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Synthesis, separation and identification of dinitropyrene isomers

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Abstract— Dinitropyrenes (DNP) were prepared by nitration of pyrene, and the product was characterized by MS and elemental analysis. Three isomers of DNP were separated by HPLC and identified by ^{1}H-NMR. The eluting sequence on the normal phase column is 1, 3-DNP, 1, 6-DNP and 1, 8-DNP, whereas is 1, 6-DNP, 1, 8-DNP and 1, 3-DNP on the reversed phase column. The separation of three DNP isomers under different chromatographic conditions was also discussed.

Keywords: dinitropyrenes; environmental pollutant; mutagen polycyclic aromatic hydrocarbon; nitropolycyclic aromatic hydrocarbon.

INTRODUCTION

Since many of the nitropolycyclic aromatic hydrocarbons (NO_2-PAHs) have been shown to be potent mutagens in Ames assay and have exhibited genotoxic activity in other tests, numerous authors have undertaken studies on the analysis of a variety of environmental mixtures for this class of compounds, which has been detected in diesel exhaust, carbon black and ambient air. It has been found that the mutagenicities of NO_2-PAHs have relation not only with the number of nitrogroups but also with their substitutional sites (Zhang, 1991; Pitts, 1981; Xu, 1982). For example, the dinitropyrenes (DNP) are more mutagenic than other nitropyrenes (mononitropyrenes, trinitropyrenes), whereas different isomers of dinitropyrenes manifest different degrees of toxicities. Now advanced studies require the definitive analysis of specific isomers. In this paper, three isomers of DNP were synthesized and their separation conditions were discussed.

EXPERIMENTAL

1. Chemicals and standards

All solvents were of analytical grade and distilled in glass before use except benzene and nitric acid, which were of chromatographic grade. Redistilled water was used. All chemical standards

(PAHs and NO_2-PAHs) were purchased from Aldrich (USA) and used without further purification. All chemicals, except acetonitrile which were purchased from America, were obtained from China.

2. Apparatus

(1) High-performance liquid chromatography

Model 1090, Hewlett & Packard, with DAD diode-array detector, USA; Model LC-3A and Model LC-5A, Shimadzu, Japan.

(2) Columns for separation and preparation

MOS C-8 10cm × 2.1 mm, 5μm, RP; ODS C-18 20 cm × 4.6mm, 5μm, RP; ODS C-18 25 cm × 4.6mm, 5μm, BP; APS 10 cm × 2.5 mm, 5μm, RP; Zorbax CN 25 cm × 4.6 mm, P. N., Zorbax Sil 25 cm × 4.6mm, P. N.; Partisil Silica 25 cm × 10mm, 10μm (for preparation).

(3) Mass spectrometry (MS)

JMS/D-300S JEOL, Japan, direct probe.

(4) Nuclear magnetic resonance

XL-200MHz, Vear Ram, USA; Solvent: $CDCl_3$; Internal standard: $CHCl_3$.

3. Synthesis of DNP

1.27 mmol pyrene in acetic acid solution was nitrated by 25 mmol 68% nitric acid at 90℃ for 10 h. The product was extracted with benzene, washed with water and purified through a silica gel column. The solid product DNP obtained after concentration and drying was identified by elemental analysis, chromatography and MS.

4. Separation, identification of three DNP isomers

The DNP product was separated on preparation column (Partisil 10 μm; silica 25cm × 10mm) by LC-5A with mobile phase C_6H_{14}/CH_2Cl_2 (70/30, v/v). The three isomers were collected, dried, and then identified by chromatography and ^{1}H-NMR.

RESULTS AND DISCUSSION

1. Synthesis of DNP

The nitration of PAH by nitric acid is an efficient and high-productive method to prepare NO_2-PAHs, in which the number of nitro groups is dependent mainly on the reaction conditions (Fig. 1), especially on the concentration of HNO_3 and reaction temperature. A small amount of mononitropyrene occurs as main impurity in the products, and is removed by eluting the benzene solution of the product through silica gel. The HPLC chromatography (Fig. 2), MS (Fig. 3) and elemental analysis (Table 1) show that the synthesis of DNP is successful.

