Separation and Characterization of Mononitro Derivatives of Benzo[a]pyrene, Benzo[e]pyrene and Benzo[ghi]perylene

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Mononitro derivatives of three polycyclic aromatic hydrocarbons with 5–6 condensed rings have been synthesized and purified to a purity of approximately 99.9 % for measurements of mutagenic properties. Structural isomers were identified from ^{1}H NMR, MS and UV spectra.

In view of the potent mutagenic properties of nitro-substituted polycyclic aromatic hydrocarbons (PAHs) and the apparent need for pure reference substances to perform Ames tests,[1] a scheme for preparation of nitro-PAH derivatives of high purity (99.9 %) has been developed. In a previous paper[2] the synthesis and purification of mononitro derivatives of various PAHs with 3–4 condensed rings were described. This report comprises further developments in preparation of nitro derivatives of PAHs with 5–6 condensed rings.

RESULTS AND DISCUSSION

Synthesis and Purification. The synthesis and crude purification of the mononitro derivatives were performed as described previously.[2] Final purification of each isomer to a purity of approximately 99.9 % was carried out by preparative HPLC. In each case the isomer distribution was determined from the HPLC chromatogram of the mononitro fraction.

According to Dewar[3] nitration of benzo[a]pyrene (BaP) yields one dominant (6-nitro-BaP) and at least one minor isomer (not identified) in a total yield of 66 %. With our synthetic procedure, however, nitrobenzo[a]pyrenes were obtained in 97 % yield as a mixture of 6-nitro-BaP, 1-nitro-BaP, and 3-nitro-BaP in a ratio of 84:12:4. 6-Nitrobenzo[a]pyrene was easily separated by column chromatography on a preparative scale. Due to a small separation factor (a=1.06) 1-nitro-BaP and 3-nitro-BaP were purified for MS and UV analyses only; NMR studies were performed on a 35:65 mixture of the two isomers.

In benzo[e]pyrene (BeP) positions 1 and 3 are expected to be significantly more reactive toward electrophilic attack than the other positions.[4] It is therefore not surprising that 1-nitro-BeP and 3-nitro-BeP are the mononitro derivatives formed when BeP is nitrated. With a hydrocarbon:nitric acid ratio of 1:3 a 1:1 isomer mixture results in 80 % yield. However, with a hydrocarbon:nitric acid ratio of 1:10 the yield increased to 93 % whereas the 1-nitro-BeP–3-nitro-BeP ratio changed to 64:36. Thus, an isomer distribution reflecting approximately the electron density of the highest occupied π-orbital was obtained with a limited excess of nitrating agent, but surprisingly enough the potentially less reactive and less accessible 1-position was preferred to the 3-position under more indiscriminate conditions.

Nitration of benzo[ghi]perylene has been reported by Hopff and Schweizer[5] to take place when the hydrocarbon is treated with nitroethylene in nitrobenzene. The product, a mononitro benzoperylene according to elemental analysis, was obtained in low yield (25 %), probably as a mixture of isomers. When benzo[ghi]perylene (BPer) was nitrated according to our procedure, a 60:40 mixture of two mononitro
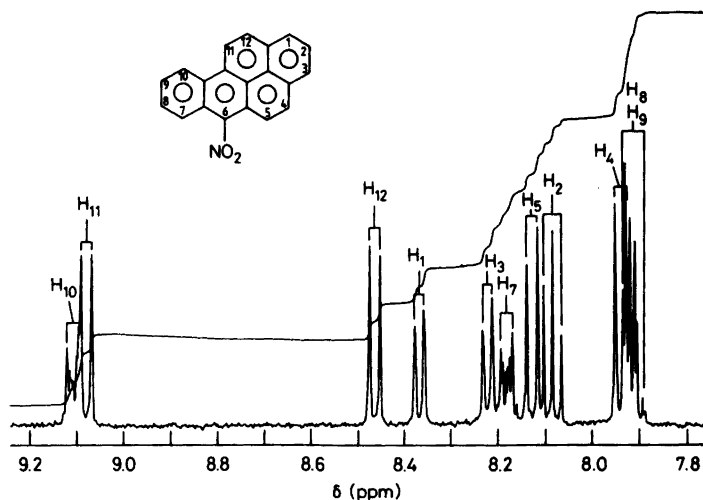
Fig. 1. The 400 MHz ^{1}H NMR spectrum of 6-nitrobenzo[*a*]pyrene in CDCl$_3$ at 24 °C relative to internal TMS.

