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The Photoreactions of Phenazines *via* a Semiquinone Radical. The Photoreductive Deacylation of 1- or 2-Acyloxyphenazine[1]

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The Photo-irradiation of a solution of 1- or 2-benzoyloxyphenazine in 2-propanol, followed by aeration, affords 1- or 2-hydroxyphenazine, benzaldehyde, isopropyl benzoate, and/or benzoic acid as the reaction products. The spectral change of the solution in the reaction process gives evidence of the fission of the acyl-to-oxygen bond. The addition of ferric chloride or dimethyl sulfate to the reaction system containing 1-benzoyloxyphenazine in 2-propanol leads to the formation of isopropyl benzoate. When methanol instead of 2-propanol is used as the solvent, methyl benzoate is produced mainly. In cyclohexene as a hydrogen-donating solvent, benzaldehyde and an oxetane are obtained. The irradiation of a solution of 1-benzoyloxyphenazine in *t*-butyl alcohol or benzene gives no product, and the starting material is recovered in a 90—100% yield. 1-Benzoyloxyphenazhydrin, a photo-reduction intermediate, does not contribute to the formation of benzaldehyde. The intermediary formation of a semiquinoid form during irradiation brings about the fission of the acyl-to-oxygen bond, thus affording the corresponding aldehyde.

The oxidation-reduction through a semiquinone radical is the subject of considerable interest in relation to that in a biological system, particularly in flavoprotein catalysis, and in oxidation-reduction by dehydrogenases involving pyridine nucleotides.[2]

The photo-reduction of phenazine in alcoholic media produces the semiquinone radical (I) or molecular complexes (II) of phenazine and 5,10-dihydrophenazine of varying stoichiometry.[3,4] We attempted to investigate a reaction which proceeds through the intermediary

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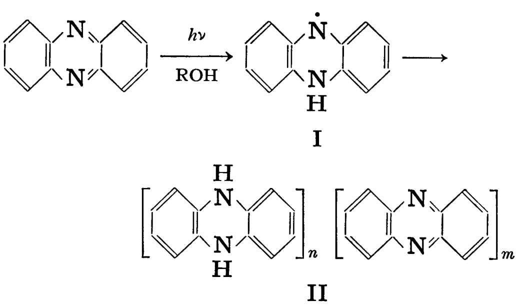
[1] Presented partly at the 22nd Annual Meeting of the Chemical Society of Japan, Tokyo, April 1969.

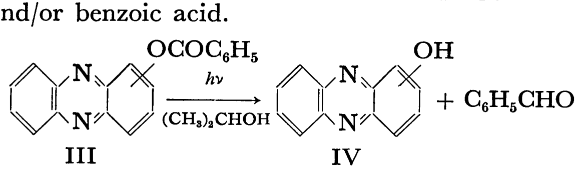
[2] H. R. Mahler and E. H. Cordes, "Biological Chemistry," Harper and Row, New York (1966), pp. 354, 575.

[3] D. N. Bailey, D. K. Roe, and D. M. Hercules, *J. Amer. Chem. Soc.*, **90**, 6291 (1968).

[4] G. A. Russell, R. Konaka, E. T. Strom, W. C. Danen, K. Y. Chang, and G. Kaupp, *ibid.*, **90**, 4646 (1968).



formation of the semiquinone radical about phenazines with a substituent. The present paper will indicate that the irradiation of a solution of 1- or 2-acyloxyphenazine in 2-propanol, followed by aeration, leads to the fission of the acyl-to-oxygen bond, thus affording 1- or 2-hydroxyphenazine (IV), and that in the case of 1- or 2-benzoyloxyphenazine (III), the benzoyl group is converted to benzaldehyde, isopropyl benzoate, and/or benzoic acid.



