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Hydroxynitrobiphenyls Produced by Photochemical Reaction of Biphenyl in Aqueous Nitrate Solution and Their Mutagenicities

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From the photoreaction products of biphenyl in aqueous nitrate solution, the following eight hydroxynitrobiphenyls were isolated by silica gel column chromatography and thin layer chromatography on silica gel and polyamide plates: 4-hydroxy-3,5-dinitrobiphenyl (F1(2)or), 4,2′-dihydroxy-3,3′-dinitrobiphenyl (F4(1)-3), 4-hydroxy-3,3′-dinitrobiphenyl (F4(1)-4), a hydroxydinitrobiphenyl (F4(1)-5a), 4,4′-dihydroxy-3,3′-dinitrobiphenyl (F4(1)-5b), a dihydroxydinitrobiphenyl (F4(1)-6), 2-hydroxy-3-nitrobiphenyl (F5(1)) and 4-hydroxy-3-nitrobiphenyl (F5(2)). Among these compounds, four dinitro compounds, F1(2)or, F4(1)-4, F4(1)-5a and F4(1)-6, exhibited mutagenicity toward *S. typhimurium* TA98 without S-9 mix. The major products in this reaction were nitrophenylphenols, F5(1) and F5(2), and these were non-mutagenic. The major mutagens, F4(1)-5a and F4(1)-6, were produced in such small amounts that the exact structures could not be determined. The mechanism of production of these hydroxynitrobiphenyls is discussed on the basis of the results of analysis of the reaction mixture by high-performance liquid chromatography.

Keywords——biphenyl; aqueous nitrate solution; UV irradiation; nitration; hydroxylation; hydroxynitrobiphenyl; mutagen

Introduction

Polycyclic nitroaromatics are noted as important environmental mutagens, because they have been found to be formed by combustion of fossil fuel[1,2] and by photolysis of polycyclic aromatics in an atmosphere containing nitrogen oxide.[3,4] In earlier studies, we also found that mutagens were formed by the photolysis of aromatic compounds in water containing nitrate or nitrite ion,[5,6] and we reported on the effects of nitrate ion, irradiation time, pH and carrier on the formation of mutagens from biphenyl.[7] We found in a preliminary study that one of the mutagens formed from biphenyl was hydroxynitrobiphenyl.[7] Further investigation revealed that many mutagenic or non-mutagenic hydroxynitrobiphenyls were produced by the photoreaction of biphenyl in aqueous nitrate solution.

This paper reports on the separation and isolation of the photoreaction products of biphenyl, the identification of many of them as hydroxynitrobiphenyls, and their mutagenicities.

Experimental

Chemicals——Biphenyl, *o*-phenylphenol, *p*-phenylphenol and 4,4′-dihydroxybiphenyl were obtained from Tokyo Kasei Co. Sodium nitrate, diethyl ether (peroxide-free) and other chemicals were obtained from Kanto Kagaku Co. All these chemicals were of the highest grade commercially available and were used without further purification. 2-Hydroxy-3-nitrobiphenyl and 2-hydroxy-3,5-dinitrobiphenyl were synthesized by the nitration (with nitric acid and glacial acetic acid) of *o*-phenylphenol[8] and purified on Merck pre-coated thin layer chromatography

(TLC) plates, polyamide 11 F254 (solvent, acetone–ether (7:3)). 4-Hydroxy-3-nitrobiphenyl and 4-hydroxy-3,5-dinitrobiphenyl were synthesized by the nitration of *p*-phenylphenol[8] and purified on Merck pre-coated TLC plates, Kiesel gel 60 F254 (solvent, benzene–ethyl acetate (4:1)). 4,4′-Dihydroxy-3,3′-dinitrobiphenyl was synthesized by the nitration of 4,4′-dihydroxybiphenyl[8] and purified on polyamide 11 F254 TLC plates (solvent, chloroform).