isomers was obtained in 96 % yield. The predominant isomer turned out to be 5-nitro-BPer whereas the other one was identified as 7-nitro-BPer. These results are in accordance with electron density calculations[4] which clearly indicate that position 5 is more easily attacked by electrophiles than position 7. The same calculations indicate that the electron density of the highest occupied π-orbital is identical in positions 7 and 4. It is therefore surprising that 4-nitro-BPer was not observed. However, the possibility that 4-nitro-BPer coelutes with 5-nitro-BPer can-
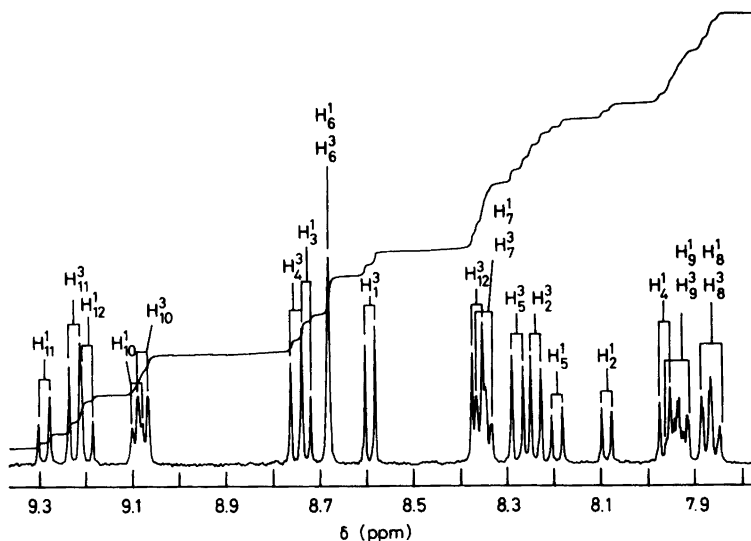

Fig. 2. The 400 MHz ^{1}H NMR spectrum of a 35:65 mixture of 1-nitrobenzo[*a*]pyrene and 3-nitrobenzo[*a*]pyrene, respectively, in CDCl$_3$ at 24 °C relative to internal TMS. The hydrogen atoms of 1–NO$_2$–BaP are denoted H^1 whereas those belonging to 3-NO$_2$-BaP are denoted H^3. For numbering, see Fig. 1.

not be disregarded, if the amount of 4-nitro-BPer is less than 10 % of the amount of 5-nitro-BPer and therefore not detectable by NMR. This suggestion is supported by the broad m.p. of 5-nitro-BPer.

^{1}H NMR Spectroscopy. The structures of the mononitro derivatives formed by nitration of the title compounds, were elucidated by NMR experiments.

The main isomer from nitration of benzo(a)pyrene gave rise to the proton spectrum shown in Fig. 1. Since the spectrum does not contain a singlet, the compound is evidently 6-nitrobenzo[a]pyrene. This is also borne out by

double resonance experiments which prove that the spectrum consists of one AA'MX, one AMX, and two AB subspectra. These experiments do not, however, allow a complete interpretation of the spectrum, but taking into account that other BaP derivatives give rise to signals for H_{10} and H_{11} around 9 ppm and for H_3 at higher field than H_1 but at lower field than H_2,[6] the interpretation of the spectrum tentatively given in Fig. 1 is the most likely one.

The two minor nitro-BaP isomers formed during the nitration of BaP were very difficult to separate and their structures were therefore elucidated by performing NMR experiments on a
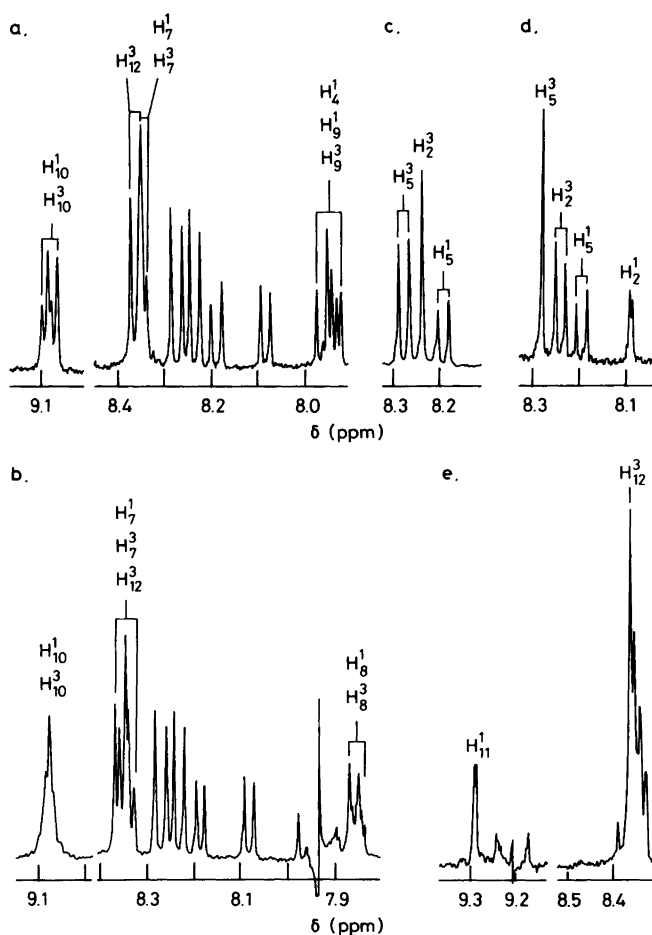

