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Formation of Mutagens by Photolysis of Aromatic Compounds in Aqueous Nitrate Solution

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In recent years, various mutagenic compounds have been found to be present in the aquatic environments, i.e., drinking water (DAVID et al. 1979), river water (MOORE et al. 1980) or river sediment (SUZUKI et al. 1982). The appearance of teratoid in aquatic animals (WELLINGS et al. 1976) may be caused by these mutagens. Mutagens in aquatic environment must originate from biological and chemical formations in the environment as well as inflow of domestic sewage or industrial waste water containing mutagens. For example, chlorination of water has been found to be responsible for the formation of mutagens (MARUOKA et al. 1980). We found that biphenyl was nitrated by the photochemical reaction in nitrate aqueous solution to give mutagenic compound (SUZUKI et al. 1982). Other aromatic compounds may also undergo a similar reaction in water containing nitrate ion.

It has been reported by many workers that the aquatic environment was polluted by various aromatic hydrocarbons (ACHESON et al. 1976; DIPAK et al. 1978; HITES et al. 1977). Seawater has been polluted with crude petroleum containing aromatics drained out in a tanker accident (PATRICK et al. 1976). On the other hand, nitrate content of surface water is 1 to 5 ppm as nitrate nitrogen (NO_3-N) and that of sewage plant effluent is more than 15 ppm(BRINKHOFF 1978). Accordingly, studying the photochemical reaction of aromatic compounds in nitrate aqueous solution from the view point of mutagenicity is very significant.

The present paper describes the occurrence of mutagenicity by photochemical reaction of some aromatic compounds such as polycyclic aromatics and mono-substituted benzenes in nitrate aqueous solution.

MATERIALS AND METHODS

Materials. All chemicals used were reagent grade from Kanto Chemicals Co. and used without further purification. Silicagel (Wakogel Q22, 200 mesh) from Wako Pure Chemical Ind. Ltd., used as carrier for dispersion of water-insoluble compounds, was 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

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Sangyo Inc.) with maximum energy distribution at 365 nm. The lamp was fitted with a quartz glass filter or a Pyrex glass (7740) filter outside the lamp for circulation of cooling water. 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 and pyrene were coated on carrier in the following manner: Ether solution of each compound (50 mg) was evaporated to dryness under reduced pressure together with carrier (1 g). The amount of the compound coated on carrier was determined colorimetrically after extraction with ether from a portion of the carrier. The compound (20-25 mg) coated on carrier was suspended in 500 mL of an aqueous solution of sodium nitrate (16.5 ppm as NO_3-N) in the reaction vessel. Other compounds (25-100 mg) were added together with 500 mg of carrier in the nitrate aqueous solution. UV irradiation was performed for 3 h at 25°C with stirring in a thermostat.

The reaction mixture containing the carrier was extracted with ether (100 mL x 2) under acidic condition (at pH 5). The ether layer was evaporated to dryness under reduced pressure at 40°C, if necessary, the residual water layer was also evaporated. The dried samples were dissolved in dimethyl sulfoxide (DMSO) for the ether extract and in sterilized water for the water-layer sample, and a 0.1 mL each of these solutions was subjected to the mutation assay.

Mutation Assay. Mutation tests were carried out by slight modification (YAHAGI 1975) of the method described by Ames et al. (1973a) using Salmonella typhimurium strain TA98. Liver microsomal fraction (S-9 mix) was prepared from PCB-treated male rats as described by Ames et al. (1973b).

The mutagenicities of the ether extracts were estimated from the dose-response curve at the dosage of 1, 10, and 100 μg per plate, and those of the water-layer samples from the assay at 1 mg per plate. Each sample was assayed using four replicate plates at each dose level. No significant lethal effect on the tester strain was observed in the presence or absence of S-9 mix at any of the dose levels tested. Spontaneous revertants are expressed as the mean of revertant colonies obtained from all the control groups in each assay. Each sample was considered to be mutagenic when the number of revertant colonies obtained at the dose level assayed was at least twice that of the control.

RESULTS AND DISCUSSION

Eleven kinds of aromatics, i.e., naphthalene, anthracene, biphenyl, pyrene, benzene, phenol, chlorobenzene, aniline, toluene, sodium benzoate, and sodium benzene sulfonate, were irradiated with a 100 W high-

Table 1. Mutagenicity of the photolytic products from aromatic compounds by UV irradiation in nitrate aqueous solution (NO_3-N, 16.5 ppm)with a 100 W high-pressure mercury lamp.

