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Preparation and Characterization of Dinitroperylenes

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Dinitroperylenes have been synthesized and purified by HPLC. Based on NMR, MS and UV data, the main isomers were identified as 3,6-, 3,7-, 3,9- and 3,10-dinitroperylene.

A scheme for preparation of nitrated polycyclic aromatic hydrocarbon (nitro-PAH) derivatives of high purity has been developed[1,2] in order to obtain pure reference substances for tests of mutagenic properties. In previous papers[1,2] the synthesis and isolation of a series of mononitrated PAHs have been described. Since dinitropyrenes have been found to be considerably more mutagenic than mononitropyrenes,[3] it was of interest to find out whether a similar relationship exists among other nitro-PAHs as well. As a part of such studies a variety of dinitroperylenes have been prepared and characterized; the results are reported here.

RESULTS AND DISCUSSION

Synthesis and purification. The nitration of perylene (Per) has mostly been performed with nitric acid in acetic anhydride[4] or in dioxane[5]. In the former case 3-nitro-Per was the predominant isomer whereas a mixture of 3-nitro-Per and 1-nitro-Per was obtained in the latter in a ratio of 70:30. The yields were 45 and 80 %, respectively. Recently, nitration with dinitrogen tetroxide was reported to yield 95 % of a 99:1 isomer mixture[6] of the same isomers. The first synthesis of dinitroperylenes was reported by Zinke *et al.*[7], but no isomers were identified. The procedure was repeated by Hopff and Schweizer,[8] who suggested that the product was a mixture of 3,9- and 3,10-dinitro-Per.

When perylene was nitrated according to our procedure, *i.e.* with 5 equivalents of nitric acid in acetic anhydride at 0 °C, a 91 % yield of 3-nitro-Per[5] and 1-nitro-Per[5] in a ratio of 97:3 was obtained. However, with 20 equivalents of nitric acid a mixture of dinitro derivatives was obtained in 60 % yield. This mixture was separated by HPLC (Fig. 1) in three fractions containing, according to NMR analysis (see below), 3,6-dinitro-Per, 3,7-dinitro-Per, and an inseparable mixture of 3,9-dinitro-Per and 3,10-dinitro-Per, respectively.

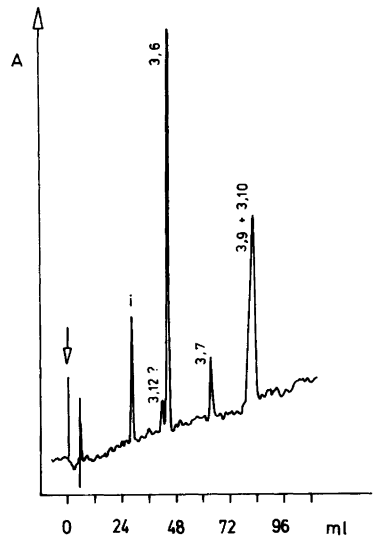

Fig. 1. Distribution of isomers of dinitroperylene on a 3 μm Hypersil silica column (300×3.9 mm) with 0.2 % CH_3CN and 1 % $CHCl_3$ in hexane, UV detection at 280 nm, flowrate 2 ml/min. (i:impurity).

