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18. **Hirotaka Otomasu** : Studies on Phenazines. XVIII.* Nitration of Phenazine and Its Derivatives. (3).

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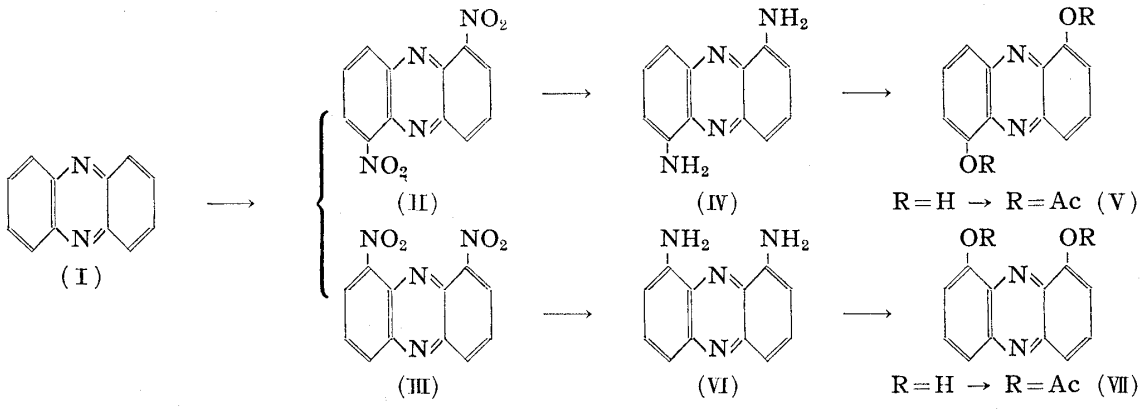
As was reported in Parts VII[1] and XI[2] of this series, among various nitrations of phenazine and some of its derivatives, phenazine was not nitrated with potassium nitrate in conc. sulfuric acid at 0°. When the temperature was raised up to 60°, a dinitrophenazine, which darkened at about 225° with sintering, was formed. This substance was described in the previous paper as 1,3-dinitrophenazine, referring to the data of Kehrman, *et al.*,[3] who gave no valid proof for this structure.

Albert and Duewell[4] examined the experiment of Kehrmann, *et al.*, for the reason that 1,3-disubstitution is improbable in view of the fact that one nitro group would prevent another from entering the same ring, because a nitro function is electrophilic and the second nitro substitution in the ring, which has already been replaced by the first, is quite unlikely. Thus, they stated that another unattacked ring should be available.

They obtained a nitration product of m.p. 177~250°, which was a mixture of mono- (mainly) and other dinitro-phenazines. On reduction, this was separated into three; 1-amino- and two diamino-phenazines, m.p. ca. 250°(decomp.) and m.p. 214°(sealed). They assumed one of the latter compound of m.p. 214° to be 1,9-diaminophenazine, for this amino compound contains only α-amino groups and was not identical with any of the four known 1,3-, 2,3-, 2,7-, and 2,8-diaminophenazines.

This time, with the purpose of verifying this assumption, nitration of phenazine was carried out, two dinitro compounds were obtained, and the positions of nitro groups substituted in the phenazine-ring was determined. Very recently, however, it was learned that 1-nitro-, and 1,6- and 1,9-dinitro-phenazines have been obtained by Maffei and Aymon[5] by direct nitration of phenazine. The disubstituted products obtained by them agree with those from the present experiments except for differences detailed below. Results of nitration of phenazine N-oxide is also described in this connection.



* Part XVII : Yakugaku Zasshi, **77**, (1957).
** Ebara 2-chome, Shinagawa-ku, Tokyo (乙益寛隆).

1) H. Otomasu : This Bulletin, **2**, 283(1954).
2) H. Otomasu : *Ibid.*, **4**, 117(1956).
3) F. Kehrmann, E. Havas : Ber., **46**, 347(1913).
4) A. Albert, H. Duewell : J. Soc. Chem. Ind. (Loudon), **66**, 11(1947).
5) S. Maffei, M. Aymon : Gazz. chim. ital., **84**, 667(1954) (C. A., **50**, 1037(1956)).

