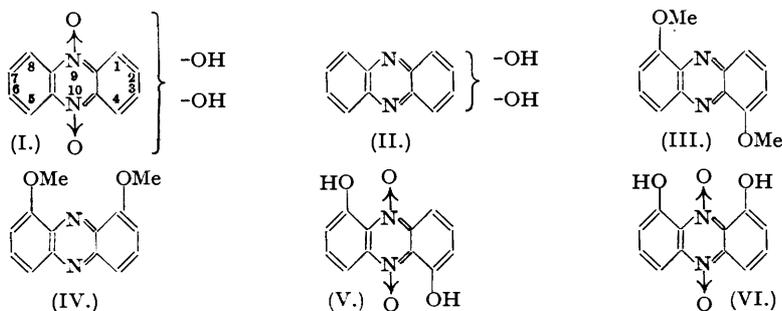


307. The Phenazine Series. Part VIII. The Constitution of the Pigment of *Chromobacterium iodinum*.

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It has been shown that the two hydroxyl groups in iodinin occupy the 1:5-positions. 1:2-, 1:5-, and 1:8-Dimethoxyphenazines have been synthesised, and the 1:5- and 1:8-isomers have been demethylated.

THE pigment of *Chromobacterium iodinum* was shown by Clemo and McIlwain (*J.*, 1938, 479) to be the di-*N*-oxide of a dihydroxyphenazine (I), and on catalytic reduction it gave a dihydroxy-dihydrophenazine which autoxidised to a dihydroxyphenazine (II).



Absorption spectra and certain qualitative tests showed that the bacterial pigment resembled alizarin but not quinizarin.

Of the ten possible dihydroxyphenazines, only the diacetyl derivatives of the 2:3- and the 2:6-isomers* had been previously described, and both differed from the derivative made from the pigment. Synthetic work towards the elucidation of the latter's constitution was interrupted in 1939, but the possibility that it was 1:2-dihydroxyphenazine was eliminated by Hegedüs (*Festschrift für E. C. Borell*, 1946), who synthesised this compound, the dimethyl ether, and the diacetate, and showed that they differed from the pigment analogues.

Independent syntheses of 1:2-dimethoxyphenazine were reported by Slack and Slack (*Nature*, 1947, **160**, 437) and Clemo and Daglish (*Nature*, 1947, **160**, 752): the melting point recorded in each case was 138—139°, whereas Hegedüs gave the melting point as 145°. In an attempt to clear up this anomaly, we sublimed a specimen of 1:2-dimethoxyphenazine, from Dr. Hegedüs to whom our best thanks are due, as well as a sample of our 1:2-dimethoxyphenazine, at 80—90° in a molecular still. The two sublimates still showed the original m. p.s, and the mixture melted at 138—144°; a crystallographic examination of the two samples has also failed to resolve this anomaly.

1:4-Dimethoxyphenazine was also described by Slack and Slack (*loc. cit.*). Instead of attempting the demethylation of this compound, we methylated the dihydroxyphenazine obtained from the pigment and obtained a compound, m. p. 245—246°, which differed from that prepared by Slack and Slack. Recently King, Clark, and Davis (*J.*, 1949, 3012) have described the demethylation of 1:4-dimethoxyphenazine using aluminium chloride in dry benzene.

The synthesis of 1:3-dihydroxyphenazine (Clema and Daglish, *J.*, 1948, 2318) completed the investigation of the four possible isomers containing two hydroxy-groups in the same ring, and as it was also different from the dihydroxyphenazine obtained from iodinin, it became certain that in the natural product the two hydroxyl groups were in different benzene nuclei.

* The numbering shown in (I) is used in this paper solely to conform with that used in previous Parts of this series.