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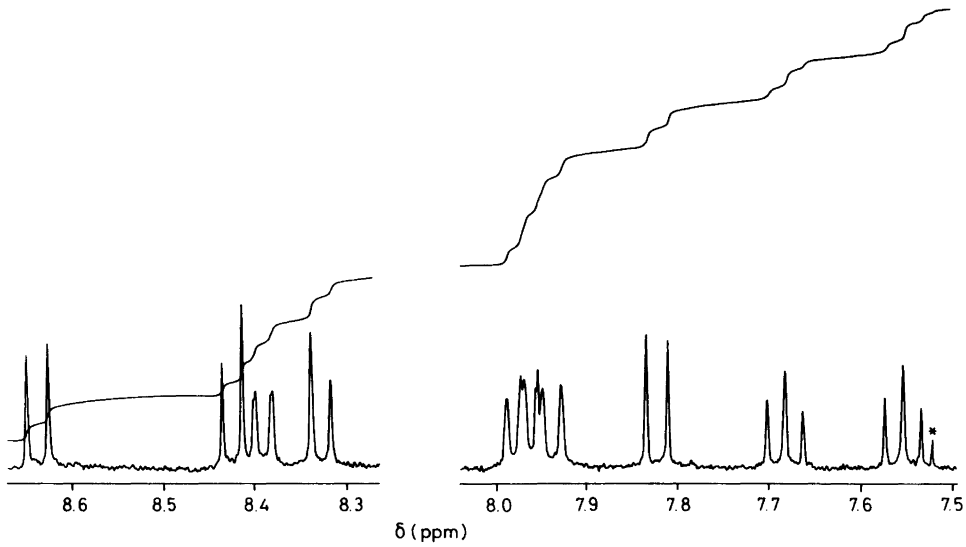


Fig. 2 The 400 MHz ^{1}H NMR spectrum of 3,6-dinitroperylene in $CDCl_3$ at 24 °C relative to internal TMS. The peak marked with an asterisk is due to a contaminant.

The ratio of the 3,6-, 3,7-, 3,9- and 3,10-dinitroperylene isomers in the reaction mixture was estimated to be approximately 5:2:1:2.

A fifth compound (Fig. 1) was formed in very low yield, and was shown by mass spectrometry to be yet another dinitro derivative, but the product was not obtained in sufficient quantities with sufficient purity to allow structure elucidation by NMR. However, we suggest that the compound is 3,12-dinitro-Per.

^{1}H NMR spectroscopyy. The dinitro isomers are formed in two-step reactions where the second step in each case involves electrophilic attack on nitroperylene. Since the mixture of

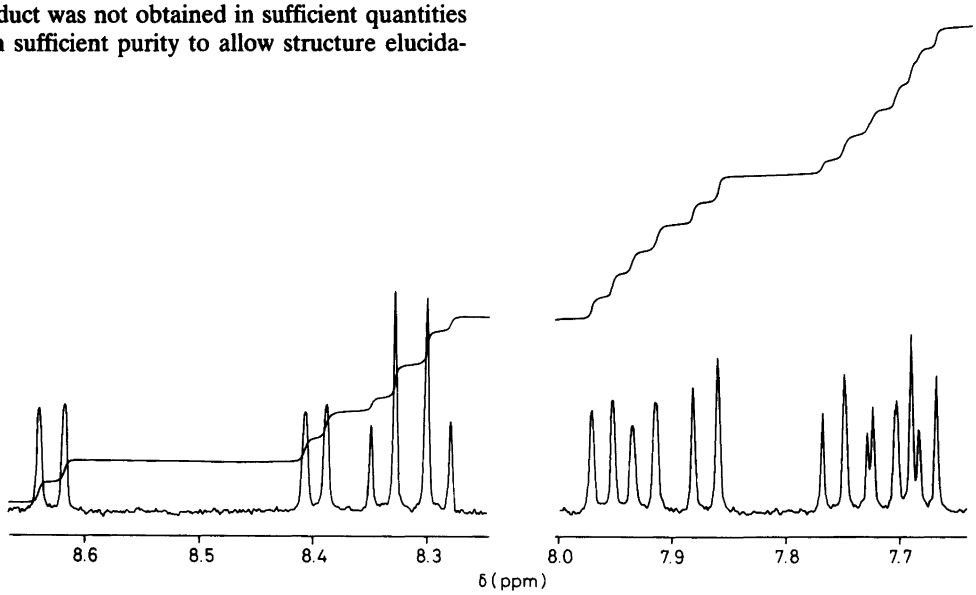


Fig. 3. The 400 MHz ^{1} H NMR spectrum of 3,7-dinitroperylene in $CDCl_3$ at 24 °C relative to internal TMS.

