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Synthesis and Structure–Mutagenicity Relationship of Benzo-Annulated Cyclopentaphenanthrenes

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Abstract—The synthesis of 2,3-dihydro-1*H*-indeno[5,4-*a*]anthracene (**2**), the fluoreno[*a*]anthracenes **3** and **4,** 2,3-dihydro-1*H*-cyclopenta[*a*]chrysene (**6**), 3,4-dihydro-2-vinylphenanthrene (**10**) and cyclopenta[*c*]chrysenes **11**, **12** has been described. Structure analysis of the new products by ^{1}H and ^{13}C NMR spectroscopy is presented. Estimates of the mutagenic activity of compounds **2**–**4**, **6** and **11**–**14** in *Salmonella typhimurium* determined by Ames' test indicate that all products are inactive for both TA 98 and TA 100 strains except 4,5-dihydro-3*H*-cyclopenta[*c*]chrysene (**12**). The mutagenic properties of these compounds have been compared with those shown by previously studied benzo[*g*]cyclopenta[*a*]phenanthrenes and cyclopenta[*c*]phenanthrenes and discussed. Some conclusions have been drawn about the effects of benzoannulation and of the carbonyl function on the mutagenicity of this class of compounds.

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are an important class of environmental pollutants which are derived from the incomplete combustion of organic matter.[1] Several of these compounds are powerful animal mutagens or carcinogens and may, therefore, pose a serious threat to human health. The relationship between the structures of mutagenic and carcinogenic PAHs and the base-pairs in nucleic acids has been a crucial problem that has attracted the attention of chemists and biologists.[2] The most complete investigation on the molecular mechanism by which PAHs elicit their biological activities was performed with the benzo-derivatives of pyrene, i.e., benzo[*a*]pyrene (B[*a*]P) and dibenzo[*a,l*]pyrene (DB[*a,l*]P).[1,3] Some years ago we began a research program to study the effects of benzo-annulation on the mutagenicity of cyclopenta[*a*]- and cyclopenta[*c*]phenanthrenes. In contrast to the structure of the cyclopenta[*a*]phenanthrenes which are characterized by the presence of a bay region, the isomeric cyclopenta[*c*]phenanthrenes contain a pseudo-fjord region. The aim of the project is to help to investigate more fully the structure–mutagenicity relationship in this class of compounds. Previous papers have reported on the effects of benzo[*g*]annulation[4] of cyclopentaphenanthrenes. We have extended the study to benzo[*h*]- and benzo[*i*]- derivatives in order to evaluate and compare the effects of benzoannulation at the different positions of the A benzene ring in both cyclopenta[*a*]-[5] and cyclopenta[*c*]phenanthrene[4a] derivatives.

The present paper reports the synthesis of six new polycyclic compounds **2**–**4**, **6**, **11** and **12,** and the mutagenic activity of compounds **2**–**4**, **6** and **11**–**14** in *Salmonella typhimurium*.

Results

Chemistry

The synthetic procedures for compounds **2**–**4**, **6**, **11** and **12** are outlined in Schemes 1 and 2. 2,3-Dihydro-1*H*-indeno[5,4-*a*]anthracene (**2**), and 12*H*-fluoreno[2,1-*a*]anthracene (**3**) and 1*H*-fluoreno[3,4-*a*]anthracene (**4**) were prepared by Diels–Alder reaction of diene **1**, subsequent $NaBH_4$ reduction of the carbonyl function of the Diels–Alder cycloadducts followed by dehydration–aromatization over 10% Pd/C[6] (Scheme 1). Similarly 2,3-dihydro-1*H*-cyclopenta[*a*]chrysene (**6**) was prepared by Diels–Alder reaction between diene **5** and 2-bromo-

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2-cyclopenten-1-one, and subsequent reduction ($NaBH_4$) of pentacyclic ketones followed by dehydration–aromatization with 10% Pd/C of the alcoholic mixture (15% overall yield) (Scheme 1). Previously,[7] compound **6** was prepared by a nine-step synthesis in 8% yield. Their structures were assigned by NMR spectroscopy. The assignment of the methylenic protons on C-12 and C-1 for hydrocarbons **3** and **4**, respectively, was based on ^1H–^1H NOE experiments. Selective pre-irradiation of the resonance due to Hs-12 for compound **3** resulted in signal enhancement of the resonances attributed to H-11 and H-13, while for hydrocarbon **4**, NOE enhancement was observed on H-2 and H-15 by irradiation of Hs-1.