The spectral data for the synthesized standard substances were as follows. 2-Hydroxy-3-nitrobiphenyl: liquid at room temp. Nuclear magnetic resonance (NMR) (in $CDCl_3$) δ: 7.02 (1H, dd, $J_{4,5}=J_{5,6}=8$ Hz, 5-H), 7.45 (5H, m, phenyl-H), 7.60 (1H, dd, $J_{5,6}=8$ Hz, $J_{4,6}=2$ Hz, 6-H), 8.10 (1H, dd, $J_{4,5}=8$ Hz, $J_{4,6}=2$ Hz, 4-H), 11.13 (1H, s, 2-OH), mass spectrum (MS) *m/e*: 215 (62%, M^+), 198 (48%, M^+-OH), 168 (100%, $M^+-OH-NO$), 152 (11%, $M^+-OH-NO_2$), 139 (44%, $M^+-OH-NO-CHO$), 115 (32%), 63 (13%), 30 (17%). 2-Hydroxy-3,5-dinitrobiphenyl: mp 211—212 °C. NMR (in $CDCl_3$) δ: 7.50 (5H, m, phenyl-H), 8.46 (1H, d, $J_{4,6}=2$ Hz, 6-H), 9.02 (1H, d, $J_{4,6}=2$ Hz, 4-H), 11.59 (1H, s, 2-OH). MS *m/e*: 260 (84%, M^+), 243 (100%, M^+-OH), 213 (50%, $M^+-OH-NO$), 197 (18%, $M^+-OH-NO_2$), 167 (33%, $M^+-OH-NO-NO_2$), 139 (81%, M^+-2NO_2-CHO), 105 (20%), 77 (28%), 63 (20%), 30 (14%). 4-Hydroxy-3-nitrobiphenyl: mp 69—71 °C. NMR (in $CDCl_3$) δ: 7.20 (1H, d, $J_{5,6}=8$ Hz, 5-H), 7.45 (5H, m, phenyl-H), 7.79 (1H, dd, $J_{5,6}=8$ Hz, $J_{2,6}=2$ Hz, 6-H), 8.28 (1H, d, $J_{2,6}=2$ Hz, 2-H), 10.58 (1H, s, 4-OH). MS *m/e*: 215 (100%, M^+), 168 (11%, $M^+-OH-NO$), 139 (26%, $M^+-OH-NO-CHO$), 115 (14%), 63 (6%), 30 (4%). 4-Hydroxy-3,5-dinitrobiphenyl: mp 195—205 °C (dec.). NMR (in $CDCl_3$) δ: 7.50 (5H, m, phenyl-H), 8.48 (2H, s, 2-, 6-H), 11.40 (1H, s, 4-OH). MS *m/e*: 260 (100%, M^+), 168 (8%, M^+-2NO_2), 139 (44%, M^+-2NO_2-CHO), 105 (22%), 63 (12%), 30 (6%). 4,4′-Dihydroxy-3,3′-dinitrobiphenyl: mp 270—274 °C. NMR (in $CDCl_3$) δ: 7.24 (2H, d, $J_{5,6}=8$ Hz, 5-, 5′-H), 7.78 (2H, dd, $J_{5,6}=8$ Hz, $J_{2,6}=2$ Hz, 6-, 6′-H), 8.26 (2H, d, $J_{2,6}=2$ Hz, 2-, 2′-H), 10.67 (2H, s, 4-, 4′-OH). MS *m/e*: 276 (100%, M^+), 230 (10%, M^+-NO_2), 184 (10%, M^+-2NO_2), 155 (12%), 126 (19%), 115 (10%), 63 (18%).

Ultraviolet (UV) Irradiation——The silica gel carrier (Wako gel Q22, 500 mg) coated with biphenyl (50 mg) was suspended in 500 ml of neutral aqueous nitrate solution ($NaNO_3$, 1%) in a reaction vessel. UV light was obtained