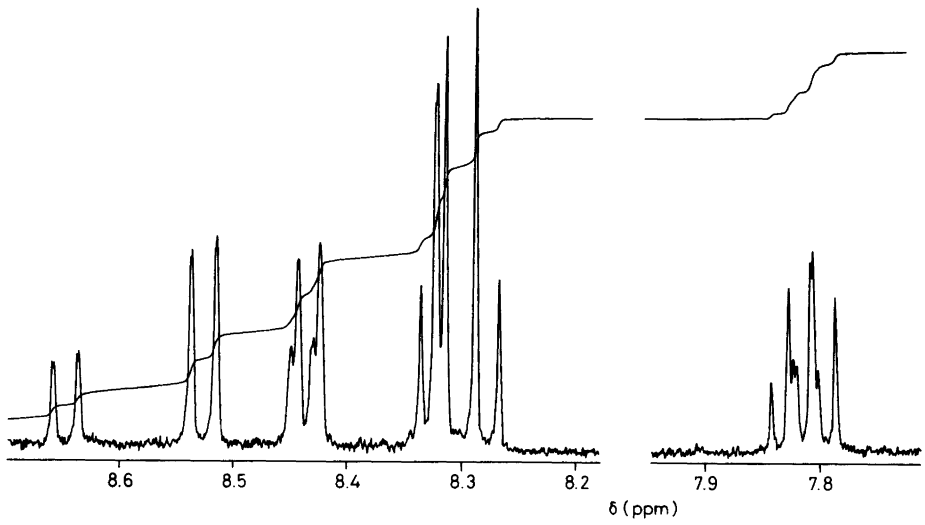


Fig. 4. The 400 MHz ^{1}H NMR spectrum of a 3:7 mixture of 3,9-dinitroperylene and 3,10-dinitroperylene, respectively.

mononitroperylenes consists almost exclusively of 3-nitro-Per, it is reasonable to believe that all the dinitroperylene isomers formed in isolable amounts have one nitro group attached to C-3.

The NMR spectra of the compounds isolated by preparative HPLC are shown in Figs. 2–4. Simple homonuclear double resonance experiments showed that all spectra contained AB and AMX subspectra only. Furthermore, all J_{AB} turned out to be *ortho* coupling constants (>8 Hz); consequently, carbon atoms 1, 2, 5, 8 and 11 can not be attached to a nitro group.

These facts result in a reduction of the number of structures for the isomers to six possibilities (Fig. 5) which ought to give rise to ^{1}H -NMR spectra of either of two types. The 3,4-, 3,9- and 3,10-dinitroperylene isomers should give spectra

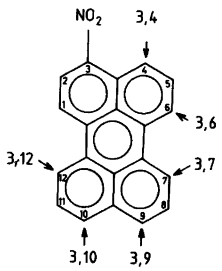


Fig. 5. The possible structures of the dinitroperylene isomers.

consisting of one AB and one AMX subspectrum due to symmetry. On the other hand, the other three dinitro derivatives, lacking a plane or a center of symmetry, should give spectra comprising two AB and two AMX subspectra.

From the NMR spectra depicted in Figs. 2 and 3, it is evident that the pure, single isomers isolated from the reaction mixture are among the latter group of compounds. Furthermore, the main product is certainly 3,6- or 3,7-dinitroperylene since NOE studies reveal a nuclear enhancement, typical for protons belonging to the same bay area, between one AB proton and one proton belonging to one of the three-spin systems (Fig. 6). Similar experiments with the other pure isomer were, strangely enough, unsuccessful as no nuclear Overhauser effect was detected. However, the structure of these compounds was established by extensive relaxation time measurements[9] which supported the conclusion that the main product is 3,6-dinitroperylene and the other unsymmetrical isomer is 3,7-dinitroperylene.