2. Separation, identification of three DNP isomers

The separation of three DNP isomer has been carried out on silica gel preparative column (Fig. 4 and Fig. 5). The ^{1}H-NMR spectra of each separated isomer are shown in Fig. 6.

From the molecular structure of 1, 3-DNP (Fig. 7), we know that the two nitro groups show the most induction effect to H_2, and make H_2 the highest, while H_7 is lowest because H_7 is far from the two nitro groups that have little effect on it, and is splitted into triplet peaks by H_6 and H_8.

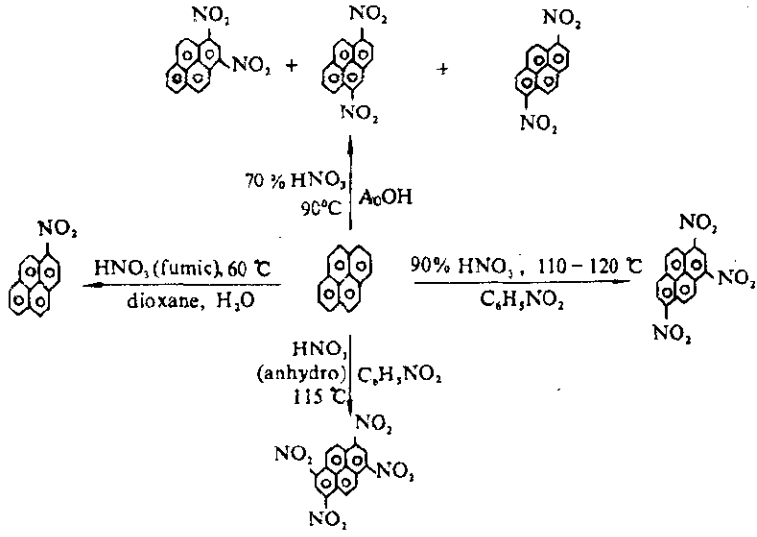


Fig. 1 Synthesis scheme for different nitro-pyrenes

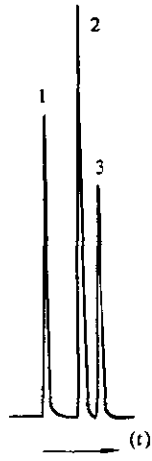
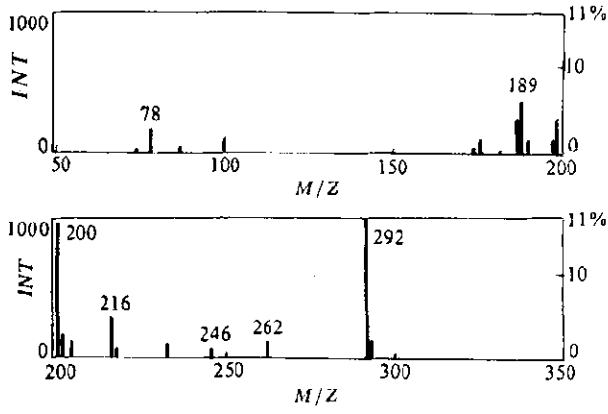


Fig. 2 HPLC chromatogram of the product DNP

1. 1, 6-DNP; 2. 1, 8-DNP; 3. 1, 3-DNP

Analysis column: ODS C-18 25 cm × 4.6mm, 5μm BP; mobile phase: $CH_3OH/H_2O = 85/15$; temperature: 30°C; detection wave length (UV): 254 nm; rate: 1ml/min.