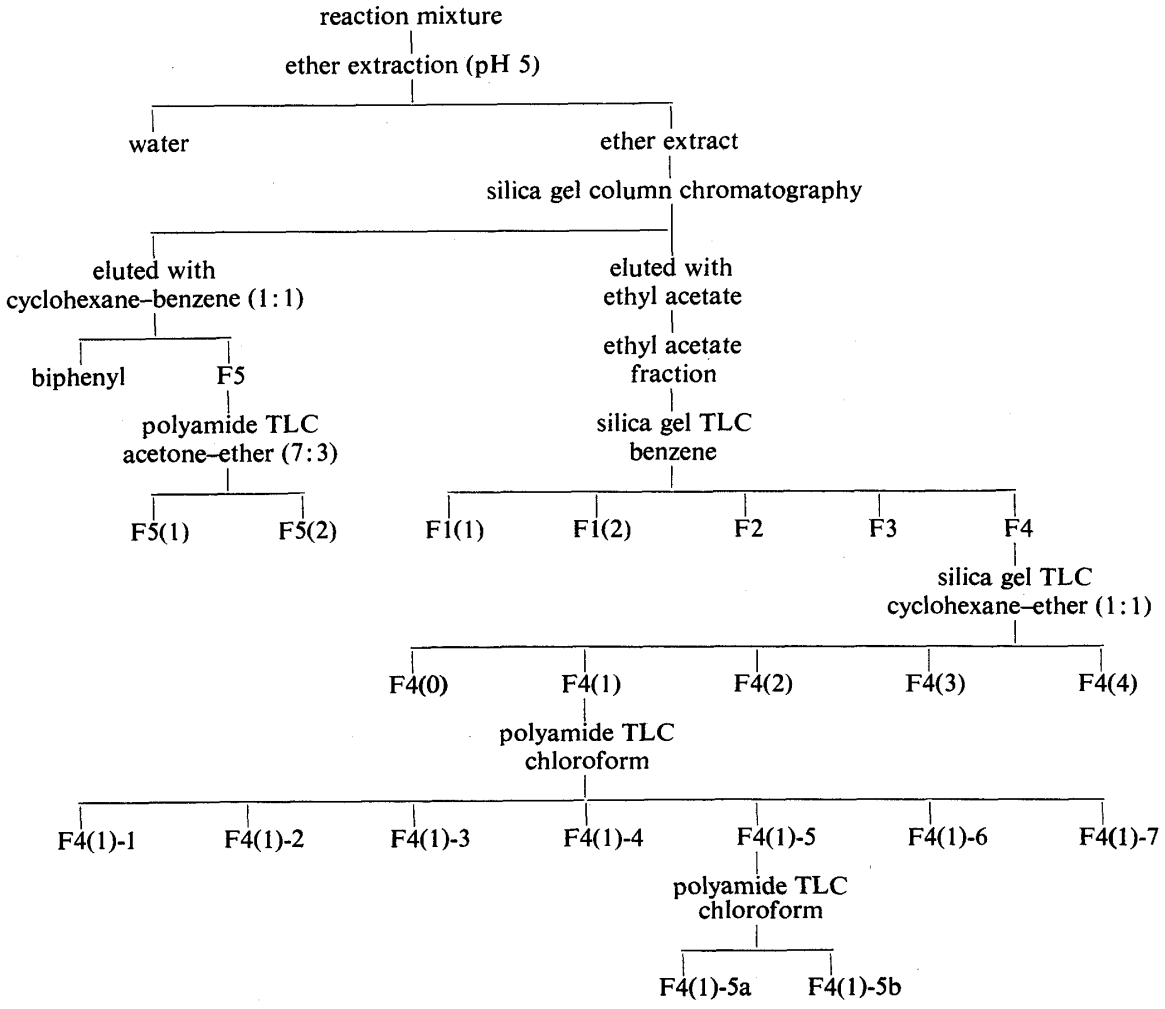


Chart 1. Fractionation Scheme for the Ether Extract of the Photoreaction Mixture of Biphenyl

from a 100 W high-pressure mercury lamp, UVL-100HA (Riko Kagaku Sangyo Inc.) with maximal energy output at 365 nm. The lamp, equipped with a quartz glass tube for circulation of cooling water, was placed inside the reaction vessel, and UV irradiation was performed at 25 °C with stirring. Other details of the UV irradiation were as described in the previous paper.[7]

Separation and Isolation of Reaction Products——The reaction mixture containing the carrier was acidified (pH 5) with 0.01 N HCl and then extracted with ethyl ether (100 ml × 2). The extract was evaporated to dryness under reduced pressure. Unreacted biphenyl and predominant yellow components (F5) in the extract were first separated chromatographically with cyclohexane–benzene (1 : 1) on a silica gel column (size 2.2 cm i.d. × 45 cm) packed with Wako gel Q22 activated for 4 h at 100 °C, and then the material retained on the column was eluted with ethyl acetate. The components of the ethyl acetate fraction and F5 were further separated and isolated by TLC using Merck pre-coated plates of Kiesel gel 60 F254 and polyamide 11 F254, as shown in Chart 1. The compounds separated on the silica gel plate and polyamide plate were recovered with ethyl acetate and chloroform, respectively.

