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Photochemical Nitrosation of Phenol in Aqueous Nitrite Solution

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Photochemical reaction of phenol with nitrite ion was investigated by ultraviolet irradiation in aqueous solution. The main product of the photochemical reaction was isolated and identified as *p*-nitrosophenol on the basis of spectral data and high performance liquid chromatography analysis. The production of *p*-nitrosophenol increased with increases in nitrite ion concentration and irradiation time. The production rate of *p*-nitrosophenol was enhanced under basic condition or under a nitrogen atmosphere, and was suppressed by the addition of sodium thiocyanate or under an oxygen atmospheres. These results suggested that the photo-nitrosation was caused by NO and OH radicals produced by the photolysis of nitrite ion.

Keywords——phenol; aqueous nitrite solution; UV irradiation; nitrosation; *p*-nitrosophenol

Polycyclic aromatic hydrocarbons in airborne particulates have been found to react photochemically with traces of nitrogen dioxide and nitric acid to give nitro derivatives.[1-3] Many nitro aromatic compounds are known to be direct acting mutagen,[4,5] and they have been shown to make a significant contribution to environmental mutagens.[6-8]

We have reported that photochemical formation of mutagens from aromatic compounds also occurs in water containing nitrite or nitrate ion, and that mutagenic compounds are formed more readily in aqueous nitrite solution than in nitrate solution.[9-11] It was found that photo-reaction of biphenyl in aqueous nitrate solution gave hydroxynitrobiphenyl.[9] However, irradiation products of aromatic compounds in aqueous nitrite solution have not been identified as yet.

The present paper is concerned with the photo-reaction of phenol with nitrite ion in aqueous solution in order to obtain information on the mechanism of mutagen formation from aromatics, and demonstrates that the main reaction is nitrosation.

Experimental

Chemicals——Phenol and sodium nitrite were obtained from Kanto Kagaku Co., and were of the highest grade. *p*-Nitrosophenol of reagent grade (obtained from Tokyo Kasei Co. as a brownish powder) was purified by silica gel column chromatography (Wakogel Q22, eluent $CHCl_3$) to give pale yellow needles. Other chemicals were of the best grade commercially available.

UV Irradiation——A distilled water or buffer solution (200 ml) containing phenol (1.1 mM) and nitrite ion (1—12 mM) was placed in a photo-reaction vessel (Riko Kagaku Sangyo Inc.). Ultraviolet (UV) light was obtained from a 100 W high-pressure mercury lamp, UVL-100 HA (Riko Kagaku Sangyo Inc.), with maximal energy output at 365 nm, and was passed through a Pyrex glass (7740) filter so as to cut off shorter wavelengths than 300 nm. The system was cooled by circulating water. The lamp was placed inside the photo-reaction vessel, and UV irradiation was performed at 25 °C with stirring.

Isolation of Reaction Product and Instrumental Analyses——The reaction mixture was extracted with ether (100 ml × 2) under acidic conditions (pH 4 with 0.1 N HCl). The main product in the ether extract was separated preparatively on Merck pre-coated thin-layer chromatography (TLC) plates, polyamide 11F254 (solvent $CHCl_3$/MeOH, 8/2) and Silica gel 60F254 (solvent benzene/MeOH, 9/1). The fractionated product was finally purified by high performance liquid chromatography (HPLC) with a Merck Lobar column, Licroprep Si60 (solvent benzene/MeOH, 9/1).

Nuclear magnetic resonance (NMR) and mass spectra of the isolated substance were obtained with a JNM-FX 100 NMR spectrometer (in $CDCl_3$, internal standard tetramethylsilane (TMS)), and a Hitachi double-focussing gas chromatography (GC)-mass spectrometer M-80 operating at 70 eV, respectively. A Hitachi 323 recording spectrophotometer was used for measurement of UV spectra.

The spectral data for the isolated product were as follows. NMR spectrum (in $CDCl_3$, internal standard TMS) δ ppm: 6.48 (2H, d, H_2 and H_6, $J_{2,3=5,6}=10$ Hz), 7.18 (1H, dd, H_3 or H_5, $J_{2,3}=10$ Hz, $J_{3,5}=3$ Hz), 7.72 (1H, dd, H_5 or H_3, $J_{6,5}=10$ Hz, $J_{5,3}=3$ Hz), 9.02 (1H, br s, OH). Mass spectrum *m/e*: 123 (100%, M^+), 95 (15%, M^+-CO), 93 (10%, M^+-NO), 65 (42%, $M^+-CO-NO$), 52 (21%), 39 (18%), 26 (11%), 18 (7%). UV $\lambda_{max}^{H_2O(pH\,6)}$ nm ($E_{1\,cm}^{1\%}$): 302 (880), 398 (840).

