers with a perpendicular nitro group had shorter retention times than if the nitro substituent was parallel to the ring system. The addition of a nitro group caused a substantial decrease in retention time when compared to its parent PAH. When using normal-phase HPLC, an additional benzo ring increased the retention time. The presence of one or two nitro groups on the molecule also increased the retention time, while saturation of a benzo ring decreased the retention time. These results suggest that the polarity of the PAH or nitro-PAH is the principal factor for determining its HPLC retention time.

INTRODUCTION

Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are a class of mutagenic and carcinogenic environmental pollutants [1–8]. Since nitro-PAHs may pose adverse effect to human health, it is necessary to identify the toxic nitro-PAHs present in the environment, to study their metabolic activation, and to determine their genotoxic effects. HPLC has been the major analytical tool in the biological stud-

* Corresponding author.

ies of nitro-PAHs, including separation of the metabolites and nucleoside adducts [5,7,8]. The metabolites separated include epoxides, *trans*-dihydrodiols, phenolic products, quinones, tetrahydrotetrols, nitroso-PAHs and amino-PAHs [7]. However, to date, HPLC has been used much less frequently for separating the parent nitro-PAHs present in environmental mixtures [2,9–11]. This may be due to the fact that geometric isomers of nitro-PAHs, such as 1-nitropyrene and 2-nitropyrene, are generally difficult to separate by conventional analytical methods, including HPLC [2,9–11]. Thus, little has been known concerning the relationships between structure and HPLC retention time of nitro-PAHs [2,11]. It has been reported that certain structural features of nitro-PAHs can affect the biological activities, such as mutagenicity and tumorigenicity, as well as the chemical properties of these compounds [5–7,12–16]. Among these, orientation of the nitro group has been found to be a decisive structural feature [6,7]. It is known that when a compound is eluted from an HPLC column, polarity and molecular size of the molecule are important factors in determining the HPLC retention time [2,11–14]. Polarity is largely related to the type, number, and location of the functional group(s) in the molecule. Nitro orientation would also alter polarity and therefore affect HPLC retention times of nitro-PAHs. In order to determine the relationships between structures and HPLC retention times, 46 nitro-PAHs were chosen for this study and several different HPLC columns (reversed-phase and normal-phase; monomeric, polymeric, and chiral stationary phase) were employed.

EXPERIMENTAL

Materials

The following PAHs and nitro-PAHs were purchased from Aldrich (Milwaukee, WI, USA): naphthalene (N), biphenyl (Bp), fluorene (F), anthracene (A), phenanthrene (Ph), 9,10-dihydrophenanthrene (H_2-Ph), pyrene (Py), 1,2,3,6,7,8-hexahydropyrene (H_6-Py), benz[*a*]anthracene (BA), chrysene (Ch), benzo[*a*]pyrene (BaP), benzo[*e*]pyrene (BeP), 1-nitronaphthalene (1-N-N), 2-nitronaphthalene (2-N-N), 4-nitrobiphenyl (4-N-Bp), 2-nitrofluorene (2-N-F), 9-nitrophenanthrene (9-N-Ph), 1-nitropyrene (1-N-Py), and 3,4-dihydrobenz[*a*]anthracen-1(2H)-one. 4,5-Dihydrobenzo[*a*]pyrene (H_2-BaP), 7,8,9,10-tetrahydrobenzo[*a*]pyrene (H_4-BaP), 1-nitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene (1-N-H_4-BaP), 3-N-H_4-benzo[*a*]pyrene, 6-N-H_4-benzo[*a*]pyrene, 1-nitrobenzo[*a*]pyrene (1-N-BaP), 3-nitrobenzo[*a*]pyrene (3-N-BaP), 6-nitrobenzo[*a*]pyrene (6-N-BaP), 9,10,11,12-tetrahydrobenzo[*e*]pyrene (H_4-Bep), 1-nitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene (1-N-H_4-BeP), 3-N-H_4-benzo[*e*]pyrene, 4-N-H_4-benzo[*e*]pyrene, 1,2,3,6,7,8,9,10,11,12-decahydrobenzo[*e*]pyrene (H_{10}-BeP), 4-nitro-1,2,3,6,7,8,9,10,11,12-decahydrobenzo[*e*]pyrene (4-N-H_{10}-BeP), 1-nitrobenzo[*e*]pyrene (1-N-BeP), 3-nitrobenzo[*e*]pyrene (3-N-Bep), 4-nitrobenzo[*e*]pyrene (4-N-Bep), 1,3-dinitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene (1,3-Di-N-H_4-BeP), 1,6-dinitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene (1,6-Di-N-H_4-BeP), 1,8-dinitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene (1,8-Di-N-H_4-BeP), 1,3-dinitrobenzo[*e*]pyrene (1,3-Di-N-BeP), 1,6-dinitrobenzo[*e*]pyrene (1,6-Di-N-BeP) and 1,8-dinitrobenzo[*e*]pyrene (1,8-Di-N-BeP) were prepared as previously described [12–15,17]. 4-Nitro-1,2,3,6,7,8-hexahydropyrene (4-N-H_6-Py) was synthesized by nitration of 1,2,3,6,7,8-hexahydropyrene (H_6-Py) with sodium nitrate in trifluoroacetic acid, a modified method of Spitzer and Stewart [18] and 4-nitropyrene (4-N-Py) was prepared by dehydrogenation of 4-N-H_6-Py with 2,3-dichloro-5,6-dicyano-1,6-benzoquinone [19,20]. 4,5,9,10-Tetrahydropyrene (H_4-Py) was synthesized by hydrogenation of pyrene (Py) catalyzed by palladium on charcoal [21]. 4,5,7,8,9,10,11,12-Octahydrobenzo[a]pyrene (H_8-BaP) was similarly synthesized from H_4-BaP [21]. 9-Nitroanthracene (9-N-A) [14], 7-nitrobenz[*a*]anthracene (7-N-BA) [22], 6-nitrochrysene (6-N-Ch), 2-nitro-9,10-dihydrophenanthrene (2-N-H_2-Ph), 2-nitro-4,5,9,10-tetrahydropyrene (2-N-H_4-Py) [23], 2-nitro-4,5,7,8,9,10,11,12-octahydro-BaP (2-N-H_8-BaP), 7-nitrodibenz[*a,h*]anthracene (7-N-DiB[*a,h*]A), and 9-nitrodibenz[*a,c*]anthracene (9-N-DiB[*a,c*]A) were similarly prepared by nitration of anthracene, benz[*a*]anthracene (BA), chrysene (Ch), 9,10-dihydrophenanthrene (H_2-Ph), 4,5,9,10-tetrahydropyrene (H_4-Py), 4,5,7,8,9,10,11,12-octahydrobenzo[*a*]pyrene (H_8-BaP), dibenz[*a,h*]anthracene (DiB[*a,h*]A), and dibenz[*a,c*]anthracene (DiB[*a,c*]A), respectively, with sodium nitrate in trifluoroacetic acid. Nitration of 4,5-dihydro-BaP gave 6-

