

DENSITY FUNCTIONAL THEORETICAL STUDY OF NITRATED POLYCYCLIC AROMATIC HYDROCARBONS

Kefa K. Onchoke
Christopher M. Hadad
Prabir K. Dutta

*Department of Chemistry, The Ohio State University,
Columbus, Ohio, USA*

Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are well-known mutagens. Correlations between the orientation of the nitro group relative to the plane of the aromatic ring and mutagenic effects of nitro-PAHs have been proposed. Synthesis of specific isomers of nitro-PAHs and elucidation of their crystal structure is required to establish the validity of the structure-function relationships. Such studies are scarce. Fortunately, electronic structure calculations can be readily done. In this study we have used density functional theory calculations to predict structure of nitro-PAHs, including 1-, 2-nitronaphthalenes; 1-, 2-, 9-nitroanthracenes; 1-, 2-, 3-, 4-, 9-nitrophenanthrenes; 1-, 2-, 4-nitropyrenes; and 6-nitrochrysene. The relationships between the calculated structures and the mutagenicity reported in the chemical literature are discussed.

Keywords biological activity, density functional theory, mutagenicity, nitrated polycyclic aromatic hydrocarbons, nitro group orientation

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are generated by combustion processes (e.g., diesel and gasoline emissions, fly

Received 4 August 2003; accepted 20 October 2003.

The authors acknowledge financial support from the Ohio State Environmental Molecular Science Institute, funded by the National Science Foundation (CHE-0090147, EMSI Program). They also thank the Ohio Supercomputer Center for generous allocations of computer time.

Address correspondence to Prabir K. Dutta, Department of Chemistry, The Ohio State University, 100 W. 18th Avenue, Columbus, OH 43210, USA. E-mail: dutta.1@osu.edu

ash), but can also be formed by atmospheric transformation of polycyclic aromatic hydrocarbons (e.g., by reactions of PAHs with nitrogen oxides (1)). Many of these nitro-PAH compounds are known to be potent genotoxins, rodent carcinogens, and mutagenic and endocrine disrupters while some have been shown to be direct-acting mutagens (2–5). Even though nitro-PAHs are present at concentrations one or two orders of magnitude lower than their parent PAH species (6), their high carcinogenic and mutagenic activities pose greater risks to human health (5). Thus, there is considerable interest in developing structure-function relationships to predict the biological activity of nitro-PAHs (7, 8). Several correlations have been developed, including the first half-wave reduction potential; orientation of the nitro substituents relative to the aromatic plane; size of the nitro-PAH, its aromaticity, and hydrophobicity as well as the number of electrons involved in the first step of the nitroreduction (9, 10). The role of these factors is evident if the metabolic activation necessary for biological activity is considered. Two common pathways that have been found in bacterial systems include nitroreduction and ring oxidation followed by nitroreduction (8).

Of particular relevance to this article is the hypothesis that the nitro group orientation is correlated with mutagenicity of nitro-PAHs. Information regarding the orientation has been obtained from X-ray crystallography, ^1H NMR, mass spectroscopy, ultraviolet-visible (UV-vis) spectroscopy, and Raman spectroscopy (11, 12). Except for X-ray crystallography, the other spectroscopic techniques do not provide values for the dihedral angle but suggest planar or perpendicular geometries of the nitro group relative to the aromatic plane. Electronic structure theory calculations provide an alternative method to predict geometry and have been used in investigations of PAHs due to the ease of using Hückel molecular orbital (MO) theory (13). In the past two decades, concerted efforts have been made in using correlated methods such as perturbation theory and density functional theory (DFT) for investigating PAHs (13). Properties investigated include electron affinities, HOMO-LUMO energy gaps, zero-point vibrational energies, heats of formation, and substituent effects. The purpose of the present study was to determine the structure of a series of isomers of different nitro-PAHs with DFT using the B3LYP functional. Prior theoretical studies have been limited to semiempirical calculations (14–16) and our objective was to examine whether DFT calculations could provide better structural information.

We chose to study nitro-aromatic PAH isomers with two to four rings whose biological mutagenicity is known. Examination of the literature shows that isomers of the same ring system perform varying biological

activities against bacterial strains and animal cell lines, and that is the focus of this article. Biological activity of other nitro-PAHs has been reported (14), but we did not include it here because systematic studies of their isomers were not reported. Based on our DFT calculations, relationships between the orientation of nitro groups in a series of isomers of the same PAH are correlated with the reported mutagenicity levels. In cases where no biological studies are available, we have made predictions of the biological activity.

COMPUTATIONAL METHODS

All of the computational calculations were performed using the Gaussian 98 suite of programs (17) at the Ohio Supercomputer Center. Becke's three-parameter hybrid (B3) functional (18) was used, along with the correlation functional of Lee-Yang-Parr (LYP) (19). The 6-31G* basis set (20) was used for the geometric optimization. The DFT methods, especially the B3LYP hybrid DFT method, have been shown to reproduce reliable geometries (21) and also require less time and computer resources (22). Optimized geometries of the molecules were calculated without any geometrical restriction, except those enforced by symmetry. Optimization with the z-matrix coordinate system resulted in many of the nitro molecules yielding one imaginary frequency, indicating a transition state. In such cases the normal mode for the imaginary vibrational frequency was examined, and the orientation of the nitro group distorted and resubmitted for further optimization without any other restriction. All optimized geometries were confirmed to be stationary points through vibrational frequency analysis. An overall scaling factor of 0.9614 as proposed by Scott and Radom (23) was used for the vibrational frequencies. This corrects for the anharmonicity, basis set deficiencies, and approximate treatments of electron correlation (24). In some of the nitro-PAHs multiple low-energy minima were found and, in the subsequent discussion, the lowest energy stationary point will be considered.

RESULTS

Computational Strategy

In the initial phase of our studies, several model aromatic systems without the nitro group were examined. These included benzene, naphthalene, phenanthrene, anthracene, chrysene, and pyrene. These well-studied systems were used as calibration compounds. Using bond

lengths, bond angles, and dihedral angles, comparisons of the calculated results (at the B3LYP/6-31G* level of theory) with the experimental data (see Supplementary Information) were made to provide confidence in the calculations. This was followed by calculations on nitro-PAHs including nitrobenzene and 1-nitronaphthalene; 2-nitronaphthalene; 1-nitroanthracene; 2-nitroanthracene; 9-nitroanthracene; 1-, 2-, 3-, 4-, and 9-nitrophenanthrenes; 1-, 2-, 4-nitropyrenes; and 6-nitrochrysene, and comparisons to structural information derived from experimental data were made wherever possible.

Aromatic Compounds

Experimental and calculated equilibrium geometries of benzene, naphthalene, anthracene, phenanthrene, chrysene, and pyrene have been studied. In making structural comparisons, we have used microwave, neutron diffraction, electron diffraction, and X-ray data. For calibration tests of the DFT method, we used benzene as a test compound for the aromatic hydrocarbons (25–27). Table 1 compares experimental and calculated parameters. It should be noted that computed equilibrium C–C, C–H bond lengths and C–C–C, C–C–H bond angle geometries are in excellent agreement with experimentally determined values. Results obtained by B3LYP/6-31G* in this study are in excellent agreement with those obtained with the larger basis sets such as 6-311G*. Results for the other PAHs, which support the conclusions reached for benzene are presented in Supplementary Tables S1-7.

TABLE 1. Geometry of Benzene and Naphthalene

	rC–C		rC–H			C–C–H Angle		C–C–C Angle	
	Calc.	Exp.	Calc.	Exp.	1,084 ^c	Calc.	Exp.	Calc.	Exp.
Benzene	1.397	1.397 ^a	1.087	1.079 ^b	1,084 ^c	120.0		120.0	120.0 ^d
Naphthalene			1.087	1.092					

Note. The computed equilibrium C–C, C–H bond lengths and C–C–C and C–C–H bond angles of benzene are in excellent agreement with experimental data. The C–H bond lengths agree to within 0.003 Å. Bond lengths are given in angstroms (Å), bond angles in degrees.

^aFrom Ref. 25.

^bFrom Ref. 25.

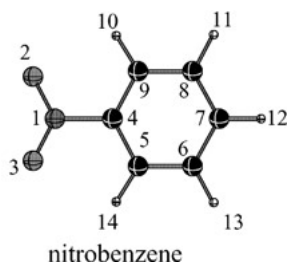
^cFrom Ref. 26.

^dFrom Ref. 27.

Nitro-Aromatic Compounds

The C—C bond lengths, N—O bond distances, and C—C—N—O dihedral angles between the aromatic moiety and the NO₂ group of nitro-PAHs were the focus of the calculations. Calculated parameters were compared to experimental data whenever available. If neutron or electron diffraction studies were not available, X-ray crystallographic data were used for comparison.

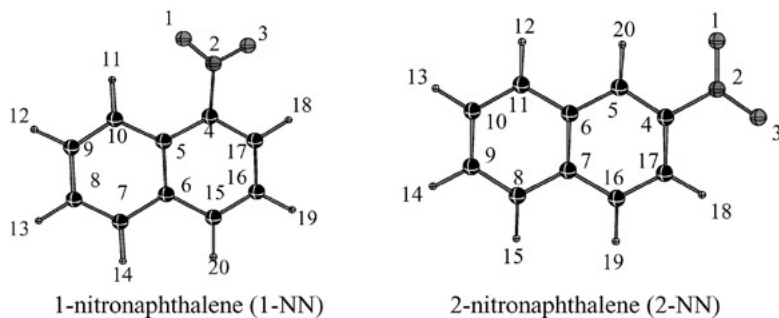
Nitrobenzene



Scheme 1

The structure of nitrobenzene was determined using neutron diffraction and electron diffraction. Tables 2 and 3 compare the present calculations with experimental data, and the agreement is excellent. For example, the C—N bond length determined by electron diffraction studies differs with the present B3LYP/6-31G* calculations by 0.01 Å (28). A planar geometry of nitrobenzene is predicted, in agreement with calculations using electron correlation and ab initio methods (29).