$+ C_6H_5COOCH(CH_3)_2 + C_6H_5COOH$

Experimental

Materials. 1- or 2-Hydroxyphenazine was synthesized by the method described in the literature.[5-7] 1-Hydroxyphenazine, mp 157—158°C. 2-Hydroxyphenazine, mp 253—254°C. The acyloxyphenazines were prepared by the reaction of acetic anhydride or benzoyl chloride with 1- or 2-hydroxyphenazine in pyridine, followed by recrystallization from ethanol.[8] 1-Acetoxyphenazine, mp 119—120°C. 2-Acetoxyphenazine, mp 151—152°C. 1-Benzoyloxyphenazine, mp 172—173°C.

Found: C, 75.95; H, 3.94; N, 9.61%. Calcd for $C_{19}H_{12}N_2O_2$: C, 75.99; H, 4.03; N, 9.33% 2-Benzoyloxyphenazine, mp 144—145°C.

Found: C, 75.95; H, 4.01; N, 9.05%. Calcd for $C_{19}H_{12}N_2O_2$: C, 75.99; H, 4.03; N, 9.33%.

The benzaldehyde, isopropyl benzoate, methyl benzoate, anhydrous ferric chloride, and dimethyl sulfate were purchased from commercial sources. The methanol, 2-propanol, *t*-butyl alcohol and cyclohexene used as solvents were purified by distilling them two times on anhydrous cupric sulfate, calcium oxide, calcium oxide and sodium respectively.

General Procedure of Photoreaction. Spectral Change: The reactions were carried out in a quartz spectrophotometer cell which was irradiated with light from a 100-W high-pressure mercury arc lamp at a distance of 6 cm and at room temperature. The lamp characteristics, and the procedure for carrying out the reactions, were the same as have been described in detail in a previous paper.[9] At the concentration of 1- or 2-acetoxyphenazine of $(1—1.5)\times 10^{-4}$ mol/*l*, the change in the visible absorption spectra corresponding to substrate-disappearance or product-appearance was followed with the period of irradiation. After the reaction, oxygen was admitted into the reaction system, and the spectra of the resulting solution were measured.

Photoreaction: The photoreactions were carried out by the methods described in a previous paper.[10] A dispersed solution of $(3—5)\times 10^{-5}$ mol of 1- or 2-benzoyloxyphenazine in 10 m*l* of 2-propanol, unless otherwise stated, was placed in a quartz or Pyrex tube with an inside diameter of 15—20 mm and a wall thickness of 1 mm, and was cooled with liquid nitrogen; the air was removed under the reduced pressure of 1 mmHg, and then the tube was filled with nitrogen gas. The procedure of the replacement by nitrogen gas was repeated 4—5 times. The tube was then sealed and irradiated with a 100-W high-pressure mercury arc lamp in a distance of 4 cm and at room temperature. In order to isolate the reaction products, another procedure was attempted on a large scale by using a cylinderical, 300-m*l* reaction vessel.

Analyses of Products. After the reaction, the visible absorption spectrum of the reaction mixture was measured. The amount of 1- or 2-hydroxyphenazine produced was determined from the optical density of the absorption at 440 mμ, where the starting material has no absorption. After measuring the spectrum, the solution was concentrated to about one-tenth of the original volume in order to analyze the products. The benzaldehyde and isopropyl benzoate were identified by a comparison of their retention times in gas chromatography with those of authentic materials. The amounts of these compounds were determined by means of gas chromatography (gc), using a Yanagimoto 5 DH gas chromatograph; a column containing polyethylene glycol-20M and/or silicone grease-30 was used. Helium gas was used as the carrier gas. Benzoic acid was converted to methyl benzoate by introducing diazomethane into a part of the concentrated solution,[11] after measuring the amounts of benzaldehyde and isopropyl benzoate, and was determined by measuring the amount of methyl benzoate by gc analysis.