The fraction with the longest retention time (Fig. 1) gave an NMR spectrum (Fig. 4) containing two sets of subspectra, each consisting of one AB and one AMX system, in a ratio of 3:7. The fraction is therefore a mixture of two of the isomers 3,4-, 3,9- and 3,10-dinitroperylene. Since the compunds were inseparable under a variety of conditions their polarity has to be almost

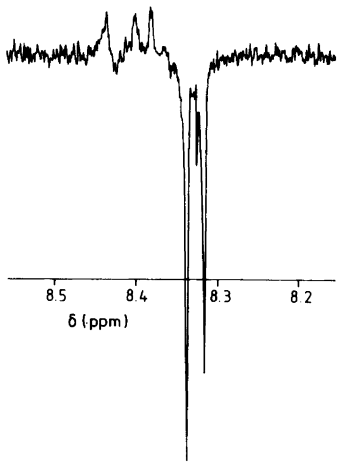


Fig. 6. The $^1H-\{^1H\}$ NOE difference spectrum of 3,6-dinitroperylene when the doublet at 8,33 ppm is irradiated. NOE effect at 8,39 ppm, INDOR at 8,42 ppm.

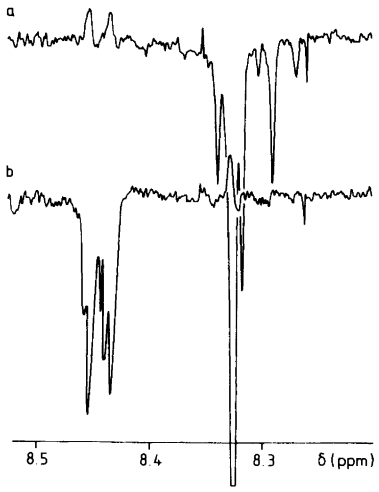


Fig. 7. $^1H-\{^1H\}$ NOE difference spectra of a 3:7 mixture of 3,9- and 3,10-dinitroperylene. *a*, Irradiation around 8.32 ppm. *b*, Irradiation around 8.45 ppm.

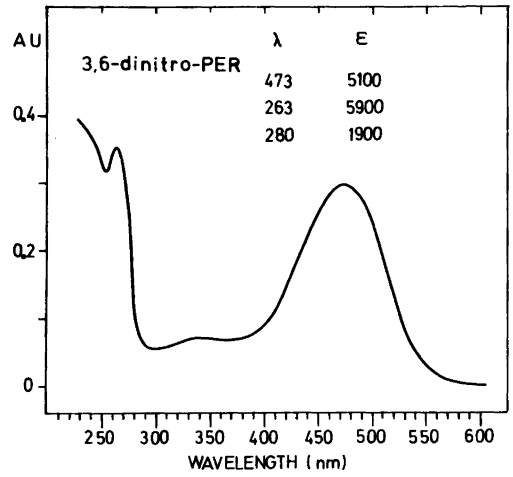
identical. This is the case with 3,9- and 3,10-dinitroperylene whose dipole moments are close to nil, drastically below the 5–6 D which is a conservative estimate of the dipole moment of 3,4-dinitroperylene.[10] The fraction under consideration is therefore most likely a mixture of 3,9- and 3,10-dinitro-Per. This conclusion is supported by NOE experiments. Thus, the major component does not show any nuclear enhancement across the bays which indicates that the protons belonging to the same bay are identical; the major component is therefore 3,10-dinitro-Per. The minor isomer, on the other hand, shows a substantial nuclear Overhauser effect between the AB and AMX spin systems (Fig. 7) which is compatible with the structure 3,9-dinitroperylene for this compound. This structure elucidation is also supported by extensive T_1 measurements.[9]

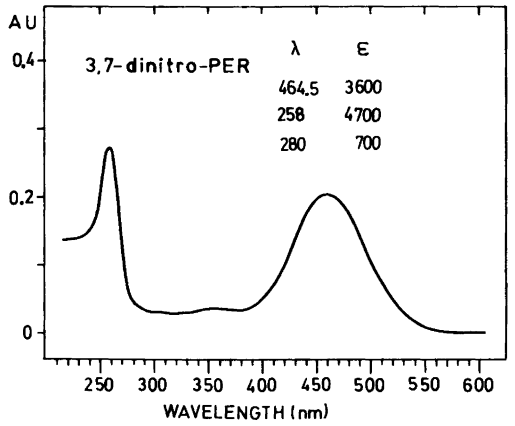
Mass spectroscopy. The mass spectra of the compounds (Table 1) contain fragments due to loss of NO and NO_2 which are generally present in such spectra of mononitro- PAHs.[1,2] In addition, major peaks owing to $M-NO_2-NO$, $M-2NO_2$, and $M-2HNO_2$ fragments are observed in all spectra. M–HNO, an abundant fragment in mass spectra of mononitro-PAHs,[1,2] was almost completely absent, whereas $M-HNO_2$, another abundant fragment from mononitro-PAHs, was present but in relatively small amounts. More important, however, is the presence of M–O and M–OH fragments in the spectra of the 3,6- and 3,7-dinitro derivatives and the absence of the same fragments in the spectra of 3,9- and 3,10-dinitro-Per. This fragmentation pattern has been found only in the mass spectra of isomers having a nitro group in a bay position.[1,2] The MS studies therefore support the structure elucidation based on NMR experiments.