Nitronaphthalenes



Scheme 2

TABLE 2. Selected Geometric Parameters of the Nitro-Polycyclic Aromatic Hydrocarbons (Nitro-PAHs)

Molecule	Bond length (Å)				Bond angle (°)	
	C—N		O—N		ONO	
	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.
Nitrobenzene	1.4729	1.486 ^a , 1.478 1.46 ^b 1.465 ^c	1.2307	1.223 ^a 1.20 ^b 1.218 ^c	124.62	125.3 ^a 123.5 ^d
1-Nitronaphthalene	1.4741		1.2326		123.80	
2-Nitronaphthalene	1.4702		1.2314		124.53	
1-Nitroanthracene	1.4727		1.2333		123.58	
2-Nitroanthracene	1.4685		1.2319		124.47	
9-Nitroanthracene	1.4718	1.482 ^e	1.2320	1.216 ^e	124.28	124 ^e
1-Nitrophenanthrene	1.4749		1.2324		123.94	
2-Nitrophenanthrene	1.4688		1.2316		124.50	
3-Nitrophenanthrene	1.4695		1.2319		124.40	
4-Nitrophenanthrene	1.4763	1.488 ^f 1.468 ^g 1.471 ^g	1.2282	1.213(3) ^f 1.230(3) ^f	125.18	123.6(3) ^f 123.4(3) ^f
9-Nitrophenanthrene	1.4748		1.2325		123.91	
1-Nitropyrene	1.4692		1.2333, 1.2341		123.55	
2-Nitropyrene	1.4726		1.2311		124.46	
4-Nitropyrene	1.4749		1.2326, 1.2319		123.81	
6-Nitrochrysene	1.4904		1.2533		123.61	

^aFrom Ref. 46, electron diffraction data from Ref. 28.

^bFrom Ref. 43, X-ray (liquid).

^cX-ray crystal data from (Cambridge Data Base).

^dFrom Ref. 47, electron diffraction data.

^eFrom Ref. 31, X-ray diffraction data.

^fFrom Ref. 33, X-ray diffraction data.

^gFrom Ref. 32, X-ray diffraction data.

Tables 2 and 3 show selected geometrical parameters of 1- and 2-nitronaphthalenes (1-NN, 2-NN, the molecules being depicted in Scheme 2). No structural data are available on these compounds. However, the experimentally determined average C(ar)—N bond length compiled from many compounds is found to be around 1.468 Å (30), consistent with that calculated for the nitronaphthalenes.

Comparisons of the structures of the isomers can be made from the calculations. 1-NN shows longer C—N and O—N bond length as compared

TABLE 3. Selected Geometric Parameters of the Nitro-Polycyclic Aromatic Hydrocarbons (Nitro-PAHs) Calculated at the B3LYP/6-31G* Level of Theory and Experimental Mutagenicity

Molecule	Dihedral angle (°) C—C—N—O		Reported at semi-empirical level of theory		Mutants per nmol for <i>S. typhimurium</i> TA98 (-S)
	Calc.	Exp.	AM1 ^a	PM3 ^c	
Nitrobenzene	0.046				0.023
1-Nitronaphthalene	25.35		59.5	28.4	0.05 ^f
2-Nitronaphthalene	0.00		0.2	0.6	0.2 ^f
1-Nitroanthracene	22.12				
2-Nitroanthracene	0.00				892 ^f
9-Nitroanthracene	51.04	85 ^b		82.5	0.5 ^f
1-Nitrophenanthrene	30.40				108 ⁱ
2-Nitrophenanthrene	0.00		16.9		443 ^j
3-Nitrophenanthrene	0.02				330 ⁱ
4-Nitrophenanthrene	53.00	72.7(7) ^e , 67.4(2) ^e			
9-Nitrophenanthrene	29.35				61 ⁱ , 297 ^j
1-Nitropyrene	23.15			55.6	237 ^g
2-Nitropyrene	0.00			1.0	2223 ^h
4-Nitropyrene	26.00			7.3	2475 ^h
6-Nitrochrysene	26.00				269 ^f

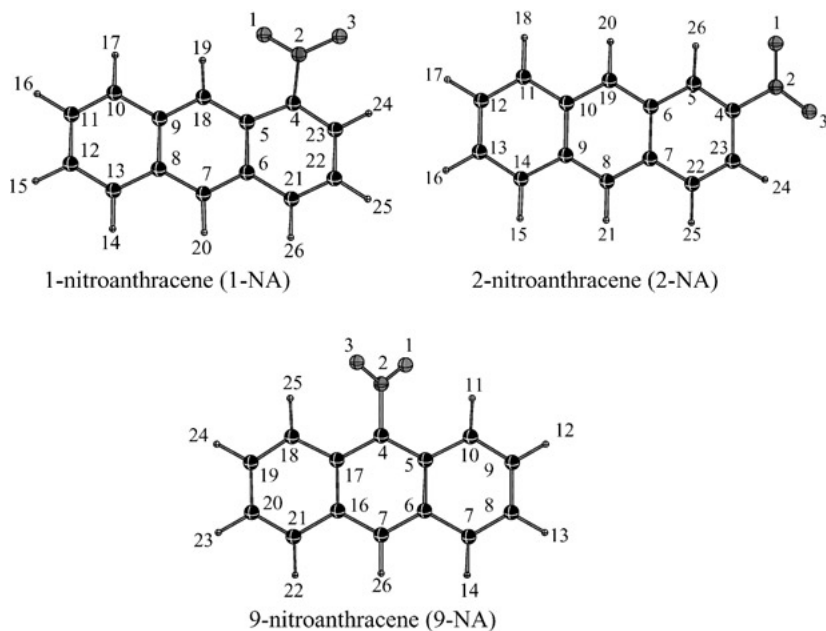
^aFrom Ref. 40.^bFrom Ref. 31.^cFrom Ref. 16.^eFrom Ref. 33, X-ray diffraction.^fFrom Ref. 37.^gFrom Ref. 48.^hFrom Ref. 37.ⁱFrom Ref. 40.^jFrom Ref. 38, dose = $\mu\text{g}/\text{plate}$, result reported as net revertants/plate.

to 2-NN by 0.004 and 0.0012 Å, respectively. The longer C—N bond length in 1-NN indicates lower NO₂ conjugation with the aromatic ring. Table 3 also shows that the nitro group in 2-NN is planar with the aromatic ring with a C—C—N—O dihedral angle of 0°, whereas the nitro group in 1-NN is out of plane with a dihedral angle of 25.4°. In rationalizing the differences in the dihedral angles the steric factors experienced by the O atoms of the NO₂ with the peri hydrogens is important, and distances to the neighboring hydrogen atoms are reported in Table 4. A

TABLE 4. O . . . H Distances in Nitro-Polycyclic Aromatic Compounds

Nitro compound	O—H Distances (Å)
1-Nitronaphthalene	O1-H11 = 2.1658
	O3-H18 = 2.3320
2-Nitronaphthalene	O1-H20 = 2.3858
	O3-H18 = 2.3910
1-Nitroanthracene	O1-H19 = 2.1447
	O3-H24 = 2.3075
2-Nitroanthracene	O1-H26 = 2.3819
	O3-H24 = 2.3916
9-Nitroanthracene	O1-H11 = 2.2835
	O3-H25 = 2.2813
1-Nitrophenanthrene	O1-H19 = 2.1604
	O3-H24 = 2.3629
2-Nitrophenanthrene	O1-H26 = 2.4064
	O3-H22 = 2.3919
3-Nitrophenanthrene	O1-H26 = 2.3532
	O3-H22 = 2.3979
4-Nitrophenanthrene	O1-H12 = 2.4669
	O3-H22 = 2.5923
9-Nitrophenanthrene	O1-H23 = 2.1607
	O3-H10 = 2.3470
1-Nitropyrene	O1-H25 = 2.3206
	O3-H15 = 2.1398
2-Nitropyrene	O1-H25 = 2.3847
	O2-H28 = 2.3847
4-Nitropyrene	O19-H23 = 2.3269
	O17-H26 = 2.1622
6-Nitrochrysene	O1-H29 = 2.1573
	O3-H24 = 2.3058

planar NO₂ group will have maximum conjugation with the aromatic ring, and therefore is the desired geometry. However, in the planar geometry, if the O atoms of the nitro group are close to the peri hydrogens, the nitro group will be forced out of the plane. Thus smaller O—H distances in the optimized geometry indicate stronger interactions with the H atoms; for example, for nonplanar 1-NN the shortest O—H distance is 2.1658 Å, compared to the planar 2-NN where the shortest O—H distance is 2.3858 Å.

