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Separation and Characterization of Mononitro Derivatives of Phenanthrene, Pyrene, Chrysene, Fluoranthene and Triphenylene

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Mononitro derivatives of selected polycyclic aromatic hydrocarbons with 3 and 4 condensed rings have been synthesized to obtain samples for measurements of mutagenic properties. Crude purification of the compounds was carried out on gravity columns prior to final purification by HPLC to a purity of approximately 99.9 %. Structural isomers were identified from NMR, MS and UV spectra.

It is well known that nitrated polycyclic aromatic hydrocarbons (nitro-PAH) which are found in carbon black,[1–3] in combustion emissions from diesel engines,[4–10] and in airborne particulate matter[11–14] may be mutagenic. However, when literature data on mutagenicity are compared, it appears that erroneous results have been published. Most probably this is due to the use of impure test substances. The preparation of nitrated PAH has mainly been reported by Dewar *et al.*,[15–19] but their work was carried out prior to the development of modern separation techniques and of powerful spectroscopic methods. Consequently, their purification procedures could not be expected to be adequate for the preparation of samples of sufficient purity for Ames tests. Small amounts of dinitro derivatives and other impurities may seriously affect the mutagenic response, and even the presence of small amounts of structural isomers can cause large deviations from the essential effect of a pure compound.[20] Furthermore, their structure elucidation was generally not supported by spectroscopic and/or spectrometric data, but mostly by predictions based on molecular-orbital theory.[21–24] It was therefore of interest to prepare nitro-PAH of high purity and to determine the structure of these compounds beyond doubt by UV, MS and NMR studies. The results for nitro-PAH with three and four condensed rings are reported here.

RESULTS AND DISCUSSIONS

Synthesis and purification. The preparation and crude purification of 9-nitrophenanthrene (9-nitro-Phe) and 1-nitropyrene (1-nitro-Pyr) were performed according to literature procedures.[15] Final purification was subsequently carried out by HPLC on silica columns. However, after storage for 3 months in the dark at −20 °C, a purity test of 1-nitro-Pyr revealed the appearance of two decomposition products, of which one was strongly mutagenic. These compounds were effectively removed by repeated purification on silica.

According to Dewar,[18] nitration of *chrysene* results in approximately 90 % of 6-nitrochrysene (6-nitro-Ch) plus one additional isomer, tentatively identified as 1-nitro-Ch from electron density calculations according to which the 1-position is more reactive than positions 4 and 5.[23,24]

The synthetic procedure of Dewar *et al.*[18] was modified in order to increase the yield of the mononitro fraction and the initial crude purification was carried out on silica instead of alumina, because silica resulted in a better group separa-

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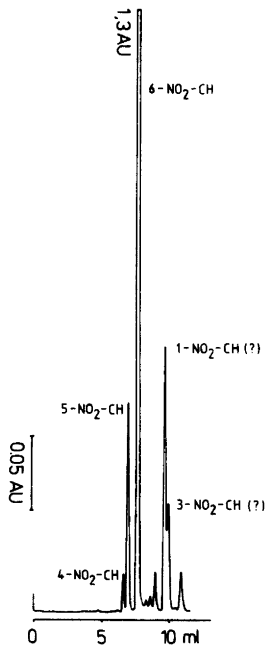


Fig. 1. Distribution of isomers of nitrochrysene (8μg) after the modified synthesis and work-up procedure, on 3μm Hypersil silica (250×4.6 mm) with 7.5 % dichloromethane and 0.1 % acetonitrile in hexane, at 2 ml/min, with UV-detection at 280 nm. The nitro group position of the three isolated isomers is marked on the figure (4,5,6), while the position of two other isomers is suggested (1 and 3).

tion of the mononitro fraction. This fraction was then analyzed by HPLC. The fraction contained five isomers of nitro-Ch. Three of the isomers were isolated, purified and identified as 6-nitro-Ch, 5-nitro-Ch and 4-nitro-Ch. The position of the nitro group in the two remaining isomers has not been determined, but based on the expected reactivity, the second largest peak in Fig. 1 is assumed to be the 1-nitro-Ch and the co-eluting shoulder peak 3-nitro-Ch. The retention of the assumed 1-nitro-Ch relative to 6-nitro-Ch on alumina was in agreement with the observations of Dewar.[18]

Nitration of *fluoranthene* (Fl) has been reported by Streitwieser to yield only four of the five possible nitro-Fl derivatives (1-, 3-, 7- and 8-nitro-Fl)[22] because position 2 should be nonreactive and impossible to nitrate directly.[6,25] When our nitration procedure was employed, however, crude purification on silica and further purification by HPLC gave Streitwieser's four products as well as a fifth nitro-Fl, which was identified (by MS) as 2-nitro-Fl (Fig. 2). Conceivably Streitwieser also got 2-nitro-Fl which, however, was not isolated due to insufficient resolution of the separation methods employed.

1-Nitrotriphenylene (1-nitro-Tri) and 2-nitrotriphenylene (2-nitro-Tri) which are the only mononitro derivatives of *triphenylene,* have been synthesized by Dewar[19] in 46 % yield and by Zinke[26] in 50 % yield as 1:1 isomeric mixtures. When our method was employed, a 59 % yield of a 1:1 mixture of 1-nitro-Tri and 2-nitro-Tri was obtained after purification on silica. Recently Radner reported that nitro-Tri was obtained in 92 % when triphenylene was nitrated with dinitrogentetroxide.[27] The nitrotriphenylene isomers comprise an outstanding example of the importance of isomer purity in tests for mutagenic effects, since the number of revertants (in the TA 98 assay) per microgram of 2-nitro-Tri is of the order of 10 000 times higher than for 1-nitro-Tri.[20] Thus, a purity of 99.9 % will not necessarily be sufficient as 0.1 % impurity of the former in the latter would lead to grossly

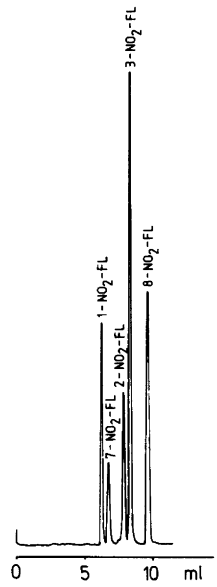


Fig. 2. Distribution of isomers of nitrofluoranthene (6μg totally). Column and conditions as in Fig. 1. The nitro group position is marked on the figure.