Fig. 3. Decoupling experiments with the 1-NO$_2$-BaP and 3-NO$_2$-BaP mixture. ^{a} Irradiation of H^1_8 and H^3_8, simplification at H^1_7, H^3_7, H^1_9, H^3_9, H^1_{10}, and H^3_{10}. ^{b} Irradiation of H^1_9 and H^3_9, elimination of coupling to H^1_7, H^3_7, H^1_8, H^3_8, H^1_{10}, and H^3_{10}. ^{c} Irradiation of H^3_1, decoupling of H^3_2. ^{d} Irradiation of H^3_4 and H^1_3, decoupling of H^3_5 and H^1_2. ^{e} Saturation of H^3_{11} and H^1_{12}, decoupling of H^3_{11} and H^1_{11}.
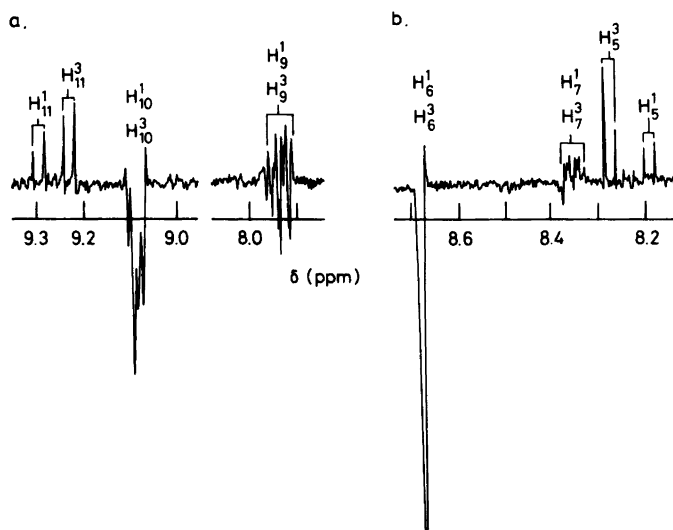
Fig. 4. ^{1}H-$\{^1$H$\}$ NOE difference spectra of a mixture of 1-NO$_2$-BaP and 3-NO$_2$-BaP. ^{a} Irradiation of H$^1_{10}$ and H$^3_{10}$. ^{b} Irradiation of H$^1_6$ and H$^3_6$.

mixture of the components in a ratio of 35:65. The proton NMR spectrum of this mixture was fairly complex (Fig. 2) but the spectrum could nevertheless be interpreted by using double resonance techniques. Decoupling experiments, summarized in Fig. 3, clearly show that both isomers give rise to a spectrum that contains one ABMX and three AB subspectra as well as a singlet. All J_{AB}>7 Hz and since there is no subspectrum caused by a three-spin system, this proves that the two isomers are 1-NO$_2$-BaP and 3-NO$_2$-BaP. The interpretations of their spectra, included in Fig. 2, are supported by NOE experiments. Thus, irradiation of H$^1_{10}$ and H$^3_{10}$ results in nuclear enhancement of H$^1_{11}$, H$^3_{11}$, H$^1_9$, and H$^3_9$ (Fig. 4a) whereas irradiation of H$^1_6$ and H$^3_6$ causes nuclear enhancement of H$^1_7$, H$^3_7$, H$^1_5$, and H$^3_5$ (Fig. 4b). In order to find out whether 1-nitro-BaP or 3-nitro-BaP predominates the binary mixture it is sufficient to compare the relative positions of the H$_{12}$ and H$_4$ signals in the spectra due to the two isomers. This is so because the *peri* interaction from the nitro group should shift H$_{12}$ downfield and H$_4$ upfield in the spectrum of 1-nitro-BaP relative to that of 3-nitro-BaP.[6,7] From Fig. 2 it is evident that the more abundant isomer gives rise to doublets for H$_4$ and H$_{12}$ which are 0.8 ppm downfield and 0.8 ppm upfield, respectively, relative to the corres-

ponding doublets due to the minor isomer. Consequently, the more abundant isomer is 3-nitro-BaP. The isomeric mixture therefore consists of 65 % 3-nitrobenzo[*a*]pyrene and 35 % 1-nitrobenzo[*a*]pyrene.

The mononitroisomers of benzo[*e*]pyrene gave rise to proton spectra (Figs. 5 and 6) which consisted of one ABMX, one AMX, and two AB subspectra according to double resonance experiments. Furthermore all J_{AB} are *ortho* coupling constants (>7.5 Hz). This proves that the isomers are 1-nitro-BeP (Fig. 5) and 3-nitro-BeP (Fig. 6). These results do not, however, allow complete interpretations of the spectra, but ^{2}H NMR studies presently under way,[8] will probably remove the remaining ambiguities.

The two mononitro derivatives obtained by nitration of benzo[*ghi*]perylene, gave ^{1}H NMR spectra (Figs. 7 and 8) which comprised one AMX and four AB subspectra with J_{AB}>7.5 Hz. Consequently, the two nitro compounds are necessarily 5-nitro-BPer and 7-nitro-BPer. The UV and MS spectra strongly indicate that the main component is 5-nitro-BPer and this conclusion is fully supported by the results of the T_1-measurements.[8]