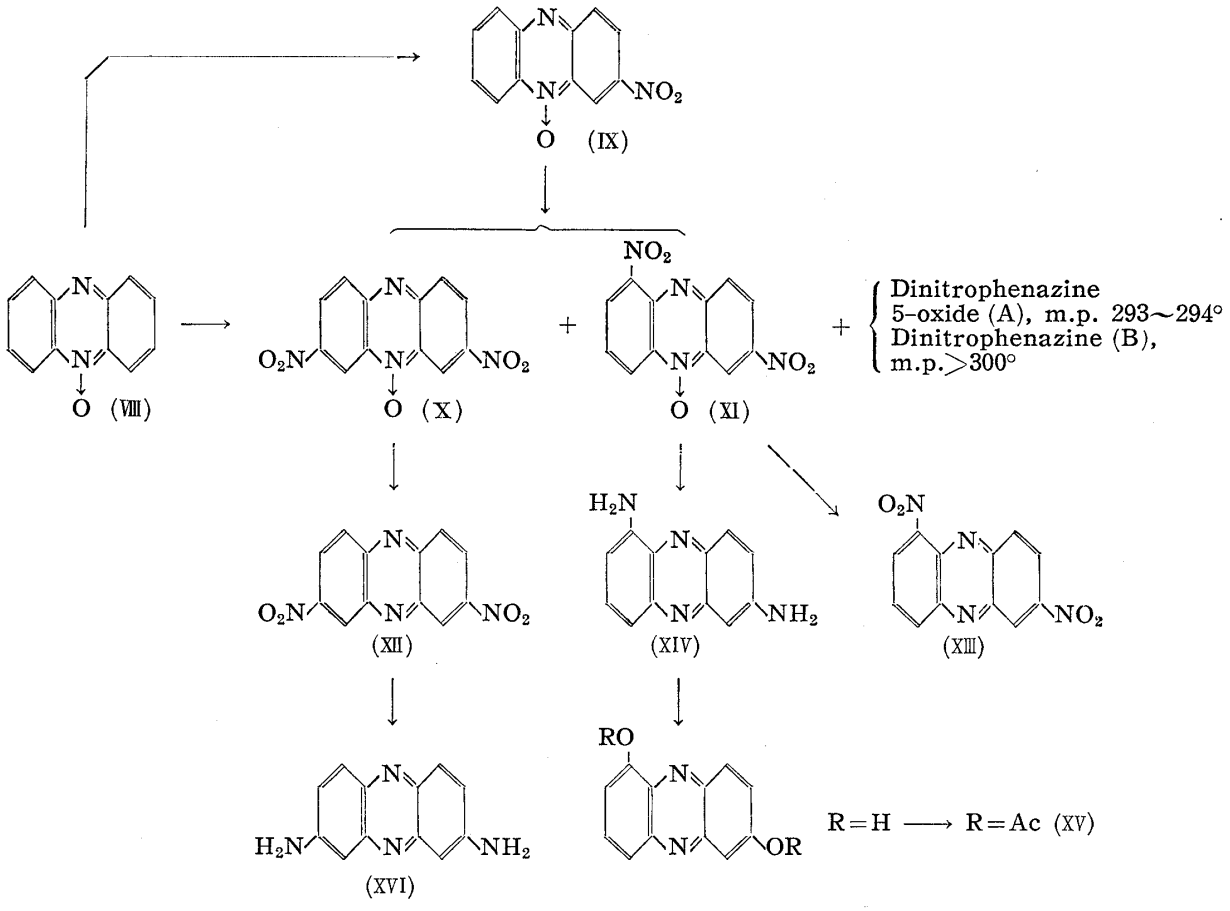
Phenazine (I) was dissolved in conc. sulfuric acid, fuming sulfuric and nitric acids were added to this, and the mixture was heated to 100°. The nitration product was treated by fractional recrystallization from glacial acetic acid and gave two isomers, 1,6- (II), m.p. over 300°, and 1,9-dinitrophenazine (III), m.p. ca. 260°(decomp.), in respective yields of 22% and 30%. By this modified method, the yield of dinitro compounds was much higher than that reported in the earlier paper.[2]

