at 3.07 (OH) and 5.96 μ (C=O, conjugated lactone); it also contained peaks at 6.16, 6.24, 6.64, 6.92, 7.03, 7.20, 7.31, 7.51, 7.82, 8.71, 9.18, 9.37, 11.01, and 12.00 μ.

Compound XXI. By Deacylation of B and C with 75% Sulfuric Acid.—A solution of B (0.6 g) in 6 ml of 75% aqueous sulfuric acid was kept at 25° for 2 days. The mixture was then poured onto ice. The solid formed was filtered and washed with water. After the solid had been dissolved in a large volume of ether, the solution was dried over magnesium sulfate and filtered. Evaporation of the ether to dryness gave 0.38 g (93%), mp 252–255°. It was identified as XXI by its infrared spectrum and by mixture melting point with a known sample of XXI prepared from orcinol and ethyl acetoacetate.[11]

When C (0.6 g) was treated with 75% sulfuric acid under the same conditions, 0.5 g of a solid melting at 160–180° was obtained. This solid was heated with toluene (30 ml) and filtered hot. The toluene-insoluble material was recrystallized from ethanol to give 0.2 g (49%) of impure XXI, mp 242–253°. A mixture of this compound with an authentic sample of XXI showed mp 247–253°. The compound was also identified by its infrared spectrum.

Conversion of B into Compound A.—A mixture of B (1.5 g), benzene (22 ml), and triethylamine (0.3 g) was refluxed, while diketene (4.5 g) was added dropwise over a 25-min period. After the addition, the mixture was refluxed 4 hr longer and then cooled to room temperature. The insoluble solid was filtered, yielding 2.0 g, mp 227–230°. The crude solid was then suspended in methanol, heated to boiling, and filtered hot. The insoluble solid amounted to 1.90 g, mp 234–236°. A mixture of this compound with a sample of A obtained by the reaction of diketene in triethylamine showed no depression in melting point. It was also identified as A by spectral analyses. Evaporation of the original benzene filtrate gave only 2.6 g of impure I as by-product, mp 102–108°.

When the experiment was repeated under the same conditions in the absence of B, only 0.15 g of A and 2.8 g of impure I could be obtained; therefore, the yield of A obtained from B and diketene was about 1.75 g (94%) based on B. When the same experiment was repeated in the presence of C instead of B, again only traces of A together with impure I and unchanged C were isolated.

Registry No.—I, 520-45-6; VI, 13444-19-4; VI diacetate, 13473-46-6; VII, 13449-09-7; VIII, 13449-10-0; XIII, 13473-47-7; XIV, 1634-34-0; XV, 13444-20-7; XVI, 13449-12-2; XVII, 13449-13-3; XVIII, 13449-14-4; XX, 13444-21-8; XXI, 6335-27-9; XXII, 13444-23-0; diketene, 6144-29-2.

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The Structure of the Orange Pigment from *Pseudomonas aureofaciens*[1]

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The orange pigment of *Pseudomonas aureofaciens* has been characterized as 2-hydroxyphenazine-1-carboxylic acid. Three new pigments were also isolated from *Pseudomonas aureofaciens* and spectral properties of two are given.

The major pigment present in cultures of a strain of *Ps. aureofaciens* is the yellow phenazine-1-carboxylic acid.[2,3] In addition to this yellow pigment, Kluyver[2] and Haynes[3] isolated a minute amount of red acidic pigment. Kluyver showed that this second pigment also had a phenazine nucleus and speculated that it was an oxidized form of phenazine-1-carboxylic acid. Toohey, *et al.*,[4] have also isolated this pigment from a strain of *Ps. aureofaciens* and concluded, on the basis of infrared and ultraviolet spectral data and a positive Folin phenol test, that it is probably 2-hydroxyphenazine-1-carboxylic acid. Because of our interest in the biosynthesis of phenazine pigments,[5] we undertook to determine the structure of this second pigment, which is orange when pure, and have shown it to be 2-hydroxyphenazine-1-carboxylic acid (I). Recently, the isolation of 2-hydroxyphenazine from *Ps. aureofaciens* has been reported.[6]

