

[Chem. Pharm. Bull.]
[36(6) 2204—2211(1988)]

Formation of Mutagens by Photochemical Reaction of 2-Naphthol in Aqueous Nitrite Solution

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(Received September 29, 1987)

The following seven compounds were isolated from the photoreaction products of 2-naphthol in aqueous nitrite solution by silica gel chromatography and high performance liquid chromatography: 1-nitro-2-naphthol (Fp-2), 1-nitroso-2-naphthol (Fp-3), isocoumarin (Fp-4(1)), 5- or 8-nitro-2-naphthol (Fp-5(1)), a monoquinonoid dimer of 2-naphthol (Fp-5(2a)), a dinitro-2-naphthol (Fp-6(1)) and 1,2-naphthoquinone. Among these compounds, the three nitro compounds, Fp-2, Fp-5(1) and Fp-6(1), and isocoumarin exhibited a weak mutagenicity towards *S. typhimurium* TA98 without S9 mix. Although no major mutagen was isolated, it was found that irradiation of the solution of non-mutagenic Fp-5(2a) with nitrite and of the mixed solution of 1,2-naphthoquinone and Fp-2 without nitrite resulted in the formation of further mutagenic compounds. From these results, it is concluded that not only nitration but also quinone production is closely related to photochemical mutagen formation.

Keywords—2-naphthol; aqueous nitrite solution; UV irradiation; mutagen; nitronaphthol; nitrosonaphthol; isocoumarin; naphthoquinone

Introduction

Formation of nitroarenes by combustion of fossil fuels^{1,2)} and by photolysis of polycyclic aromatics in the atmosphere containing nitrogen oxide^{3,4)} has been pointed out as an important cause of mutagen formation in the environment.

In previous studies, we found that the photolysis of many aromatic compounds in water containing nitrate or nitrite ion also resulted in mutagen formation.^{5,6)} In particular, mutagen formation in water containing nitrite has been confirmed to occur by exposure to sunlight.⁷⁾ In addition, it was found that photochemical mutagen formation could be observed not only in polycyclic aromatics but also in a wide variety of aromatic compounds such as benzene, phenol, chlorobenzene, benzoate⁶⁾ and aromatic amino acids.⁸⁾

We have identified hydroxynitrobiphenyls from biphenyl,^{7,9)} 1-nitropyrene from pyrene¹⁰⁾ and *p*-nitrosophenol from phenol¹¹⁾ as products of photoreactions in aqueous nitrite solution. The data indicated that the major photoreaction in aqueous nitrite solution consists of hydroxylation, nitration and nitrosation.⁷⁾ However, mutagen formation from a wide variety of aromatics appears not to be explained by these three reactions only, because none of the simple nitro aromatics other than polycyclic aromatic compounds exhibit a very strong mutagenicity.^{12–14)} In fact the mutagenicity of phenol irradiated in aqueous nitrite solution could not be explained by the identified *p*-nitrosophenol.¹¹⁾ In order to resolve this problem, we further studied photochemical reactions for mutagen formation from some other aromatic compounds. In the course of these studies, we found that photochemical quinone production is a key step in the mutagen formation from 2-naphthol. The compound 2-naphthol has been found to exhibit a very strong mutagenicity on irradiation,⁶⁾ despite the lack of potent mutagens among known nitro-naphthols.¹⁴⁾ Accordingly, quinone production is also pre-

sumed to be an important reaction for such photochemical mutagen formation from other aromatic compounds that cannot be interpreted only by simple nitration of the aromatic ring.

In this paper, we report the production of some compounds from 2-naphthol by ultraviolet (UV) irradiation in aqueous nitrite solution, and present evidence that the photochemical oxidation of 2-naphthol to 1,2-naphthoquinone as well as nitration plays an important role in the mutagen formation.