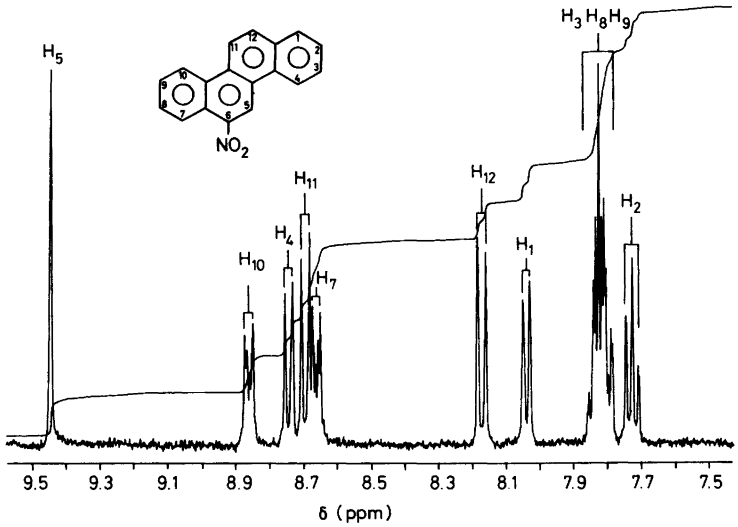


Fig. 3. The 400 MHz ^{1}H NMR spectrum of 6-nitrochrysene in $CDCl_3$ at 24 °C relative to internal TMS.

erroneous data for 1-nitro-Tri.

NMR spectroscopy. Except for 4-nitrochrysene which was identified from MS and UV spectra, and 1-nitropyrene, the structure of which was elucidated by ^{13}C NMR spectroscopy,[28] all the mononitro derivatives were conveniently identified by ^{1}H NMR spectroscopy. 1-Nitrotriphenylene, and 2-nitrotriphenylene gave proton spectra equivalent to those reported in the literature.[29] The ^{1}H NMR spectrum of the nitro-Phe isomer consisted of a sharp singlet at 8.61 ppm and a complex multiplet at 8.15–7.46 ppm in a ratio of 1:8 which is compatible only with the structure 9-nitrophenanthrene for this compound.

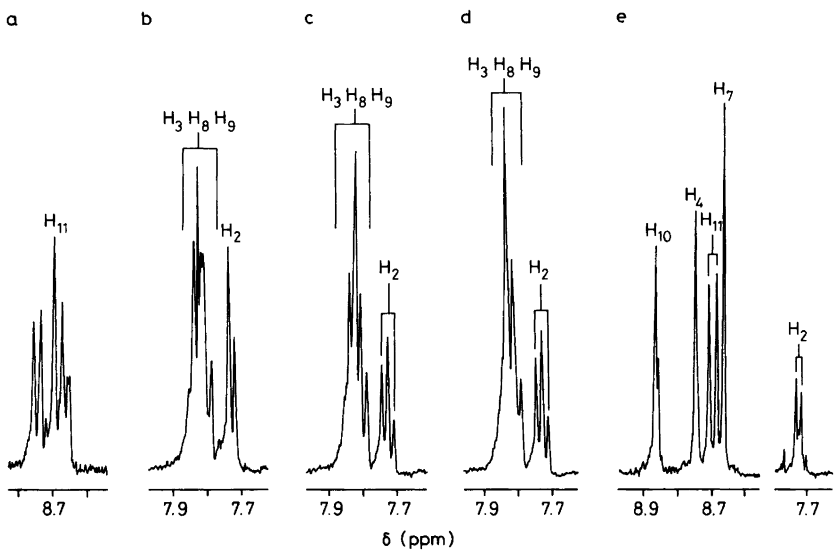


Fig. 4. Decoupling experiments with 6-nitrochrysene. *a,* Irradiation of H-12. *b,* Irradiation of H-1. *c,* Irradiation of H-7. *d,* Irradiation of H-10. *e,* Irradiation of H-3, H-8 and H-9.

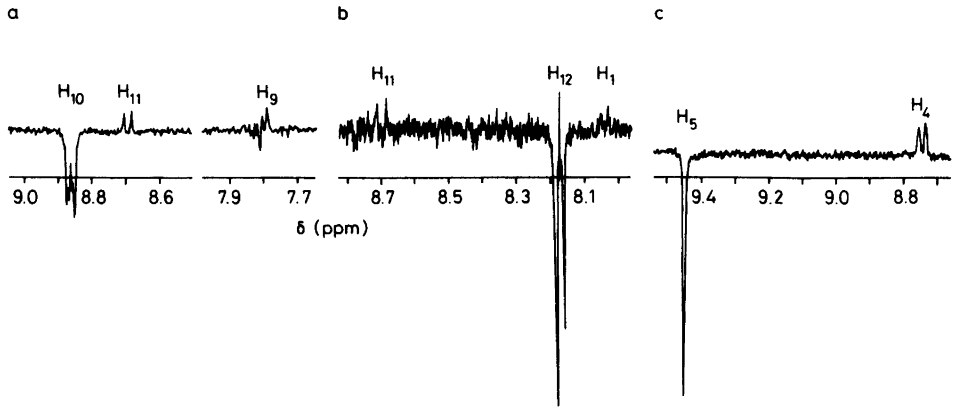


Fig. 5. ^{1}H- [^{1}H] NOE difference spectra of 6-nitrochrysene. *a,* Irradiation of H-10. *b,* Irradiation of H-12. *c,* Irradiation of H-5.