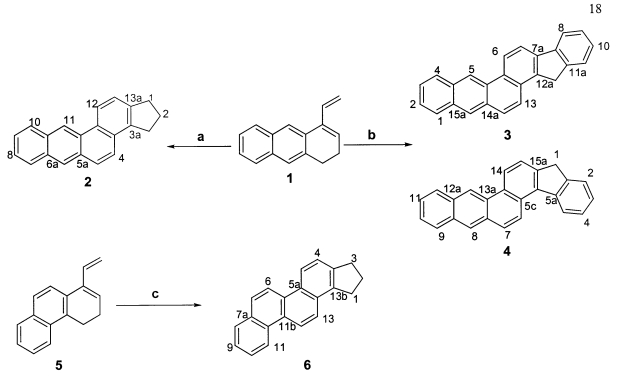
3,4-Dihydro-2-vinylphenanthrene (**10**) was prepared in 56% overall yield by acetylation of 3,4-dihydro-2*H*-phenanthren-1-one (**7**)[8] with acetic anhydride-boron trifluoride system, and subsequent reduction ($NaBH_4$) of diketone **9** followed by dehydration with $POCl_3$ in pyridine of the crude mixture of diols.[9]

3,4-Dihydro-5*H*-cyclopenta[*c*]chrysen-5-one (**11**) was prepared in 53% overall yield by a $EtAlCl_2$- catalyzed Diels–Alder reaction of diene **10** with 4-acetoxy-2-cyclopenten-1-one[6,10] and subsequent treatment of the products with Pd/C catalyst. The determined structure was based on selective ^1H–$\{^1H\}$ NOE experiments. Selective pre-irradiation of the resonance due to the C-3 hydrogens resulted in signal enhancement of resonances attributed to Hs-2 and Hs-4, while selective saturation of H-1 enhanced the signal of H-2, H-13 and saturation of H-6 gave NOE enhancement of H-7, thus indicating the regiochemistry of the carbonyl function.

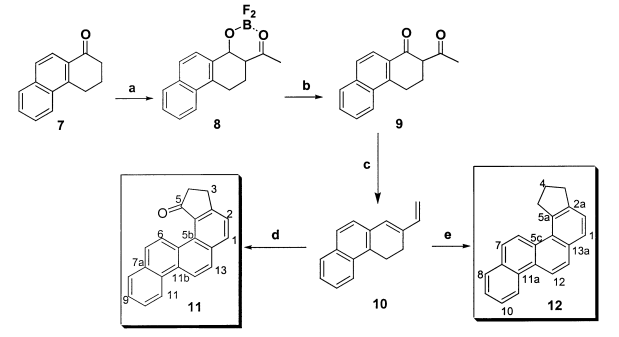
When the products of the above-described Diels–Alder reaction were submitted to $NaBH_4$ reduction followed by dehydration–aromatization over 10% Pd/C, 4,5-dihydro-3*H*-cyclopenta[*c*]chrysene (**12**) was obtained in 47% overall yield.

The assignment of structure to hydrocarbon **12** was based on the outcome of the reactions used to prepare it, and on the analysis of ^1H–$\{^1H\}$ NOE effects observed in NMR spectra. Selective pre-irradiation of the resonances attributed to the Hs-5 protons in hydrocarbon **12** resulted in signal enhancement of the resonance attributed to Hs-4 and H-6 protons, while saturation of H-1 proton enhanced the signals of H-2 and H-13 and the saturation of H-11 enhanced the signals of H-10 and H-12.

Compounds **13** and **14** (Fig. 1) were obtained as described in previous paper.[8]



Scheme 1. (a) (1) 4-acetoxy-2-cyclopenten-1-one, $EtAlCl_2$, CH_2Cl_2, 0 °C; (2) $NaBH_4$, $MeOH/CH_2Cl_2$, rt; (3) 10% Pd/C, triglyme, reflux; (b) (1) 3-bromo-indan-1-one, Et_3N, CCl_4, reflux; (2) $NaBH_4$, $MeOH/CH_2Cl_2$, rt; (3) 10% Pd/C, triglyme, reflux; (c) (1) 2-bromo-2-cyclopenten-1-one, $EtAlCl_2$, CH_2Cl_2, 0 °C; (2) $NaBH_4$, $MeOH/CH_2Cl_2$, rt; (3) 10% Pd/C, triglyme, reflux;.



Scheme 2. (a) $BF_3.Et_2O$, Ac_2O, 60 °C, (99%); (b) CH_3CO_2Na, acetone/H_2O, reflux, (82%); (c) (1) $NaBH_4$, EtOH, reflux; (2) Pyridine, $POCl_3$, reflux, (68%); (d) (1) 4-acetoxy-2-cyclopenten-1-one, $EtAlCl_2$, CH_2Cl_2, 0 °C; (2) 10% Pd/C, triglyme; reflux, (53%); (e) (1) 4-acetoxy-2-cyclopenten-1-one, $EtAlCl_2$, CH_2Cl_2, 0 °C; (2) $NaBH_4$, MeOH/CH_2Cl_2, rt; (3) 10% Pd/C, triglyme, reflux, (47%).

Biology

The compounds **2**–**4**, **6** and **11**–**14** were tested for reversion of the base pair substitution mutated *his* revertant strain TA 100 and of the frameshift mutated *his* revertant TA 98 strain of *Salmonella typhimurium*. The mutagenic activities of the compounds are reported in Table 1.

