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Formation of Mutagen by Photolysis of Aromatic Compounds in Water Containing Nitrite Ion

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Various mutagenic compounds have been known to be present in drinking water (SCHWARTZ et al. 1979), river water (MOORE et al. 1980) and river sediment (SUZUKI et al. 1982a). A part of these mutagens in aquatic environments seems to be formed by biological and chemical conversions of non-mutagenic pollutants. For example the chlorination of water has been found to be responsible for the formation of mutagens (MARUOKA et al. 1980).

In our earlier works (SUZUKI et al. 1982b, c), aromatic compounds were found to give mutagenic compounds by the UV irradiation in aqueous nitrate solution. Aquatic environments have been known to be polluted with various aromatic hydrocarbons, and surface water and sewage plant effluent contain nitrate nitrogen (BRINKHOFF 1978). Nevertheless, mutagen seemed to be not formed appreciably with sunlight in natural environment, because the photochemical formation of mutagen in aqueous nitrate solution was hard to occur by the irradiation of long wavelength light (λ>300 nm), though easy by short wavelength light (SUZUKI et al. 1982c).

Further investigations, however, revealed that aromatic compounds readily give mutagenic compounds even by the long wavelengths light near sunlight if the aqueous solution contains nitrite ion. The present paper deals with the formation of mutagen from polycyclic aromatic hydrocarbons and mono-substituted benzenes by UV irradiation in water containing nitrite ion.

MATERIALS AND METHODS

Materials. All chemicals were reagent grade from Kanto Chemicals Co. and used without further purification. Silica gel used as a carrier for dispersion of water-insoluble compounds was purchased from Wako Pure Chemical Ind. Ltd. (Wakogel Q22, 200 mesh), washed with distilled water and dried at 100°C prior to use.

Apparatus. Ultraviolet light was derived from a 100 W high-pressure mercury lamp, UVL-100 HA (Riko Kagaku Sangyo Inc.) with maximum energy distribution at 365 nm. The lamp was equipped with a Pyrex glass (7740) filter so as to cut off shorter wavelengths than 300 nm. The reaction vessel used was a glass cylinder (7 cm id x 21 cm) and the UV lamp was set on the inside.

Photochemical Reaction. Water-insoluble naphthalene, biphenyl, anthracene, fluorene and pyrene were coated on a carrier (silica gel) by evaporating the ether solution of each compound (50 mg) to dryness under reduced pressure together with a carrier (1 g). The amount of the compound coated on a carrier was determined colorimetrically by extraction with ether from a portion of the carrier. The compound (20-25 mg) coated on a carrier was suspended in 500 mL of a neutral aqueous solution of sodium nitrite (16.2 ppm as NO_2-N) in a reaction vessel. Other compounds were dissolved or suspended together with 500 mg of carrier in the aqueous nitrite solution. UV irradiation was performed for 3 h at 25°C with stirring in a thermostated reaction vessel.

The reaction mixture containing the carrier was extracted with ether (100 mL x 2) under acidic conditions (at pH 5). The ether layer was evaporated to dryness under reduced pressure at 40°C. The dried samples were weighed accurately, dissolved in dimethyl sulfoxide (DMSO) in the prescribed concentration, and a 0.1 mL each of these solution was subjected to the mutation assay.

Mutation Assay. Bacterial strain used was Salmonella typhimurium TA98. The mutation test was performed by the same method as described in the previous paper (SUZUKI et al. 1982c).

RESULTS AND DISCUSSION

Benzene, polycyclic aromatic hydrocarbons, and monosubstituted benzenes were irradiated for 3 h with a 100 W high-pressure mercury lamp in aqueous nitrite solution (NO_2-N, 16.2 ppm). The yields of ether extract obtained from the reaction mixtures and their mutagenicities to S. typhimurium TA98 were shown in Table 1. The ether extracts of reaction mixture of all compounds were mutagenic except for aniline and benzenesulfonate with the S-9 mix. No mutagenicity was observed for the products obtained by photolysis in the nitrite- and nitrate-free aqueous solution as described in the previous paper (SUZUKI et al. 1982c). It is apparent that not only nitrate but also nitrite play an indispensable role in the formation of mutagen by photo-reaction of aromatic compounds in aqueous solution.

