

## Isolation of Nitrophenols from Diesel Exhaust Particles (DEP) as Vasodilatation Compounds

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Received December 20, 2002; accepted January 31, 2003; published online February 3, 2003

**The compounds in diesel exhaust particles (DEP) that are responsible for vasodilatation were isolated and characterized for the first time. From benzene extract of DEP, 2-methyl-4-nitrophenol, 3-methyl-4-nitrophenol and 4-nitrophenol were isolated, and their vasodilatation activities were confirmed. 3-methyl-4-nitrophenol caused dilatation of rat thoracic artery, and the other two nitrophenols, also showed vasodilatation activities.**

**Key words** diesel exhaust particle; nitrophenol; vasodilatation

Diesel exhaust particles (DEP) contain a vast number of organic compounds such as polyaromatic hydrocarbons, nitroaromatic hydrocarbons, heterocycles, quinones, aldehydes, and aliphatic hydrocarbons.<sup>1–4</sup> On the health effect of DEP it has been reported that lung cancer,<sup>5,6</sup> allergic rhinitis,<sup>7,8</sup> and bronchial asthma-like disease,<sup>9,10</sup> could be caused by DEP.

With regard to effects of DEP on the cardiovascular system, it has been reported that DEP extract could induce arrhythmia and cardiovascular mortality in guinea pigs.<sup>11</sup> Furthermore, it has been reported that DEP cause low blood pressure and arrhythmia in rats.<sup>12</sup> These findings suggest that DEP contain some chemical substances that affect cardiovascular function. No such chemical compounds, however, have yet been found in DEP.

In order to clarify the mechanism of the effect of DEP on cardiovascular function, we attempted to isolate compounds in DEP that are responsible for vasodilatation in rats by repeating bioassays and fractionations on the basis of the chemical properties of compounds.

Here we report that the compounds in DEP that have vasodilatation activity are nitrophenols.

### MATERIALS AND METHODS

**Reagents** Nitrophenols, 2-methyl-4-nitrophenol, 3-methyl-4-nitrophenol and 4-nitrophenol, used as authentic samples for GC-MS and <sup>1</sup>H-NMR measurement were purchased from Tokyo Kasei Kogyo Co. Ltd., Japan.

**DEP** DEP were collected from the diesel exhaust of a 4JB1-type engine manufactured by Isuzu Automobile Company, Tokyo, Japan, as described previously.<sup>13</sup> The particles were produced from the diesel exhaust of light oil containing

0.05% sulfur. The DEP were kept in a sealed bottle at –20 °C in the dark.

**Isolation and Identification of Nitrophenols** DEP were extracted successively with hexane, benzene, dichloromethane, methanol, 1 M ammonia and 1 M HCl, as described previously.<sup>14</sup> The extract of benzene was fractionated to acidic, phenolic, and neutral portions, following to the method described previously.<sup>15</sup> The compounds in the phenolic fraction were further fractionated by column chromatography on silica gel, and the vasodilatation activity of each fraction was measured. Three nitrophenols were isolated as compounds that have relaxation activities toward the rat thoracic artery. The structures of these compounds were identified by comparison of their GC-MS and <sup>1</sup>H-NMR spectra with those of the authentic samples.

**Measurement of Vascular Relaxation** Seven-month-old SPF F344 rats were used in the experiments. The excised thoracic artery of each rat was cut into 3 mm ring segments, and isometric force was measured in Locke-Ringer's solution (NaCl, 153.8; KCl, 5.63; CaCl<sub>2</sub>, 3.17; glucose, 5.55; NaHCO<sub>3</sub>, 2.38; (mM), pH 7.4) at 37 °C under the condition of aeration with 95% O<sub>2</sub>:5% CO<sub>2</sub>. After contraction with 10<sup>–6</sup> M phenylephrine (PE), the nitrophenols dissolved in PBS containing 0.05% Tween 80 were accumulatively added, and changes in tension were recorded.