Isolation of 1-Hydroxyphenazine. A dispersed solution of 0.4 g of 1-benzoyloxyphenazine in 250 m*l* of 2-propanol, containing partially the crystal of 1-benzoyloxyphenazine, was placed in a cylinderical, 300-m*l* reaction vessel, and then the vessel was irradiated with a 100-W high-pressure mercury arc lamp for 100 hr after nitrogen gas had been bubbled into the solution. The resulting solution was concentrated up to a half volume *in vacuo* at room temperature, and the concentrated solution was poured into about 500 m*l* of water. A white precipitate deposited (0.15 g) was collected with filtration; subsequent recrystallization from ethanol afforded 1-hydroxyphenazine (mp 152—154°C), the IR spectrum of which was identical with that of the authentic meterial.

1-Benzoyloxy-5,10-dihydrophenazine (VI) and Its Phenazhydrin (V). An aqueous saturated solution of sodium hydrosulfite was added to a solution of 30 mg of 1-benzoyloxyphenazine in ethanol. A white powder precipitated. The precipitate (VI) (25 mg) was filtered off, washed repeatedly with water, and dried *in vacuo*. Immediately after the powder had been dried, it was submitted to the reaction, for it was unstable in air. The IR spectrum of the powder (KBr) is shown in Fig. 4. When the powder stood for 2—3 days, it changed to a greenish powder (V), the IR spectrum of which

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[5] A. R. Surrey, "Organic Syntheses," Coll. Vol. III, p. 753 (1955).

[6] I. Yosioka, *J. Pharm. Soc. Jap.*, **72**, 1128 (1952).

[7] E. Matsumura and H. Takeda, *Nippon Kagaku Zasshi*, **81**, 515 (1960).

[8] F. Wrede and E. Strack, *Z. Physiol. Chem.*, **140**, 1 (1924); F. Kehrmann and F. Cherpillod, *Helv. Chim. Acta*, **7**, 973 (1924).

[9] H. Inoue, K. Tamaki, N. Komakine, and E. Imoto, This Bulletin, **39**, 1577 (1966).

[10] H. Inoue, K. Komakine, and E. Imoto, *ibid.*, **41**, 2726 (1968).

[11] M. Maruyama, "Zoku Jikken Kagaku Koza," Vol. 9, Maruzen, Tokyo (1965), p. 340.

had the absorptions at 3300 and 3400 cm^{-1} characteristic of phenazhydrin.[12]

Results and Discussion

Spectral Aspects. 1-Acetoxyphenazine was used for the measurement of the spectral change under irradiation with ultraviolet light, since it is more easily soluble in 2-propanol than 1-benzoyloxyphenazine. A solution of 1-acetoxyphenazine in 2-propanol, which had been placed in a quartz cell, was irradiated with light after degassing under the reduced pressure of 10^{-5} mmHg. As is shown in Fig. 1, the absorption of 1-acetoxyphenazine at 363 mμ disappeared rapidly and a new broad band appeared in the 490—500 mμ region. The new broad band also disappeared gradually. Immediately after irradiating for 5 min, oxygen gas was admitted to the system. The visible absorption spectrum of the resulting solution had absorptions at 368 and 420—430 mμ, which agreed with those of 1-hydroxyphenazine, as is shown in Fig. 1. The results show that 1-hydroxyphenazine is obtained quantitatively. This means that the acetyl group is cleaved, but does not rearrange on the phenazine ring during the photo-reduction or the oxidation process. Furthermore, the absorption in the 490—500 mμ region may be attributed to that of a phenazhydrin-type compound, such as a molecular complex between 1-hydroxyphenazine or its keto form and 1-hydroxy-5,10-dihydrophenazine. The irradiation of 1-acetoxyphenazine in methanol also showed a spectral change similar to that in the case of 2-propanol.