The main product from nitration of *chrysene* gave a proton NMR spectrum (Fig. 3) which turned out to be due to 6-nitrochrysene. This structure was partly deduced by double resonance studies. Irradiation at the frequencies of H-12 and H-1 causes the H-11 signal to collapse to a singlet (Fig. 4a) and the multiplet due to H-2, H-3, H-8 and H-9 to simplify considerably (Fig. 4b). Simplification of the H-3, H-8 and H-9 multiplets also results when the frequencies of H-7 (Fig. 4c) and H-10 (Fig. 4d) are saturated. Finally, irradiation of the complex multiplet from H-3, H-8 and H-9 simplifies the H-2, H-4, H-7 and H-10 signals (Fig. 4e). These experiments clearly show that this spectrum consists of one singlet and three subspectra of which there is one AB, one ABMX, and one AA'MX system. The nitro group is therefore attached to either of the positions 5 and 6, and to determine the substitution pattern NOE studies were carried out. Fig. 5 shows NOE difference spectra of the compound which confirm that (1) H-10 and H-11 belong to the same bay area of the molecule (Fig. 5a), (2) H-12 is in a *peri* position relative to H-1 (very little nuclear Overhauser enhancement) (Fig. 5b), and (3) the singlet is due to a hydrogen atom

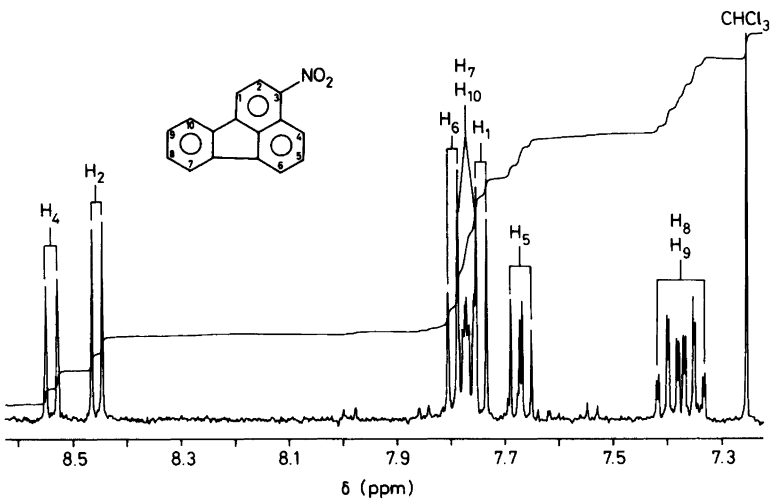


Fig. 6. The 400 MHz ^{1}H NMR spectrum of 3-nitrofluoranthene in $CDCl_3$ at 24 °C relative to internal TMS.

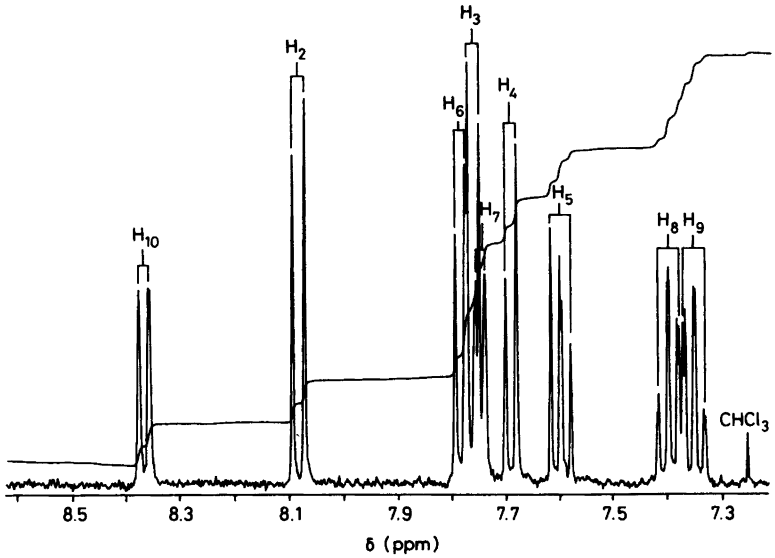


Fig. 7. The 400 MHz ^{1}H NMR spectrum of 1-nitrofluoranthene in $CDCl_3$ at 24 °C relative to internal TMS.

which belongs to the same "bay" as H-4 (a strong nuclear Overhauser effect) (Fig. 5c). The most abundant mononitro-chrysene derivative is therefore 6-nitrochrysene.

^{1}H NMR studies were also carried out on one of the minor mononitrochrysene derivatives, but the compound did not permit a complete analysis of the spectrum due to decomposition under the experimental conditions. However, a sharp singlet at 9.33 ppm strongly indicates that the isomer is 5-nitrochrysene which is the only nitrochrysene isomer, except 6-nitrochrysene, that will give rise to a singlet in the proton spectrum. This conclusion is also supported by the good agreement between the proton spectrum of this compound and that of 5-acetylchrysene (Table 1).[30]

Four of the five mononitro isomers obtained by nitration of *fluoranthene* were analyzed by ^{1}H NMR spectroscopy.