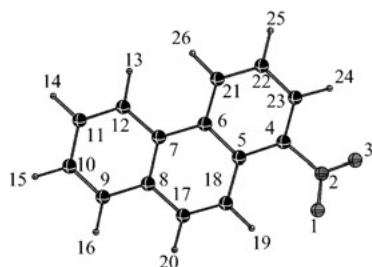
Nitroanthracenes**Scheme 3**

Tables 2 and 3 show the geometrical parameters of 1-, 2-, and 9-nitroanthracenes (1-NA, 2-NA, and 9-NA, the molecules being depicted in Scheme 3). Experimental data from X-ray diffraction is available only on 9-NA, and the bond lengths and the O–N–O bond angles show a satisfactory match between calculation and experiment. Slight differences (e.g., the C–N bond length being shorter by 0.01 Å compared to X-ray crystallographic data [31]) could be due to crystal packing forces inherent in solid crystals. The experimental and calculated dihedral angle of the nitro group versus the aromatic ring for 9-NA is 85° and 51°, respectively. Trotter suggested that the plane of the nitro group in 9-NA may be free to oscillate between angles of 64° and 116° in solution or the vapor phase (31).

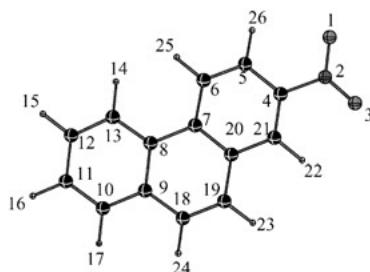
As for the trends between the isomers, the C–N bond lengths follow the order: 2-NA < 9-NA < 1-NA. The shortening of the C–N bond in 2-NA is indicative of increased conjugation of the NO₂ group with the aromatic ring. The calculated C–C–N–O dihedral angles are 0.0°, 22.1°, and 51.0° for 2-NA, 1-NA, and 9-NA respectively. The planar geometry in 2-NA enhances maximum π - π orbital interactions between the aromatic ring and the nitro group. The steric factors experienced by the O atoms of the NO₂ with the peri hydrogens is responsible for the

out-of-plane arrangement of the nitro group as is evident from the $O \cdots H$ lengths reported in Table 4. In 1-NA, the O_1-H_{19} , and O_3-H_{24} distances are calculated as 2.1447 and 2.3075 Å respectively. In 2-NA, the O_1-H_{26} and O_3-H_{24} lengths are determined to be 2.3819 and 2.3916 Å respectively. In 9-NA, the O_1-H_{11} and O_3-H_{25} lengths were determined to be 2.2835 Å and 2.2813 Å respectively. Thus the O atoms in 2-NA in general, experience, less repulsion from the adjacent hydrogens, compared to the other two isomers.

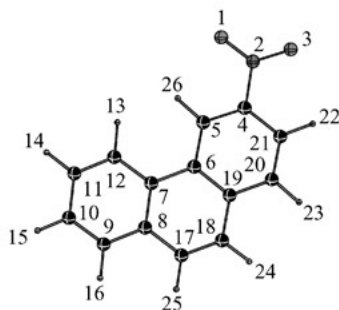
Nitrophenanthrenes



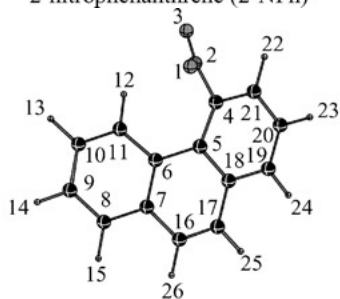
1-nitrophenanthrene (1-NPh)



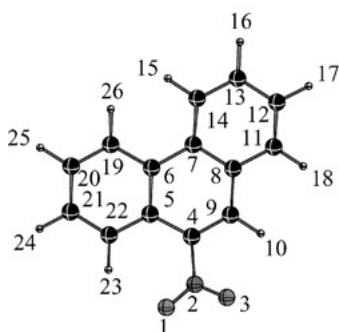
2-nitrophenanthrene (2-NPh)



3-nitrophenanthrene (3-NPh)



4-nitrophenanthrene (4-NPh)



9-nitrophenanthrene (9-NPh)

Scheme 4

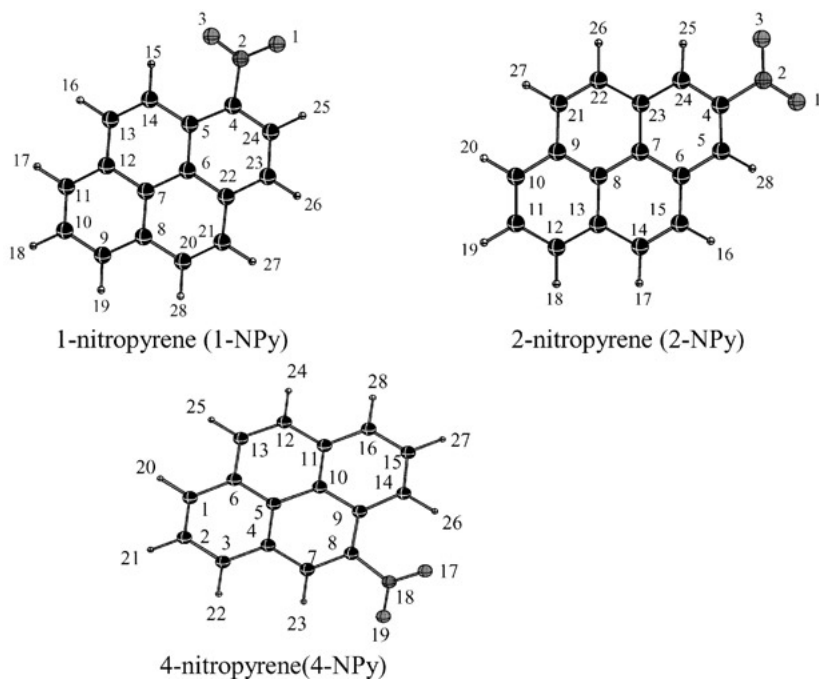
Tables 2–4 summarize the geometric parameters for 1-, 2-, 3-, 4-, and 9-nitrophenanthrenes (1-NPh, 2-NPh, 3-NPh, 4-NPh, 9-NPh, the molecules being depicted in Scheme 4). X-ray crystallographic data is available only for 4-NPh (32, 33) and shows excellent agreement with calculation. The bond lengths in 4-NPh reveal a lengthening of the C–N bond as compared to 1-NPh, 2-NPh, 3-NPh, and 9-NPh. The dihedral angle between the NO₂ and the phenanthrene planes in 4-NPh is 53.0°, while X-ray crystallography showed this to be 72.2° (32). Other studies (33) established the dihedral angles between the NO₂ groups in two polymorphic forms of 4-NPh and the phenyl rings as 70.3(2) and 67.4(2)° (33). A reported calculation showed this angle to be 60.7° (level of theory not given in Ref. [32]).

The calculations make possible structural comparison of the isomers. The C–N bond lengths follows the order: 2-NPh < 3-NPh < 9-NPh < 1-NPh < 4-NPh. The N–O bond length is the shortest in 4-NPh, which is consistent with the longest C–N bond. 4-NPh shows a larger C–C–N–O dihedral angle (53.0°) than the other isomers. This can be attributed to lowered conjugation between the ring and the nitro group. 1-NPh and 9-NPh show similar dihedral angles. The 2-NPh and 3-NPh bond lengths are predicted to be planar systems with C–C–N–O dihedral angles of 0.00° and 0.02°, respectively. A consideration of the calculated interatomic distances between oxygen and neighboring hydrogens sheds light on the nonplanarity of the nitro group. From Table 4, the longer O–H distances for 2- and 3-NPh are consistent with the planarity of the NO₂ group. Both 1- and 9-NPh have short O–H distances and nonplanar nitro groups. 4-NPh, however, does not follow the predicted trend since it has longer O–H distances (>2.4 Å), yet has a nonplanar NO₂ group.

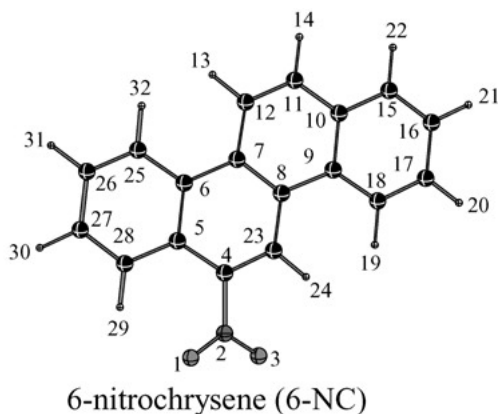
Nitropyrenes

Tables 2–4 show the calculated bond lengths, and O–N–O and C–C–N–O torsional angles of 1-nitro-, 2-nitro-, and 4-nitropyrene (1-NPy, 2-NPy, 4-NPy, the molecule being depicted in Scheme 5). There is no reported experimental data on the pyrenes to compare with the calculations. Thus, we analyzed the trends in the isomers based on the calculations.

The C–N bond lengths follow the trend: 1-NPy < 2-NPy < 4-NPy. The C–C–N–O dihedral angle of 0° in 2-NPy indicates conjugation of NO₂ to the aromatic ring. Compared to 1-NPy and 4-NPy, the 2-NPy isomer shows the shortest O–N bonds. The O–H distances shown in Table 4 are also consistent with the planarity of 2-NPy and the nonplanarity of the nitro group in 1- and 4-NPy.



Scheme 5

6-Nitrochrysene

Scheme 6

Tables 2–4 show the calculated bond lengths, and O–N–O and C–C–N–O torsional angles of 6-nitrochrysene, with the molecule shown in Scheme 6. The O–N–O and C–C–N–O bond angles are calculated as 123.6° and 26.0° , respectively. There are no structural data available

for this compound. The dihedral angle shows that the oxygen atoms experience steric hindrance from the peri hydrogens, as is evident from the short bond length of O₁–H₂₉ (2.1573 Å).