Mass spectroscopy. All the nitro PAH derivatives gave mass spectra that are typical for this class of compound;[2] major peaks which can be
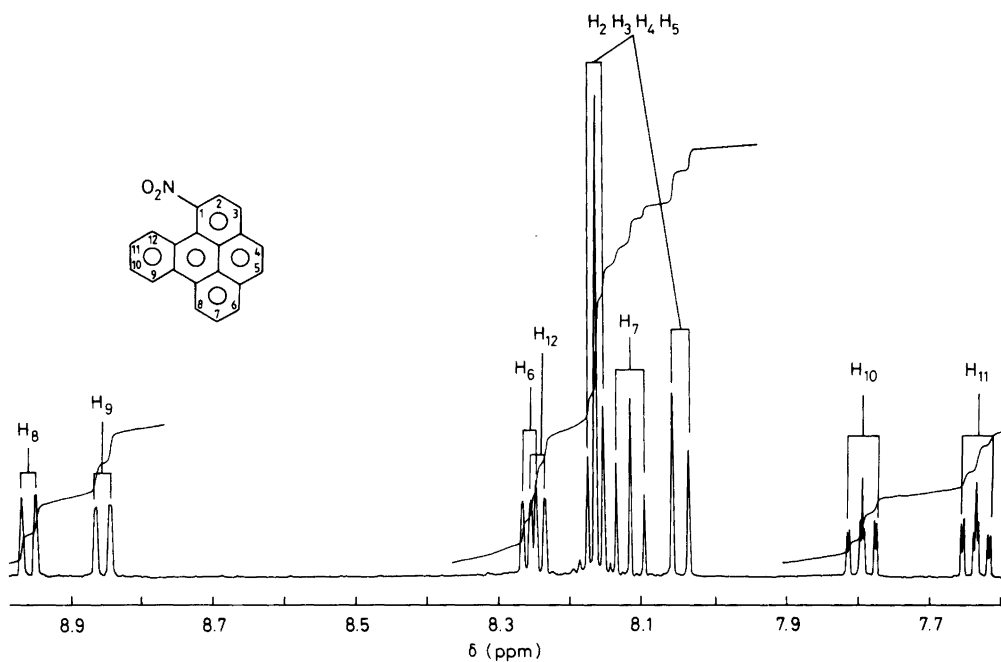
Fig. 5. The 400 MHz ^{1}H NMR spectrum of 1-nitrobenzo[*e*]pyrene in CDCl$_3$ at 24 °C relative to internal TMS.
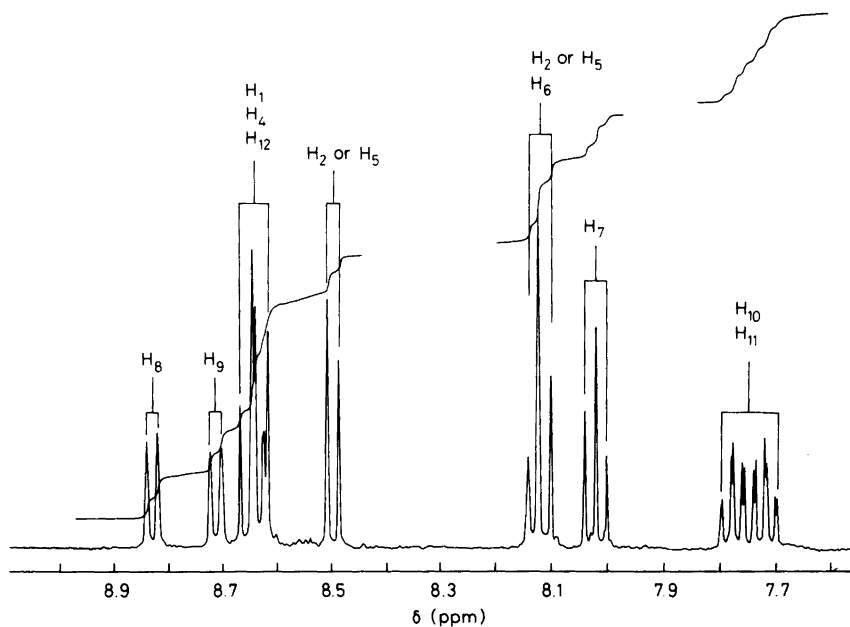

Fig. 6. The 400 MHz ^{1}H NMR spectrum of 3-nitrobenzo[*e*]pyrene in CDCl$_3$ at 24 °C relative to internal TMS. For numbering, see Fig. 5.
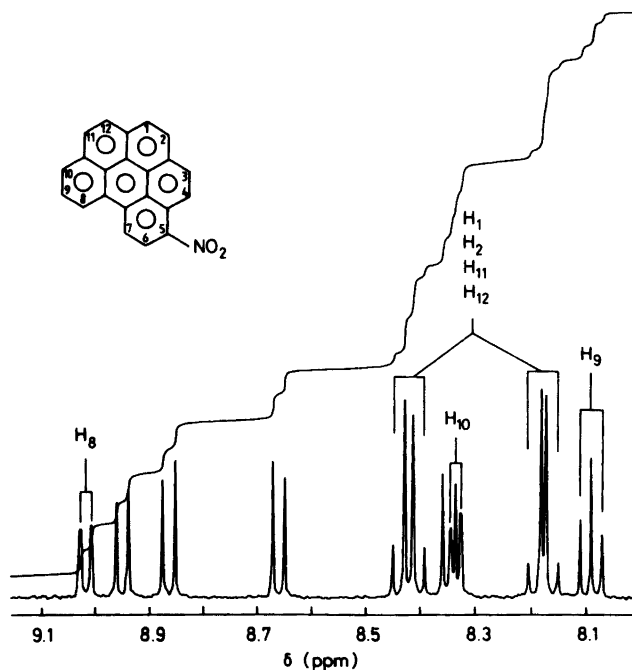
Fig. 7. The 400 MHz ¹H NMR spectrum of 5-nitrobenzo[*ghi*]perylene in CDCl₃ at 24 °C relative to internal TMS.

ascribed to loss of NO, NO₂, and HNO₂ are observed in all spectra (Table 1). More significant, however, are the M−OH and M−HNO fragments which have been found simultaneously only in the mass spectra of isomers with the nitro group in a bay region.[2] The mass spectra therefore confirm the structure elucidation based on the NMR experiments.