Table 2 shows the mutagenicities of the ether extracts of the reaction mixture when the light source was derived from the 100 W high-pressure mercury lamp equiped with a Pyrex glass filter. This light is cut off shorter wavelengths than 300 nm, and hence its wavelength characteristics is analogous to that of sunlight. As can be seen in Table 2, most of the ether extracts were mutagenic, except for biphenyl, toluene and benzenesulfonate. The dose response curves of mutagenicity for ether extracts of the representative compounds are shown in Fig. 1.

Table 1. Mutagenicity of the photolytic products from aromatic compounds by UV irradiation (3 h) in aqueous nitrite solution (NO_2-N, 16.2 ppm) with a 100 W high-pressure mercury lamp.

Compounds	Original amount (mg)	Yield of ether extract (mg)	Mutagenicity (TA98) Revertants/100µg/plate	
			S-9 mix(-)	S-9 mix(+)
Benzene	86	4.5	142	111
Naphthalene	23	4.9	749	371
Biphenyl	16	10.1	141	125
Anthracene	22	27.9	382	225
Pyrene	23	20.8	2162	1100
Phenol	25	5.4	68	130
Chlorobenzene	49	8.1	267	205
Aniline	36	6.3	54	129
Toluene	39	2.9	91	131
Sodium benzoate	25	7.3	207	147
Sodium benzene sulfonate	25	12.9	26	223
Spontaneous revertants			26 ± 5	55 ± 18

The ether extracts of the reaction mixtures of naphthalene, β-naphthol, fluorene and pyrene showed very strong mutagenicity in the absence of S-9 mix. The large difference in mutagenicity between α-naphthol and β-naphthol seems to be due to the difference in reactivities of these isomers or due to that in mutagenicities of isomeric products. The mutagenicities of the ether extracts of mono-substituted benzenes reaction mixture were liable to be lower than those of polycyclic compounds. However, biphenyl and anthracene were exceptional compounds in the weak mutagenicities of their ether extracts. The extract of biphenyl reaction mixture was not mutagenic as shown in Table 2, but longer irradiation (6-9 h) caused the extract to become mutagenic. One of the causes of the unusually weak mutagenicity in these compounds seems to be poor reactivity under irradiation of long wavelength light.

In aqueous nitrate solution, the aromatic compounds that produced mutagen by irradiation of long wavelengths light were a minority and their mutagenicities were very weak as described in the previous paper (SUZUKI et al. 1982c). However, the results in Table 2 indicated that

Table 2. Mutagenicity of the photolytic products from aromatic compounds by UV irradiation (3 h) in aqueous nitrite solution (NO_2-N, 16.2 ppm) with a Pyrex-filtered mercury lamp.

Compounds	Original amount (mg)	Yield of ether extract (mg)	Mutagenicity (TA98) Revertants/100µg/plate	
			S-9 mix(-)	S-9 mix(+)
Benzene	88	4.4	107	110
Naphthalene	25	15.9	615	133
α-Naphthol	25	8.8	99	59
β-Naphthol	25	8.5	1221	644
Biphenyl	17	20.0	23	82
Anthracene	25	27.6	99	81
Fluorene	25	22.0	345	100
Pyrene	25	24.8	2796	383
Phenol	25	4.2	81	58
Chlorobenzene	54	3.6	130	118
Aniline	62	13.6	47	139
Toluene	37	4.3	23	82
Sodium benzoate	25	24.5	78	40
Sodium benzene sulfonate	25	6.9	27	77
Spontaneous revertants			26 ± 5	55 ± 18

mutagens were very easily formed even by irradiation of long wavelengths light in aqueous nitrite solution in contrast the nitrate solution.

Some workers have reported that nitrate and nitrite ion are activated photochemically in water to give free radicals (NO, NO_2, OH) (TREININ et al. 1970; DANIELS et al. 1968; ZAFIRIOU et al. 1979). These active fragments in the photolysis of nitrate and nitrite are presumed to react with aromatics in the present photoreaction systems to give a mutagen. This presumption is supported by the fact that the main product and one of mutagens in the photo-reaction of biphenyl in nitrate solution were hydroxy-nitro-biphenyls (SUZUKI et al. 1982b).