Experimental

Chemicals—2-Naphthol, sodium nitrite and diethyl ether were obtained from Kanto Kagaku Co. 1-Nitro-2-naphthol and 1,2-naphthoquinone were obtained from Tokyo Kasei Co. and 1-nitroso-2-naphthol from Nakarai Chemicals Co. All these chemicals and other compounds were of the best grade commercially available and were used without further purification.

UV Irradiation—A distilled water solution (500 ml) containing 40 mg of sodium nitrite and a methanol solution (1 ml) containing 25 mg of 2-naphthol were placed in a photoreaction vessel (Riko Kagaku Sangyo Inc.). UV light was obtained from a 100 W high-pressure mercury lamp, UVL-100HA (Riko Kagaku Sangyo Inc.), with maximal energy output at 365 nm, and was passed through a Pyrex glass filter so as to cut off wavelengths shorter than 300 nm. The lamp, which was cooled by circulating water, was placed inside the photoreaction vessel, and UV irradiation was performed at 25°C with stirring.

Separation and Isolation of Reaction Products—The reaction solution was extracted with ethyl ether (100 ml $\times$ 2) under neutral conditions so as to prevent any nitrosation reaction under acidic conditions. The extract was evaporated to dryness under reduced pressure. The dried extract was first separated chromatographically into eight fractions on a silica gel column (2 i.d. $\times$ 20 cm, packed with Kiesel gel 60, Art. 9385 (Merck)) with the following eluents: cyclohexane, cyclohexane–benzene (1:1), benzene, benzene–chloroform (1:1), chloroform–ethylacetate (1:1), ethylacetate–methanol (1:1), methanol and methanol–acetic acid (99:1). Each fraction obtained was, after evaporation, further separated with the same silica gel packed column and/or finally purified by high performance liquid chromatography (HPLC) with a Merck Lobar column, Licroprep Si 60 or Licroprep Rp-8 and by recrystallization.

Instrumental Analyses—Nuclear magnetic resonance (NMR) and mass spectrum (MS) of the isolated substances and of commercial specimens were obtained with a JNM-FX 100 NMR spectrometer (in CDCl_3 , internal standard tetramethylsilane) and a Hitachi double-focussing mass spectrometer, RMU-7M, operating at 70 eV, respectively. UV and infrared (IR) spectra (KBr disk) were obtained with a Hitachi 330 recording spectrophotometer and a Hitachi 225 IR spectrophotometer, respectively. Elemental analysis was performed with the kind cooperation of the Laboratory of Organic Micro Analyses at the Institute of Physical and Chemical Research.

Mutation Assay—The bacterial strain used was *Salmonella typhimurium* TA98. The mutation tests were performed by Ames' method with some modifications including a step of preincubation of the test materials with tester strain for 20 min at 37°C.¹⁵⁾ The extracts, separated fractions and isolated compounds were dissolved in dimethyl sulfoxide after evaporation and then subjected to the assay. Each sample was assayed with 4 replicate plates at each dose level. The mutagenicities of the isolated compounds (Table II) were estimated from the dose-response curves at three to four doses.

Results

When 2-naphthol solution (50 mg/l) containing nitrite ion (NaNO_2 , 80 mg/l) was irradiated under neutral conditions, the mutagenicity of the ether extract from the solution increased in proportion to the irradiation time (Fig. 1). The ether extract (20 mg) obtained from the solution irradiated for 3 h was fractionated by silica gel chromatography. The elution pattern and the eluents employed in the chromatography are shown in Fig. 2. The yields and the mutagenicities of the fractions obtained are summarized in Table I. As can be seen from Table I, about 70% of the produced mutagen was eluted into F-5 and F-6, although the other fractions also showed a weak mutagenicity. On the other hand, the quantitatively major portion of the ether extract was eluted into F-2, F-4, F-5 and F-6. The large value for the yield of F-8 seems to be due to deterioration of the silica gel caused by acetic acid in the eluent.