DISCUSSION

Correlation between Nitro Group Orientation and Biological Activity

Previous studies have shown nitro-PAHs to be potent bacterial mutagens. The Ames test, based on the bacterium *Salmonella typhimurium*, has been used extensively for short-term mutation assays (34). Animal models using rodents (Sprague-Dawley [CD]) also have been developed (35). Efforts are being made to find human cell lines for xenobiotic metabolism that can be routinely tested for mutagenicity (34).

Table 3 shows the mutagenicity studies of nitro-PAHs tested against *S. typhimurium* TA98. A general observation is that nitro-PAHs with the NO₂ group planar to the phenyl ring are more mutagenic than their isomers, consistent with predictions made two decades ago (9, 10, 12). However, since the calculations discussed in this article provide the geometry of a large number of isomers of the same nitro-PAHs, we are in a position to establish the validity of the correlations between orientation of the nitro group and biological activity, and this forms the focus of our discussion. In Figure 1A–D, we show the correlation between the calculations and the biological activity separated according to PAH ring size, because of the large differences in the biological activities of the PAHs.

Nitronaphthalenes

Figure 1A shows the correlation between the experimental results of mutagenicity studies of nitronaphthalenes as measured by exposure to *S. typhimurium* TA98 and the C–C–N–O dihedral angle. There is a fourfold mutagenic increase of 2-nitronaphthalene (2-NN) compared to 1-nitronaphthalene (1-NN). Sasaki et al. (3, 36) found that both intragenic and chromosomal scale mutational events were higher for 2-nitronaphthalene in comparison to 1-nitronaphthalene (36). 2-NN induces cancer in animals while 1-NN does not (37). Well correlated to the higher mutagenicity of 2-nitronaphthalene is the fact that the calculated dihedral angles show a coplanar orientation of the NO₂ group relative to the naphthalene ring.

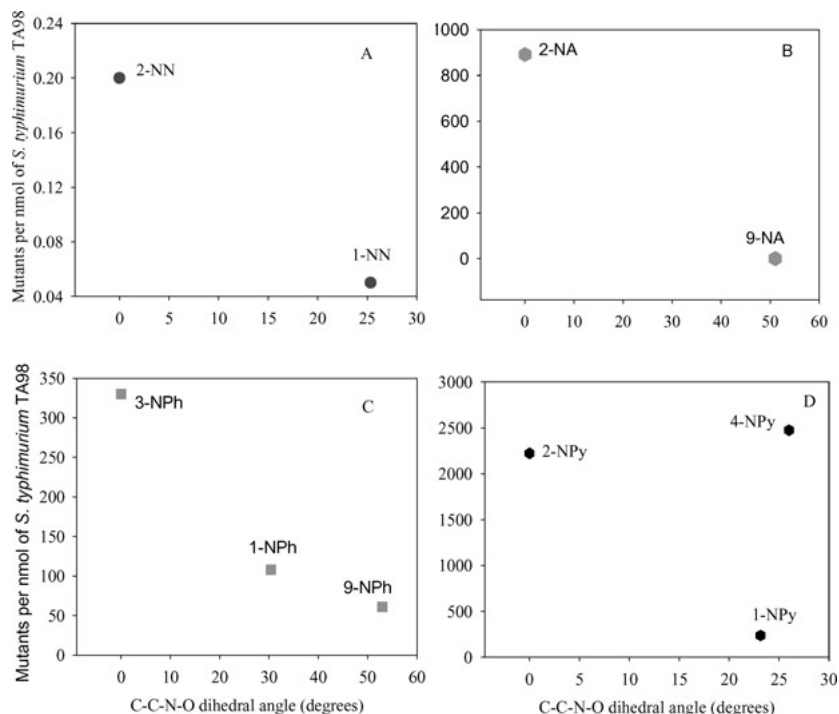


FIGURE 1. C—C—N—O dihedral angle of nitro-PAHs (*x*-axis) versus mutants per nmol of *Salmonella typhimurium* TA98 (*y*-axis). A, 1- and 2-nitronaphthalene (1-NN, 2-NN); B, 2- and 9-nitroanthracene (2-NA and 9-NA); C, 1-, 3-, and 9-nitrophenanthrene (1-NPh, 3-NPh and 9-NPh); D, 1-, 2-, and 4-nitropyrene (1-NPy, 2-NPy, and 4-NPy). Data from Refs. (8, 37, 48).

Nitroanthracenes

Bacterial mutagenicity of 2- and 9-nitroanthracene (NA) as well as their effects on rat liver microsomal metabolism has been reported by Fu et al. (38). 2-NA was metabolized by rat liver microsomes under aerobic conditions to produce diols. Assay of 2-NA and the two trans-dihydrodiol metabolites with the *S. typhimurium* TA98 strain in the presence and absence of S9 enzymes showed them to be mutagenic. However, mutagenic tests of 9-NA and its metabolites (namely, 9-NA trans-1,2- and 3,4-dihydrodiols for *S. typhimurium* TA98) were found to exhibit very low mutagenicity (39). The extent of out-of-plane orientation

of the nitro group follows the order 2-NA \gg 9-NA and this trend matches the reported mutagenicity (Figure 1B). 1-NA is predicted to have lower mutagenicity.

Nitrophenanthrenes

Table 3 and Figure 1C shows that for the nitrophenanthrenes, 3-NPh is the most mutagenic. Using another strain *S. typhimurium his* (strains TA100 that were nitroreductase deficient), it was observed that 1-, 3-, and 9-nitrophenanthrenes induced 329, 620, and 438 revertants per nmol respectively (40). 3-NPh, in general, showed increased mutagenicity levels toward *S. typhimurium his* YG1021, YG1026, and the O-acetyltransferase-overproducing tester strains of TA98 (40). Based on the calculations, the degree of nonplanarity of the nitro group follows the order 4-NPh > 1-, 9-NPh > 3-, 2-NPh. The coplanar geometry of the nitro group with the phenanthrene ring in 3-NPh is consistent with the greater mutagenic effects. Data reported by Hirayama et al. (41) showed 2-NPh to have greater mutagenic effects than 9-NPh against *S. typhimurium* TA98, consistent with the nitro group orientation. However, few studies are available in the literature that clearly compare the mutagenic effects between 2-NPh and 3-NPh against bacterial strains. Decreased levels of mutagenicity are predicted for 4-NPh.

Nitropyrenes

The relative order of bacterial mutagenicities in *S. typhimurium* strains TA1538 and TA98 was determined as 1-NPy \ll 2-NPy < 4-NPy in both the presence and absence of rat liver supernatant (42, 43). A similar order of carcinogenicity has been observed following direct administration of each isomer into the mammary pads of weanling female Sprague-Dawley (CD) rats. The DNA adducts formed with 4-NPy were investigated by Chae et al. (44) in order to understand the differences in mutagenicities observed among the three isomers. The rate of 4-NPy binding to rat mammary DNA was at least threefold higher than that of 1-NPy and 2-NPy.

Calculations indicated that the nitro group in 2-NPy is coplanar with the ring, but not so in case of 4-NPy (Figure 1D). Thus, in the nitropyrenes, the correlation between nitro group orientation and mutagenicity fails. The metabolic pathways that nitro-PAHs undergo in an in vivo system to express mutagenicity is very complex. Different mechanisms are involved when nitro-PAHs induce mutagenic effects on prokaryotic cells vis-à-vis eukaryotes. Experimental records have revealed that in many mammalian and human cell lines, multiple routes are followed in

the reduction and oxidation of the nitro group (8). This is due to the complexities of the reactions occurring in the cells. The cytochrome P450 system triggers many differently structured monooxygenases that are involved in the oxidation, peroxidative, and reduction metabolism of the environmental pollutants. For example, studies have indicated differences in the metabolic pathways for the reduction of 4-NPy vis-à-vis 1-NPy and 2-NPy (8). The charge distribution around the PAH ring system, the stereochemical strain on the PAH ring system, or the stereochemical strain on its activated metabolite as well as the complementary shapes also influence reactivity and could be the reason that, as four rings are considered, the trend based on NO₂ group orientation begins to fail.

LUMO Energy Levels

Nitro-PAHs require metabolic activation to exhibit mutagenic, carcinogenic, or both effects. Both the oxidation of the aromatic ring and reduction of the nitro group are relevant in the metabolic activation. The hypothesis is that the orientation of the nitro group determines the reducibility by the various nitroreductases (45). In support of the model involving reduction of the nitro group, a correlation between the LUMO energies and the ease with which they are reduced in bacterial strains is expected. Indeed, LUMO energies (E_{LUMO}) have been used previously as predictors of mutagenicity (14) using the semiempirical AM1 method (15). E_{LUMO} provides a measure of the negative electron affinity via the application of the Koopman's theorem (15). Decreased LUMO energies are indicative of the electron-withdrawing nature of the nitro compounds compared to their parent PAHs.

Following this approach, we have examined the correlation between the planarity of the NO₂ group and the LUMO energies of the nitro-PAHs. Table 5 reports the LUMO values for each class of PAH. For example, among the nitroanthracenes, the order of LUMO is 1-NA < 2-NA ≪ 9-NA, suggesting that 9-NA should be less mutagenic, in agreement with experiment.

For the nitrophenanthrenes, the LUMO order is 3-NPh < 2-NPh < 1-NPh < 9-NPh < 4-NPh, and 3-NPh is known to have the highest mutagenicity.

Among the pyrene isomers, the E_{LUMO} energies follow the trend of 1-NPy < 4-NPy < 2-NPy. This trend correlates well with the biological activities of 2-NPy but does not explain the increased mutagenicity of 4-NPy.