Attention was then directed to the preparation of 1 : 5- and 1 : 8-dimethoxyphenazine. Thus, 3-methoxy-*o*-benzoquinone and 2 : 3-diaminoanisole condensed together under the conditions used for 1-methoxyphenazine (*Org. Synth.*, 1946, **26**, 86) to give a mixture of 1 : 5- and 1 : 8-dimethoxyphenazines (III and IV) which were separated chromatographically on alumina. One of these compounds provided to be identical with the dimethoxyphenazine from iodinin, and both the isomers were smoothly demethylated by hydrobromic acid-acetic acid.

After many unsuccessful attempts to prepare either the 1 : 5- or 1 : 8-dimethoxyphenazine by an unambiguous synthesis, the 1 : 6-compound was eventually obtained by condensing 3-bromo-2-nitroanisole (Hodgson and Moore, *J.*, 1926, 155) and 3-nitro-2-aminoanisole (Blanksma, *Chem. Weekblad*, 1908, **5**, 789) to give 2 : 2'-dinitro-3 : 6'-dimethoxydiphenylamine. On reduction and ring closure of this amine under mild oxidising conditions (cf. Tomlinson, *J.*, 1939, 160, and Elderfield, Gensler, and Birstein, *J. Org. Chem.*, 1946, **2**, 817), the 1 : 5-dimethoxyphenazine was obtained ; it was found to be identical to the dimethoxyphenazine derived from the pigment.

2 : 2'-Dinitro-3 : 6'-dimethoxydiphenylamine exists in three crystalline forms : a stable form (*A*), which forms prisms from alcohol or benzene, m. p. 118°; and two unstable forms (*B*) and (*C*), which crystallise from benzene-light petroleum (b. p. 60—80°) in yellow needles and red prisms, respectively. Both unstable forms melt at 104°, and when allowed to solidify change to the stable form. All three forms can be interchanged by seeding the solution in benzene.

Malaviya and Dutt (*Proc. Acad. Sci. Agra Oudh*, 1935, **4**, 319) claimed that 1 : 5-dimethoxyphenazine was formed when *o*-anisidine was exposed to tropical sunlight. Their product melted, however, at above 300°, and our attempts to reproduce their results have been unsuccessful. Following Wohl's phenazine synthesis (*Ber.*, 1901, **34**, 2446; 1903, **36**, 4135), attempts were made to prepare the 1 : 5-dimethoxy-derivative by condensing *o*-anisidine and *o*-nitroanisole in the presence of potassium hydroxide, but the only product isolated was phenazine itself. Attempts to prepare phenazine-1 : 5-dicarboxylic acid from anthranilic acid and *o*-nitrobenzoic acid (cf. Kögl and Postowsky, *Ann.*, **480**, 280) were also unsuccessful.

Amongst the unsuccessful attempts to prepare by the Ullmann reaction substituted diphenylamines which might be subsequently ring-closed to give either 1 : 5- or 1 : 8-dimethoxyphenazine, are the following :

(a) 3-Chloro-2-nitroanisole and 3-nitro-2-aminoanisole; (b) 2-chloro-3-nitroanisole and 3-nitro-2-aminoanisole; (c) 2-chloro-3-nitroanisole and 2-chloro-3-aminoanisole; (d) 3-chloro-2-nitroanisole and 3-chloro-2-aminoanisole; (e) 4-chloro-3 : 5-dinitrobenzoic acid and 4-chloro-3 : 5-bisacetamidobenzoic acid; (f) 4-chloro-3 : 5-dinitrobenzoic acid and 2 : 3-dinitroaniline.