292 : $(M)^+$; 262 $(M\text{-}NO)^+$;

246 : $(M\text{-}NO_2)^+$;

216: $(M\text{-}NO_2\text{-}NO)^+$;

200: $(M\text{-}NO_2\text{-}NO_2)^-$

Fig. 3 MS spectrum of the product DNP

Table 1 Elemental composition of the product DNP (w%)

Molecular form	Molecular weight	Calculation value C	H	N	Measurement value C	H	N
Mononitro-Py $C_5H_5NO_2$	247	77.7	3.6	5.7			
Dinitro-Py $C_{10}H_8N_2O_4$	292	65.75	2.74	9.59	65.86	2.87	9.05
Trinitro-Py $C_{10}H_7N_3O_6$	337	57.0	2.1	12.5			

Notes: Analysis method: GC Carlo Erba 1102

H_4 (same as H_{10}) has higher δ than H_5 (same as H_9) because of deshielding effect of the two nitro groups. The total effect of induction and deshielding from two nitro groups makes δ H of 1, 3-DNP as follows:

δ	H_2	> H_4-H_{10}	> H_5-H_9	> H_6-H_8	> H_7
Peak No.	s	d	d	d	t
H No.	1	2	2	2	1

The above analysis is coincident with data in Fig 6A (Paputa-Peck, 1983). From the molecular structure of 1, 6-DNP, H_2 (same as H_7) has higher δ because of induction effect of nitro groups, H_5 (same as H_{10}) has highest δ by deshielding effect of nitro groups, and is splitted into doublet by $H_4(H_9)$. So, the ^{1}H-NMR spectrum of 1, 6-DNP can be explained as follows:

δ	H_5-H_{10} >	H_2-H_7 >	$H_3-H_8 \geqslant$	H_4-H_9
Peak No.	d	d	d	d
H No.	2	2	2	2

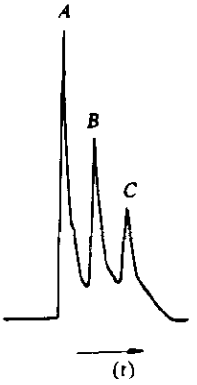


Fig. 4 HPLC chromatogram of the product DNP on preparation column
A: 1, 3-DNP; B: 1, 6-DNP; C: 1, 8-DNP; preparative column: partsil 10 μm silica 25 cm × 10mm, 20℃; $C_6H_{14}/CH_2Cl_2 = 70/30$; rate: 3 ml / min; UV: 254nm.

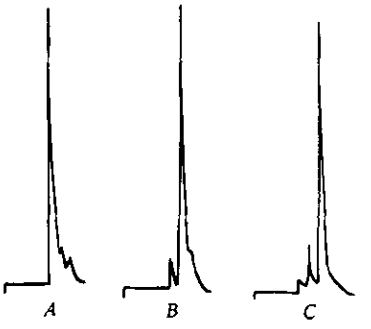


Fig. 5 The HPLC chromatograms of each separated isomer separation with the same conditions to Fig. 4

