H, 5.51; N, 12.28. Found (prisms): C, 74.82; H, 5.40; N, 12.41. The two kinds of substances gave no melting point depression on admixture.

Summary

Griseolutein-B and -A were purified by the counter-current distribution method and their properties were examined. Diacetylgriseolutein-B was easily purified and crystallized. It had one methoxyl group, two acetyl groups, and one carboxyl group, but no C-methyl group or N-oxide group. Its methyl ester was obtained. Alkaline hydrolysis of diacetylgriseolutein-B gave griseoluteic acid which was crystallized as its methyl ester or as monomethyl ester of monoacetylgriseoluteic acid. Distillation of griseoluteic acid with zinc dust gave phenazine, 1-methoxyphenazine, and another compound. This compound was identified as 1-methoxy-4-methylphenazine by comparison with the synthesized sample. 1-methoxy-4-methylphenazine was synthesized from aniline and 3-nitro-4-methoxytoluene and its two crystal forms were observed.

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109. Shoshiro Nakamura: Studies on the Structure of Griseolutein-B, a Streptomyces Antibiotic. II[1]. Decarboxylation and Periodic Acid Oxidation.

(*Institute of Applied Microbiology,* University of Tokyo*)

As described in the preceding paper,[1] griseolutein-B, $C_{17}H_{16}O_6N_2$, was easily purified and crystallized as diacetylgriseolutein-B, $C_{21}H_{20}O_8N_2 \cdot H_2O$, in which one methoxyl group, two acetyl groups, one carboxyl group, and no C-methyl group were determined. Alkaline hydrolysis of diacetylgriseolutein-B gave an acid, named griseoluteic acid. Monomethyl ester of griseoluteic acid, $C_{16}H_{14}O_4N_2$, and monomethyl ester of monoacetylgriseoluteic acid, $C_{18}H_{16}O_5N_2$, were crystallized. The zinc-dust distillation of griseoluteic acid gave phenazine, 1-methoxyphenazine, and 1-methoxy-4-methylphenazine. In this paper, results of decarboxylation of diacetylgriseolutein-B and periodic acid oxidation of griseolutein-B are described, and on the basis of these results partial structures of griseoluteic acid and griseolutein-B are presented.

Diacetylgriseolutein-B was decarboxylated in quinoline by heating at 230° for 2 hours, using copper dust as a catalyst. The reaction product was subjected to alumina chromatography for purification and the fraction obtained by elution with benzene gave yellow crystals, $C_{16}H_{14}O_3N_2$, m.p. 139°, U.V. λ_{max}^{MeOH} $m\mu(E_{1cm}^{1\%})$: 260~261 (1660), 361~362 (265) (Fig. 1). Another crop was obtained from the effluent with ethyl acetate as yellow crystals, $C_{14}H_{12}O_2N_2$, m. p. 196°, U.V. λ_{max}^{MeOH} $m\mu(E_{1cm}^{1\%})$: 263 (1945), 362 (324) (Fig. 1). No difference was found between the former and the acetylated product of the latter on admixture and by comparison of their infrared spectra. The latter was presumed to be 1-methoxy-4-hydroxymethylphenazine because of the following facts: 1-Methoxy-4-methylphenazine[1] was obtained as one of zinc-dust distillation

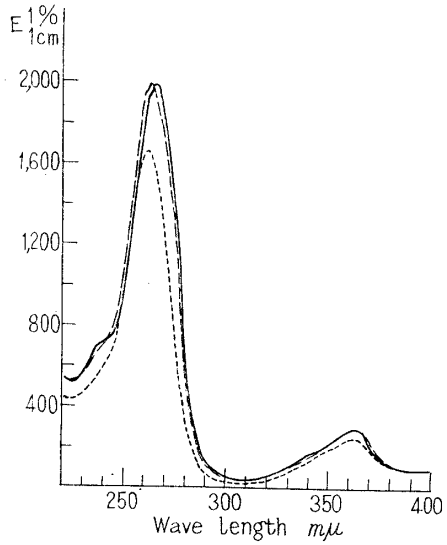

Fig. 1. Ultraviolet Absorption Spectra (in MeOH)
——— 1-Methoxyl-4-methylphenazine
— — — Decarboxylation product $C_{14}H_{12}O_2N_2$, m. p. 196°
- - - - - Decarboxylation product $C_{16}H_{14}O_3N_2$, m. p. 139°