The F-2 with a high yield was found to consist essentially of a single component on silica

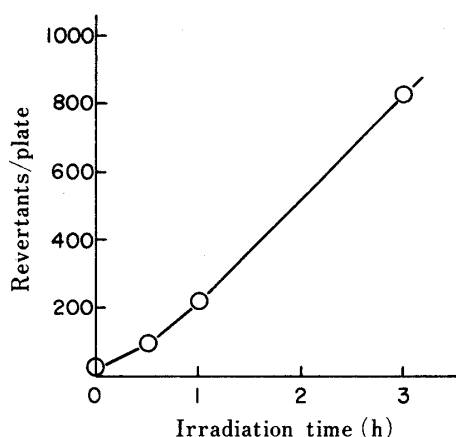


Fig. 1. Time Course of Mutagen Formation from 2-Naphthol by Irradiation in Aqueous Nitrite Solution (2-Naphthol 50 mg/l, NaNO_2 80 mg/l)

Mutation assay was performed using *S. typhimurium* TA98 without S9 mix at a dosage of the ether extract corresponding to 0.5 ml of the reaction mixture per plate.

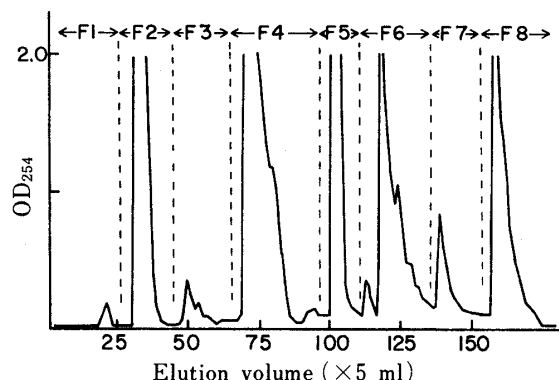


Fig. 2. Elution Pattern on Column Chromatography (Kiesel Gel 60) for Photoreaction Products (20 mg of Ether Extract) of 2-Naphthol Irradiated for 3 h in Aqueous Nitrite Solution

Eluents used: F-1, cyclohexane; F-2, cyclohexane-benzene (1:1); F-3, benzene; F-4, benzene-chloroform (1:1); F-5, chloroform-ethylacetate (1:1); F-6, ethylacetate-methanol (1:1); F-7, methanol; F-8, methanol-acetic acid (99:1). The absorbance (OD) at 254 nm of the fraction containing benzene or ethylacetate was measured in chloroform or methanol after evaporation of the eluent.

TABLE I. Mutagenicity of Each Fraction Separated by Column Chromatography

Fraction	Yield (mg)	Mutagenicity to TA98 without S9 mix ^{a)} (Revertants/plate)	Proportion of activity ^{b)} (%)
F-1	0.9	16	1
F-2	4.4	35	2
F-3	0.3	81	5
F-4	6.2	74	5
F-5	3.7	496	33
F-6	8.2	515	34
F-7	2.6	141	9
F-8	78.7	153	10
Original extract	20.0	1521	—

a) Spontaneous revertants have been subtracted. The dose per plate in the mutation assay was 1/100 of the yield of each fraction. b) This value shows the proportion of the mutagenic activity of each fraction to the whole mutagenicity of the extract.

gel thin layer chromatography (TLC) using benzene, although the mutagenicity was very weak, indicating that the F-2 was a major product of this photoreaction. The F-2 was therefore purified by re-chromatography on a silica gel column (solvent, cyclohexane-benzene (1:1)) to give a single compound Fp-2.

The minor F-3 was also purified by re-chromatography on a silica gel column (solvent, benzene) and then recrystallized from *n*-hexane to give a single yellow compound Fp-3.

The F-4 was found to be composed of unreacted 2-naphthol and at least one other major product on silica gel TLC using chloroform. The F-4 was therefore separated and purified by HPLC with a Si 60 Lobar column (solvent, cyclohexane-chloroform (1:1)) to give a single

compound Fp-4(1).