Discussion

The analysis of the results for the mutagenicity test of compounds **2**–**4**, **6** and **11**–**14** (Table 1), shows that only compound **12** was mutagenic for strain TA 100 and the effect was low (9.3 rev/nmol). A comparison of these results with those reported in previous papers[4] for compounds **15**–**23** (Fig. 2), provides information on the effects of the benzoannulation at the [*h*]-position of cyclopenta[*a*]phenanthrenes and at the [*i*]-position of cyclopenta[*a*]- and cyclopenta[*c*]phenanthrenes. It should be noted that we name benzo[*h*]-annulated cyclopentaphenanthrenes as indeno- and fluorenoanthracenes **2**–**4, 13** and **14**, while benzo[*i*]annulated cyclopentaphenanthrenes as cyclopentachrysenes **6**, **11**

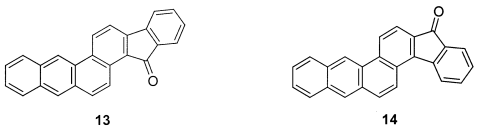


Figure 1.

Table 1. Mutagenicity of polycyclic aromatic compounds **2**–**4**, **6** and **11**–**14** in Ames' test with *Salmonella typhimurium* strains TA 98 and TA 100 in the presence of microsomal activating fraction S9

Compound	Total revertants (nmol)		Range tested
	Mutagenicity TA 98	Mutagenicity TA 100	(μg)
2	NM[a]	NM	1–100
3	NM	NM	1–50
4	NM	NM	1–40
6	NM	NM	1–100
11	NM	NM	1–100
12	NM	9.3	1–100
13	NM[a]	NM	1–40
14	NM	NM	1–50

[a]NM, non-mutagenic.

and **12**; this terminology has been used to facilitate the discussion (Fig. 3).

Comparison of the mutagenic activity of hydrocarbons **2**–**4** (NM) and ketones **13, 14** (NM) (see Table 1) with that of the related hydrocarbons **21**, **17** and **18** (NM) and indeno[*a*]phenanthrenones **15, 16** (M) (Fig. 2) respectively, point out that benzo[*h*]annulation (Fig. 3) seems to decrease the mutagenicity of the ketones, while it does not affect the activity of hydrocarbons. This observation is also supported by comparing the mutagenicity of compounds **3**, **4, 13** and **14** (NM) with that exhibited by cyclopenta[*a*]phenanthrene (**21**) (NM), cyclopenta[*a*]phenanthren-15-one (**19**) (M) and cyclopenta[*a*]phenanthren-17-one (**20**) (M) respectively. Simultaneous benzoannulation at the [*h*]-position and at the cyclopentane ring decreases mutagenicity in the case of the carbonyl compounds, but is ineffective in the case of hydrocarbons. This result was expected in view of the fact that it was observed recently[4b] that benzoannulation at the cyclopentane ring of cyclopentaphenanthrenes does not affect mutagenicity. It was also previously[4b] observed that benzo[*g*]-annulation seems to markedly decrease mutagenicity. It is well-known that PAHs must be metabolically activated into electrophilic intermediates in order to show mutagenicity and/or carcinogenicity and this activation occurs by the formation of vicinal *syn*-and *anti*-diol-epoxides that are normally formed adjacent to a bay or fjord region. Whereas the decreased mutagenicity due to benzoannulation at [*h*]-position of cyclopenta[*a*]phenanthrenes (Fig. 3) could be justified in view of the impossible formation of the *syn*-and *anti*-diol-epoxide adjacent to the bay region, the loss of activity in the benzo[*g*]-derivatives[4b] is difficult to explain.

In the case of the cyclopentachrysenes **6**, **11** and **12**, only hydrocarbon **12** is mutagenic. Comparing the data reported in Table 1 for cyclopenta[*a*]chrysene **6** and cyclopenta[*c*]chrysenes **11, 12** with the mutagenicity exhibited by the related cyclopenta[*a*]phenanthrene **21** and cyclopenta[*c*]phenanthrenes **22**, **23** (Fig. 2), some information is obtained on the effect of benzoannulation at the [*i*]-position of cyclopentaphenanthrenes. Whereas both hydrocarbons **12** and **23** are active mutagens, pentacyclic compounds **6**, **11** and the related tetracyclic **21** and **22** are inactive. Benzo[*i*]annulation, therefore, is ineffective in both cyclopenta[*c*]phenanthrenes (ketones and hydrocarbons) and cyclopenta[*a*]-phenanthrenes.

Comparison of the biological activity exhibited by chrysene[1] and benzo[*c*]chrysene[1] with that of cyclopenta[*c*]chrysene **12**, shows that the fusion of cyclopentane or benzene rings at [*c*]-position of chrysene has a comparable effect on the mutagenic activity. This observation is in agreement with the biological activity shown by benzo[*c*]phenanthrene[1] and cyclopenta[*c*]phenanthrene (**23**)[4a] in respect to that of phenanthrene.

Figure 3 summarizes the effects of benzo- and dibenzoannulation and of the carbonyl function in the cyclopenta[*a*]- and cyclopenta[*c*]phenanthrenes.

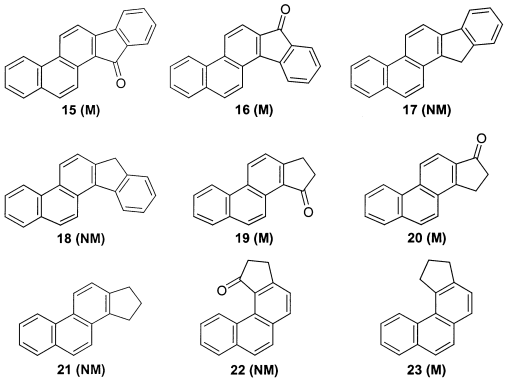


Figure 2. (M) = Mutagenic; (NM) = non-mutagenic.