On the other hand, it has been also pointed out that nitrate undergoes photolysis with shorter wavelengths light than 340 nm, while nitrite with longer wavelengths light than 300 nm (ZAFIRIOU et al. 1979). The above mentioned ready conversion of aromatics to mutagens in

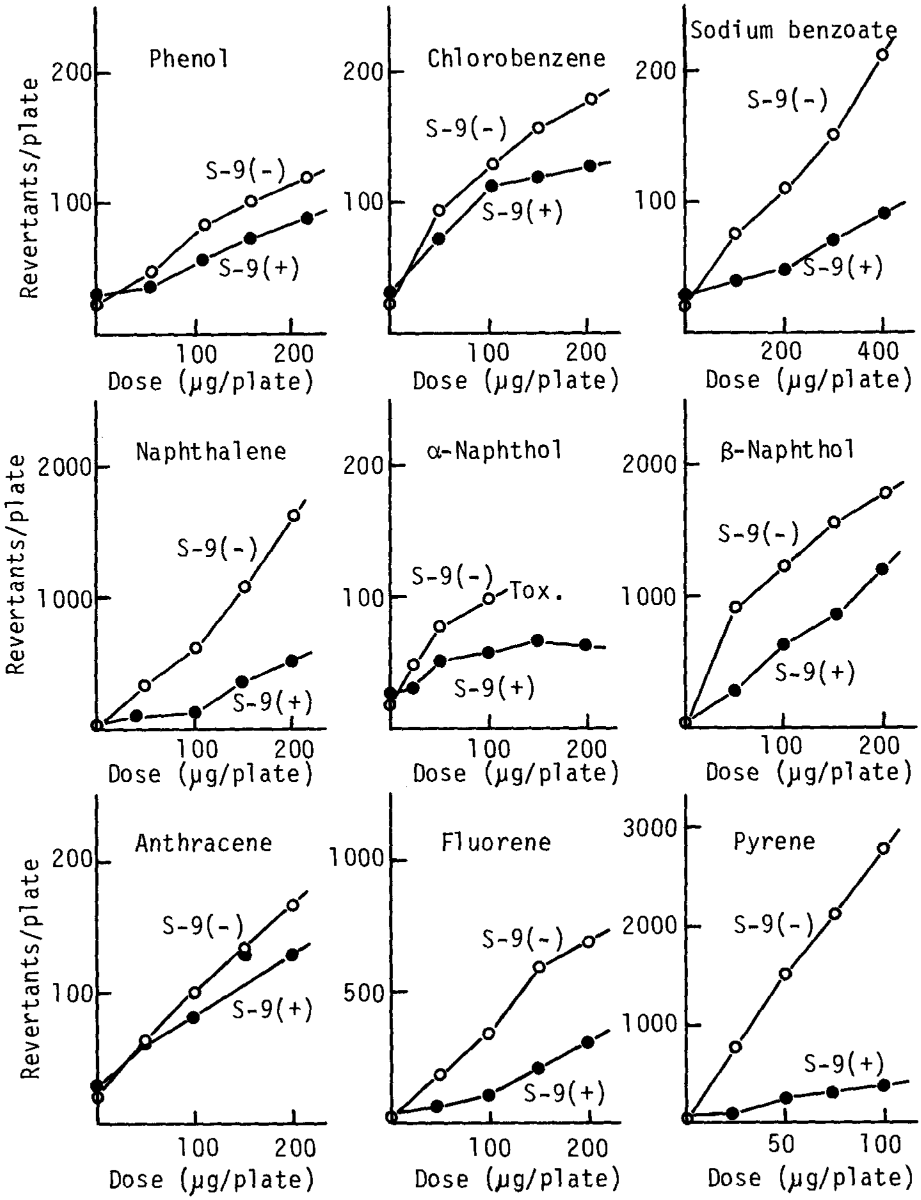

Fig. 1. Dose-response curves of mutagenicity toward S. typhimurium TA98 of photolytic products (ether extracts) from aromatic compounds by UV irradiation (3 h) in aqueous nitrite solution (NO_2-N, 16.2 ppm) with a Pyrex-filtered mercury lamp.

aqueous nitrite solution seems to be due to such wavelength characteristics in the photo-activation of nitrite.

Nitrite ion is contained at low concentration in surface water (OKADA et al. 1979) and at relatively high concentration in the effluent from night soil treatment plant (MIZUNO et al. 1981). It also is supplied from nitrate ion through biological (CARLUCCI et al. 1970) and photochemical reactions (PETRICONI et al. 1978). Based on our data, the formation of mutagens from organic compounds in aquatic environments is probably catalyzed by sunlight.

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