The spectral change of 2-acetoxyphenazine in 2-propanol by irradiation with ultraviolet light was similar to that of 1-acetoxyphenazine. However, the absorption in the 490—500 mμ region in the case of 2-acetoxyphenazine became larger than that of 1-acetoxyphenazine, indicating the easier formation of a phenazhydrin-type compound, as is shown in Fig. 2. Immediately after the large peak appeared, the introduction of oxygen resulted in an absorption spectrum represented by the sum of the spectra of 2-acetoxyphenazine and 2-hydroxyphenazine. For example, the introduction of oxygen after irradiating for 11 min, resulted in the molar ratio of 2-acetoxyphenazine to 2-hydroxyphenazine of 3/7. This must mean that the phenazhydrin-type compound contains either 2-acetoxyphenazine or its dihydrophenazine. The difference between the optical densities at 490—500 mμ in the spectral changes of 1- and 2-acetoxyphenazine may be caused by the difference in the ease of the formation of their phenazhydrin-type compounds. The oxidation after the absorption at 490—500 mμ region disappeared completely resulted in the generation of the absorption spectrum of the keto form of 2-hydroxyphenazine, which has been reported by Badger, Pearce and Pettit,[13] as is shown in Fig. 2. Thus, also in the case of 2-acetoxyphenazine, it was confirmed that the fission of the acyl-to-oxygen bond occurs during irradiation with ultraviolet light. However, the acetyl group of 1-acetoxyphenazine was removed more readily than that of 2-acetoxyphenazine. This was caused by the greater difficulty of reducing a semiquinone radical of 1-acetoxyphenazine to its dihydrophenazine as compared with the case of 2-acetoxyphenazine because of the steric retardation of the acetoxy group of 1-acetoxyphenazine.

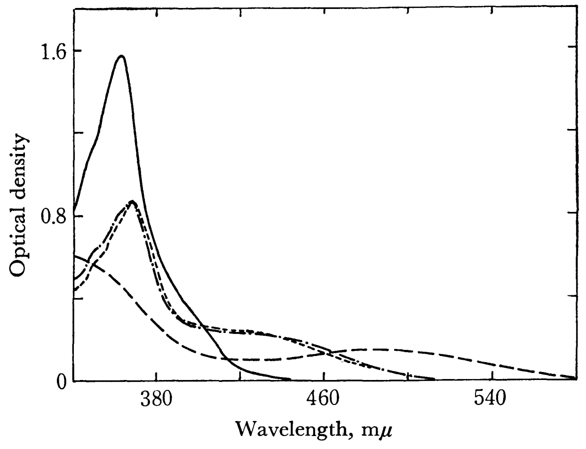

Fig. 1. Spectral change of 1-acetoxyphenazine in 2-propanol under irradiation of ultraviolet light: Concentration of 1-acetoxyphenazine; 1.22 × 10^{-4} mol/*l*.
—— Before irradiation, - - - after 5 min of irradiation, —·— after 1 hr of oxidation after irradiating for 5 min and ------ spectrum of 1-hydroxyphenazine after 15 min of irradiation, followed by oxidation.

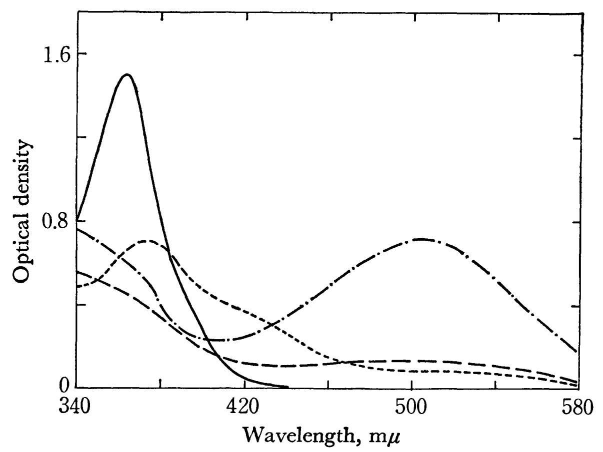

Fig. 2. Spectral change of 2-acetoxyphenazine in 2-propanol under irradiation of ultraviolet light: Concentration of 2-acetoxyphenazine; 1.18 × 10^{-4} mol/*l*.
—— Before irradiation, —·— after 11 min of irradiation, — — after 3 hr of irradiation and ------ after 1 hr of oxidation after irradiating for 3 hr.
The shape of the absorption spectrum of ----- was similar with that of the keto form of 2-hydroxyphenazine which has the extension of the absorption as far as about 540 mμ.