The formula $C_{13}H_8O_3N_2$ inconclusively assigned by Kluyver[2] to the second pigment was found to be consistent with our microanalyses of the pigment and its derivatives. The pK_a of the pigment in 66.8% dioxane water was found[7] to be 7.61, a value which is reasonable for a carboxyl group attached to a phenazine ring system. The ultraviolet spectrum of the pigment in neutral ethanol is similar to that of phenazine-1-carboxylic acid.

Titration of the orange pigment in 66.8% dioxane water gave a neutralization equivalent of about 240; however, the red color did not appear at this end point but rather when almost twice the equivalent amount of base had been added. Hence the phenazine carboxylate anion is not responsible for the red color of the orange pigment in base. Since 1- and 2-phenazinol form red solutions in base, the presence of the hydroxyl group on the phenazine ring system was inferred. This was further confirmed by a positive ceric nitrate and ferric chloride test.

The presence of a carboxyl group is also supported by the strong band in the infrared spectrum at 1676 cm^{-1}. In chloroform the orange pigment shows no sharp OH stretching frequencies above 3000 cm^{-1} from which we assume that hydrogen of the hydroxyl group must be strongly hydrogen bonded to a neighboring carboxyl group. This has been confirmed by an X-ray structure determination of the orange pigment.[8] An intense carbon hydrogen out-of-plane bending absorp-

(1) Supported by Grant No. GM 10218 from the National Institute of General Medical Sciences of the U. S. Public Health Service.

(2) A. J. Kluyver, *J. Bacteriol.*, **72**, 406 (1956).

(3) W. C. Haynes, F. H. Stodola, J. M. Locke, T. G. Pridham, H. F. Conway, V. E. Sohns, and R. W. Jackson, *ibid.*, **72**, 412 (1956).

(4) J. I. Toohey, C. D. Nelson, and G. Krotkov, *Can. J. Botany*, **43**, 1055 (1965).

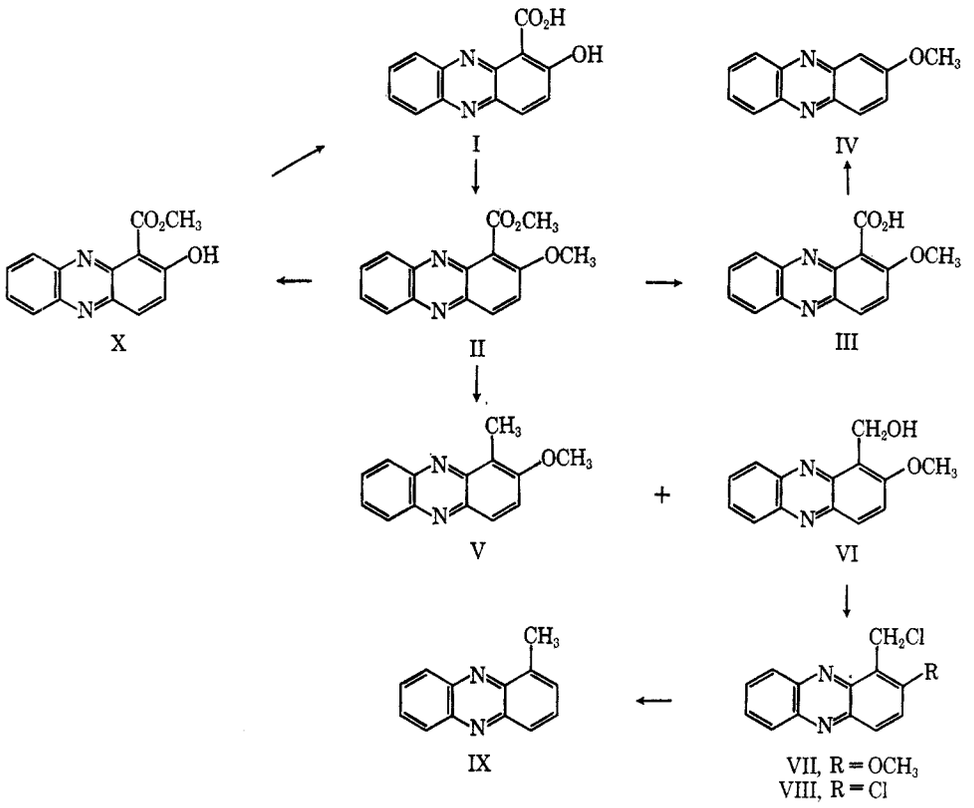
(5) R. E. Carter and J. H. Richards, *J. Am. Chem. Soc.*, **83**, 481 (1961).

