

FACILE SYNTHESIS OF 1-NITROPYRENE- d_9 OF HIGH ISOTOPIC PURITY

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SUMMARY

1-Nitropyrene- d_9 , 1,3-dinitropyrene- d_8 , 1,6-dinitropyrene- d_8 , and 1,8-dinitropyrene- d_8 , used as internal standards for the determination of nitro-polycyclic aromatic hydrocarbons (NPAH) in simple and complex mixtures, and required for GC/MS measurements used in the certification of NBS Standard Reference Material (SRM) 1596 (a mixture of nitropyrenes), were synthesized in one step from commercially available pyrene- d_{10} and nitric acid- d_1 . The electron impact mass spectra and isotopic purity of 1-nitropyrene- d_9 and the deuterated dinitropyrenes were determined; the compounds were characterized by gas chromatography-mass spectrometry (GC-MS) and by high performance liquid chromatography (HPLC).

Key Words: 1-nitropyrene- d_9 , 1,3-dinitropyrene- d_8 , 1,6-dinitropyrene- d_8 , 1,8-dinitropyrene- d_8 , deuterium labeling, mass spectra, gas chromatography-mass spectrometry, high performance liquid chromatography.

INTRODUCTION

Nitrated polycyclic aromatic hydrocarbons are a class of potent direct-acting mutagens (1-3) that are produced in trace concentrations from combustion processes (4-6) or by the reaction of smog or nitrogen oxide with

polycyclic aromatic hydrocarbons (PAH) which are adsorbed on particulates (1). Because these nitrated species, particularly 1-nitropyrene, are so important in environmental (3,5,6) and biological (7,8) studies, precise analytical methods are needed which can determine their concentrations accurately at the sub-part-per-million level. Recently, new procedures for the analysis of nitropyrenes using liquid chromatography with electrochemical and fluorescence detection were developed in this laboratory (9).

Mechanistically, the nitration of pyrene can be regarded as aromatic electrophilic substitution involving ipso attack by the nitronium NO_2^+ ion (10); however, formation of the radical cation from an ArH/NO_2^+ reaction via an electron-transfer mechanism with NO^+ as the oxidant (11) or participation of ion radical pairs $[\text{Ar}^{\cdot+}, \text{NO}_2^{\cdot-}]$ as reactive intermediates (12,13) are not excluded. The literature reports various methods for synthesis of 1-nitropyrene; these include the reaction of pyrene with nitric acid in acetic acid (14-16), nitric acid in acetic anhydride (17), nitric acid in p-dioxane (18), sodium nitrite-peroxydisulfate in acetonitrile (15), silver nitrate-iodine in acetonitrile (20) and nitrogen dioxide in dichloromethane (21). An application of the use of nitric acid on silica gel as a nitrating agent for phenols, which was recently reported (22), looks promising for nitration of polycyclic aromatic hydrocarbons (23); additionally, silica gel-supported cerium(IV) ammonium nitrate may be employed for a selective nitration of polynuclear arenes (24).

Pure 1-nitropyrene- d_9 as well as deuterated dinitropyrenes were needed as internal standards for the GC-MS analysis of SRM 1596 (which has been issued as a dichloromethane solution containing a mixture of 1-nitropyrene, and 1,3-, 1,6- and 1,8-dinitropyrenes). We report here the synthesis of 1-nitropyrene- d_9 of high isotopic purity.

RESULTS AND DISCUSSION

The nitration of pyrene with nitric acid under ionic conditions (acetic acid) can lead to a mixture of isomers, including mono-, di-, tri-, and tetra-nitropyrenes (14,15,16,25). Since the reaction is kinetically controlled (12), the amount of each isomer formed is temperature and nitric acid con-

centration dependent. At low to moderate temperatures and limited amount of nitric acid, 1-nitropyrene is the predominant product; with excess nitric acid and higher temperature a mixture of 1-nitro-, 1,3-, 1,6-, 1,8-dinitro-, 1,3,6-trinitro-, and 1,3,6,8-tetranitropyrenes is formed. Separation of the crude nitropyrene reaction mixture is difficult, however (14,16,25).

Our synthesis of 1-nitropyrene-d₉ is a modification of a reported procedure (14) that involves the controlled nitration of pyrene-d₁₀. In developing this procedure, the aims were to (1) offer a rapid and efficient method with little or no deuterium scrambling, and (2) offer a good yield of product of high isotopic purity. Because of the cost of deuterated starting materials, the procedure must be simple. By selection of the reaction conditions (temperature, solvent and nitric acid concentration) it was possible to obtain 1-nitropyrene-d₉ of 99% isotopic purity; on further purification, an analytical sample of 99.44 atom percent deuterium was obtained, in 75% overall yield. This is a considerable improvement of a reported synthesis of 1-nitropyrene-d₉ that involved a multiple-step procedure and gave a product of 95.7% isotopic purity with no overall yield of the product given (26).