Photo-reduction of 1-Benzoyloxyphenazine. The benzoyl group was selected as the acyl group in order to make easier analyses of the photoreaction products. A dispersed solution of 1-benzoyloxyphenazine in 2-propanol was degassed in a Pyrex vessel, irradiated with light at room temperature, and then aerated. The yields of the reaction products are shown in Table 1.

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[12] A. Sugimoto, Y. Yokota, S. Kusaka, H. Inoue, and E. Imoto, to be published.

[13] G. M. Badger, R. S. Pearce, and R. Pettit, *J. Chem. Soc.*, **1951**, 3204.

TABLE 1. PHOTO-REDUCTION OF 1-BENZOYLOXYPHENAZINE IN ALCOHOLS AND SUBSEQUENT OXIDATION[a)]

ROH	Irradiated time, hr	Yield of product (%)			
		1-HOPh[b)]	C_6H_5-CHO	C_6H_5-COOR	C_6H_5-COOH
$(CH_3)_3COH$	160	10	0	0	0
$(CH_3)_2CHOH$	260	72	14	9	20—30
CH_3OH	140	55	0	40	5—10

a) The amount of 1-benzoyloxyphenazine was (4.4—4.6) × 10^{-5} mol in 10 m*l* of alcohols. Irradiated distance: 4 cm. Reaction vessel: Pyrex glass.

b) 1-HOPh means 1-hydroxyphenazine.

The spectral change in 1-benzoyloxyphenazine was similar to the results obtained by directly irradiating a solution of 1-acetoxyphenazine in 2-propanol with ultraviolet light for a short time, as has been described above. The spectral yield of 1-hydroxyphenazine, as calculated from the optical density of the absorption at 440 mμ, was 72%; the analytical yields of benzaldehyde, isopropyl benzoate, and benzoic acid by gc analysis were 14, 9, and 20—30% respectively. The generation of gas was not observed. In addition to these products, the presence of pinacol and acetone was confirmed by gc. The formation of pinacol and acetone indicates the occurence of the photo-reduction of 1-benzoyloxyphenazine. Furthermore, 1-hydroxyphenazine was isolated from the residue after distilling out the resulting solution, and was identified by a comparison with the IR spectrum of the authentic material. The formation of bezaldehyde means that the homolytic fission of the acyl-to-oxygen bond occurs in the photo-reduction process.

The yields of benzaldehyde and benzoate changed depending on the kinds of alcohols used as the solvents, as Table 1 shows. When methanol was used as the solvent, methyl benzoate was obtained as the main product. On the irradiation of 1-benzoyloxyphenazine in *t*-butyl alcohol for 160 hr, benzaldehyde and *t*-butyl benzoate were not obtained, but 1-benzoyloxyphenazine was recovered in a 90—100% yield. That is, the requirement for the formation of benzaldehyde is that the solvent is a hydrogen donor. Furthermore, a protic solvent tended to decrease the yield of benzaldehyde and, contrarily, to increase that of the corresponding ester.

When 1-benzoyloxyphenazine in cyclohexene with a hydrogen-donating property was irradiated with light, an oxetane (8-phenyl-7-oxabicyclo[4.2.0]octane),[14)] benzaldehyde, bicyclohexenyl, and 1-hydroxyphenazine were confirmed by gc analysis, though the amount of the oxetane could not be analyzed quantitatively because it is unstable to heat. The results are shown in Fig. 3. The formation of the oxetane supports the idea that benzaldehyde is produced during the irradiation of 1-benzoyloxyphenazine in a hydrogen-donating solvent.