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1) Part I. S. Nakamura: This Bulletin, 6, 539(1958).

products of griseoluteic acid; its ultraviolet absorption spectrum (Fig. 1) was very similar to that of 1-methoxy-4-methylphenazine; the absorption bands[2] (2.85, 7.08, 9.24, and 10.10μ)

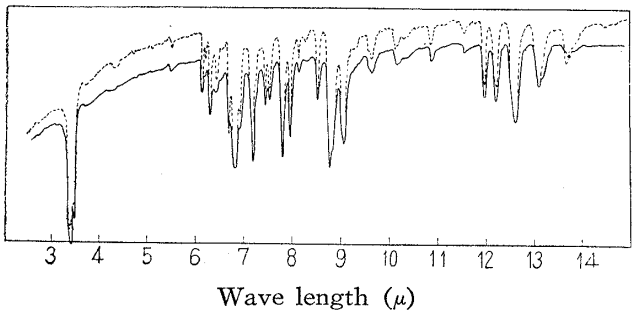
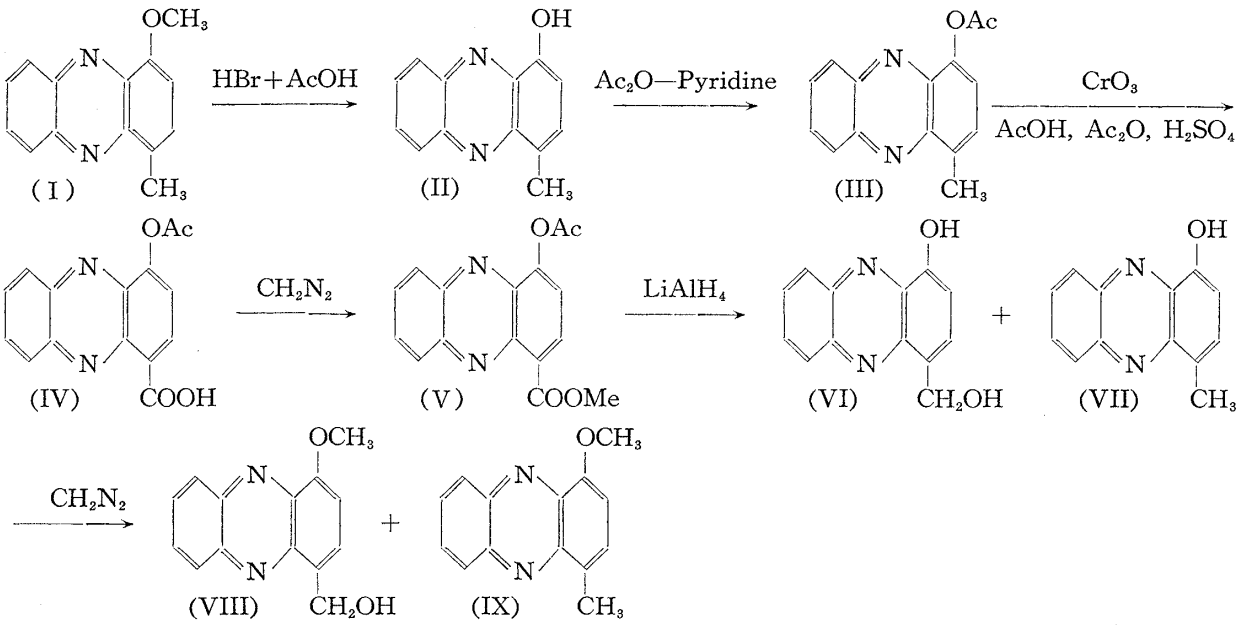


Fig. 2. Infrared Spectra (in Nujol)
——— 1-Methoxy-4-hydroxymethylphenazine
- - - - - Decarboxylation Product $C_{14}H_{12}O_2N_2$, m. p. 196°

in its infrared spectrum (Fig. 2) suggested the presence of hydroxymethyl group; the strong bands[3] resulting from four adjacent, free hydrogen atoms (13.26 μ) and two adjacent hydrogen atoms (11.92, 12.05, or 12.48 μ) were observed in the out-of-plane C-H vibration; it was not soluble in aqueous sodium hydroxide and did not react with diazomethane, but was acetylated with acetic anhydride-pyridine.