(II) and (III) were catalytically reduced with palladised charcoal to diamino compounds, 1,6- (IV), m.p. 265°(decomp.), and 1,9-diaminophenazine (VI), m.p. ca. 250°. Therfore, Albert and Duewell obtained diaminophenazines, m.p. ca. 250°(decomp.) and m.p. 214°(sealed), and they must be 1,6- and 1,9-diaminophenazines, respectively, though there is some discrepancy in the melting point of these compounds.

By heating with 3*N* sulfuric acid solution in an autoclave at 180°, these diamino compounds, (IV) and (VI), were converted into 1,6- and 1,9-dihydroxyphenazines and acetylated to 1,6- (V)[6] and 1,9-diacetoxyphenazines (VII),[7] respectively.

Thus, the nitration product assumed to be 1,3-dinitrophenazine was proved to be a mixture of 1,6- and 1,9-dinitrophenazines.

Subsequently, nitration of phenazine N-oxide was carried out. On nitration of phenazine N-oxide, Wohl[8] reported the formation of two kinds of dinitro compounds but he did not determine the position of nitro groups. The reaction was followed in more detail and the same products, m.p. 269°(decomp.) (X) and m.p. 240° (XI), were obtained in respective yields of 23% and 41%, along with a small amount of another



6) I. Yosioka, Y. Kidani: Yakugaku Zasshi, **72**, 848(1952).
7) I. Yosioka: This Bulletin, **3**, 25(1954).
8) A. Wohl: Ber., **36**, 4139(1903).

isomer, m.p. 293~294°, and a deoxygenated dinitro product of m.p. over 300°. Both (X) and (XI) were also obtained by the nitration of 3-nitrophenazine 5-oxide (IX).

When (X) and (XI) were refluxed with phosphorus trichloride in chloroform solution, deoxygenation occured and the corresponding dinitrophenazines were obtained. (XI) was reduced catalytically to a diamino compound (XIV) of m.p. 223°, converted into dihydroxyphenazine by heating with 3*N* sulfuric acid in an autoclave, and followed by acetylation to 1,7-diacetoxyphenazine (XV).[8] The afore-mentioned autoclaving is generally made at 4~6 kg./cm², but in this case, the inner pressure was required to be kept at 16 kg./cm².

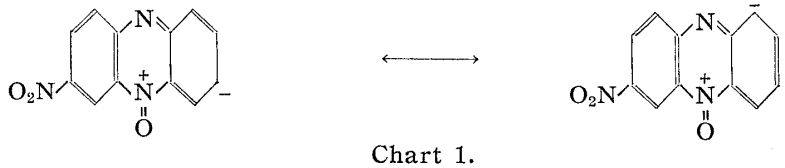


Chart 1.

From the above results, the structure of (XI) must therefore be 1,7-dinitrophenazine 5-oxide.

The other dinitrophenazine N-oxide of m.p. 269°(decomp.)[9] (X) was assumed to be 3,7-dinitrophenazine 5-oxide, because, as seen in Chart 1, 1- and 3-positions of phenazine 5-oxide are active against electrophilic reagents by the polarization of N-oxide group and, in fact, the reduction product of (X) was a diaminophenazine of m.p. ca. 283~285°, which agreed well with the data for 2,8-diaminophenazine (XVI) by Nietzki, *et al.*[10]

The author is very grateful to Prof. Dr. M. Ishidate, University of Tokyo, for his encouragement and to Prof. Dr. A. Albert, Department of Medical Chemistry, Australian National University, for his kind advice. The author is indebted to the members of microanalytical room of the University of Tokyo for the microanalyses reported herein.