The mutagenic F-5 was further fractionated into two fractions, F-5(1) and F-5(2), by silica gel chromatography using chloroform–ethyl acetate (9:1). The mutagenic F-5(1) was found to be a single product on silica gel TLC (solvent, chloroform). It was therefore purified by HPLC with a Si 60 Lobar column (solvent, chloroform) and recrystallized from methanol containing some water to give a single compound Fp-5(1). The F-5(2) was further separated into a major component F-5(2a) and other components by silica gel chromatography using chloroform–methanol (99:1). The F-5(2a) was purified by HPLC with a RP-8 Lobar column (solvent, methanol–water (7:3)) and recrystallized from methanol containing some water to give a brown single compound Fp-5(2a).

The mutagenic F-6 was further fractionated into F-6(1) and F-6(2) by silica gel chromatography using ethyl acetate. Most mutagens of the F-6 were eluted in F-6(2). The major mutagenic fraction F-6(2) was confirmed to contain many components by means of HPLC. In spite of the application of various chromatographic techniques, however, attempts to isolate a major mutagenic component were unsuccessful. On the other hand, the F-6(1), which was also mutagenic and a single product on silica gel TLC (solvent, benzene–methanol (4:1)), was purified by HPLC with a RP-8 Lobar column (solvent, acetonitrile–water (1:1)) and recrystallized from methanol containing some water to give a single compound Fp-6(1).

The six isolated compounds, *i.e.*, Fp-2, Fp-3, Fp-4(1), Fp-5(1), Fp-5(2a) and Fp-6(1) were identified from their NMR, MS and elemental analysis as follows. Fp-2 (mp 102.5–103 °C) was determined as 1-nitro-2-naphthol, because the following spectral data coincided with those of the commercial standard (mp 103 °C): NMR (in CDCl_3) δ : 7.27 (1H, d, $J_{3,4}$ = 8 Hz, 3-H), 7.42–7.90 (3H, m, 5-, 6-, 7-H), 8.02 (1H, d, $J_{3,4}$ = 8 Hz, 4-H), 8.94 (1H, dd, $J_{8,7}$ = 8 Hz, $J_{8,6}$ = 2 Hz, 8-H), 12.19 (1H, s, 2-OH). MS m/z : 189 (100%, M^+), 172 (4%, M^+ – OH), 159 (9%, M^+ – NO), 143 (17%, M^+ – NO_2), 131 (34%, M^+ – NO – CO), 115 (74%, M^+ – NO_2 – CO), 103 (30%, M^+ – NO – 2CO), 89 (33%), 77 (26%), 63 (33%).

Fp-3 (mp 104–108 °C) was determined as 1-nitroso-2-naphthol, because the following spectral data coincided with those of the commercial standard (mp 109–110 °C): NMR (in CDCl_3) δ : 6.54 (1H, d, $J_{3,4}$ = 10 Hz, 3-H), 7.38–7.57 (3H, m, 5-, 6-, 7-H), 7.66 (1H, d, $J_{4,3}$ = 10 Hz, 4-H), 8.28 (1H, d, $J_{8,7}$ = 8 Hz, 8-H), 17.48 (1H, s, 2-OH). MS m/z : 173 (91%, M^+), 156 (76%, M^+ – OH), 145 (15%, M^+ – CO), 143 (19%, M^+ – NO), 128 (67%, M^+ – OH – CO), 115 (100%, M^+ – NO – CO), 101 (26%), 89 (26%), 75 (29%), 62 (31%), 50 (24%), 38 (16%), 29 (9%).