Instrumental Analyses——NMR and MS of the synthesized standard substances and the isolated substances were obtained with a JNM-FX 100 NMR spectrometer (in $CDCl_3$, internal standard tetramethylsilane), and a Hitachi M-80 double-focussing gas chromatograph (GC)-mass spectrometer operating at 70 eV, respectively.

Mutation Assay——Bacterial strains used were *Salmonella typhimurium* TA98 and TA100. The mutation test was performed by Ames' method with some modifications including a step of preincubation of the test materials with S-9 mix and tester strain for 20 min at 37 °C.[9,10] The S-9 fraction was prepared from the liver of PCB-treated male rats as described by Ames *et al.*[11] Each sample was assayed with 4 replicate plates at each dose level. Methanol was used as a solvent for all samples. Each sample was considered to be mutagenic when the number of revertant colonies observed at the dose level assayed was at least twice that of spontaneous revertants. The mean numbers of spontaneous revertants in each assay system were as follows: TA98 without S-9 mix (28 ± 11), TA98 with S-9 mix (38 ± 10), TA100 without S-9 mix (89 ± 20), TA100 with S-9 mix (106 ± 22).

High-Performance Liquid Chromatography (HPLC) Analysis of Reaction Products——After the irradiation, the reaction mixture was extracted with ether at pH 5. The ether layer was dried over anhydrous sodium sulfate, evaporated to dryness on a rotary evaporator and then dissolved in acetonitrile. After addition of 1 ml of internal standard solution (0.1% α-naphthoquinone in acetonitrile), the solution was made up to 10 ml with acetonitrile and subjected to HPLC analysis on a Shimadzu LC-4A. Biphenyl and reaction products were analyzed under the following HPLC conditions: column (at 50 °C), Zorbax ODS (4.6 mm i.d. × 150 mm); solvent, CH_3OH–H_2O gradient (CH_3OH 45—70%/20 min); flow rate, 1 ml/min; wavelength for detection, 254 nm.

Results

Separation of Reaction Products and Mutagenicity

The ether extract obtained from the reaction mixture was first separated into biphenyl, yellow F5 and ethyl acetate fractions by silica gel column chromatography. The ethyl acetate

Table I. Mutagenicities of the Six Fractions Obtained from the Ether Extract of the Photoreaction Mixture of Biphenyl by Silica gel Column Chromatography and TLC

Fraction	*Rf* value in TLC[b]	Yield[c] (mg)	Mutagenicity (Revertants/10 μg/plate)[a]			
			TA98		TA100	
			S-9(−)	S-9(+)	S-9(−)	S-9(+)
F5	0.58	6.8	4	2	3	2
F4	0.28—0.48	1.6	1505	1876	2957	1387
F3	0.18—0.20	3.8	27	96	231	302
F2	0.05—0.10	1.4	277	53	535	725
F1(2)	0.04	2.4	130	81	457	739
F1(1)	0 —0.03	7.5	345	423	744	789

a) Spontaneous revertants have been subtracted. *b*) Plate, Kieselgel 60 F254 (precoated plate); solvent, benzene. *c*) The original amount of ether extract, which was obtained from 5 reactions, was 64.3 mg. The low yields are due to unreacted biphenyl.

TABLE II. Mutagenicities of the Five Fractions Separated from F4 by TLC

Fraction	*Rf* value in TLC[b]	Yield[c] (mg)	Mutagenicity toward TA98 without S-9 mix (Revertants/10 μg/plate)[a]
F4(4)	0.75	0.7	7
F4(3)	0.63	1.0	52
F4(2)	0.48	1.2	164
F4(1)	0.06—0.40	2.8	1132
F4(0)	0	1.8	17

a) Spontaneous revertants have been subtracted. *b*) Plate, Kieselgel 60 F254 (precoated plate); solvent, cyclohexane–ether (1 : 1). *c*) The original amount of F4 fraction was 8.2 mg.

TABLE III. Mutagenicities of Seven Components Separated from F4(1) by TLC

Fraction	*Rf* value in TLC[b]	Yield[c] (mg)	Mutagenicity toward TA98 without S-9 mix (Revertants/10 μg/plate)[a]
F4(1)-1	0.86	1.0	0
F4(1)-2	0.67	1.5	8
F4(1)-3	0.59	1.0	0
F4(1)-4	0.55	1.1	78
F4(1)-5	0.33—0.40	0.8	675
F4(1)-6	0.20	1.2	1900
F4(1)-7	0.03	0.8	313

a) Spontaneous revertants have been subtracted. *b*) Plate, polyamide 11 F254 (precoated plate); solvent, chloroform. *c*) The original amount of F4(1) fraction was 10.3 mg.

fraction was further separated into five fractions by silica gel TLC. Table I shows the yields, the mutagenicities and the *Rf* values in TLC (solvent, benzene) of the six fractions (except the biphenyl fraction) which were obtained from 64.3 mg of the ether extract (reacted 5 times). Five fractions other than F5 had mutagenic activity. The F4 fraction (*Rf* 0.28—0.48) exhibited the highest mutagenic activity.