Experimental

Nitration of Phenazine : 1,6- (II) and 1,9-Dinitrophenazines (III)—To a solution of phenazine (I) (9 g.) in conc. H_2SO_4(90 cc.), fuming H_2SO_4(50%, 45 cc.) and fuming HNO_3(d=1.52, 90 cc.) were added under cooling. The temperature was gradually raised to 100° during a period of 1 hr. and held there for 30 mins. After cool, the reaction mixture was poured into cold water, the product that precipitated was collected, and dried. The precipitate was refluxed with glacial AcOH (900 cc.) to complete solution, concentrated to a volume of about 600 cc., and cooled to room temperature. The crystals that separated out were collected and recrystallized from acetone to pale yellow needles, m.p. >300°(darkening at ca. 230°) (Maffei, *et al.*[5] recorded m.p. 343°). Yield, 3 g. (22%). *Anal.* Calcd. for $C_{12}H_6O_4N_4$(II) : C, 53.33; H, 2.22; N, 20.78. Found : C, 53.51; H, 2.58; N, 20.53.

The mother liquor left after separation of (II) was evaporated to dryness after purification with activated carbon and the dark residue was washed with 2% methanolic NaOH solution to remove some alkali-soluble substance. The product was recrystallized from Ac_2O to yellow needles, m.p. ca. 260°(decomp., darkening at ca. 200°) (Maffei, *et al.*[5] recorded m.p. 273°). Yield, 4 g. (30%). *Anal.* Calcd. for $C_{12}H_6O_4N_4$(III) : C, 53.33; H, 2.22; N, 20.78. Found : C, 53.33; H, 2.02; N, 20.99.

Reduction of Dinitrophenazine : 1,6-Diaminophenazine (IV)—1,6-Dinitrophenazine (II) (1 g.) was dissolved in acetone (500 cc.) and shaken with Pd-C in H_2 stream, at atmospheric pressure and room temperature. When the absorption of H_2 ceased, the reaction mixture was filtered and the filtered catalyst was washed with acetone. The filtrate rapidly turned red in air. This was concentrated to dryness and the crude product obtained was recrystallized from MeOH to orange-red blades, m.p. 265°(decomp.) (Maffei, *et al.*[5] recorded m.p. 245°). *Anal.* Calcd. for $C_{12}H_{10}N_4$: C, 68.57; H, 4.75; N, 26.66. Found : C, 68.83; H, 4.59; N, 26.18.

1,6-Diacetoxyphenazine (V)—(IV) (0.8 g.) was heated with 3*N* H_2SO_4(80 cc.) in an autoclave at 180°

9) Recently, Maffei and Bettinetti (Ann. Chim. (Rome), **45**, 1031(1955) [C. A., **50**, 12063(1956)]) reported that one of the dinitro products, m.p. 269°, which was consistent with Wohl's results, was reduced to 2,8-diaminophenazine, but no details of the experimental process and procedure are given.

10) R. Nietzki, O. Ernst : Ber., **23**, 1852(1890).

for 6 hrs. (gauge pressure: 4~5 kg./cm^2). The reaction mixture was basified with 20% NaOH solution, the precipitate formed was collected, and neutralized with AcOH. Brown crystals of 1,6-dihydroxyphenazine that deposited were collected and dried. Yield, 0.5 g. This was acetylated with Ac_2O in pyridine to 1,6-diacetoxyphenazine as pale yellow needles (from MeOH), m.p. 235~236°, not depressed on admixture with the authentic specimen.[6]

1,9-Diaminophenazine (VI)—(III) was reduced with Pd-C in acetone. Dark red needles (from MeOH), m.p. ca. 250° (Maffei, *et al.*[5] recorded m.p. 264~265°). *Anal.* Calcd. for $C_{12}H_{10}N_4$: C, 68.57; H, 4.75; N, 26.66. Found: C, 68.46; H, 4.80; N, 26.68.