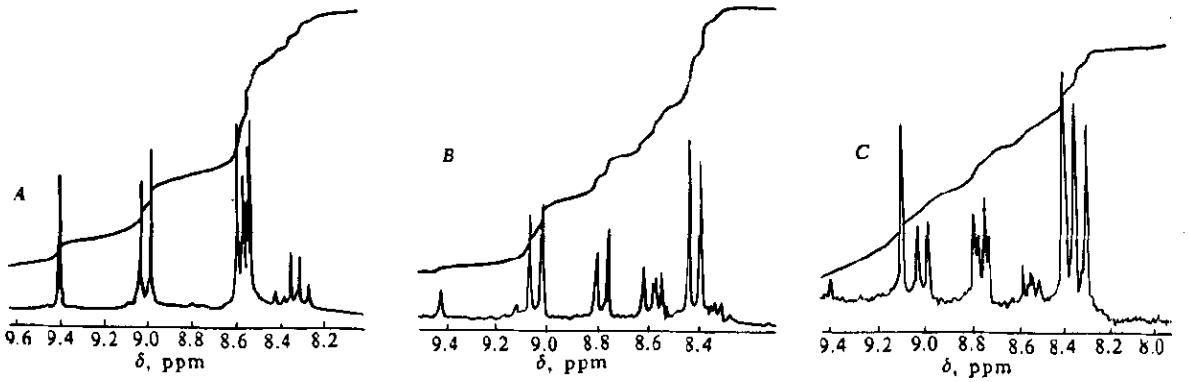


Fig. 6 The ^1H NMR spectra of three DNP isomers

A: 1, 3-DNP

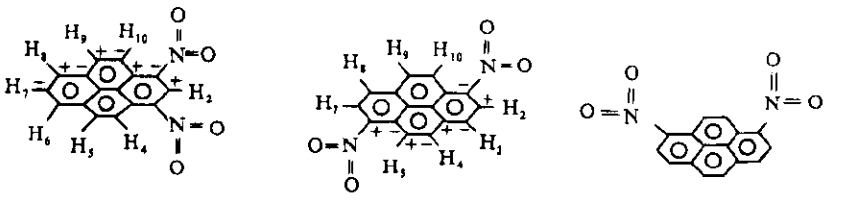
δ	$C_2H > C_4H \sim C_{10}H > C_5H \sim C_9H > C_1H \sim C_7H > C_6H$				
	9.4	9.01	8.58	8.54	8.33
	s/1	d/2	d/2	d/2	t/1

B: 1, 6-DNP

δ	$C_5H \sim C_{10}H > C_2H \sim C_4H > C_8H \sim C_9H \geqslant C_3H \sim C_7H$			
	9.04	8.78	8.42	8.42
	d/2	d/2	d/2	d/2

C: 1.8-DNP δ: $C_9H \sim C_{10}H > C_2H \sim C_7H > C_3H \sim C_6H > C_4H \sim C_5H$

9.10	8.76	8.38	8.30
s/2	d/2	d/2	s/2



1,3-DNP 1, 6 -DNP 1,8-DNP

Fig. 7 The molecular structure of DNP isomers
δ: $C_9H \sim C_{10}H > C_2H \sim C_7H > C_8H \sim C_6H > C_4H \sim C_5H$

It is also coincident with data in Fig. 6*b*, in which $H_3(H_8)$ has same value with $H_4(H_9)$ (Poputa-peck, 1983), while δ9.4, δ8.56, δ8.54, δ8.33 are impurity peaks from 1, 3-DNP.

Similarly, data of Fig. 6*c* is for 1, 8-DNP. From retention time on reversed phase column, isomer peaks 1, 2, and 3 are correspondent to 1, 6-DNP, 1, 8-DNP and 1, 3-DNP, respectively (Fig. 8), while on normal phase column, the eluting sequence is 1, 3-DNP (A), 1, 6-DNP (B) and 1, 8-DNP(C). The different eluting sequence on different columns may be explained from their molecular structures of the three DNP isomers (Fig. 7) and their different affinities to stationary phase. For example, 1, 3-DNP, the aromatic ring of which is most exposed, is easy to be eluted out by nonpolar mobile phase on normal phase column, but is difficult to be eluted out on reversed phase column as it has strong attraction with stationary phase.

From UV spectra (Fig. 9) of the three isomers λ_{max} of 1, 6-DNP has a little bit red-shift because of its nonpolarization, and this also proves the correction of above identification.