UV spectroscopy. The UV spectra support the conclusions which were drawn as a result of the NMR and MS data. The high-wavelength band of 5-nitrobenzo[*ghi*]perylene is almost absent in 7-nitrobenzo[*ghi*]perylene (Fig. 9), in accordance with our previous findings for isomers with nitro groups in *bay* positions.[2] Exactly the same tendency, but less marked, is seen in the difference between the spectra of 1-nitrobenzo[*e*]pyrene (*bay*) and 3-nitrobenzo[*e*]pyrene (Fig. 10). Of the three nitrobenzo[*a*]pyrene isomers containing no *bay* substituents, the UV spectra (Fig. 11) demonstrate the extended chromophore found in 1- and 3-nitro-BaP resulting in a high-wavelength band at 438 nm not found in 6-nitro-BaP which contains a less extended conjugated system. Since 280 nm was
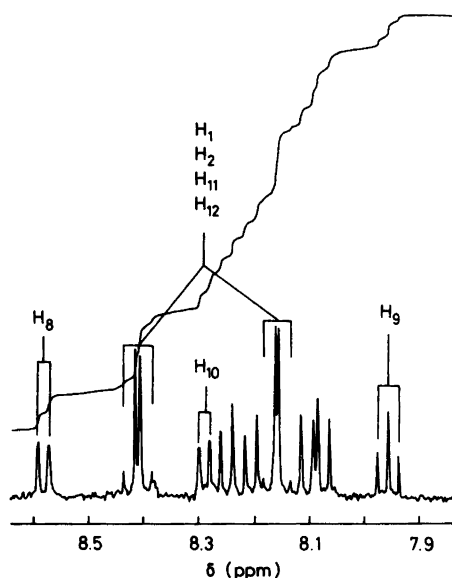

Fig. 8. The 400 MHz ¹H NMR spectrum of 7-nitrobenzo[*ghi*]perylene in CDCl₃ at 24 °C relative to internal TMS. For numbering, see Fig. 7.
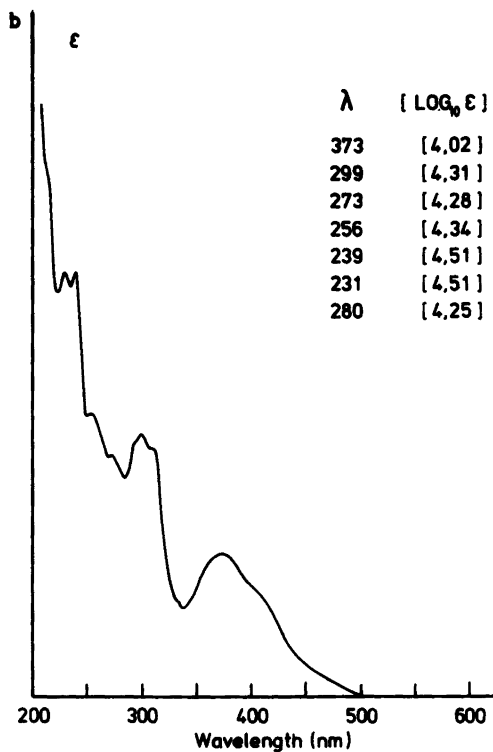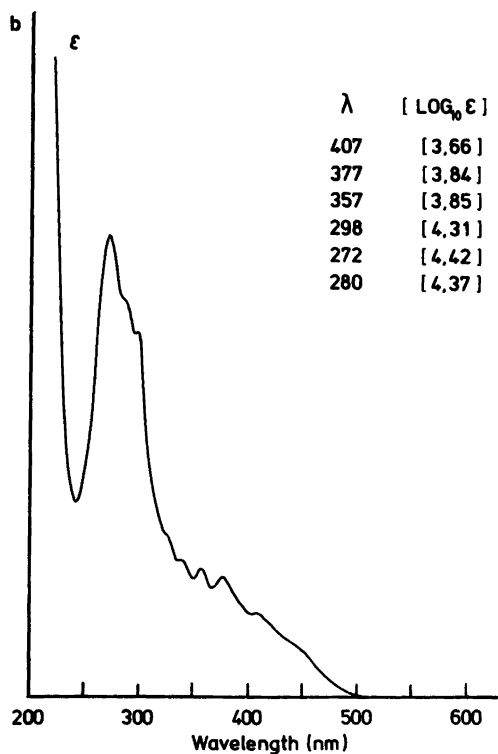
a

ε

λ	[$LOG_{10}\varepsilon$]
421	[3,96]
278	[4,25]
218	[4,51]
280	[4,25]

Wavelength (nm)

a

ε

λ	[$LOG_{10}\varepsilon$]
376	[3,66]
306	[4,15]
271	[4,47]
225	[4,50]
280	[4,33]

Wavelength (nm)

b

ε

λ	[$LOG_{10}\varepsilon$]
407	[3,66]
377	[3,84]
357	[3,85]
298	[4,31]
272	[4,42]
280	[4,37]

