

Communications to the Editor

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ISOLATION AND STRUCTURAL DETERMINATION OF MUTAGENIC SUBSTANCES IN COAL TAR

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Eleven compounds that were mutagenic to *Salmonella typhimurium* TA98 were isolated from a basic fraction of coal tar. These were six amino-polycyclic aromatic hydrocarbons, 1-aminofluoranthene, 1- and 3-aminophenanthrene, 1- and 2-aminopyrene and 5-aminobenz[*a*]anthracene, and five aza-polycyclic aromatic hydrocarbons, phenaleno[1,9-*gh*]quinoline, pyrido[3,2-*c*]carbazole, quino[3,2-*c*]carbazole, benzo[*h*]naphtho[2,1,8-*def*]quinoline and benzo[*de*]naphtho[1,8-*gh*]quinoline.

KEYWORDS—coal tar; mutagenic substance; isolation; *Salmonella typhimurium* TA98; amino-polycyclic aromatic hydrocarbon; aza-polycyclic aromatic hydrocarbon

Cancer was first experimentally produced in 1915 by Yamagiwa and Ichikawa using coal tar.¹⁾ In 1932, Cook *et al.* found benzo[*a*]pyrene as a carcinogen in coal tar.²⁾ It was isolated from the acid/neutral fraction of a distillate of the pitch of coal tar. Later, coal tar was also shown to contain other potent carcinogenic hydrocarbons.³⁾

Since coal was produced under high pressure and high temperature from plants which contain proteins and amino acids, we thought that coal tar might contain basic mutagens produced from those proteins and amino acids. As we expected, the basic fraction of coal tar was highly mutagenic, so we tried to isolate mutagens from this basic fraction.

Mutagenicity was tested by a modification of Ames' method.⁴⁾ Namely, the test material dissolved in dimethylsulfoxide was preincubated at 37°C for 20 min with bacteria (*Salmonella typhimurium* TA98) and S9 mix.

The material was isolated as shown in Chart 1. Coal tar (12kg) was distilled under reduced pressure (2 mmHg) at 315°C, and the residue (6.8kg) was dissolved in 34 liters of chloroform and extracted with 68 liters of 20% sulfuric acid. The basic fraction (82.3g), which had high mutagenic activity, was then subjected to

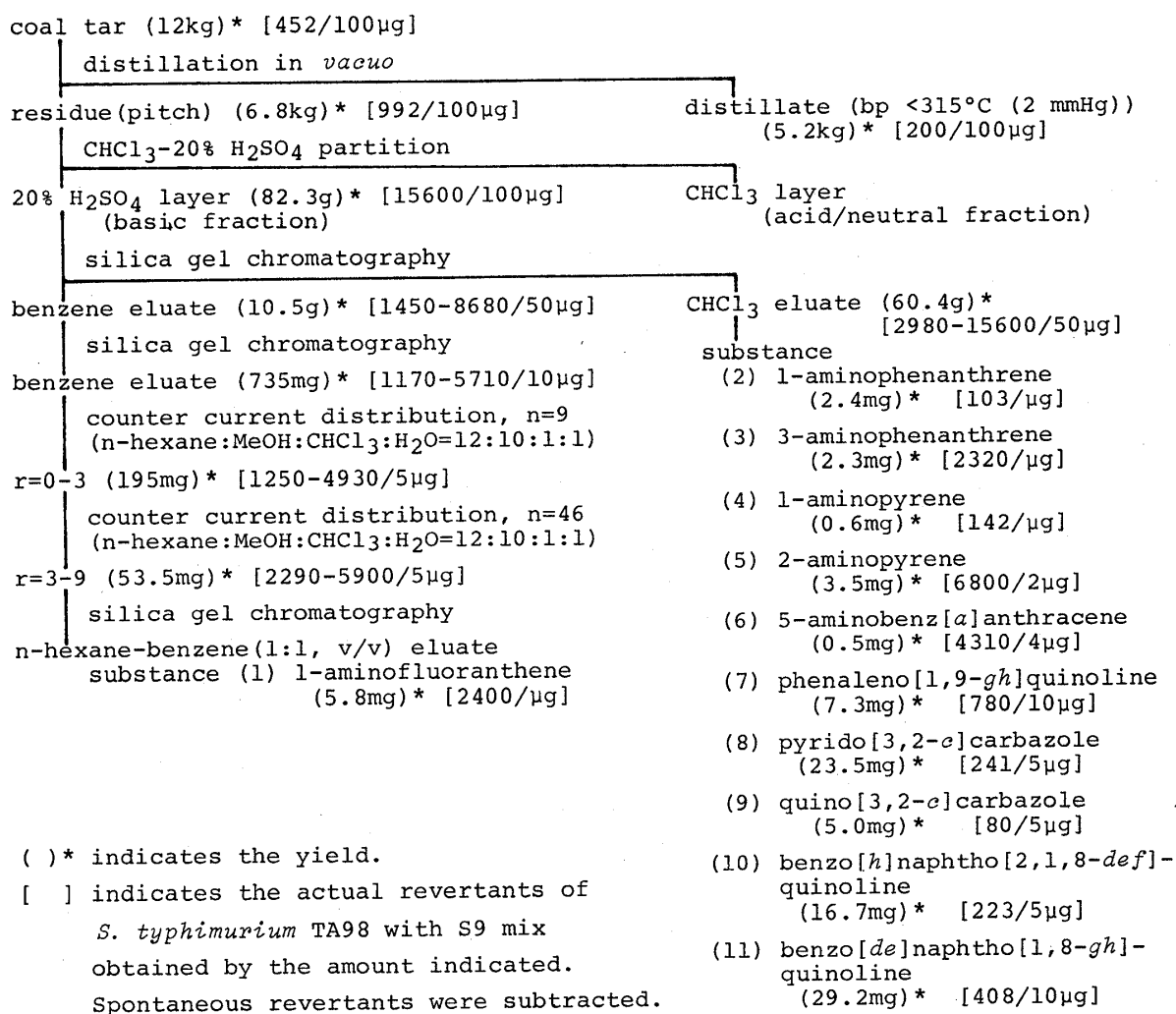


Chart 1. Isolation of Mutagens from the Basic Fraction of Coal Tar

silica gel column chromatography.