The most and the least abundant isomers gave rise to the spectra shown in Figs. 6 and 7, respectively. The structures of these compounds were partly deduced by double resonance experiments. For the most predominant isomer separate irradiation at the frequence of H-4 or H-6 causes the H-5 double doublet to collapse to a doublet. Similarly, the H-1 doublet collapses to a singlet when the H-2 frequency was saturated.

Table 1. The proton NMR spectra of 5-acetylchrysene (5-Ac-Ch)[30] and 5-nitrochrysene (5-nitro-Ch) in ppm in $CDCl_3$ relative to internal TMS.

5-Ac-Ch	5-nitro-Ch
7.4–8.0 (m,7H)	7.6–8.2 (m,7H)
8.07–8.27 (m,H-10, H-11)	8.35–8.53 (m,2H)
8.66 (s,H-6)	8.84 (m,1H)
8.82 (m,H-4)	9.33 (s,1H)

Finally, partial saturation of the H-7, H-10 multiplet almost converts the H-8, H-9 multiplet into an AB quartet. Analogous experiments for the least predominant isomer gave the following results: Partial saturation of the double doublet from H-5 causes the H-4 and H-6 doublets to collapse essentially to singlets. Furthermore, irradiation at the H-10 frequency removes a *para* coupling to H-7, a *meta* coupling to H-8 and an *ortho* coupling to H-9. Similarly, when the frequency of H-7 is saturated, a *para* coupling to H-10, a *meta* coupling to H-9 and an *ortho* coupling to H-8 are eliminated. Finally, irradiation of the doublet from H-2 causes the H-3 doublet to collapse to a singlet. It is therefore evident that both NMR spectra consist of one

Table 2. Chemical shifts in ppm relative to internal TMS of the hydrogen atoms in the naphtalene moiety of fluoranthene, 1-nitrofluoranthene and 3-nitrofluoranthene.

Proton	Fl[a]	1-nitro-Fl[b]	3-nitro-Fl[b]
H-1	7.82	–	8.00
H-2	7.52	8.30	8.35
H-3	7.72	7.84	–
H-4	7.72	7.87	8.51
H-5	7.52	7.74	7.87
H-6	7.82	7.99	8.02

[a] From Ref. 32. [b] These values have been calculated from the observed values of fluoranthene by using the deshielding parameters of Wells.[31]

AB, one AMX and one four-spin subspectrum.

Since J_{AB} (>7.5 Hz) is an *ortho* coupling constant in both cases, the two isomers are 1-nitrofluoranthene (1-nitro-Fl) and 3-nitrofluoranthene (3-nitro-Fl). In order to determine which isomer corresponds to which structure, the chemical shifts of the hydrogen atoms in the naphtalene moiety of the alternative structures were calculated[31–33] (Table 2) and compared with the experimental data (Figs. 6 and 7). This comparison clearly reveals that only 3-nitro-Fl is expected to give signals matching the two doublets observed at low field in the spectrum of the most abundant nitrofluoranthene derivative (Fig. 6). Consequently, this isomer is 3-nitrofluoranthene, whereas the least abundant one is 1-nitrofluoranthene. This conclusion is supported by the different four-spin subspectra observed in the NMR spectra of the two compounds. The nitro group in 3-nitro-Fl has very little influence on the disubstituted benzene moiety which therefore appears as an ABMM′ subspectrum (Fig. 6). In 1-nitro-Fl, on the other hand, the nitro group deshields H-10 considerably more than H-7, H-8 and H-9 and the four-spin system results in an ABMX.

The isomer with the longest retention time on Hypersil silica gave the ^{1}H NMR spectrum shown in Fig. 8. As evidenced by a simple decoupling experiment this spectrum contains an AMX subspectrum which is characteristic for a 1,2,4-trisubstituted benzene derivative. This isomer is therefore 8-nitrofluoranthene.

The fourth nitrofluoranthene isomer gave the ^{1}H NMR spectrum shown in Fig. 9a. Unfortunately, the compound was unstable and decomposed before a complete series of decoupling experiments had been performed, rendering a detailed analysis of the spectrum impossible.

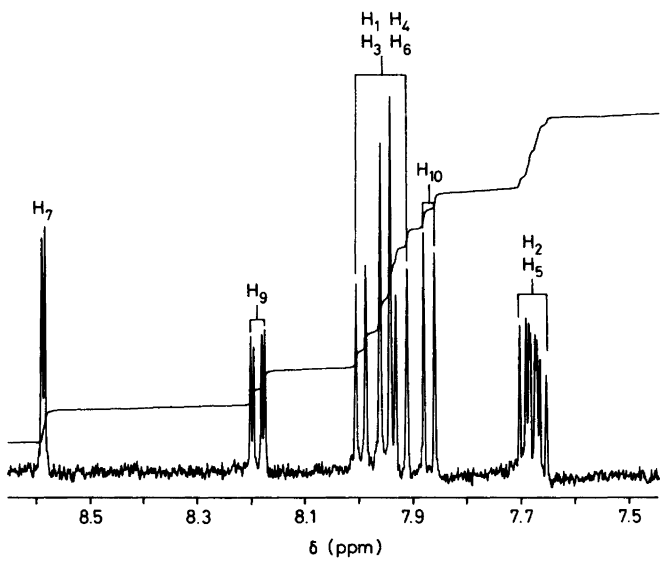


Fig. 8. The 400 MHz ^{1}H NMR spectrum of 8-nitrofluoranthene in $CDCl_3$ at 24 °C relative to internal TMS. The interpretation is based on decoupling experiments and by taking the relative positions of H-1–H-6 in fluoranthene into consideration.[32]

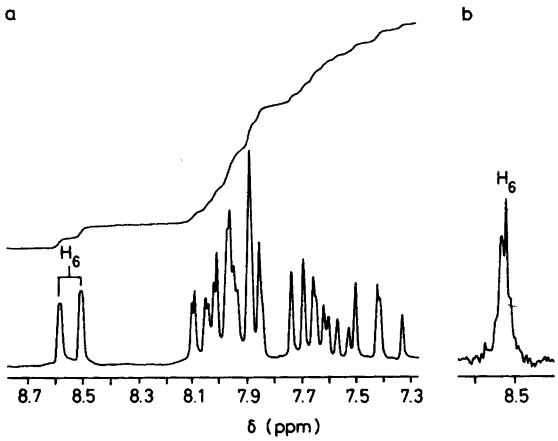


Fig. 9. a, The 90 MHz ^{1}H NMR spectrum of 7-nitrofluoroanthene in $CDCl_3$ at 29 °C relative to internal TMS. *b,* Irradiation at 7.7 ppm, decoupling at 8.54 ppm.