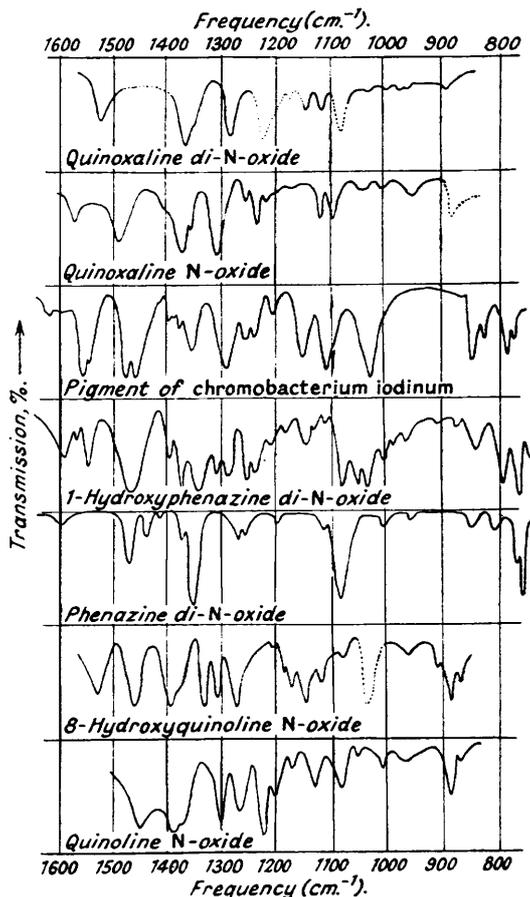
In an attempt to prepare 1 : 8-diaminophenazine, di-(2 : 6-dinitro-4-carbomethoxyphenyl)-amine was synthesised, but catalytic reduction gave an unstable blue hydrochloride which readily changed to a black polymeric product.

Although phenazine is only pale yellow, its di-*N*-oxide is deep orange. This is surprising in view of the absence of colour from most tertiary amine oxides, and the fact that azoxy-compounds are lighter in colour than the corresponding azo-derivatives. It is also surprising that the permanganate-coloured iodinin (*V*) is insoluble in water and gives an intense blue solution in alkali, whereas the yellow 1 : 5-dihydroxyanthraquinone which could have a similar bond structure gives only a red solution.

Pending a successful synthesis of either 1 : 5- or 1 : 8-dimethoxyphenazine it was considered, in view of the work of Flett (*J.*, 1948, 1441) which showed that infra-red absorption spectra differentiated between isomeric dihydroxyanthraquinones, that the same method might be expected to distinguish between 1 : 5- and 1 : 8-dihydroxyphenazines and their di-*N*-oxides. Thus, 1 : 8-dihydroxyphenazine di-*N*-oxide (*VI*) should show two N→O frequencies because the strengths of the two N→O bonds are unequal, a hydrogen bond being formed in only one case. However, in 1 : 5-dihydroxyphenazine di-*N*-oxide (*V*), both N→O groups interact with the adjacent hydroxyl group and only one N→O frequency should result.

The absorption spectra of iodinin, phenazine di-*N*-oxide and its 1-hydroxy-derivative, quinoline *N*-oxide and its 8-hydroxy-derivative, and quinoxaline *N*-oxide and di-*N*-oxide were kindly determined by Dr. Flett through the courtesy of Imperial Chemical Industries Limited, Dyestuffs Division. All aromatic nitro-compounds have an absorption band at 1350 cm.⁻¹, and this and another at 1075 cm.⁻¹ are shown by phenazine di-*N*-oxide. Quinoxaline di-*N*-oxide has a band at 1370 cm.⁻¹ and (possibly) the 1075 cm.⁻¹ band. In the mono-*N*-oxide the latter band is much weaker and is at 1100 cm.⁻¹, while the 1370 cm.⁻¹ band (including the small inflexion at longer wave-length) persists. It is very doubtful if either band is present in the

quinoline *N*-oxide spectrum (the 1390 cm^{-1} band may be present—note the inflexion), but both are probably shown by that of its 8-hydroxy-derivative (at 1395 cm^{-1} —note the inflexion—and at 1040 cm^{-1}). 1-Hydroxyphenazine di-*N*-oxide does not show either band clearly, and it is barely possible that iodinin shows them at 1290 cm^{-1} and 1020 cm^{-1} , but in this case the characteristic inflexion of the shorter wave-length band is concealed by new bands. As a



diagnostic test for the presence of a 1-hydroxy-group in phenazine this method therefore fails. The complete change in character of the absorption spectrum of phenazine di-*N*-oxide on the introduction of a hydroxyl group indicates that a more profound electronic change has taken place than is evident from the formula (V) for the pigment.