The F4 fraction was further fractionated into five fractions by silica gel TLC using cyclohexane–ether (1 : 1). Table II shows the *Rf* values, the yields and the mutagenicities of the five fractions obtained from 8.2 mg of F4. Three fractions, F4(1), F4(2) and F4(3) had mutagenic activity. Among them, the mutagenic activity of F4(1) was the strongest.

The mutagenic F4(1) was further separated into seven components by polyamide TLC using chloroform. Table III shows the *Rf* values, the yields and the mutagenicities of the seven components obtained from 10.3 mg of F4(1). Four components, F4(1)-4, F4(1)-5, F4(1)-6 and F4(1)-7 had mutagenic activity. Among them, F4(1)-6 showed the highest mutagenicity, that is, 1900 revertant colonies per 10 μg in *S. typhimurium* TA98 without S-9 mix.

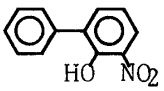
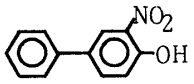
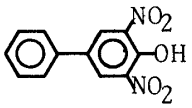
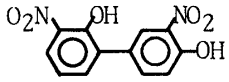
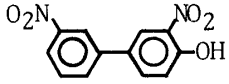
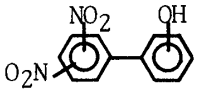
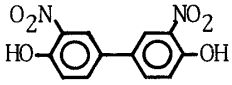
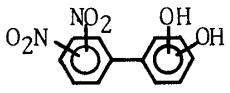
Identification of Isolated Products

Three components in Table III, F4(1)-3, F4(1)-4 and F4(1)-6, were each found to be a single compound on TLC using several solvent systems. From F4(1)-5, two compounds, F4(1)-5a (*Rf*, 0.40) and F4(1)-5b (*Rf*, 0.33), were isolated by re-development on polyamide TLC (solvent, chloroform). F4(1)-5a was a potent mutagen but was obtained in extremely small amount. F4(1)-5b accounted for a large portion of F4(1)-5 but was non-mutagenic. On the other hand, the F5 fraction (Table I), a major but non-mutagenic fraction of the reaction

mixture, was separated into two components, F5(1) (*Rf*, 0.82) and F5(2) (*Rf*, 0.61), by polyamide TLC (solvent, acetone–ether (7 : 3)). F1(2) in Table I was also purified by silica gel TLC (solvent, benzene–ethyl acetate (1 : 1)) to give a single compound F1(2)or.