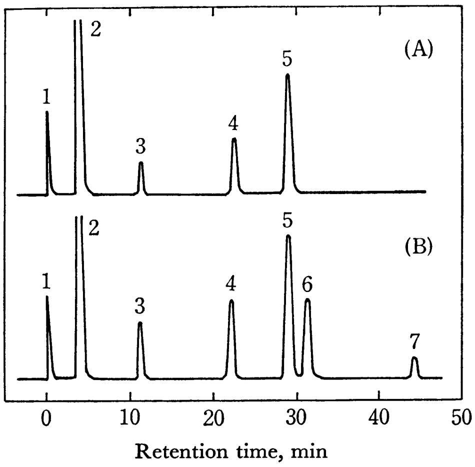
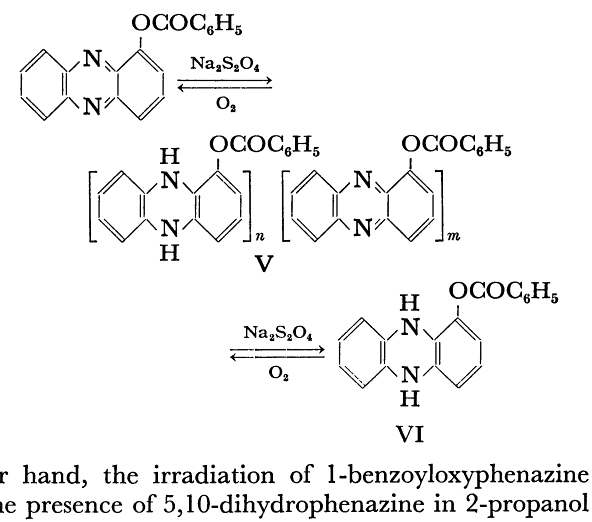


Fig. 3. Gas chromatogram of the products in the photoreaction of 1-benzoyloxyphenazine(A) or benzaldehyde(B) in cyclohexene: Conditions; absorbent, 150 cm, high-vacuum silicone grease-30; carrier gas, helium gas; programmed temperature rate, 6°C/min and temperature range, 80—320°C.

1 air, 2 cyclohexene, 3 benzaldehyde, 4 bicyclohexenyl, 5 8-phenyl-7-oxabicyclo[4.2.0]octane, 6 cyclohexenylphenylcarbinol, and 7 1,2-diphenyl-1,2-ethanediol.

The reduction of 1-benzoyloxyphenazine with aqueous sodium hydrosulfite did not result in the fission of the acyl-to-oxygen bond. That is, 1-benzoyloxyphenazine was reduced to 1-benzoyloxy-5,10-dihydrophenazine (VI) by an aqueous sodium hydrosulfite solution, and then the resulting solution was heated at about 80°C under a nitrogen atmosphere for 6 hr. The color of the solution changed from yellow to greenish-black. The oxidation of the solution with oxygen resulted in the recovery of 1-benzoyloxyphenazine. Furthermore, 1-benzoyloxyphenazhydrin (V) was prepared by the reduction of 1-benzoyloxyphenazine with an aqueous sodium hydrosulfite solution,[12)] and was characterized by its IR spectrum (Fig. 4), which has the absorptions of the –NH– group at 3300 and 3400 cm^{-1} and of the –CO–O– group at 1725 and 1740 cm^{-1}. The oxidation of V in 2-propanol with oxygen did not give benzaldehyde and isopropyl benzoate, but resulted in the quantitative recovery of 1-benzoyloxyphenazine. On the other hand, the irradiation of 1-benzoyloxyphenazine in the presence of 5,10-dihydrophenazine in 2-propanol for 140 hr gave 1-hydroxyphenazine and isopropyl benzoate in 35 and 5% yields respectively. A considerable amount of 1-benzoyloxyphenazine remained, although the amount was not obvious because of the contamination of phenazine. Thus, the formation of a phenazhydrin- or dihydrophenazine-type compound (V or VI), which is considered to be one of the photo-reduction