EXPERIMENTAL.

(M. p.s are uncorrected. Microanalyses are by Mr. W. A. Campbell and Drs. Weiler and Strauss, Oxford.)

1-Hydroxy-2-methoxy-5 : 6 : 7 : 8-tetrahydrophenazine.—A solution of 5 : 6-dinitroguaiacol (0.5 g.) (Pollecöff and Robinson, *J.*, 1918, 648) in boiling alcohol (10 ml.) was mixed with concentrated hydrochloric acid (5 ml.) and excess of zinc dust (2 g.) was added. When the vigorous reaction had subsided, the solution was diluted with water, filtered, and a solution of cyclohexane-1 : 2-dione (0.25 g.) in aqueous sodium hydrogen sulphite solution, containing sodium acetate (2 g.), was added. The mixture was boiled for five minutes, cooled, and filtered, yielding a red zinc complex which was suspended in chloroform and decomposed with hydrogen sulphide. The chloroform solution yielded the *tetrahydrophenazine* as orange-red prisms (0.1 g., 17%), m. p. 100° (Found : C, 67.7; H, 6.4; N, 12.7. $\text{C}_{13}\text{H}_{14}\text{O}_2\text{N}_2$ requires C, 67.8; H, 6.1; N, 12.2%).

1 : 2-Dimethoxy-5 : 6 : 7 : 8-tetrahydrophenazine.—A solution of 3 : 4-dinitroveratrole (11.7 g.) (Pollecöff and Robinson, *loc. cit.*) in boiling alcohol was reduced with zinc and hydrochloric acid, and then boiled with cyclohexane-1 : 2-dione (5.6 g.) for two hours. The alcohol was removed, and the solution was basified with ammonium hydroxide and extracted continuously with ether. The tetrahydrophenazine was purified by extraction from ethereal solution with hydrochloric acid (5%), precipitation

with aqueous sodium hydroxide (5%), and re-extraction with ether. Removal of the solvent, followed by crystallisation from light petroleum (b. p. 40–60°), gave straw-coloured needles (6 g., 48%), m. p. 82–83° (King, Clark, and Davis, *loc. cit.*, give m. p. 82–83°) (Found: C, 69.0; H, 6.6; N, 11.9. Calc. for $C_{14}H_{10}O_2N_2$: C, 68.9; H, 6.6; N, 11.4%).

1 : 2-Dimethoxyphenazine.—The tetrahydro-compound (3 g.) and palladium-charcoal (0.9 g.; 10% palladium) were heated under reflux in *p*-cymene (30 ml.) for three hours. The cooled product was filtered and the solvent removed under reduced pressure, leaving the phenazine which was purified by extraction with hydrochloric acid (5%) and recrystallised from light petroleum (b. p. 80–100°), yielding orange-yellow blades (1.3 g., 44%), m. p. 138–139° (Found: C, 70.0; H, 5.4. Calc. for $C_{14}H_{12}O_2N_2$: C, 70.0; H, 5.0%).

Methylation of the Hydroxyphenazine prepared from Iodinin.—The dihydroxyphenazine (0.03 g.) was mixed with methyl alcohol (3 ml.) and a freshly prepared ethereal solution of diazomethane (0.166 g. in 10 ml.). After 48 hours the precipitated dimethoxyphenazine was filtered off and recrystallised from benzene yielding yellow needles (0.016 g., 47%), m. p. 245–246° (Found: C, 69.9; H, 4.4. $C_{14}H_{12}O_2N_2$ requires C, 70.0; H, 5.0%). A further amount, (making 56% in all) was obtained by removal of the solvent and extraction of the residue with sodium hydroxide solution (3%).