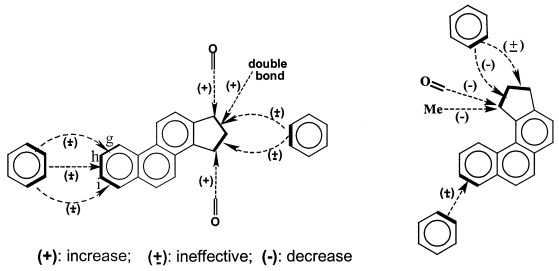


Figure 3. Effects of benzoannulation and of the carbonyl function on the mutagenicity of cyclopenta[*a*]- and cyclopenta[*c*]phenanthrenes.

Conclusions

The mutagenic properties of the compounds reported in this study compared with those previously obtained indicate that benzoannulation at the [*g*]-, [*h*]- and [*i*]-positions of the terminal benzene ring, does not affect the mutagenicity of cyclopenta[*a*]phenanthrenes and decreases the mutagenicity of cyclopenta[*a*]phenanthrenones. In contrast, benzo[*i*]-annulation of both cyclopenta[*c*]phenanthrenes and cyclopenta[*c*]phenanthrenones is ineffective. Therefore, while the presence of the carbonyl function increases the mutagenicity of the cyclopenta[*a*]phenanthrenes, it decreases the mutagenicity of the cyclopenta[*c*]phenanthrenes.

Experimental

Chemistry

Material and methods. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Infrared spectra of $CHCl_3$ solutions were recorded on a Perkin–Elmer 983 spectrometer and mass spectra on a Hewlett Packard 5970 GC–MS instrument, calibrated with perfluorotributylamine for 70 eV operation. All operations for preparing mixtures of the Diels–Alder reactions were executed in a dry-box. Absorption chromatography was carried out on a Merck silica gel (0.040–0.063 mm, 230–400 mesh ASTM). The extracts were dried over anhydrous Na_2SO_4. All new compounds gave correct elemental analyses. The NMR spectra were recorded on a Varian Associates VXR-400 multinuclear instrument (internal Me_4Si) in $CDCl_3$ solution. Proton assignments for all compounds were based on ^1H–^1H decoupling, COSY and NOE experiments. Connectivities between identified protons and protonated carbons were obtained by HETCOR experiments. A commercially available 1 M hexane solution of $EtAlCl_2$ was used; CH_2Cl_2 was distilled from CaH_2. Microanalyses, unless indicated, were in agreement with calculated values within $\pm 0.4\%$. Previously reported procedures were followed to prepare 12*H*-fluoreno[2,1-*a*]anthracen-12-one (13)[8] and 1*H*-fluoreno[3,4-*a*]anthracen-1-one (14).[8]

2,3-Dihydro-1*H*-indeno[5,4-*a*]anthracene (2). A 1 M *n*-hexane solution of $EtAlCl_2$ (0.5 ml, 0.5 mmol) was added to a solution of 4-acetoxy-2-cyclopenten-1-one[10] (0.62 g, 4.4 mmol) in dry CH_2Cl_2 (14 mL) under nitrogen and the mixture was stirred at 0 °C for 30 min.[11,12] Then, a solution of 1-vinyl-3,4-dihydroanthracene (**1**)[8] (0.44 g, 2.2 mmol) in dry CH_2Cl_2 (16 mL) was added and the resulting mixture stirred at 0 °C for 1.5 h. After that, the reaction mixture was poured into a saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined extracts were washed with water and dried. Evaporation of the solvent under reduced pressure gave a residue that was chromatographed on column (silica gel). Elution with 9:1 hexane/EtOAc, gave a product (1 g) which was shown (GC–MS analysis) to be a 1:1 mixture of cycloadducts. $NaBH_4$ (1.3 g) was added to a solution of the reaction mixture in 1:1 MeOH/CH_2Cl_2 (300 mL) and the resulting mixture was stirred at room temperature for 3.5 h under nitrogen.[6] After usual work up, a crude product was obtained and used directly in the next step.