V

VI

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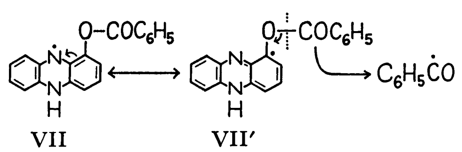
14) J. S. Bradshaw, *J. Org. Chem.*, **31**, 237 (1966).

products of 1-benzoyloxyphenazine, was ineffective in the fission of the acyl-to-oxygen bond. Therefore, the intermediary formation of a semiquinone radical during the photo-reduction must exert a profound effect on the fission of the acyl-to-oxygen bond. That is, the formation of benzaldehyde can be explained by a homolytic β-fission of the acyl-to-oxygen bond from a canonical structure (VII') of the semiquinone (VII):



VII VII'

The mechanism of the formation of the benzoate ester is not yet clear. However, the possibility of the formation of isopropyl benzoate from benzaldehyde diisopropyl acetal (VIII)[15] can be ruled out, since benzaldehyde and VIII in 2-propanol do not change to isopropyl benzoate under similar conditions. The mechanism of the formation of benzoic acid will be reported later.

$$C_6H_5CHO \longrightarrow \underset{\text{VIII}}{C_6H_5CH(OCH(CH_3)_2)_2} \xrightarrow{h\nu} C_6H_5COOCH(CH_3)_2$$

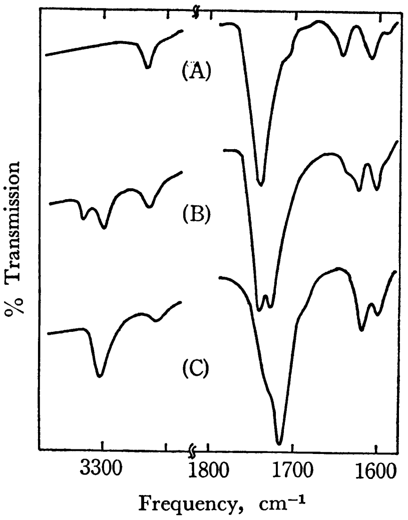


Fig. 4. Infrared spectra of 1-benzoyloxyphenazine (A), 1-benzoyloxyphenazhydrin (B), and 1-benzoyloxy-5,10-dihydrophenazine (C).

Effects of Ferric Chloride and Dimethyl Sulfate. When a solution of 1-acetoxyphenazine in 2-propanol containing ferric chloride with the molar ratio of 1: 1 was irradiated, the absorption of 1-acetoxyphenazine at 363 mμ disappeared rapidly; after irradiation for 2—3 min, a broad new peak appeared at 440 mμ similar to that of the semiquinoid form of pyocyanine.[16] This new band also disappeared upon further irradiation, leading to the appearance of the absorption at 480—490 mμ. After the absorption in the visible region had disappeared completely, oxidation was accomplished by oxygen and resulted in the appearance of a major peak at 368 mμ, as well as a tail up to about 660 mμ, which corresponds to the absorption of the mixture of 1-hydroxyphenazine and ferric chloride. However, there was no spectral change without irradiation of light. Thus, the occurence of the fission of the acyl-to-oxygen bond was established by investigating the spectral change of the reaction system. Also, in the case of dimethyl sulfate, the abosrption at 440 mμ was observed during the photo-reduction, and oxidation by oxygen resulted in the appearance of an absorption spectrum having absorptions at 368 and 430 mμ, absorptions which corresponded to those of 1-hydroxyphenazine, as is shown in Fig. 5.