2 : 3-Diaminoanisole.—A solution of 2 : 3-dinitroanisole (2.5 g.) (Wrede and Strack, *Ber.*, 1929, 62, 2053) in glacial acetic acid (50 ml.) was shaken with hydrogen at room temperature and atmospheric pressure in the presence of Adams's catalyst (0.1 g.) for six hours. The colourless solution was filtered, diluted with water (50 ml.), and just basified with ammonia. Extraction with ether (4 × 60 ml.) followed by distillation gave a colourless oil (1.22 g., 70%), b. p. 130°/5 mm. (Found: C, 60.9; H, 7.8. Calc. for $C_7H_{10}ON_2$: C, 60.9; H, 7.2%).

2-Chloro-3-aminoanisole.—A solution of 2-chloro-3-nitroanisole (5.4 g.) in aqueous acetic acid (54 ml., 50%) was stirred and heated on the steam-bath while iron powder (4 g.) was added during one hour; heating was continued for another hour. The product was diluted with hot water (100 ml.), basified with sodium carbonate, and steam-distilled. The distillate was extracted with ether, yielding a colourless oil (4.2 g., 93%), b. p. 125°/3 mm. The oil solidified when kept for several months, and was recrystallised from light petroleum (b. p. 40–60°), affording colourless needles of the anisole, m. p. 50° (Found: C, 53.6; H, 5.1. C_7H_8ONCl requires C, 53.3; H, 5.1%).

1 : 5- and 1 : 8-Dimethoxyphenazine.—Lead dioxide (27 g.) was added to a solution of pyrogallol 1-methyl ether (1.35 g.) (*Org. Synth.*, 1946, 26, 90) in dry benzene (270 ml.). After being shaken for 20 minutes, the red solution was filtered and mixed with a solution of 2 : 3-diaminoanisole (1.03 g.) in benzene (26.8 ml.) and glacial acetic acid (10.7 ml.). After 90 minutes, the dark red solution was washed thoroughly with sodium hydroxide solution (5%), followed by water, and dried ($MgSO_4$), diluted with light petroleum (400 ml.; b. p. 60–80°), and adsorbed on alumina. The chromatogram was developed with benzene and a broad yellow band containing both isomers was gradually eluted. The first 150 ml. of eluent gave yellow needles, m. p. 247°, not depressed by admixture with the dimethoxyphenazine prepared from iodinin; extinction angle 42–44° (Found: C, 70.4; H, 4.7. Calc. for $C_{14}H_{12}O_2N_2$: C, 70.0; H, 5.0%). The last 150 ml. of eluent gave yellow needles, m. p. 253–254°, showing straight extinction (Found: C, 70.2; H, 4.7). The middle portion of the eluent contained a mixture of the two isomers, and was re-chromatographed.

1 : 5- and 1 : 8-Dihydroxyphenazine.—Dimethoxyphenazine (0.2 g.) (1 : 5-, 1 : 8-, or a mixture) was heated under reflux with hydrobromic acid (2.5 ml.; *d* 1.49) and glacial acetic acid (2.5 ml.) for 24 hours. The cooled product was diluted with water and treated with sodium hydroxide (5%) until the colour changed sharply from red to yellow (pH 4–5), whereupon a yellow precipitate formed and was removed and dried.

(a) The product from the dimethoxyphenazine, m. p. 247°, on recrystallisation from ethyl acetate yielded golden needles (0.02 g., 11%), m. p. 274°, not depressed by admixture with the dihydroxyphenazine prepared from iodinin (Found: C, 67.4; H, 3.9; N, 13.0. Calc. for $C_{12}H_8O_2N_2$: C, 67.9; H, 3.8; N, 13.2%).

(b) The product from the dimethoxyphenazine melting at 253–254° was recrystallised from benzene and gave orange-red plates (0.02 g., 11%), m. p. 295° (Found: C, 67.5; H, 3.9; N, 12.9%).