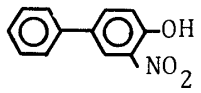
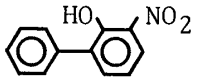
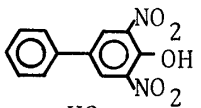
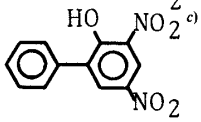
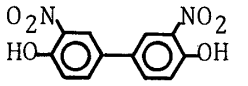
The eight isolated compounds, *i.e.*, F1(2)or, F4(1)-3, F4(1)-4, F4(1)-5a, F4(1)-5b, F4(1)-6, F5(1) and F5(2), were identified from their NMR and MS as follows. F1(2)or was determined to be 4-hydroxy-3,5-dinitrobiphenyl, because the spectral data coincided with those of the synthesized standard. The mutagenic activity was also confirmed to be the same as that of the standard. F4(1)-3 was determined to be 4,2′-dihydroxy-3,3′-dinitrobiphenyl from the following data: NMR (in $CDCl_3$) δ: 7.06 (1H, dd, $J_{4',5'}=J_{5',6'}=8$ Hz, 5′-H), 7.24 (1H, d, $J_{5,6}=8$ Hz, 5-H), 7.62 (1H, dd, $J_{5',6'}=8$ Hz, $J_{4',6'}=2$ Hz, 6′-H), 7.82 (1H, dd, $J_{5,6}=8$ Hz, $J_{2,6}=2$ Hz, 6-H), 8.14 (1H, dd, $J_{4',5'}=8$ Hz, $J_{4',6'}=2$ Hz, 4′-H), 8.30 (1H, d, $J_{2,6}=2$ Hz, 2-H), 10.67 (1H, s, 4-OH), 11.19 (1H, s, 2′-OH). MS *m/e*: 276 (100%, M^+), 260 (14%), 230 (7%, M^+-NO_2), 213 (6%, M^+-NO_2-OH), 184 (8%, M^+-2NO_2), 167 (3%, M^+-2NO_2-OH), 155 (12%), 126 (21%), 77 (9%), 30 (11%). The mutagenic F4(1)-4 was determined to be 4-hydroxy-3,3′-dinitrobiphenyl from the following data: NMR (in $CDCl_3$) δ: 7.28 (1H, d, $J_{5,6}=8$ Hz, 5-H), 7.62 (1H, dd, $J_{5',6'}=J_{4',5'}=8$ Hz, 5′-H), 7.84 (1H, dd, $J_{5,6}=8$ Hz, $J_{2,6}=2$ Hz, 6-H), 7.86 (1H, ddd, $J_{5',6'}=8$ Hz, $J_{4',6'}=J_{2',6'}=2$ Hz, 6′-H), 8.22 (1H, ddd,

TABLE IV. Mutagenicities and Structures of Compounds Isolated from the Ether Extract of the Photoreaction Mixture

Fraction	Structure	Mutagenicity toward TA98 without S-9 mix (Revertants/10 μg/plate)[a]
F5(1)		3
F5(2)		5
F1(2)or		15
F4(1)-3		0
F4(1)-4		78
F4(1)-5a		675[b]
F4(1)-5b		0
F4(1)-6		1900

a) Spontaneous revertants have been subtracted. *b*) The yield of F4(1)-5a fraction was too small to permit accurate determination of the mutagenicity.

TABLE V. Mutagenicities and Structures of Standard Compounds Synthesized from Phenylphenols

Compound	Mutagenicity (Revertants/100 μg/plate)[a]			
	TA98		TA100	
	S-9(−)	S-9(+)	S-9(−)	S-9(+)
	0	13	Tox[b]	0
	Tox	2	Tox	33
	99	35	320	323
	Tox	8	21	28
	2	11	5	0

a) Spontaneous revertants have been subtracted. *b*) Tox indicates toxic effect toward tester strains. *c*) This compound, 2-hydroxy-3,5-dinitrobiphenyl, was not found in the reaction products.

$J_{4',5'}=8$ Hz, $J_{4',6'}=J_{2',4'}=2$ Hz, 4′-H), 8.34 (1H, d, $J_{2,6}=2$ Hz, 2-H), 8.39 (1H, dd, $J_{2',4'}=J_{2',6'}=2$ Hz, 2′-H), 10.65 (1H, s, 4-OH). MS *m/e*: 260 (100%, M^+), 214 (5%, M^+-NO_2), 168 (16%, M^+-2NO_2), 139 (42%, M^+-2NO_2-CHO), 63 (7%), 30 (5%). The strongly mutagenic compound, F4(1)-5a, was considered from the MS to be a hydroxydinitrobiphenyl: MS *m/e*: 260 (100%, M^+), 230 (14%, M^+-NO), 184 (6%, $M^+-NO-NO_2$), 168 (15%, M^+-2NO_2), 139 (35%, M^+-2NO_2-CHO). F4(1)-5a was obtained in so small an amount that its NMR spectrum could not be obtained, and accurate estimation of the specific mutagenic activity was impossible. F4(1)-5b was determined to be 4,4′-dihydroxy-3,3′-dinitrobiphenyl, because its spectral data coincided with those of the synthesized standard. The most strongly mutagenic compound, F4(1)-6, which seemed to be the major mutagen in the reaction products, was determined from the MS to be a dihydroxydinitrobiphenyl: MS *m/e*: 276 (100%, M^+), 246 (15%, M^+-NO), 218 (14%), 184 (8%, M^+-2NO_2), 155 (10%, M^+-2NO_2-CHO), 149 (14%), 126 (15%, M^+-2NO_2-2CHO), 57 (23%), 30 (30%). However, the NMR spectrum was so unclear that unambiguous assignment of the molecular structure was difficult. The major products F5(1) and F5(2) were determined to be 2-hydroxy-3-nitrobiphenyl and 4-hydroxy-3-nitrobiphenyl, respectively, because their NMR and MS coincided with those of the synthesized standards. The structures and the mutagenicities of the identified compounds are summarized in Table IV. For reference, those of the synthesized standards are given in Table V.