A solution of the residue in triglyme (5 mL) was treated with 10% Pd/C (2 g) at reflux temperature,[6] under nitrogen and stirring, for 13 h. Usual work up gave a residue which was purified by column chromatography (silica gel). Elution with petroleum ether afforded 0.46 g of pure **2** (40% overall yield): mp 227–228 °C (Et_2O); ^1H NMR δ 2.28 (m, 2H, Hs-2), 3.16 (t, 2H, $J=7.4$ Hz, Hs-1), 3.32 (t, 2H, $J=7.4$ Hz, Hs-3), 7.50–7.57 (m, 3H, H-9, H-8, H-13), 7.68 (d, 1H, $J=9.1$ Hz, H-4), 7.81 (d, 1H, $J=9.1$ Hz, H-5), 8.03 (m, 1H, H-7), 8.11 (m, 1H, H-10), 8.36 (s, 1H, H-6), 8.68 (d, 1H, $J=8.0$ Hz, H-12), 9.14 (s, 1H, H-11); ^{13}C NMR δ 25.0 (C-2), 31.3 (C-3), 33.5 (C-1), 121.4 (C-11, C-12), 123.3 (C-13), 123.9 (C-4), 125.5 (C-9), 125.6 (C-8), 126.8 (C-6), 127.3 (C-5), 127.7 (C-7), 128.5 (C-10), 128.3, 128.8, 129.5, 130.3, 131.6, 132.0, 141.2, 142.9 (quaternaries Cs); MS, *m*/*z* (rel. intensity) 268 (M^+, 100), 252 (23), 239 (7), 133 (11), 120 (6).

12*H*-Fluoreno[2,1-*a*]anthracene (3) and 1*H*-fluoreno[3,4-a]anthracene (4). Et_3N (0.58 g, 5.6 mmol) was added dropwise to a solution of 3-bromo-indan-1-one[13] (0.88 g, 4.2 mmol) and 1-vinyl-3,4-dihydroanthracene (**1**) (1.1 g, 5.4 mmol) in CCl_4 (28 mL) heated at reflux temperature.[8,14] The reaction mixture was then refluxed for 5 h. The mixture was poured into 5% aqueous H_2SO_4 solution, extracted with $CHCl_3$ and the combined extracts washed with aqueous $NaHCO_3$ solution and brine, dried and evaporated under reduced pressure. A 2:1:1:1 mixture of cycloadducts was obtained as shown by GC–MS analysis; it was used directly in the next step.

$NaBH_4$ (1.4 g) was added to a solution of the cycloadducts (0.8 g) in 1:1 CH_2Cl_2/MeOH (100 mL) under stirring. The reaction mixture was stirred at room temperature for 6 h and then worked up as usual[4a] to give a mixture of alcohols which was directly used in the next step.

A solution of the alcohols (0.8 g) in triglyme (40 mL) was treated with 10% Pd/C (1.6 g) under nitrogen and the mixture heated at reflux temperature for 20 h. After usual work up, a 2:1 mixture of hydrocarbons **3** and **4,** respectively, was obtained. Medium pressure column chromatography on Merck lichoprep Si 60 prepacked column (elution with toluene) afforded 0.22 g (16% overall yield) of pure **3** and 0.13 g (10% overall yield) of pure **4**.

3: mp 303–305 °C (dec., $CHCl_3$); ^1H NMR δ 4.25 (br s, 2H, Hs-12), 7.54–7.67 (m, 5H, H-2, H-3, H-9, H-10, H-11), 7.86 (d, 1H, $J=9.2$ Hz, H-13), 7.89 (d, 1H, $J=9.2$ Hz, H-14), 7.91 (m, 1H, H-8), 8.05 (m, 1H, H-1), 8.10 (d, 1H, $J=8.3$ Hz, H-7), 8.14 (m, 1H, H-4), 8.39 (s, 1H, H-15), 8.90 (d, 1H, $J=8.3$ Hz, H-6), 9.22 (s, 1H, H-5); ^{13}C NMR δ 35.8 (C-12), 118.7 (C-7), 119.9 (C-8), 121.7 (C-5), 122.4 (C-6), 123.3 (C-13), 125.0 (C-11), 125.7 (C-3), 125.8 (C-2), 126.7 (C-10), 126.9 (C-9), 127.0 (C-15), 127.7 (C-1), 128.0 (C-14), 128.4 (C-4), 128.7 (C-12b),

129.5 (C-5b), 130.4 (C-14a), 131.7 (C-5a), 132.1 (C-4a, C-15a), 140.4 (C-7a), 140.7 (C-12a), 142.1 (C-7b), 143.4 (C-11a); MS, *m*/*z* (rel. intensity) 316 (M^+, 100), 287 (2), 158 (23), 143 (4).

4: mp 236–237 °C ($CHCl_3$); ^1H NMR δ 4.08 (br s, 2H, Hs-1), 7.38–7.59 (m, 4H, H-3, H-4, H-10, H-11), 7.67 (m, 1H, H-2), 7.88 (d, 1H, $J=8.4$ Hz, H-15), 7.98 (d, 1H, $J=9.4$ Hz, H-7), 8.07 (m, 1H, H-9), 8.15 (m, 1H, H-12), 8.43 (s, 1H, H-8), 8.45 (dd, 1H, $J=7.9$, 1.2 Hz, H-5), 8.69 (d, 1H, $J=9.4$ Hz, H-6), 8.87 (d, 1H, $J=8.4$ Hz, H-14), 9.24 (s, 1H, H-13); MS, *m*/*z* (rel. intensity) 316 (M^+, 100), 287 (2), 158 (23), 143 (5).