(c) The product obtained from the mixture of the two isomers was dissolved in benzene and adsorbed on alumina. The less strongly adsorbed, purple band contained the dihydroxyphenazine, m. p. 295°, and the more strongly adsorbed, red band contained the isomer, m. p. 274°.

2 : 2'-Dinitro-3 : 6'-dimethoxydiphenylamine.—3-Bromo-2-nitroanisole (0.78 g.) (Hodgson and Moore, *J.*, 1926, 155), 3-nitro-2-aminoanisole (0.565 g.), anhydrous potassium carbonate (0.67 g.), freshly reduced copper powder (0.1 g.), nitrobenzene (2 ml.), and a trace of water were stirred and heated at 220° for 2 hours (longer or shorter heating periods gave decreased yields). The cooled product was dissolved in boiling benzene (20 ml.) and filtered, light petroleum (30 ml.; b. p. 60–80°) added, and the mixture allowed to crystallise, affording a yellow solid which was recrystallised from alcohol yielding orange-red prisms of the amine (0.13 g., 12%), m. p. 118° (Found: C, 52.7; H, 4.2. $C_{14}H_{13}O_2N_3$ requires C, 52.7; H, 4.1%).

The same compound was obtained in 10% yield when the reagents were heated in the absence of solvent at 180° for 6 hours. The cooled product was extracted with ether; removal of the solvent gave a brown powder which was recrystallised from benzene-light petroleum (b. p. 60–80°), yielding either yellow needles, m. p. 104° (Found: C, 52.9; H, 4.1; N, 13.0. Calc. for $C_{14}H_{13}O_2N_3$: C, 52.7; H, 4.1; N, 13.2%), or red prisms, m. p. 104° (Found: C, 52.9; H, 4.0; N, 12.9%).

1 : 5-Dimethoxyphenazine.—A solution of 2 : 2'-dinitro-3 : 6'-dimethoxydiphenylamine (0.1 g.) in ethanol (8 ml.) was shaken with hydrogen at room temperature and atmospheric pressure for one hour in the presence of Adams's catalyst (0.05 g.). The colourless solution was filtered and acidified with concentrated hydrochloric acid (0.1 ml.), and the alcohol was removed, yielding a white hydrochloride; this was dissolved in water (5 ml.) and oxidised with a solution of ferric chloride (0.5 g.) in water (1 ml.). A dark red colour developed immediately, changing to brown with the formation of a yellow precipitate. After 12 hours, water (20 ml.) was added and the precipitated solid was recrystallised from benzene

yielding yellow needles (0.068 g., 90%), m. p. 247° not depressed by admixture with the *dimethoxyphenazine* prepared from iodinin (Found: C, 70.3; H, 5.2. $C_{14}H_{12}O_2N_2$ requires C, 70.0; H, 5.0%).

2 : 6-Dinitro-4 : 4'-dicarboxydiphenylamine.—A solution of 4-chloro-3 : 5-dinitrobenzoic acid (8.6 g.), *p*-aminobenzoic acid (4.8 g.), and sodium carbonate (2.6 g.) in water (340 ml.) was boiled for two hours. The cooled product was acidified and filtered, and the residue recrystallised from acetic acid, yielding orange prisms (12.8 g., 95%), m. p. 309° (Found: C, 47.2; H, 3.4. Calc. for $C_{14}H_8O_8N_4$: C, 47.2; H, 3.2%).

This compound (1.5 g.) was dried at 120° for three hours, added in small portions to a mixture of nitric acid (7.5 g.; *d* 1.5) and sulphuric acid (7.5 g.; *d* 1.825) and left for six hours, whereupon a crystalline precipitate of di-(2 : 4 : 6-trinitrophenyl)amine was formed; this was washed with water and recrystallised from acetic acid yielding yellow prisms (2.5 g., 75%), m. p. 241° (Found: C, 33.2; H, 1.1. Calc. for $C_{12}H_8O_{12}N_7$: C, 32.8; H, 1.1%).