HPLC Analysis of Reaction Products

Figure 1 shows the HPLC chromatogram of the ether extract obtained from the reaction mixture of biphenyl irradiated for 3 h in aqueous nitrate solution (biphenyl coated on silica gel carrier, 50 ppm; sodium nitrate, 100 ppm). The retention times of peaks a (18.0 min), b

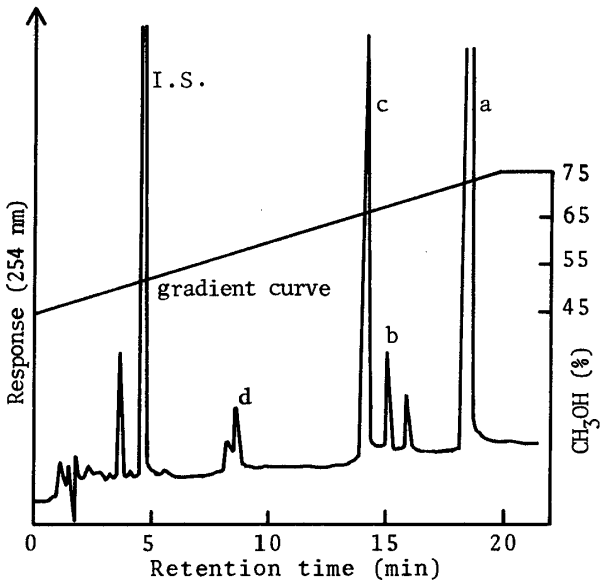


Fig. 1. HPLC Chromatogram of the Ether Extract of the Reaction Mixture of Biphenyl Irradiated for 3 h in Aqueous Nitrate Solution

Column, Zorbax ODS (4.6 mm i.d. × 15 cm); solvent, CH_3OH–H_2O gradient (CH_3OH 45—75%/20 min); I.S., internal standard.

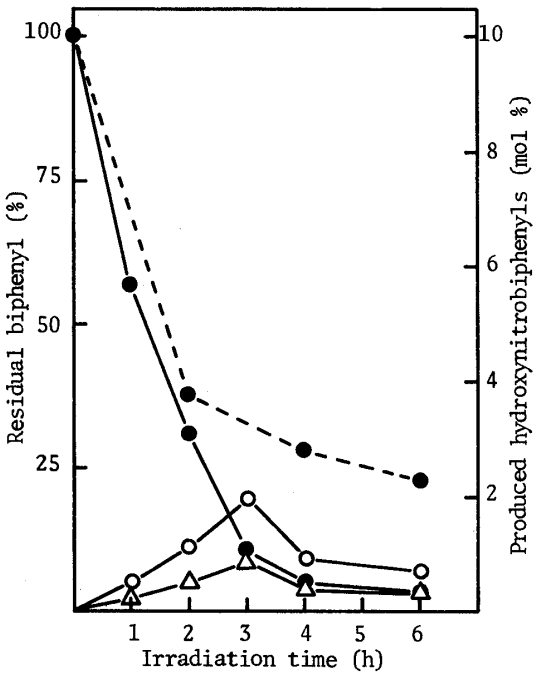


Fig. 2. Time Course of Photoreaction of Biphenyl in Neutral Aqueous Nitrate Solution ($NaNO_3$, 100 ppm)

The proportion (%) of residual biphenyl with respect to the original amount (0.3 mM) (●), and the proportions (mol%) of 4-hydroxy-3-nitrobiphenyl (○) and 2-hydroxy-3-nitrobiphenyl (△) produced with respect to the original biphenyl are shown. The broken line shows the photoreaction of biphenyl in nitrate-free aqueous solution.