However, partial saturation of the multiplet at 7.7 ppm caused the two low-field signals to collapse essentially to a broad singlet (Fig. 9b) which proves that a doublet arising from an *ortho* coupling (*J* 7.3 Hz) is situated at 8.54 ppm. This isomer is therefore 7-nitrofluoranthene which is the only remaining alternative that will give rise to such a doublet at low field.

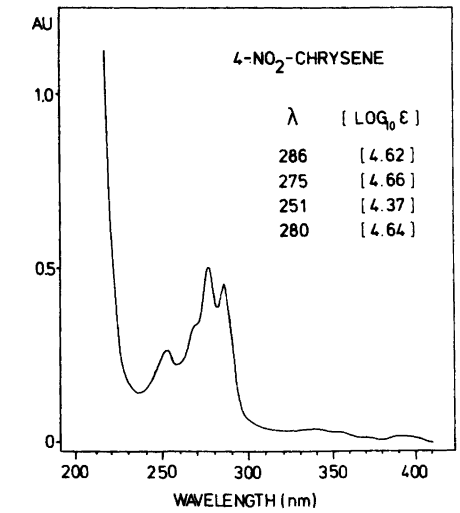
Mass spectroscopy. Two of the most common fragmentation processes after electron impact ionization of aromatic nitro compounds are loss of NO and NO_2. The loss of NO may occur after rearrangement of the nitro group to a nitrite moiety, or through a three-centered cyclic transition state.[34,35] Furthermore, loss of HNO_2 may also take place, most probably as NO_2+H. The same cleavage reactions are also observed in the mass spectra of nitro-PAH, *e.g.* 9-nitroanthracene, 2-nitropyrene, 6-nitrobenzo(a)pyrene and 3-nitroperylene,[36,37] and these processes can be used to elucidate the structures of the nitro-PAH derivatives reported here from characteristic peaks in their mass spectra (Table 3).

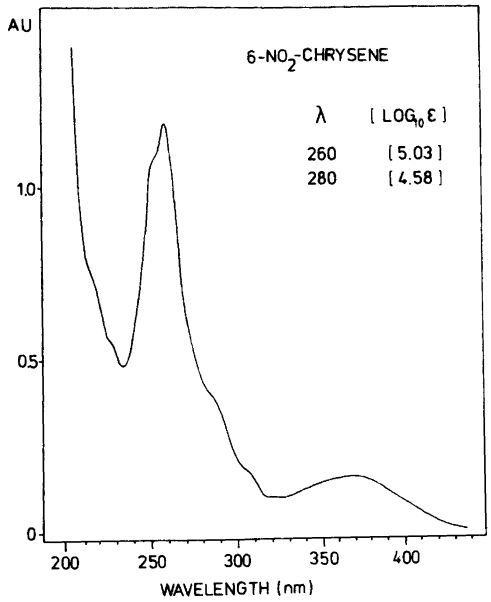
The M–OH fragment was exclusively found in the spectra of nitro-PAH with the nitro group in a *bay* position, *e.g.* of 5-nitro-Ch and 1-ni-

Table 3. 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]	Substituent position
9-NO_2-Phe	100	–	63	1	57	68	59	*peri*
1-NO_2-Pyr	100	–	76	5	96	55	53	*peri*
4-NO_2-Ch	56	7	58	100	57	90	54	*bay*
5-NO_2-Ch	100	2	47	3	44	80	91	*bay*
6-NO_2-Ch	100	–	19	1	48	78	62	*peri*
1-NO_2-Fl	90	–	30	3	100	53	29	"*bay*"
2-NO_2-Fl	81	–	100	7	89	48	39	*peri*
3-NO_2-Fl	100	–	39	1	81	67	48	*peri*
7-NO_2-Fl	78	–	12	–	100	47	22	"*bay*"
8-NO_2-Fl	100	–	27	1	89	56	29	*peri*
1-NO_2-Tri	21	6	100	12	17	58	90	*bay*
2-NO_2-Tri	100	–	5	–	51	70	29	–

[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 contribution.





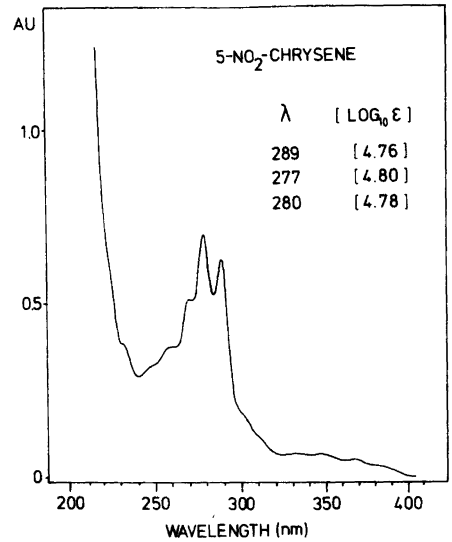


Fig. 10. The UV-spectra of 4-, 5- and 6-nitrochrysene in acetonitrile.

troperylene, 1-nitrobenzo[*e*]pyrene and 5-nitrobenzo[*ghi*]perylene.[38] The appearance of such a fragment is in accordance with the M–OH peak in the mass spectrum of *o*-nitrobiphenyl,[34] another compound with a nitro group in a *bay* position. It was therefore reasonable to believe that 4-nitrochrysene was the third nitro-Ch isomer isolated after nitration of chrysene. The intensity of the M–OH fragment is generally low and this explains the lack of M–OH fragmentation in 1-nitro-Fl and 7-nitro-Fl where abstraction of a *bay* hydrogen atom is significantly less probable due to the longer distance between the nitro group and the hydrogen atom across the *bay*.