1-Methoxy-4-hydroxymethylphenazine was synthesized by the following route:



Birkofer[4] obtained 1-hydroxymethylphenazine by the reduction of 1-methoxycarbonylphenazine with lithium aluminum hydride in ether, but he did not obtain 1-methylphenazine. However, in the present experiment, reduction of 1-acetoxy-4-methoxycarbonylphenazine (V) with lithium aluminum hydride in ether produced 1-hydroxy-4-hydroxymethylphenazine (VI) and 1-hydroxy-4-methylphenazine (VII) simultaneously. The reaction products were methylated with diazomethane and separated by alumina chromatography into 1-methoxy-4-hydroxymethylphenazine (VIII) and 1-methoxy-4-methylphenazine (IX), and the latter was comfirmed by comparison with an authentic sample synthesized by another method described in the preceding paper.[1]

One of the products (m. p. 196°) obtained by the decarboxylation of diacetylgriseolutein-B was identified with synthetic 1-methoxy-4-hydroxymethylphenazine by the mixed melting point test and comparison of the infrared spectra (Fig. 2).

2) L. J. Bellamy: "The Infrared Spectra of Complex Molecules," Methuen & Co., London, 83 (1954).
3) L. J. Bellamy: *Ibid.* 54(1954).
4) L. Birkofer, A. Birkofer: Chem. Ber., **85**, 286(1952).
5) D. Glick: "Methods of Biochemical Analysis," **3**, 111.

Griseolutein-B was dissolved in sodium hydrogen carbonate solution and oxidized[5] with 0.1*N* periodic acid at room temperature for 1 hour. One mole of periodic acid was consumed and griseoluteic acid,[1] formaldehyde,[5] and formic acid[5] were produced. The unreacted periodic acid was reduced with an excess of standard sodium arsenite solution, the aqueous solution was adjusted to pH 5.4~5.6 with 2*N* acetic acid, the same volume of buffer solution of sodium acetate-hydrochloric acid (containing equal volumes of 2*N* sodium acetate and *N* hydrochloric acid) was added, and the mixture was filtered to remove reddish brown precipitate of griseoluteic acid. The filtrate, on addition of 0.4% dimedone solution, was allowed to stand over night. The white crystals (0.88 mole) of methylene-bismethone, m.p. 186°, were obtained and identity was confirmed by mixed melting point test with authentic sample. The methyl ester of the acid obtained as reddish brown precipitate (0.90 mole) was identified as methyl griseoluteate by mixed melting point test with an authentic sample and comparing their infrared spectra (Fig. 3).

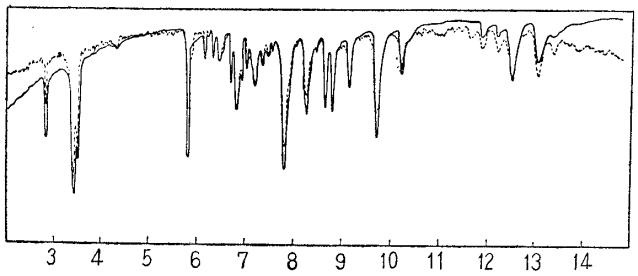


Fig. 3. Infrared Spectra of Methyl Griseoluteate (in Nujol)

——— from alkaline hydrolysis
- - - - - from periodic acid oxidation

Griseolutein-B was oxidized by the same method and excess periodic acid was decomposed with ethylene glycol. The reaction mixture was lyophilized after pH was adjusted to 7.4~7.6. The resulting product was dissolved in water, and saturated sodium acetate solution and 4*N* hydrochloric acid were added. The precipitate of griseoluteic acid was removed by filtration, 10% mercuric chloride solution was added to the filtrate, and the mixture was boiled in the dark for 1 hour. The precipitate of mecrurous chloride equivalent to 0.41 mole of formic acid was determined gravimetrically.

The periodic acid oxidation of griseolutein-B to produce griseoluteic acid, formic acid, and formaldehyde after consuming 1 mole of the acid indicates the following structure: The carbon atom carrying secondary hydroxyl, adjacent to the terminal carbon atom carrying primary hydroxyl, is linked to griseoluteic acid by oxygen bridge as shown in Chart 1.