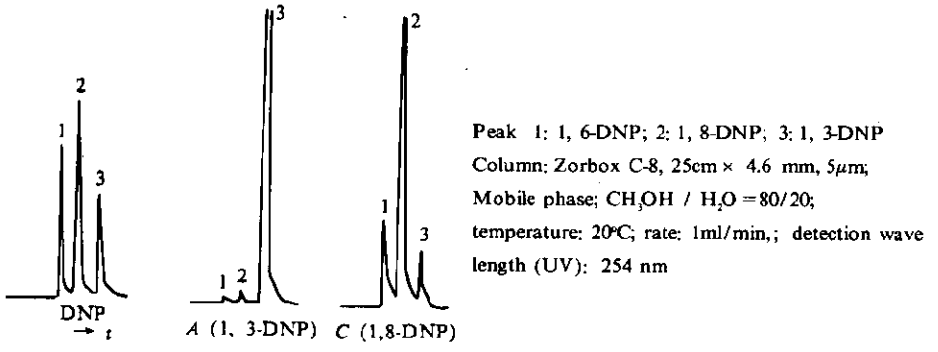


Fig. 8 The identification of DNP isomers on the reversed phase column with retention time

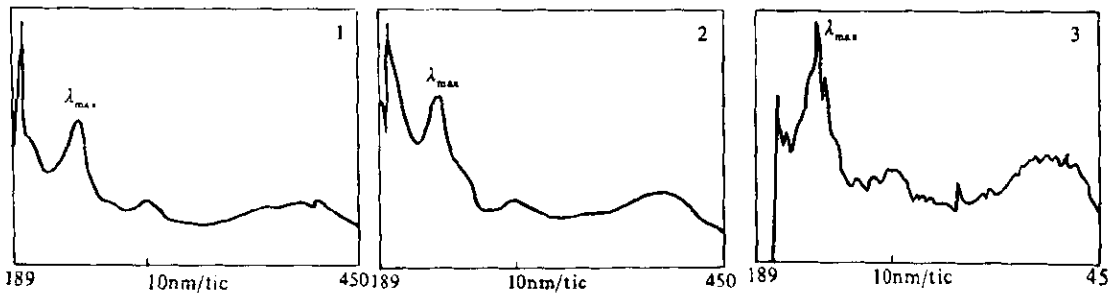
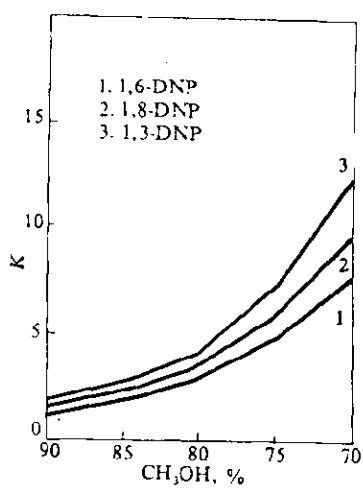


Fig. 9 The UV spectra of the DNP isomers (from H&P HPLC)
1: 1, 6-DNP; 2: 1, 8-DNP; 3: 1, 3-DNP

3. Selection of separation conditions of DNP isomers

For better separation of the three DNP isomers, different columns with different mobile phases were compared. From Fig. 10 and Fig. 11, the DNP isomers can be well separated on C-8 and C-18 columns (reversed columns) with mobile phase CH_3OH/H_2O, in which, the separation effect is related to the polarity of mobile phase. Also the separation effect becomes better with longer carbon chain for C-8 and C-18 columns. In Fig. 10, 11, we know that more nonpolar stationary phase and more polar mobile phase make better separation. In Fig. 11 and 12, the mobile system CH_3OH/H_2O is better than CH_3CN/H_2O in separation because of less polarity of the latter mobile system. Better separation is between isomer 1 (1, 6-DNP) and 2 (1, 8-DNP) in CH_3OH/H_2O mobile system, and between isomers 2 (1, 8-DNP) and 3 (1, 3-DNP) in CH_3CN/H_2O.



Column: MOS C-8, 10 cm × 2.1 mm, 5μm, RP;
CH_3OH/H_2O, 20°C, 1ml/min, UV : 254 nm

Fig. 10 The effect of the concentration of methanol in the mobile phase on the capacity factor *k*

On normal phase column (Fig. 13), DNP isomers can be separated well with C_6H_{14}/CH_2Cl_2, in which better separation is correspondent to nonpolarity of mobile phase.