(6) M. E. Levitch and P. Reitz, *Biochemistry*, **5**, 689 (1966).

(7) C. Tanford and S. Wawzonek, "Technique of Organic Chemistry," Vol. I, A. Weissburger, Ed., 3rd ed, Interscience Publishers, Inc., New York, N. Y., 1960, part 4, p 2942.

(8) R. Marsh, N. Jones, and J. H. Richards, unpublished results.

SCHEME I



tion (probably due to four adjacent hydrogens on one of the aromatic rings) occurs at 760 cm^{-1} and a weaker peak (presumably due to two adjacent hydrogens) occurs at 875 cm^{-1}.

The orange pigment (I) formed a bright yellow derivative (II) with the formula $C_{15}H_{12}O_3N_2$ on treatment with diazomethane in ether. The nmr spectrum of this compound indicates the presence of two methoxyl groups. The carbonyl peak in the infrared spectrum has shifted to 1737 cm^{-1}. If the carbonyl group of the orange pigment had, in fact, been strongly hydrogen bonded to a neighboring phenolic hydrogen, methylation of such a hydroxyl group would account for this large hypsochromic shift.

On refluxing in 10% sodium hydroxide, the dimethyl derivative (II) was converted to the methoxyphenazine carboxylic acid (III) which on decarboxylation gave 2-methoxyphenazine (IV), the identity of which was confirmed by mixture melting point and infrared spectral comparisons.

Reduction of the dimethyl derivative (II) with lithium aluminum hydride in tetrahydrofuran gave two compounds which were separated by chromatography on alumina. A yellow, fluorescent band was eluted with benzene–ether and from it bright yellow crystals were obtained. The nmr spectrum indicates the presence of one methyl and one methoxyl group. The compound has neither OH nor carbonyl stretching bands in the infrared and, hence, was presumed to be methyl-2-methoxyphenazine (V). Reduction of a carbomethoxyphenazine to a methylphenazine with lithium aluminum hydride in tetrahydrofuran has been previously reported by Nakamura.[9]

The second band, eluted with acetone, gave yellow crystals of a hydroxymethyl-2-methoxyphenazine (VI). This compound was treated with thionyl chloride and an inseparable mixture believed to be composed of chloromethyl-2-methoxyphenazine (VII) and chloromethyl-2-chlorophenazine (VIII) was obtained. Reduction of the mixture with lithium aluminum hydride in ether and chromatography of the products gave pale yellow crystals of 1-methylphenazine (IX) (Scheme I). From this evidence, the orange pigment must be 2-hydroxyphenazine-1-carboxylic acid (I). This structure has been confirmed by X-ray diffraction studies.[8]