nitro-4,5-dihydrobenzo[*a*]pyrene (6-N-H_2-BaP) as a major product, and 12-nitro-4,5-dihydrobenzo[*a*]pyrene (12-N-H_2-BaP) and 1,6-dinitro-4,5-dihydrobenzo[*a*]pyrene (1,6-Di-N-H_2-BaP) as minor products [24]. Nitration of 7,8,9,10-tetrahydrobenzo[*a*]pyrene with two equivalent molar ratio of sodium nitrate in trifluoroacetic acid yielded 1,3-dinitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene (1,3-Di-N-H_4-BaP), 1,6-dinitro-7,8,9,10-tetrahydrobenzo[*a*]-pyrene (1,6-Di-N-H_4-BaP) and 3,6-dinitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene (3,6-Di-N-H_4-BaP) as major products and 1,3,6-trinitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene (1,3,6-Tri-N-H_4-BaP) as a minor product [24]. 2-Nitroanthracene (2-N-A) was obtained as a gift from Dr. F. A. Beland. 1,3-Dinitropyrene (1,3-Di-N-Py), 1,6-dinitropyrene (1,6-Di-N-Py), and 1,8-dinitropyrene (1,8-Di-N-Py) were prepared by nitration of pyrene with two equivalent amounts of sodium nitrate in trifluoroacetic acid, and were separated by a normal-phase HPLC system employing a Zorbax SIL semi-preparative column (250 × 9.4 mm I.D.), eluted with hexane–methanol–acetonitrile (72:2:1, v/v/v) at a flow-rate of 5.6 ml/min. Under these conditions, 1,3-dinitropyrene, 1,6-dinitropyrene, and 1,8-dinitropyrene eluted at 10.6, 12.8, and 17.0 min, respectively. All the known compounds, either purchased or synthesized, were characterized by comparison of their UV–visible absorption and mass spectra with the published data. For the identification of the new compounds, high-resolution nuclear magnetic resonance spectral analysis was employed. The orientation of the nitro substituents of each compound were also characterized by spectral analysis [12–16,23,24].

Chromatography

The HPLC system was composed of two Beckman/Altex (Fullerton, CA, USA) Model 100 pumps, a Beckman/Altex 420 gradient controller, a Beckman/Altex Model 210 injector, a Waters Assoc. (Milford, MA, USA) Model 440 absorbance detector set at 254 nm, a Kipp & Zonen (Delft, Netherlands) Model BD41 dual-pen strip-chart recorder, and optionally, a Hewlett-Packard (Palo Alto, CA, USA) Model 3390A reporting integrator, or with a Hewlett-Packard 1040A detection system with the Data Processing Unit option. The columns used were: Zorbax ODS (250 × 4.6 mm I.D.) (DuPont Medical Products, Wilmington, DE, USA); Zorbax SIL (250 × 4.6 mm I.D.); Vydac 201TP54 ODS (250 × 4.6 mm I.D.) (The Separations Group, Hesperia, CA, USA) and a Pirkle-type chiral stationary phase column (250 × 4.6 mm I.D.) (Regis, Morton Grove, IL, USA) packed with (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine covalently bonded to spherical particles of γ-aminopropylsilanized silica. All columns have a particle size of 5 μm. All mobile phases, as described in Tables I and II, were HPLC-grade solvents and were degassed before use. Each injection was 20 μl or less in volume. The retention time was recorded by the 1040A detection system, and the ultra violet absorption spectrum of the material in each chromatographic peak was recorded, so that the identity of the chromatographic peak could be confirmed. After the retention time of each compound had been determined, a mixture of two or more compounds was chromatographed in order to confirm the relative elution order and reproducibility of retention times. To eliminate the possible UV photolytic decomposition of the compounds, the laboratory was equipped with UV-absorbing films placed above the light diffusion panels.