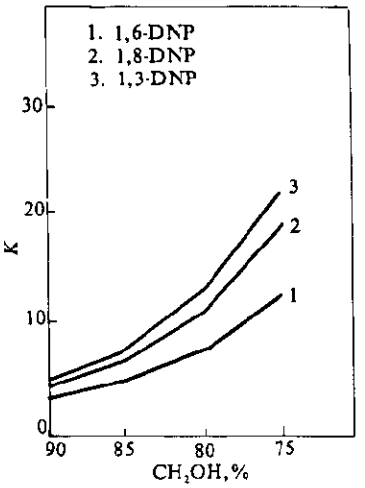


Fig. 11 The effect of the concentration of methanol in the mobile phase on the capacity factor k
Column: DOS C-18, 25 cm × 4.6 mm, 5μm
CH_3OH/H_2O, 20°C; 1ml/min; UV: 254 nm

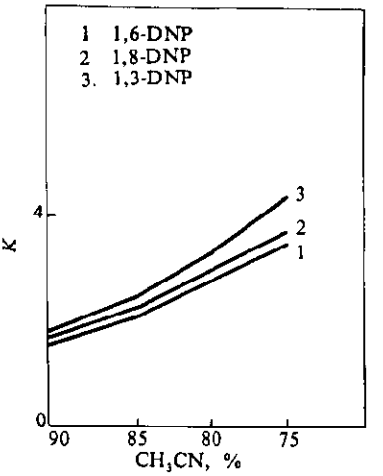


Fig. 12 The effect of the concentration of acetonitrile in the mobile phase on the capacity factor k
Column: ODS C-18, 25cm × 4.6mm, 5μm
CH_3CN/H_2O, 20°C; 1 ml/min; UV: 254nm

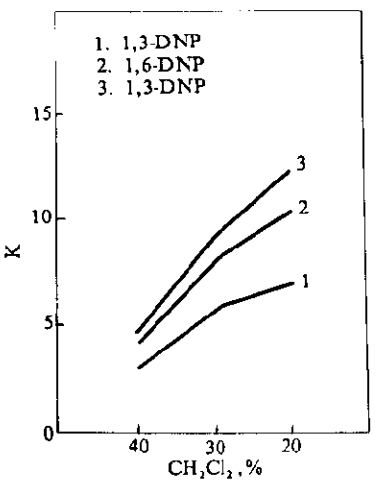


Fig. 13 The effect of the concentration of methylene dichlorides in the mobile phase on the capacity factor k
Column: Zorbax sil 25 cm × 4.6 mm, P.N
CH_2Cl_2/C_6H_{14}, 20°C; 1ml/min; UV: 254nm

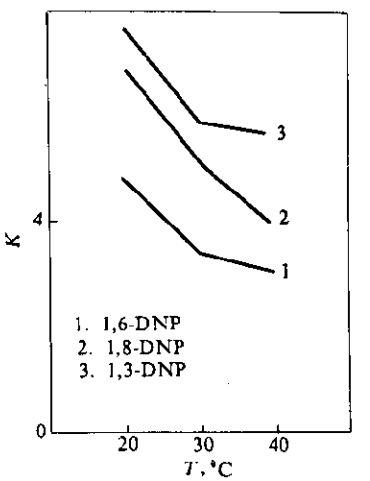


Fig. 14 The effect of the temperature on the capacity factor k
1. 1, 6-DNP; 2. 1, 8-DNP; 3. 1, 3-DNP
Column: ODS C-18 25 cm × 4.6 mm, 5μm
CH_3OH/H_2O = 85/15; 1ml/min; UV: 254nm

However, good separation has not been obtained on Zorbax CN column (containing CN) with mobile phase CH_3OH/H_2O and APS column (containing NH_2) with CH_3OH/H_2O. This may be resulted from insufficient difference in polarity between mobile phase and stationary phase. From Fig. 14, temperature of column has not shown significant effect on separation.

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