1-Methyl-2-methoxyphenazine was synthesized and shown to be identical with the product (V) obtained by reduction of the dimethyl derivative of the orange pigment by lithium aluminum hydride. 3-Chloro-2-methyl-2′-nitrodiphenylamine, prepared by heating 3-chloro-2-methylaniline with *o*-chloronitrobenzene and sodium acetate at 250°, was reduced with ferrous oxalate at 270° to give 1-methyl-2-chlorophenazine. An N-oxide was obtained by oxidation with peracetic acid at 50°. The N-oxide function provides sufficient activation for a nucleophilic displacement of the chlorine attached to the aromatic nucleus that refluxing 1-methyl-2-chlorophenazine N-oxide with potassium hydroxide in methanol yielded 1-methyl-2-methoxyphenazine N-oxide which was reduced by lithium aluminum hydride in ether to 1-methyl-2-methoxyphenazine identical in all respects with the degradation product (V) of orange pigment. These results confirm that the orange pigment of *Ps. aureofaciens* is 2-hydroxyphenazine-1-carboxylic acid (I).

Hydrolysis of 1-carbomethoxy-2-methoxyphenazine with concentrated hydrochloric acid cleaves the methyl ether and yields 1-carbomethoxy-2-hydroxyphenazine (X) with a carbonyl band in the infrared at 1665 cm^{-1}, indicating again strong hydrogen bonding of the ester carbonyl group at C-1 to the hydroxyl group at C-2.

(9) S. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **6**, 539 (1958).

Saponification of the ester group regenerates the original orange pigment (I).

Other pigments were also isolated but in insufficient amount for characterization. One substance is rose, one violet, and one brown. These substances probably are more highly oxidized derivatives of the parent phenazine ring system.

Experimental Section

Pigment Production.—Strain NRRL-B-1543 P of *Ps. aureofaciens* was cultured by the method of Haynes, *et al.*[3]

Isolation and Purification of the Orange Pigment.—The culture medium (800 ml) was acidified with concentrated hydrochloric acid (5.0 ml) and Celite (10 g) was added. After standing at least 1 hr, the suspension was filtered and the Celite, containing the pigments, allowed to dry. The Celite pads from five cultures were combined and boiled with chloroform (250 ml). After filtering the chloroform, the Celite pads were boiled again with chloroform (100 ml) and this process was repeated six times. The combined chloroform filtrates were extracted with three 25-ml portions of 5% sodium hydroxide. The red, basic solutions were acidified with concentrated hydrochloric acid and the pigments were extracted with three 75-ml portions of chloroform. The chloroform solutions were dried over anhydrous sodium sulfate, filtered, and evaporated to give about 3.5 g of crude pigment.

Sodium hydroxide (10%) was added to the crude pigments so as to form a thin paste which was filtered by suction. Acidification of the red filtrate with 1 *N* hydrochloric acid, extraction with three 50-ml portions of chloroform, and evaporation of the chloroform after drying over anhydrous sodium sulfate yielded about 35 mg of crude red pigment. The crude pigment was crystallized twice from benzene, giving fine orange needles which darkened at about 220° and melted, with decomposition, at 225–226°. Above the decomposing pigment, yellow crystals formed which decomposed themselves at 236–237°. Further treatment of the original mixture of pigments with sodium hydroxide yielded an additional 25 mg of crude pigment from which orange pigment was obtained by repeated crystallization from benzene.

The orange pigment formed a gold-brown solution with ferric chloride and pyridine[10] and a brown solution with ceric nitrate in dioxane.[11] The pure orange pigment gave a negative Tollens test; however, the impure red pigment formed a silver mirror with ammoniacal silver nitrate.

The orange pigment (2.93 mg) was dissolved in 66.8% dioxane water (10 ml) and titrated with 0.02343 *N* sodium hydroxide on a pH-Stat.[12] An end point was observed at 0.52 ml of base, giving a neutralization equivalent of ~240. The pK_a was 7.60 ± 0.01; λ_{max}^{EtOH} 252 mμ (log ε 4.66), 363 (4.04); $\lambda_{max}^{basic\ EtOH}$ 287 mμ (log ε 4.66), 363 (397), 490 (3.90); $\nu_{max}^{CHCl_3}$ 1675, 1608, 1567, 1483, 1445, 1365, 1300, 1275, 1140, 1125, 1009, 990, 961, 910, 865, and 845 cm^{-1}; $\nu_{max}^{CS_2}$ 3005, 2955, 2845, 1212, 1122, 925, 875, 760, and 670 cm^{-1}.