HPLC Analysis of Reaction Products——After the irradiation, 1 ml of internal standard solution was added to 4 ml of the reaction solution and the mixture was subjected to HPLC analysis on a Shimadzu LC-4A or a Nihon Seimitsu NSLC-100 instrument. *p*-Nitrosophenol, the main product, was analyzed under the following HPLC conditions: column, Zorbax ODS (4.6 mm i.d. × 150 mm); solvent, MeOH/distilled water (40/60); flow rate, 1 ml/min; wavelength for detection, 280 nm; internal standard, *p*-cresol (250 ppm). Nitrate and nitrite ions were analyzed by the method of Thayer[12] with a modified solvent under the following HPLC conditions: column, Partisil-10 SAX (4.6 mm i.d. × 250 mm); solvent, pH 6.4 KH_2PO_4–Na_2HPO_4 buffer (0.01 M); flow rate, 1 ml/min; wavelength for detection, 215 nm; internal standard, *p*-hydroxybenzoic acid (1000 ppm).

Results

Neutral aqueous solution containing phenol and nitrite (1.1 and 2.9 mM, respectively) readily acquired a yellowish-brown color upon UV irradiation. The reaction products were analyzed by reversed-phase HPLC. A representative chromatogram is shown in Fig. 1; peak III corresponded to phenol. The retention time of peak II coincided with that of authentic *p*-nitrosophenol. No peaks corresponding to nitrophenols were observed (t_R of *p*-nitrophenol and *o*-nitrophenol were 5.4 and 8.5 min, respectively). Peak I seemed to be due to other reaction products, including degradation products of *p*-nitrosophenol, but we could not isolate them.

For isolation of the product corresponding to peak II, the reaction mixture was extracted with ether. The ether extract was separated by preliminary polyamide TLC, and the yellow band at *Rf* 0.5—0.7 was collected. This fraction was further separated by silica gel TLC, and the pale yellow substance at *Rf* 0.16 was collected. After purification by silica gel HPLC, the product was determined from the NMR, mass and UV spectra to be *p*-nitrosophenol.

Figure 2 shows the production of *p*-nitrosophenol from phenol by irradiation (3 h) at various nitrite concentrations. Both phenol degradation and *p*-nitrosophenol production

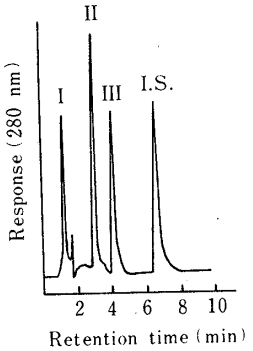

Fig. 1. HPLC Chromatogram (Zorbax ODS) of the Reaction Mixture of Phenol (1.1 mM) Irradiated for 3 h in Neutral Aqueous Nitrite Solution (2.9 mM)

Details of HPLC conditions are given in the text. I.S. is the peak of *p*-cresol (internal standard).

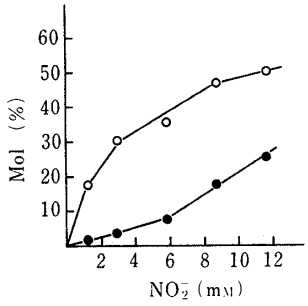

Fig. 2. Effect of Nitrite Ion Concentration on the Photo-Nitrosation of Phenol by Irradiation for 3 h under Neutral Conditions

Proportion (mol%) of phenol lost to original amount (1.1 mM) (○), proportion (mol%) of *p*-nitrosophenol produced to original phenol (●).

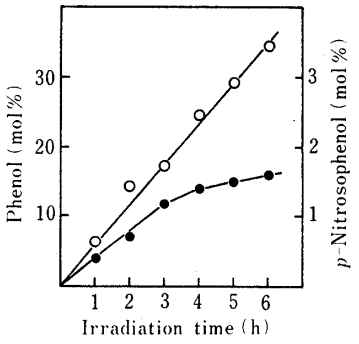

Fig. 3. Time Course of Photo-Nitrosation of Phenol by Irradiation in Neutral Aqueous Nitrite Solution (1.2 mM)

Proportion (mol%) of phenol lost to original amount (1.1 mM) (○), proportion (mol%) of *p*-nitrosophenol produced to original phenol (●).