Fragments due to loss of HNO were found in the mass spectra of all the nitro-PAH derivatives, but with higher intensity in the spectra of the compounds with the nitro group in a *bay* position. It is interesting to note that the M–HNO fragments are more intense than the corresponding M–OH fragments. When the temperature in the ion source was increased, remarkably intense peaks due to oxygen loss appeared in the mass spectra of the compounds with a nitro group in a *bay* position. So far only preliminary experiments have been performed, but a thermal degradation and rearrangement of the nitro derivatives to carbazoles accommodates the observations.

UV-spectroscopy. The UV-spectra of the isomers of nitro-Ch (Fig. 10), nitro-Fl (Fig. 11) and nitro-Tri (Fig. 12) demonstrated the lack of a strong high-wavelength band in the spectra of isomers with a *bay* region nitro group. This is suggested to be caused by a less efficient π-electron overlap from a nitro group forced out-of-plane in a *bay* position. The effect is less marked in the fluoranthenes, where the distance between two "bay"substituents is increased, due

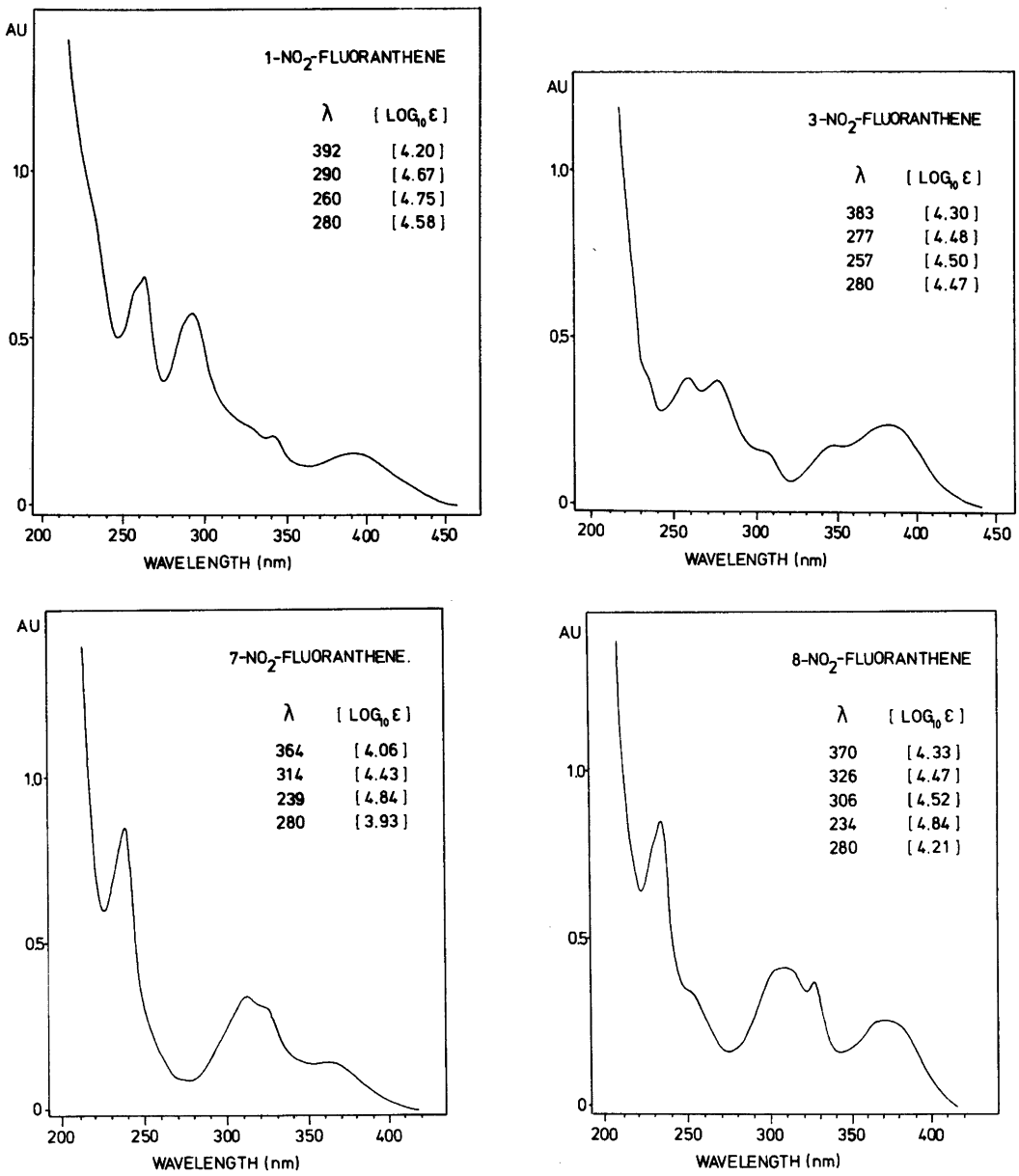
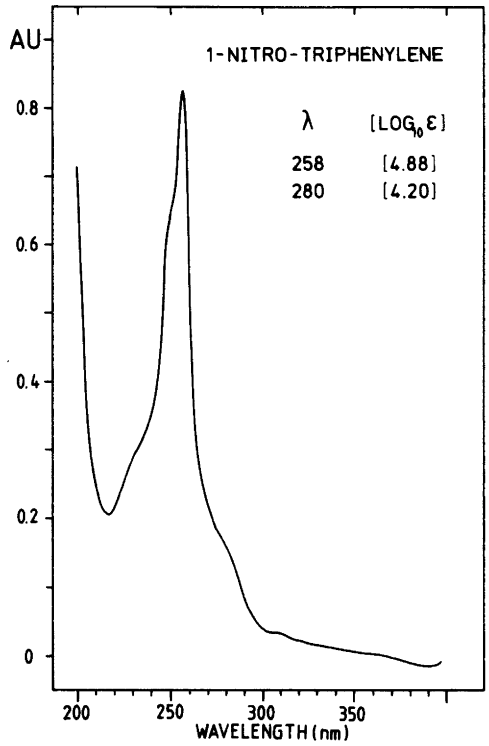