Anal. Calcd for $C_{13}H_8O_3N_2$: C, 65.00; H, 3.36; N, 11.67. Found: C, 64.88; H, 3.41; N, 11.75.

Methylation of the Orange Pigment (1-Carbomethoxy-2-methoxyphenazine).—A solution of the orange pigment (100 mg) in benzene (50 ml) was methylated with ethereal diazomethane. Evaporation of solvent left a yellow powder which was chromatographed on an alumina (Merck) column. A bright yellow band was eluted with benzene–ether and recrystallized from carbon tetrachloride to give 96 mg of yellow crystals, mp 127–128°. Further purification by sublimation from 110–120° at 1 mm and recrystallization from carbon tetrachloride gave material melting at 129°: λ_{max}^{EtOH} 257 mμ (log ε 4.82), 360 (3.95); $\nu_{max}^{CHCl_3}$ 1737, 1634, 1608, 1492, 1468, 1387, 1350, 1328, 1302, 1158, 1136, 1125, 1093, 969, and 905 cm^{-1}; $\nu_{max}^{CS_2}$ 3060, 3010, 2950, 2845, 828, 820, 800, 780, and 760 cm^{-1}.

Anal. Calcd for $C_{15}H_{12}O_3N_2$: C, 67.15; H, 4.51; N, 10.44. Found: C, 67.09; H, 4.74; N, 10.30.

The nuclear magnetic resonance spectrum in carbon tetrachloride showed two nmr absorptions, one for each O–CH_3 group.

2-Methoxyphenazine-1-carboxylic Acid.—A mixture of the dimethyl derivative of the orange pigment (44 mg) and 10% aqueous sodium hydroxide (25 ml) was heated at 95° in an oil bath for 7 hr. The orange, basic solution was cooled, washed with three 25-ml portions of chloroform, and acidified with dilute hydrochloric acid. Extraction of the resulting suspension with chloroform and evaporation of the solvent after drying over anhydrous sodium sulfate gave a yellow powder which was recrystallized from benzene, yielding 25 mg of 2-methoxyphenazine-1-carboxylic acid, mp 204–205°, $\nu_{max}^{CHCl_3}$ 1717 cm^{-1}.

Anal. Calcd for $C_{14}H_{10}O_3N_2$: C, 66.13; H, 3.96; N, 11.02. Found: C, 66.30; H, 4.01; N, 11.16.

2-Methoxyphenazine (by Decarboxylation of 2-Methoxyphenazine-1-carboxylic Acid).—Decarboxylation of 20 mg of the methoxy acid (2-methoxyphenazine-1-carboxylic acid) was effected by heating in diphenyl ether (25 ml) containing precipitated copper powder (0.5 g) at 250° for 4 hr. The mixture was cooled and chromatographed on alumina (Merck). A yellow band was separated on the column with benzene and eluted from the column with chloroform. Evaporation of the chloroform left a yellow powder which was crystallized from water, giving 16 mg of light yellow crystals, mp 121–122°. The infrared spectrum was identical with that of authentic 2-methoxyphenazine and a mixture melting point was not depressed.

By Synthesis.—2-Nitro-4-methoxydiphenylamine prepared from *o*-chloronitrobenzene and *p*-anisidine was reduced to 2-methoxyphenazine by the method of Vivian[13] without modification. The 2-methoxyphenazine was purified as above by chromatography on alumina (Merck) and crystallization from water yielding yellow crystals, mp 122–123°.