RESULTS AND DISCUSSION

In this paper, we employ a series of structurally-related nitro-PAHs to study the relationships between structure and HPLC retention. For determining the effect of the nitro substituent on HPLC retention time, the HPLC retention times of the corresponding parent PAHs were also determined. The structures and abbreviations of the parent PAHs and nitro-PAHs used in this study are shown in Fig. 1.

It has been previously reported that, depending on the geometric location of the nitro substituent, one of two orientations will predominate [6,7,12]. Orientation of the nitro group can be well determined by spectral analysis of the compounds, including uv-visible absorption, mass and proton nuclear magnetic resonance (NMR) spectra [6,7,25–27]. Compounds of the first type have their nitro substituents perpendicular or nearly perpendicular to the aromatic moiety so that steric hindrance with the adjacent protons can be minimized. Nitro-PAHs of the second type have their nitro substituents parallel (co-planar) or nearly parallel to the

TABLE I

HPLC RETENTION TIMES OF POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR PARTIALLY SATURATED DERIVATIVES ON DIFFERENT REVERSED- AND NORMAL-PHASE COLUMNS

The eluents for the HPLC analyses are: methanol–water (90:10, v/v) at a flow-rate of 1.5 ml/min for the Zorbax ODS column; methanol–water (90:10, v/v) at a flow-rate of 1.0 ml/min for the Vydac ODS column; tetrahydrofuran–hexane (3:97) at a flow-rate of 3.0 ml/min for the Zorbax SIL column; and ethanol–acetonitrile–hexane (2:1:27, v/v/v) at a flow-rate of 1.0 ml/min for the Pirkle column.

Compound	Retention time (min)			
	Zorbax ODS	Vydac ODS	Zorbax SIL	Pirkle Ph-Gl
Naphthalene (N)	3.8	3.4	4.0	4.5
Biphenyl (Bp)	4.6	3.8	3.9	4.6
Fluorene (F)	5.7	4.4	4.3	4.9
Anthracene (A)	6.2	5.0	4.3	5.6
Phenanthrene (Ph)	5.8	4.4	4.1	5.8
9,10-Dihydrophenanthrene (H_2-Ph)	6.3	4.9	5.0	5.0
Pyrene (Py)	8.2	6.3	6.5	6.8
4,5,9,10-Tetrahydropyrene (H_4-Py)	9.0	5.8	3.97	4.3
1,2,3,6,7,8-Hexahydropyrene (H_6-Py)	14.0	6.7	3.99	4.1
Chrysene (Ch)	10.6	11.1	5.0	7.1
Benz[*a*]anthracene (BA)	10.5	11.2	4.9	6.9
Benzo[*a*]pyrene (BaP)	16.3	12.3	3.9	7.9
4,5-Dihydrobenzo[*a*]pyrene (H_2-BaP)	17.7	16.2	2.9	6.2
7,8,9,10-Tetrahydro-BaP (H_4-BaP)	27.4	22.7	3.98	6.4
4,5,7,8,9,10,11,12-Octahydro-BaP (H_8-BaP)	28.7	23.7	3.96	4.0
Benzo[*e*]pyrene (BeP)	14.7	13.9	6.4	8.3
9,10,11,12-Tetrahydro-BeP (H_4-BeP)	27.0	18.5	3.79	7.9
1,2,3,6,7,8,9,10,11,12-Decahydro-BeP (H_{10}-BeP)	43.6	18.8	3.78	7.7
Dibenz[*a,c*]anthracene (DiB[*a,c*]A)	18.5	14.2	5.2	9.2
Dibena[*a,h*]anthracene (DiB[*a,h*]A)	19.0	14.3	5.0	9.3

TABLE II

HPLC RETENTION TIMES OF NITRO-POLYCYCLIC AROMATIC HYDROCARBON DIHYDRODIOLS AND THEIR PARTIALLY SATURATED DERIVATIVES ON DIFFERENT REVERSED AND NORMAL PHASES

The eluents for the HPLC analyses are: methanol–water (90:10, v/v) at a flow-rate of 1.5 ml/min for the Zorbax ODS column; methanol–water (90:10, v/v) at a flow-rate of 1.0 ml/min for the Vydac ODS column; tetrahydrofuran–hexane (3:97, v/v) at a flow-rate of 3.0 ml/min for the Zorbax SIL column; and ethanol–acetonitrile–hexane (2:1:27, v/v/v) at a flow-rate of 1.0 ml/min for the Pirkle column.