Fig. 11. The UV-spectra of 1-, 3-, 7- and 8-nitrofluoranthene in acetonitrile.

to the five-membered central ring. The UV-spectra therefore support the conclusion that 4-nitro-Ch is a minor product when chrysene is nitrated with nitric acid in acetic anhydride.

EXPERIMENTAL

The HPLC equipment consisted of Waters M 6000 A pumps, a Waters U6K injector, a Waters 440 UV detector with 2 flow cells, a Perkin Elmer LC-55 variable wavelength UV detector and a



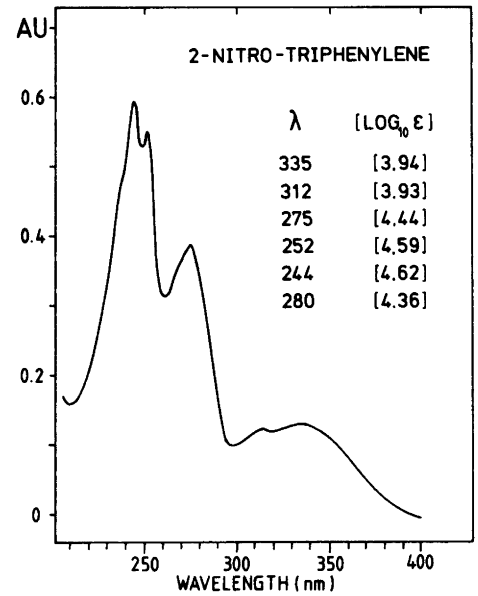


Fig. 12. The UV-spectra of 1-nitro- and 2-nitro-triphenylene in acetonitrile.

Kontron SFM 23LC spectrofluorometric detector.

The organic solvents used for the crude purification on gravity columns were of *pro analysi* quality (Merck). All the HPLC work was performed with solvents of HPLC quality (Rathburn), except chloroform (Fluka, *p.a.*).

The UV spectra were recorded on a Cary Recording Spectrophotometer, model 14, with a scan speed of 6.25 A/s. The molar absorbances at the maxima were measured on a Cary Spectrophotometer, model 16.

The mass spectra (70 eV) were obtained on a Micromass 7070 F double focusing magnetic instrument with electron impact ionization and a ion-source temperature of 200 °C.

The ^{1}H NMR spectra were recorded on a Bruker WH 400 (400.13 MHz) spectrometer at 24 °C or on a Jeol FX 90 Q (89.55 MHz) instrument at 29 °C. The samples were 0.1–0.3 % by weight in $CDCl_3$ which provided the deuterium signal for the NMR field lock. The spectra were run with a spectral width of 4000 Hz, a pulse width of 3 μs (25°) and a repetition time of 1 s. A Lorentzian-Gaussian conversion was applied to the FID before Fourier transformation. The computer operation conditions gave a digital resolution of 1.0 Hz at 400 MHz and of 0.5 Hz at 90 MHz.

Procedure for nitration and crude purification. 10–50 mg of the aromatic hydrocarbon were dissolved in acetic anhydride (20–200 ml) at room temperature or by careful heating, and cooled to 0 °C. Nitric acid (100 %, density 1.52) in acetic anhydride was added dropwise with stirring at 0 °C and the reaction mixture was stirred in the dark. Details are given in Table 4. The reactions were followed by TLC on silica (Merck Kieselgel 60–F_{254}), with dichloromethane–hexane (30:70) as eluent. When the mononitro fraction appeared to be at a maximum concentration, the reaction was quenched by adding water (40 ml) and concentrated (95–97 %) sulfuric acid (0.5 ml) at 0 °C. The hydrolysis of the acetic anhydride was completed by stirring for approximately 9 h at 0 °C. The solution or suspension was extracted with dichloromethane and the organic phase was washed with an aqueous sodium bicarbonate solution (4 g in 60 ml water). The organic phase was evaporated to dryness under vacuum. The crude product was dissolved in dichloromethane (5–10 ml), diluted with hexane (10–20 ml) in order to reduce the elution strength and applied on a silica (Merck Kieselgel 60, 0.063–0.200 mm) column. The first fractions contained unreacted starting material (if present). The next fractions contained mononitro derivatives (Table 4). Further elution resulted in small amounts of

Table 4. Experimental data on synthesis and crude purification of mononitro PAH.