Reduction of 1-Carbomethoxy-2-methoxyphenazine.—A solution of 1-carbomethoxy-2-methoxyphenazine (100 mg) in purified tetrahydrofuran (3 ml) was added to a stirred suspension of lithium aluminum hydride (100 mg) (Metal Hydrides, Inc.) in diethyl ether (20 ml). The mixture was refluxed for 3 hr. The ether was evaporated and the residue chromatographed on alumina (Merck). A yellow band, brightly fluorescent in ultraviolet light, was eluted with benzene; a second yellow band was eluted with ether–acetone. Sublimation of the yellow material from the first band at 60° and 1 mm removed a small amount of yellow impurity. Further sublimation at 80° at 1 mm gave a yellow solid which was recrystallized from 95% ethanol, giving 22 mg of yellow crystals, mp 123–124°. A mixture melting point with synthetic 1-methyl-2-methoxyphenazine was not depressed and both had identical infrared spectra. The nuclear magnetic resonance spectrum in chloroform showed singlets at δ 4.25 and 2.93 of equal area. Sublimation of the material in the second yellow band eluted with ether–acetone at 100° and 1 mm yielded yellow crystals of 1-hydroxymethyl-2-methoxyphenazine. Recrystallization from carbon tetrachloride gave 61 mg of the product, mp 173–174°.

Anal. Calcd for $C_{14}H_{12}O_2N_2$: C, 69.99; H, 5.03; N, 11.66. Found: C, 70.09; H, 5.06; N, 11.60.

Chlorination of 1-Hydroxymethyl-2-methoxyphenazine.—1-Hydroxymethyl-2-methoxyphenazine (50 mg) was warmed with 3 ml of thionyl chloride on a hot plate for 10 min. The solution was heated to boiling and the thionyl chloride evaporated. Petroleum ether (bp 30–60°) was added to the residue and then also evaporated. The residue was sublimed at 100° and 1 mm, giving some yellow material (7 mg) which was recrystallized from carbon tetrachloride. As the bath temperature was raised to 140°, more material (36 mg) was obtained. This was also recrystallized from carbon tetrachloride. Both recrystallized sublimates melted at 197–198°. The product seemed to decompose on attempted chromatography on alumina or silicic acid columns and on recrystallization from ethanol. Consistent analyses were not obtained; however, the chlorine percentage indicated the presence of both mono- and dichlorinated products.

Reduction of Mixture from Chlorination.—A solution of 25 mg of the above mixture obtained by chlorination of 1-hydroxymethyl-2-methoxyphenazine with thionyl chloride was added dropwise to a stirred suspension of 50 mg of lithium aluminum hydride in 20 ml of diethyl ether and the mixture refluxed for 2 hr. After decanting, the ether was evaporated and the residue chromatographed on alumina. Elution with benzene separated

(10) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed, John Wiley and Sons, Inc., New York, N. Y., 1956, p 110.

(11) See ref 10, p 112.

(12) J. B. Nielands and M. D. Cannon, *Anal. Chem.*, **27**, 29 (1955).

(13) D. L. Vivian, J. Hartwell, and H. Waterman, *J. Org. Chem.*, **19**, 1136 (1957).

a pale yellow band and sublimation of this band gave light yellow crystals of 1-methylphenazine (7 mg), mp 109°, undepressed on admixture with an authentic sample. No other crystalline products could be obtained by further elution of the column.

1-Carbomethoxy-2-hydroxyphenazine.—To a red solution of 1-carbomethoxy-2-methoxyphenazine (50 mg) in 20 ml of concentrated hydrochloric acid were added 2 drops of a solution of 0.5 g of stannous chloride in 2 ml of concentrated hydrochloric acid (warmed with tin until clear) at 0°. A dark green complex formed immediately and, after 2 min, the solution turned yellow. The yellow solution was diluted with 30 ml of water and extracted with three 40-ml portions of chloroform. The combined chloroform extracts were shaken with 25 ml of 5% sodium hydroxide solution, giving an orange aqueous solution. This was acidified with hydrochloric acid and extracted with three 40-ml portions of chloroform. The extract was washed with water, dried over anhydrous sodium sulfate, and the chloroform evaporated, giving an orange powder which was crystallized from a small amount of carbon tetrachloride and sublimed at 110° and 1 mm. The yellow sublimate was recrystallized from methanol (with the use of Norit), giving 36 mg of yellow crystals, mp 140–141°. The compound was slightly soluble (about 0.5%) in sodium bicarbonate solution, but was easily extracted from the bicarbonate solution with chloroform. A ferric chloride test in water was negative; however, in chloroform, a red complex formed with ferric chloride, confirming the phenolic nature of the product which showed $\nu_{max}^{CHCl_3}$ 1665 cm^{-1}.