The confirmation of the structure and chemical and isotopic purity of the product was obtained by GC-MS and HPLC analyses. The mass spectrum of 1-nitropyrene-d₉ is shown in Figure 1. The molecular ion at m/z 256 is the base

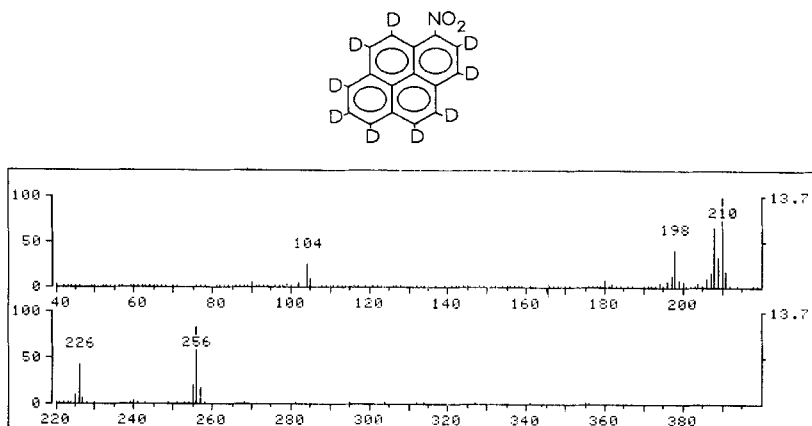


Figure 1. Mass Spectrum of 1-Nitropyrene-d₉

peak in the spectrum, and it confirms the molecular weight. Major fragments in the spectrum are observed at m/z 226 ($M - NO$), m/z 210 ($M - NO_2$), m/z 208 ($M - DNO_2$), and m/z 198 ($M - NO - CO$). The only other significant ion in the spectrum appears at m/z 104 and is presumably the doubly charged ($M - DNO_2$) species. An isotopic purity of 99.44 atom percent deuterium was calculated from the molecular ion peak.

The mass spectra of the deuterium labeled 1,3- 1,6- and 1,8-dinitropyrene isomers are similar. The mass spectrum for the 1,6-dinitropyrene- d_8 is shown in Figure 2. The mass spectrum for the 1,6-dinitropyrene- d_8 is shown in Figure 2. The molecular ion at m/z 300 is the base peak in the spectrum and represents the fully deuterium labeled species $C_{16}D_8O_4N_2$. Other significant ions in the spectrum appear at m/z 270 ($M - NO$), m/z 254 ($M - NO_2$), m/z 240 [$M - 2(NO)$], m/z 224 ($M - NO_2 - NO$), m/z 208 ($M - 2NO_2$), and m/z 196 ($M - NO_2 - NO - CO$). The ion at m/z 299 is due to the incompletely labeled $C_{16}D_7HN_2O_4$ species. The isotopic purity for each of the dinitropyrenes was calculated to be 98.8 atom% D.

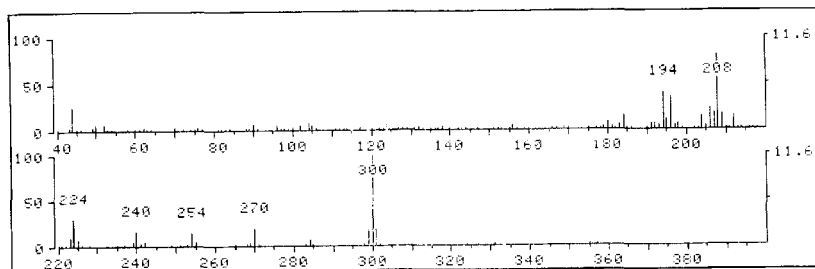
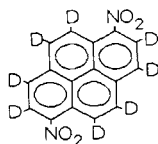


Figure 2. Mass Spectrum of 1,6-dinitropyrene- d_8

EXPERIMENTAL

Warning: 1-nitropyrene (e.g., 1-nitropyrene- d_9) is a potent mutagen (1-3,27,28); it is readily absorbed through the skin, consequently all operations require use of gloves and an efficient laboratory hood.

The deuterium labeled starting materials were obtained from commercial

sources and were used as received. The pyrene-*d*₁₀ was 99 ± 1 atom percent deuterium; the nitric acid-*d*₁, a 68 percent solution was 99.5+ atom percent deuterium; glacial acetic acid-*d*₄ was 99.5+ atom percent deuterium, and deuterium oxide-*d*₂ was 99.8+ atom percent deuterium. Nitric acid in acetic acid or acetic anhydride proved to be a suitable reagent under controlled conditions for the synthesis of 1-nitropyrene. Moreover, acetic anhydride and chlorobenzene were found to be excellent solvents for purification of the nitropyrenes (labeled and unlabeled).

