

Isolation and Characterization of 1-Nitronaphthalene from Coarse Tea Leaves (Bancha, in Japanese) and 3-Nitrostyrene from Roasted Coffee Beans

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Abstract

Extraction of 1-nitronaphthalene from coarse tea leaves (bancha, in Japanese) and of 3-nitrostyrene from roasted coffee beans (coffee) and characteristics of these purified compounds were described.

Commercially obtained bancha and coffee were pulverized and extracted with chloroform/methanol (1:1, v/v). The two extracts were fractionated by silicic acid column chromatography using first, cyclohexane/benzene (1:1, v/v) and next, chloroform as elution solvents. Each fraction was purified by thin-layer chromatography developing with cyclohexane/chloroform (1:1, v/v). The purified eluate was analyzed by colour reaction, high pressure liquid chromatography and gas chromatography-mass spectrometry. The content of 1-nitronaphthalene in the bancha and 3-nitrostyrene in the coffee was 32.0ppb and 2.0ppb, respectively. However, because the latter compound has a low melting point (-5°C), it is likely that a significant quantity of the compound was lost during purification steps. The toxicities of these materials were not tested in the paper.

Introduction

Many nitrated polycyclic aromatic hydrocarbons (nitro-PAH) are distributed in the environment, e. g. extract of diesel engine emission, cigarette smoke condensate, wood burning smoke, roasted foods etc. The nitrogen atom of nitro-PAH originates from the atmospheric nitrogen producing NOx

during fuel combustion. Of these nitro-PAH, a considerable number have direct mutagenicity and carcinogenicity for animals¹⁾.

In spite of numerous studies, low molecular weight nitrated aromatic hydrocarbons, such as nitronaphthalenes and nitrostyrenes except β -nitrostyrene reported already²⁾ have not been described in foods up to the present.

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One of the nitronaphthalenes, 1-nitronaphthalene (1-NN) has mutagenicity toward *Salmonella typhimurium* TA 100 and 98³¹, although, the toxicity and distribution of 3-nitrostyrene (3-NS) have not been reported in the environment. In this connection, the authors observed the above two compounds in coarse tea leaves (bancha, in Japanese) and roasted coffee beans (coffee).

The results are presented in this paper.

Materials and Methods

All organic solvents were used after distillation. Sodium borohydride was obtained from MERCK; 9-nitroanthracene, 1-NN, 3-NS, β -nitrostyrene, 1-nitrostyrene and 2-nitrofluorene from Aldrich Chem. Co.; pentacyanoammine ferroate from Sigma Chem. Co.; silicic acid (100mesh, AR) from Mallinckrodt Co. Bancha (heated at 98°C for 1 hr) and coffee (roasted at 170–200°C for 17 min) were obtained from commercial sources.

Each sample (200 g) was pulverized and extracted separately with chloroform/methanol (2:1, v/v, 600ml) three times in a sonicator (45 kHz, for 10 min). The extracts were combined and evaporated to dryness under reduced pressure. The residue was suspended in cyclohexane/benzene (1:1, v/v) and applied to silicic acid column chromatography (50 times of the residue used). Elution solvents were first, cyclohexane/benzene (1:1, v/v, 10 times of silicic acid volume) to obtain nonpolar lipid and PAH fraction, and next, chloroform (10 times) for mononitro-PAH fraction. Each eluate was purified by thin-layer chromatography (TLC) using silica gel plate (MERCK, precoated silica gel 60). The developing solvent system was cyclohexane/chloroform (1:1, v/v).

Nitro-PAH on the plate were detected qualitatively by spraying first with reducing

reagent (0.3 g of sodium borohydride dissolved in 18ml of 83% methanol containing 0.5mg of copper (II) chloride) and then with 1% pentacyanoammine ferroate. The spots which corresponded to 1-NN and/or 3-NS standards were scrapped off from the plates, eluted with chloroform/methanol (2:1, v/v) using a sonicator and treated again with the same TLC to obtain purified compounds (PE-1 for bancha, PE-2 for coffee).

High pressure liquid chromatography (HPLC) was carried out by Hitachi Model 638 equipped with ERC-ODS-1171 column (6.0×200mm). The elution system was acetonitrile/water (7:3, v/v) without solvent programming. The wavelength monitored was 254 nm.

Gas chromatography-mass spectrometry (GC-MS) was performed by Shimadzu Model QP-1000 using capillary silica column (0.33mm×25m) coated with CBP-1. Operating conditions were as follows; column temperature for 1-NN, 180–270°C at 4°C/min, and for 3-NS, 120°C without temperature programming. Ion source and molecular separator temperature was 250°C. Flow rate of carrier gas was 1.5 kg/cm². All measurements were operated at 70 eV.

Recovery tests of added 1-NN (15.8, 23.7 and 31.6 μ g/100 g) to bancha and 3-NS (100 μ g/100 g) to coffee (each case was three runs) were done, the former by GC-MS and the latter by HPLC.

Results and Discussion

The average recoveries of added varying quantities of 1-NN to bancha are shown in Table 1. Quite sufficient values were obtained (102–108%, average 105.9%), however the values were more than 100% in all case. In the case of 3-NS to coffee, on the other hand, scattering and low recovery values of 3-NS were detected (69.8, 66.5 and 46.4%,

Table 1. Recoveries of added 1-Nitronaphthalene to Coarse Tea Leaves.

Added 1-nitronaphthalene	Average recovery (three runs)
15.8 $\mu\text{g}/100\text{ g tea}$	109.5 %
23.7	107.0
31.6	102.3

Methods and conditions: see text.

Recoveries of added 3-nitrostyrene to coffee: see text.

average 60.9%).