Anal. Calcd for $C_{14}H_{10}O_3N_2$: C, 66.13; H, 3.96. Found: C, 65.87; H, 3.89.

2-Hydroxyphenazine-1-carboxylic Acid.—A solution of 1-carbomethoxy-2-hydroxyphenazine (15 mg) in 10% sodium hydroxide (25 ml) was refluxed for 1 hr. The solution was then cooled, acidified, and extracted with chloroform which was dried over anhydrous sodium sulfate and evaporated, yielding an orange powder. Recrystallization from benzene afforded 3 mg of orange needles, mp 218–221°, of 2-hydroxyphenazine-1-carboxylic acid (the mixture melting point was not depressed on admixture with the orange pigment and the infrared spectra of the two samples were identical).

Synthesis of 1-Methyl-2-methoxyphenazine. 2-Nitro-3′-chloro-2′-methyldiphenylamine.—*o*-Chloronitrobenzene (25 g, 0.16 mole) was heated with 3-chloro-2-methylaniline (35 g, 0.25 mole) and anhydrous sodium acetate (25 g) in a flask with a condenser at 270° for 20 hr. The resulting black mixture was steam distilled until no more organic material appeared in the distillate. The black oil remaining in the distillation flask was chromatographed on alumina (Merck). Elution with petroleum ether–benzene gave some orange material which on recrystallization from ethanol yielded yellow crystals (7.4 g, 18%) of 2-nitro-3′-chloro-2′-methyldiphenylamine, mp 92–93°.

Anal. Calcd for $C_{13}H_{11}O_2N_2Cl$: C, 59.42; H, 4.22; N, 10.65; Cl, 13.50. Found: C, 59.75; H, 4.41; N, 10.90; Cl, 13.73.

1-Methyl-2-chlorophenazine.—A mixture of 5 g (0.19 mole) of 2-nitro-3′-chloro-2′-methyldiphenylamine, 6.6 g of ferrous oxalate (prepared by stirring together equimolar solutions of ferrous sulfate and oxalic acid and drying the precipitate in air), and 50 g of granulated lead was heated to 270° for 30 min. The mixture was cooled to 100° and sublimed at 1-mm pressure. The yellow sublimate was chromatographed on alumina (Merck); petroleum ether–benzene eluted 2.4 g of unreacted 2-nitro-3′-chloro-2′-methyldiphenylamine. The desired product was eluted with ether and crystallized from ethanol, yielding 1.6 g (70% based on unrecovered 2-nitro-3′-chloro-2′-methyldiphenylamine) of yellow, crystalline 1-methyl-2-chlorophenazine, mp 134°.

Anal. Calcd for $C_{13}H_9N_2Cl$: C, 68.29; H, 3.96; N, 12.24; Cl, 15.51. Found: C, 68.36; H, 4.04; N, 12.22; Cl, 15.45.

1-Methyl-2-chlorophenazine N-oxide.—1-Methyl-2-chlorophenazine (1.5 g, 6.5 mmole) was dissolved in 75 ml of warm acetic acid and 7.5 ml of 30% hydrogen peroxide was added. The solution was heated at 50° for 24 hr. On addition of 75 ml of water, the N-oxide crystallized and was collected. Recrystallization from ethanol gave 1.4 g (88%) of bright yellow needles of 1-methyl-2-chlorophenazine N-oxide, mp 175–176°, $\nu_{max}^{CHCl_3}$ 1342 cm^{-1}.