Elemental analysis of Fp-4(1) (mp 45.5–46.5 °C) yielded C, 73.43%; H, 4.13%; and O, 22.29% (found); corresponding to C, 73.96%; H, 4.14% and O, 21.90% (Calcd for $\text{C}_9\text{H}_6\text{O}_2$). The MS data were as follows: m/z : 146 (20%, M^+), 118 (100%, M^+ – CO), 90 (95%, M^+ – 2CO), 89 (93%, M^+ – CO – CHO), 74 (14%), 63 (79%), 50 (28%), 39 (50%), 29 (23%). The NMR revealed a characteristic doublet of doublets (J = 0.7 and 6 Hz) at 6.54 ppm, which changed to a doublet on irradiation of a doublet of triplets at 8.35 ppm, and was assigned as follows: NMR (in CDCl_3) δ : 6.54 (1H, dd, $J_{4,3}$ = 6 Hz, $J_{4,8}$ = 0.7 Hz, 4-H), 7.33 (1H, d, $J_{3,4}$ = 6 Hz, 3-H), 7.47 (1H, dd, $J_{5,6}$ = 8 Hz, $J_{5,7}$ = 2 Hz, 5-H), 7.56 (1H, ddd, $J_{7,6}$ = $J_{7,8}$ = 8 Hz, $J_{7,5}$ = 2 Hz, 7-H), 7.78 (1H, ddd, $J_{6,5}$ = $J_{6,7}$ = 8 Hz, $J_{6,8}$ = 2 Hz, 6-H), 8.35 (1H, tt, $J_{8,7}$ = 8 Hz, $J_{8,6}$ = 2 Hz, $J_{8,4}$ = 0.7 Hz, 8-H). On the basis of these spectral data, Fp-4(1) was identified as isocoumarin (mp 46–47 °C).¹⁶⁾

Fp-5(1) (mp 144–146 °C) was identified as 5-nitro-2-naphthol (mp 147–149 °C)¹⁷⁾ or 8-nitro-2-naphthol (mp 144–145 °C)¹⁸⁾ from the following data: MS m/z : 189 (95%, M^+), 160 (9%, M^+ – CHO), 143 (64%, M^+ – NO_2), 131 (30%, M^+ – NO – CO), 115 (100%, M^+ – NO_2 – CO). NMR (in CDCl_3) δ : 5.60 (1H, s, 2-OH), 7.29 (1H, dd, $J_{3,4}$ = 8 Hz, $J_{3,1}$ = 2 Hz, 3-H), 7.42 (1H, dd, $J_{7,6}$ = $J_{7,8}$ = 8 Hz, 7-H), 7.92 (1H, d, $J_{4,3}$ = 8 Hz, 4-H), 8.09 (1H, dd, $J_{8,7}$ = 8 Hz, $J_{8,6}$ = 1 Hz, 8-H), 8.12 (1H, d, $J_{1,3}$ = 2 Hz, 1-H), 8.35 (1H, dd, $J_{6,7}$ = 8 Hz,

$J_{6,8} = 1$ Hz, 6-H) or 5.60 (1H, s, 2-OH), 7.29 (1H, dd, $J_{3,4} = 8$ Hz, $J_{3,1} = 2$ Hz, 3-H), 7.42 (1H, dd, $J_{6,5} = J_{6,7} = 8$ Hz, 6-H), 7.92 (1H, d, $J_{4,3} = 8$ Hz, 4-H), 8.09 (1H, dd, $J_{5,6} = 8$ Hz, $J_{5,7} = 1$ Hz, 5-H), 8.12 (1H, d, $J_{1,3} = 2$ Hz, 1-H), 8.35 (1H, dd, $J_{7,6} = 8$ Hz, $J_{7,5} = 1$ Hz, 7-H). The assignments in the NMR spectrum were made on the basis of the spin-spin-decoupling data by irradiations at 7.42, 7.92 and 8.35 ppm (δ).