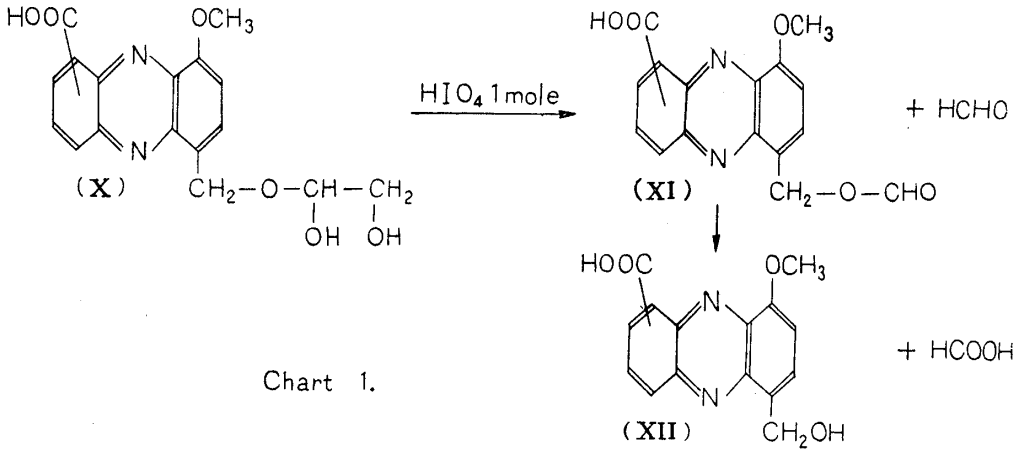


Chart 1.

O-Formylgriseoluteic acid (XI) was not stable in alkaline solution and hydrolyzed to griseoluteic acid (XII) and formic acid immediately. About 0.6 mole of oxalic acid was obtained when diacetylgriseolutein-B was fused with potassium hydroxide at 250° for 40 minutes. Oxalic acid was expected to be produced by oxidation of 1,2-diacetoxyethoxy

group. As described above, 1-methoxy-4-hydroxymethylphenazine and its acetate were obtained as the decarboxylation products of diacetylgriseolutein-B. It was assumed that 1, 2-diacetoxyethoxymethyl group was decomposed to hydroxymethyl and 1, 2-diacetoxyethanol with decarboxylation, and the acetyl group of the latter moved to the former as shown in Chart 2.

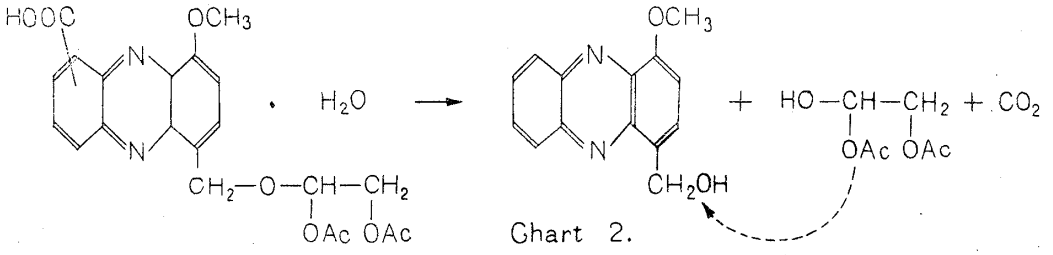


Chart 2.

The total structure of griseolutein-B, whose partial structure (X) is shown here, will be presented in the following report.

The author expresses his sincere thanks to Prof. H. Umezawa and Prof. Y. Sumiki of this Institute for their kind guidances and directions in this study. He also expresses his deep thanks to Prof. S. Sugasawa for valuable advices. The author is also indebted to Mr. Maeda and Mr. Osato, National Institute of Health, Tokyo, for their generous supply of griseolutein-B.

Experimental

Decarboxylation of Diacetylgriseolutein-B—A mixture of diacetylgriseolutein-B (500 mg.), Cu dust (50 mg.), and quinoline (5 cc.) was heated at 230° for 2 hrs. in an oil bath. After quinoline was removed by steam distillation, the residual precipitate was extracted with $CHCl_3$ and chromatographed on alumina. Yellow crystals (13 mg.), m.p. 139° (from hexane), were obtained from the effluent with benzene. *Anal.* Calcd. for $C_{16}H_{14}O_3N_2$: C, 68.07; H, 5.00; N, 9.92. Found: C, 68.24; H, 5.07; N, 9.99.

Further crop of yellow needles (25 mg.) was obtained from the effluent with AcOEt and recrystallized from hexane, m.p. 196°. *Anal.* Calcd. for $C_{14}H_{12}O_2N_2$: C, 69.99; H, 5.03; N, 11.66. Found: C, 69.79; H, 5.11; N, 11.44.