UV-spectroscopy. The structure elucidation is also supported by the UV spectra of the compounds (Fig. 8). Thus, the UV spectrum of the mixture of 3,9- and 3,10-dinitroperylene showed the strongest band at the highest wavelength.

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

Compound	M	M–16	M–17	M–30	M–31	M–46	M–47	M–76	M–77	M–92	M–94
3,6-dinitro-Per	95	8	26	28	–	20	16	83	25	100	54
3,7- "	100	5	17	42	4	16	4	67	17	95	50
3,9+3,10- "	100	–	–	30	–	27	11	35	8	61	27





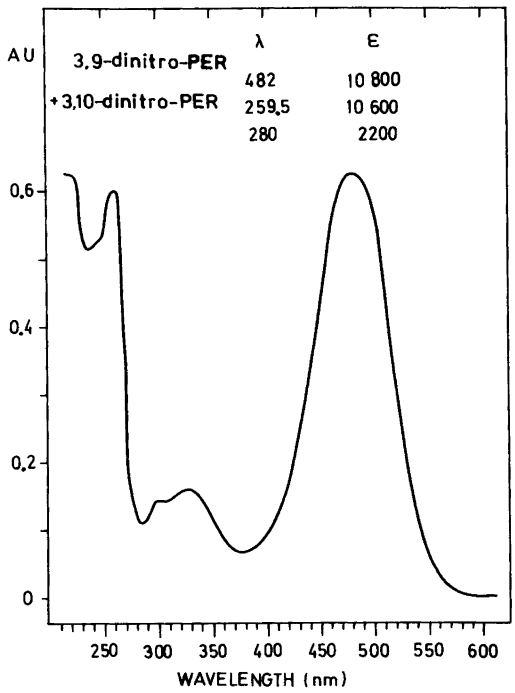


Fig. 8. UV spectra of dinitroperylenes in acetonitrile. The molecular absorbances at 280 nm are included since the isomer ratios were determined from absorbance measurements at this wavelength.

This observation is in accordance with our previous findings for nitro-PAH derivatives without a nitro group in a bay position.[1,2]

Mutagenic properties. The details of the study of the mutagenic properties have been published,[11] but the main conclusions are the following.

In the TA-98 Ames test, without addition of a metabolic activator, the 3,9- + 3,10-dinitro-Per mixture was considerably more mutagenic than the other two isomers. The mutagenic activity of the 3,9- + 3,10-mixture was determined to around 50 revertants per nanogram, which is more than 100 times the effect of 3-nitroperylene and comparable to some of the most strongly mutagenic compounds.[12]

These results add strength to the impression that dinitro PAHs are significantly more mutagenic than the corresponding mononitro analogues.