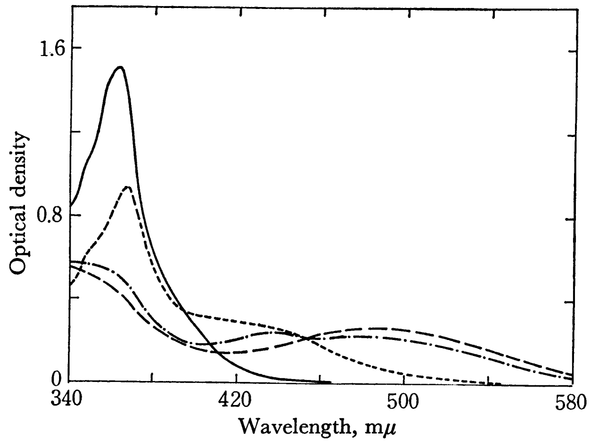


Fig. 5. Spectral change of 1-acetoxyphenazine in the presence of dimethyl sulfate under irradiation of ultraviolet light: Concentration of 1-acetoxyphenazine and dimethyl sulfate; 1.02×10^{-4} mol/*l* and 1.4×10^{-4} mol/*l*.
—— Before irradiation, —·— after 2 min, —— 3 min of irradiation, and ------ after 1 hr of oxidation after irradiating for 3 min.

The photo-irradiation of a solution of 1-benzoyloxyphenazine in 2-propanol, which has been placed in a Pyrex vessel, resulted in the formation of isopropyl benzoate, as is shown in Table 2. The spectral change in the reaction mixture was similar to the results in the case of 1-acetoxyphenazine described above. Although the irradiation of 1-benzoyloxyphenazine in *t*-butyl alcohol did not result in the fission of the acyl-to-oxygen bond, the addition of dimethyl sulfate to the reaction system

TABLE 2. EFFECT OF THE ADDED COMPOUNDS ON THE PHOTOREACTION OF 1-BENZOYLOXYPHENAZINE IN 2-PROPANOL[a)]

Added compound	Irradiated time, hr	Yield of product (%)			
		1-HOPh	C_6H_5-CHO	C_6H_5-COOCH-$(CH_3)_2$	C_6H_5-COOH
None	260	72	14	9	20—30
$(CH_3)_2SO_4$	120	—	0	30	—
$FeCl_3$	95	—	0	45	—
Ph-H_2[b)]	140	35	0	5	—

a) The starting amounts of 1-benzoyloxyphenazine, dimethyl sulfate, ferric chloride and 5,10-dihydrophenazine were $(4.3—4.6) \times 10^{-5}$, 4.9×10^{-4}, 4.9×10^{-5}, and 1.3×10^{-4} mol, respectively. The volume of 2-propanol was 10 m*l*. Irradiated distance: 4 cm. Reaction vessel: Pyrex glass.
b) Ph-H_2 means 5,10-dihydrophenazine.

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15) D. Elad and R. D. Youssefyeh, *Tetrahedron Lett.*, **1963**, 2189.
16) W. S. Zaugg, *J. Biol. Chem.*, **239**, 3964 (1964).

gave 1-hydroxy- phenazine and *t*-butyl benzoate as the products. The yield of 1-hydroxyphenazine was 70%. The amount of *t*-butyl benzoate could not be measured because it decomposed so readily in gc analysis.

Photo-reduction of 2-Benzoyloxyphenazine. A solution of 2-benzoyloxyphenazine in 2-propanol was irradiated with light for 50 hr according to the procedure used in the case of 1-benzoyloxyphenazine. 2-Hydroxyphenazine, benzaldehyde, and isopropyl benzoate were obtained in 66, 6, and 2% yields respectively. No other products derived from the benzoyl group could be detected by gc analysis in spite of recording up to about 300°C. Furthermore, the photochemical Fries rearrangement[17] of the benzoyl group to the phenazine ring was not observed. The benzoyl group would be converted to the dark brown polymeric material by a secondary photochemical reaction.

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17) D. A. Plank, *Tetrahedron Lett.*, **1968**, 5423; R. A. Finnegan and D. Knutson, *ibid.*, **1968**, 3429; D. Bellus and P. Hrdlovic, *Chem. Rev.*, **67**, 599 (1967).