Wavelength (nm)

b

ε

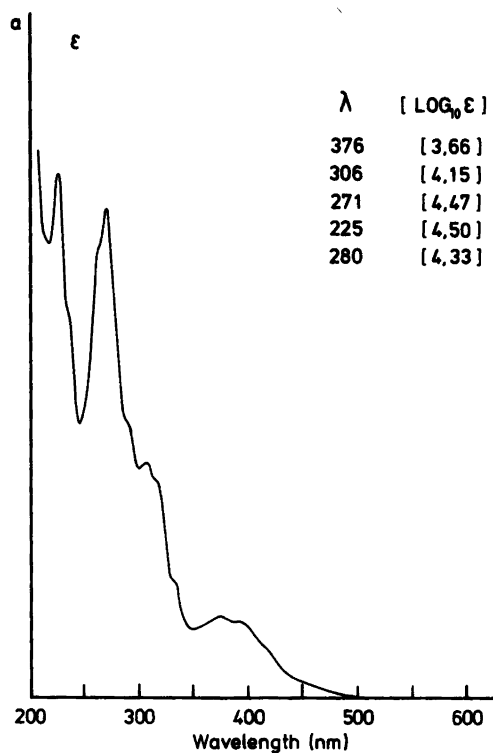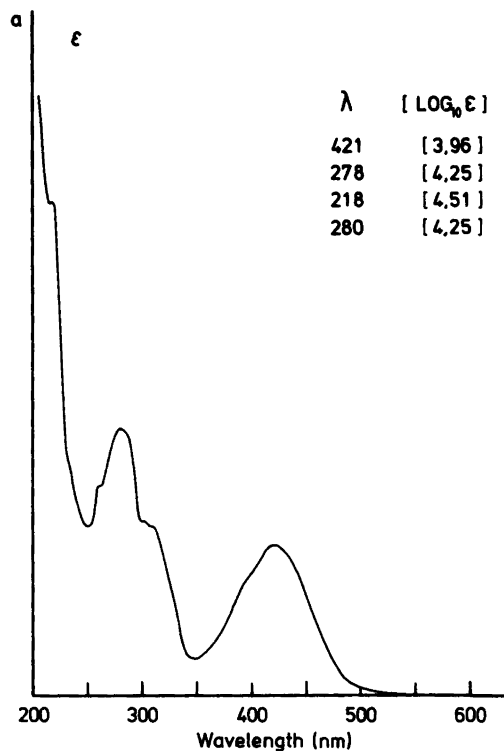| λ	[$LOG_{10}\varepsilon$]
373	[4,02]
299	[4,31]
273	[4,28]
256	[4,34]
239	[4,51]
231	[4,51]
280	[4,25]

Wavelength (nm)

Fig. 9. UV spectra of 5- and 7-nitro-benzo[*ghi*]perylene in methanol.

Fig. 10. UV spectra of 1- and 3-nitro-benzo[*e*]pyrene in methanol.

Table 1. Mass spectrometric fragments, in % of the base peak.

Compound	M	M−17[a]	M−30[b]	M−31[c]	M−46[d]	M−47[e]	M−58[f]	Sub-stituent position
1-nitro-BaP	96	−	100	5	92	68	51	peri
3-nitro-BaP	93	−	80	6	100	71	45	peri
6-nitro-BaP	100	2	88	−	69	66	54	peri
1-nitro-BeP	55	8	83	18	39	100	68	bay
3-nitro-BeP	100	−	35	−	53	86	52	peri
5-nitro-BPer	99	−	43	−	100	62	42	peri
7-nitro-BPer	81	21	70	25	100	97	53	bay

[a] M−OH. [b] M−NO. [c] M−HNO. [d] M−NO$_2$. [e] M−HNO$_2$. [f] M−NOCO; not adjusted for the ^{13}C-isotope contributions.

chosen as the common wavelength for the quantitative determination of the isomer distribution and for the purity tests, the absorbance data at 280 nm has been included in the figures in addition to the λ_{max}.

EXPERIMENTAL

General. The instruments employed have been described elsewhere.[2] The nitration of the PAHs and the crude purification of the reaction mixtures were carried out essentially as previously described. Experimental details are summarized in Table 2.

Purification by HPLC. The purification was carried out in accordance with published procedures.[2] Three different HPLC columns were utilized:

A. 250×10 mm, packed with 3 μm Hypersil silica (Shandon)
B. 250×7.7 mm, packed with 5 μm Hypersil silica (Shandon)

C. 250×22 mm, packed with 10 μm Perkin Elmer ODS (Perkin Elmer)

6-Nitro-BaP eluted in front of the mixture of 1- and 3-nitro-BaP at the crude purification on silica (Table 2). A purity better than 99.9 % was obtained on column C with methanol/water (85:15) at 9 ml/min. The *1-nitro-BaP* and *3-nitro-BaP* mixture was purified on column C as above. Separation of 1-nitro-BaP from 3-nitro-BaP was performed on column A with 5 % CH$_2$Cl$_2$ in hexane, collection of front (3-nitro-BaP) and tail (1-nitro-BaP) and rechromatography of each on column A, whereby samples with an isomer purity of 99 % were obtained.