The latter was identified as 1-methoxy-4-hydroxymethylphenazine on admixture with a synthesized sample and by comparison of their infrared spectra. The former crystals (m. p. 139°) did not show any depression of the melting point when mixed with the acetate (m. p. 139°) of 1-methoxy-4-hydroxymethylphenazine.

Synthesis of 1-Methoxy-4-hydroxymethylphenazine—i) 1-Hydroxy-4-methylphenazine[1] (II): 1-Methoxy-4-methylphenazine (760 mg.) was refluxed in HBr (sp. gr. 1.45) (10 cc.) and AcOH (7 cc.) for 15 hrs. in an oil bath. After cool, water was added and the solution was neutralized with NH_4OH. The yellow precipitate was collected and recrystallized from benzene to 410 mg. of orange needles, m. p. 196°. *Anal.* Calcd. for $C_{13}H_{10}ON_2$: C, 74.27; H, 4.79; N, 13.30. Found: C, 74.11; H, 4.82; N, 13.15.

ii) 1-Acetoxy-4-methylphenazine (III): 1-Hydroxy-4-methylphenazine (360 mg.) was acetylated with Ac_2O (5 cc.) and pyridine (2 cc.). The reaction mixture was diluted with water, the yellow precipitate was collected, and it was recrystallized from hexane to 420 mg. of yellow needles, m.p. 170~171°. *Anal.* Calcd. for $C_{15}H_{12}O_2N_2$: C, 71.41; H, 4.80; N, 11.11. Found: C, 71.21; H, 4.81; N, 11.02.

iii) 1-Acetoxy-4-carboxyphenazine (IV): 1-Acetoxy-4-methylphenazine (110 mg.) was dissolved in a mixture of AcOH (2 cc.), Ac_2O (0.5 cc.), and H_2SO_4 (0.25 cc.) under cooling with ice water, then CrO_3 (200 mg.) was added in small portions with stirring. While the stirring was continued for 1.5 hrs., the temperature was maintained at 15~20°. The mixture was diluted with water, the yellow precipitate was collected, and 48 mg. of yellow needles were obtained after recrystallization from MeOH, m.p. 197~199°. *Anal.* Calcd. for $C_{15}H_{10}O_4N_2$: C, 63.83; H, 3.57; N, 9.93. Found: C, 64.06; H, 3.64; N, 9.71.

iv) 1-Acetoxy-4-methoxycarbonylphenazine (V): CH_2N_2 was added to MeOH solution of 1-acetoxy-4-carboxyphenazine. Yellow needles, m.p. 161~162°, were obtained after recrystallization from MeOH. *Anal.* Calcd. for $C_{16}H_{12}O_4N_2$: C, 64.86; H, 4.08; N, 9.46. Found: C, 64.59; H, 4.06; N, 9.63.

v) 1-Methoxy-4-hydroxymethylphenazine (VI): A solution of 1-acetoxy-4-methoxycarbonylphenazine (60 mg.) dissolved in ether (60 cc.) was dropped into the ether solution (10 cc.) of $LiAlH_4$ (30 mg.) with stirring at room temperature. The reaction mixture colored reddish-violet. This was refluxed gently for 2 hrs., cooled, and water was added slowly. The ether layer was separated and the aqueous phase was extracted twice with benzene. The extracts were combined with the ether phase and distilled *in vacuo*. A mixture of crystals (40 mg.) of 1-hydroxy-4-hydroxymethylphenazine (VI) and 1-hydroxy-4-methylphenazine (VII) was obtained. The mixture was methylated with CH_2N_2 and separated on alumina. The yellow needles, m.p. 149° (20 mg.), obtained from the fraction eluted with benzene, were

identified as 1-methoxy-4-methylphenazine (IX) on admixture with an authentic specimen. The second crop of yellow crystals (VIII), m. p. 196° (13 mg.), obtained from the fractions eluted with AcOEt was recrystallized from hexane. *Anal.* Calcd. for $C_{14}H_{12}O_2N_2$: C, 69.99; H, 5.03. Found: C, 70.22; H, 4.98.