Nitro-PAH	Nitro orientation	Retention time (min)			
		Zorbax ODS	Vydac ODS	Zorbax SIL	Pirkle Ph-Gly
1-N-N	Parallel	3.2	3.1	4.3	8.0
2-N-N	Parallel	3.4	3.5	4.2	7.7
4-N-H_6-Py	Parallel	11.2	5.3	6.43	4.9
4-N-H_{10}-BeP	Parallel	26.2	13.5	6.44	10.1
4-N-Bp	Parallel	3.8	3.6	6.15	7.6
2-N-F	Parallel	4.5	4.0	4.9	8.6
2-N-H_2-Ph	Parallel	5.3	4.2	3.24	5.0
2-N-H_4-Py	Parallel	6.6	5.3	11.2	6.8
2-N-H_8-BaP	Parallel	17.1	13.6	12.3	6.4

TABLE II (*continued*)

Nitro-PAH	Nitro orientation	Retention time (min)			
		Zorbax ODS	Vydac ODS	Zorbax SIL	Pirkle Ph-Gly
2-N-A	Parallel	5.3	4.7	4.45	8.8
9-N-A	Perpendicular	2.0	2.6	4.55	6.3
9-N-Ph	Parallel	5.8	4.0	5.9	10.2
6-N-Ch	Parallel	8.7	7.6	5.6	3.4
7-N-BA	Parallel	7.5	6.9	5.7	4.5
6-N-H_2-BaP	Perpendicular	8.5	9.2	3.86	7.8
12-N-H_2-BaP	Parallel	12.2	12.4	10.2	15.6
9-N-DiB[*a*,*c*]A	Perpendicular	10.5	9.3	9.3	12.8
7-N-DiB[*a*,*h*]A	Perpendicular	12.5	9.4	8.0	11.6
4-N-Py	Parallel	7.0	5.9	6.7	9.6
1-N-Py	Parallel	6.8	5.5	7.8	8.0
1-N-H_4-BaP	Parallel	20.1	20.5	5.0	10.5
3-N-H_4-BaP	Parallel	20.0	22.8	5.2	10.7
6-N-H_4-BaP	Perpendicular	16.6	15.0	5.54	10.8
1-N-H_4-BeP	Perpendicular	15.2	9.6	5.4	8.4
3-N-H_4-BeP	Parallel	23.5	16.2	4.63	10.8
4-N-H_4-BeP	Parallel	24.6	16.6	4.3	12.4
1-N-BaP	Parallel	14.0	9.4	8.02	14.0
3-N-BaP	Parallel	14.2	9.7	8.03	10.9
6-N-BaP	Perpendicular	11.9	8.4	6.99	14.5
1-N-BeP	Perpendicular	8.3	7.1	8.35	12.1
3-N-BeP	Parallel	13.0	12.6	6.98	13.0
4-N-BeP	Parallel	12.6	11.9	6.57	12.4
1,3-Di-N-Py	Parallel	6.6	5.4	10.6	4.6
1,6-Di-N-Py	Parallel	5.3	4.7	10.9	3.4
1,8-Di-N-Py	Parallel	5.5	4.9	13.3	3.2
1,3-Di-N-H_4-BaP	Parallel	14.0	10.5	4.0	12.5
1,6-Di-N-H_4-BaP	Mixed[a]	15.3	14.0	2.8	12.2
3,6-Di-N-H_4-BaP	Mixed	13.1	15.0	4.3	12.0
1,3-Di-N-H_4-BeP	Mixed	16.6	11.6	7.65	12.9
1,6-Di-N-H_4-BeP	Mixed	12.4	9.9	8.76	15.3
1,8-Di-N-H_4-BeP	Mixed	8.8	7.6	9.3	12.2
1,3,6-Tri-N-H_4-BaP	Mixed	16.6	15.1	3.76	9.2
1,6-Di-N-H_2-BaP	Mixed	8.7	9.9	10.1	15.5
1,3-Di-N-BeP	Mixed	6.7	10.1	8.06	11.8
1,6-Di-N-BeP	Mixed	7.4	6.6	5.0	12.3
1,8-Di-N-Bep	Perpendicular	5.5	4.4	5.2	12.0

[a] "Mixed" indicates that the nitro-PAH compound contains at least one nitro substituent with a parallel orientation and one nitro substituent with a perpendicular orientation.

aromatic moiety. Based on the analysis of the spectral data, the orientations of the nitro-PAHs are shown in Table II. In order to find out how nitro orientation can affect HPLC retention time, 46 nitro-PAHs of both types were chosen for this study. The compounds were selected on a structure comparison basis. For example, there are four sets of geometric mononitro-PAH isomers: (1) 1-N-BaP, 3-N-BaP, and 6-N-BaP; (2) 1-N-BeP, 3-N-BeP, and 4-N-BeP; (3) 1-N-H_4-BaP, 3-N-H_4-BaP, and 6-N-H_4-BaP; and (4) 1-N-H_4-BeP, 3-N-H_4-BeP, and 4-N-H_4-BeP (see Fig. 1). Each set contains the iso-

mers with different nitro orientations, and therefore, is relevant for determining the effect of nitro orientation on HPLC retention. To examine the effect of the nitro group(s) on HPLC retention, some representative parent PAHs were included for study.