1-Nitro-BeP and *3-nitro-BeP* were partially separated during the crude purification (Table 2). After rechromatography on large particle silica, the less retained compound, 1-nitro-BeP, was purified on column A with 5 % CH$_2$Cl$_2$ in hexane at 4 ml/min and finally on column C with 85 % methanol in water. 3-Nitro-BeP was purified under identical conditions. Both compounds were 99.9 % pure. M.p. (1-nitro-BeP):

Table 2. Experimental data on synthesis and crude purification on silica of mononitro PAH.

PAH	Molar ratio HNO$_3$−PAH	Nitration Time (h)	Temp. (°C)	Column size (cm)	CH$_2$Cl$_2$/hexane	Elution volume (ml)	Main isomer	Total yield %
BaP	5	15	0	26×1.5	30/70	101−180	6-nitro	97
						181−305	1+3-nitro	
BeP	10	90	0	26×1.5	30/70	101−180	1-nitro	93
						181−280	3-nitro	
BPer	10	90	0	26×1.5	30/70	101−180	7-nitro	96
						181−400	5-nitro	

a

ε

λ	$[\text{LOG}_{10}\varepsilon]$
438	[4,04]
307	[4,11]
264	[4,50]
224	[4,09]
280	[4,02]

Wavelength (nm)

b

ε

λ	$[\text{LOG}_{10}\varepsilon]$
438	[4,04]
398	[4,24]
379	[4,04]
299	[4,14]
268	[4,52]
250	[4,54]
280	[4,31]

Wavelength (nm)

c

ε

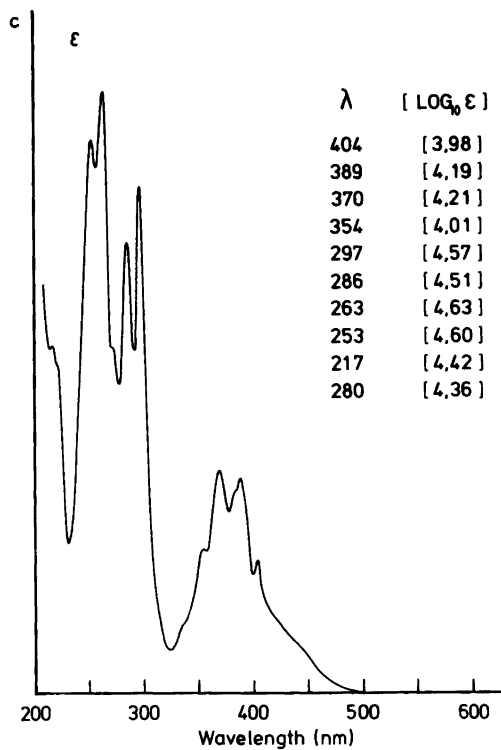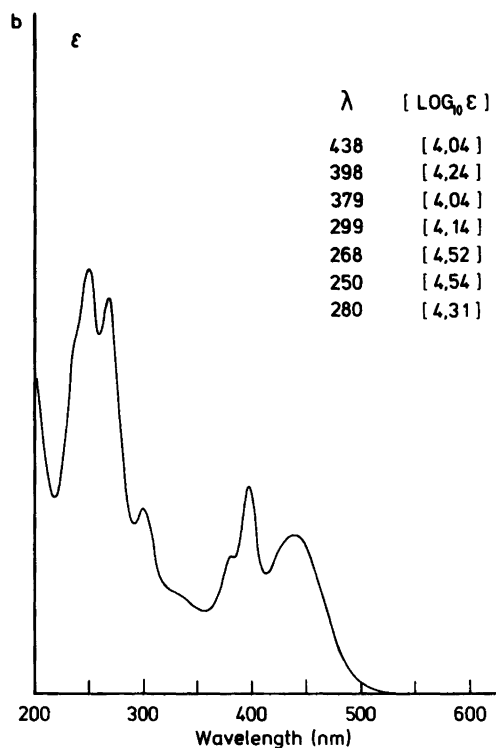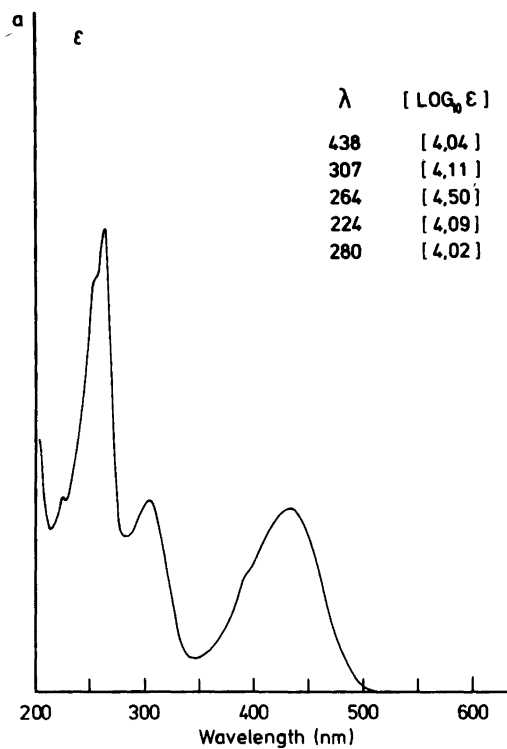| λ	$[\text{LOG}_{10}\varepsilon]$
404	[3,98]
389	[4,19]
370	[4,21]
354	[4,01]
297	[4,57]
286	[4,51]
263	[4,63]
253	[4,60]
217	[4,42]
280	[4,36]

Wavelength (nm)

Fig. 11. UV spectra of 1-, 3- and 6-nitro-benzo[a]pyrene in methanol.