Periodic Acid Oxidation of Griseolutein-B—i) Determination of periodic acid[5]: To a solution of griseolutein-B (49.60 mg.$=0.1440\times10^{-3}M$) dissolved in N $NaHCO_3$ (5 cc.) and water (15 cc.), 0.1N HIO_4 solution (10 cc.) was added and the mixture was allowed to stand for 1 hr. at room temperature. From this reaction mixture 5.0 cc. aliquot was pipetted out, 5 cc. of a saturated $NaHCO_3$ solution, 5 cc. of 0.048M arsenite solution, and 1 cc. of $NaHCO_3$ solution containing 20% KI were added with 1 cc. of a 1% soluble starch solution as indicator. 3.06 cc. of 0.045M iodine solution was required to titrate the excess arsenite. On the other hand, 2.53 cc. of 0.045M iodine solution was consumed by the blank test carried out by the same procedure. Therefore,

$$(3.06-2.53)\times0.045\times30/5\times10^{-3}M=0.1431\times10^{-3}M$$

ii) Determination of formaldehyde[5] and griseoluteic acid[1]: Griseolutein-B (64.32 mg.$=0.1868\times10^{-3}M$) was dissolved in N $NaHCO_3$ (0.7 cc.) and water (4.5 cc.), 0.1N HIO_4 solution (5 cc.) was added and the mixture was allowed to stand 1 hr. at room temperature. After the solution was adjusted to pH 7.4, standard arsenite solution (6 cc.) was added, and the mixture was allowed to stand at room temperature for 1 hr. The solution was acidified with 2N AcOH to pH 5.6 and AcONa-HCl buffer (10 cc.) was added. The brown red precipitate was removed by filtration. 0.4% dimedone solution (6 cc.) was added and the mixture was allowed to stand for 24 hrs. The white needles of methylene-bismethone were collected, washed with water, and dried. m.p. 186°. Yield, 48.5 mg. (Calcd. for $0.1868\times10^{-3}M=55.10$ mg.). It was proved to be methylene-bismethone by admixture with authentic sample. The reddish brown precipitate (47 mg.) (Calcd. for $0.1868\times10^{-3}M=52.05$ mg.) was methylated with CH_2N_2 and orange yellow needles of m. p. 188~189° were obtained. It was identified as methyl griseoluteate on admixture and by comparison of their infrared spectra.

iii) Determination of formic acid[5]: Griseolutein-B (62.45 mg.$=0.1814\times10^{-3}M$) was dissolved in N $NaHCO_3$ (0.7 cc.) and water (4.5 cc.), 0.1N HIO_4 solution (5 cc.) was added, and the mixture was allowed to stand for 1 hr. at room temperature. 0.04 cc. of ethylene glycol was added to the solution to decompose the excess HIO_4, and the mixture was allowed to stand for 1 hr. The solution was adjusted to pH 7.4~7.6 and lyophilized. The residue was dissolved in water (15 cc.) and was added with 1 cc. of a saturated AcONa solution and 0.2 cc. of 4N HCl. After the precipitate of griseoluteic acid was filtered off, 2 cc. of 10% $HgCl_2$ solution was added to the filtrate, and the mixture was heated in a boiling water bath in the dark for 1 hr. After cooling to 5°, Hg_2Cl_2 was collected, washed with water, and dried. Yield, 35 mg. (Calcd. for $0.1814\times10^{-3}M=85.44$ mg.).

Summary

Decarboxylation of diacetylgriseolutein-B in qinoline with copper gave 1-methoxy-4-hydroxymethylphenazine. The periodic acid oxidation of griseolutein-B gave griseoluteic acid, formic acid, and formaldehyde. From these results a partial structure of griseolutein-B was presented. The reduction of 1-acetoxy-4-methoxycarbonylphenazine with lithium aluminum hydride which gave 1-hydroxy-4-hydroxymethylphenazine and 1-hydroxy-4-methylphenazine was described.

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110. Shoshiro Nakamura: Studies on Structure of Griseolutein-B, a Streptomyces Antibiotic. III.[2] The Complete Structure.

(*Institute of Applied Microbiology,* University of Tokyo*)

Diacetylgriseolutein-B[1] ($C_{21}H_{20}O_8N_2\cdot H_2O$) can be easily purified and crystallized. It has one methoxyl, two acetyl, and one carboxyl groups but no C-methyl group. Its alkaline hydrolysis gave an acid, named griseoluteic acid.[1] which was crystallized as its methyl ester ($C_{16}H_{14}O_4N_2$) or as a monomethyl ester of its monoacetyl derivative ($C_{18}H_{16}O_5N_2$). Zinc dust

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1) Part I. S. Nakamura: This Bulletin, **6**, 539(1958).
2) Part II. S. Nakamura: This Bulletin, **6**, 543(1958).