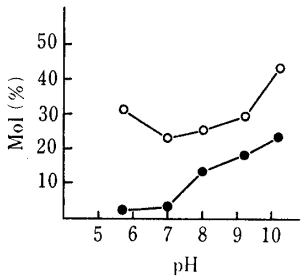

Fig. 4. Effect of pH on Photo-Nitrosation of Phenol by Irradiation for 3 h in Aqueous Nitrite Solution (2.9 mM)

pH 5.7, 7, and 8 (0.01 M-phosphate buffer), pH 9.2 and 10.2 (0.01 M-carbonate buffer). Proportion (mol%) of phenol lost to original amount (1.1 mM) (○), proportion (mol%) of *p*-nitrosophenol produced to original phenol (●).

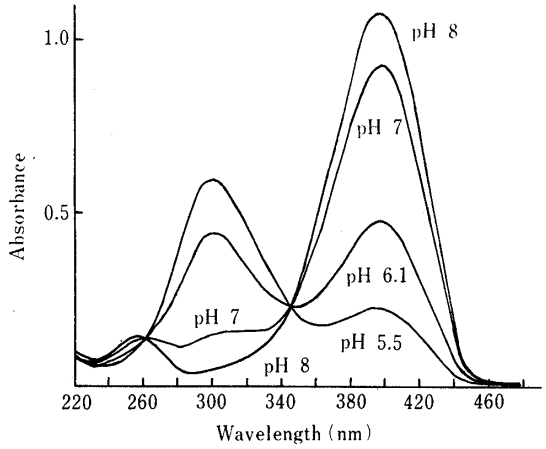

Fig. 5. UV Spectra of *p*-Nitrosophenol (0.04 mM) under Various pH Conditions (in 0.01 M Phosphate Buffer)

increased with increase in the concentration of nitrite. Phenol was scarcely degraded by irradiation in nitrite-free aqueous solution. On the other hand, the amount of *p*-nitrosophenol formed relative to that of phenol degraded increased with increase in nitrite concentration, and reached 50% at 12 mM nitrite, indicating that *p*-nitrosophenol was the main product at high concentrations of nitrite.

Figure 3 shows the time courses of *p*-nitrosophenol production and phenol degradation on irradiation at a low concentration of nitrite (1 mM). The amount of degraded phenol increased linearly with irradiation time. However, the production of *p*-nitrosophenol was depressed by prolonged irradiation, and the amount of *p*-nitrosophenol formed relative to that of phenol lost was only about 5%.

The effect of pH on the photo-nitrosation of phenol is shown in Fig. 4. The degradation rate of phenol was higher under acidic and basic conditions than neutral conditions. The production rate of *p*-nitrosophenol, however, was higher under basic conditions than other conditions. On the other hand, *p*-nitrosophenol was found to be stable under basic conditions and unstable under acidic conditions as shown in Table II. This indicates that the differences in the production rate of *p*-nitrosophenol under basic and acidic conditions are attributable to the differences in its stability. The UV spectrum of *p*-nitrosophenol is altered by change of pH, as shown in Fig. 5. The absorption maximum at 300 nm disappears under basic conditions in which *p*-nitrosophenol is stable. Therefore, excitation based on UV absorption at 300 nm

TABLE I. Some Factors Affecting Photo-Nitrosation of Phenol

Conditions	Nitrite lost (mol%)[b]	Nitrate produced (mol%)[b]	Phenol lost (mol%)[c]	*p*-Nitrosophenol produced	
				(mol%)[c]	Ratio[d]
Control[a]	13.9	5.4	28.5	4.9	1.00
In N_2	19.3	0.0	34.7	19.7	4.02
In O_2	9.1	9.0	27.0	2.6	0.53
NaSCN (1 mM) added	10.6	2.3	25.7	2.5	0.51

a) Neutral distilled water solution containing phenol (1.1 mM) and nitrite (2.9 mM) was irradiated for 3 h.
b) Proportions of nitrite lost and nitrate produced to original nitrite (2.9 mM).
c) Proportions of phenol lost and *p*-nitrosophenol produced to original phenol (1.1 mM).
d) Ratio of the amount of *p*-nitrosophenol produced under each condition to that of the control.

TABLE II. Degradation Rate of *p*-Nitrosophenol by UV Irradiation in Nitrite-Free Aqueous Solution under Various Conditions

Conditions	Degradation rate of *p*-nitrosophenol (mol%)
Control[a]	59.9
In N_2	63.0
In O_2	81.9
NaSCN (1 mM) added	75.8
pH 10 carbonate buffer (0.01 M)	0.6
pH 5.5 phosphate buffer (0.01 M)	69.7

a) Neutral distilled water solution containing *p*-nitrosophenol (0.4 mM) was irradiated for 2 h.

seems to be responsible for the photochemical instability.