202–203 °C, m.p. (3-nitro-BeP): 194–195 °C.

5-Nitro-BPer and *7-nitro-BPer* were partially separated by the crude purification. Both compounds were then purified separately on column C with 85 % methanol in water to an apparent purity of 99.9 %. 7-Nitrobenzo[*ghi*]perylene is the less retained isomer of the two under these conditions. M.p. (7-nitro-BeP): 301–303 °C (subl.), m.p. (5-nitro-BPer): 306–332 °C (subl.).

Proton NMR spectra. The spectra were recorded as described previously.[2] The samples were 0.3–0.5 % by weight in deuteriochloroform (99.9 %) which provided the deuterium signal for the NMR field lock. The spectra of the nitro-BaP derivatives were run on solutions in ordinary 5 mm tubes. The spectra of the other nitro compounds were run on solutions in 1 mm capillary tubes which were fastened in the middle of 5 mm NMR tubes. The spectra are summarized below.

1-Nitro-BaP. ^{1}H NMR (400 MHz): δ 7.86 (1H,m), 7.93 (1H,m) 7.96 (1H,d, *J* 9.0 Hz), 8.08 (1H,d, *J* 8.0 Hz), 8.19 (1H,d, *J* 9.0 Hz), 8.34 (1H,m), 8.68 (1H,s), 8.72 (1H,d, *J* 8.0 Hz), 9.08 (1H,m), 9.20 (1H,d, *J* 9.7 Hz), 9.28 (1H,d, *J* 9.7 Hz).

3-Nitro-BaP. ^{1}H NMR (400 MHz): δ 7.86 (1H,m), 7.93 (1H,m), 8.23 (1H,d, *J* 8.6 Hz), 8.28 (1H,d, *J* 9.5 Hz), 8.35 (1H,m), 8.36 (1H,d, *J* 9.1 Hz), 8.59 (1H,d, *J* 8.6 Hz), 8.68 (1H,s), 8.74 (1H,d, *J* 9.5 Hz), 9.07 (1H,m), 9.22 (1H,d, *J* 9.1 Hz).

6-Nitro-BaP. ^{1}H NMR (400 MHz): δ 7.92 (2H,m), 7.94 (1H,d, *J* 9.4 Hz), 8.08 (1H,t, *J* 7.6 Hz), 8.12 (1H,d, *J* 9.4 Hz), 8.18 (1H,m), 8.21 (1H,d, *J* 7.5 Hz), 8.36 (1H,d, *J* 7.7 Hz), 8.46 (1H,d, *J* 9.2 Hz), 9.08 (1H,d, *J* 9.2 Hz), 9.11 (1H,m).

1-Nitro-BeP. ^{1}H NMR (400 MHz): δ 7.63 (1H,m), 7.79 (1H,m), 8.04 (1H,d, *J* 8.8 Hz), 8.11 (1H,t, *J* 7.8 Hz), 8.16 (1H,d, *J* 8.8 Hz), 8.16 (2H, ABq, *J* 8.3 Hz), 8.25 (2H,m), 8.35 (1H,m) 8.95 (1H,m).

3-Nitro-BeP. ^{1}H NMR (400 MHz): δ 7.72 (1H,m), 7.76 (1H,m), 8.01 (1H,t, *J* 7.8 Hz), 8.10 (1H,d, *J* 9.4 Hz), 8.12 (1H,m), 8.49 (1H,d, *J* 8.8 Hz), 8.62 (1H,d, *J* 9.5 Hz), 8.63 (1H,m), 8.65 (1H,d, *J* 8.8 Hz), 8.71 (1H,m), 8.82 (1H,m).

5-Nitro-BPer. ^{1}H NMR (400 MHz): δ 8.09 (1H,t, *J* 7.8 Hz), 8.16 (1H,d, *J* 8.8 Hz), 8.18 (1H,d, *J* 8.8 Hz), 8.33 (1H,m), 8.35 (1H,d, *J* 9.3 Hz), 8.40 (1H,d, *J* 8.3 Hz), 8.43 (1H,d, *J* 8.3 Hz), 8.66 (1H,d, *J* 8.7 Hz), 8.86 (1H,d, *J* 9.3 Hz), 8.95 (1H,d, *J* 8.7 Hz), 9.01 (1H,m).

7-Nitro-BPer. ^{1}H NMR (400 MHz): δ 7.95 (1H,t, *J* 7.9 Hz), 8.07 (1H,d, *J* 8.4 Hz), 8.10 (1H,d, *J* 8.8 Hz), 8.15 (1H,d, *J* 8.2 Hz), 8.16 (1H,d, *J* 8.2 Hz), 8.20 (1H,d, *J* 8.4 Hz), 8.24

(1H,d, *J* 8.8 Hz), 8.28 (1H,m) 8.40 (1H,d, *J* 8.3 Hz), 8.41 (1H,d, *J* 8.3 Hz), 8.58 (1H,m).

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