Gas chromatographic-mass spectrometric analyses were performed on a commercial quadrupole GC-MS system. Chromatographic separations were carried out on a 30m x 0.25 mm i.d. fused silica capillary column coated with a 0.25µm film of a bonded, non-polar liquid phase. The gas chromatographic column was interfaced directly to the ion source of the mass spectrometer through a 30 cm length of 1.55 mm stainless steel tubing which served as a conduit for the column. The mass spectrometer was operated in the electron impact mode under the following conditions: ion source temperature = 200 °C, electron energy = 70 eV, emission = 300 µA. Mass spectra were recorded during each chromatographic run by scanning the mass spectrometer from m/z 40 to m/z 400 at a scan speed of 200 u per second.

High performance liquid chromatographic analyses were performed under the following conditions: column, Zorbax ODS 5 µm diameter, 25 cm long; solvent 95% MeOH, flow rate 1 mL/min; detection: UV at 250 nm.

Pyrene-*d*₁₀ (106 mg, 0.5 mmol) was placed in a 25 mL round-bottom flask that had been pre-dried and flushed with nitrogen. A magnetic stirring bar was added to the flask, followed by glacial acetic acid-*d*₄ (10 mL). The flask was placed in a water bath at 55 °C. After the pyrene crystals were dissolved with gentle stirring, the greenish, strongly fluorescent solution was placed in a water bath at 25 °C, and a solution of nitric acid-*d*₁ (3.5 mL, 50 mmol) mixed in acetic acid-*d*₄ (2 mL) was added dropwise from a separatory funnel over three minutes with stirring. The solution was stirred for an additional three minutes, followed by dropwise addition of deuterium oxide-*d*₂ (0.5 mL) which caused the product to crystallize. The yellow crystals were collected by filtration on a sintered glass filter, washed with 1:1 acetic acid-

d_4 :hexane (2 mL) and dried in vacuo; yield 110 mg, 86%, chemical purity 99% (HPLC). The material was recrystallized from acetic anhydride (5 mL) by gentle heating on a hot plate until completely dissolved; the solution was cooled to 5-10 °C, mixed with hexane (5 mL) and kept for an additional 30 min at room temperature. 1-Nitropyrene- d_9 crystallized as long yellow needles; yield: 102 mg, 80%, chemical purity 99.2% (HPLC). This material is suitable for research use without further purification. The analytical sample was prepared by chromatography on silica gel (100 x 200 mesh, 25g) with chlorobenzene:hexane (1:2) as the eluant. Recrystallization from acetic anhydride:hexane (1:1) gave 1-nitropyrene- d_9 ; yield: 85 mg, 75%; the isotopic purity was 99.44 atom %D (GC-MS, HPLC). The physical and spectroscopic data for the compound were as follows: m.p. 153-154 °C (heating rate 2 °C/min), literature value for unlabeled material 153-154 °C (14), 151-152 °C (19,26); UV_{max} (95% C_2H_5OH) 241 nm (ϵ 28000); 288 nm (ϵ 14000); 375 nm (ϵ 11700); 399 nm (ϵ 11850). The required unlabeled 1-nitropyrene was prepared similarly; the material was purified by recrystallization from chlorobenzene--hexane and then from acetic anhydride or acetic anhydride--hexane mixture; yield 85%, purity 99.8% (GC/MS, HPLC); m.p. 152-153 °C; UV_{max} (95% C_2H_5OH) 241 nm (ϵ 28700); 287 nm (ϵ 14500); 375 nm (ϵ 11550); 399 nm (ϵ 11700).

The required mixture of deuterated 1-nitro-, 1,3-dinitro-, 1,6-dinitro-, and 1,8-dinitropyrenes was prepared by nitration of pyrene- d_{10} (100 mg) in acetic acid- d_4 (5 mL) with nitric acid- d_1 (5 mL) at 85° for 15 min with stirring; the cooled deep-yellow mixture was filtered and recrystallized from chlorobenzene; yield 160 mg, m.p. > 305° (d). The GC-MS analysis showed the product to be a mixture of 1-nitropyrene- d_9 (18%), 1,3-dinitropyrene- d_8 (21%), 1,6-dinitropyrene- d_8 (30%), and 1,8-dinitropyrene- d_8 (31%). Similarly the required mixture of undeuterated 1-nitro-, 1,3-dinitro-, 1,6-dinitro-, and 1,8-dinitropyrenes was prepared and analyzed by GC-MS.

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