2,3-Dihydro-1*H*-cyclopenta[*a*]chrysene (6). A 1 M solution of $EtAlCl_2$ (12 mL, 12 mmol) was added to a solution of 2-bromo-2-cyclopenten-1-one[6,15] (3.56 g, 21.6 mmol) in CH_2Cl_2 (60 mL) and the mixture stirred at 25 °C for 40 min under nitrogen. Then a solution of diene **5** (2.4 g, 12 mmol) in CH_2Cl_2 (60 mL) was added and the resulting mixture stirred at 0 °C for 5.5 h. After usual work up, the residue was chromatographed on column (SiO_2) and elution with gradient from 6:4 CH_2Cl_2/hexane to CH_2Cl_2 gave a residue (1.08 g) shown, by GC–MS, to be a mixture of two products derived from the Diels–Alder products by dehydrobromination. This mixture was submitted to reduction with $NaBH_4$ (1.08 g) and then treated with 10% Pd/C (1.08 g) according to the procedure described above for compound **2**. The residue was purified by column chromatography (SiO_2). Elution with 9:1 hexane/EtOAc afforded 0.104 g of pure 2,3-dihydro-1H-cyclopenta[*a*]-chrysene (**6**) (15% overall yield): mp 260–261 °C (EtOH) [lit.[7] 260–261 °C (EtOH)]; ^1H NMR (DMSO) δ 2.25 (m, 2H, H-2), 3.17 (br t, 2H, $J=7.6$ Hz, H-3), 3.34 (br t, 2H, $J=7.6$ Hz, H-1), 7.63 (d, 1H, $J=8.3$ Hz, H-4), 7.67 (dd, 1H, $J=8.4$, 7.0 Hz, H-9), 7.73 (dd, 1H, $J=8.3$, 7.0 Hz, H-10), 8.02 (d, 1H, $J=9.1$ Hz, H-7), 8.71 (d, 1H, $J=8.3$ Hz, H-5), 8.82 (d, 1H, $J=9.0$ Hz, H-12), 8.84 (d, 1H, $J=9.1$ Hz, H-6), 8.87 (d, 1H, $J=8.3$ Hz, H-11); ^{13}C NMR δ 25.0 (C-2), 31.6 (C-1), 33.8 (C-3), 122.0 (C-6), 122.4 (C-12), 122.6 (C-5), 123.9 (C-11), 124.5 (C-4), 124.6 (C-13), 127.0 (C-8), 127.5 (C-5a), 127.6 (C-10), 127.7 (C-5b), 128.0 (C-7), 129.1 (C-8), 129.2 (C-11a), 129.5 (C-11b), 130.9 (C-13a), 132.3 (C-7a), 140.8 (C-13b), 142.4 (C-3a); MS *m*/*z* (rel. intensity) 268 (M^+, 100), 267 (37), 252 (23).

3,4-Dihydro-2-vinylphenanthrene (10). A mixture of $BF_3.Et_2O$ (1.8 mL, 14.1 mmol), 3,4-dihydro-2*H*-phenanthren-1-one (**7**)[8] (2.0 g, 10.2 mmol) and anhydrous Ac_2O (3.7 mL, 39.2 mmol) was heated at 60 °C for 2 h and then at reflux temperature for 2 h according to a previous procedure.[9] Usual work up gave a yellow crystalline complex BF_2-diketone (**8**) (2.9 g, 99% yield): mp 242–244 °C (dec). ^1H NMR δ 2.36 (s, 3H, CH_3), 2.78 (t, 2H, $J=7.6$ Hz, Hs-3), 3.37 (t, 2H, $J=7.6$ Hz, Hs 4), 7.52–7.75 (m, 4H, Arom. Hs), 8.06–8.12 (m, 2H, Arom. Hs).

The complex **8** was then hydrolyzed in 1:1 acetone-saturated aqueous sodium acetate solution (64 mL) at reflux temperature for 20 h. After usual work up, the product was purified by column chromatography on silica gel: elution with 4:1 hexane/EtOAc afforded 2 g of pure diketone (**9**) (82%); mp 120–122 °C (Et_2O) [lit.[16] 120–121 °C]; IR 1730, 1598 cm^{-1}; ^1H NMR δ 2.26 (s, 3H, CH_3), 2.75 (t, 2H, $J=5.7$ Hz, Hs-3), 3.31 (t, 2H, $J=7.5$ Hz, Hs-4), 7.53–8.14 (m, 6H, Arom. Hs).

A solution of $NaBH_4$ (2.1 g, 55.5 mmol) in water (41 mL) under nitrogen was added to a refluxing solution of diketone **9** (2 g, 8.4 mmol) in EtOH (85 mL). After 3 h at reflux temperature, the mixture was worked up as usual[9] to afford a mixture of diols which was used, without purification, for the next step.

A solution of diols (2 g, 8.3 mmol) in dry pyridine (190 mL) and $POCl_3$ (1.3 mL, 14.0 mmol) was refluxed for 4 h under nitrogen. After usual work up,[9] chromatography of the residue on neutral alumina, eluting with hexane, yielded 1.17 g (68%) of pure diene **10**: mp 55–57 °C (hexane); ^1H NMR δ 2.57 (br t, 2H, Hs-3), 3.23 (br t, 2H, Hs-4), 5.12 (d, 1H, $J=10.5$ Hz, H-12), 5.33 (d, 1H, $J=17.4$ Hz, H-12), 6.48 (br s, 1H, H-1), 6.56 (dd, 1H, $J=10.5$, 17.4 Hz, H-11), 7.33–7.44 (m, 3H, H-6, H-7, H-10), 7.59–7.69 (m, 2H, H-8, H-9), 7.97 (d, 1H, $J=8.0$ Hz, H-5).