The eluate with benzene was further separated by silica gel column chromatography and counter current distribution. In this way, a mutagenic substance (1) that induced 2400 revertants/µg was obtained and identified as 1-aminofluoranthene by X-ray crystallographic analysis.

The eluate from the silica gel column with chloroform was separated by alternate and repeated Sephadex LH-20 gel filtration, silica gel and alumina column chromatographies and counter current distribution. In this way, ten mutagenic substances (2)-(11) were obtained and (2), (3), (4), (5), (6) and (7) were identified as 1- and 3-aminophenanthrene, 1- and 2-aminopyrene, 5-aminobenz[*a*]anthracene and phenaleno[1,9-*gh*]quinoline, respectively, by mass, UV and NMR spectroscopy by comparison with authentic samples. Substances (8), (9), (10) and (11) were identified by X-ray crystallographic analysis as pyrido[3,2-*c*]carbazole, quino[3,2-*c*]carbazole, benzo[*h*]naphtho[2,1,8-*def*]quinoline and benzo[*de*]naphtho[1,8-*gh*]quinoline, respectively. The yield of these substances and their mutagenic activities are shown in Chart 1, and the structure of these compounds are given in Chart 2. Details of

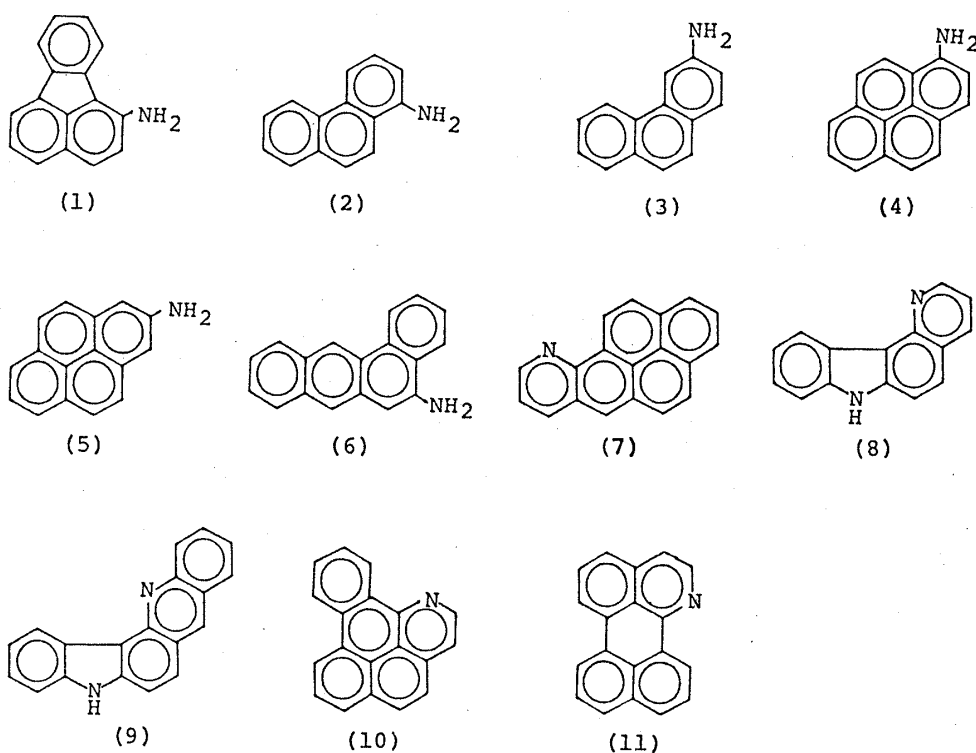


Chart 2. Chemical Structures of Mutagens isolated from the Basic Fraction of Coal Tar

the procedures used for isolation of substances (2)-(11) and X-ray crystallographic analyses of substances (1), (8), (9), (10) and (11) will be described elsewhere.

The mutagenicity of the basic fraction accounted for 24% of the total mutagenicity of coal tar. Eleven mutagenic compounds, amino- and aza-polycyclic aromatic hydrocarbons, were isolated from the basic fraction of coal tar, but they were all different from the mutagenic compounds previously isolated from the basic fraction of the pyrolysates of various amino acids and protein.⁵⁾ Among the compounds isolated from coal tar in this experiment, pyrido[3,2-*c*]carbazole, quino[3,2-*c*]carbazole, benzo[*h*]naphtho[2,1,8-*def*]quinoline and benzo[*de*]naphtho[1,8-*gh*]quinoline are newly identified compounds. 1- and 3-Aminophenanthrene⁶⁾ and phenaleno[1,9-*gh*]quinoline⁷⁾ were previously reported to be carcinogenic, but the carcinogenicities of the other eight compounds have not yet been tested. There is also no information available yet on whether the basic fraction of coal tar is carcinogenic. The role of other basic compounds besides polycyclic hydrocarbons in the carcinogenicity of coal tar should be considered.

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