1,9-Diacetoxyphenazine (VII)—(VI) (0.7 g.) was heated with 3*N* H_2SO_4 solution (70 cc.) in an autoclave at 180° for 6 hrs. (gauge pressure, 5~6 kg./cm^2). The reaction mixture was treated as for (V) and 0.44 g. of 1,9-dihydroxyphenazine was obtained. This was refluxed with Ac_2O in pyridine to yield 1,9-diacetoxyphenazine as pale yellow flat needles (from MeOH), m.p. 258~259°, not depressed by mixed fusion with the authentic specimen.[7]

Nitration of Phenazine N-Oxide: 3,7-Dinitrophenazine 5-Oxide (X) and 1,7-Dinitrophenazine 5-Oxide (XI)—To a solution of phenazine N-oxide (6 g.) in conc. H_2SO_4(60 cc.), HNO_3(d=1.42, 60 cc.) was added. The mixture was treated as in the case of phenazine and 7 g. of orange-yellow product was obtained. This was refluxed with acetone (700 cc.) for 30 mins. and allowed to stand at room temperature. The crystals that separated were collected, together with some insoluble substance, and recrystallized from acetone to orange-yellow needles, m.p. 269°(decomp.).[7] Yield, 2 g. (23%). *Anal.* Calcd. for $C_{12}H_6O_5N_4$(X): N, 19.58. Found: N, 19.54.

The mother liquor obtained after separation of (X) was concentrated to dryness, the orange-yellow residue was washed with MeOH, dissolved completely in a small volume of Ac_2O with heating, and the solution was allowed to stand at room temperature. Orange-yellow microblades of m.p. 240°[7] were obtained. Yield, 3.6 g. (41%). *Anal.* Calcd. for $C_{12}H_6O_5N_4$(XI): N, 19.58. Found: N, 19.30.

The mother liquor was poured into water, the product that separated was recrystallized from benzene, and 0.3 g. of orange needles (A), m.p. 293~294°, and 0.4 g. of yellow blades (B), m.p. >300°, were obtained. *Anal.* Calcd. for $C_{12}H_6O_5N_4$(A): C, 50.35; H, 2.10; N, 19.58. Found. C, 50.79; H, 2.01; N, 19.68. *Anal.* Calcd. for $C_{12}H_6O_4N_4$(B): C, 53.33; H, 2.22; N, 20.78. Found: C, 53.06; H, 2.36; N, 20.90.

Nitration of 3-Nitrophenazine 5-Oxide (IX)—To a solution of (IX) (1 g.) in conc. H_2SO_4(10 cc.), HNO_3(d=1.42, 10 cc.) was added. The mixture was treated as for phenazine and 0.45 g. of (X) and 0.35 g. of (XI) were obtained.

Deoxygenation of (X): 2,8-Dinitrophenazine (XII)—To a solution of (X) (0.5 g.) in $CHCl_3$(250 cc.), PCl_3(4 cc.) was added with cooling and shaking. The mixture was refluxed for a while and filtered from a black precipitate. The $CHCl_3$ solution was washed with water, dried over anhyd. Na_2SO_4, and purified by chromatography on Al_2O_3. The solvent was removed from the effluent and the residue was recrystallized from Ac_2O to yellow needles, m.p. 230° (Maffei, *et al.*[9] recorded m.p. 232°). Yield, 0.36 g. *Anal.* Calcd. for $C_{12}H_6O_4N_4$: C, 53.33; H, 2.22; N, 20.78. Found: C, 53.13; H, 2.66; N, 20.42.

Deoxygenation of (XI): 1,7-Dinitrophenazine (XIII)—To a solution of (XI) (0.4 g.) in $CHCl_3$(150 cc.), PCl_3 (2 cc.) was added with cooling and shaking. The mixture was treated as above and yellow crystalline powder, m.p. ca. 200°, was obtained. Yield, 0.22 g. *Anal.* Calcd. for $C_{12}H_6O_4N_4$: C, 53.33; H, 2.22; N, 20.78. Found: C, 53.12; H, 2.27; N, 20.72.