### RESULTS AND DISCUSSION

Vasodilatation compounds in the phenolic fraction of the benzene extract of DEP were fractionated by column chromatography on silica gel, and the chemical structures of these compounds were analyzed using GC-MS and <sup>1</sup>H-NMR.

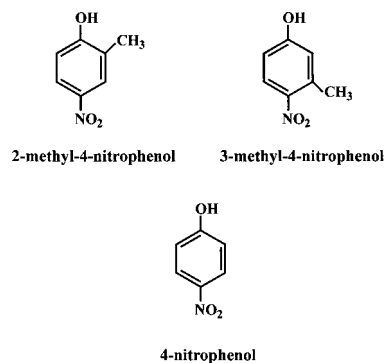


Fig. 1. Chemical Structures of 2-Methyl-4-nitrophenol, 3-Methyl-4-nitrophenol, and 4-Nitrophenol

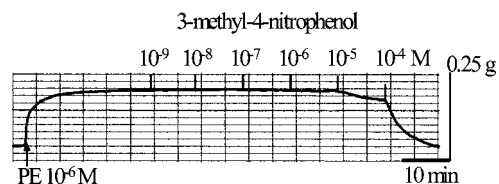


Fig. 2. Relaxation of Thoracic Artery by 3-Methyl-4-nitrophenol

The excised thoracic artery from each rat was cut into 3 mm ring segments, and isometric force was measured as described in Materials and Methods. After contraction with 10<sup>–6</sup> M phenylephrine (PE), 3-methyl-4-nitrophenol dissolved in PBS containing 0.05% Tween 80 was added accumulatively from 10<sup>–9</sup> M and changes in tension were recorded.

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Thus, three nitrophenol derivatives, 2-methyl-4-nitrophenol, 3-methyl-4-nitrophenol and 4-nitrophenol, were identified as vasodilatation compounds (Fig. 1). The contents of 2-methyl-4-nitrophenol, 3-methyl-4-nitrophenol, and 4-nitrophenol were estimated by GC-MS as 34, 28 and 15 mg/kg DEP, respectively.

Figure 2 shows the vasodilatation activity of 3-methyl-4-nitrophenol toward an isolated rat thoracic artery. Vasodilatation of the thoracic artery was observed from  $10^{-5}$  M of 3-methyl-4-nitrophenol, after vasoconstriction with  $10^{-6}$  M PE. No relaxation was observed with the vehicle containing 0.05% Tween 80. The magnitude of relaxation of the artery caused by 3-methyl-4-nitrophenol was not dependent on cytotoxic effect, because after vasodilatation with this compound, the artery was constricted again by the addition of PE or KCl. The other nitrophenols, 2-methyl-4-nitrophenol and 4-nitrophenol, also showed vasodilatation activity.

This is the first report on the isolation of compounds responsible for vasodilatation, *i.e.*, three nitrophenols (2-methyl-4-nitrophenol, 3-methyl-4-nitrophenol and 4-nitrophenol) from DEP.

Dockery *et al.* epidemiologically found that cardiovascular mortality and morbidity are associated with exposure concentration of particulate matter in air.<sup>16,17</sup> Most of the particulate matter in air pollutants in Japan and developing countries is thought to consist of DEP. It is thought that these nitrophenol compounds contained in DEP could cause adverse human health by affecting cardiovascular functions.

Nishioka *et al.* reported that such nitrophenols are present in the air particulate as mutagenic compounds.<sup>18</sup> It is also known that 3-methyl-4-nitrophenol is a degradation product of the insecticide fenitrothion,<sup>19</sup> which is used widely in many countries and is being accumulated in air.<sup>18,20</sup> Furthermore, it is known that 4-nitrophenol is a degradation product of the insecticide parathion.

The results of the present study indicate that accumulation of nitrophenols, including 3-methyl-4-nitrophenol, in air and on the earth from diesel exhaust and from degradation of fenitrothion used on farms could have serious effect on

human health due to disturbance of the cardiovascular system.

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