3,4-Dihydro-5H-cyclopenta[*c*]chrysen-5-one (11). A 1 M hexane solution of $EtAlCl_2$ (1.1 mL, 1.1 mmol) was added to a solution of 4-acetoxy-2-cyclopenten-1-one[6,10] (0.34 g, 2.4 mmol) in CH_2Cl_2 (15 mL) and the mixture stirred at 25 °C for 40 min under nitrogen. Then a solution of diene **10** (0.56 g, 2.7 mmol) in CH_2Cl_2 (7 mL) was added and the resulting mixture stirred at 0 °C for 2 h. After that, a CH_2Cl_2 solution (2 mL) of diene **10** (0.21 g, 1 mmol) was again added and stirring continued for another 2h at 0 °C. Usual work up[6] gave a residue which was purified by column chromatography on silica gel. Elution with a gradient from 3:2 hexane/EtOAc to EtOAc gave 0.98 g of a 4:1 mixture of two products shown, by GC–MS, to be the tetrahydroderivatives of **11** formed from the cycloadduct by acetic acid elimination. The reaction mixture (0.95 g) was aromatized by treating it with 10% Pd/C (0.95 g) in triglyme (35 mL) at reflux temperature for 17 h under stirring and nitrogen,[4] to give a crude product which was purified by column chromatography (SiO_2). Elution with hexane gave 0.4 g of pure **11** (53%): mp 163–164 °C (EtOAc); IR 1694 (s, C=O) cm^{-1}; ^1H NMR δ 2.94 (t, 2H, $J=7.4$ Hz, Hs-4), 3.34 (br t, 2H, Hs-3), 7.61 (d, 1H, $J=8.1$ Hz, H-2), 7.64 (m, 1H, H-9), 7.68 (m, 1H, H-10), 7.93 (d, 1H, $J=9.2$ Hz, H-7), 7.98 (d, 1H, $J=8.4$ Hz, H-8), 7.99 (d, 1H, $J=9.0$ Hz, H-13), 8.15 (d, 1H, $J=8.1$ Hz, H-1), 8.78 (m, 1H, H-11), 8.80 (d, 1H, $J=9.0$ Hz, H-12), 9.11 (d, 1H, $J=9.2$ Hz, H-6); ^{13}C NMR δ 26.3 (C-3), 37.2 (C-4), 122.5 (C-12), 123.4 (C-11), 124.7 (C-2), 125.4 (C-7), 126.3 (C-10), 126.9 (C-9), 127.0 (C-13), 127.5 (C-11a), 127.8 (C-6), 128.9 (C-8), 129.3 (C-5c), 130.0 (C-11b), 130.7 (C-5b), 132.5 (C-7a), 132.8 (C-13a), 133.7 (C-5a), 136.2 (C-1), 158.6 (C-2a), 205.3 (C-5); MS *m*/*z* (rel. intensity) 282 (M^+, 100), 252 (39), 250 (14), 239 (12), 226 (15), 126 (18).

4,5-Dihydro-3*H*-cyclopenta[*c*]chrysene (12). The starting materials were the product mixture of the above-described

Diels-Alder reaction between diene **10** and 4-acetoxy-2-cyclopenten-1-one.

$NaBH_4$ (0.45 g) was added portionwise to a solution of the Diels–Alder reaction products (0.456 g) in 1:1 v/v CH_2Cl_2/MeOH (318 mL) under stirring. The reaction mixture was stirred for 4 h at 30 °C and then worked up as usual[4] to afford a mixture of diols (0.408 g) which was submitted to dehydration–aromatization reaction by treatment with 10% Pd/C (0.408 g) in triglyme (5 mL) at reflux temperature for 20 h. Usual work up gave a residue which was purified by column chromatography on silica gel. Elution with 95:5 hexane/EtOAc gave 0.18 g of **12** (47%): mp 185–187 °C (EtOH); ^{1}H NMR δ 2.29 (m, 2H, H-4), 3.14 (br t, 2H, J = 7.4 Hz, H-3), 3.84 (br t, 2H, J = 7.4 Hz, H-5), 7.56 (d, 1H, J = 7.9 Hz, H-2), 7.63 (dd, 1H, J = 8.8, 7.0 Hz, H-9), 7.69 (dd, 1H, J = 8.3, 7.0 Hz, H-10), 7.83 (d, 1H, J = 8.9 Hz, H-13), 8.67 (d, 1H, J = 8.9 Hz, H-12), 8.81 (d, 1H, J = 8.3 Hz, H-11), 8.90 (d, 1H, J = 9.1 Hz, H-6); ^{13}C NMR δ 26.4 (C-4), 33.9 (C-3), 38.3 (C-5), 120.6 (C-12), 123.8 (C-11), 124.0 (C-2), 125.6 (C-6), 126.3 (C-7), 126.7 (C-9), 126.8 (C-10), 128.2 (C-1), 128.6 (C-8), 128.8 (C-13), 129.7 (C-11b), 129.8 (C-11a), 129.9 (C-5c), 130.9 (C-5b), 132.1 (C-13c), 132.6 (C-7b), 139.9 (C-5a), 144.8 (C-2a); MS m/z (rel. intensity) 268 (M^+, 100), 265 (20), 252 (35), 239 (22), 126 (15).