(15.0 min) and c (14.0 min) coincided with those of authentic biphenyl, 2-hydroxy-3-nitrobiphenyl (2H3NBP) and 4-hydroxy-3-nitrobiphenyl (4H3NBP), respectively. Hydroxydinitrobiphenyls or dihydroxydinitrobiphenyls were eluted in the neighborhood of peak d. However, they were not separated from each other, and hence it was impossible to determine them. The results of determination of biphenyl and nitrophenylphenols (2H3NBP and 4H3NBP) are shown in Fig. 2. Biphenyl disappeared rapidly with increase of the irradiation time, and nitrophenylphenols were gradualy accumulated up to 3 h and decreased during further irradiation. The production rate of 4H3NBP was about twice that of 2H3NBP. However, the amount of both nitrophenylphenols was only a few percent of the degraded biphenyl even at the time of maximal production, suggesting that the produced nitrophenylphenols were further hydroxylated and nitrated. As shown in Fig. 2, biphenyl disappeared on irradiation even in nitrate-free aqueous solution. A small amount of phenylphenols was detected by HPLC, although these compounds were not detected in the photoreaction in aqueous nitrate solution.

Discussion

As can be seen in Table IV, the identified mutagens were all dinitro compounds, although the molecular structures of two mutagenic dinitro compounds, F4(1)-5a and F4(1)-6, could not be determined. However, 4,2′-dihydroxy-3,3′-dinitrobiphenyl (F4(1)-3) and 4,4′-dihydroxy-3,3′-dinitrobiphenyl (F4(1)-5b) exhibited no mutagenicity even though they apparently resemble F4(1)-4, 4-hydroxy-3,3′-dinitrobiphenyl. This indicates that the ad-

ditional hydroxyl group decreases the mutagenicity. On the other hand, synthesized 2-hydroxy-3,5-dinitrobiphenyl did not exhibit mutagenicity, unlike 4-hydroxy-3,5-dinitrobiphenyl, F1(2)or (see Tables IV and V). This indicates that the mutagenicities of hydroxynitrobiphenyls are also affected by the position of the hydroxyl group. The major products, 4-hydroxy-3-nitrobiphenyl and 2-hydroxy-3-nitrobiphenyl, were not mutagenic, and two dihydroxydinitrobiphenyls, F4(1)-3 and F4(1)-5b, which are composed of the same substituted phenyl groups, were also not mutagenic. In addition, none of the nitro compounds synthesized from *o*-phenylphenol, *p*-phenylphenol and 4,4′-dihydroxybiphenyl exhibited such strong mutagenicity as the photoreaction products. Accordingly, the most strongly mutagenic dihydroxydinitrobiphenyl, F4(1)-6, is presumed to contain substituted phenyl groups different from those of the major products, *e.g.*, possibly a dinitrophenyl group and a dihydroxyphenyl group.

On the other hand, it is noteworthy that all the substances identified in this study were hydroxynitrobiphenyls and that no simple nitrobiphenyl was observed. Fukui *et al.* have reported that the photoreaction of biphenyl with nitrogen monoxide in the gas phase gave mono- and dinitrobiphenyls.[4] The production of hydroxynitrobiphenyls may be a characteristic of the photochemical reaction in the aqueous phase.

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.[12,13] These radicals may be responsible for the production of hydroxynitrobiphenyl. As reported previously, the major product in the photoreaction of phenol in aqueous nitrite solution was *p*-nitrosophenol, and the reaction could be explained in terms of nitrosation with NO radical after hydrogen abstraction by OH radical.[14] If the hydrogen abstraction by OH radical occurred first in the present reaction of biphenyl in aqueous nitrate solution, a simple nitrobiphenyl should have been found among the reaction products. However, the reaction products identified were all hydroxynitrobiphenyls.

On the other hand, biphenyl was found to be degraded by UV irradiation even in nitrate-free aqueous solution, as shown in Fig. 2. Furthermore, the production of phenylphenols was confirmed by HPLC. This result indicates that the hydroxylation of biphenyl occurred in preference to nitration with NO_2 radical in aqueous nitrate solution. Although the mechanism of the hydroxylation of biphenyl in aqueous solution is unclear, the produced phenylphenol should be nitrated smoothly to hydroxynitrobiphenyl by the OH and NO_2 radicals formed by the photolysis of NO_3^- in aqueous nitrate solution.

The production of hydroxynitroaromatics has not previously been reported in the photoreaction of polycyclic aromatics with nitrogen oxides in the gas phase. However, hydroxynitroaromatics may be produced by photoreaction not only in aqueous solution but also in the atmosphere, because the atmosphere contains some moisture. Accordingly, hydroxynitroaromatic compounds must be considered as possible environmental mutagens, as well as nitroaromatics.

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