2 : 6-Dinitro-4 : 4'-dicarbomethoxydiphenylamine.—A solution of the acid (5 g.) in methanol (80 ml.) containing sulphuric acid (4 ml.; *d* 1.825) was heated under reflux for two hours and cooled, and the solid filtered off. Recrystallisation from alcohol yielded yellow needles of the *ester* (5.9 g., 92%), m. p. 269° (Found: C, 51.3; H, 3.9. $C_{16}H_{11}O_{12}N_5$ requires C, 51.2; H, 3.5%).

Di-(2 : 6-dinitro-4-carbomethoxyphenyl)amine.—The dinitro-ester (3.6 g.) was added in small portions during one hour to nitric acid (18 ml.) (prepared by distilling nitric acid, *d* 1.5, with two volumes of sulphuric acid, *d* 1.825). After 24 hours, the mixture was poured on ice (500 g.) and filtered, and the residue crystallised from ethyl acetate, yielding yellow prisms of the *tetranitro*-compound (5.7 g., 78%), m. p. 246° (Found: C, 41.1; H, 2.5. $C_{18}H_{11}O_{12}N_5$ requires C, 41.3; H, 2.4%).

Wohl Reactions.—*o*-Nitroanisole (10 g.), *o*-aminoanisole (10 g.), and potassium hydroxide pellets (40 g.) were stirred and heated at 150° for 15 minutes. The product was diluted with water and steam-distilled. The solid residue was purified by extraction with acid, sublimed at 160° at 2 mm. pressure, and recrystallised from light petroleum (b. p. 40–60°), yielding phenazine (0.02 g., 0.2%), m. p. 172–173° not depressed by admixture with an authentic specimen (Found: C, 79.6; H, 4.5; N, 15.4. Calc. for $C_{12}H_8N_2$: C, 80.0; H, 4.4; N, 15.6%).

The same product was obtained by using powdered potassium hydroxide at 15–100°. The use of powdered zinc chloride at 180° (cf. Wohl and Lange, *Ber.*, 1910, 43, 2186) gave no identifiable product.

Ultra-violet Irradiation of Amines.—Dilute solutions of *o*-anisidine in hydrochloric acid (0.5%) and anthranilic acid in water were exposed at a distance of six inches to an ultra-violet lamp for 24 hours. The dark brown solution was basified and extracted with benzene, and the extract adsorbed on a column of alumina. No trace of phenazine or substituted phenazine was found.

4-Chloro-3 : 5-bisacetamidobenzoic Acid.—4-Chloro-3 : 5-dinitrobenzoic acid (2 g.) was added during one hour to a solution of stannous chloride (11 g.) in hydrochloric acid (11.3 ml.; *d* 1.18), and the solution was stirred and heated on the steam-bath. Next morning the product was diluted with water, treated with hydrogen sulphide to remove tin, filtered and evaporated to dryness, yielding the crude *hydrochloride* (1.0 g.); this was heated under reflux for one hour with sodium acetate (0.8 g.) and acetic anhydride (0.8 g.) and poured into water, yielding white plates of the *bisacetamido*-compound (0.8 g., 36.5%), m. p. 187–188° (Found: C, 48.6; H, 4.5. $C_{11}H_{11}O_4N_2Cl$ requires C, 48.8; H, 4.1%).

3-Chloro-2-benzamidophenol.—3-Chloro-2-nitrophenyl benzoate (4 g.) was added during one hour to a stirred suspension of iron filings (13.4 g.) in aqueous acetic acid (14.5 ml.; 5%) on the steam-bath. The product was liberated with sodium carbonate, filtered off, and extracted with boiling alcohol, yielding white plates of the *benzamido*-compound (2.0 g., 56.1%), m. p. 155° (Found: C, 62.7; H, 4.4. $C_{18}H_{10}O_2NCl$ requires C, 63.0; H, 4.0%). The product showed the normal reactions of a phenol and an amide, indicating the migration of the benzoyl group during the reduction.

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