EXPERIMENTAL

The instrumentation has been described elsewhere.[1]

Perylene (25 mg, 10^{-4} mol) in acetic anhydride (75 ml) was reacted with nitric acid ($2 \cdot 10^{-3}$ mol) at 0 °C, as described earlier.[1] After 8 h at 0 °C, the reaction mixture was hydrolyzed, extracted and evaporated. The crude, red product (35 mg) was dissolved in CH_2Cl_2 (200 ml) and diluted with hexane (70 ml). A 20 ml (2.6 mg) sample was applied on a gravity silica column (25×2.5 cm) and eluted with 0.08 % methanol plus 40 % dichloromethane in hexane. The 450–650 ml fraction contained mainly a mixture of 3,6-dinitro-Per and 3,7-dinitro-Per. The 651–1000 ml fraction contained mainly the 3,9- + 3,10-mixture, but also a smaller amount of the first two isomers. Samples (50 μg) of each fraction, dissolved in CH_2Cl_2 (200 μl) were purified on a 10 μm Perkin Elmer ODS column (250×22 mm) with methanol–water (85:15). The 240–270 ml fraction contained a mixture of 3,6-dinitro-Per and 3,7-dinitro-Per, and the 280–310 ml fraction contained the 3,9- + 3,10-isomer mixture. With 0.2 % acetonitrile plus 7 % CH_2Cl_2 in hexane on a 3 μm Hypersil silica column (250×9 mm), the isomers were eluted in the order of 3,6, 3,7 and 3,9 + 3,10. Each isomer was collected and rechromatographed on the silica column with 0.2 % acetonitrile plus 1.5 % chloroform (3,6-dinitro-Per), 0.2 % acetonitrile plus 2 % chloroform (3,7-dinitro-Per) and with 0.2 % acetonitrile plus 25 % chloroform (3,9- + 3,10- dinitro-Per), after which the fractions had a purity of at least 99.5, 99.5 and 98 %, respectively. Smaller amounts of impurities could not be detected due to the low solubility of the compounds. The >98 % purity of the last fraction must be considered an appearing purity, since the NMR showed a mixture of the 3,9 and 3,10 isomers.

Safety precautions. Due to the mutagenic properties nitro-PAHs should be handled with care. Disposable surgical gloves have been used in all procedures involving solutions of the products. Gloves which can be disposed of with frequent intervals are strongly recommended compared with normal use of standard laboratory gloves which results in contamination of the whole laboratory and allows penetration of the products through the gloves.

Proton NMR spectra. The spectra were run in 5 mm NMR tubes on samples which were 0.2–0.4 % by weight, in deuteriochloroform (99.9 %). The spectra are summarized below with chemical shifts given in ppm relative to internal tetramethylsilane (TMS).

3,6-Dinitroperylene. ^{1}H NMR (400 MHz): δ 7.55 (1 H, t, J 7.9 Hz), 7.68 (1H, t, J 7.9 Hz), 7.83 (1H, d, J 9.3 Hz), 7.92–7.99 (3H, m), 8.33 (1H, d, J 8.5 Hz), 8.39 (1H, dd, $J \sim 7.5$ and 1.0 Hz), 8.42 (1H, d, J 8.5 Hz), 8.63 (1H, d, J 9.3 Hz).

3,7-Dinitroperylene. ^{1}H NMR (400 MHz): δ 7.68 (1H, d, J 8.8 Hz), 7.70 (1H, dd, J 7.7 and 8.7 Hz), 7.75 (1H, dd, J 7.7 and 8.1 Hz), 7.86 (1H, d, J 8.8 Hz), 7.92 (1H, d, J 8.1 Hz), 7.96 (1H, d, J 7.7 Hz), 8.29 (1H, d, J 8.4 Hz), 8.33 (1H, d, J 8.4 Hz), 8.39 (1H, d, J 7.7 Hz), 8.63 (1H, d, J 8.7 Hz).

3,9-Dinitroperylene. ^{1}H NMR (400 MHz): 7.83 (2H, dd, J 7.7 and 8.5 Hz), 8.32 (4H, ABq, J 8.4 Hz), 8.45 (2H, m, J 7.7 Hz), 8.65 (2H, dd, J 0.9 and 8.5 Hz).

3,10-Dinitroperylene. ^{1}H NMR (400 MHz): 7.81 (2H, dd, J 7.6 and 8.6 Mz), 8.29 (2H, d, J 8.4 Hz), 8.32 (2H, d, J 8.4 Hz), 8.44 (2H, m, J 7.6 Hz), 8.53 (2H, m, J, 8.6 Hz).

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