The C/M (2 : 1, v/v) extracts from bancha and coffee yielded 6.6% and 10.9%, respectively. The cyclohexane/benzene (1 : 1, v/v) eluates by silicic acid column chromatography of the C/M extracts were 11.1% from the former and 48.5% from the latter sample, while following the chloroform eluates, recovered reduced values than of the above results, 4.1% and 2.3% of the C/M extracts.

With qualitative colour reaction on the

Table 2. R_f Values and Colours of Thin-layer Chromatography of PE-1, PE-2, Nitrostyrenes and Some Nitro-PAH.

No.	Compound	R_f value	Colour
1	PE-1*	0.51	Violet
2	PE-2**	0.53	Violet
3	9-Nitroanthracene	0.54	Green
4	3-Nitrostyrene	0.52	Violet
5	1-Nitronaphthalene	0.51	Light green
6	1-Nitrostyrene	0.44	Violet
7	2-Nitrofluorene	0.43	Violet
8	β -Nitrostyrene	0.43	Purple

Conditions: developing solvent, cyclohexane/chloroform (1 : 1, v/v); coloured reagent, see text; also spots were detected with iodine vapour; *, purified extract from coarse tea leaves; **, purified extract from roasted coffee beans.

TLC plate, 2 spots, one corresponding to references 1-NN from bancha and the other 3-NS from coffee, were detected.

The R_f values and colour of the PEs and related standard materials are shown in Table 2. The characteristics (retention times of HPLC and maximum absorption wavelength) of PE-1 and PE-2 were almost the same as references 1-NN and 3-NS (Table 3).

Table 3. Retention Times on HPLC and Maximum Absorption Wavelength of PE-1, PE-2, Nitrostyrenes and Some Nitro-PAH.

No.	Compound	Retention time (min)	Maximum absorption wavelength (nm)
1	PE-1*	4.9	342.6
2	PE-2**	4.7	242.6
3	9-Nitroanthracene	6.1	252.4
4	3-Nitrostyrene	4.7	242.6
5	1-Nitronaphthalene	4.8	342.2
6	1-Nitrostyrene	7.2	290.0
7	2-Nitrofluorene	6.1	334.4
8	β -Nitrostyrene	5.1	315.2

Conditions: see text: * and **, see Table 2.

Mass fragments were identical with those of 1-NN for PE-1 and 3-NS for PE-2, i. e. 173 (M^-), 143 ($M-NO$), 127 ($M-NO_2$) of 1-NN and 149 (M^+), 103 ($M-NO_2$), 91 ($M-NO-CO$) and 77 (C_8H_8-1) of 3-NS (Table 4).

The concentration of 1-NN in the bancha was 32.0ppb, while 3-NS in the coffee was about 2.0ppb. The latter value, however may be incorrect, because it is possible that since 3-NS has a low melting point (-5°C), then considerable amounts of this material might be lost during extraction, purification and particularly during the evaporation process.

Table 4. Mass Spectra of PE-1, PE-2 and Reference Compounds

Compound	Retention times of ion monitor (min)	Mass fragment M (% of base peak)
PE-1	1.2	173 (71.7), 153 (19.0) 127 (100.0), 115 (52.7)
1-Nitronaphthalene	1.2	173 (57.5), 153 (11.9) 127 (100.0), 115 (85.6)
PE-2	1.9	149 (62.0), 103 (67.0) 91 (23.0), 77 (100.0)
3-Nitrostyrene	1.9	149 (62.0), 103 (79.0) 91 (26.0), 77 (100.0)

Condisions: see text and abbreviation, see Table 2.

The toxicity of 1-NN has mutagenicity toward *Salmonella typhimurium* TA 100 and 98³⁾, but for 3-NS it is still unidentified. However, because of styrene a nervous toxicity is shown (LC₅₀ 316mg/mouse), so an increase of toxicities to animals by nitration of styrene may be probable. Roasted beverages such as teas and coffee beans always contain PAH⁴⁾. In relation to this, some nitro-PAH and/or low molecular weight nitrated aromatic compounds⁵⁾ exist in these beverages.

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焙焼した番茶およびコーヒー豆からの
1-ニトロナフタレンおよび3-
ニトロスチレンの分離とその特性

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摘 要

番茶の葉（番茶）およびコーヒー豆からそれぞれ1-ニトロナフタレンおよび3-ニトロスチレンを抽出し、それらの化合物の特性について記載した。

市販されている番茶およびコーヒー豆を粉碎し、クロロホルム/メタノール（1：1，v/v）で抽出した。それぞれの抽出物はケイ酸カラムクロマトグラフィーにより、シクロヘキサン/ベンゼン（1：1，v/v）、ついでクロロホルムを用いて、順次溶出、分画した。各画分をケイ酸薄層クロマトグラフィーに供し（展開溶媒、シクロヘキサン/クロロホルム，1：1，v/v）、低分子量ニトロPAHを単離・精製後、呈色反応、高速液体クロマトグラフィーおよびガスクロマトグラフィー質量分析（GC-MS）により同定、定量した。

番茶およびコーヒー豆から得られた脂溶性物質の収量は、それぞれ6.6%および10.9%であった。脂溶性物質には多環芳香族炭化水素としてアントラセン、フェナントレン、フルオランテンおよびビレンなどが検出された他に、GC-MSの結果から、番茶の葉より1-ニトロナフタレン（32.0ppb）が、またコーヒー豆から3-ニトロスチレン（約2.0ppb）の存在が確認された。しかしながら3-ニトロスチレンは融解点（-5℃）が低いため、分離・精製段階でいくらかの損失を招いた可能性が考えられた。毒性試験については行わなかった。