Biology

Mutagenicity assay. The mutagenicity tests were carried out by *Salmonella*/mammalian microsome plate assay using *Salmonella thyphimurium* TA 98 and TA 100 strains obtained directly from Dr Ames (Berkeley, CA, USA). The assays were carried out in the presence and absence of rat liver homogenate S9 fraction, obtained from Sprague–Dawley male rats induced with Aroclor 1254 (Moltox Laboratories, Annapolis, MD, USA), used at the optimum concentration as suggested by Ames. The bacteria were stored at −80 °C and their stability was periodically verified by demonstrating sensitivity to UV light and crystal violet, resistance to ampicillin and histidine dependence.

Prior to the tests, the bacterial strains derived from an isolated colony on a master plate were cultured overnight in nutrient broth (Oxoid nO. 2). The concentration of the overnight culture was 1.10^9 cfu/mL, as determinated by both plate count and nephelometry (at 600 nm). Two standard solutions of different compounds in DMSO were prepared at concentrations of 0.4 μg/μL and 0.04 μg/μL for **4**, **13** and **14**; 0.1 μg/μL and 1 μg/μl for **2, 6, 11** and **12**; and 0.5 μg/μL and 0.005 μg/μL for **3**.

The lowest compound doses (1–10 μg/plate) were prepared from 0.1 μg/μL for **2, 6**, **11** and **12**, 0.05 μg/μL solution for **3**; and 0.04 μg/μL solution for **4**. The highest doses (20-100 μg/plate) were prepared from 0.4 μg/μL solution for **4** and **14**; 1 μg/μL solution for **2**, **6**, **11** and **12;** and 0.5 μg/μL solution for **3**.

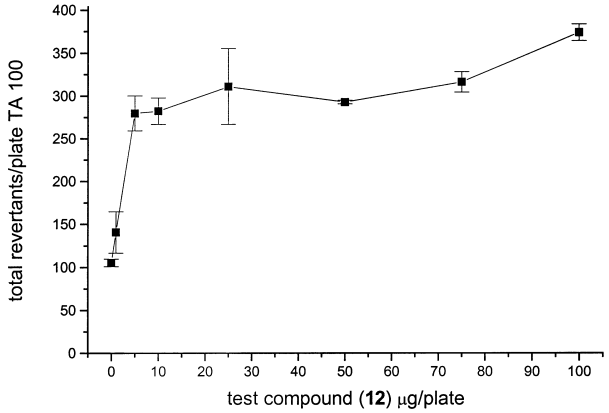


Figure 4. Dose–response curve of the reversion of *his Salmonella typhimurium* TA 100 strain by mutagenic compound **12**. Values are mean ± SD of triplicate tests. Where error bars are not visible, they fall within the symbol.

For the test, 100 μL of the overnight-cultured bacterial strain, 0.5 mL of S9 mix and increasing amounts of DMSO in which the compound to be tested was dissolved were added sequentially to a test tube containing 2 mL of top agar (0.6% Difco agar, 0.5% NaCl, 10 mL of solution of 0.5 mmol of 1-histidine and 0.5 mmol of biotin). The standard S9-mix was composed of 8 mmol $MgCl_2$, 33 mmol KCl, 5 mmol glucose 6-phosphate, 4 mmol NADP, 100 mmol NaH_2PO_4/Na_2HPO_4 buffer (pH 7.4), and S9 at a concentration of 0.04 μg/mL mix. The top agar was maintained at 45 °C, vortexed and rapidly added to 30 mL minimal agar plates containing 1.5% Bacto Difco agar, 2% glucose and 2% 50× Vogel Bonner salts, consisting of 1% $MgSO_4 \cdot 7H_2O$, 10% citric acid monohydrate, 50% anhydrous K_2HPO_4 and 17.5% $NH_4NaHPO_4 \cdot H_2O$ in distilled water. The plates were incubated for 48 h at 37 °C and revertant colonies were counted. Negative controls included DMSO only (100 μL/plate) to provide the spontaneous background mutation rate. The average number of spontaneous revertants were 26.1 ± 4.3 (−S9) and 34.7 ± 3.9 (+S9) for TA 98 and 132.2 ± 17.7 (−S9) and 138.2 ± 25.7 (+S9) for TA 100. Positive controls containing benzo[*a*]pyrene (1 μg/plate) and 2-aminofluorene (2.5 μg/plate), yielded the following numbers of revertants respectively 116.3 ± 26.0 and 505.2 ± 82.9 for TA 98 strain and 485.2 ± 40.3 and 535.0 ± 137.2 for TA 100.

Mutagenic activity was calculated from the linear ascending portion of the dose–response curve (Fig. 4) and expressed as the number of total revertants/nmol.

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