TABLE 5. LUMO Values of Nitro-Polycyclic Aromatic Hydrocarbons (Nitro-PAHs)

Nitro-PAH	LUMO (eV)
Nitrobenzene	-0.08923
1-Nitronaphthalene	-0.0907
2-Nitronaphthalene	-0.09053
1-Nitroanthracene	-0.09611
2-Nitroanthracene	-0.09586
9-Nitroanthracene	-0.09098
1-Nitrophenanthrene	-0.08787
2-Nitrophenanthrene	-0.08971
3-Nitrophenanthrene	-0.09019
4-Nitrophenanthrene	-0.08015
9-Nitrophenanthrene	-0.08878
1-Nitropyrene	-0.09508
2-Nitropyrene	-0.08727
4-Nitropyrene	-0.09233
6-Nitrochrysene	-0.10829

CONCLUSIONS

In this study, DFT calculations on a series of nitro-PAHs are reported. Comparisons with known structural parameters for the isomers show that DFT is an excellent method for calculating geometry and energy parameters. Based on the calculations, we have been able to examine in detail the correlations between mutagenicity and the orientation of the NO₂ group relative to the aromatic plane. The correlation works well for nitro-PAHs such as nitronaphthalenes, nitroanthracenes, and nitrophenanthrenes with some differences noted in nitropyrene compounds (four aromatic rings).

REFERENCES

1. W. A. MacCrehan, W. E. May, S. D. Yang, and B. A. Benner Jr., Determination of Nitro Polynuclear Aromatic Hydrocarbons in Air and Diesel Particulate Matter using liquid chromatography with Electrochemical and Fluorescence Detection, *Analytical Chemistry* 60 (1988):194–199.
2. T. Enya, H. Suzuki, T. Watanabe, T. Hirayama, and Y. Hisamatsu, 3-Nitrobenzanthrone, a Powerful Bacterial Mutagen and Suspected Human Carcinogen Found in Diesel Exhaust and Airborne Particulates, *Environmental Science and Technology* 31 (1997):2772–2776.

3. J. C. Sasaki, J. Arey, D. A. Eastmond, K. K. Parks, P. T. Phousongphouang, and A. J. Grosovsky, Evidence for Oxidative Metabolism in the Genotoxicity of the Atmospheric Reaction Product 2-Nitronaphthalene in Human Lymphoblastoid Cell Lines, *Mutation Research* 445 (1999):113–125.
4. K. Fukuhara, M. Kurihara, and N. Miyata, Photochemical Generation of Nitric Oxide from 6-Nitrobenzo[a]pyrene, *Journal of the American Chemical Society* 123 (2001):8662–8666.
5. M. Iwanari, M. Nakajima, R. Kizu, K. Hayakawa, and T. Yokoi, Induction of CYP1A1, CYP1A2, and CYP1B1 mRNAs by Nitropolycyclic Aromatic Hydrocarbons in Various Human Tissue-Derived Cells: Chemical-, Cytochrome P450 Isoform-, and Cell-Specific Differences, *Archives of Toxicology* 76 (2002):287–298.
6. J. L. Durant, W. F. Busby Jr., A. L. Lafleur, B. W. Penman, and C. L. Crespi, Human Cell Mutagenicity of Oxygenated, Nitrated and Unsubstituted Polycyclic Hydrocarbons Associated with Urban Aerosols, *Mutation Research* 371 (1996):123–157.
7. P. P. Fu, L. S. V. Tungeln, L.-H. Chiu, D.-J. Zhan, J. Deck, T. Bucci, and C.-J. Wang, Structure, Tumorigenicity, Microsomal Metabolism, and DNA Binding of 7-Nitrodibenz[a,h]Anthracene, *Chemical Research in Toxicology* 11 (1998):937–945.
8. V. Purohit and A. K. Basu, Mutagenicity of Nitroaromatic Compounds, *Chemical Research in Toxicology* 13 (2000):673–692.
9. W. A. Vance and D. E. Levin, Structural Features of Nitroaromatics that Determine Mutagenic Activity in *Salmonella typhimurium*, *Environmental Mutagenesis* 6 (1984):797–811.
10. H. Jung, A. U. Shaikh, R. H. Heflich, and P. P. Fu, Nitro Group Orientation, Reduction Potential, and Direct-Acting Mutagenicity of Nitro-Polycyclic Aromatic Hydrocarbons, *Environmental and Molecular Mutagenesis* 17 (1991):169–180.
11. Y. S. Li, P. P. Fu, and J. S. Church, The Conformation of Some Nitro-Polycyclic Aromatic Hydrocarbons, *Journal of Molecular Structure* 550–551 (2000):217–223.
12. P. P. Fu, M. W. Chou, D. W. Miller, G. L. White, R. H. Heflich, and F. A. Beland, The Orientation of the Nitro Substituent Predicts the Direct-Acting Bacterial Mutagenicity of Nitrated Polycyclic Aromatic Hydrocarbons, *Mutation Research* 143 (1985):173–181.
13. J. C. Rienstra-Kiracofe, C. J. Barden, S. T. Brown, and H. F. Schaefer III, Electron Affinities of Polycyclic Aromatic Hydrocarbons, *The Journal of Physical Chemistry* A105 (2001):524–528.
14. A. K. Debnath, R. L. Lopez de Compadre, G. Debnath, A. J. Shusterman, and C. Hansch, Structure-Activity Relationship of Mutagenic Aromatic and Heteroaromatic Nitro Compounds: Correlation with Molecular Orbital Energies and Hydrophobicity, *Journal of Medicinal Chemistry* 34 (1991):786–797.
15. A. Kitti, M. Harju, M. Tysklind, and B. V. Bavel, Multivariate Characterization of Polycyclic Aromatic Hydrocarbons Using Semi-Empirical Molecule Orbital Calculations and Physical Data, *Chemosphere* 50 (2003):627–637.
16. S.-T. Lin, Y.-F. Jih, and P. P. Fu, Mass Spectral Analysis of Nitropolycyclic Aromatic Hydrocarbons with Torsion Angle Obtained from Semiempirical Calculations, *Journal of Organic Chemistry* 61 (1996):5271–5273.

17. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. Strain, et al., Gaussian packages, Gaussian 98, Revision A. and Revision A.9, Gaussian, Inc., Pittsburgh, Pa. (1998).
18. A. D. Becke, A New Mixing of Hartree-Fock and Local Density-Functional Theories, *Journal of Chemical Physics* 98 (1993):1372–1377.
19. C. Lee, W. Yang, and R. G. Parr, Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density, *Physical Review B* 37 (1988):785–789.
20. W. J. Hehre, L. Radom, P. V. R. Scheyes, and J. A. Pople, *Ab Initio Molecular Orbital Theory* (New York: John Wiley and Sons Inc., 1986).
21. E. A. Momany and J. L. Willett, Computational Studies on Carbohydrates: I. Density Functional *Ab Initio* Geometry Optimization on Maltose Conformations, *Journal of Computational Chemistry* 21 (2000):1204–1219.
22. C. J. Cramer, *Essentials of Computational Chemistry: Theories and Models* (New York: John Wiley & Sons, 2002).
23. A. P. Scott and L. Radom, Harmonic Frequencies: An Evaluation of Hartree-Fock, Moller-Plesset, Quadric Configuration Interaction, Density Functional Theory, and Semiempirical Scale Factors, *The Journal of Physical Chemistry* 100 (1996):16502–16513.
24. S. Y. Lee, Density Functional Theory Calculation of Molecular Structure and Vibrational Spectra of Dibenzothiophene in the Ground and the Lowest Triplet State, *The Journal of Physical Chemistry A* 105 (2001):8093–8097.
25. P. Swiderek, G. Hohlneicher, S. A. Maliendes and M. Dupis, Theoretical Prediction of the Vibrational Spectrum of Naphthalene in the First Excited Singlet State, *Journal of Chemical Physics* 98(1993):974–987.
26. E. Cané, A. Miani, and A. Trombetti, Geometry of Benzene From the Infrared Spectrum, *Journal of Chemical Education* 76(1999):1288–1290.
27. L. E. Sutton (Ed.), *Tables of Interatomic Distances and Configuration in Molecules and Ions*, M19 (London: The Chemical Society, 1958).
28. S. Irle, T. M. Krygowski, J. E. Niu, and H. E. Schwarz, Substituent Effects of -NO and NO₂ Groups in Aromatic Systems, *Journal of Organic Chemistry* 60 (1995):6744–6755.
29. M. Staikova and I. G. Csizmadia, *Ab Initio* Investigation of Internal Rotation in Conjugated Molecules and the Investigation of NO₂ in Nitroaromatics: Nitrobenzene, *O*-monofluoro- and *O*,*O'*-Difluoro-Nitrobenzenes, *Journal of Molecular Structure (Theochem.)* 467 (1999):181–186.
30. F. H. Allen, O. Kennard, D. G. Watson, L. Brammer, A. G. Orpen, and R. Taylor, Tables of Bond Lengths Determined by X-Ray and Neutron Diffraction: I. Bond Lengths in Organic Compounds, *Journal of the Chemical Society Perkin Transaction II* 2 (1987):S1–S19.
31. J. Trotter, The Crystal Structure of Some Anthracene Derivatives: V. 9-Nitroanthracene, *Acta Crystallographica* 12 (1959):237–242.
32. M. R. Taylor and M. J. Thompson, 4-Nitrophenanthrene, *Acta Crystallographica* E57 (2001): 9–10.