PAH	mg PAH	mmol HNO_3	molar ratio HNO_3:PAH	Nitration Time (h)	Temp. (°C)	Column size (cm)	Ch_2Cl_2/ hexane	Elution volume (ml)	Yield %
Ch	18.0	0.7	9	48	0				
				120	20	26×1.5	30/70	110–250	77
Fl	20.7	0.05	0.6	168	0	26×1.5	30/70	110–250	38
Tri	50.0	2.4	11	48	60	25X2.5	40/60	160–210 [a]	
								211–290 [b]	59
								291–340 [c]	

[a] 1-nitro-Tri. [b] Mixture of 1- and 2-nitro-Tri. [c] 2-nitro-Tri.

dinitro derivatives.

Purification by HPLC. A 99.9 % purity was considered the practical limit for the preparative purification procedures, and this purity has turned out to be necessary for some compounds since the mutagenic response in TA-98 assays may vary with a factor of up to 10 000 within one group of isomers.[20]

The purity measurements were carried out with two different HPLC systems, one by adsorption chromatography and one by reversed phase chromatography, with UV-detection at 2 wavelengths. The 280 nm wavelength was chosen as a standard for all compounds, while a second higher wavelength was chosen according to the various absorption maxima. Thus, the purity measured was based on the assumption of approximately equal molar absorbance of possible contaminants, an assumption which turned out to be essentially valid when controlled by glass capillary gas chromatography. Gas chromatography alone was found less satisfactory for purity testing, since the amounts of thermal decomposition products (of the higher nitro-PAH) increased with increasing temperature and increasing time on the columns. A fluorescence detector was found to be valuable to spot minor amounts of certain decomposition products formed by evaporation of solutions in the light or by prolonged storage.

Three HPLC columns were utilized in the purification procedures: A. 250×10 mm, packed with 3 μm Hypersil silica (Shandon). B. 250×7.7 mm, packed with 5 μm Spherisorb silica (Phase Sep.). C. 250×10 mm, packed with 5 μm Hypersil-ODS (Shandon).

The nitro-PAH were applied in CH_2Cl_2 or in CH_2Cl_2–hexane (1:1). The application volumes were 25–150 μl. The flow rates were 4–5 ml/min.

9-Nitro-Phe was purified first on column B with 5 % CH_2Cl_2 in hexane, than rechromatographed on column A with 3 % CH_2Cl_2 in hexane.

1-Nitro-Pyr was purified on column A with 5 % CH_2Cl_2 in hexane. Decomposition products were removed on Kieselgel 60 with 40 % CH_2Cl_2 in hexane.

4-, 5- and 6-Nitro-Ch were separated on column A with 5 % CH_2Cl_2 in hexane. 6-Nitro-Ch was purified on column A with 0.05 % isopropanol in hexane. 4- and 5-Nitro-Ch were purified on column A with 3 % CH_2Cl_2 in hexane.

1-, 2-, 3-, 7- and 8-Nitro-Fl were separated on column B with 5 % CH_2Cl_2 in hexane. 7- and 3-Nitro-Fl needed rechromatography on column B with 3 % CH_2Cl_2 in hexane.

1- and 2-Nitro-Tri separated on column A with 0.8 % acetonitrile in hexane (2-nitro-Tri eluted prior to 1-nitro-Tri). With 10 % CH_2Cl_2 in hexane the order of elution was reversed. An even better resolution was obtained on column C with 75–80 % methanol in water.

The purity of the products was examined on 5 μm Hypersil silica (300×4,6 mm) with 3–5 % CH_2Cl_2 in hexane and on Hypersil-ODS (250×4,6 mm) with 80 % methanol in water.

Proton NMR spectra. The proton spectra of 6-nitrochrysene and four of the nitrofluoranthene derivatives are summarized below. The chemical shifts are given in ppm relative to internal tetramethylsilane (TMS).

6-Nitro-Ch. ^{1}H–NMR (400 MHz): δ 7.69–7.76 (1H,m), 7.78–7.85 (3H,m), 8.03 (1H,d,J 7.8 Hz), 8.17 (1H,d,J 8.8 Hz), 8.64–8.69 (1H,m), 8.69 (1H,d,J 8.8 Hz), 8.72–8.76 (1H,m), 8.83–8.88 (1H,m), 9.44 (1H,s).

1-Nitro-Fl. ^{1}H NMR (400 MHz): δ 7.31–7.42 (2H,m), 7.60 (1H,dd,J 6.9 Hz, J 8.2 Hz), 7.69 (1H,d,J 8.2 Hz), 7.74 (1H,d,J 7.3 Hz), 7.76 (1H,d,J 8.8 Hz), 7.78 (1H,d,J 6.9 Hz), 8.08 (1H, d, J 8.2 Hz), 8.36 (1H, m, J 7.6 Hz).

3-Nitro-Fl. ^{1}H NMR (400 MHz): δ 7.31–7.42 (2H,m), 7.67 (1H,dd,J 7.0 Hz, J 8.6 Hz), 7.74 (1H,d,J 7.7 Hz), 7.75–7.79 (2H,m), 7.79 (1H,d,J

7.0 Hz), 8.45 (1H,d,*J* 7.7 Hz), 8.54 (1H,d,*J* 8.6 Hz).

7-Nitro-Fl. ^{1}H NMR (90 MHz): δ 7.32–7,73 (3H,m), 7.85–8.10 (5H,m), 8.54 (1H,d,*J* 7.3 Hz).

8-Nitro-Fl. ^{1}H NMR (400 MHz): δ 7.64–7.71 (2H,m), 7.87 (1H,d,*J* 8.3 Hz), 7.92 (1H,d,*J* 8.2 Hz), 7.95 (2H,d,*J* 7.5 Hz), 7.99 (1H,d,*J* 7.0 Hz), 8.19 (1H, dd,*J* 2.2 Hz, *J* 8.3 Hz), 8.59 (1H,d,*J* 2.2 Hz).

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