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Determination of 3,6-dinitrobenzo[*e*]pyrene in surface soil and airborne particles by high-performance liquid chromatography with fluorescence detection

Tomohiro Hasei *, Tetsushi Watanabe, Teruhisa Hirayama

Kyoto Pharmaceutical University, 5 Nakauchi-cho Misasagi Yamashina-ku, 607-8414 Kyoto, Japan

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Abstract

We developed a sensitive analytical method and an efficient clean-up method to quantify 3,6-dinitrobenzo[*e*]pyrene (3,6-DNBeP) in surface soil and airborne particles. After purification using a silica gel column and two reversed-phase columns, 3,6-DNBeP was reduced to 3,6-diaminobenzo[*e*]pyrene by a catalyst column and analyzed by high-performance liquid chromatography (HPLC) with a fluorescence detector. 3,6-DNBeP was detected in all of the soil samples and airborne particles examined. The concentration of 3,6-DNBeP in surface soil and airborne particles was determined in the ranges of 347–5007 pg/g of soil and 137–1238 fg/m^3, respectively.

Keywords: 3,6-Dinitrobenzo[*e*]pyrene; Surface soil; Airborne particles; Fluorescence detection; 3,6-Diaminobenzo[*e*]pyrene; On-line reduction

1. Introduction

Numerous chemicals are emitted into air from anthropogenic sources such as motor vehicles [1,2], industrial power plants [3,4], and municipal incinerators [5]. Many epidemiological studies have shown that outdoor air pollution tends to be associated with the incidence of lung cancer and cardiopulmonary mortality [6–11]. Therefore, measuring airborne contaminants is necessary to estimate potential environmental risks to our health. On the other hand, atmospheric compounds are thought to descend to the ground and accumulate on the surface. Since, surface soil samples are readily available for chemical analysis and biological assays without any special equipment, such as a high-volume air sampler, or power source, surface soil seems to be a promising material for monitoring environmental pollution. In previous studies [12,13], we found that the mutagenicity levels of surface soils in Osaka Prefecture, which has high age-adjusted mortality rates for lung cancer in both males and females in Japan [14], were markedly higher than those in other regions in Japan and other countries. Moreover, the direct-acting mutagenicity of the soil extracts showed a strong empirical relationship with the concentrations of 1,3-, 1,6-, and 1,8-dinitropyrene (DNP) isomers, which are strong mutagens and carcinogens [15,16] and are representative airborne contaminants [17–19], in the extracts.

Recently, we identified a novel chemical, 3,6-dinitrobenzo[*e*]pyrene (3,6-DNBeP, Fig. 1), from large amounts of surface soil samples collected at Takatsuki in Osaka Prefecture and four other cities by the bioassay directed fractionation method [20]. Extracts from these surface soils showed potent mutagenicity in the Ames/*Salmonella* assay. 3,6-DNBeP is highly mutagenic in *Salmonella typhimurium* TA98 in the absence of the mammalian metabolic system (S9 mix), inducing 285,000 revertants/nmol in TA98, and the potency is comparable to those of 1,6- and 1,8-DNP, which are the most potent mutagens reported to date [15]. As it has extremely potent mutagenicity, clarifying the levels of 3,6-DNBeP in the environment is quite important to assess its potential health risk, however, there has been no report on the analytical method of 3,6-DNBeP.

The purpose of this study was to develop an analytical method to quantify trace amounts of 3,6-DNBeP in soil and airborne particles. High-performance liquid chromatography (HPLC) [18,19,21–23,30], gas chromatography [21,24,25], or capillary electrophoresis [26] has been used to analyze the

* Corresponding author. Tel.: +81 75 595 4650; fax: +81 75 595 4769.
E-mail address: hasei@mb.kyoto-phu.ac.jp (T. Hasei).

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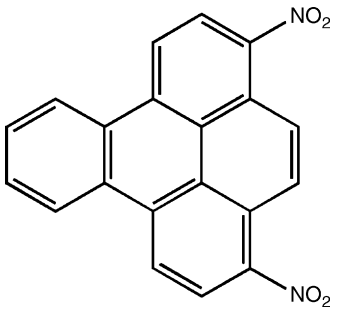

Fig. 1. Structure of 3,6-DNBeP.

complex mixtures such as airborne particles and surface soil. Although HPLC with a chemiluminescence detector [18,19] and liquid chromatography with a mass spectrometer [30] are used because of their selectivity and sensitivity, these instruments have not been spread and readily available in most laboratories. On the other hand, it is well known that fluorescence detectors have high sensitivity. In this study, it was found that 3,6-DNBeP had no fluorescence, but its reduced derivative, i.e. 3,6-diaminobenzo[*e*]pyrene (3,6-DABeP), had strong fluorescence. Trace amounts of 3,6-DNBeP could be quantified by fluorescence detection. After cleaning-up organic extracts from surface soil and airborne particles using silica gel column chromatography and HPLC, 3,6-DNBeP was detected as 3,6-DABeP by reversed-phase HPLC with on-line reduction. This method was applied to several soil samples and airborne particles collected in residential areas in several different regions.