33. A. Sekine, H. Uekusa, Y. Ohashi, K. Yoshimura, M. Yagi, and J. Higuchi, 4-Nitro-9-10-Dihydrophenanthrene and a Polymorphic Form of 4-Nitrophenanthrene, *Acta Crystallographica C57* (2001):1462–1464.
34. J. L. Durant, A. L. Lafleur, E. F. Plummer, K. Taghizadeh, W. F. Busy Jr., and W. G. Thilly, Human Lymphoblast Mutagens in Urban Airborne Particles, *Environmental Science and Technology* 32 (1998):1894–1906.
35. K. El-Bayoumy, D. Desai, T. Boyiri, J. Rosa, J. Krzeminski, A. K. Sharma, B. Pittman, and S. Amin, Comparative Tumorigenicity of the Environmental Pollutant 6-Nitrochrysene and Its Metabolites in the Rat Mammary Gland, *Chemical Research in Toxicology* 15 (2002):972–978.
36. J. C. Sasaki, J. Arey, D. A. Eastmond, K. K. Parks, and A. J. Grosovsky, Genotoxicity Induced in Human Lymphoblasts by Atmospheric Reaction Products of Naphtahlene and Phenanthrene, *Mutation Research* 393 (1997):23–35.
37. H. S. Rosenkranz and R. Mermestein, in *Nitrated Polycyclic Aromatic Hydrocarbons*, ed. C. M. White (Heidelberg: Dr. Alfred Huethig Verlag, Heidelberg, 1985), pp. 267–297.
38. P. P. Fu, R. H. Heflich, L. S. V. Tungeln, D. T. Yang, E. K. Fifer, and F. A. Beland, Effect of the Nitro Group Conformation on the Rat Liver Microsomal Metabolism and Bacterial Mutagenicity of 2- and 9-Nitroanthracene, *Carcinogenesis* 7 (1986):1819–1827.
39. P. P. Fu, L. S. V. Tungeln, and M. W. Chou, Metabolism of 9-Nitroanthracene by Rat Liver Microsomes: Identification and Mutagenicity of Metabolites, *Carcinogenesis* 6 (1985):753–757.
40. N. Sera, K. Fukuhara, N. Miyata, and H. Tokiwa, Mutagenicity of Nitrophenanthrene Derivatives for *Salmonella typhimurium*: Effects of Nitroreductase and Acetyltransferase, *Mutation Research* 349 (1996):137–144.
41. T. Hirayama, K. Iguchi, and T. Watanabe, Metabolic Activation of 2,-Dinitrophenyl Derivatives for Their Mutagenicity in *Salmonella typhimurium* TA 98, *Mutation Research* 243 (1990):201–206.
42. K. Imaida, M. Hirose, L. Tay, M. S. Lee, C. Y. Wang, and C. M. King, Comparative Carcinogenicities of 1-, 2-, and 4-Nitropyrene and Structurally Related Compounds in the Female CD Rat, *Cancer Research* 51 (1991):2902–2907.
43. L. Zhou and B. P. Cho, Synthesis, Characterization, and Comparative Conformational Analysis of N-(Deoxyguanosine-8-yl)aminopyrene Adducts Derived from the Isomeric Carcinogens 1-, 2-, and 4-nitropyrene, *Cancer Research in Toxicology* 11 (1998): 35–48.
44. Y.-H. Chae, B.-Y. Ji, J.-M. Lin, P. P. Fu, B. P. Cho, and K. El-Bayoumy, Nitroreduction of 4-Nitropyrene is Primarily Responsible for DNA Adduct Formation in the Mammary Gland of Female CD Rats, *Cancer Research in Toxicology* 12 (1999):180–186.
45. C. Ioannides (Ed.), *Enzyme Systems that Metabolise Drugs and Other Xenobiotics* (New York: John Wiley, 2002).
46. P. Urbanowicz, T. Kupka, R. Wrzalik, and K. Pasterny, Molecular Orbital Studies of Harmonic Vibrations of Nitrobenzene in the Gas Phase and Solution Using Semi-Empirical, Ab Initio and Density Functional Theory Calculations, *Journal of Molecular Structure* 482–483 (1999):409–414.

47. I. Hargittai and M. Hargittai (Eds.), *Stereochemical Applications of Gas-Phase Electron Diffraction. Part B: Structural Information for Selected Classes of Compounds*, (New York: VCH Publishers, 1988).
48. E. K. Fifer, P. C. Howard, R. H. Heflich, and F. A. Beland, Synthesis and Mutagenicity of 1-Nitropyrene 4,5-Oxide and 1-Nitropyrene 9,10-Oxide, Microsomal Metabolites of 1-Nitropyrene, *Mutagenesis* 1 (1986):433–438.

SUPPLEMENTARY INFORMATION

TABLE S1. Methods, Basis Sets, Optimization Levels of Theory, and Experimental Studies in the Literature

Aromatic hydrocarbon	Levels of theory used in past studies	Theoretical studies (References)	Experimental studies (References)
Benzene	B3LYP with basis sets 6-31G*, TZP, 6-311G*, aug-cc-pVDZ, PVDZ, CCSD(T), DZP	Miani (2000) ^a Wiberg (1997) ^b Swiderek (1993) ^c	Cane (1999) ^d
Naphthalene	B3LYP with 6-311G, pVDZ, aug-cc-pVDZ basis sets	Wiberg (1997) ^b Millefiori (1998) ^e	Ketkar (1981) ^f
Anthracene	B3LYP/6-311G*, BLYP/6-311G*	Wiberg (1997) ^b Cioslowski (1996) ^g Cioslowski (1999) ^h	Chaplot (1982) ⁱ Ketkar (1981) ^j Wiberg (1997) ^b
Phenanthrene	HF/4-21G*, B3LYP/cc-pVDZ	Bandyopadhyay (2000) ^k Martin (1996) ^m	Kay (1971) ^l Bandyopadhyay (2000) ^k Martin (1996) ^m
Chrysene	B3LYP/6-311G*	Wiberg (1997) ^b	Burns (1960) ⁿ
Pyrene	B3LYP/6-311G*	Wiberg (1997) ^b	Hazel (1972) ^o

^aA. Miani, *J. Chem. Phys.* 112, 248–259 (2000); ^bK. B. Wiberg, *J. Org. Chem.* 62, 5720–5727 (1997); ^cP. Swiderek, G. Hohlneicher, A. M. Sergio, M. Dupuis, *J. Phys. Chem.* 98, 974–987 (1993); ^dE. Cane, A. Miani, A. Trombetti, *J. Chem. Educ.* 76, 1288–1290 (1999); ^eS. Millefiori, A. Alparone, *J. Mol. Struct. (Theochem)* 422, 179–190 (1998); ^fS. N. Ketkar, M. Fink, *J. Mol. Struct.* 77, 139–147 (1981); ^gJ. Cioslowski, G. Liu, M. Martinov, P. Piskorz, D. Moncrieff, *J. Am. Chem. Soc.* 118, 5261–5264 (1996); ^hJ. Cioslowski, M. Schimeczek, P. Piskorz, D. Moncrieff, *J. Am. Chem. Soc.* 121, 3773–3778 (1999); ⁱS. L. Chaplot, N. Lehner, G. S. Pawley, *Acta Cryst.* B38, 483–487; ^jS. N. Ketkar, M. Fink, R. C. Ivey, *J. Mol. Struct.* 77, 127–138 (1981) (electron diffraction); ^kI. Bandyopadhyay, S. Manogaram, *J. Mol. Struct. (Theochem.)* 496, 107–119 (2000); ^lM. I. Kay, Y. Okaya, D. E. Cox, *Acta Cryst.* B27, 26–33 (1971); ^mJ. M. Martin, J. El-Yazal, J.-P. Francois, *J. Phys. Chem.* 100, 15358–15367 (1996); ⁿD. M. Burns, *J. Ball J. Proc. Royal Soc. (London), Ser. A, Math. Phys. Sci.* 257, 491–514 (1960); ^oC. A. Hazel, F. K. Larsen, M. S. Lehmann, *Acta Cryst.*, B28, 2977–2984 (1972).