To compare the separation capability of each type of column, a monomeric Zorbax ODS column, a polymeric Vydac ODS column, a normal-phase Zorbax SIL column, and a Pirkle-type chiral stationary-phase column were employed. The retention times of the parent PAHs and the nitro-PAHs

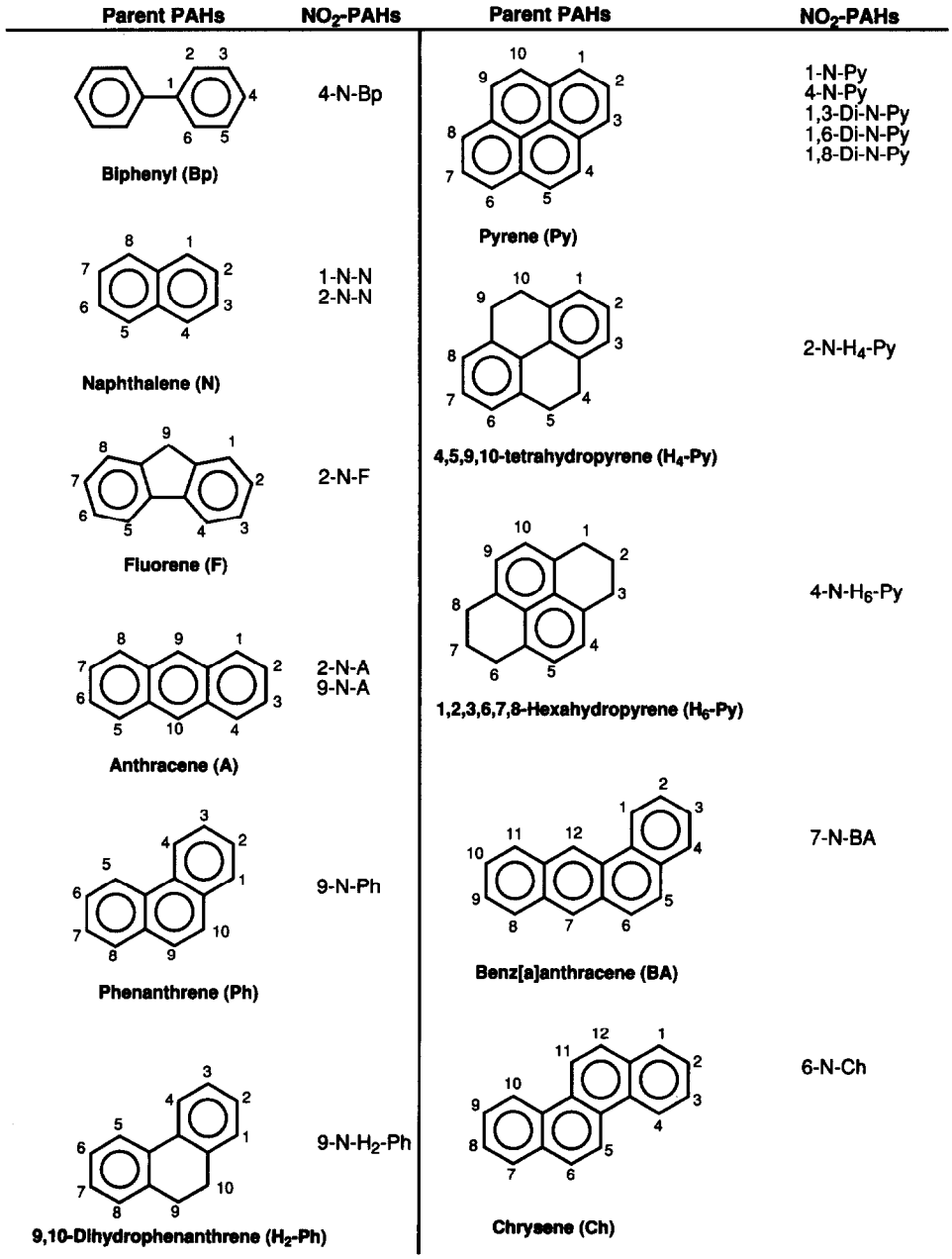


Fig. 1.