Anal. Calcd for $C_{13}H_9ON_2Cl$: C, 63.76; H, 3.71; N, 11.52; Cl, 14.48. Found: C, 63.87; H, 3.62; N, 11.36; Cl, 14.60.

1-Methyl-2-methoxyphenazine N-Oxide.—To a hot solution of 1-methyl-2-chlorophenazine N-oxide (1.7 g, 6.5 mmoles) in 125 ml of methanol was added 25 ml of an aqueous solution containing 9.0 g of potassium hydroxide. The solution was stirred and refluxed for 24 hr. The resulting greenish solution was extracted three times with ether. The ether was evaporated and the residue chromatographed over alumina (Merck). Elution with benzene–ether separated the material into three bands. The first band yielded 120 mg (8%) of 1-methyl-2-chlorophenazine. The second band, which had a green fluorescence, was unreacted starting material (565 mg, 34%). Th third band, which had a yellow fluorescence, gave, after recrystallization from ethanol, 282 mg (17%) of yellow needles of 1-methyl-2-methoxyphenazine oxide, $\nu_{max}^{CHCl_3}$ 1342 cm^{-1}.

Anal. Calcd for $C_{14}H_{12}O_2N_2$: C, 69.99; H, 5.03. Found: C, 69.75; H, 5.31.

1-Methyl-2-methoxyphenazine.—Reduction of 1-methyl-2-methoxyphenazine N-oxide (100 mg, 0.39 mmole) with lithium aluminum hydride was carried out in the same manner as in the case of the dimethyl derivative of the orange pigment described above. After chromatographic purification, sublimation and recrystallization from ethanol yielded 35 mg (38%) of 1-methyl-2-methoxyphenazine, mp 125°.

Anal. Calcd for $C_{14}H_{12}ON_2$: C, 74.99; H, 5.38; N, 12.49. Found: C, 75.16; H, 5.57; N, 12.57.

Isolation of Additional Pigments.—A particularly dark red batch of the impure orange pigment (50 mg) was treated with diazomethane in the manner described previously and the product chromatographed on alumina (Merck). First a small amount of 1-carbomethoxyphenazine (5 mg) was eluted, then 35 mg of 1-carbomethoxy-2-methoxyphenazine. Elution with methanol–ether separated the remaining material into three bands. Not enough of these pigments were obtained for crystallization or analysis, hence, only spectral data were determined. First to be eluted was a rose pigment (4 mg): $\nu_{max}^{CS_2}$ 3495, 3470, 3380, 3290, 2950, 2880, 1740, 1660, 1380, 1365, 1322, 1295, 1196, 1110, 1086, 1075, 1025, 955, 948, 875, 828, 818, and 796 cm^{-1}; λ_{max}^{EtOH} 257 mμ (log ε 4.9), 300 (sh) (3.9), 485 (2.8). Next, a violet pigment (3 mg) was obtained: $\nu_{max}^{CHCl_3}$ 3625, 3520, 3470, 3340, 2975, 2945, 2890, 1725, 1653, 1605, 1567, 1384, 1297, 1135, 1075, 1042, 875, 836, and 826 cm^{-1}; λ_{max}^{EtOH} 940 mμ (log ε 4.6), 255 (4.6), 282 (4.3), 293 (4.3), 364 (4.2), and 535 (2.8). A third, brown pigment was also obtained but in insufficient quantity even for spectral determination.

Registry No.—I, 4075-25-6; II, 13392-00-2; III, 13392-01-3; IV, 2876-18-8; V, 13392-03-5; VI, 13392-04-6; VII, 13389-18-9; VIII, 13421-42-6; IX, 1016-59-7; X, 13389-20-3; 2-nitro-3′-chloro-2′-methyldiphenylamine, 13389-21-4; 1-methyl-2-chlorophenazine, 7495-32-1.