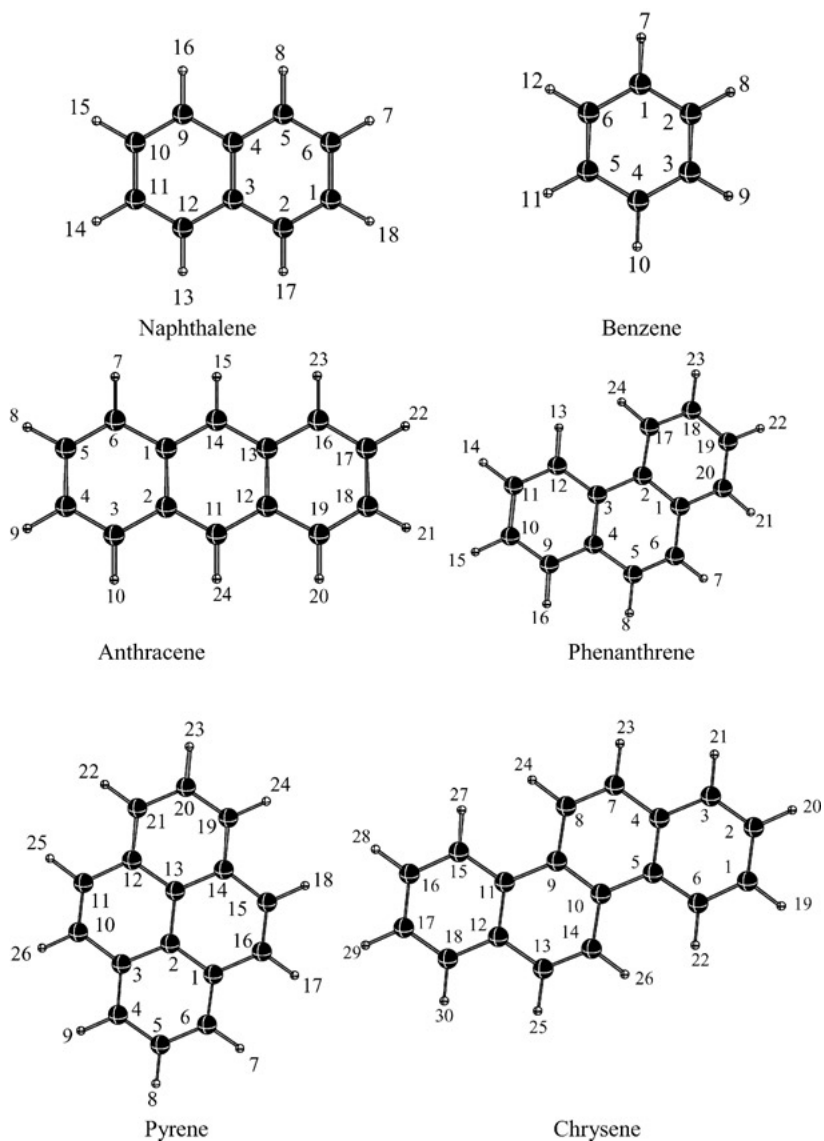


FIGURE S1. Structures of polycyclic aromatic hydrocarbons used for calibration.

TABLE S2. Bond Lengths (Å) and Bond Angles (°) of Benzene

Parameter	Calc.	Expt. ^a	Parameter	Calc.	Expt.
C1–C2	1.3967	1.397	C1–C2–C3	120.000	120 ^c
C2–C3	1.3967	1.397	C2–C3–C4	120.000	120 ^c
C3–C4	1.3966	1.397	C3–C4–C5	120.000	120 ^c
C4–C5	1.3967	1.397	C4–C5–C6	120.000	120 ^c
C5–C6	1.3967	1.397	C3–C4–H10	119.999	
C6–C1	1.3967	1.397	C1–C6–C5	119.997	
C1–H7	1.087	1.079 ^a , 1.084 ^b	C5–C6–H12	120.002	
C2–H8	1.087	1.079 ^a , 1.083 ^b			
C3–H9	1.087	1.079 ^a , 1.083 ^b			
C4–H10	1.087	1.079 ^a , 1.083 ^b			
C5–H11	1.087	1.079 ^a , 1.083 ^b			
C6–H12	1.087	1.079 ^a , 1.083 ^b			

^aSwiderek, Petra et al. *J. Phys. Chem* 98, 974–987 (1993)—Derived from rotational constants of C₆H₆ by ultrahigh resolution resolution spectroscopy.

^bElisabeth Cane, Andrea Miani, Agostino Trombetti, *J. Chem Educ.* 76, no. 9, 1288–1290 (1999) (microwave spectroscopy).

^cSutton, L. E. Tables of Interatomic distances, The chemical society, London 1958.

TABLE S3. Bond Lengths (Å) and Bond Angles (°) in Naphthalene

Parameter	Calc.	Expt.(e-diffr.) ^a	Parameter	Calc.	Expt.
C1–C2	1.3766	1.377(2) ^b	C2–C1–C6	118.597	119.5(3)
C2–C3	1.4211	1.411(2) ^a	C2–C1–C14	119.087	119.5(3)
C3–C4	1.4339	1.420	C6–C1–C14	122.316	119.5(3)
C4–C5	1.4211	1.422(3)	C1–C2–C3	118.594	119.5(3)
C5–C6	1.4169	1.381(2) ^a			
C6–C1	1.4169	1.412(8)	C2–C3–C4	120.970	119.5(3)
C6–H7	1.0868	1.092(1)	C1–C2–C11	119.089	119.5(3)
C5–H8	1.0877	1.092(1)	C2–C3–H10	118.453	119.7 ^b
C4–C9	1.4211	1.422	C4–C3–H10	120.577	119.7 ^b
C9–C10	1.3766	1.377(2) ^b	C3–C4–C5	120.434	119.5(3) ^b
C10–C11	1.4169	1.412 ^b	C3–C4–H9	120.156	119.7 ^b
C3–C12	1.4211	1.424(2) ^a	C4–C5–C6	120.436	119.5(3)
C12–H13	1.0877	1.092(1) ^b	C4–C5–H8	119.411	119.7 ^b
C10–H15	1.0868	1.092(1) ^b	C6–C5–H8	120.153	119.7 ^b
C9–H16	1.0877	1.092(1) ^b			
C2–H17	1.0877	1.092(1) ^b			
C1–H18	1.0868	1.092(1) ^b			

^aK. B. Wiberg, *J. Org. Chem.* 62, 5720–5727 (1997).

^bS. N. Ketkar, M. Fink, *J. Mol. Struct.* 77, 139–147 (1981) (electron diffraction).

TABLE S4. Bond Lengths (Å) and Bond Angles (°) in Anthracene

Parameter	Calc.	Expt. ^a	Expt. ^b	Parameter	Calc.	Electron.	Neutr.
		(Electron.)	(Neutr.)			diff. ^a	diff. ^b
C1–C2	1.4453	1.437	1.444	C2–C1–C6	118.597	118.86	
C1–C14	1.4005	1.392	1.407	C2–C1–C14	119.087		
C2–C3	1.43		1.436	C6–C1–C14	122.316		
C2–C11	1.4005			C1–C2–C3	118.594		118.7
C3–C4	1.3699	1.422		C1–C2–C11	119.089		119.4
C4–C5	1.4262	1.432		C3–C2–C11	122.317		121.9
C5–C6	1.3699	1.397	1.378	C2–C3–C4	120.97		121
C6–C1	1.43	1.437	1.436	C2–C3–H10	118.453		118.4
C6–H7	1.0876	1.085	1.087	C4–C3–H10	120.577		120.6
C5–H8	1.0867	1.085	1.093	C3–C4–C5	120.434		120.3
C4–H9	1.0867	1.085		C3–C4–H9	120.156		120
C3–H10	1.0876	1.085		C5–C4–H9	119.409		119.7
C11–C12	1.4005			C4–C5–C6	120.436	120.09	
C11–H24	1.084	1.085		C4–C5–H8	119.411		
C12–C13	1.4453			C6–C5–H8	120.153		
C12–C19	1.43			C1–C6–C5	120.969	121.07	
C13–C14	1.4005			C1–C6–H7	118.456		
C13–C16	1.43			C5–C6–H7	120.575		
C14–H15	1.0884	1.085	1.097	C2–C11–C12	121.824	112	121.2
C16–C17	1.3699			C2–C11–H24	119.089		119.4
C17–C18	1.4262			C12–C11–H24	119.087		
C18–C19	1.3699			C11–C12–C13	119.087		
C19–H20	1.0876	1.085		C11–C12–C19	122.316		
C18–H21	1.0867	1.085		C13–C12–C19	118.597		
C17–H22	1.0867	1.085		C12–C13–C14	119.089		
C16–H23	1.0876	1.085		C12–C13–C16	118.594		
				C14–C13–C16	122.317		
				C1–C14–C13	121.824		
				C1–C14–H15	119.087		
				C13–C14–H15	119.089		
				C13–C16–C17	120.97		
				C13–C16–H23	118.453		
				C17–C16–H23	120.577		
				C16–C17–C18	120.434		

^aS. N. Ketkar, M. Fink, R. C. Ivey, *J. Mol. Struct.* 77, 127–138 (1981) (electron diffraction); K. B. Wiberg, *J. Org. Chem.* 1997, 62, 5720–5727.

^bS. L. Chaplot, *Acta Cryst.* B38, 483–487 (1982) (neutron diffraction); K. B. Wiberg, *J. Org. Chem.* 62, 5720–5727 (1997).