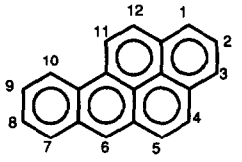
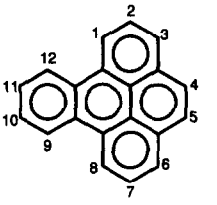
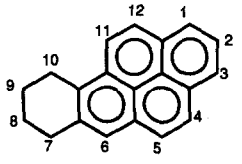
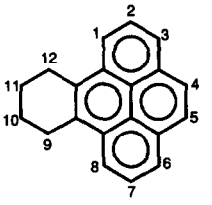
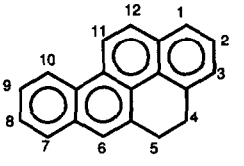
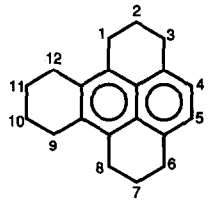
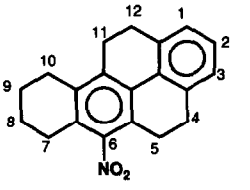
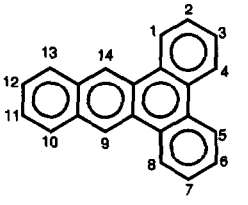
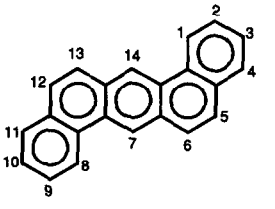
Parent PAHs	NO_2-PAHs	Parent PAHs	NO_2-PAHs
 Benzo[a]pyrene (BaP)	1-N-BaP 3-N-BaP 6-N-BaP	 Benzo[e]pyrene (BeP)	1-N-BeP 3-N-BeP 4-N-BeP 1,3-Di-N-BeP 1,6-Di-N-BeP 1,8-Di-N-BeP
 7,8,9,10-Tetrahydrobenzo[a]pyrene (H_4-BaP)	1-N-H_4-BaP 3-N-H_4-BaP 6-N-H_4-BaP 1,3-Di-N-H_4-BaP 1,6-Di-N-H_4-BaP 3,6-Di-N-H_4-BaP 1,3,6-Tri-N-H_4-BaP	 9,10,11,12-Tetrahydro-benzo[e]pyrene (H_4BeP)	1-N-H_4-BeP 3-N-H_4-BeP 4-N-H_4-BeP 1,3-Di-N-H_4-BeP 1,6-Di-N-H_4-BeP 1,8-Di-N-H_4-BeP
 4,5-Dihydrobenzo[a]pyrene (H_2-BaP)	6-N-H_2-BaP 12-N-H_2-BaP 1,6-Di-N-H_2-BaP	 1,2,3,6,7,8,9,10,11,12-Decahydrobenzo[e]pyrene (H_{10}-BeP)	4-N-H_{10}-BeP
 4,5,7,8,9,10,11,12-Octahydro-BaP (H_8-BaP)	2-N-H_8-BaP	 Dibenz[a,c]anthracene (DiB[a,c]A)	9-N-DiB[a,c]A
		 Dibenz[a,h]anthracene (DiB[a,h]A)	7-N-DiB[ah]A

Fig. 1. Structures and abbreviations of the polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs used in this study.

were determined accordingly (Tables I and II). In order to obtain suitable retention times for nitro-PAHs, a number of different solvent systems and solvent flow-rates have been tested. The final conditions chosen are shown in Tables I and II.

Separation of the parent PAHs by reversed-phase and normal-phase HPLC

To determine the effect of the nitro substituent on HPLC retention, the HPLC chromatographic conditions chosen for eluting the parent PAHs were identical to those used for nitro-PAHs. Thus, optimum conditions were not chosen for separation of these compounds. As expected, due to the low polarity of the PAH molecules, the monomeric Zorbax ODS column and the polymeric Vydac ODS column provided much better separation than the normal-phase Zorbax SIL column (Table I). Both the Zorbax SIL and the Pirkle-type CSP columns separated the parent PAHs poorly (Table I). Between the two ODS columns, the monomeric Zorbax ODS column provided better separation (retention times ranging from 3.8 to 43.6 min) than the Vydac column (retention times ranging from 3.3 to 22.7 min). These results contrast to the previous finding that the polymeric ODS Vydac column had better separation for the phenolic derivatives of BA, BaP and chrysene than the monomeric Zorbax ODS column [28]. In general, in these two reversed-phase HPLC systems, PAHs with a larger molecular size had a longer retention time. For example, the increased retention times of naphthalene, anthracene, pyrene, chrysene, and BaP (Table I) is in accord with the increased molecular size of the compounds. For the Zorbax ODS column, another general phenomenon is that retention time increases when one or more aromatic rings are saturated (Table I). An example is the large increase of the retention time from BeP (14.7 min) to H_4-BeP (27.0 min) and H_{10}-BeP (43.6 min). However, this phenomenon is not prominent with the polymeric Vydac ODS column.

Separation of nitro-PAHs by the reversed-phase and normal-phase HPLC

The order of the nitro-PAHs listed in Table II is based on the identity of the aromatic moiety of the molecules. For instance, 1-N-N, 2-N-N, 4-N-H_6-Py, and 4-N-H_{10}-BeP all have a naphthalene aromatic ring system, and thus, are grouped together. Such an arrangement facilitates the determination of the relationship between structural features and HPLC retention order.