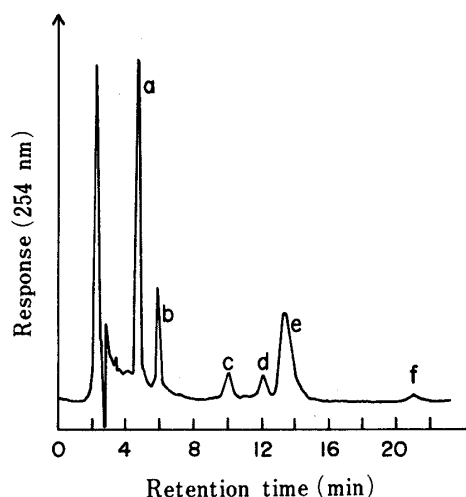


Fig. 3. HPLC Chromatogram of Ether Extract of the Reaction Mixture of 2-Naphthol Irradiated for 1 h in Aqueous Nitrite Solution

HPLC conditions: column, SSC-ODS 262 (6 mm i.d. $\times$ 10 cm); solvent, MeOH-H₂O (3:2). The retention times of peaks a (t_R , 4.6 min), b (t_R , 6.4 min), c (t_R , 9.9 min), e (t_R , 13.3 min) and f (t_R , 20.9 min) coincided with those of 1,2-naphthoquinone, isocoumarin, 2-naphthol, 1-nitro-2-naphthol and an isolated monoquinonoid dimer of 2-naphthol, respectively. The compound corresponding to peak d was not isolated.

TABLE II. Mutagenicities and Structures of Compounds Isolated from Ether Extract of the Photoreaction Mixture and 1,2-Naphthoquinone

Fraction	Compound Structure	Mutagenicity to TA98 ^{a)} without S9 mix (Revertants/10 μ g/plate)
Fp-2		4
Fp-3		Toxic
Fp-4(1)		27
Fp-5(1)		52
Fp-5(2a)		0
Fp-6(1)		190
1,2-Naphthoquinone		Toxic ^{b)}

a) Spontaneous revertants have been subtracted. b) This mutagenicity was assayed using commercial standard.

Fp-5(2a) (mp 221—226 °C) showed a molecular ion peak and fragmentation peaks at MS m/z 302 (12%, $M^+ + 2H$), 300 (13%, M^+), 272 (100%, $M^+ - CO$), 255 (29%, $M^+ - CO - OH$), 243 (64%, $M^+ - CO - CHO$), 215 (96%, $M^+ - 2CO - CHO$), 213 (50%, $M^+ - CO - CHO - CH_2O$) and 189 (24%, $M^+ - 2CO - CHO - C_2H_2$). The fragment ion peak ($M^+ + 2H$) is known to be observed characteristically in 1,2-naphthoquinone.¹⁹⁾ The NMR spectrum (in $CDCl_3$) revealed a hydroxyl proton at 5.64 ppm (δ) (a broad singlet) which disappeared on addition of D_2O , although assignment of the other complex signals was impossible. Fp-5(2a) also had an absorption at ν_{max} (KBr disk) 3100—3500 cm^{-1} (hydroxyl group) and 1650 and 1695 cm^{-1} (carbonyl group) in the IR spectrum and λ_{max} (ethanol) 228, 251, 274, 286, 331 and 400 nm in the UV spectrum. The UV spectrum could be explained by summation of the spectra of 1,2-naphthoquinone and 2-naphthol, and was also analogous to that of 4-(2-hydroxy-1-naphthyl)-1,2-naphthoquinone (mp 148—149 °C).²⁰⁾ On the basis of these results, Fp-5(2a) was considered to be a monoquinonoid dimer of 2-naphthol (NP-NQ).

The mutagenic Fp-6(1) (mp 149—155 °C) was determined from the following MS to be a dinitro-2-naphthol: MS m/z : 234 (70%, M^+), 188 (40%, $M^+ - NO_2$), 160 (10%, $M^+ - NO_2 - CO$), 132 (29%, $M^+ - NO_2 - 2CO$), 130 (31%, $M^+ - NO_2 - CO - NO$), 102 (100%, $M^+ - NO_2 - 2CO - NO$). Fp-6(1) was obtained in such a small amount that its NMR spectrum could not be evaluated.