In order to obtain information on the mechanism of photonitrosation of phenol, aqueous nitrite-phenol solutions were irradiated for 3 h under various conditions, and the amounts of phenol lost and *p*-nitrosophenol produced were determined. Nitrite and nitrate were also determined at the same time, since nitrite is known to be photochemically converted to nitrate.[13] These results are summarized in Table I. Under a nitrogen atmosphere, the production of *p*-nitrosophenol was enhanced, whereas the conversion of nitrite to nitrate was completely suppressed owing to the lack of oxygen. Under an oxygen atmosphere, most of the nitrite lost was converted to nitrate, and the production of *p*-nitrosophenol was suppressed.

The photochemical nitrosation of phenol was also suppressed by addition of sodium thiocyanate. Such suppression indicates that the hydroxy radical participates in this photo-nitrosation, since sodium thiocyanate is known to be a scavenger of the hydroxy radical.[14]

On the other hand, Table II shows the degradation rate of *p*-nitrosophenol on UV irradiation in nitrite-free aqueous solution under the above conditions. *p*-Nitrosophenol was found to be degraded at approximately the same rate, except under basic conditions. The degradation rates were not affected by the coexistence of phenol. These results indicate that the differences in the production rate of *p*-nitrosophenol in Table I are attributable to differences in photo-nitrosation, although the pH effect on the production rate of *p*-nitrosophenol is partially explicable in terms of the pH dependence of the product stability.

Discussion

The results presented here show that phenol is nitrosated by irradiation with UV light (>300 nm) in aqueous nitrite solution. Furthermore, this nitrosation was found to depend solely on the photo-excitation of nitrite. Nitrite ions in aqueous solution show a UV absorption with a maximum at 355 nm. The UV absorption causes excitation and subsequent photolysis of nitrite ions as reported previously by Treinin *et al.*[13] and Zafiriou *et al.*[15] The proposed reaction scheme can be summarized as follows:

$$NO_2^- \xrightarrow{h\nu\ (>300\,nm)} {}^*NO_2^- \quad (1)$$

$${}^*NO_2^- + 1/2O_2 \longrightarrow NO_3^- \quad (2)$$

$${}^*NO_2^- + HOH \longrightarrow \cdot NO + \cdot OH + OH^- \quad (3)$$

Our present results indicate that the nitrosation of phenol is caused by both radicals, NO and OH, produced in reaction 3. Phenol may be nitrosated by the NO radical after hydrogen abstraction by the OH radical. The production of both radicals (scheme 3) should be enhanced if reaction (2) is suppressed under oxygen-deficient conditions. This seems to be one of the causes of the enhancement of *p*-nitrosophenol production under a nitrogen atmosphere.

On the other hand, nitrate ions produced in reaction (2) may also be presumed to take part in the photo-reaction of phenol. Nitrate ions in aqueous solution show a UV absorption with a maximum at 300 nm. Nitrate ions have been found to be excited upon UV absorption to give NO_2 and OH radicals.[15,16] Therefore, the participation of nitrate in photo-reaction should result in the production of nitro compounds. In the present reaction of phenol, however, no nitrophenols were observed, indicating that the photo-reaction related to nitrate ion is negligible. The lack of nitrophenols also indicates that *p*-nitrosophenol was not oxidized to nitrophenol under these experimental conditions. However, this seems to be specific for *p*-nitrosophenol, because general nitroso compounds are easily oxidized to nitro compounds.

We reported previously that the ether extract obtained from the reaction mixture of phenol irradiated under the same conditions as used in the present experiments exhibited mutagenicity toward *Salmonella typhimurium* TA98.[11] Gilbert *et al.* have reported that *p*-nitrosophenol has a direct mutagenic effect toward TA1538.[17] However, the mutagenicity toward TA98 was so weak that the mutagenicity of the ether extract could not be explained by that of *p*-nitrosophenol alone. An unknown degradation product contained in the ether extract also seems to contribute to the mutagenicity.

It is likely that the NO and OH radicals produced by photolysis of nitrite ions in aqueous solutions cause nitrosation of other aromatic compounds as well as phenol. There is a high probability that the resulting nitroso compounds are mutagenic, since nitroso aromatics are well-known to be metabolic intermediates of mutagenic nitro aromatics,[4,5] and many aromatic compounds are known to be converted to mutagens by UV irradiation in aqueous nitrite solution.[11] Accordingly, the photochemical nitrosation caused by photolysis of nitrite ion may play an important role in the formation of mutagens in aquatic environments.

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