Reversed-phase HPLC. Both the monomeric Zorbax and the polymeric Vydac ODS column show similar efficiency on the separation of nitro-PAHs. In general, retention time increased when molecular size increased (Table II). For example, 4-N-H_6-Py has a longer retention time than 1- and 2-N-N, but has a shorter retention time than 4-N-H_{10}-BeP (Table II). Because of higher polarity, nitro-PAHs have shorter retention times than their corresponding parent PAHs. In addition, nitro-PAHs with a perpendicular orientation have a shorter retention time than the geometric isomers which have a parallel orientation. For example, 6-N-BaP and 6-N-H_4-BaP have shorter retention times than 1- and 3-N-BaP, and 1- and 3-N-H_4-BaP, respectively (Table II). Similarly, 1-N-BeP and 1-N-H_4-BeP have shorter retention times than their geometric isomers, 3- and 4-N-BeP and 3- and 4-N-H_4-BeP. Since a nitro-PAH with a perpendicular orientation is more polar than the geometric isomer which has a parallel (co-planar) orientation [29], our results suggest that polarity of the nitro-PAHs is an important factor in determining the HPLC retention time.

With an additional nitro substituent, dinitro-PAHs have shorter retention time than the mononitro analogue (Table II). When a dinitro-PAH has both nitro groups adopting a perpendicular or nearly perpendicular orientation (*e.g.*, 1,8-Di-N-BeP and 1,8-Di-N-H_4-BeP), it has a shorter retention time than the dinitro-PAH isomer(s) which have only one or no nitro group with a perpendicular orientation (*e.g.*, 1,3- and 1,6-Di-N-BeP, and 1,3- and 1,6-Di-N-H_4-BeP). These results further indicate that polarity of a molecule is a decisive factor in determining the HPLC retention time (order).

Normal-phase HPLC. Separation of nitro-PAHs by the normal-phase Zorbax SIL column is much less efficient than by the reversed-phase HPLC column. Retention time increases when molecular size increases (Table II). When a benzo ring is saturated, retention time is also decreased. Contrary to the reversed-phase HPLC, due to polar character of the nitro substituent, mononitro-PAHs have longer retention times than the parent PAHs. Similarly, a

TABLE III

RELATION OF STRUCTURAL FEATURES AND HPLC RETENTION OF NITRO-POLYCYCLIC AROMATIC HYDROCARBONS

The symbols "+" and "−" denote the HPLC retention time increased and decreased, respectively.

Structural feature	Effect on retention time			
	Reversed-phase		Normal-phase	Pirkle-type
	Zorbax ODS	Vydac ODS		
Increase in molecular size	+++	++	+	+
Addition of a benzo ring	++	++	+	++
Addition of a tetrahydrobenzo ring	++	++	±	++
Saturation of a benzo ring	−−	−	−	±
Addition of a nitro group				
(parallel orientation)	−−	−−	+	±
(perpendicular orientation)	−−−	−−−	++	±
Addition of two nitro groups				
(parallel orientation)	−−−	−−−	++	±
(perpendicular orientation)	−−−−	−−−−	+++	±

dinitro-PAH has a much longer retention time than its mononitro analogues (Table II). As expected, a mononitro-PAH with a perpendicular nitro group has a longer retention time than the isomer with a parallel nitro group. The same phenomenon is observed for dinitro-PAHs (Table II).

Chiral Pirkle HPLC. The Pirkle-type chiral stationary phase column employed has been shown to efficiently resolve enantiomers of a large number of compounds, including the ring-oxidized derivatives of PAHs [30–32]. However, although it was reported that several nitro-PAHs were better separated by chiral Pirkle HPLC than by the other columns [33], our study indicates that the large number of nitro-PAHs used in this study were not well separated. In most cases, but not all, retention time increased when the molecular size increased. Nitro orientation does not significantly affect the retention order. Thus, the chiral character of the stationary phase does not facilitate the separation of nitro-PAHs.

Relationships between structures and HPLC retention times of nitro-PAHs

Greibrokk *et al.* [11] reported the separation of nitro-PAHs and found that, eluted from a normal-phase silica HPLC column with dichloromethane–hexane, the retention order was dependent on (i) the location of the nitro group, (ii) the number of protons *peri* to the nitro substituent, and (iii) the length/breadth ratio of the molecule. We previously reported a study on the separation of ring-oxidized derivatives of nitro-PAHs and found that polarity is the major factor for determining HPLC retention time [29]. We have now examined the relationships between structure and HPLC retention time employing the parent nitro-PAHs. The relationships are summarized in Table III. For both reversed-phase HPLC systems, a larger molecule results in a longer retention time. In contrast, saturation of an aromatic ring shortens the retention time. Among the isomeric nitro compounds, the isomer with a perpendicular nitro group has a shorter retention time than the isomer with a parallel nitro substituent. Comparison of the retention times of the nitro-PAHs and their parent PAHs indicates that a nitro group decreases the retention time substantially.

For the normal-phase chromatography, an additional benzo ring results in an increase of retention time. Saturation of a benzo ring also leads to a shorter retention time (*e.g.*, H_4-BeP *vs.* BeP). The presence of one or two nitro groups to the molecules causes a longer retention time on a normal-phase HPLC system. The opposite effect of a nitro

group on the retention time to the reversed- and normal phase HPLC systems clearly indicate that polarity of the molecules is an important factor in determining the HPLC retention time.

As described previously, certain structural features, particularly the nitro orientation, are important factors in determining the biological activities of nitro-PAHs. In this paper, we have found the relationships between several of the structural features and the HPLC retention order of nitro-PAHs. Thus, our results provide useful information concerning both the HPLC chromatography and the biological significance of the environmental nitro-PAHs.