Figure 3 shows the HPLC elution pattern of an ether extract of the reaction mixture of 2-naphthol irradiated for 1 h in aqueous nitrite solution. The retention times (t_R) of peaks b (t_R , 6.4 min), c (t_R , 9.9 min), e (t_R , 13.3 min) and f (t_R , 20.9 min) were coincident with those of the isolated isocoumarin, original 2-naphthol, 1-nitro-2-naphthol, and a monoquinonoid dimer of 2-naphthol (NP-NQ), respectively. A large peak a was observed only in the solution during the initial irradiation period and was determined to be due to 1,2-naphthoquinone, because the retention time coincided with that of the commercial standard.

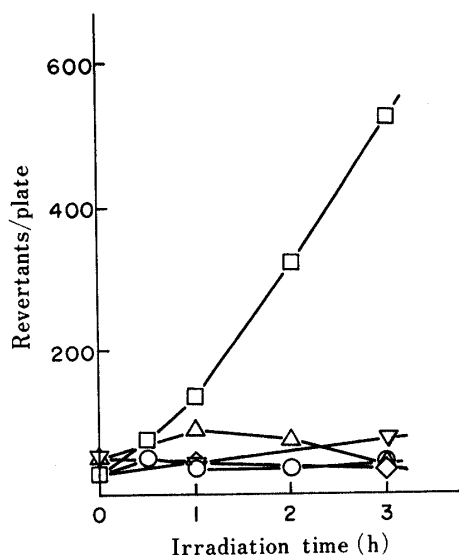


Fig. 4. Formation of Further Mutagenic Compounds from the Isolated Compounds by Irradiation in Aqueous Nitrite Solution ($NaNO_2$, 80 mg/l; Each Compound, 10 mg/l)

Mutation assay was performed using *S. typhimurium* TA98 without S9 mix at a dosage of the ether extract corresponding to 1 ml of the reaction mixture per plate: 1,2-naphthoquinone (○), isocoumarin (△), 1-nitro-2-naphthol (▽), 1-nitroso-2-naphthol (◇), and a monoquinonoid dimer of 2-naphthol (□).

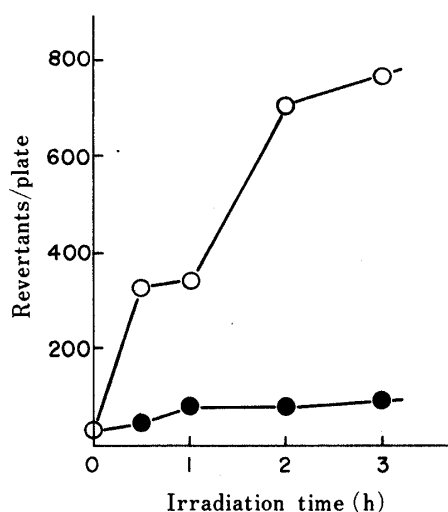


Fig. 5. Formation of Mutagen from a Mixture of 1,2-Naphthoquinone (20 mg/l) and 1-Nitro-2-naphthol (20 mg/l) by Irradiation

The solution was irradiated in the presence (●) and absence (○) of nitrite. Mutation assay was performed using *S. typhimurium* TA98 without S9 mix at a dosage of the ether extract corresponding to 1 ml of the reaction mixture per plate.

The structures and mutagenicities of the identified compounds are summarized in Table II. 1-Nitroso-2-naphthol, 1,2-naphthoquinone and a monoquinonoid dimer of 2-naphthol, NP-NQ, exhibited no mutagenicity. The other four compounds displayed mutagenicity towards *S. typhimurium* TA98 in the absence of S9. Among them, the F-6(1), a dinitronaphthol, was the most mutagenic. However, all of them, even the F-6(1), were weak mutagens and accounted merely for only part of the mutagenicity of the irradiated solution of 2-naphthol. Attempts to isolate and identify any major mutagens were unsuccessful as mentioned above.