TABLE S5. Bond Lengths (Å) and Bond Angles (°) in Phenanthrene

Parameter	Calc.	Expt. (x-ray) ^a	Neutron diff. ^{a,b}	Parameter	Calc.	Expt. ^{a,b}
C1—C2	1.4273	1.412	1.427	C2—C1—C6	119.703	120.7
C1—C20	1.4143	1.433	1.427	C2—C1—C20	119.626	120.6
C2—C3	1.4578	1.464	1.465	C6—C1—C20	120.671	118.6
C3—C4	1.4273	1.420	1.419	C1—C2—C3	119.103	119.2
C4—C5	1.4351	1.453	1.474	C1—C2—C17	117.846	118.3
C5—C6	1.3597	1.341	1.358	C3—C2—C17	123.052	122.6
C5—H8	1.0874		1.04	C2—C3—C4	119.104	118.5
C6—C1	1.4351	1.446	1.435	C2—C3—C12	123.05	122.2
C6—H7	1.0875		1.07	C4—C3—C12	117.846	119.2
C9—C4	1.4144	1.424	1.406	C3—C4—C5	119.703	120.2
C10—C9	1.3808	1.382	1.389	C3—C4—C9	119.625	120.5
C10—C11	1.4073	1.379	1.409	C5—C4—C9	120.672	119.2
C12—C3	1.4147	1.416	1.395	C4—C5—C6	121.193	120.5
C12—H13	1.0848		1.10	C4—C5—H8	118.278	116.4
C11—H14	1.0867		1.10	C6—C5—H8	120.529	123.2
C10—H15	1.0866		1.11	C1—C6—C5	121.194	120.8
C9—H16	1.0876		1.11	C1—C6—H7	118.277	118.8
C17—C2	1.4147	1.408	1.416	C5—C6—H7	120.529	120.3
C17—C18	1.3832	1.391	1.397	C4—C9—C10	121.125	120.3
C19—C18	1.4073	1.393	1.393	C4—C9—H16	118.539	118.6
C20—C1	1.4143	1.433	1.427	C10—C9—H16	120.335	121.0
C20—H21	1.0876		1.10	C9—C10—C11	119.518	119.1
C19—H22	1.0866		1.05	C9—C10—H15	120.335	123.5
C18—H23	1.0867		1.11	C11—C10—H15	120.147	117.4
C17—H24	1.0848		1.07	C10—C11—C12	120.388	121.2
C12—C11	1.3832	1.406	1.407	C10—C11—H14	119.926	119.8
C19—C20	1.3808	1.365	1.407	C12—C11—H14	119.686	119.0
				C3—C12—C11	121.497	119.7
				C3—C12—H13	119.906	123.5
				C11—C12—H13	118.597	116.8
				C2—C17—C18	121.498	120.5
				C2—C17—H24	119.908	120.5
				C18—C17—H24	118.595	119.0
				C17—C18—C19	120.388	121.3
				C17—C18—H23	119.686	117.4
				C19—C18—H23	119.926	121.3
				C18—C19—C20	119.519	120.2
				C18—C19—H22	120.146	118.6
				C20—C19—H22	120.335	121.2
				C1—C20—C19	121.124	119.1
				C19—C20—H21	120.337	121.6

^aI. Bandyopadhyay, S. Manogaram, *J. Mol. Struct. (Theochem.)* 496, 107–119 (2000).^bM. I. Kay, Y. Okaya, D. E. Cox, *Acta Cryst. B*27, 26 (1971) (neutron diffract).

TABLE S6. Bond Lengths (Å) and Bond Angles (°) in Chrysene

Parameter	Calc.	X-ray ^a diffraction	Parameter	Calc.	X-ray. diff. ^a
C1–C2	1.4096		C2–C1–C6	120.559	
C2–C3	1.3785		C2–C1–H19	119.828	
C3–C4	1.4162		C6–C1–H19	119.613	
C4–C5	1.4278		C1–C2–C3	119.465	
C5–C6	1.3813		C1–C2–H20	120.106	
C6–C1	1.4183		C3–C2–H20	120.429	
C7–C4	1.4266		C2–C3–C4	121.01	
C7–C8	1.3638		C2–C3–H21	120.497	
C8–C9	1.4312		C4–C3–H21	118.494	
C9–C10	1.4184	1.401	C3–C4–C5	119.93	
C10–C5	1.4531		C3–C4–C7	121.011	
C11–C9	1.4531	1.468	C5–C4–C7	119.059	
C11–C12	1.4278	1.406	C4–C5–C6	117.521	
C12–C13	1.4266	1.421	C4–C5–C10	119.261	
C13–C14	1.3638	1.369	C6–C5–C10	123.217	
C14–C10	1.4312	1.428	C1–C6–C5	121.514	
C15–C11	1.4183	1.409	C1–C6–H22	118.355	
C15–C16	1.3813	1.379	C5–C6–H22	120.13	
C16–C17	1.4096	1.394	C4–C7–C8	121.308	
C17–C18	1.3785	1.363	C4–C7–H23	118.536	
C18–C12	1.4162	1.428	C8–C7–H23	120.156	
C1–H19	1.0867		C7–C8–C9	121.775	
C2–H20	1.0865		C7–C8–H24	118.581	
C3–H21	1.0876		C9–C8–H24	119.644	
C6–H22	1.0841		C8–C9–C10	118.667	
C7–H23	1.0874		C8–C9–C11	121.403	120.5
C8–H24	1.0837		C10–C9–C11	119.929	119.8
C13–H25	1.0874		C5–C10–C9	119.929	
C14–H26	1.0837		C5–C10–C14	121.403	
C15–H27	1.0841		C9–C10–C14	118.667	119.7
C16–H28	1.0867		C9–C11–C12	119.261	118.4
C17–H29	1.0865		C9–C11–C15	123.217	122.9
C18–H30	1.0876		C12–C11–C15	117.521	118.7
			C11–C12–C13	119.059	120.1
			C11–C12–C18	119.93	119.4
			C13–C12–C18	121.011	120.3
			C12–C13–C14	121.308	121.2
			C12–C13–H25	118.536	
			C14–C13–H25	120.156	
			C10–C14–C13	121.775	120.8

(Continued on next page)

TABLE S6. Bond Lengths (Å) and Bond Angles (°) in Chrysene
(Continued)

Parameter	Calc.	X-ray ^a diffraction	Parameter	Calc.	X-ray. diff. ^a
			C10–C14–H26	119.644	
			C13–C14–H26	118.581	
			C11–C15–C16	121.514	120.9
			C11–C15–H27	120.13	
			C16–C15–H27	118.355	
			C15–C16–C17	120.559	120.3
			C15–C16–H28	119.613	
			C17–C16–H28	119.828	
			C16–C17–C18	119.465	120.5
			C16–C17–H29	120.106	
			C18–C17–H29	120.429	
			C12–C18–C17	121.01	120.5
			C12–C18–H30	118.494	
			C17–C18–H30	120.497	

^aD. M. Burns, J. Iball, *Proceedings of the Royal Society of London, Series A, Mathematical and Physical Sciences* 257, 491–514 (1960).

^bK. B. Wiberg, *J. Org. Chem.*, 62, 5720–5727 (1997).

TABLE S7. Bond Lengths (Å) and Bond Angles (°) in Pyrene

Parameter	Calc.	Expt.(Neutr.) ^a	Parameter	Calc.	Neutr. diff. ^a
C1–C2	1.4286	1.426	C2–C1–C6	119.024	119.35
C2–C3	1.4285	1.425	C2–C1–C16	118.562	118.84
C2–C13	1.4266	1.43	C6–C1–C16	122.413	121.82
C3–C4	1.4041	1.414	C1–C2–C3	119.858	120.04
C3–C10	1.4377	1.435	C1–C2–C13	120.071	120.11
C4–C5	1.3939	1.396	C3–C2–C13	120.071	119.85
C5–C6	1.3939	1.393	C2–C3–C4	119.025	118.76
C6–C1	1.4041	1.392	C2–C3–C10	118.562	118.73
C10–C11	1.3616	1.365	C4–C3–C10	122.413	122.52
C11–C12	1.4377	1.444	C3–C4–C5	120.76	120.36
C12–C13	1.4286	1.421	C3–C4–H9	119.145	119.15
C13–C14	1.4286	1.429	C5–C4–H9	120.095	120.49
C14–C15	1.4377	1.433	C4–C5–C6	120.572	120.71
C16–C1	1.4377	1.439	C4–C5–H8	119.715	118.31
C14–C19	1.4041	1.409	C6–C5–H8	119.713	120.98
C20–C12	1.4041	1.407	C1–C6–C5	120.761	120.79

(Continued on next page)

TABLE S7. Bond Lengths (Å) and Bond Angles (°) in Pyrene (*Continued*)

Parameter	Calc.	Expt.(Neutr.) ^a	Parameter	Calc.	Neutr. diff. ^a
C15–C16	1.3636	1.369	C1–C6–H7	119.145	118.23
C20–C19	1.3939	1.392	C5–C6–H7	120.094	120.98
C20–C21	1.3939	1.398	C3–C10–C11	121.368	121.66
C6–H7	1.0874	1.099	C3–C10–H26	118.275	118.42
C5–H8	1.0867	1.1	C11–C10–H26	120.357	119.92
C4–H9	1.0874	1.113	C10–C11–C12	121.365	120.69
C11–H25	1.0875	1.091	C10–C11–H25	120.358	120.34
C10–H26	1.0875	1.126	C12–C11–H25	118.277	118.97
C15–H18	1.0875	1.097	C11–C12–C13	118.563	118.82
C16–H17	1.0875	1.098	C11–C12–C21	122.413	121.97
C21–H22	1.0874	1.098	C13–C12–C21	119.024	119.22
C20–H23	1.0867	1.074	C2–C13–C12	120.071	120.26
C19–H24	1.0874	1.109	C2–C13–C14	120.071	119.7
			C12–C13–C14	119.858	120.05
			C13–C14–C15	118.563	119.13
			C13–C14–C19	119.024	118.94
			C15–C14–C19	122.413	121.94
			C14–C15–C16	121.366	121.17
			C14–C15–H18	118.276	118.38
			C16–C15–H18	120.358	120.25
			C1–C16–C15	121.366	121.06
			C1–C16–H17	118.276	118.99
			C15–C16–H17	120.358	119.95
			C14–C19–C20	120.761	120.66
			C14–C19–H24	119.144	118.72
			C20–C19–H24	120.095	120.63
			C19–C20–C21	120.572	120.66
			C19–C20–H23	119.715	120.28
			C21–C20–H23	119.713	119.07
			C12–C21–C20	120.761	120.46
			C12–C21–H22	119.146	119.32
			C20–C21–H22	120.093	120.22

^aC. A. Hazel, F. K. Larsen, M. S. Lehmann, *Acta Crysta.* B28 2977–2984 (1972).

Copyright of Polycyclic Aromatic Compounds is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.