1,7-Diaminophenazine (XIV)—This was obtained by the catalytic reduction of (XI) with Pd-C. Red needles (from MeOH), m.p. 223°. *Anal.* Calcd. for $C_{12}H_{10}N_4$: C, 68.53; H, 4.75; N, 26.66. Found: C, 68.31; H, 4.61; N, 26.24.

1,7-Diacetoxyphenazine (XV)—(XIV) (1 g.) was heated with 3*N* H_2SO_4(80 cc.) in an autoclave at 180° for 6 hrs. In this case, it was desirable to keep the inner pressure higher than usual. For this purpose, 30 cc. of 20% HCl was sealed together with the raction vessel in the autoclave, when the gauge indicated 16 kg./cm^2. The reaction mixture was treated as for (V) and 1,7-dihydroxyphenazine was obtained as a brown crystalline powder. Yield, 0.75 g. This was acetylated with Ac_2O in pyridine to 1,7-diacetoxyphenazine as pale yellow needles (from MeOH), m.p. 153°, not depressed by admixture with other specimen.[7]

2,8-Diaminophenazine (XVI)—To a suspension of (X) (1 g.) in MeOH (10 cc.), $SnCl_2$(3 g.) in conc. HCl (6 cc.) was added in small portions with shaking. The reaction mixture was warmed on a water bath for a while, the amino salt that separated was collected, and dissolved in dil. HCl solution with heating. The solution was filtered and neutralized with NH_4OH to separate the free amino derivative. The brown precipitate obtained was recrystallized from water to brownish yellow needles, m.p. 283~284°.[10] *Anal.* Calcd. for $C_{12}H_{10}N_4$: N, 26.66. Found: N, 26.14.

Catalytic reduction of (X) with Pd-C in acetone solution was attempted, but the corresponding diaminophenazine was not obtained.

Summary

The nitration product of phenazine, which was previously described as 1,3-dinitrophenazine, was proved to be a mixture of 1,6- and 1,9-dinitrophenazines. These structures were determined by their conversion into dihydroxyphenazines by way of diaminophenazines.

From the nitration of phenazine N-oxide under high temperature, two isomers, 1,7-dinitrophenazine 5-oxide and 3,7-dinitrophenazine-5-oxide were obtained.

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19. Takeo Tsukamoto,* Tetsuya Komori,* Nadao Kinoshita,* Naoki Toida,** and Hirosi A. Kuriyama**: Chemical Studies on Visual Function. V.*** On the Chemical Nature of Rhodopsin. (1). Purification of Scotopsin and Its Amino Acid Component.

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Rhodopsin, the photo-sensitive pigment in the outer segment of the rods, has been studied in detail by Wald,[1] Robeson,[2] Oroshnik,[3] Hubbard,[4] and others so far as the structure of each isomer of its prosthetic group, retinene, is concerned. However, the protein fraction of rhodopsin has never been clarified except in a few details since Mirsky[5] pointed out that the bleaching and regeneration of rhodopsin should be considered as a result of reversible denaturation of proteins. Wald's theory[6] of thioacetal binding and Morton's theory[7] of Schiff-base binding have been advanced for the study of the active radical with which retinene may combine. Yet it remains unexplained what kind of amino acid of its protein portion combines with retinene, or whether it is combined with some non-protein substance. According to the result of our investigation[8] on the influence of arecoline compounds on the toad E.R.G., it is inferable that the substances related to arecoline which accelerate the rhodopsin regeneration also augment the height of b-wave in the retinal action potential with a few exception and such substances have one double bond in their molecular structure. However, it is necessary all the same that the relationship of various chemicals to rhodopsin and the chemical nature of rhodopsin should be examined for elucidation of the first stage in the agitation of the sense of sight in the retina. We are chiefly making a series of studies in scotopsin, the protein portion of rhodopsin kept in the dark.

Purification of Scotopsin from Cattle Retina—For the purification of scotopsin a modification of the method of Hubbard[9] is used. The retina extracted from fresh cattle eyeball and kept

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