We therefore tested whether the identified compounds produced further mutagenic compounds on irradiation or not, in order to elucidate the route of formation of the major mutagens. As shown in Fig. 4, 1,2-naphthoquinone, isocoumarin, 1-nitroso-2-naphthol and 1-nitro-2-naphthol produced no more mutagenic compounds in the presence of nitrite ion. In the absence of nitrite, they also produced no mutagen. Only the dimerized compound, NP-NQ, produced a strong mutagen on irradiation in the presence of nitrite. On the other hand, Fig. 5 shows the change in mutagenicity of a mixed solution of 1,2-naphthoquinone and 1-nitro-2-naphthol on irradiation. The results demonstrate that coexistence of both compounds resulted in mutagen formation by irradiation in the absence of nitrite. Such mutagen formation appears to be caused by the photochemical reaction of 1,2-naphthoquinone with 1-nitro-2-naphthol, because neither of these compounds produced mutagen independently by irradiation, as mentioned above.

Discussion

In this study, seven compounds, *i.e.*, 1-nitroso-2-naphthol, 1-nitro-2-naphthol, 5- or 8-nitro-2-naphthol, a dinitro-2-naphthol, isocoumarin, 1,2-naphthoquinone and a monoquinonoid dimer of 2-naphthol, were determined as photoreaction products from 2-naphthol by irradiation in the presence of nitrite. The production of the former four compounds is thought to be attributable to simple nitration or nitrosation and is predictable from the results of previous work on phenol¹¹⁾ and pyrene.¹⁰⁾ On the other hand, the later three compounds are considered to be produced by oxidation of the original 2-naphthol. The production of a quinone from aromatics by irradiation has been already suggested in the case of pyrene.¹⁰⁾ Also, the hydroxylation observed in the case of biphenyl⁹⁾ is considered to be a kind of oxidation. Accordingly, the photoreaction of aromatics in the presence of nitrite can be said to consist of oxidation and nitration or nitrosation. Differences in the reactivities of the aromatics used and in the stabilities of the products seem to give rise to the differences in the compounds identified from the respective aromatics.

On the other hand, among the isolated compounds, 1-nitro-2-naphthol, 5- or 8-nitro-2-naphthol, a dinitro-2-naphthol and isocoumarin, exhibited only a weak mutagenicity towards *S. typhimurium* TA98, and we were unable to isolate any major mutagen formed from 2-naphthol by irradiation. However, it was found that the irradiation of a solution of a monoquinonoid dimer of 2-naphthol in the presence of nitrite and of a mixed solution of 1,2-naphthoquinone and 1-nitro-2-naphthol in the absence of nitrite resulted in the formation of further mutagenic compounds. These findings suggest that the formation of a major mutagen is closely related to the nitration of NP-NQ. Consequently, it is concluded that the photochemical mutagen formation from 2-naphthol in the presence of nitrite is caused by both reactions of oxidation to quinone and nitration.

In previous work, we revealed that the mutagen formation from biphenyl and from pyrene is caused by both hydroxylation and nitration⁹⁾ and by nitration only,¹⁰⁾ respectively. Production of quinone was not observed in the photoreaction of biphenyl.⁹⁾ In the case of pyrene, the production of a pyrenequinone was suggested, but it appeared to be almost independent of the formation of the major mutagen.¹⁰⁾ This may have been because the

mutagenicity of the major mutagen, 1-nitropyrene, was too strong. This implies that quinones play an important role only in the photochemical mutagen formation from such aromatics, and their simple nitro derivatives do not exhibit a very strong mutagenicity. Accordingly, quinone production is also presumed to be closely related to the photochemical mutagen formation in the case of such single ring aromatics as benzene and phenol. Further detailed research is needed on the role of quinones in mutagen formation, since mutagen formation from a wide variety of aromatics is important as a source of mutagens in the environment including the atmosphere.

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