2. Experimental

2.1. Reagents

3,6-DNBeP (CAS 847862-64-0) was synthesized as described previously [20]. HPLC-grade acetonitrile and methanol were purchased from Nacalai Tesque (Kyoto, Japan). Silica gel (63–200 μm particle size) was purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

2.2. Instrumentation

Electron impact mass spectrum (EI-MS) was measured at 70 eV using a Shimadzu QP5050A mass spectrometer (Shimadzu, Kyoto, Japan) with a direct inlet system. The melting point was determined on a Yanagimoto hot-stage apparatus (Yanaco LID, Kyoto, Japan) and is uncorrected. Fluorescence spectra were measured with a Jasco FP-1520S fluorescence detector (Jasco, Tokyo, Japan). Airborne particles were collected with a high volume air sampler (Shibata Science Technology, Tokyo, Japan).

2.3. Synthesis of 3,6-DABeP

3,6-DNBeP (2 mg), dissolved in tetrahydrofuran (1 ml), was reduced by raney nickel (5 mg) and hydrazine (0.1 ml) for 4 h at 60 °C. After removing raney nickel by filtration, the filtrate was poured into distilled water. Crude 3,6-DABeP was filtered off and dissolved in methanol. The methanol solution was applied to an Inertsil ODS-3 column (5 μm, 250 mm × 10 mm I.D., GL Sciences, Tokyo, Japan), eluted with 75% methanol, and the fraction corresponding to 3,6-DABeP was evaporated to dryness. 3,6-DABeP (1.5 mg) was obtained as yellow powder. The melting point of 3,6-DABeP was 222–223 °C. The structure of 3,6-DABeP was confirmed by electron ionization mass spectra. EI-MS *m/z* (%): 226 (7), 254 (13), 282 (100).

2.4. Sampling and extraction

Surface soil was collected at five sampling sites in residential areas in Kyoto and Osaka Prefectures, Japan. The soil was dried at room temperature for two days and screened through a 60 mesh sieve. Fifteen grams of the sieved soil was extracted ultrasonically with 200 ml methanol twice for 10 min each time. The extract was filtered through an Advantec Toyo (Tokyo, Japan) No. 5C filter paper, and the filtrate was evaporated to dryness.

Airborne particles were collected on glass fiber filters for 24 h at a flow rate of 1 m^3/min with a high volume air sampler in residential areas in Kyoto, Osaka, and Tokyo, Japan, and the particles on the filters were extracted with 120 ml methanol with an ultrasonic apparatus for 20 min. The extracts were filtered and evaporated to dryness.

2.5. Clean-up

Organic extracts from surface soil and airborne particles were dissolved in 1 ml chloroform and three aliquots (0.3 ml each) were applied to three silica gel open columns (220 mm × 10 mm I.D.). The silica gel was activated for 18 h at 160 °C and then deactivated with distilled water (7.4%, w/w) prior to use. The material was eluted with 20 ml hexane, 20 ml hexane/toluene (9/1, v/v), 20 ml hexane/toluene (2/1, v/v), 20 ml hexane/toluene (1/1, v/v), and 30 ml toluene. 3,6-DNBeP was eluted with 30 ml toluene. The toluene fractions were evaporated to dryness and the residue was dissolved in 0.5 ml of 50% ethanol. Then, 0.4 ml of this solution was applied to a Cosmosil 5C_{18}-MS-II column (250 mm × 4.6 mm I.D., Nacalai Tesque) for HPLC with 75% acetonitrile at a flow rate of 0.7 ml/min. The HPLC system consisted of a Shimadzu LC-10AS pump, a Rheodyne (Cotati, CA, USA) 7125 sample injector with 1 ml loop, a Shimadzu CTO-10Avp column oven, and a Shimadzu SPD-M10Avp photodiode array detector. Since 3,6-DNBeP was eluted at a retention time of 21.3 min, the eluate from 19.3 to 23.3 min was collected and evaporated to dryness. The residue was dissolved in 0.5 ml of 50% ethanol and 0.4 ml of the solution was applied to a Luna 5μ Phenyl-Hexyl column (250 mm × 4.6 mm I.D., Phenomenex, Torrance, CA, USA), eluting with 90% methanol at a flow rate of 0.7 ml/min. Since 3,6-DNBeP was eluted at 25.3 min, the eluate from 23.3 to 27.3 min was collected and evaporated to dryness. HPLC procedures were carried out at 30 °C, and the eluates were monitored for UV absorption spectra.