Compounds	Original amount (mg)	Yield of ether extract (mg)	Residue of water-layer (mg)	Mutagenicity (TA98) Ether-layer Revertants/100μg/plate S-9 mix(-)	Ether-layer Revertants/100μg/plate S-9 mix(+)	Water-layer Revertants/1 mg/plate S-9 mix(-)
Benzene	98	3.9	75	246	102	84
Naphthalene	23	8.4	40	385	89	125
Biphenyl	21	9.5	66	520	316	145
Anthracene	22	33.1	42	575	186	262
Pyrene	23	35.3	64	941	216	1184
Phenol	25	7.0	71	136	102	62
Chlorobenzene	40	4.2	44	342	126	75
Aniline	74	27.6	85	121	213	71
Toluene	41	3.4	59	154	101	56
Sodium benzoate	25	3.7	71	146	167	200
Sodium benzene sulfonate	25	1.6	76	143	152	68
Spontaneous revertants				28	76	34

pressure mercury lamp fitted with a quartz glass filter in the nitrate aqueous solution. The yields of the ether extract and the water-layer obtained from the reaction mixture and their mutagenicities are shown in Table 1. All of the ether extracts were mutagenic in the absence of S-9 mix, and their mutagenicities were depressed in the presence of S-9 mix, indicating that they comprised direct-acting mutagen. No mutagenicity was observed in the products obtained by photolysis in the nitrate-free aqueous solution (the data not shown). Accordingly, it is evident that nitrate ion played an indispensable role in the formation of direct-acting mutagens by this photochemical reaction. The direct-acting mutagen produced from biphenyl in this photo-reaction system has been found to be a nitro compound as described previously (SUZUKI et al. 1982). In addition, a number of nitro aromatic compounds have been known to be a direct-acting mutagen (McCANN et al. 1975; CHIU et al. 1978; PITTS JR et al. 1982). The direct-acting mutagens produced from the aromatics other than biphenyl are probably nitro compounds.

The mutagenicities in Table 1 are given in revertant colonies per 100 ug for the ether fraction and per 1 mg for the water fraction. Therefore, the specific mutagenicities of the water fractions were ascertained to be much weaker than those of the corresponding ether fractions. The net mutagenicities of most water fractions, which were estimated from the yields and the numbers of revertants colonies per plate, were 10 to 20% of the net mutagenicities of corresponding ether fractions. It is apparent that a large portion of the mutagenic products were ether extractable. In the case of sodium benzoate and sodium benzenesulfonate, the net mutagenicities of their water fractions were higher than those of the ether fractions. The mutagenicities of the water-layer fractions may be due to basic products or highly polar products remaining after ether extraction.

The mutagenicities of the ether extract from polycyclic aromatics were significantly higher than those from benzene mono-substituents. The highest mutagenicity was observed in the ether extract from pyrene. Both the specific mutagenicity and the net mutagenicity of the ether extract increased in order of benzene, naphthalene, biphenyl, anthracrne, and pyrene. This result indicates that the production rate of mutagenic compound and/or the mutagenic activity of the product in this photochemical reaction increased in proportion to the number of aromatic ring of original compound.

The light source in the above-mentioned experiment was comprised of the wavelengths of 250 to 577 nm, and the energy of shorter wavelengths than 300 nm comprised 15% of the total energy. On the other hand, the sunlight on the surface of the earth is comprised of longer wavelengths than 300 nm.

Table 2. Mutagenicity of the photolytic products from aromatic compounds by UV irradiation in nitrate aqueous solution (NO_3-N, 16.5 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	86	3.6	36	42
Naphthalene	23	6.6	72	64
Biphenyl	20	2.8	40	47
Anthracene	22	14.7	27	59
Pyrene	23	30.3	72	50
Phenol	25	3.6	142	111
Chlorobenzene	40	3.8	34	85
Aniline	31	10.1	60	226
Toluene	30	2.1	49	92
Sodium benzoate	25	5.1	24	68
Sodium benzene sulfonate	25	4.1	31	88
Spontaneous revertants			28	76

In order to evaluate the possibility of such formation of mutagen as mentioned above in a natural aquatic environment, the same experiment was carried out using the high-pressure mercury lamp fitted with a Pyrex glass filter so as to cut off shorter wavelengths than 300 nm. Table 2 shows the mutagenicities of the ether extracts obtained from the Pyrex filter reaction system. Compared with the mutagenicities of the ether extracts in Table 1, those in Table 2 are weaker or not observed. These results indicate that the photochemical formation of mutagens in nitrate aqueous solution is easy to occur by the irradiation of the light of short wavelengths rather than long wavelengths. A low-pressure mercury lamp with maximum energy distribution at 254 nm is generally used for sterilization. In such cases, the production probability of mutagen must be much higher as described above.

On the other hand, the ether extracts from naphtharene, pyrene, phenol and aniline were mutagenic in spite of irradiation for only 3 h in Pyrex filter system. This suggests that a similar reaction is possible to occur also with sunlight in natural aquatic environments.

Accordingly, more attention should be given to the photochemical formation of mutagens.

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