NOTE

The opinion or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Food and Drug Administration.

REFERENCES

1 H. S. Rosenkranz and R. Mermelstein, *Mutat. Res.*, 114 (1983) 217.
2 C. M. White (Editor), *Nitrated Polycyclic Aromatic Hydrocarbons*, Hüthig, Heidelberg, 1985.
3 H. Tokiwa and Y. Ohnishi, *CRC Crit. Rev. Toxicol.*, 17 (1986) 23.
4 C. M. King, L. J. Romano and D. Schuetzle (Editors), *Carcinogenic and Mutagenic Responses to Aromatic Amines and Nitroarenes*, Elsevier, Amsterdam, New York, 1988.
5 F. A. Beland, R. H. Heflich, P. C. Howard and P. P. Fu, in R. G. Harvey (Editor), *Polycyclic Hydrocarbons and Carcinogenesis (ACS Symposium Series*, No. 283), American Chemical Society, Washington, DC, 1985, pp. 371–396.
6 P. P. Fu, M. W. Chou and F. A. Beland, in S. K. Yang and B. D. Silverman (Editors), *Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure–Activity Relationships*, Vol. II, CRC Press, Boca Raton, FL, 1988, pp. 37–65.
7 P. P. Fu, *Drug Met. Rev.*, 22 (1990) 209.
8 P. C. Howard, S. S. Hecht and F. A. Beland (Editors.) *Nitroarenes: Occurrence, Metabolism and Biological Impact*, Plenum Press, New York, 1991.
9 H. Svendsen, H.-P. Ronningsen, L. K. Sydnes and T. Greibrokk, *Acta Chem. Scand. B.*, 37 (1983) 833.
10 E. Johansen, L. K. Sydnes and T. Greibrokk, *Acta Chem. Scand. B.*, 38 (1984) 309.
11 T. Grebrokk, B. Iversen, E. J. Johansen, H.-P. Ronningsen and H. Svendsen, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 671.
12 P. P. Fu, M. W. Chou, D. W. Miller, G. L. White, R. H. Heflich and F. A. Beland, *Mutat. Res.*, 143 (1985) 173.
13 M. W. Chou, R. H. Heflich, D. A. Casciano, D. W. Miller, J. P. Freeman, F. E. Evans and P. P. Fu, *J. Med. Chem.*, 27 (1984) 1156.
14 P. P. Fu, L. S. Von Tungeln and M. W. Chou, *Carcinogenesis*, 6 (1985) 753.
15 P. P. Fu, Y.-C. Ni, Y. M. Zhang, R. H. Heflich, Y. K. Wang and J. S. Lai, *Mutat. Res.*, 225 (1989) 121.
16 H. Jung, A. U. Shaikh, R. H. Heflich and P. P. Fu, *Environ. Mol. Mut.*, 17 (1991) 169.
17 Y. S. Wu, Y. K. Wang, C. C. Lai, J. S. Lai, L. E. Unruh, F. E. Evans and P. P. Fu, in W. M. Cooke, K. Loening and J. Merritt (Editors), *Polynuclear Aromatic Hydrocarbons: Measurements, Means, and Metabolism*, Battelle Press, Columbus, OH, 1991, pp. 1083–1095.
18 U. A. Spitzer and R. Stewart, *J. Org. Chem.*, 39 (1974) 3936.
19 P. M. G. Bavin, *Can. J. Chem.*, 37 (1959) 1614.
20 P. P. Fu and R. G. Harvey, *Chem. Rev.*, 78 (1978) 317.
21 P. P. Fu and R. G. Harvey, *Tetrahedron Lett.*, (1977) 415.
22 P. P. Fu and S. K. Yang, *Biophys. Res. Commun.*, 115 (1983) 123.
23 D. W. Miller, D. Herreno-Saenz, K. H. Huang, T. M. Heinze and P. P. Fu, *J. Org. Chem.*, 57 (1992) 3746.
24 Unpublished results.
25 M. Mushtag, Z. Bao and S. K. Yang, *J. Chromatogr.*, 385 (1987) 293.
26 P. P. Fu, L. S. Von Tungeln, D. T. C. Yang, E. K. Fifer and F. A. Beland, *Carcinogenesis*, 7 (1986) 1819.
27 P. P. Fu, R. H. Heflich, L. S. Von Tungeln, H. Z. Miranda and F. E. Evans, *Carcinogenesis*, 9 (1988) 951.
28 D. W. Miller, F. E. Evans and P. P. Fu, *Spectroscopy Int. J.*, 4 (1985) 91.
29 L. S. Von Tungeln and P. P. Fu, *J. Chromatogr.*, 461 (1989) 315.
30 S. K. Yang, H. B. Weems, M. Mushtaq and P. P. Fu, *J. Chromatogr.*, 316 (1984) 569.
31 S. K. Yang, M. Mushtaq and P. P. Fu, *J. Chromatogr.*, 371 (1986) 195.
32 H. B. Weems, M. Mushtaq, P. P. Fu and S. K. Yang, *J. Chromatogr.*, 371 (1986) 211.
33 M. W. Chou, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 304.